Age-dependent Glomerular Damage in the Rat

Dissociation between Glomerular Injury and both Glomerular Hypertension and Hypertrophy. Male Gender as a Primary Risk Factor

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Abstract

The glomerulus develops progressive injury with advancing age which is particularly pronounced in males and is not the result of any specific disease process. In the present studies conducted in rats, glomerular function and structure were examined in adult (8 mo), elderly (12 mo), and old (19 mo) Munich Wistar rats. Intact males and females and castrated rats of both sexes were studied to determine the role of the sex hormones in mediating age-dependent glomerular damage. Intact males developed glomerular injury and proteinuria whereas females, both intact and ovariectomized, and castrated males were protected from injury. Glomerular blood pressure did not increase with advancing age in any group and did not correlate with glomerular damage. Glomerular volume did increase with advancing age in all groups but did not correlate with glomerular damage. We found that the presence of the androgens rather than the absence of the estrogens provide the risk factor for development of age-dependent glomerular damage. Neither glomerular hypertension nor glomerular hypertrophy provide the primary mechanism by which age-dependent glomerular injury occurs in the intact male. (*J. Clin. Invest.* 1994. 94:1823–1829.) Key words: ageing • glomerulus • glomerular blood pressure • glomerular volume • glomerular injury

Introduction

In men, glomerular filtration rate (GFR)[1] begins a slow decline of $\sim 1\%$ per year after the age of 40 (1, 2). Reductions in renal blood flow and renal plasma flow also occur with advancing

1. *Abbreviations used in this paper:* BP, mean arterial blood pressure; BW, body weight; GFR, glomerular filtration rate; K_f, glomerular ultrafiltration coefficient; KW, kidney weight; P_{GC}, glomerular capillary blood pressure; P_T, proximal tubule hydrostatic pressure; Q_A, glomerular plasma flow; R_A, preglomerular arteriolar resistance; R_E, efferent arteriolar resistance; RPF, renal plasma flow; SN, single nephron; U_pV, urinary protein excretion; V_g, mean glomerular volume.

age (3, 4) and morphologic changes include focal and segmental glomerular sclerosis and a reduction in the number of functioning glomeruli (1, 2). This age-dependent decline in glomerular function occurs in the absence of any specific underlying renal disease and the rate of decline is sufficiently slow enough that it does not compromise overall renal function (1, 2). Because of these decremental hemodynamic changes, however, the aging kidney is at increased risk to develop toxic or ischemic acute renal failure, or chronic renal disease due to multiple causes including systemic hypertension (2). Spontaneous declines in glomerular function and structural injury also appear in the aging rat. Chronic renal disease is a major cause of death in some rat strains, although the incidence of age dependent glomerular damage is variable between strains (5–10). As with man, the kidney of the aging rat also shows an increased susceptibility to renal damage due to other causes (5).

There is good evidence that increases in glomerular capillary blood pressure (P_{GC}) play a pathogenic role in a number of primary glomerular diseases (10). Certainly the presence of systemic hypertension in man accelerates the rate of age-dependent reductions in GFR (1, 2). In rats, modification of diet influences the rate of progression of primary glomerular diseases. Dietary protein restriction protects, and high dietary protein feeding exacerbates glomerular injury, by reducing and increasing P_{GC} respectively (10). It is also well established that caloric and specifically protein restriction attenuate the rate of development of glomerular lesions in aging rat colonies (5, 10). Based on these observations, Anderson and Brenner (10) have suggested that age dependent glomerulopathy occurs secondary to age dependent increases in P_{GC} (10).

In addition to diet, gender is another major determinant of the rate at which glomerular structural injury and derangements in renal hemodynamics occur with advancing age, since renal functional declines are delayed in females compared with males (3, 4, 7). To further investigate the mechanisms of age-dependent glomerular injury we conducted the present studies in Munich Wistar rats aged from 7–20 mo. Measurements were made of glomerular hemodynamics, 24-h protein excretion, glomerular histologic injury and morphometric analysis of glomerular volumes in intact male rats. To investigate the sex difference, similar studies were also conducted in intact female rats. To gain further insight into the role of sex hormones in the pathogenesis of age dependent glomerular injury, additional studies were conducted in castrated rats of both sexes.

Methods

Studies were conducted on 95 Munich Wistar rats derived from a mycoplasma-free colony at Simonsen Laboratories (Gilroy, CA). Virgin rats of both sexes were selected at 8–10 wk of age and 50% of males were castrated and 50% of females were ovariectomized. Rats were then aged up to 20 mo, under barrier conditions to eliminate air-borne diseases.

Cohorts of rats were established over a 6-mo period and subgroups were taken out for study at the following ages; 7–9 mo (~ 8 mo), 11–13 mo (~ 12 mo), and 18–20 mo (~ 19 mo). Rats were shipped to our laboratory 2–4 wk before study where they were maintained in positive pressure laminar flow hoods. Throughout their lives rats had ad libitum access to drinking water and standard rat chow containing ~ 24% protein and 0.4% Na.

Before acute study, rats were placed in metabolic cages for collection of urine which was analyzed for total protein concentration, by the Bradford assay (11). On the day of micropuncture study, rats were anesthetized with ip thiobarbiturate (100–120 mg/kg body weight) and placed on a temperature-regulated table to maintain core temperature at 36–38°C throughout the experiment. The left femoral artery was catheterized and used for periodic blood sampling and to monitor arterial blood pressure, by a pressure transducer connected to a recorder. The left femoral vein was cannulated for infusion of isoncotic artificial serum at 1% body weight per hour during the preparatory surgery and thereafter at 0.15% body weight/h, to maintain plasma volume (12). A tracheostomy was performed and the left jugular vein was catheterized for infusion of ^{3}H-inulin (100 μCi/ml 0.9% NaCl, NEN, Boston, MA) at ~ 40 μCi/100 g body weight per hour. A midline abdominal incision was made and the left kidney was prepared for micropuncture as described earlier (13). The left ureter was cannulated with PE10 for urine collection directly into graduated tubes. For measurement of P_{GC} by the indirect, stop-flow pressure method, tubule fluid flow was stopped by insertion of paraffin wax blocks into 5–7 randomly selected, mid-proximal surface nephron segments.

After a 45–60-min equilibration period at the end of surgery, two (20–30 min) urine collections were made and midpoint femoral arterial blood samples were taken. Simultaneously, the following measurements and collections were made by micropuncture in the superficial renal cortex: P_{GC} was measured by direct puncture of accessible superficial glomeruli ($n = 2$–4) and indirect P_{GC} in deeper glomeruli was calculated from the stop-flow pressure + afferent arteriolar oncotic pressure (π_A). The stop-flow pressure was measured in the earliest segment proximal to the wax block in the 5–7 blocked tubules. In addition, hydrostatic pressure was measured in 2–3 efferent arterioles and in 10 proximal tubule segments (P_T) by direct puncture with micropipettes (2–4 μm tip diameter), containing 1.2 M NaCl solution. These micropipettes formed part of a servonull micropressure measuring system (model 4a; Instruments for Physiology and Medicine, San Diego, CA) coupled to a pressure transducer and recorder. In addition, 5–6 exactly timed (2–4 min) samples of fluid from superficial proximal tubules and samples of blood from 3–5 superficial efferent arterioles were collected. At the end of the experiment the left kidney was removed, weighed, sliced longitudinally, and fixed in 10% buffered formalin for later histology and morphometry studies.

Activity of ^{3}H-inulin was counted in a liquid scintillation counter in the entire tubular fluid sample, in 5 μl plasma samples and in 1-μl urine samples. Total protein concentrations in postglomerular (efferent) arteriolar and systemic (femoral) arterial plasma samples were measured by a microadaption of the Lowry method (14). From these measurements, calculations were made of GFR, single nephron (SN) GFR, single nephron filtration fraction (SNFF), glomerular plasma flow (Q_A), afferent and efferent arteriolar oncotic pressures (π_A and π_E), the glomerular ultrafiltration coefficient (K_f), and preglomerular and efferent arteriolar resistances (R_A and R_E) as have been described previously (13). All statistical analyses of functional studies were performed using unpaired t test or the appropriate analysis of variance (see Results). Statistical significance is defined as $P < 0.05$, throughout. All values are expressed as mean±SEM.

After fixation, blocks of kidney tissue were dehydrated, embedded in paraffin, and 3-μm sections were cut and stained with periodic acid schiff with Harris hematoxylin counterstain. The tissues were coded and examined on a blinded basis by light microscopy for the degree of glomerular sclerosis. Injury was assessed using a 0–4 + scale (15) on 100 cortical glomeruli/section and each section was scored 2–3 times

and the average used. Statistical evaluation of histologic data was by Wilcoxon rank sum analysis.

Mean glomerular volume (V_g) was calculated for each rat, from the planar cross-sectional area of 50 undamaged cortical glomeruli, measured on a blinded basis. Details of this technique were reported previously (12). These V_g measurements were made on immersion fixed tissue. It had previously been reported that immersion fixation with the slowly penetrating EM fixative (glutaraldehyde) gave erroneously low values for V_g and that perfusion fixation was preferred (16). To test the validity of our use of immersion fixed tissue, using the rapidly penetrating fixative, formalin, we conducted additional studies in five young adult Sprague Dawley males. After induction of general anesthesia, one kidney was clamped at the pedicle, removed, sliced longitudinally, and placed in 10% buffered formalin. The other kidney was perfused for several minutes at 100 mmHg with 10% buffered formalin, then removed and placed in 10% formalin. The average V_g was measured on both kidneys from each of the rats. The mean value of V_g for the group was similar in immersion fixed and perfusion fixed kidneys (11.96±0.44 and 11.71±0.62 $\mu m^3 \times 10^5$, respectively), validating our use of kidneys fixed by immersion in formalin for V_g measurement.

Results

Body weight (BW) remained constant in intact male Munich Wistar rats from 8–19 mo of age, whereas the wet left kidney weight (KW) increased and was significantly higher at both 12 and 19 vs 8 mo (Table I). GFR and renal plasma flow (RPF) remained relatively stable with advancing age although a slight tendency to decline was noticed which was only significant at 12 vs 8 mo of age for GFR. When factored for kidney weight, however, GFR and RPF were lower at 19 vs 8 mo (0.83±0.03 vs 1.02±0.04 ml/min, $P < 0.05$ and 2.9±0.1 vs 3.8±0.4 ml/min, $P = 0.05$, respectively). The filtration fraction remained unchanged with advancing age (not shown). Mean arterial blood pressure (BP) did not change and remained in the normotensive range up to 19 mo of age. The P_{GC} is shown for both superficial (directly measured) and deeper (indirectly measured) glomeruli in Fig. 1. There were no systematic differences between direct and indirectly measured values of P_{GC}, and there was no systematic change in either measure of P_{GC} with advancing age. The average P_{GC} from direct and indirect values, is shown in Table I and did not increase from 8 to 19 mo of age. P_T also remained unchanged and therefore the transglomerular hydrostatic pressure difference was also constant (not shown). SNGFR and Q_A tended to decline slightly with advancing age but this was only statistically significant at 12 vs 8 mo for SNGFR. Both R_A and R_E increased at 19 vs 8 mo of age but K_f remained unchanged. There was a significant increase in total urinary protein excretion (U_pV) at 19 mo of age which was accompanied by histologic evidence of glomerular damage, since the number of normal glomeruli declined. The V_g increased substantially between 8 and 12 mo of age and remained elevated at 19 mo in intact males (Table I).

Table II summarizes studies conducted in normal intact female Munich Wistar rats. Both BW and KW increased slightly with advancing age. GFR and RPF did not change, even when factored for KW and similarly mean arterial blood pressure (BP), direct and indirect P_{GC} (Fig. 1), and average P_{GC} and P_T remained unchanged up to 19 mo of age. SNGFR remained constant although a small increase occurred in Q_A at 19 months and values of R_A, R_E, and K_f did not change with age although a trend towards renal vasodilation was noted. Urinary protein excretion was low and remained constant with aging in intact

Table I. Summary of Whole Kidney and Single Nephron Variables in Normal, Intact Male Munich Wistar Rats at ~ 8, ~ 12, and ~ 19 mo of Age

	BW	KW	GFR	RPF	BP	P_{GC}	P_T	SNGFR	Q_A	R_A	R_E	K_f	U_pV	NG	V_g
		g		mL/min		mmHg			nl/min		$dyn.cm^{-5} \times 10^{10}$		nl/min/mmHg	mg/24 h	%	$\mu^3 \times 10^5$
~ 8 mo (n = 6)															
Mean	387	1.34	1.4	5.2	98	52	13	55	225	0.88	0.80	0.063	29	93	11.22
±SE	14	0.05	0.1	0.6	2	2	1	3	26	0.12	0.12	0.003	9	1	1.52
~ 12 mo (n = 8)															
Mean	396	1.51*	1.2*	4.5	93	49	12	44*	207	0.87	0.97	0.063	41	91	17.98*
±SE	7	0.08	0.1	0.3	7	1	1	2	39	0.20	0.13	0.010	9	2	1.26
~ 19 mo (n = 8)															
Mean	402	1.52*	1.3	4.5	103	52‡	12	51	165	1.30*	1.18*	0.074	98*‡	82*‡	16.02*
±SE	5	0.02	0.1	0.2	3	1	1	3	7	0.07	0.06	0.008	22	2	0.48

* Differs from 8 mo $P < 0.05$; ‡ differs from 12 mo $P < 0.05$ by one way ANOVA.

females, as did the number of normal glomeruli, whereas V_g increased at 12 vs 8 mo and this increase was maintained at 19 mo. In comparing the data derived from intact males vs intact females (by two-way ANOVA), males were heavier and KW was greater at all ages vs females ($P < 0.001$ throughout). GFR and RPF were always higher in males vs females ($P < 0.001$ and $P < 0.005$, respectively) and SNGFR and Q_A were also higher in males at all ages ($P < 0.001$ and $P < 0.02$ respectively). There were no differences in BP or P_T although the average value of P_{GC} was slightly higher in males vs females at 19 mo of age ($P = 0.05$) (Tables I and II). At 8 mo of age, R_A and R_E were lower in males vs females (both $P < 0.001$) as reported previously by us (17). This difference persisted at 12 mo of age (both $P < 0.005$) but by 19 mo there was no difference in R_A and R_E between intact males and females.
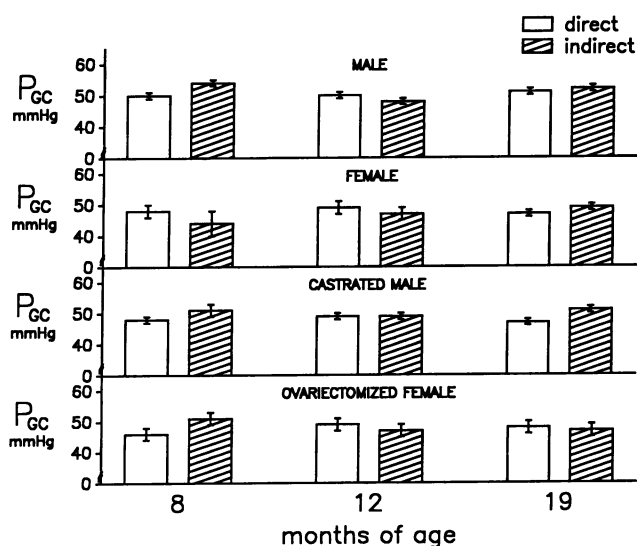

Figure 1. Glomerular blood pressure (P_{GC}) measured by direct puncture in superficial glomeruli (□) and by the indirect stop flow method sampling from deeper cortical glomeruli (■) are given for rats at 8, 12, and 19 mo of age, respectively. Data are given for intact male (upper panel), intact female (second panel), castrated male (third panel), and ovariectomized female (fourth panel). Data given as mean±SE.

There were no differences in the value of K_f between the sexes at any age. Female rats were always less proteinuric than males ($P < 0.05$ at 8 mo; $P < 0.005$ at 12 and 19 mo), and had more normal glomeruli at 19 mo of age compared with males ($P < 0.002$). V_g was not different at 8 mo but was larger in males vs females at 12 and 19 mo of age ($P < 0.05$ and < 0.01, respectively).

Table III summarizes data obtained in castrated male rats. No age-dependent changes were seen in BW, KW, or whole kidney hemodynamics. BP fell slightly at 12 mo of age but was not different between 8 and 19 mo of age and was in the normotensive range throughout. Similarly, P_{GC} and P_T remained at normal values over the aging period (Table III, Fig. 1). There were no changes in SNGFR, Q_A, R_A, or R_E with advancing age in castrated male rats. K_f may have fallen slightly after 8 mo of age, however, since the mean K_f was calculated from minimum and unique values this reduction may have no biological significance. There was no increase in U_pV or histologic evidence of glomerular damage over the entire aging period. However, V_g increased significantly between 8 and 12 mo of age and remained elevated at 19 mo. In comparison between castrated and intact males, kidney weight was always higher in intact males ($P < 0.001$) although body weights were similar at all ages. In general, whole kidney and single nephron hemodynamics were similar in castrated and intact males at all ages with the exception that GFR was slightly lower and R_A was slightly higher in castrated vs intact males at 8 mo of age ($P < 0.02$ and $P < 0.05$; respectively). Importantly, P_{GC} was similar in intact and castrated males at all ages. The most striking difference was at 19 mo of age when castrated rats had significantly less proteinuria and less glomerular damage compared to intact males ($P < 0.005$ for both variables). The values of V_g were similar between castrated and intact males at all ages.

Table IV summarizes data from ovariectomized female Munich Wistar rats. BW and KW remained stable up to 12 mo of age but increased slightly by 19 mo. BP, whole kidney, and single nephron glomerular hemodynamics remained unchanged in ovariectomized female rats over the entire aging period with the single exception that SNGFR was lower at 12 vs 8 mo (Table IV, Fig. 1). U_pV remained low and the percentage of undamaged or normal glomeruli was unaffected by aging. V_g increased from 8 to 12 mo of age and remained constant thereaf-

Table II. Summary of Whole Kidney and Single Nephron Variables in Normal, Intact Female Munich Wistar Rats at ~ 8, ~ 12, and ~ 19 mo of Age

	BW	KW	GFR	RPF	BP	P_{GC}	P_T	SNGFR	Q_A	R_A	R_E	K_f	U_pV	NG	Vg
		g		ml/min		$mmHg$			nl/min	$dyn.cm^{-5} \times 10^{10}$		$nl/min/$ $mmHg$	$mg/24\ h$	%	$\mu^3 \times 10^5$
~ 8 mo ($n = 8$)															
Mean	207	0.80	0.64	2.2	96	48	12	33	104	1.93	1.67	0.052	7	96	8.37
±SE	5	0.03	0.04	0.1	5	1	1	2	6	0.12	0.08	0.004	1	2	0.62
~ 12 mo ($n = 9$)															
Mean	229*	0.97*	0.70	2.5	88	47	12	30	103	1.74	1.71	0.053	11	93	11.26*
±SE	4	0.04	0.04	0.3	2	1	1	2	7	0.15	0.14	0.003	2	1	0.59
~ 19 mo ($n = 8$)															
Mean	242*	1.00*	0.77	3.1	94	48	13	33	133*‡	1.51	1.38	0.051	16	92	12.36*
±SE	5	0.03	0.07	0.4	3	1	1	3	10	0.10	0.11	0.006	4	1	0.67

* Differs from 8 mo $P < 0.05$; ‡ differs from 12 months $P < 0.05$ by one way ANOVA.

ter. In comparison between intact and ovariectomized females one striking difference was that ovariectomized females were heavier than intact females at all ages (all $P < 0.001$) but there were no differences in KW. At 8 mo of age GFR and RPF were higher in ovariectomized vs intact females ($P < 0.005$ and 0.01, respectively) and although SNGFR was not different, Q_A was also higher in ovariectomized females ($P < 0.01$). Both R_A and R_E were lower in ovariectomized vs intact females at 8 mo of age ($P < 0.05$ and 0.01, respectively) as reported by us earlier (17) but this difference disappeared with advancing age. All other variables including BP, P_{GC}, and U_pV were similar at all ages in intact and ovariectomized females. The glomeruli were structurally normal in ovariectomized as well as intact females and values of V_g were similar in intact and ovariectomized females at each age.

A four-way comparison at 19 mo of age showed that there was significantly more glomerular damage and proteinuria in intact males compared with all other groups. Table V summarizes histologic data for 19-mo-old rats and shows the severity of glomerular damage in the four groups. Glomerular injury was generally focal and segmental in all groups. There were fewer undamaged glomeruli in intact males compared with the other three groups. The 1+ and 2 + grades of injury (which correspond to < 25 and 50% injury, respectively) were similar in all four groups, however, the most severe levels of glomerular injury, 3+ and 4+ appeared more frequently in intact males compared to the other three groups. By four-way analysis, values of P_{GC} were similar in the four groups at 19 mo of age (Tables I–IV, Fig. 1), and V_g was larger in intact males vs intact and ovariectomized females (both $P < 0.05$) but not different to the castrated male.

Discussion

In this study we have demonstrated that intact male Munich Wistar rats develop a slowly evolving glomerular structural injury by 19 mo of age. It is well established that glomerular capillary hypertension plays a causal role in the development of various glomerulopathies and Anderson and Brenner (10) have suggested that age dependent glomerular damage results from high P_{GC}. In the present study however, we measured glomerular hemodynamics at intervals during aging and found no evidence that increased P_{GC} preceded or was causally related to the development of glomerular structural injury. This finding

Table III. Summary of Whole Kidney and Single Nephron Variables in Castrated Male Munich Wistar Rats at ~ 8, ~ 12, and ~ 19 mo of Age

	BW	KW	GFR	RPF	BP	P_{GC}	P_T	SNGFR	Q_A	R_A	R_F	K_f	U_pV	NG	Vg
		g		ml/min		$mmHg$			nl/min	$dyn.cm^5 \times 10^{10}$		$nl/min/$ $mmHg$	$mg/24\ h$	%	$\mu^3 \times 10^5$
~ 8 mo ($n = 7$)															
Mean	377	1.05	1.14	4.08	99	49	13	50	176	1.24	1.04	0.082	15	93	9.17
±SE	5	0.03	0.03	0.25	2	1	1	2	18	0.11	0.09	0.008	3	1	0.99
~ 12 mo ($n = 8$)															
Mean	371	1.09	1.05	4.39	91*	49	13	43	182	1.06	1.05	0.058*	21	92	15.76*
±SE	9	0.06	0.10	0.41	2	1	1	3	22	0.11	0.11	0.004	5	2	0.92
~ 19 mo ($n = 8$)															
Mean	387	1.12	1.10	4.83	98‡	50	12	44	196	1.14	1.08	0.058*	23	90	13.65*
±SE	6	0.04	0.07	0.57	3	1	1	3	26	0.09	0.14	0.006	3	1	0.86

* Differs from 8 mo $P < 0.05$; ‡ differs from 12 mo $P < 0.05$ by one way ANOVA.

Table IV. Summary of Whole Kidney and Single Nephron Variables in Ovariectomized Female Munich Wistar Rats at ~ 8, ~ 12, and ~ 19 mo of Age

	BW	KW	GFR	RPF	BP	P_{GC}	P_T	SNGFR	Q_A	R_A	R_F	K_f	U_pV	NG	Vg
		g		mL/min		$mmHg$			nL/min		$dyn.cm^5 \times 10^{10}$	$nL/min/mmHg$	$mg/24\ h$	$\%$	$\mu^3 \times 10^5$
~ 8 mo (n = 9)															
Mean	278	0.82	0.79	2.79	97	50	13	38	140	1.50	1.28	0.053	10	93	8.02
±SE	6	0.03	0.03	0.16	2	2	1	1	10	0.15	0.10	0.010	3	1	0.65
~ 12 mo (n = 8)															
Mean	279	0.86	0.69	2.74	92	48	13	30*	121	1.79	1.60	0.038	8	93	13.01*
±SE	3	0.03	0.06	0.25	2	2	1	2	14	0.36	0.16	0.008	1	1	0.45
~ 19 mo (n = 8)															
Mean	302*‡	0.92*	0.81	3.66	92	48	14	32	150	1.53	1.39	0.061	13	91	12.89*
±SE	7	0.03	0.09	0.43	2	2	1	3	25	0.24	0.17	0.013	2	1	0.68

* Differs from 8 mo $P < 0.05$; ‡ differs from 12 mo $P < 0.05$ by one way ANOVA.

agrees with earlier observations by us in male Sprague Dawley rats studied at 20–22 mo of age; these rats exhibited substantial glomerular damage but had no increase in P_{GC} (12). An unusual substrain of the Munich Wistar, the MWF/ZTM exhibits rapidly, spontaneously developing glomerular injury in the male which apparently represents an accelerated kidney aging process and in these rats P_{GC} is normal (18). By 24 mo of age, P_{GC} is elevated in superficial glomeruli of male Munich Wistar rats and chronic treatment with converting enzyme inhibitor prevents the high P_{GC} and attenuates the glomerular injury (19). These observations have been interpreted as support for a primary role of increased P_{GC} in development of age dependent glomerulopathy. However, given the present findings and earlier observations (12, 18), it seems more likely that the late rise in P_{GC} is a secondary, compensatory response to glomerular damage, and that CEI evokes its beneficial effects via other, nonhemodynamic mechanisms (19).

In the Sprague Dawley rat, glomerular injury generally develops at a more accelerated pace than in Wistars (7) and in Sprague-Dawley's, small increases in P_{GC} have been reported at 13–15 and 18 mo of age (9, 20), (although not at 20–22 mo, [12]) which could contribute to the increased tendency of this strain to develop glomerular injury. In the Nagase strain of analbuminemic rats, there is no evidence of age dependent glomerular injury by 18 mo of age and these animals also exhibit lower than normal values of P_{GC} (9). This, together with the wealth of evidence suggesting that systemic hypertension worsens age-dependent glomerular injury (1, 2, 5, 10), implies that

when increases in P_{GC} do occur, they will exacerbate the underlying development of age-dependent glomerular injury. However, the present studies in the male Munich Wistar rat show that glomerular damage will develop in the absence of glomerular capillary hypertension, thus high P_{GC} cannot be the primary cause.

Rather than implicating a role for increases in P_{GC} in age dependent glomerulopathy, perhaps exposure of the delicate glomerular capillary to normal P_{GC}, over a lifetime, provides the damaging stimulus? We gained further insight into this question by investigating the sex difference in rate of kidney aging. In the present study we observed that female Munich Wistar rats aged over a similar time period to males did not develop glomerular damage or proteinuria. Similar findings have been reported by others (7, 21) and clinical studies suggest that female human kidneys are also protected against age-dependent declines in renal hemodynamics and appearance of glomerular injury (3, 4). This sex-specific protection of the glomerulus is not limited to age-dependent glomerulopathy but is evident in the greater susceptibility of the male to develop ablation-induced glomerular damage (22, 23). Also, males but not females develop the spontaneous glomerular lesion seen in the MWF/ZTN substrain of Munich Wistar rats (18, 24). This sex difference is clinically relevant since the rate of progression towards end stage renal failure in autosomal dominant polycystic kidney disease is significantly slower in women vs men (25). Men are at increased risk to develop end stage renal disease secondary to diabetes and hypertensive complications (26, 27) and long

Table V. Summary of Histologic Glomerular Injury Data for 19-mo-old Rats

Injury grade	0	1+	2+	3+	4+
Intact male (n = 8)	82.4±1.5	7.0±0.9	2.8±0.3	2.4±0.4	5.1±0.6
Intact female (n = 8)	91.9±1.3*	5.8±0.8	1.1±0.4	0.5±0.2*	0.8±0.3*
Castrated male (n = 8)	90.2±1.1*	5.3±0.5	2.1±0.6	0.5±0.2*	1.9±0.6*
OVX female (n = 8)	90.5±0.9*	6.3±0.5	2.1±0.4	0.6±0.2*	0.6±0.1*

* $P < 0.001$ vs intact male by Wilcoxon rank sum analysis. This data is expressed in terms of the 0 − 4 + glomerular injury scale (17): 0, no damage; 1+, focal damage involving < 25% of the glomerulus; 2+, focal damage, between 25–50% of the glomerulus; 3+, widespread damage involving 50–75% of the glomerulus; 4+, widespread damage involving 75–100% of the glomerulus and includes obsolescent glomeruli.

term followup on kidney donors have indicated a greater incidence of hypertension and proteinuria in males vs female donors (28). We had earlier reported that the kidney of the young adult female is vasoconstricted relative to that of the male and in particular has a higher value of R_A (17). At that time we speculated that this elevated R_A might protect the female from increased P_{GC} thus accounting for the sex dependent protection exhibited by females in aging, ablation, etc. induced glomerular injury (17). In an earlier study we reported that P_{GC} was higher (5 mmHg) in male vs female rats several months after uniehrectomy and high dietary protein feeding (22). In the present study, however, we found that despite the higher values for R_A in females, P_{GC} was similar in females and males at 8 and 12 mo and only slightly elevated in males at 19 mo of age. In castrated males and ovariectomized females there were no differences in P_{GC} vs intact males at any age and yet only intact males developed glomerular damage. Thus, our present observations argue against a primary hemodynamic mechanism for development of age-dependent glomerular injury.

It has been suggested that hypertrophy of the glomerular tuft provides a separate risk factor for development of glomerular injury by increased intramural tension (29–31). In the present study we measured glomerular volumes and observed an increase in volume in intact male rats from 8–12 mo of age, which preceeded the development of the injury. Also, we found that the glomerular volumes of female rats were substantially lower than those of males at all ages. In the substrain of Munich Wistar rats MWF/ZTN where the males develop spontaneous injury and proteinuria, morphometric analysis showed an increase in glomerular volume in male but not female glomeruli (24). Thus, there is circumstantial evidence to suggest a role of glomerular hypertrophy in development of age- and sex-dependent glomerular injury. However, we also conducted studies on castrated males and observed that glomerular volumes increased in castrated as in intact males, but that this increase in volume was not associated with the development of injury in castrated rats. This persistent increase in V_g with age in castrated males was unexpected since the androgens promote kidney growth (31, 32). However, the renotrophic action of testosterone appears to be specific for tubular elements in rats with two kidneys (33) although androgens do contribute to the glomerular hypertrophy that occurs after reduction of renal mass (34). In the setting of reduction in renal mass, androgen-induced glomerular hypertrophy correlates with increased injury (34). The observations from the present studies suggest that aging-induced glomerular injury is caused by a different mechanism to glomerulopathy due to ablation.

Thus, whereas it is clear that both glomerular hypertension and glomerular hypertrophy can produce glomerular injury in some situations, the present studies in the aging Munich Wistar rat suggest that neither of these risk factors provide the primary mechanism for the development of age-dependent glomerulopathy in intact males. Based on our castration data and since ovariectomy does not alter the protected status of the intact female, it seems that either directly or indirectly, the presence of the androgens, rather than the absence of ovarian hormones, causes glomerular damage in intact males. Castration has previously been reported to protect male rats from development of glomerular injury following uninephrectomy, although this maneuver was also associated with a reduction in glomerular volume (35).

The cause of age-dependent glomerular damage in intact males has not been determined by the present studies. There is evidence to suggest that mesangial matrix accumulation plays a pathogenic role in immune-mediated and ablation-induced glomerular injury (36, 37). The increased susceptibility of the male to development of glomerular sclerosis after subtotal nephrectomy correlates with mesangial expansion and higher levels of glomerular procollagen mRNA levels in male vs female kidneys (23). Earlier observations by others suggest that aging is associated with mesangial hypertrophy and/or an excessive accumulation of mesangial matrix products. Mesangial lesions (38) and mesangial expansion (39) preceed the development of focal glomerular sclerosis due to aging and increases in mesangial matrix products have been reported in aging male but not female Wistar rats (21). In the Wistar/Lou strain, no age dependent glomerular injury develops and morphometric studies show no increase in mesangial matrix or mesangial cellularity in males up to 36 mo of age and females up to 42 mo of age (8). Mesangial matrix products can accumulate both because of increased synthesis and/or reduced rates of degradation (38–40). We have recently reported that a neutral glomerular metalloprotease, one of the enzymes responsible for degradation of mesangial matrix products, increases markedly with aging in intact and ovariectomized females and castrated male rats all of whom are protected from injury. In contrast, there is no increase in metalloprotease activity in intact males who do exhibit damage (41). In addition to accumulation of mesangial matrix material, Remuzzi and colleagues have suggested that defects in glomerular permeability to macro-molecules cause proteinuria which leads to injury (42) and this may be the basis for the spontaneous injury that develops in male (but not female) MWF/ZTN rats (18).

In summary, our studies demonstrate that age-dependent glomerulopathy proceeds and develops in the absence of increases in P_{GC}. Further, glomerular hypertrophy is unlikely to provide the primary explanation for development of age dependent injury. The frequently reported sex difference in development of age-dependent glomerular damage (males susceptible) is the result of male gender since castration of the male is protective. The relative protection in females is unlikely to result from female gender since ovariectomy has no effect on glomerular structure with advancing age.

Acknowledgments

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