Accelerated age-related decline in renal and vascular function in female rats following early-life growth restriction

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Black MJ, Lim K, Zimanyi MA, Sampson AK, Bubb KJ, Flower RL, Parkington HC, Tare M, Denton KM. Accelerated age-related decline in renal and vascular function in female rats following early-life growth restriction. Am J Physiol Regul Integr Comp Physiol 309: R1153–R1161, 2015. First published September 16, 2015; doi:10.1152/ajpregu.00403.2014.—Many studies report sexual dimorphism in the fetal programming of adult disease. We hypothesized that there would be differences in the age-related decline in renal function between male and female intrauterine growth-restricted rats. Early-life growth restriction was induced in rat offspring by administering a low-protein diet (LPD; 8.7% casein) to dams during pregnancy and lactation. Control dams were fed a normal-protein diet (NPD; 20% casein). Mean arterial pressure (MAP) and renal structure and function were assessed in 32- and 100-wk-old offspring. Mesenteric artery function was examined at 100 wk using myography. At 3 days of age, body weight was ~24% lower (P < 0.0001) in LPD offspring; this difference was still apparent at 32 wk but not at 100 wk of age. MAP was not different between the male NPD and LPD groups at either age. However, MAP was greater in LPD females compared with NPD females at 100 wk of age (~10 mmHg; P < 0.001). Glomerular filtration rate declined with age in the NPD male, LPD male and LPD female offspring (~45%, all P < 0.05), but not in NPD female offspring. Mesenteric arteries in the aged LPD females had reduced sensitivity to nitric oxide donors compared with their NPD counterparts, suggesting that vascular dysfunction may contribute to the increased risk of disease in aged females. In conclusion, females growth-restricted in early life were no longer protected from an age-related decline in renal and arterial function, and this was associated with increased arterial pressure without evidence of renal structural damage.

EPIDEMIOLOGICAL STUDIES CLEARLY demonstrate a link between intrauterine growth restriction and increased risk of renal and cardiovascular disease in adulthood (1, 21, 49, 60), and this programming response to an adverse intrauterine environment can differ between male and female offspring (16, 26, 37). In our laboratory, we have a well-established model of maternal protein restriction in rats, which leads to early-life growth restriction in the offspring. In this model, there is ~25% reduction in nephron endowment at weaning (63, 64), but there is no difference between intrauterine growth restriction (IUGR) and control offspring in blood pressure and glomerular filtration rate (GFR), when adjusted to body weight, at 24 wk of age (64). However, at 24 wk of age, there is evidence of upregulation of early markers of renal pathology in the IUGR kidneys compared with controls (64).

It is well known that there is a progressive loss of nephrons during normal ageing and that this results in an age-dependent fall in renal function (2, 6, 14, 59). This age-related decline in renal function is accelerated in males (2, 5, 59). Hence, the question arises: since IUGR rats already have a decreased complement of nephrons at the beginning of life, are their kidneys more vulnerable to the aging process and will the response differ between males and females? In this study, we explored how renal function and blood pressure are affected in our maternal protein restriction model, as the offspring grow to old age. We comprehensively examined renal function in both male and female offspring in middle age (32 wk of age) and then in old age (100 wk of age). We also examined vascular function in mesenteric arteries of our aged rats and explored whether there were sex differences in the aging of the vascular lumen of the IUGR and non-IUGR offspring.

METHODS

Animals and Diet Treatment

Ten-week-old female and male Wistar-Kyoto (WKY) rats were obtained from the Australian Resource Centre (Perth, Australia). The female rats were divided into two groups and fed, ad libitum, either a normal-protein diet (NPD; 20% casein) or a low-protein diet (LPD; 8.7% casein) for 2 wk prior to mating, during pregnancy, and 2 wk after birth (as nephrogenesis in the rat continues in the first 2 wk postnatally) (32). After this time, all dams were fed normal laboratory chow. The offspring were weaned at 3 wk of age. The nutrient content of the NPD and LPD semipurified diets was equivalent, except for starch, which was varied to ensure that the diets were close to isocaloric (32). The rats were housed individually and maintained at a temperature of 21°C with a 12:12-h day-night cycle. To prevent stress to the dams, the pups were not handled until 3 days after birth, when all litters were reduced to eight pups per dam. Litter size ranged from 8 to 12 pups per litter, and there was no difference in litter size between groups. From weaning, the offspring were fed normal rat chow; food and water were provided ad libitum. One cohort of offspring was grown to 32 wk of age and another cohort to 100 wk of age. Experimental groups were derived from 1 male and 1 female pup per litter. In all female offspring, serial vaginal smears were taken for 3–5 days prior to the day of experiment to determine estrous status, and uterine weight was measured at the end of the study. Animal experiments were approved by the School of Biological Sciences Animal Ethics Committee, Monash University. Treatment and care of the animals were in accordance with the Australian Code of Practice for the care and use of animals for scientific purposes.

Measurement of Conscious Mean Arterial Pressure and Heart Rate

At 32 or 100 wk of age (± 2 wk), rats were briefly anesthetized with isoflurane (~10 min), and a catheter was inserted into the tail...
artery, as previously described (13). The rats were then allowed to waken and following an hour recovery, mean arterial pressure (MAP) and heart rate were measured for 30 min in awake freely moving rats. All animals were acclimatized to handling since vaginal smears were taken for the week before the experimental day, and the males were handled during this week to match the females.

### Determination of Effective Renal Plasma Flow and Glomerular Filtration Rate

Following measurement of arterial pressure via the tail artery, the rats were reanesthetized using Inactin (150 mg/kg body wt). Catheters were inserted into the jugular vein and bladder. The tail artery catheter was used to record arterial pressure. [3H]-insulin (20 μCi·mL⁻¹·min⁻¹) and [14C]-para-aminohippurate (0.1 μCi·mL⁻¹·min⁻¹) were intravenously infused via the left jugular vein, and these infusions were allowed to equilibrate for 1 h. GFR and effective renal plasma flow (RPF) were determined using clearance techniques (52). Urine flow rate, renal vascular resistance, and filtration fraction were subsequently calculated (52). All of the renal function data was adjusted for body weight. Estrous status was equally distributed between the groups (NPD, 3 estrus and 3 nonestrous; LPD, 4 estrus and 3 nonestrous). Examination of the data revealed no significant effect of estrous stage and, therefore, the data for females in estrus and nonestrous were combined for statistical analysis.

### Histological Analysis of Kidneys

At the end of the experiment, the kidneys were collected and weighed. Then a 1-mm longitudinal middle slice of the kidney, taken through the hilum, was embedded in paraffin and sectioned at 5 μm. The sections were stained with Masson’s trichrome and periodic acid-Schiff (PAS) reagent. The sections stained with Masson’s trichrome were systematically sampled, and levels of interstitial collagen were measured using image analysis (Image-Pro Plus Version 6.0). In the PAS-stained sections, 100 glomeruli were systematically sampled and levels of glomerulosclerosis graded (from 0 to 4) (62). Normal glomeruli were given a grade of 0, glomeruli with 1–25% glomerulosclerosis were given a score of 1, those with 26–50% glomerulosclerosis scored a 2, those with 51–75% glomerulosclerosis scored a 3, and those with 76–100% glomerulosclerosis scored a 4.

### Assessment of Vascular Function at 100 Weeks of Age

Second-order branches of the mesenteric artery were isolated. Ring segments were mounted onto a wire myograph to enable assessment of endothelial and smooth muscle function as described previously (61). Arteries were continuously superfused with physiological saline (PSS, in mM: 120 NaCl, 5 KCl, 25 NaHCO₃, 1 KH₂PO₄, 11 glucose, 1.2 MgSO₄, and 2.5 CaCl₂) bubbled with 95% O₂ and 5% CO₂ at 35°C. To assess contraction, phenylephrine was added cumulatively (10⁻⁸ to 10⁻⁵ M), and contractions were expressed as a percentage of contraction to high K⁺ PSS (isotonic replacement of Na⁺ with 100 mM K⁺). Smooth muscle relaxation was tested using cumulative addition of the nitric oxide (NO) donor, sodium nitroprusside (SNP 10⁻⁴ to 10⁻⁵ M) in arteries submaximally constricted with phenylephrine. To test endothelial function, increasing concentrations of ACh (ACh, 10⁻¹⁰ to 10⁻⁷ M) were applied cumulatively in arteries submaximally constricted with phenylephrine. ACh concentration-response curves were repeated following blockade of NO synthase with N⁶-nitro-L-arginine methyl ester (l-NNAME, 2 × 10⁻⁴ M) and the cyclooxygenase inhibitor indomethacin (10⁻⁶ M). Relaxation remaining in the presence of l-NNAME and indomethacin was attributed to endothelium-derived hyperpolarizing factor (EDHF) (61). Functional responses in arteries from the females in estrus were analyzed separately to those in nonestrous, to account for the effects of the estrous cycle on vascular function (NPD, 5 estrus and 4 nonestrus; LPD, 5 estrus and 5 nonestrus) (56).

### Testing Passive Wall Properties

Five-milliliter lengths of mesenteric artery were mounted onto the cannula of a pressure myograph for the determination of passive mechanical wall properties, as described previously (61). Segments were superfused with zero-Ca²⁺ PSS containing 1 mmol/l EGTA. Intraluminal pressure was raised in 10-mmHg increments (10–200 mmHg), and dimensions were measured at each pressure to enable the calculation of wall stress and wall strain, as described previously (61). For analysis, the data for females in estrus and nonestrous were combined as the estrous cycle was without effect on passive stiffness.

### Statistical Analysis

All data are expressed as means ± SE. Statistical analyses were undertaken using GraphPad Prism (version 5.00; GraphPad Software, San Diego, CA). Body weight at 3 days of age was analyzed using an unpaired Student’s t-test. Body weight, hemodynamic, and renal function data collected at 32 and 100 wk of age were analyzed using a two-way ANOVA with factors: maternal diet group (P group; 32 wk-LPD; 32 wk-NPD, 100 wk-LPD, or 100 wk-NPD), sex (P sex; male or female) and their interaction (P group × sex). Tukey post hoc analysis was performed to determine differences between specific groups and time points. For vascular experiments conducted in offspring at 100 wk of age, sigmoidal curves were fitted to the dose-response data using GraphPad Prism. The concentration for half-maximal response (EC₅₀), the pD₂ (-log EC₅₀), and maximal response (Eₘₐₓ) were determined and compared between groups. Stress–strain relationships were generated, as described previously (61). Data were analyzed using a two-way ANOVA with factor group (100 wk LPD, 100 wk NPD), dose, and their interaction, with Bonferroni post hoc analysis or unpaired t-test, as appropriate. Statistical significance was accepted at the level of P ≤ 0.05.

### RESULTS

#### Body and Organ Weights

Body weight was significantly lower (~24% reduction, P < 0.0001) at 3 days of age in the LPD offspring compared with NPD offspring. There was no difference in body weight at 3 days of age between male and female offspring. Both male and female adult offspring exhibited an increase in body weight with age (all P < 0.05; Fig. 1A). However, adult female offspring had a significantly lower body weight compared with male offspring (~40% lower, all P < 0.0001; Fig. 1A). At 32 wk of age, the body weights of the LPD offspring remained lower (~10% reduction in both sexes; P < 0.05; Fig. 1A) compared with the NPD offspring. At 100 wk of age, body weight was no longer different between the NPD and LPD offspring. With age, body weight increased by ~15% (P < 0.001) in male NPD, ~11% (P < 0.05) in female NPD, ~27% (P < 0.001) in male LPD and ~29% (P < 0.001) in female LPD compared with offspring at 32 wk of age (Fig. 1A). Absolute kidney weight was lower in LPD compared with NPD male and female offspring at 32 wk of age (Fig. 1B). At 100 wk of age, absolute kidney weight had increased in male LPD offspring, such that values were no longer significantly different to age-matched male NPD offspring. However, differences in absolute kidney weight between the LPD and NPD offspring remained present in the 100 wk compared with the 32-wk aged females (Fig. 1B). Kidney-to-body weight ratio was greater in 32-wk-old females compared with age-matched males, irrespective of diet; however, this difference was no longer apparent at 100 wk of age (Fig. 1C). Relative kidney...
RENAL FUNCTION IN AGED GROWTH-RESTRICTED OFFSPRING

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Fig. 1. Body weights (A), kidney weights (B), and kidney-to-body weight ratio (C) in male and female rats at 32 and 100 wk of age of mothers fed a normal (open bars) or low-(solid bars) protein diet throughout pregnancy and lactation. n = 5–8 per group. The symbols represent Tukey post hoc analysis: *P < 0.05 **P < 0.01 compared with sex and treatment matched group; †† P < 0.01 compared with aged and treatment matched group; # P < 0.05, ## P < 0.01 compared with age and sex matched group.

Renal Function in Anesthetized Rats

Mean arterial pressure and renal vascular resistance. MAP in the anesthetized animals was similar to the values obtained in the conscious rats, albeit the values were ~5 mmHg lower, the significant age- and treatment-related differences observed were still apparent (32 wk: NPD-male, 98 ± 2 mmHg; NPD-female, 97 ± 1 mmHg; LPD-male, 99 ± 2 mmHg; LPD-female, and 101 ± 2 mmHg. 100 wk: NPD-male, 120 ± 1 mmHg; NPD-female, 116 ± 2 mmHg; LPD-male, 120 ± 3 mmHg; and LPD-female, 128 ± 4 mmHg). In male offspring, renal vascular resistance (RVR) was greater at 100 wk compared with 32 wk of age (P < 0.001). However, RVR was not different between NPD and LPD groups at 32 or 100 wk of age in the males (Fig. 3A). In contrast, while RVR was not different between the NPD and LPD female offspring at 32 wk of age, at 100 wk of age, RVR was significantly greater in the female LPD compared with the NPD group (P < 0.05). Thus, RVR was markedly lower in the female NPD 100 wk offspring compared with the male NPD, male LPD and female LPD age matched groups (Fig. 3A).

Renal plasma flow. At 32 wk of age, RPF corrected for body weight was greater in female compared with male offspring (all P < 0.001, Fig. 3B); however, there was no effect of maternal diet on RPF in male or female offspring. At 100 wk of age, RPF was significantly lower in both NPD (~60%; P < 0.001) and LPD (~68%; P < 0.001) male offspring compared with male offspring at 32 wk of age. In contrast, while RPF was reduced by a similar extent in the LPD female offspring (~76%; P < 0.001) compared with the 100-wk-old LPD males, the reduction in the 100-wk NPD female offspring was

MAP was not significantly different between the sexes in the NPD offspring. However, MAP was greater in the 100 wk compared with the 32-wk age groups (~20 mmHg; all P < 0.0001; Fig. 2A). Moreover, while MAP was not different between the male NPD and LPD groups at either age, MAP was significantly greater in the LPD (132 ± 4 mmHg) compared with the NPD (121 ± 3 mmHg) females at 100 wk of age (P < 0.001; Fig. 2A). Heart rate was not different between the groups at 32 wk of age, but increased with age in both sexes (all P < 0.05). Heart rate was significantly greater in female offspring at 100 wk of age compared with males, irrespective of maternal diet (all P < 0.05; Fig. 2B).

Conscious MAP and Heart Rate

MAP was not significantly different between the sexes in the NPD offspring. However, MAP was greater in the 100 wk compared with the 32-wk age groups (~20 mmHg; all P < 0.0001; Fig. 2A). Moreover, while MAP was not different between the male NPD and LPD groups at either age, MAP was significantly greater in the LPD (132 ± 4 mmHg) compared with the NPD (121 ± 3 mmHg) females at 100 wk of age (P < 0.001; Fig. 2A). Heart rate was not different between the groups at 32 wk of age, but increased with age in both sexes (all P < 0.05). Heart rate was significantly greater in female
not as great (~41%; \( P < 0.01 \)). Thus, while RPF declined in the female NPD 100-wk-old offspring, RPF was not reduced to the same degree as in the male NPD, male LPD, and female LPD age-matched groups (Fig. 3B).

**Glomerular filtration rate.** GFR corrected for body weight was greater in NPD female compared with NPD male offspring (\( P < 0.05 \); Fig. 3C). At 32 wk of age, there was no difference in GFR in male or female NPD and LPD offspring (Fig. 3C). GFR was significantly lower at 100 wk of age in both NPD and LPD male groups (both \( P < 0.05 \); Fig. 3C). GFR was also significantly reduced in the LPD females at 100 wk of age compared with the LPD females at 32 wk of age (\( P < 0.01 \)). However, GFR was not significantly different between the 32-wk and 100-wk-old NPD female offspring (Fig. 3C). Thus, GFR was reduced with age in male NPD (~40%; \( P < 0.05 \)), male LPD (~36%; \( P < 0.01 \)), and female LPD (~58%; \( P < 0.01 \)) offspring, but age did not alter GFR in NPD female offspring (Fig. 3C).

**Filtration fraction.** Filtration fraction was greater in the older animals (all \( P < 0.0001 \), Fig. 3D), but was not different between the sexes. Filtration fraction was not different between the LPD or NPD groups, of either sex, at 100 wk of age (Fig. 3D). However, post hoc analysis (two-way ANOVA factors diet, sex, and their interaction) of the 32-wk data demonstrated a main effect of diet and sex. This indicated that at 32 wk of age, filtration fraction was greater (~5%, \( P_{\text{sex}} = 0.005 \)) in male than female offspring and that filtration fraction was increased in the LPD groups (~5%, \( P_{\text{diet}} = 0.03 \)); however, the effect was not different between the sexes (\( P_{\text{diet} \times \text{sex}} = 0.6 \)).

**Urine flow rate.** UFR was significantly greater in NPD females compared with males (\( P < 0.0001 \); Fig. 3E). In male offspring, while UFR tended to fall with age, this did not reach statistical significance (Fig. 3E; \( P = 0.07 \)). In female NPD offspring, UFR was not significantly different between 32 and 100 wk of age, but in female LPD offspring, UFR was reduced at 100 wk of age compared with 32 wk of age (\( P < 0.001 \)) to a level similar to that observed in males at 100 wk of age (Fig. 3E).

**Renal histology.** Kidney sections were analyzed for evidence of renal injury. At 32 wk of age, very little evidence of renal structural damage was observed with a glomerular sclerosis index score of zero observed in ~96% of glomeruli examined in all groups (Fig. 4A). Glomerular sclerosis index was observed to increase with age, with >20% receiving a score of one or greater at 100 wk of age, but there was no significant difference between the sexes or dietary groups (Fig. 4B). The degree of renal interstitial fibrosis, as estimated by % PAS staining was low (<2%) and was not different between the ages, sexes, or dietary groups (data not shown).

**Vascular Function at 100 wk of Age**

**Smooth muscle contraction and relaxation.** There was no difference in the sensitivity or maximal contraction of mesenteric arteries to the \( \alpha_1 \)-adrenoceptor agonist phenylephrine between the LPD and NPD groups in male rats or female rats in estrus. Sensitivity to phenylephrine was reduced in mesenteric arteries from LPD females not in estrus compared with those from NPD females (Fig. 5A; \( P = 0.03 \); \( \text{pD}_2 \), 5.6 ± 0.1, \( n = 5 \) vs. 5.8 ± 0.1; \( n = 4 \)).

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Fig. 3. Renal function variables renal vascular resistance (RVR; A), renal perfusion flow (RPF; B), glomerular filtration rate (GFR; C), filtration fraction (D), and urine filtration rate (UFR; E), measured in male and female rats at 32 and 100 wk of age of mothers fed a normal- (open bars) or low- (solid bars) protein diet throughout pregnancy and lactation. \( n = 5–8 \) per group. The symbols represent Tukey post hoc analysis: * \( P < 0.05 \), ** \( P < 0.01 \) compared with sex- and treatment-matched group; \( \dagger \) \( P < 0.05 \), \( \ddagger \) \( P < 0.01 \) compared with age- and treatment-matched group; \( \# P < 0.05 \), \( \#\# P < 0.01 \) compared with age- and sex-matched group.
The sensitivity to SNP was reduced in mesenteric arteries of LPD males \((P = 0.003; \text{pD}_2 7.9 \pm 0.1, n = 4 \text{ vs. } 8.3 \pm 0.1; n = 4)\) and nonestrous females \((P = 0.01; \text{pD}_2 8.0 \pm 0.1, n = 5 \text{ vs. } 8.6 \pm 0.1, n = 4); \text{Fig. 5B}\). The maximal smooth muscle relaxation evoked by SNP was not different between LPD and NPD groups in male or female rats.

**Endothelium-dependent relaxation.** Total endothelium-dependent relaxation to ACh was not different in arteries from male and female (estrus and nonestrus) LPD and NPD offspring. Likewise, the response attributed to EDHF was not different between LPD and NPD offspring (Fig. 5C).

**Passive mechanical wall properties.** There was no difference in the stress-strain relationship for mesenteric arteries between the LPD and NPD groups in the male \((P = 0.7)\) or female \((P = 0.057)\) offspring (Fig. 5D).

**DISCUSSION**

Our findings clearly demonstrate that females were protected from the age-related decline in renal function observed in males, an effect that was lost in females that were growth restricted in early life. In male rats, there was no difference in the age-related decline in renal function between IUGR and non-IUGR offspring; in contrast, there was an exaggerated age-related impairment in renal function in females that were growth-restricted in early life compared with their non-growth-restricted counterparts. In aged rats, we also observed impaired vascular function in male rats and more extensive vascular dysfunction in female rats (not in estrus) that had been growth-restricted in early life. Overall, our findings suggest that females, growth-restricted in early life, are more vulnerable to renal and vascular aging when compared with those that were not growth-restricted and that this was associated with an increase in arterial pressure without evidence of renal structural damage.

**Enhanced Age-Related Increase in Arterial Pressure in Growth-Restricted Females**

Arterial pressure was similar between groups at 32 wk of age. This is in agreement, with our previous reports in this model in which no difference in arterial pressure was observed at 24 and 32 wk of age in IUGR offspring (30, 31, 64). However, MAP increased between 32 and 100 wk of age by \(\sim 25\) mmHg in NPD male and in both male and female LPD offspring but only by \(\sim 10\) mmHg in NPD female offspring. In other studies, the effects of IUGR on blood pressure differ widely. As we have comprehensively reviewed, some studies report no effect on blood pressure, whereas others report an elevation in blood pressure in adulthood in models of IUGR (65). Possible explanations for these disparities include lack of catch-up growth as observed in our model (65) or may reflect stress induced by the method employed to measure arterial pressure (25, 26). Indeed, intra-arterial pressure measurement in acutely instrumented rats, as employed in the current study, may be associated with a degree of stress (12, 25). Nevertheless, in the current study, there were clear differences in blood pressure with age, and it appeared that control females were protected against the age-related increase in arterial pressure observed in males, but that IUGR females were not. Other studies in humans and various programming models have also shown an association between low birth weight and increased arterial pressure in aged females (see Refs. 65, 66). Therefore, our data support the contention that IUGR females have an increased risk of developing hypertension with age.

**Better Renal Function in Females Versus Males in NPD-Control Rats**

It is well described that the progression of chronic renal disease is more rapid in males than females and that the prevalence of renal failure is higher in males than females (5, 42, 48, 53). Reports of the rate at which renal function declines with age in animal models are highly variable and depend on genetic background and environmental factors (4). Our findings in the NPD control group support previously published studies in both animal models and humans of better renal function in aged females than males (3, 4, 34). In our study, GFR and RPF were markedly greater in control NPD females at both 32 and 100 wk of age compared with males. Previously, we reported no sex difference in GFR (corrected for body weight) in young adult rats (11–12 wk of age) (19), in agreement with previous reports (40). However, in the current study by 32 wk in middle-aged offspring, relative GFR was lower in NPD males compared with NPD females, indicative of a decline in renal function in males relative to females, even at 32 wk of age. This is in agreement with a report demonstrating that GFR fell between 10 and 40 wk of age in male WKY rats (9). In Wistar (WAG/Rij) rats, it has been demonstrated that GFR fell progressively between 6 and 30 mo of age in males (18), but fell between 20 and 30 mo in females (11). Therefore, we suggest that in NPD males compared with females that the onset of the age-related decline in renal function occurs earlier in males.
The mechanisms leading to the increased risk of age-related renal impairment in males has been widely studied and is thought to be mediated via differences in the hormonal milieu and related differences in body mass index, rather than developmental differences in renal structure (34, 44, 53). Interestingly nephron endowment is not different in Wistar or Sprague-Dawley strains of rats (39), and yet greater structural damage has been reported to evolve in Sprague-Dawley rats with age (4). Certainly, our previous stereological analyses in male vs. female rats (in either NPD or LPD groups) suggest that there is no sex difference in nephron number in early life (63, 64), a finding supported by others in humans and animal models (22, 33, 39, 44). Importantly, in an autopsy study of middle-aged subjects, there were no differences in nephron number between men and women aged 55–60 years, although glomerular volume was larger, and this was directly dependent on the greater body surface area in men (44). Finally, in our study, it is unlikely that there has been significant loss of nephrons with age, given the very limited histological evidence of renal injury observed at 100 wk of age. Thus, taken together, these data suggest functional rather than structural changes account for the observed sex differences in the decline in renal function with age, in agreement with the literature (3).

Fig. 5. Vascular reactivity and stiffness measured in mesenteric arteries of male and female rats at 100 wk of age of mothers fed a normal- (open symbols) or low- (solid symbols) protein diet. A: contraction to phenylephrine (PE). B: smooth muscle relaxation evoked by sodium nitroprusside (SNP). C: endothelium-dependent relaxation evoked by ACh in the absence (no blockers) and the presence of nitric oxide synthase (NAME) and cyclooxygenase (Indomethacin, Indo) inhibitors. D: stress-strain relationships. *pD2, P < 0.05; n = 4 or 5 per group.

Age-Related Decline in Renal Function in IUGR and Non-IUGR Males and IUGR Females

It has been postulated that in the presence of a congenital nephron deficit (as occurs in LPD offspring) there is subsequent compensatory glomerular hypertrophy and, thus, glomerular hyperfiltration (8). Certainly, the normalized GFR and increased filtration fraction in LPD offspring at 32 wk of age likely reflects glomerular hyperfiltration associated with increased glomerular capillary pressure and/or glomerular en-
largement; although there was no marked evidence histologically of renal injury. Importantly, in middle-aged LPD offspring, the kidneys (although hyperfiltering compared with controls) were able to maintain overall renal function, such that neither total GFR nor blood pressure were adversely affected. Moreover, we have previously shown that fat and lean muscle mass were proportional to body weight in 32-wk-old DEXA offspring compared with age-matched controls via DEXA body composition analysis (30). Thus, the lack of catch-up growth postnatally in the LPD offspring up to 32 wk of age likely played a beneficial role, as the functional demands on the kidneys would be commensurate to body size. Certainly, the presence of catch-up growth and IUGR has been linked to disease risk (10).

There was an age-related decline in GFR and RPF in all male rats from 32 to 100 wk of age, with the decline similar in both male NPD and LPD offspring. Interestingly, GFR did not decline from 32 to 100 wk of age in control NPD female rats. In contrast, GFR was 57% lower in IUGR female LPD rats at 100 wk compared with 32 wk of age and had declined to a level matching that seen in males at this age. Whereas, RPF fell with age in both NPD and LPD female offspring, though the fall was greater in the LPD female offspring. At 100 wk of age, there was also a significant increase in MAP in LPD females compared with NPD females, which is in accordance with the significantly reduced GFR. The observation of a greater fall in RPF than GFR with aging is compatible with previous reports in aged rats (2) and also supports a role for functional rather than structural alterations being responsible for the age-related renal dysfunction observed in the current study.

The question therefore arises: Why were all males (LPD and NPD) vulnerable to an age-related decline in renal function, whereas only IUGR LPD females were vulnerable and non-IUGR NPD females are not? Certainly, it is well established that sex hormones directly influence renal function; testosterone has been shown to be detrimental, whereas estrogen is reported to exert a protective role (20, 43, 53). Hence, in the case of males, both the LPD and NPD males have been subjected from puberty through to old age to the adverse effects of testosterone, and this is one possible reason for the fall in renal function in males. Under these circumstances, a greater nephron complement in NPD males did not appear to confer any functional advantage. This is in contrast to the study of Saez et al. (50), in which a greater age-related decline in renal function was observed in a model of low-nephron number in both sexes. However, in this model of IUGR, induced via maternal angiotensin receptor blocker administration, the renal phenotype in the offspring is more severe. Therefore, it is possible that the severity of the initial nephron deficit may influence future renal and cardiovascular disease risk.

In apposition to this, estrogen has been shown to be renoprotective in females of reproductive age (20, 43, 53). The timing of reproductive senescence in rats is strain-dependent, with reports varying from 6 to 20 mo of age in rats (58). In the current study, both control and IUGR females at 100 wk of age were still undergoing estrous cycling, as determined by vaginal smear, compatible with a previous report in Wistar rats (58). Hence, it is likely that in the aged NPD females, the estrogen levels were sufficient to preserve GFR in the aged cohort. In the IUGR LPD females, it is possible that the initial congenital deficit combined with aging has markedly depleted renal functional reserve and, thus, counteracted the beneficial hormonal effects of circulating estrogen. In support of this idea, a congenital deficit in nephron number has been linked to an age-related decline in renal function in females in other animal programming models [including placental insufficiency (24, 38), fetal uninephrectomy (54), and maternal nicotine (55)]. It has been suggested that kidneys with fewer nephrons are more sensitive to some stimuli (ANG II, renal ischemia reperfusion injury), which may increase their vulnerability to renal injury (23).

An alternative explanation for the accelerated decline in renal function in the IUGR females relative to the controls is that the age-related decline in estrogen levels is greater in the aged LPD females relative to the aged NPD females, thus, attenuating the protective effects of estrogen. Certainly, there are a number of studies that have shown that IUGR can affect sexual development in IUGR offspring (7, 41, 47) and lead to delayed onset of puberty (17, 29) and premature reproductive aging (increase in cycle length and decline in fertility rates) (17). Hence, this, in turn, may adversely impact on blood pressure and renal function. In support of this idea, it has been shown in reproductively senescent spontaneously hypertensive rats (where regular estrous cycling ceases at 10 mo of age) that there is a rise in arterial pressure by 18 mo of age to match that measured in males (15). In contrast, although available evidence supports delayed onset of puberty, and premature aging of the reproductive system in IUGR offspring, circulating levels of estradiol may not be reduced. For example, in a study by Guzman et al. (17), using a similar rat model of maternal growth restriction, the estradiol levels were reported to be higher in aged IUGR females at 1 yr of age compared with controls. In another study, estradiol levels were reported not to be different in IUGR and non-IUGR female rats at 18 mo of age; in that study, IUGR was induced by bilateral uterine artery ligation (35). We examined the influence of estrous stage on renal function in the current study but observed no significant effect. It is possible that small differences may not have been detected in the current study. Santmyire et al. (51) demonstrated an increase in RPF (~20%), but not GFR, during estrus utilizing a within-animal experimental design. Another, limitation of the current study was that plasma estradiol levels were not determined. Therefore, we can only speculate that the greater fall in renal function with age in the IUGR female offspring is likely due to the combined effect of the initial congenital deficit in nephron number (without evidence of a further loss of nephrons with age) and loss of the functionally protective effects of estrogen. Certainly, evidence suggests that estrogen, via its interaction with nitric oxide, angiotensin, and endothelin could contribute to age-related changes in renal function in females, including in programming models (3, 23, 36).

**Vascular Function Is Impaired in Aged IUGR Rats**

In support of this conjecture compared with NPD controls, we observed reduced sensitivity to the NO donor nitroprusside in the mesenteric resistance vessels of LPD males and females that were not in estrus. A similar impaired response to NO donors has been described in the cerebral arteries of LPD offspring; this is associated with reduced cGMP levels and expression of guanylate cyclase (27). In addition, in our study,
there was reduced sensitivity to the vasoconstrictor phenylephrine in the mesenteric vessels of LPD female rats that were not in estrus. Therefore, our findings suggest that increased levels of estrogen (during estrus) may prevent these abnormal vascular responses. Many experimental studies have described adverse programming of vascular function in response to IUGR (46) and an interaction between estrous cycle and the extent of vascular dysfunction has been demonstrated (56, 57). In addition, low birth weight in human subjects has been linked to vascular dysfunction in infants, children, and adults (28, 45). Thus, in women, estrogen may attenuate the expression of vascular abnormalities arising from early-life insults that may be unmasked after menopause leading to an increased risk of renal and cardiovascular disease.

In conclusion, the findings of this study demonstrate that female rats that are growth-restricted in early life are more vulnerable to renal and vascular aging. In contrast, in males, there is marked decline in renal function with age, regardless of whether they have or have not experienced early-life growth restriction. Thus, the age-related decline in renal and vascular function may be accelerated in IUGR women following menopause.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


