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Effect of prenatal programming and postnatal rearing on glomerular filtration rate in adult rats

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Lozano G, Elmaghrabi A, Salley J, Siddique K, Gattineni J, Baum M. Effect of prenatal programming and postnatal rearing on glomerular filtration rate in adult rats. Am J Physiol Renal Physiol 308: F411–F419, 2015. First published December 23, 2014; doi:10.1152/ajprenal.00593.2014.—The present study examined whether a prenatal low-protein diet programs a decrease in glomerular filtration rate (GFR) and an increase in systolic blood pressure (BP). In addition, we examined whether altering the postnatal nutritional environment of nursing neonatal rats affected GFR and BP when rats were studied as adults. Pregnant rats were fed a normal (20%) protein diet or a low-protein diet (6%) during the last half of pregnancy until birth, when rats were fed a 20% protein diet. Mature adult rats from the prenatal low-protein group had systolic hypertension and a GFR of 0.38 ± 0.03 versus 0.57 ± 0.05 ml·min⁻¹·100 g body wt⁻¹ in the 20% group (P < 0.01). In cross-fostering experiments, mothers continued on the same prenatal diet until weaning. Prenatal 6% protein rats cross-fostered to a 20% mother on day 1 of life had a GFR of 0.53 ± 0.05 ml·min⁻¹·100 g body wt⁻¹, which was not different than the 20% group cross-fostered to a different 20% mother (0.45 ± 0.04 ml·min⁻¹·100 g body wt⁻¹). BP in the 6% to 20% group was comparable with the 20% to 20% group. Offspring of rats fed either 20% or 6% protein diets during pregnancy and cross-fostered to a 6% mother had elevated BP but a comparable GFR normalized to body weight as the 20% to 20% control group. Thus, a prenatal low-protein diet causes hypertension and a reduction in GFR in mature adult offspring, which can be modified by postnatal rearing.

prenatal programming; postnatal programming; glomerular filtration rate; hypertension

BRENNER AND COLLEAGUES put forth the hypothesis that low-birth weight infants have fewer nephrons, resulting in both systemic and glomerular hypertension and in an increased risk of developing chronic kidney disease (4, 6, 7, 48). In support of this hypothesis, offspring of mothers who suffered through the Dutch famine (hunger winter) during midgestation were at increased risk of developing albuminuria compared with those born before or after the famine (31). Similarly, Australian aborigines, a population with a high prevalence of chronic kidney disease, are at increased risk of developing albuminuria if born small for gestational age (16). Several other studies in humans have found results consistent with the hypothesis that small for gestational age infants are at risk for chronic kidney disease in later life (10, 15, 16, 20–22, 34, 42, 43). A meta-analysis found that low birth weight was associated with a 70% increased likelihood of developing albuminuria, chronic kidney disease, or end-stage renal disease (43); however, these studies by and large included patients that were premature at birth, obscuring the impact of prenatal programming leading to small for gestational age infants on the development of progressive renal injury.

Several studies have examined the effect of prenatal insults on offspring when they were studied as adults. Pregnant rats that have been treated with glucocorticoids, fed a low-protein diet, or have uterine artery ligation have offspring with a reduction in nephron number. Some studies have shown these prenatal insults result in hypertension in adult offspring (1, 29, 41, 47). A reduced nephron endowment is a potential risk factor for the development of hyperfiltration, leading to glomerular hypertension and progressive renal disease (7, 24). Offspring of rats where the mother had a prenatal insult can develop hypertension, which, in part, may be due to the reduced glomerular surface area leading to Na⁺ retention, another risk factor for progressive renal disease (24). While the reduction in nephron number and hypertension seen with prenatal programming would be potential risk factors for chronic kidney disease, the preponderance of evidence shows that prenatal insults do not lead to a reduction in glomerular filtration rate (GFR) (2, 3, 29, 30), unless the insult is extremely severe and throughout pregnancy (23, 47).

In addition to prenatal insults, many neonates, such as those born very prematurely, are nutritionally deprived postnatally. While there has been a focus on prenatal programming, most glomerulogenesis in the rat actually occurs after birth in the first 7–10 days of life (19). This would be comparable with a premature neonate born before nephrogenesis is complete at 34–36 wk of gestation. Thus, it is possible that postnatal insults in the rat may have a greater effect on renal function than prenatal insults. Indeed, we have recently shown that postnatal dietary deprivation during lactation results in hypertension (36). The purpose of the present study was to determine if prenatal programming results in a progressive decline in GFR and hypertension. The second goal was to examine if improving the postnatal environment during the time of nephrogenesis prevents the decline in GFR and increase in blood pressure by cross-fostering neonates from mothers that were fed a low-protein diet during pregnancy to mothers that were fed a normal protein diet. Finally, the third aim was to examine the effect...
of a maternal postnatal low-protein diet while nursing on rats whose mothers were fed a normal prenatal environment to determine if suboptimal postnatal nutrition, which causes failure to thrive (36), results in hypertension and a reduction in GFR in adult offspring.

METHODS

Animals

Pregnant Sprague-Dawley rats were fed either a control 20% protein diet or a 6% (low) protein diet from the 12th day of gestation until the time of birth using the same protocol as previously described (9, 13, 14, 25). The mothers had all been pregnant previously and were 4–6 mo of age. Diets contained the same caloric density and vitamin and mineral content. Our laboratory has previously shown that the number of neonatal rats born to mothers who were fed the two diets was not different (11.4 ± 0.6 pups in the control group vs. 13.0 ± 0.5 pups in the low-protein group, P = not significant) (14). In all experiments, mothers were removed at 25 days, at which time all rats were placed on a 20% protein diet. Rats were studied as adults and were from at least four different litters in each group. In the experiments measuring GFR at 17 mo of age, there were at least six litters used, and in all experiments, no more than two rats were used from the same litter. Male rats were studied since they are affected by prenatal programming more severely than female rats and to reduce variability (2, 26, 30, 46). These experiments were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

There were two groups of experiments. In prenatal programming experiments, the mother that gave birth to the litter and reared the neonates and all mothers were fed a 20% protein diet after birth. In the second set of experiments, we examined if the postnatal environment affected blood pressure and GFR in 20% protein control rats and in rats whose mothers were fed a 6% protein diet during the last half of pregnancy. In cross-fostering experiments, litters were transferred to a different mother on day 1 of life and the mother was fed the same diet as she was fed during the last half of pregnancy until weaning. The following four cross-foster groups were used.

- **20% to 20% group.** Rats from the 20% protein group were cross-fostered to a different 20% mother that continued on the 20% protein diet to serve as a cross-fostering control group.
- **6% to 20% group.** Offspring of mothers fed a 6% protein diet during the last half of pregnancy were cross-fostered to mothers that were fed a 20% protein diet throughout gestation and while nursing to determine if improving the postnatal environment affected blood pressure and GFR.
- **20% to 6% group.** Litters of 20% mothers were cross-fostered to rats whose mothers were fed a 6% protein diet while pregnant and nursing to examine if a suboptimal postnatal environment affected blood pressure and GFR in rats that had a normal prenatal environment.
- **6% to 6% group.** Litters of 6% mothers were fostered to a different 6% mother that continued to be fed a 6% protein diet while nursing to determine the effect of an abnormal prenatal and postnatal environment on blood pressure and GFR.

After weaning, all rats were fed a 20% protein diet.

Urine Collections

At 1 yr of age, rats were placed in metabolic cages for the collection of urine for creatinine clearance and urinary protein excretion. Rats were first acclimatized to the metabolic cages for 48 h before collections. Urine was then collected for 24 h, and specimens were frozen at −80°C until assays were performed. At the end of the urine collection, blood was obtained by retroorbital puncture after rats had been anesthetized with isoflurane. Total urine protein excretion was measured using the Bradford assay (Bio-Rad Laboratories, Hercules, CA). Urine albumin was measured using a Nephrat rat urinary albumin enzyme immunoassay kit (Exocell, Philadelphia, PA). Serum and urine creatinine were measured by capillary electrophoresis.

Blood Pressure Measurements

Systolic blood pressure was measured using the tail-cuff method at 12 mo of age with a CODA Blood Non-Invasive Pressure Analyzer (Kent Scientific, Torrington, CT). This technique uses volume pressure recording, which correlates well with blood pressure measurements obtained by telemetry (12). Rats were trained by placing the rats in a Lucite tube and inflating a tail cuff several times daily for 4 days before the measurement of blood pressure to acclimatize the animals to the procedure. The investigator that measured blood pressure was blinded to the group and a different investigator analyzed the data. While the CODA system accurately measures blood pressure, the rats are nonetheless in a tube with a tail cuff and they are thus under some stress while blood pressure is measured.

GFR Measurements

Rats were either 2.9 ± 0.1 or 17.0 ± 0.3 mo when GFR was measured. They were given an intraperitoneal injection of Inactin (100 mg/kg). The skin on the ventral side of the neck, right hindleg, and chest were shaved. Rats were placed on a servo-controlled heated table to maintain a constant body temperature of 37°C. Catheters (polyethylene tubing) were placed in the femoral vein and carotid artery, and a tracheostomy was performed. A vesicostomy was performed, and a bladder catheter placed to allow free flow of urine that could be quantitated. GFR was measured as previously described by our laboratory (29). Briefly, 6 μCi [1Hmethionylulin was administered as a bolus followed by 16 μCi/h in normal saline solution at 0.36 ml-100 g body weight−1 h−1. After 1 h of equilibration, at least four 30-min urine samples were collected. A blood sample was collected at the midpoint of the urine collection for the measurement of inulin. [1Hmethionylulin was measured using a liquid scintillation counter (Beckman Coulter LS 6500 Scintillation Counter). After the last sample was collected, the heart and kidney were removed and weighed, and the kidney was placed in 10% buffered formalin for further histology analysis. A sample was saved for the assessment of collagen abundance.

Measurements of Interstitial Fibrosis and Glomerular Injury

Kidney slices (5 μm) were stained with picrosirius red to assess interstitial fibrosis in the total field (minus glomeruli and large blood vessels) at 250-fold magnification (28). Picrosirius red with and without polarization were analyzed separately to assess fibrosis (11). Slides were labeled so that the person evaluating the degree of fibrosis was blinded to the origin of the tissue. Slides were photographed using an Axioscan-2 Zeiss microscope with a Zeiss AxioCam MRC3 camera (Carl Zeiss, Thornwood, NY). For experiments where picrosirius red was used assessed under polarized light to measure interstitial collagen by determining collagen type I and III abundance (11, 17, 18, 28), slides were photographed with a Nikon Eclipse TE 2000-U microscope and a DS-U3 digital camera (Nikon Instruments). At least 10 images of the cortex and 10 images of the outer medulla were photographed, and images were analyzed using NIS-Elements BR 3.2 software to quantify fibrosis (8). The percentage of the image analyzed that was stained with picrosirius red and picrosirius red with polarized light was compared between groups.

The amount of collagen was estimated in 17-mo-old rats by measuring hydroxyproline using a Hydroxyproline Colorimetric Assay Kit (BioVision, Milpitas, CA). Approximately 10 mg of the frozen kidney cortex were weighed and assayed for hydroxyproline per the manufacturer’s instructions. Briefly, homogenized tissue was digested with 6 N hydrochloric acid and heated at 120°C for 3 h. Samples were evaporated to dryness and assayed per instructions. Collagen abun-
dance was extrapolated from hydroxyproline content assuming that collagen contained 12.7% hydroxyproline by weight. Results were expressed as micrograms of collagen per milligram of kidney.

Glomerular injury was assessed with slides stained with periodic acid-Schiff at 300-fold magnification. Glomeruli were assessed for the degree of mesangial matrix expansion and glomerulosclerosis using the same definition and scale previously described by Raji et al. (32). Briefly, mesangial matrix expansion was graded from 0 to 4 for each glomerulus based on the amount of periodic acid-Shiff staining in the mesangium. Glomerulosclerosis was assessed by the percentage of the glomerulus sclerosed where a glomerulus was scored from 0 to 4 based on the amount of glomerular involvement, where sclerosis of 25% of the glomerulus was scored 1, 50% was scored as 2, etc. There were 10 glomeruli assessed on each slide in a blinded fashion by 2 independent investigators at different times. The average score for each slide for mesangial matrix expansion and glomerulosclerosis was multiplied by 100 to give a minimum score of 0 and maximum score of 400 for both parameters. The scores for each investigator were averaged by a third investigator.

Statistical Analysis

Data are reported as means ± SE. Comparisons between two groups were made by a Student’s t-test. Comparisons between the cross-fostered groups were assessed using ANOVA with a post hoc Student-Newman-Keuls test.

RESULTS

Effect of a Prenatal Low-Protein Diet on GFR and Blood Pressure

In the first series of experiments, we examined the effect of a prenatal low-protein diet on blood pressure in rats at 1 yr of age. As shown in Fig. 1A, offspring of rats whose mothers were fed a 6% protein diet during the last half of pregnancy had a higher blood pressure than rats whose mothers were fed a 20% protein diet. Rats were then placed in metabolic cages, and 24-h urine collections were obtained for protein, creatinine, and albumin. These results are shown in Table 1. The creatinine clearance was somewhat less in the 6% group compared with the 20% group, but this did not reach statistical significance (P = 0.06), and the values were comparable when normalized for body weight. There were no differences in urinary protein or albumin excretion when expressed as the total amount per 24 h or if normalized for body weight in any of the groups. Thus, despite the tendency for a decrease in renal function, there was no significant increase in proteinuria or albuminuria in the 6% group compared with the 20% group.

GFR measured using inulin clearance was comparable in 20% and 6% groups at 3 mo of age whether corrected for body weight or not, as shown in Fig. 2, A and B. We next measured GFR using inulin clearance in rats that averaged 17 mo of age, which is shown in Fig. 3. GFR was significantly lower in the 6% group compared with the 20% group when results were not factored for body weight (Fig. 3A), when GFR was normalized per 100 g body weight (Fig. 3B), and when results were normalized for kidney weight (Table 2).

Effect of Postnatal Rearing on Blood Pressure and GFR in Rats Whose Mothers Were Fed a Control Diet or Low-Protein Diet

We next examined if the early postnatal environment affected GFR and blood pressure on offspring whose mothers were fed either a 20% or 6% protein diet during pregnancy and maintained on the same diet while nursing. Neonatal rats were cross-fostered to a different mother on day 1 of life. As shown in Fig. 1B, rats fed a 6% diet that were cross-fostered to a mother that was fed a 20% diet during pregnancy and while nursing (6% to 20% group) had comparable blood pressures at 1 yr of age as rats fed a 20% diet cross-fostered to a 20% mother (20% to 20% group). The 20% to 6% group was hypertensive at 1 yr of age, as was the 6% to 6% group. Thus, improving the postnatal lactational environment prevented the
increase in blood pressure in offspring of mothers fed a low-protein diet and postnatal maternal dietary protein deprivation while nursing causes hypertension in offspring at 1 yr of age.

As shown in Table 1, in the experiments examining cross-fostered rats, the 6% to 6% group had a lower creatinine clearance than the 20% to 20% group and tended to have a lower creatinine clearance than the 20% to 20% group, although the latter was not statistically significant (P = 0.08). When the creatinine clearance was normalized for body weight, the 6% to 6% group had a statistically lower GFR than the 6% to 20% group and the 20% to 6% group. The creatinine clearance in the 20% to 20% group was not significantly different from the 6% to 6% group when corrected for body weight. Interestingly, 24-h urine albumin excretion was higher in the 20% to 20% group than in the other groups, and

Fig. 2. Effect of a prenatal low-protein diet and postnatal rearing on glomerular filtration rate (GFR) at 3 mo. A: GFR (in ml/min) was measured using inulin clearance in 3-mo-old offspring of mothers that were fed a 20% or 6% protein diet during the last half of pregnancy. Rats were all fed a 20% protein diet after weaning. B: GFR corrected for body weight (in ml·min⁻¹·100 g body wt⁻¹) in 20% and 6% groups. C: GFR (in ml/min) rats whose mothers were fed a 20% or 6% protein diet during the last half of pregnancy and then cross-fostered to a different mother that continued on the same diet until weaning. GFR was measured using inulin clearance at 3 mo of age. D: GFR corrected for body weight (in ml·min⁻¹·100 g body wt⁻¹) in cross-fostered rats at 3 mo of age.

Table 1. Effect of maternal diet and postnatal rearing on creatinine clearance and urinary protein and albumin excretion at 1 yr of age

<table>
<thead>
<tr>
<th>Number of Rats/Group</th>
<th>Creatinine Clearance, ml/min</th>
<th>Creatinine Clearance, ml/min · 100 g body wt⁻¹</th>
<th>Urinary Protein, mg/24 h · 100 g body wt⁻¹</th>
<th>Urinary Albumin, mg/24 h · 100 g body wt⁻¹</th>
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<tbody>
<tr>
<td><strong>Prenatal programming experiments</strong></td>
<td></td>
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</tr>
<tr>
<td>20% group</td>
<td>10 2.18 ± 0.22</td>
<td>0.39 ± 0.04</td>
<td>58.9 ± 9.1</td>
<td>10.2 ± 1.6</td>
</tr>
<tr>
<td>6% group</td>
<td>9 1.49 ± 0.26*</td>
<td>0.30 ± 0.05</td>
<td>41.4 ± 11.2</td>
<td>7.9 ± 2.1</td>
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<tr>
<td><strong>Cross-fostering experiments</strong></td>
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<tr>
<td>20% to 20% group</td>
<td>11 2.10 ± 0.19†</td>
<td>0.38 ± 0.03</td>
<td>70.3 ± 20.7</td>
<td>12.9 ± 3.9</td>
</tr>
<tr>
<td>6% to 6% group</td>
<td>7 1.45 ± 0.15</td>
<td>0.31 ± 0.03</td>
<td>41.5 ± 8.4</td>
<td>7.6 ± 1.6</td>
</tr>
<tr>
<td>6% to 20% group</td>
<td>8 2.31 ± 0.18†</td>
<td>0.44 ± 0.03b</td>
<td>42.7 ± 6.8</td>
<td>8.7 ± 1.4</td>
</tr>
<tr>
<td>20% to 6% group</td>
<td>10 1.89 ± 0.12†</td>
<td>0.47 ± 0.03b</td>
<td>17.2 ± 4.2*</td>
<td>4.9 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. In prenatal programming experiments, measurements were made from rats whose mothers were fed a 20% or 6% protein diet and reared by the same mother fed a 20% protein diet after birth. After weaning, all rats were fed a 20% protein diet. In cross-fostering experiments, rats were divided into the following groups: 1) rats whose mothers were fed a 20% protein diet and cross-fostered to a different mother fed a 20% diet during pregnancy, and while nursing (20% to 20% group), 2) rats whose mothers were fed a 6% protein diet and cross-fostered to a different mother fed a 6% diet during pregnancy and while nursing (6% to 6% group), 3) rats whose mothers were fed a 6% protein diet during pregnancy and cross-fostered to a mother fed a 20% diet during pregnancy and while nursing (6% to 20% group), and 4) rats whose mothers were fed a normal 20% protein diet during pregnancy and cross-fostered to a mother fed a 6% diet during pregnancy and while nursing (20% to 6% group). After weaning, all rats were fed a 20% protein diet. aP < 0.01 vs. 20% to 20%, 6% to 6%, and 6% to 20% groups; bP < 0.05 vs. the 6% to 20% group; cP < 0.01 vs. the 20% to 20% group, dP < 0.01 vs. 20% to 20% and 6% to 20%; eP < 0.05 vs. the 6% to 20% group.
urinary albumin excretion per 100 g body wt in the 20% to 6% group was less than the 20% to 20% group. The 24-h urine protein excretion was also higher in the 20% to 20% group than in the 20% to 6% group.

Figure 2C shows GFRs of the groups that were cross-fostered by different mothers at 3 mo of life. The 20% to 20% group and the 6% to 6% group had higher GFRs than the 20% to 6% group and the 6% to 6% group. However, all GFRs were comparable when GFRs were normalized for body weight (Fig. 2D). Table 2 shows GFR data when analyzed per gram of kidney weight. The results were comparable with those examining per gram body weight except that GFR per gram kidney weight was significantly higher in the 20% to 20% group than in the 20% to 6% group at 3 mo of age. At 17 mo of age, GFR in the 20% to 6% group was lower than the 20% to 20% group and the 6% to 20% group (Fig. 3C). GFR of the 6% to 6% group was lower than the 6% to 20% group and lower than the 20% to 20% group; however, the latter did not reach statistical significance (P = 0.06). As shown in Fig. 3D, GFRs of cross-reared rats were not different when corrected for body weight. Thus, providing improved postnatal nutrition by cross-fostering a 6% rat to a 20% mother resulted in a GFR comparable with the 20% to 20% group when corrected for body weight. In addition, despite the fact that most of glomerulogenesis is postnatal in the rat, 20% rats nursed by mothers fed a 6% protein diet during the last half of pregnancy and while nursing resulted in a comparable GFR with the 20% to 20% group when corrected for body weight. The 6% to 6% group did not have a lower corrected GFR than the 20% to 20% group when GFR was corrected for body weight. As shown in Table 2, there were also no differences in GFRs in cross-fostered rats at 17 mo when the results were normalized for kidney weight. Table 3 shows weights of the rats at 3 and 17 mo as well as kidney and heart weights normalized per body weight. Body weights of 6% and 20% groups were comparable at 3 mo, but the 6% group weighed less at 17 mo, which may be the result of renal insufficiency. There were no differences in kidney and heart weights except that heart weight in the 6% group at 3 mo was less than that in the 20% group. Prenatal programming has been shown to affect myocardocyte number, which may be the

Table 2. Effect of prenatal programming and postnatal rearing on GFR normalized for kidney weight

<table>
<thead>
<tr>
<th>GFR per g Kidney Wt</th>
<th>3 mo</th>
<th>17 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal programming experiments</td>
<td></td>
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<tr>
<td>20% group</td>
<td>2.77 ± 0.24</td>
<td>2.52 ± 0.20</td>
</tr>
<tr>
<td>6% group</td>
<td>2.67 ± 0.18</td>
<td>1.64 ± 0.16*</td>
</tr>
<tr>
<td>Cross-fostering experiments</td>
<td></td>
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<tr>
<td>20% to 20% group</td>
<td>3.21 ± 0.27</td>
<td>1.93 ± 0.18</td>
</tr>
<tr>
<td>6% to 6% group</td>
<td>2.82 ± 0.15</td>
<td>1.95 ± 0.09</td>
</tr>
<tr>
<td>6% to 20% group</td>
<td>2.79 ± 0.14</td>
<td>2.16 ± 0.21</td>
</tr>
<tr>
<td>20% to 6% group</td>
<td>2.42 ± 0.09†</td>
<td>2.00 ± 0.18</td>
</tr>
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</table>

Values are means ± SE. GFR, glomerular filtration rate. *P < 0.01 vs. the 20% group; †P < 0.05 vs. the 20% to 20% group.
cause for the reduction in heart weight in the 6% group at 3 mo, although the weights were comparable at 17 mo (5). We have previously shown that cross-fostering a neonatal rat to a mother that was fed a 6% protein diet results in weight gain that is less than both 6% and 20% neonatal rats cross-fostered to a 20% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36). In our previous study, we studied rats until 2 mo of age, but, as shown here, 20% rats cross-fostered to a 6% mother (36).

Table 3. Effect of maternal diet and postnatal rearing on body, kidney, and heart weights at 3 and 17 mo of age

<table>
<thead>
<tr>
<th></th>
<th>3 mo</th>
<th>17 mo</th>
<th>3 mo</th>
<th>17 mo</th>
<th>3 mo</th>
<th>17 mo</th>
<th>3 mo</th>
<th>17 mo</th>
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<tbody>
<tr>
<td></td>
<td>Body Weight, g</td>
<td>Kidney Weight, g</td>
<td>Kidney Weight, g/100 g body wt</td>
<td>Hear weight, g/100 g body wt</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>20% group</td>
<td>378 ± 5</td>
<td>613 ± 14</td>
<td>1.15 ± 0.04</td>
<td>1.40 ± 0.05</td>
<td>0.31 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.27 ± 0.01</td>
</tr>
<tr>
<td>6% group</td>
<td>390 ± 21</td>
<td>542 ± 27*</td>
<td>1.09 ± 0.06</td>
<td>1.27 ± 0.08</td>
<td>0.29 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.27 ± 0.01</td>
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Cross-fostering experiments

| 20% to 20% group    | 90 ± 12   | 143 ± 33  | 0.92 ± 0.23 | 1.11 ± 0.21 | 0.32 ± 0.01 | 0.26 ± 0.01 | 0.32 ± 0.01 | 0.26 ± 0.01 |
| 6% to 6% group      | 57 ± 7    | 91 ± 23   | 0.87 ± 0.17 | 1.09 ± 0.17 | 0.32 ± 0.01 | 0.24 ± 0.01 | 0.34 ± 0.02 | 0.28 ± 0.03 |

Effect of Prenatal Programming and Postnatal Rearing on Interstitial Fibrosis and Glomerular Injury

The results examining interstitial fibrosis and glomerular injury in 17-mo-old rats are shown in Table 4. We assessed interstitial fibrosis using picrosirius red and picrosirius red under polarized light. There were no differences in percentages of interstitial picrosirius red staining with and without polarized light in the 20% group compared with the 6% group. In experiments quantitating hydroxyproline to estimate collagen, we found no differences in collagen between 20% and 6% groups at 17 mo of age. We assessed interstitial fibrosis and glomerular injury at 17 mo of age. The collagen content assayed by measuring hydroxyproline was less in the in the 6% to 20% group compared with the other groups in the outer medulla. This difference between the cross-rearing groups was not present in the picrosirius red staining without polarization. There was little mesangial matrix expansion or glomerulosclerosis in any of the groups at 17 mo of age. The collagen content assayed by measuring hydroxyproline was less in the in the 6% to 20% group compared with the other groups. The 6% to 6% group had greater collagen content than the other cross-fostered groups.

DISCUSSION

The focus of the present study was to examine if a prenatal low-protein diet during the last half of pregnancy results in a progressive decline in GFR and hypertension and whether the decline in GFR and hypertension can be affected by modulating the postnatal environment. We show that while there were no differences in GFRs in 6% and 20% rats at 3 mo of age, there was a significant reduction in GFR in rats whose mothers were fed a low-protein diet during pregnancy and fed a normal diet to their offspring. What was surprising is that despite the marked difference in GFR between the 6% and 20% groups, programmed rats had relatively little interstitial fibrosis and glomerular injury at 17 mo of age.

We also examined the extent of interstitial fibrosis and glomerular damage in the 20% to 20% group and compared the results with the 6% to 20%, 20% to 6%, and 6% to 6% groups. There was a small but significantly less interstitial picrosirius red staining under polarized light in the 6% to 20% group compared with the other groups in the outer medulla. This difference between the cross-rearing groups was not present in the picrosirius red staining without polarization. There was little mesangial matrix expansion or glomerulosclerosis in any of the groups at 17 mo of age. The collagen content assayed by measuring hydroxyproline was less in the in the 6% to 20% group compared with the other groups. The 6% to 6% group had greater collagen content than the other cross-fostered groups.

Table 4. Effect of maternal diet and postnatal rearing on renal collagen content, interstitial fibrosis, mesangial matrix expansion, and glomerulosclerosis

<table>
<thead>
<tr>
<th></th>
<th>Collagen, µg/mg tissue</th>
<th>Interstitial Picrosirius Red, %</th>
<th>Interstitial Picrosirius Red (Polarized), %</th>
<th>Glomerular Mesangial Matrix Expansion</th>
<th>Glomerulosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cortex</td>
<td>Outer medulla</td>
<td>Cortex</td>
<td>Outer Medulla</td>
</tr>
</tbody>
</table>
| Prenatal programming experiments
| 20% group           | 4.7 ± 0.7              | 9.9 ± 0.6 | 7.7 ± 0.8 | 1.7 ± 0.2 | 2.5 ± 0.3 | 133.5 ± 16.8 | 14.3 ± 4.2 |
| 6% group            | 3.6 ± 0.7              | 9.3 ± 0.5 | 8.7 ± 1.2 | 2.3 ± 0.2 | 2.2 ± 0.2 | 100.9 ± 14.3 | 6.3 ± 2.2 |

Cross-fostering experiments

| 20% to 20% group    | 3.8 ± 0.4              | 7.1 ± 0.9 | 6.1 ± 0.7 | 1.1 ± 0.1 | 2.2 ± 0.2 | 105.5 ± 14.5 | 28.2 ± 6.7 |
| 6% to 6% group      | 5.2 ± 0.5*             | 7.7 ± 0.7 | 7.1 ± 0.7 | 1.4 ± 0.3 | 2.4 ± 0.4 | 128.9 ± 17.7 | 23.4 ± 5.2 |
| 6% to 20% group     | 2.4 ± 0.1†             | 8.1 ± 1.0 | 5.8 ± 0.8 | 0.9 ± 0.1 | 1.4 ± 0.2* | 134.2 ± 22.5 | 17.8 ± 5.4 |
| 20% to 6% group     | 3.7 ± 0.4              | 8.7 ± 1.4 | 7.8 ± 0.8 | 1.4 ± 0.2 | 2.8 ± 0.2 | 133.9 ± 17.7 | 18.6 ± 8.6 |

Values are means ± SE; n = 6–7 rats/group. *P < 0.05 vs. other groups; †P < 0.05 vs. other groups (20% to 6% group and 6% to 6% group) and P = 0.056 vs. the 20% to 20% group.
20% protein diet while nursing when GFR was assessed at 17 mo of age. In cross-fostering experiments, offspring of the 6% rats cross-fostered to a 20% mother at day 1 of age prevented the decrease in GFR. Surprisingly, offspring of rats whose mothers were fed either 6% or 20% protein diets and cross-fostered to a rat fed a 6% protein diet during the last half of pregnancy and while nursing did not have a decrease in GFR per 100 g body wt compared with the 20% to 20% control group despite the fact that most of glomerulogenesis occurs in the first 7–10 days postnatally in rats (19). At 1 yr of age, the 6% group had higher blood pressures than the 20% group, whereas cross-fostered 6% to 20% rats did not have hypertension. Offspring of rats in the 20% to 6% group and 6% to 6% group had higher blood pressures than the 20% to 20% control group. Thus, the postnatal environment can modulate the effect of a prenatal insult on GFR and blood pressure.

Previous studies have examined whether a maternal prenatal insult would program a reduction in GFR in adult offspring. The results have been conflicting. We have previously measured GFR using inulin clearance in both 2- and 6- to 9-mo-old offspring after the administration of prenatal dexamethasone (29, 30). Despite the fact that rats developed hypertension and had a reduction in nephron number, there were no differences in GFRs between dexamethasone-treated offspring and control animals at either age. Similarly, in a model of uteroplacental insufficiency, offspring develop hypertension, but there was no reduction in GFR at 3 mo of age (2). The effect of a prenatal low-protein diet on GFR has been studied as well. The severity of the in utero protein restriction appears to affect GFR in later life. While an 8.5% protein diet did not affect GFR at 5 mo of age, a 5% protein diet during the last half of pregnancy was accompanied by a 15% reduction in GFR in male offspring at 4 mo of age. However, this small difference in GFR was not significantly different from control animals when corrected for body weight (45, 47). More severe insults with maternal dietary protein or caloric deprivation throughout pregnancy have been shown to affect weight-corrected GFR in mature rats (33).

The effect of prenatal maternal insults on the fetus are not set in stone and can be modified by changes in the early postnatal environment (5, 14, 44). We have previously examined the effect of cross-fostering rats whose mothers were fed a 6% protein diet and a 20% protein diet during pregnancy and while nursing (36). Offspring were weaned and fed a 20% diet and studied at 2 mo of age. Rats in the 20% to 6% group and 6% to 6% group did not gain weight at the same rate as rats in the 6% to 20% group and 20% to 20% group, which was still apparent in the present study when rats were studied at 3 and 17 mo of age. We also found that optimizing the postnatal environment by cross-fostering neonatal pups on day 1 of life whose mothers were fed a low-protein diet during pregnancy to a mother that was fed a 20% control diet during pregnancy and while nursing (6% to 20% group) prevented the reduction in nephron number (14, 36). Only 6% to 6% rats had a decrease in nephron number (36). Similarly, others have found that rats that were the product of mothers that had uteroplacental insufficiency also had a normalization of nephron number when cross-fostered to a control mother (44). We have previously found that the 6% to 6% group and 20% to 6% group had elevated blood pressures compared with the 20% to 20% group and 6% to 20% group when studied at 2 mo of age (36). Interestingly, we found that the cause for the hypertension was likely different in that the 6% to 6% group had elevated plasma renin activity and ANG II levels compared with the other three cross-fostered groups, whereas the 20% to 6% group had elevated renal Na+/K+ -2Cl− and Na+/Cl− protein abundance compared with the 20% to 20% group as assessed by immunoblot analysis. The results from the present study show that the hypertension persisted at 1 yr of age in the 6% to 6% group and 20% to 6% group and that despite the elevated blood pressure and reduction in glomerular number in the 6% to 6% group there was no decrease in GFR when corrected for body weight.

In the present study, offspring of mothers fed a low-protein diet and nursed by their own mother that was fed a normal protein diet after birth had hypertension and a reduction in GFR. However, cross-fostering a rat whose mother was fed a low-protein diet to a mother that was fed a normal protein diet prevented the decrease in GFR. The mechanism whereby an improvement in the postnatal environment normalizes many of the adverse effects of a prenatal insult is not clear. While prenatal insults, such as uteroplacental insufficiency, affect the kidney, administration affects branching morphogenesis and fetal renal development. Administration of glucocorticoids to pregnant rats has been useful to determine the time during pregnancy when glucocorticoids result in a reduction in nephron number and hypertension in offspring. Administration of corticosterone on days 14 and 15 or dexamethasone on days 15 and 16 or 17 and 18 in rats that have a 22-day gestation results in a reduction in nephron number and hypertension in adult offspring (29, 30, 38). The hypertension and reduction in nephron number were not seen if dexamethasone was administered at a later or an earlier date during pregnancy (29, 30). Other studies have found that improved postnatal rearing can mitigate the effect of a prenatal insult on blood pressure, glomerular number, glucose intolerance, and myocardocyte number (5, 14, 37, 44). The mechanism by which improving postnatal rearing improves a prenatal insult is not clear. It is possible that the perinatal period can affect epigenetic changes caused by an adverse prenatal insult.

The most surprising finding of the present study was that offspring of either the 6% group or 20% group raised by a mother fed a 6% protein diet had a normal GFR when corrected for body weight at 17 mo of age. Our findings are congruent with a previous study that compared adult offspring of rats whose mothers were fed a 20% diet and nursed by their own mother with those fed a 20% diet and nursed by a mother fed an 8% diet while pregnant and nursing. Rats reared by a mother fed a low-protein diet while lactating had a longer life, an increase in renal antioxidant expression, and a reduction in urinary albumin excretion compared with offspring of mothers fed a control diet (40); the lower urinary albumin excretion was confirmed in the present study. The authors attribute the results to a slower growth rate of neonates nursed by a mother fed a low-protein diet, which we have also previously demonstrated in the 20% to 6% group and 6% to 6% group (36). The hypothesis that slow growth during lactation may have a protective effect may be pertinent to the findings in our switched groups, where we have previously demonstrated that
cross-fostering either a 20% or 6% rat to a mother that was fed a 6% diet and continues on that diet while nursing results in growth retardation that persists into adulthood (36). Despite the fact that these rats are nursed by a mother fed a low-protein diet during much of nephrogenesis, we show that these rats do not develop renal insufficiency when GFR is corrected for body weight.

We assessed interstitial fibrosis as well as glomerulosclerosis and mesangial matrix expansion in the 6% and 20% group at 17 mo of age. Surprisingly, there were no differences between 6% and 20% groups when mesangial matrix expansion, glomerulosclerosis, and interstitial fibrosis were compared between the two groups despite the fact that there was a very significant decrease in GFR in programmed rats. The only difference noted between the 20% to 20%, 20% to 6%, and 6% to 20% groups was in the lower percentage of picrosirius red under polarized light in the outer medulla in the 6% to 20% group compared with the other groups. In addition, the 6% to 20% group also had less collagen, as assessed by measuring hydroxyproline. This again provides evidence that optimizing the postnatal environment can have a salutatory effect after a prenatal insult. While GFR corrected for body weight was not lower in the 6% to 6% group, there was more collagen per milligram of tissue weight in this group compared with the other groups. However, this was not apparent in our assessment using picosirius red with or without polarization.

A previous study (33) has examined the effect of intrauterine food restriction to 50% of control in pregnant rats throughout pregnancy on renal fibrosis and glomerulosclerosis. Offspring of mothers on a restricted diet had greater interstitial fibrosis and glomerulosclerosis than the control group at 18 mo. The cause for the disparity in the previous study and the present study is not clear but may be related to the severity of the prenatal insult. A study (45) that examined the effect of a low-protein diet throughout gestation (8.5% compared with 19% protein) did not find evidence for a difference in interstitial fibrosis or glomerulosclerosis in rats that were 2 wk of age, consistent with the finding in the present study. Neither of these studies examined the effect of postnatal rearing on GFR, interstitial fibrosis, or glomerulosclerosis.

A previous study (35) has implicated aging as a second insult with programming. Administration of an ANG II receptor blocker during postnatal days 1–14, a time of active nephrogenesis in the rat, resulted in sex- and age-dependent increase in blood pressure and renal injury. Rats were studied at 10–11 and 16–17 mo of age. At 10–11 mo of age, only male rats had a reduction in GFR of ~30%, whereas female rats did not have a significant reduction in GFR. At 16–17 mo of age, female rats had a reduction in GFR and male rats had a significant progressive decline in GFR to ~25% of the vehicle-treated control group. Our results are consistent with these results as we found no reduction in GFR at 3 mo but a significant reduction in prenatal programmed rats at 17 mo. Thus, aging could be considered a second insult in our experiments.

There were a few limitations in the present study. We studied as many as 2 rats/litter and, ideally, only 1 rat/litter should be analyzed in programming studies. While rats were trained for several days before the measurement of blood pressure and the CODA Blood Non-Invasive Pressure Analyzer was used to measure blood pressure, we do not know if programmed rats would be hypertensive if blood pressure was measured without any stress using telemetry. Finally, the present study only studied male rat offspring and the effect of prenatal or postnatal programming in this model affected female rats. Nonetheless, this study demonstrates that postnatal rearing can have a significant effect on a prenatal insult and prevent the reduction in GFR in rats whose mothers were fed a low-protein diet during the last half of pregnancy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


