Effect of Postnatal Maternal Protein Intake on Prenatal Programming of Hypertension

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Abstract

This study examined whether postnatal maternal dietary protein deprivation during the time of nursing can program hypertension when the offspring are studied as adults. Rats were fed either a 6% or 20% protein diet during the second half of pregnancy and continued on the same diet while rats were nursing their pups. The neonates of all of the rats were cross-fostered to a different mother and studied as adults. Adult rats that had a normal prenatal environment but were reared by mothers fed a low-protein diet until weaning (20%-6%) were hypertensive, had a higher renal Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2) and Na⁺-Cl⁻ cotransporter (NCC) protein abundance yet a comparable number of glomeruli, and had higher plasma renin and angiotensin II levels compared to control (20%-20%). Rats whose mothers were fed a 6% protein diet and cross-fostered to a different rat fed a 6% protein diet until weaning (6%-6%) were hypertensive, had elevated plasma renin and angiotensin II levels, and had a reduction in nephron number but had NKCC2 and NCC levels comparable to 20% to 20% offspring. The 6% to 20% had blood pressure and glomerular numbers comparable to 20% to 20% rats. The hypertension resulting from prenatal dietary protein deprivation can be normalized by improving the postnatal environment. Combined prenatal and postnatal maternal dietary protein deprivation and maternal dietary protein deprivation while nursing alone (20%-6%) results in hypertension, but the mechanism for the hypertension in these groups is different.

Keywords

dietary protein deprivation, blood pressure, glomerular number, barker hypothesis, postnatal programming

Introduction

Prenatal insults are a risk factor for hypertension and cardiovascular disease in adult offspring.¹⁻³ Many animal studies have shown that prenatal insults leading to small for gestational age offspring result in hypertension and a reduction in glomerular number when offspring are studied as adults.⁴⁻¹² The pathogenesis of the hypertension resulting from prenatal insults has been shown to be multifactorial and involves more than just a reduction in nephron number.¹²⁻¹⁵ Factors such as activation of the renin-angiotensin system, an increase in renal sodium transport, and an increase in sympathetic tone may contribute to the hypertension with prenatal programming.^{8,10,12,15-19}

Many infants, such as those that are born very prematurely, are appropriate size for gestational age and have a normal prenatal environment but have caloric deprivation and poor weight gain after birth.²⁰⁻²² These infants are at risk of developing hypertension as adolescents and adults.²³⁻²⁹ Some premature neonates are also small for gestational age and have both prenatal and postnatal nutritional insults. Since premature infants born before 34 to 36 weeks gestation are still making nephrons, postnatal insults may affect renal development.

The effect of postnatal maternal dietary protein intake during lactation on rats whose mothers were fed either a control

or a low-protein diet during pregnancy is not clear. Since renal development continues until 7 to 10 days of postnatal age,^{30,31} it is our hypothesis that postnatal insults could program hypertension in rats. To this end, this study examined whether cross-fostering neonates born to mothers fed a normal or low-protein diet during the last half of pregnancy to mothers fed a normal or low-protein diet during lactation affected growth, nephron number, blood pressure, and renal function. We find that postnatal maternal dietary protein deprivation causes hypertension when the nursing rats are adults. Adult offspring whose mothers were protein deprived during the last half of pregnancy and during lactation are also hypertensive but the pathogenesis of the hypertension rats with appears to be different than that with postnatal maternal dietary protein deprivation alone.

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Methods

Animals

Pregnant Sprague Dawley rats were fed either a 20% protein diet or an isocaloric 6% protein diet from the 12 days of gestation until the time of birth as previously described.^{12,32-34} The diets were isocaloric and contained equal amounts of vitamins and minerals. At the time of birth, all litters were reduced to 8 to 10 rats to reduce variability. Neonates were randomly removed and there was no selection bias as to which rats were chosen. We have previously shown that the number of neonates born to mothers who were fed the 2 diets was not different (11.4 ± 0.6 pups in the control and 13.0 ± 0.5 pups in the low-protein group, $P = \text{ns}$).³⁴ Mothers fed either a 6% or a 20% diet were maintained on this diet until their pups were weaned. After weaning, all rats were placed on a 20% diet. In all of the data presented, there were rats from at least 4 different litters in each group and at most 2 rats were used from the same litter. Rats were weighed weekly. Only males were studied since they are affected by prenatal programming more severely than females.^{7,11,35,36} These studies were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

On day 1 of life, the neonatal rats were cross-fostered to a different mother. There were 4 groups:

1. Neonates that were born to a mother that was fed a 20% diet were reared by another mother that was fed a 20% diet (20%-20%).
2. Neonates born to mothers who were fed a 20% diet and reared by a mother that was fed a 6% diet during the last half of pregnancy and then remained on that diet until the rats were weaned (20%-6%).
3. Neonates that were born to mothers that were fed a 6% diet and were reared by a different mother that remained on 6% until the rats were weaned (6%-6%).
4. Neonates that were born to mothers that were fed a 6% diet during pregnancy and were reared by a mother that was fed a 20% diet during pregnancy until the cross-fostered rats were weaned (6%-20%).

Measurement of Blood Pressure

Blood pressure was measured at 2 months of age by tail-cuff with a CODA Blood Pressure Analyzer (Kent Scientific Corporation, Torrington, Connecticut). This technique uses volume pressure recording and correlates well with blood pressure measurements obtained by telemetry.³⁷ Rats were trained daily for at least 4 days prior to the actual measurement of their blood pressure by placing them in a Lucite tube and inflating the blood pressure cuff several times. The blood pressure was measured on the fifth day. The investigator who measured the blood pressure was blinded until all of the measurements in each of the groups were completed. A different investigator then analyzed the data.

Glomerular Number

Glomerular number was determined using Alcian blue to stain the glomeruli as previously described by our laboratory and by others.^{6,7,10,38,39} Briefly, 2-month-old rats were anesthetized with 100 mg/kg body weight Inactin (Sigma Chemical Company, St Louis, Missouri). A femoral artery catheter was inserted to a level above the renal arteries. A 5% solution of Alcian blue (Sigma) dissolved in isotonic saline was infused ($0.3 \text{ cm}^3/100 \text{ g}$ body weight over 30 seconds) followed a few minutes later by a second dose.⁶ Five minutes after the second administration of Alcian blue, the kidney was removed and decapsulated. The kidney was minced and placed in 1% ammonium chloride solution (Sigma) and incubated at room temperature for 5 minutes. The tissue was then incubated in 5 mL of 50% HCl (6 N) in a water bath at 37° C for 90 minutes and agitated until the tissue was dissolved. The sample was centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded. The pellet was dissolved in 50 mL of water and this tube was gently shaken to ensure that the glomeruli were dispersed uniformly. Thirty 10- μL samples of glomeruli were counted and the total number of glomeruli in the kidney was then determined from the mean 30 determinations. The investigator who counted the glomeruli was blinded from the origin of the samples.

Plasma Renin Activity, Plasma Angiotensin II, and Serum Aldosterone Levels

Rats were administered intraperitoneal ketamine (100 mg/kg; Sigma) and xylazine (10 mg/kg, VEDCO INC; St Joseph, Missouri) after anesthesia blood was quickly withdrawn from the abdominal aorta. Plasma and serum were frozen at -80°C until the assays were performed. A Gamma Coat Plasma Renin Activity ¹²⁵I RIA kit (DiaSorin; Stillwater, Minnesota) was used to measure plasma renin activity by assaying angiotensin I generation per the manufacturer's instructions. Serum aldosterone was measured using an enzyme immunoassay kit per the manufacturer's instructions (Cayman Chemical; Ann Arbor, Michigan). For measurement of angiotensin II, blood was placed in a cooled tube (4°C) containing EDTA (GIBCO; Grand Island, New York) and enalaprilat (Bedford Laboratories; Bedford, Ohio) at a final concentration of 5 mmol/L and 20 $\mu\text{mol/L}$, respectively. The tube was centrifuged at 1500g at 4°C for 30 minutes. Plasma angiotensin II was extracted and assayed as described previously by our laboratory using the angiotensin II enzyme immunoassay (Spi-Bio; Paris, France).

Clearance and Studies and Urine Collection

Rats were acclimatized to metabolic cages for 48 hours before the first urine collection. Urine was then collected for 24 hours for measurement of angiotensinogen and creatinine. The urine receptacle contained 50 μg pepstatin (Sigma), 10 mg sodium azide (Sigma), 300 nmol enalaprilat (Bedford Laboratories), and 125 μmol EDTA (GIBCO) to prevent protein degradation.

Figure 1. Effect of prenatal protein and postnatal rearing on postnatal weight gain: cross-fostering the 6% rats to a 20% mother that continued to be fed a 20% diet resulted in catch-up weight gain comparable to the 20% raised by the 20% group by 1 week of age. Fostering the neonatal rats from a 20% to a 6% mother that continued to be fed a 6% diet resulted in poor growth comparable to the 6% reared by the 6% mother. Note that neither the 20% to 6% nor the 6% to 6% had catch up growth to levels comparable to the groups raised by a 20% mother even after they were switched to a 20% diet when weaned. There were 9 to 17 rats in each group.

The collected urine was then stored at -80°C until angiotensinogen was measured using an ELISA (Immuno-Biological Laboratories, Japan).

For measurement of urine, total protein and albumin rats were placed in metabolic cages with free access to food and water. The urine was collected without preservative and quantitated. Urine was frozen at -80°C until measurement of urine total protein and albumin. Prior to assay, the urine samples were allowed to come to room temperature. The urine total protein was measured by Bradford assay (Bio-Rad Laboratories; Hercules, California). The urine albumin was measured on a 96-well plate by a rat urinary albumin enzyme immunoassay kit Nephtrac (Exocell; Philadelphia, Pennsylvania).

Protein Isolation, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, and Immunoblotting

The kidney was placed in buffer that contained 250 mmol/L sucrose and 10 mmol/L triethanolamine, a protease inhibitor cocktail (1 $\mu\text{L}/\text{mL}$; Sigma), and phenylmethylsulfonyl fluoride (100 $\mu\text{g}/\text{mL}$, Calbiochem; La Jolla, California).¹⁴ The kidney was homogenized with a polytetrafluoroethylene-glass homogenizer at 4°C and then centrifuged at 1000g for 15 minutes. The supernatant protein concentration was estimated using the Bradford method using bovine albumin as the standard.⁴⁰

Protein samples (100 μg) were denatured at 65°C for 5 minutes before loading on a polyacrylamide gel as described previously.⁴¹ Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to separate the proteins that were

then transferred to polyvinylidene difluoride membrane at 400 mA for 1 hour. The membranes were blocked with Blotto (5% nonfat dry milk in phosphate-buffered saline) for 1 hour before incubation with the primary antibody followed by incubation with the primary antibody at 4°C overnight. The primary rabbit polyclonal antibodies to $\text{Na}^{+}\text{-K}^{+}\text{-2Cl}^{-}$ cotransporter (NKCC2) was used at a 1:1500 dilution (a gift from H. Moo Kwon at the University of Maryland)⁴² and primary rabbit polyclonal antibodies to $\gamma\text{-ENaC}$ (epithelial sodium channel; Millipore; Billerica, Massachusetts) was used at a 1:600 dilution.^{43,44} The antibody to $\text{Na}^{+}/\text{H}^{+}$ exchanger 3 (NHE3) was a mouse monoclonal antibody used at a 1:5 dilution (a gift of OW Moe at UT Southwestern).⁴⁵ The antibodies to $\text{Na}^{+}\text{-Cl}^{-}$ cotransporter (NCC; Millipore), $\alpha\text{-ENaC}$ (Santa Cruz Biotechnology; San Francisco, California), and $\beta\text{-ENaC}$ (Origene; Rockville, Maryland) were all rabbit polyclonal antibodies used at a 1:600 dilution. All membranes were also probed with an antibody to $\beta\text{-actin}$ at a 1:15 000 dilution to ensure equal loading (Sigma). After incubation, the blots were washed extensively with Blotto, and donkey antirabbit or anti-mouse secondary antibodies conjugated to horseradish peroxidase were added at a 1:15 000 dilution (Santa Cruz Biotechnology). Bound antibody was detected by chemiluminescence and quantitated using densitometry. There were at least 6 blots analyzed from 6 different rats in each group.

Statistical Analysis

Data are expressed as the mean $\pm$ standard error of the mean. Analysis of variance with post hoc Student-Newman-Keuls test was used to determine statistical significance in comparisons of more than 2 groups. A post hoc Student *t* test was used where designated.

Results

Neonatal rats born to mothers on a protein-restricted diet (6%) weighed 4.58 ± 0.07 g, which was significantly less than 5.72 ± 0.10 g measured in rats born to mothers fed a 20% control diet on day 1 of life ($P < .001$). All neonatal rats were weighed and there was no selection bias. On day 1 of life, all of the rats were fostered to different mothers and there was a significant effect on weight gain as shown in Figure 1. By 7 days of age, the 6% group raised by 20% mothers had a comparable weight as 20% pups raised by 20% mothers. The low-protein group raised by the 20% control mothers (6%-20%) continued to gain weight at a comparable rate as that of the 20% to 20% group, although there was a relatively small but significant difference on days 21, 28, 35, and 42 of life. This difference was not noted at earlier or later time points. Rats born to mothers that were protein restricted and cross-fostered to protein-restricted mothers that continued on the 6% diet while nursing weighed about half of the 20% to 20% at 7 days of age and gained weight poorly throughout life. Of interest was the weight gain of the rats born to mothers that were fed a 20% diet and then cross-fostered to rats that were fed a 6% diet during pregnancy and

Figure 2. Effect of prenatal protein and postnatal rearing on glomerular number: glomeruli were counted in adult rats in a blinded fashion. Only 1 rat per litter was used in these experiments. The rats born to mothers that were fed a 6% diet and fostered to mothers that continued to be fed a 6% diet while the pups were nursing had fewer glomeruli than all of the other groups. * designates $P < .05$ versus other groups.

Figure 3. Effect of prenatal protein and postnatal rearing on blood pressure: systolic blood pressures were measured in a blinded fashion. The rats that were cross-fostered to a mother that was fed a 6% diet were hypertensive. Note that the blood pressures of offspring whose mothers were fed a 6% protein diet but were cross-fostered to a mother that was fed a 20% protein diet had comparable blood pressures to the 20% to 20% group. * designates $P < .002$ versus other groups.

postnatally until the time of weaning (20%-6%). By 1 week of age, the 20% neonates raised by 6% rats weighed significantly less than the 20% to 20% rats and had comparable weights to the 6% to 6% group. At weaning, all rats were fed a 20% protein diet. Nonetheless, there was no catch up growth in the 6% to 6% and 20% to 6% groups and both weighed far less than 6% to 20% to 20% groups.

We have previously shown that a maternal low-protein diet during pregnancy results in offspring with a reduction in nephron number when the mothers were fed a 6% diet during pregnancy and were fed 20% diet after birth compared to rats

whose mothers were fed a 20% diet before and after birth: $22\,111 \pm 627$ versus $29\,666 \pm 654$ glomeruli/kidney ($P < .001$).³³ These findings were comparable to that found by others.³² The number of glomeruli in each of the cross-fostered groups is shown in Figure 2. The number of glomeruli in the 6% to 6% group was less than all other groups. However, there was no difference between the 20% to 6% and the 6% to 20% groups compared to that of the 20% to 20% group. Thus, an optimal prenatal or postnatal maternal dietary protein intake can prevent a reduction in nephron number and a reduction in nephron number does not contribute to the hypertension in the 20% to 6% group.

We measured blood pressure in the cross-fostered rats. The results are shown in Figure 3. The blood pressure of the 6% group reared by the 20% mothers had comparable blood pressures to the 20% group reared by a 20% mother. The offspring of rats whose mothers were fed a 6% protein diet and were reared by a mother that was fed a 6% protein diet were hypertensive. Rats that were the product of a mother that were fed a 20% protein diet during pregnancy and cross-fostered to a mother fed a low-protein diet during pregnancy and while nursing were also hypertensive at 2 months of age. Thus, isolated maternal postnatal dietary deprivation can cause an increase in blood pressure when the offspring are adults.

We next examined whether there was evidence of impaired renal function in the rats raised by mothers fed a 6% diet. These results are shown in Table 1. The 20% to 6% group had a higher creatinine clearance than the rats that were nursed by mothers fed a 20% diet. The 6% to 6% group had a somewhat higher creatinine clearance as well but this did not reach statistical significance. There was no difference in the urinary protein excretion in any group although the 6% to 6% group was somewhat higher than the other groups. However, the urinary albumin excretion was higher in the 6% to 6% group than the other groups although it was only significantly different from the 20% to 6% group.

To begin to examine the cause of hypertension, we measured plasma renin activity, plasma angiotensin II, serum aldosterone, and urinary angiotensinogen/creatinine. The results of plasma renin activity and plasma angiotensin II are shown in Figure 4. The 6% to 6% group had an elevated renin and angiotensin II levels that were greater than the other 3 groups. There was no difference in serum aldosterone concentration (pg/mL) in the 20% to 20% (536.7 ± 77.5), 6% to 6% (710.6 ± 91.3), 6% to 20% (569.7 ± 178.8), or 20% to 6% (509.3 ± 107.7) groups. There was also no difference in urinary angiotensinogen/Cr (ng/mg) in the 20% to 20% (19.4 ± 3.8), 6% to 6% (43.5 ± 14.1), 6% to 20% (26.6 ± 7.5), or 20% to 6% (16.6 ± 1.9) groups, which is a measure of the intrarenal renin angiotensin II system.^{46,47}

Finally, previous studies have shown that maternal dietary protein deprivation programs an increase in NKCC2 and NCC in adult offspring and prenatal dexamethasone increases these transporters as well as NHE3.^{8,12,14,18} In the previous studies, the mothers were reared by the same mothers administered a low-protein diet or prenatal dexamethasone. As shown in

Table 1. Creatinine Clearance, 24-Hour Protein, and Albumin Excretion.

2-Month-Old Rats	Creatinine Clearance, mL/min/100 g	24-Hour Urine Protein Excretion, mg/24 h/100 g	24-Hour Urine Albumin Excretion, mg/24 h/100 g
20%-20%	0.43 ± 0.03	6.22 ± 0.68	0.81 ± 0.15
6%-6%	0.53 ± 0.08	8.60 ± 1.00	1.16 ± 0.29 ^a
6%-20%	0.39 ± 0.03	6.42 ± 1.04	0.63 ± 0.10
20%-6%	0.64 ± 0.09 ^b	6.47 ± 1.06	0.47 ± 0.09

^a 6%-6% greater than 20% to 6% at $P < .05$.

^b 20%-6% different from 20%-20% and 6%-20% at $P < .05$.

Figure 4. Effect of prenatal protein and postnatal rearing on plasma renin activity plasma angiotensin II and serum aldosterone levels and urinary angiotensinogen/Cr: the plasma renin activity and angiotensin II levels were higher in the 6% to 6% group than the other groups. * indicates 6% to 6% greater than all other groups at $P < .05$.

Figure 5, we found that NHE3 was comparable in the 20% to 20%, 6% to 6%, and 20% to 6% groups which suggests that increased NHE3 protein abundance does not contribute to the hypertension with prenatal or postnatal protein deprivation. Interestingly, NHE3 protein abundance was higher in the 20% to 6% group than in the 6% to 6% and the 6% to 20% groups. Neither prenatal nor postnatal protein deprivation affected any of the ENaC subunits (data not shown). The NKCC2 and NCC protein abundance was comparable in the 6% to 6% group compared to the 20% to 20% group. NCC and NKCC2 were higher in the 20% to 6% group than in the 20% to 20% group; however the latter was only significant using a post hoc Student *t* test. These data are consistent with the hypertension with postnatal dietary protein deprivation being mediated by a different mechanism than when there are both prenatal and postnatal insults.

Discussion

The present study examined the effect of postnatal rearing on rats that were born to mothers fed a normal protein or a low-protein diet during the last half of pregnancy. The neonates were then cross-fostered to a different mother that continued

on the same diet they were fed during the last half of gestation until pups were weaned. The rats born to mothers fed a 6% diet during the last half of pregnancy and reared by a mother that was fed a 6% diet were growth retarded and had hypertension associated with elevated renin and angiotensin II levels but had renal transporter abundance comparable to the 20% to 20% controls. The rats that were born to a mother that was fed a 20% protein diet and cross-fostered to a mother that was fed a 6% diet during pregnancy and until the time of weaning were also severely growth retarded and hypertensive and had an increase in NKCC2 and NCC compared to the 20% to 20% group but had comparable renin and angiotensin II levels to that of the 20% to 20% group. However, rats born to mothers that were fed a 6% protein diet and were cross-fostered to a mother that was fed a 20% diet during and after pregnancy were not growth retarded or hypertensive, indicating that the sequela of a prenatal insult can be ameliorated by an optimal postnatal environment.

In the present study, all mothers cared for a normal complement of cross-fostered pups. We found that there was approximately one-third the weight gain from day 7 to 21 in neonates reared by mothers fed a low-protein diet (6%-6% and 20%-6%) during lactation compared to the 20% to 20% control. In stark contrast, rats born to mothers fed a 6% protein diet weighed

	20% to 20%	6% to 6%	6% to 20%	20% to 6%	
NHE3	0.65 ± 0.08	0.40 ± 0.11	0.35 ± 0.08	0.85 ± 0.07 ^{#+}
NKCC	0.33 ± 0.07	0.59 ± 0.14	0.48 ± 0.15	0.72 ± 0.10 [*]
NCC	0.23 ± 0.07	0.40 ± 0.13	0.63 ± 0.09 [*]	0.93 ± 0.04 ^{**#+}

Figure 5. Effect of prenatal protein and postnatal rearing on tubular transporter abundance: transporter protein abundance was measured by immunoblot for the designated transporters. NCC protein abundance was greater in the 20% to 6% group than all other groups. Although NKCC2 was not different by analysis of variance (ANOVA), the 2-fold difference in NKCC in the 20% to 6% group compared to the 20% to 20% group was different using a Student *t* test ($P = .01$).

[#] > 6%-20%, $p < .01$.

⁺ > 6%-6%, $p < .01$.

^{*} > 20%-20%, $p < .01$.

less at birth than the 20% group, but by 7 days of age, the 6% group cross-fostered to the 20% mother had virtually the same weight as the 20% group cross-fostered to another 20% group. Thus, rearing a neonate by a mother that was fed a low-protein diet caused severe growth retardation, while providing a normal lactational environment can cause rapid catch-up growth in neonates born small for gestational age because of maternal dietary protein deprivation.

The present study measured the number of glomeruli in adult rats that had a prenatal insult and were cross-fostered to a mother that was fed a 20% diet during pregnancy and after birth. This study confirmed that improving the postnatal lactational environment can result in the normalization in the number of glomeruli.^{34,48} This begs the question of why would rats whose mothers were administered dexamethasone prenatally or were fed a prenatal low-protein diet and a 20% protein diet while nursing have a reduction in nephron number? The most likely explanation is that since the majority of nephron development occurs postnatally in the rat, the prenatal insult must affect the lactating mother postnatally. Postnatal growth is dependent upon mammary development with delivery of milk of the appropriate composition of nutrients. In the absence of a prenatal insult, postnatal maternal dietary protein intake can affect mammary protein and lipid synthesis, the quantity of milk produced, and weight gain of the rat pups.⁴⁹⁻⁵¹

The primary focus of the current study was to test the hypothesis that a postnatal insult on neonates where nephrogenesis is still occurring, which is a potential model for the nutritional insult and poor growth seen in very premature human neonates, causes hypertension.⁵² To this end, we cross-fostered rats whose mothers were fed a normal 20% protein diet to rats that were fed a 6% protein diet during pregnancy and continued to be fed a low-protein diet after birth until the rats were weaned (20%-6%). Surprisingly, there was not a decrease in nephron number compared to the 20% to

20% group. However, the 20% to 6% group did develop significant hypertension. Thus, a reduction in nephron number was not a significant factor in the generation of the increased blood pressure in the 20% to 6% group. However, the 6% to 6% group which may prove to be a model of small for gestational age neonates with a suboptimal postnatal environment did have a reduction in nephron number and was hypertensive.

Our laboratory had previously attempted to examine whether an adverse postnatal environment would result in hypertension or a decrease in nephron number by switching neonates at the time of birth from mothers that were fed a 20% protein diet to those that were fed a 6% protein diet during pregnancy.³⁴ However, unlike the present study, all nursing mothers were placed on a 20% protein diet after birth. The neonates from 20% rats fostered to a mother that was fed a 6% protein diet during pregnancy but was then fed a 20% protein diet while nursing did not have hypertension or a reduction in nephron number. Thus, the postnatal insult in our previous study was much less severe than that in the present study, where neonates from the 20% group were nursed by 6% mothers that continued on the 6% diet.

The present study examined 2 hypertensive groups that may have clinical relevance. We examined a 6% to 6% group where there was a prenatal insult causing small for gestational age rats that were continued with suboptimal nutrition while nursing and a 20% to 6% group where there was only suboptimal nutrition postnatally. We find that the postnatal weight gain and blood pressure of the 20% to 6% group were not different than the 6% to 6% group. However, there were significant differences between the 6% to 6% group and the 20% to 6% group. We found that the 6% to 6% group had the greatest urinary albumin excretion but this was only significantly different from the 20% to 6% group. The mechanism for the hypertension in the 6% to 6% group is likely different than that of the 20% to 6% group. The 20% to 6% group had an increase in NCC and

NKCC2 which may result in sodium-sensitive hypertension, a question to be addressed in future studies. On the other hand, the 6% to 6% group had normal transporter abundance but a decrease in glomerular number and an increase in plasma renin activity and angiotensin II level compared to the 20% to 20% group. Higher renin levels have been found in 4-month-old offspring of mothers fed a 6% diet.¹⁶ Thus, prenatal insults but not postnatal insults alone likely cause the increase in renin and angiotensin II, which can be reprogrammed by optimizing the postnatal environment. It is surprising that the renin and angiotensin II levels are elevated in the 6% to 6% group but the aldosterone levels are not different for the 20% to 20% group. However, in a previous study examining the cause for the hypertension with prenatal dietary protein deprivation, a study previously found that the low-protein offspring had elevated plasma renin activity but normal aldosterone levels as adults.¹⁶

Previous studies have looked at the effect of postnatal caloric or protein deprivation in nursing mothers on offspring studied as adults. One group restricted the caloric intake by 50% either prenatally or during lactation and compared the results to controls.⁵³ The rats that had a prenatal insult had hypertension, a reduction in nephron number, and significant proteinuria, while the calorically deprived rats during lactation only had mild proteinuria but no reduction in nephron number or increase in blood pressure. The difference between that study and the present one where maternal postnatal dietary protein deprivation caused hypertension is likely due to the fact the rat chow in the previous study had 23% protein and even at feeding 50% of a normal amount of food, they were likely less protein deprived than the 6% protein diet in the present study. In addition, all rats in the present study were offered a chow with comparable calories. In the other study looking at protein deprivation, Luzardo et al examined the effect of an 8% protein diet administered to mothers nursing pups and compared the result to those whose mothers continued on a 20% protein diet.⁵⁴ As with the present study, they found an increase in creatinine clearance in the postnatal low-protein group compared to the control when normalized for 100 g body weight. The factors that are causing the increase in glomerular filtration rate with a postnatal low-protein diet in the previous study and the current one are unclear. Luzardo et al also showed that there was a decrease in area of Bowman capsule and glomerular tuft and an increase in collagen deposition in the cortex and medulla,⁵⁴ which were not assessed in the current study. The previous study also found significant postnatal growth retardation in the offspring of mothers fed a low-protein diet compared to the control. However, there were significant differences between the previous study and this one. Unlike the present study, they also found that the 8% group had comparable blood pressures to the control group. However, they found a 30% reduction in nephron number in the postnatal low-protein group compared to the control group and the 8% group also had proteinuria. The reason for the difference between our study and the one by Luzardo et al is likely due to the fact that they reduced the litter size of all mothers to 6 rats.⁵⁴ A reduction in nursing pups to 5 pups has been shown to result in a

reduction in nephron number when the rats were studied as adults.^{55,56} Thus, the reduction in litter number in conjunction with postnatal dietary protein deprivation may be a factor accounting for the proteinuria and reduction in nephron number in the study by Luzardo et al.⁵⁴

In summary, postnatal maternal dietary deprivation in rats during lactation leads to hypertension in adult offspring. Premature humans often have suboptimal postnatal nutrition with inadequate protein intake⁵² and are at risk of developing hypertension as adults.²³⁻²⁹ The present study may serve as a model to examine the mechanism for the generation and maintenance of hypertension due postnatal nutritional deprivation.

Declaration of Conflicting Interests

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