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ORIGINAL ARTICLE

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Nephron number and blood pressure in rat offspring with maternal high-protein diet

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Abstract This study investigated the effects of a high-protein diet during pregnancy on nephron endowment and subsequent levels of blood pressure in the offspring. Female WKY rats were fed either a normal (20%, NPD) or a high (54%, HPD) protein diet during pregnancy. Male offspring were paired at birth. At 4 weeks of age, 1 of the pair was randomly chosen for perfusion fixation, and total glomerular number, and thereby nephron number, was estimated using an unbiased stereological technique. The other rat of the pair was allowed to grow to 30 weeks of age, during which time tail cuff systolic blood pressure was monitored twice weekly. There was no effect of the HPD on birth weight (NPD 4.23±0.53 g, HPD 4.26±0.45 g, mean±SD), kidney weight (NPD 0.372±0.049 g, HPD 0.337±0.090 g), or total nephron number (NPD 27,191±3,512, HPD 26,738±4,735). Systolic blood pressure at 30 weeks was 170±14 mmHg in NPD and 169±14 in HPD offspring. These findings show that a HPD during pregnancy did not lead to an increase in birth weight, kidney weight, or nephron endowment, nor did the HPD affect adult blood pressure.

Keywords Birth weight · Nephron endowment · Hypertension · Stereology

Introduction

Epidemiological studies have demonstrated a link between low birth weight and increased incidence of hypertension later in life [1, 2, 3]. There are a number of factors during pregnancy that can contribute to intrauterine growth retardation leading to low birth weight. These include smoking [4], pre-eclampsia [5], and poor maternal nutrition [6].

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In humans, maternal undernutrition during pregnancy as a result of limited protein intake can lead to low birth weight infants [6]. Similarly, in animals, administration of a low-protein diet (LPD) during pregnancy leads to low birth weight offspring [7, 8, 9, 10, 11]. In some studies this was shown to be followed by a significant elevation in systolic blood pressure later in life [12, 13]. However, the mechanisms by which this occurs are not fully understood.

It has been postulated that a reduced nephron endowment in low birth weight offspring may be a contributing factor in the observed elevations in blood pressure later in life. Indeed, in human and animal studies, low birth weight is accompanied by reductions in organ size, including kidney size, with significant decreases in nephron endowment [9, 11, 13]. Reduced nephron endowment is postulated to lead to reduced renal filtration surface area, which can then lead to renal sodium retention and subsequent systemic hypertension [14]. In order to maintain renal filtration surface area, the kidney can compensate by increasing glomerular size, with some studies showing an inverse relationship between glomerular size and glomerular number [15]. However, this relationship is not always evident [16, 17]. It is hypothesized that with loss of glomeruli through aging [18], lifestyle insults, and disease, there will ultimately be a breakdown in function of glomeruli and the proposed mechanisms will come into play. Alternatively, it is likely that individuals with supranormal glomerular filtration surface area (due to an increase in the number of glomeruli and/or increase in filtration surface area per glomerulus) will be relatively protected from the development of hypertension via these mechanisms.

Nephrogenesis is complete in humans by about 36 weeks gestation [19] and in rats by about postnatal day 8 [20]. Since no new nephrons are formed after this time and with the expected loss of glomeruli during aging and disease [18], it is important to maximize glomerular endowment at birth. It is conceivable that if administration of an LPD during pregnancy leads to reduced nephron endowment, the reverse may apply with the ad-

ministration of a high-protein diet (HPD) during pregnancy, leading to enhanced nephron endowment. Hence, the aim of this study was to determine whether administration of an HPD during pregnancy leads to high birth weight offspring with increased nephron endowment, and/or whether this is associated with reduced levels of blood pressure in adulthood. In the event that there was no effect on nephron endowment following exposure to an HPD in utero, the effect on adult blood pressure was still investigated, as the effects of an LPD in reducing nephron endowment may not be causally related to elevation in blood pressure.

Materials and methods

Animals and diets

Adult male and female Wistar-Kyoto rats were obtained from the Australian Resource Center (Perth, Australia) and mated to generate the offspring used in this study.

The female breeder rats were divided into two groups (n=5 per group) and had free access to either a normal (20% casein, NPD) or a high (54% casein, HPD) protein diet obtained from Glen Forrest Stockfeeders Perth, Australia. As the protein content of the HPD was high, the amount of carbohydrate was significantly reduced (Table 1); however, the diets were not isocaloric. The female breeders were fed their respective diets for 2 weeks prior to mating to habituate the females to their diets, during pregnancy, and for a further 2 weeks after the birth of the offspring to ensure that nephrogenesis was complete in the offspring. After this time, rats were taken off the HPD in exchange for the NPD.

After giving birth, litters were reduced to 8 pups by preferentially culling abnormally small or large pups and/or females. Litters with fewer than 8 pups were not used. Remaining pups were housed with the mother until the weaning age of 4 weeks, during which time body weight was measured daily. After weaning, rats were housed 2–3 per cage and fed an NPD until sacrifice. At 4 weeks of age, male offspring were paired, and female littermates were excluded from further experimentation. At 4 weeks, 1 of the male offspring was randomly chosen from the pair for perfusion fixation and the other rat of the pair was allowed to grow to adulthood (30 weeks of age), during which time body weight and blood pressure were determined twice weekly.

Animal care and experimental procedures conformed with the NH and MRC Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University, Department of Anatomy and Cell Biology Animal Ethics Committee.

Table 1 Composition of synthetic diets fed to breeder rats prior to mating, during pregnancy, and for 2 weeks after birth of the offspring (percentages refer to weight) (*NPD* normal protein diet, *HPD* high-protein diet)

	NPD	HPD
Sucrose (%)	10	10
Starch (%)	39.75	5.91
Dextrinized starch (%)	13.2	13.2
Cellulose (%)	5	5
Casein (%)	20	54
Safflower oil (%)	7	7
Methionine (%)	0.3	0.14
Minerals (%)	3.5	3.5
Vitamins (%)	1	1
Choline chloride 50% w/w (%)	0.25	0.25
Total ingredients	100	100

Perfusion fixation

Rats sacrificed at 4 weeks of age were perfusion fixed using 2.5% glutaraldehyde in 0.1 M phosphate buffer at a perfusion pressure of 100 mmHg. The two kidneys were excised, decapsulated, weighed, and stored in buffered glutaraldehyde until use. One of the kidneys was used to estimate glomerular number (and thereby nephron number) and the other to estimate total glomerular capillary length and surface area, and total renal filtration surface area. Perfusion-fixed left and right kidneys of 4-week-old rats were sliced at 1-mm thickness using a razor blade cutting device.

Estimation of total number of glomeruli

All kidney slices from the left kidney were embedded in glycolmethacrylate resin (Technovit 7100 resin, Haraeus Kulzer, Germany). Blocks were exhaustively sectioned at 20 µm and every tenth section was collected (with the first section chosen at random) and stained with hematoxylin and eosin.

Every tenth section was used to estimate kidney volume, but only complete kidney sections were used for the estimation of total glomerular number. The total number of glomeruli in the kidneys was estimated using a physical dissector/fractionator technique. Detailed methods of glomerular stereology have been described elsewhere [11, 21].

Estimation of glomerular capillary dimensions

For estimation of glomerular capillary length and surface area, cubes of cortex were cut out of the slices of the perfused right kidneys. From these, eight cubes were randomly selected, and embedded in Epon-Araldite. Blocks were then sectioned at 1-µm thickness (using a Leica 2065 supercut microtome, Germany) and stained with toluidine blue. Using unbiased stereological techniques, length and surface area of capillaries within glomeruli and total renal filtration surface area in the kidneys of 4-week-old rats were estimated. For detailed methods see Bertram [21].

Determination of body weight and blood pressure

The rats allowed to grow to 30 weeks of age (n=14 NPD and n=10 HPD) had body weight determined twice weekly. Systolic blood pressure was determined twice weekly in pre-warmed, restrained rats using tail cuff plethysmography.

Statistics

Data are expressed as means±SD. Analyses were carried out using Graphpad Prism, version. 2, U.S.A. Student's t-test was used to detect statistically significant differences in data between the two groups of 4-week-old rats. A two-way analysis of variance was used to determine if there were differences between groups in body weight and blood pressure over time in the rats allowed to grow to adulthood (30 weeks of age). A probability of less than 5% was considered significant.

Results

Birth weight

The amount of food consumed during pregnancy by the female breeders on the HPD was significantly lower

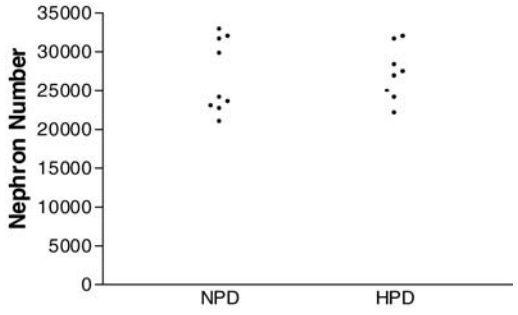

Fig. 1 Effect of a high-protein diet (*HPD*) during pregnancy on total glomerular number in offspring. Each *dot* represents a kidney (*NPD* normal protein diet)

Table 2 Birth weight, body weight, kidney weight, kidney weight/birth weight ratios in 4-week-old offspring (n=8 NPD and n=9 HPD) and body weight and blood pressure in 30-week-old rats exposed to an NPD or an HPD in utero (n=14 NPD and n=10 HPD)

	NPD	HPD
Birth weight (g)	4.23±0.53	4.26±0.45
Weight at 4 weeks (g)	55.7±6.1	52.5±10.1
Kidney weight at 4 weeks (g)	0.372±0.049	0.337±0.090
Kidney weight/birth weight (mg/g)	0.670±0.069	0.634±0.074
Adult weight at 30 weeks (g)	377.21±21.54	381.90±32.77
Adult blood pressure at 30 weeks (mmHg)	170±14	169±14

(P<0.05) than the amount consumed by the rats on the NPD. As a result, the HPD rats consumed approximately 49.75% protein instead of 54%. Neither body weight gain nor litter size were significantly affected by diet. Average body weight gain during pregnancy in the NPD (n=5) and HPD (n=6) groups was 77.8±7.0 g and 86.3±12.1 g, respectively. Litter sizes were 12±2 and 13±3 pups/litter, respectively. Birth weight of offspring exposed to an HPD was not significantly different from that of offspring exposed to the NPD. Likewise, body weights of 4-week-old weanling rats were similar (P=0.27) in both groups (Table 2).

Kidney weight

Exposure of rat pups to a HPD in utero did not appear to affect kidney growth, with kidney weight and kidney weight/body weight ratio in 4-week-old rats similar in the NPD and HPD offspring (Table 2).

Total number of glomeruli and glomerular volume

The total number of glomeruli in the kidneys of the 4-week-old rats were similar in both the NPD and HPD groups (Fig. 1). Similarly, there was no statistically significant difference in glomerular volume between the two groups (Table 3).

Table 3 Effect of diet during pregnancy on glomerular volume and glomerular capillary dimensions in 4-week-old offspring of rats exposed to an NPD (n=8) or HPD (n=7) in utero

	NPD	HPD
Average glomerular volume ($\times 10^{-4}$ mm^3)	3.09±0.60	3.61±0.79
Average renal corpuscle volume ($\times 10^{-4}$ mm^3)	3.48±0.63	3.98±0.89
Length of capillaries per average glomerulus (mm)	3.25±0.84	3.81±0.95
Surface area of capillaries per average glomerulus (mm^2)	5.46±1.32	6.73±1.64
Total renal filtration surface area (mm^2)	1,470±370	1,720±460

Glomerular capillary length and surface area and total renal filtration surface area

Average capillary length and filtration surface area per glomerulus were not significantly different in kidneys of offspring exposed to either the NPD or the HPD in utero (Table 3). Similarly, no significant differences were observed in total renal filtration surface area per kidney between rats exposed to the NPD and HPD in utero (Table 3).

Body weight gain and blood pressure

Body weight gain in the rats allowed to grow to adulthood was similar in the NPD and HPD offspring (Table 2). Tail cuff systolic blood pressure from 5 to 30 weeks of age in offspring exposed to either NPD or HPD during intrauterine development was not significantly different (Table 2).

Discussion

In this study, the administration of an HPD during pregnancy had no apparent adverse effect on the development of the resulting offspring, with no significant effect on birth weight, kidney size, nephron endowment, or systolic blood pressure in adulthood. Although it is well described that maternal protein restriction leads to reduced kidney weight and nephron endowment, when maternal dietary protein was high (double the amount consumed by controls) no differences in birth weight, kidney weight, or nephron number were observed.

According to the hypothesis proposed by Brenner et al. [14], a reduced number of glomeruli and/or reduced renal filtration surface area can lead to the development of hypertension by increasing renal sodium retention. As filtration surface area is dependent on both glomeru-

lar number and size, in this study the volume of glomeruli was also estimated. There were no significant differences in glomerular size, glomerular capillary length, glomerular capillary surface area, or total renal filtration surface area between the offspring of any of the diet treatment groups, perhaps explaining the lack of differences in adult blood pressure in weight-matched siblings.

In animal and human studies a direct correlation between nephron endowment and birth weight has been reported [9, 22]. Based on these findings, it is not surprising that in the present study nephron endowment was not significantly increased in the offspring of rats fed an HPD during pregnancy, as birth weight in the offspring was not affected.

As maternal LPD has been shown to be associated with significant reductions in birth weight, it was conceivable that in the present study, administration of an HPD during pregnancy would lead to a significant increase in birth weight. However, this was not the case. In contrast, in humans, Kramer [23] noted a general decrease in weight gain in mothers on protein supplementation during pregnancy, and a decrease in birth weight of their babies. In contrast, in a clinical trial, women who were judged to be at high risk of delivering a low birth weight infant were assigned a low- or a high-protein beverage during pregnancy. Whilst not found to be significant, there was a trend towards increased birth weight with higher protein intake [24]. Effects of dietary protein consumption during pregnancy on fetal growth are not clear-cut and are influenced by many factors, including the level of carbohydrate in the diet [25, 26]. In some studies, relative levels of carbohydrate and protein in the diet during pregnancy have been linked to changes in blood pressure in the offspring [27, 28].

In order to address the hypothesis that supernumerary nephron endowment leads to reductions in adult blood pressure, alternative strategies need to be developed to enhance nephron endowment. Lelievre-Pegorier et al. [29] suggested that dietary supplementation with vitamin A may be an alternative approach. In their studies, administration of retinoic acid (the active vitamin A derivative) on day 11 of gestation in pregnant Sprague-Dawley rats led to offspring with a 21% increase in nephron number. Furthermore, they reported that vitamin A restored nephron endowment in offspring exposed to an LPD in utero [30]. In future studies using this model it would be interesting to monitor blood pressure of offspring into adulthood.

In conclusion, administration of a high-protein, low-carbohydrate diet during pregnancy does not lead to an increase in birth weight, kidney weight, or nephron endowment in rats, nor does it affect blood pressure in adulthood. Alternative approaches other than that used in this study would need to be utilized in order to investigate whether an increase in birth weight and/or nephron endowment leads to a reduction in blood pressure later in life.

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References

1. Barker DJP, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. BMJ 301:259–262
2. Laor A, Stevenson DK, Shemer J, Gale R, Seidman DS (1997) Size at birth, maternal nutritional status in pregnancy, and blood pressure at age 17: population based analysis. BMJ 315:449–453
3. Law CM, Barker DJP, Bull AR, Osmond C (1991) Maternal and fetal influences on blood pressure. Arch Dis Child 66:1291–1295
4. Windham GC, Hopkins B, Fenster L, Swan SH (2000) Prenatal active or passive tobacco smoke exposure and the risk of preterm delivery or low birth weight. Epidemiology 11:427–433
5. Himmelmann A, Svensson A, Hansson L (1994) Relation of maternal blood pressure during pregnancy to birth weight and blood pressure in children. The hypertension in pregnancy offspring study. J Intern Med 235:347–352
6. Godfrey K, Robinson S, Barker DJP, Osmond C, Cox V (1996) Maternal nutrition in early and late pregnancy in relation to placenta and fetal growth. BMJ 312:410–414
7. Rosso P, Streeter MR (1979) Effects of food or protein restriction on plasma volume expansion in pregnant rats. J Nutr 109:1887–1892
8. Langley SC, Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci (Colch) 86:217–222
9. Merlet-Benichou C, Gilbert T, Muffat-Joly M, Lelievre-Pegorier M, Leroy B (1994) Intrauterine growth retardation leads to a permanent nephron deficit in the rat. Pediatr Nephrol 8:175–180
10. Desai M, Crowther NJ Lucas A, Hales CN (1996) Organ-selective growth in the offspring of protein-restricted mothers. Br J Nutr 76:591–603
11. Zimanyi MA, Bertram JF, Black MJ (2000) Nephron number in the offspring of rats fed a low protein diet during pregnancy. Image Anal Stereol 19:219–222
12. Langley-Evans SC, Welham SJM, Jackson AA (1999) Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci 64:965–974
13. Vehaskari VM, Aviles DH, Manning J (2001) Prenatal programming of adult hypertension in the rat. Kidney Int 59:238–245
14. Brenner BM, Garcia DL, Anderson S (1988) Glomeruli and blood pressure – less of one, more the other? Am J Hypertens 1:335–347
15. Bertram JF, Johnson K, Hughson MD, Hoy WE (2001) Renal glomerular number and size in Australian Aborigines, African Americans and white populations from the same locations: a preliminary report. Image Anal Stereol 20:153–156
16. Skov K, Nyengaard JR, Korsgaard N, Mulvany MJ (1994) Number and size of renal glomeruli in spontaneously hypertensive rats. J Hypertens 12:1373–1376
17. Nyengaard JR, Bendtsen TF (1990) A practical method to count the number of glomeruli in the kidney as exemplified in various animal species. Acta Stereol 9:243–258
18. Nyengaard JR, Bendtsen TF (1992) Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 232:194–201
19. Hinchliffe SA, Sargent PH, Howard CV, Chan YF, Van Velzen D (1991) Human intrauterine renal growth expressed in absolute number of glomeruli assessed by the dissector method and Cavalieri principle. Lab Invest 64:777–784
20. Larsson L, Aperia A, Wilton P (1980) Effect of normal development on compensatory renal growth. Kidney Int 18:29–35

21. Bertram JF (1995) Analyzing renal glomeruli with the new stereology. Int Rev Cytol 161:111–172
22. Hinchliffe SA, Lynch MRJ, Sargent PH, Howard CV, Van Velzen D (1992) The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol 99:296–301
23. Kramer MS (2000) Isocaloric balanced protein supplementation in pregnancy. Cochrane Database of Systematic Reviews (computer file). 2:CD000118
24. Adams SO, Barr GD, Huenemann RL (1978) Effect of nutritional supplementation in pregnancy. I. Outcome of pregnancy. J Am Diet Assoc 72:144–147
25. Cobrin M, Koski KG (1995) Maternal dietary carbohydrate restriction and mild-to-moderate exercise during pregnancy modify aspects of fetal development in rats. J Nutr 125:1617–1627
26. Godfrey KM, Barker DJ, Robinson S, Osmond C (1997) Maternal birthweight and diet in pregnancy in relation to the infant's thinness at birth. Br J Obstet Gynaecol 104:663–667
27. Campbell DM, Hall MH, Barker DJP, Cross J, Shiell AW, Godfrey KM (1996) Diet in pregnancy and the offspring's blood pressure 40 years later. Br J Obstet Gynecol 103:273–280
28. Shiell AW, Campbell-Brown M, Haselden S, Robinson S, Godfrey KM, Barker DJ (2001) High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. Hypertension 38:1282–1288
29. Lelievre-Pegorier M, Vilar J, Ferrier M-L, Moreau E, Freund N, Gilbert T, Merlet-Benichou C (1998) Mild vitamin A deficiency leads to inborn nephron deficit in the rat. Kidney Int 54:1455–1462
30. Lelievre-Pegorier M, Vilar J, Ferrier M-L, Moreau E, Freund N, Gilbert T, Merlet-Benichou C (1998) Vitamin A prevents nephron deficit in growth-retarded fetal rats (abstract). J Am Soc Nephrol 9:501