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ORIGINAL ARTICLE

Effects of Maternal Undernutrition on Glomerular Ultrastructure in Rat Offspring

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Background: Intrauterine growth restriction (IUGR) can reduce glomerular number and increase blood pressure in rats. The aim of this study was to assess the effects of maternal undernutrition during late gestation on glomerular ultrastructure in adult rat offspring.
Methods: Timed pregnant Sprague-Dawley rats were used. Control dams received regular food throughout pregnancy, while experimental dams received 50% of control food intake from days 15–21 of gestation. Glomerular ultrastructure was quantified in male offspring at 16 weeks of age.
Results: The ultrastructure of the filtration apparatus in the IUGR rat glomeruli was indistinguishable from that in the control rat glomeruli. The relative volumes of the glomerulus occupied by podocytes, capillaries, and mesangium, and the thickness of the glomerular basement membrane (GBM), and width of the filtration slit were comparable between control and IUGR rats.
Conclusion: These results indicate that glomerular ultrastructure is not affected by maternal undernutrition and suggest that altered glomerular ultrastructure is not a contributory factor to the pathogenesis of hypertension following maternal undernutrition.

1. Introduction

Studies in humans have shown that intrauterine growth restriction (IUGR) is a risk factor for the development of hypertension, diabetes mellitus, and ischemic heart disease in adult life.[1–3] Animal studies have demonstrated that maternal nutrition can determine the future development of cardiovascular and renal dysfunction in later life.[4] Various mechanisms have been implicated in this process, including reduced glomerular number, alteration of the renin-angiotensin system, and disturbance of the hypothalamic-pituitary-adrenal axis.[4–6] IUGR rats produced by maternal undernutrition showed significant reductions in glomerular numbers and glomerular filtration rates.[7] These findings were supported by the observation that human IUGR neonates also had significantly reduced glomerular numbers and were prone to develop hypertension in adulthood.[8] We found that maternal undernutrition during late gestation decreased glomerular number and induced hypertension in adult rat offspring.[9] Despite the link between reduced glomerular number and adult hypertension, little is known about glomerular ultrastructure in rats exposed to maternal undernutrition. We hypothesized that

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hypertension following maternal undernutrition was caused by altered glomerular ultrastructure in the rat offspring.

2. Materials and Methods

2.1. Animals

This study was approved by the Institutional Animal Use Committee at Taipei Medical University and was performed using timed pregnant Sprague-Dawley rats. Control dams received regular rat chow throughout their pregnancies. Experimental animals (maternal undernutrition) received 50% of the control food intake from 15–21 days gestation. The dams delivered spontaneously at term and were then immediately switched back to standard rat chow.

2.2. Electron microscopy

Each rat was euthanized with an intraperitoneal injection of pentobarbital (100 mg/kg), and its kidneys were removed. The kidneys were immersed and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. The kidney cortices were minced into 1-mm^3 blocks. Ultrathin sections (50–60 nm) were double-stained with uranyl acetate and lead citrate, and then examined using a Hitachi 600 electron microscope (Tokyo, Japan). Photomicrographs were taken in a systematic random manner.

2.3. Volume fraction

The relative volumes occupied by the glomerular components (volume fractions) were estimated by point counting.[10] The following components were assessed: podocytes, capillaries (endothelial cells and lumen), and mesangium (interstitial cells and matrix). Electron micrographs were printed at a final magnification of about 7500× and covered with a transparent test lattice (5 × 5-point grid) with points 20 mm apart. The number of points that fell on podocytes, capillaries, and mesangium were counted. The volume fraction was equal to Pi/Pt, where Pi represents the number of test points hitting the structure of interest, and Pt is the total number of points hitting the reference space of the glomerulus.

2.4. Thickness of glomerular basement membrane (GBM)

The thickness of the GBM was measured using the harmonic mean of the orthogonal intercept.[11] Photographs were developed to a final magnification of 7500× and were then covered by a transparent 3-cm^2 grid. Where the gridlines transected the endothelial surface of the GBM, measurements were taken of the shortest distance from the endothelial cytoplasmic membrane to the cytoplasmic membrane of the epithelial foot process. Measurements were made using a transparent logarithmic ruler using 0.75 as a multiplier of the harmonic value for each division, with calibration undertaken when no measured areas of the GBM lay within the initial division. The apparent harmonic mean thickness was calculated (lh), from which the true harmonic mean thickness (Th) was estimated using the formula: $\mathrm{Th} = 8/3\pi \times 10^6/\mathrm{magnification} \times l\mathrm{h}$.

2.5. Width of filtration slit

The width of the filtration slit was measured on photomicrographs at a magnification of 16,000× (Figure 1). The measurements were performed at the level of the filtration slit membrane where the membranes of adjacent podocytes exhibited a trilaminar cell membrane.[10]

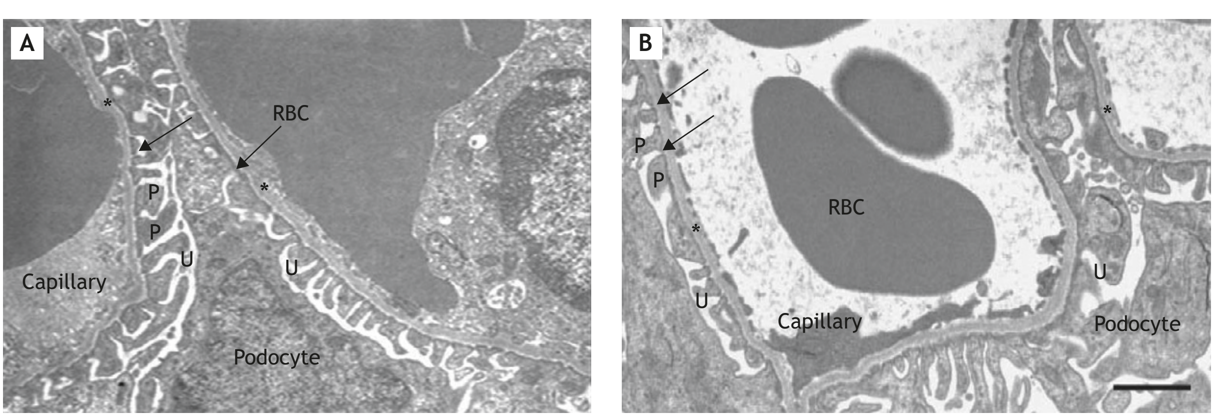

Figure 1 Electron micrographs of glomerulus and associated structures in (A) control and (B) intrauterine growth restriction rats at 16 weeks of age. The slit diaphragm lies between the two podocyte processes (arrow). * = glomerular basement membrane; P = podocyte processes; U = urinary space. Bar = 1 μm.

2.6. Statistical analysis

Data are expressed as means±SD. Between-group comparisons were made using Student's *t*-tests. Differences were considered significant at $p<0.05$.

3. Results

Thirty-five rats were born from three dams in the control group, and 32 rats from three dams in the undernourished group. There was no significant difference in litter sizes between the two study groups. Eight male offspring were randomly selected from each of the control and undernourished groups at 16 weeks of age. IUGR rats exhibited significantly higher systolic blood pressures and significantly lower glomerular numbers than control rats at 16 weeks of age.[9] The systolic blood pressures were 167.8±12.9 mmHg and 190.6±5.2 mmHg in the control and IUGR rats, respectively. The number of glomeruli in IUGR rats was $20.8\pm1.8\times10^6$/kidney, 40% lower than the $35.6\pm2.9\times10^6$/kidney of the control rats.

3.1. Effects of undernutrition on maternal and offspring body weights

The mean body weight of the undernourished dams was lower than that of control dams on gestational day 21 (Table 1). Mean body weight was significantly lower in IUGR offspring than in the control offspring at 16 weeks of age.

3.2. Effects of maternal undernutrition on glomerular ultrastructure in offspring

The ultrastructure of the filtration apparatus in the IUGR rat glomeruli was indistinguishable from that in the control rat glomeruli (Figure 1). The volumes fractions of podocytes, capillaries, and mesangium were comparable between control and IUGR rats at 16 weeks of age (Figure 2A). Capillaries occupied about 50% of the volume, while podocytes and interstitium occupied the remaining 50%. No obsolete or distorted glomeruli were found in the control or IUGR groups. The thickness of the GBM and width of the filtration slit were comparable between control and IUGR rats at 16 weeks of age (Figures 2B and 2C).

Table 1 Maternal body weight on gestational day 21 and offspring body weight at 16 weeks of age in control and intrauterine growth restriction groups

	Maternal body weight (g)	Offspring body weight (g)
Control	420±75	475±41
IUGR	350±43	408±46*

Values are means±SD. *$p<0.01$ *vs*. control. IUGR = intrauterine growth restriction.

4. Discussion

The effects of maternal undernutrition on glomerular ultrastructure during the postnatal period are largely unknown. Here, late gestational exposure

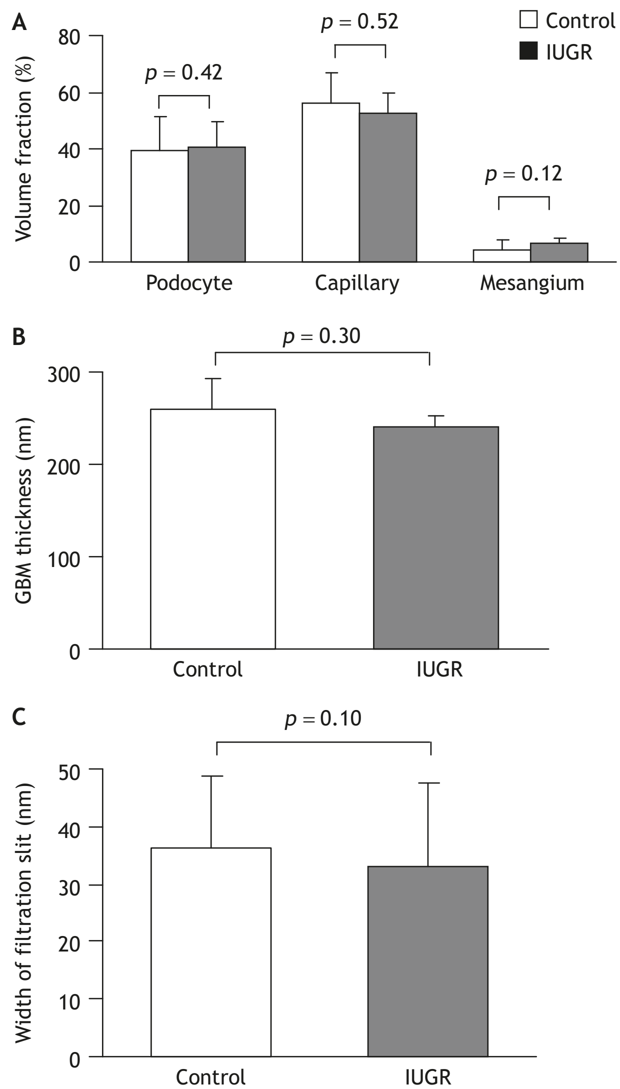

Figure 2 Effects of maternal undernutrition on (A) relative volumes of podocytes, capillaries, and mesangium, (B) thickness of glomerular basement membrane (GBM), and (C) width of filtration slit in control and intrauterine growth restriction (IUGR) rats. The relative volumes of glomerular components and thickness of GBM and width of filtration slit were comparable between control and IUGR rats.

of rat pups to maternal undernutrition was found to have no effect on the ultrastructure of glomeruli at 16 weeks of age. We used maternal undernutrition of all dietary components to investigate the long-term consequences of being born small, because maternal dietary restriction is unlikely to be limited to deficiencies in individual components. Introduction of maternal undernutrition in rats from day 15 of pregnancy (term = day 22) coincided with nephrogenesis in the rat, though $<20\%$ of nephrons are formed at birth. Nephrogenesis continues up to postnatal day 10, when the remaining 80% of nephrons are formed.[12]

The nephron is the principal functional unit of the kidney. Each rat kidney contains about one million nephrons, though they are not necessarily all uniform. We therefore used systematic, uniformly random sampling to alleviate bias in this study. The pathogenesis of hypertension induced by intrauterine dietary restriction is not clear. Several theories have been proposed to explain how the fetus is programmed to develop hypertension, including reduced glomerular number, alteration of the renin-angiotensin system, and disturbance of the hypothalamic-pituitary-adrenal axis.[4–6] This study focused on the role of glomerular ultrastructure in hypertension induced by maternal undernutrition. Hypertension can result from reduced nephron number due to impaired renal sodium excretion and natriuresis, leading to increased blood pressure.[13] Rats exposed to 50% intrauterine food restriction exhibit reduced nephron number, decreased glomerular filtration rate, and increased blood pressure.[9,14] The primary urine in the kidney is formed by ultrafiltration of plasma components through the glomerular capillary wall into the urinary space. This glomerular filtration barrier is composed of a fenestrated endothelium, GBM, and epithelial podocyte layer.[15] The glomerular filtration rate has been shown to be inversely related to relative mesangial volume and GBM thickness in diabetic nephropathy.[16,17] The structural basis of the decreased glomerular filtration rate in hypertension induced by maternal undernutrition is not clear. In the present study and our previous studies, we found that rats exposed to maternal undernutrition exhibited hypertension and reduced glomerular number, but that the glomerular ultrastructure, including the mesangial volume fraction, thickness of the GBM, and width of the filtration slit, were not altered at 16 weeks of age.[9]

Our findings indicate that glomerular ultrastructure is not affected by maternal undernutrition, and suggest that altered glomerular ultrastructure is therefore not a contributory factor to the pathogenesis of hypertension in rat offspring following maternal undernutrition.

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