Postnatal food restriction in the rat as a model for a low nephron endowment

Michiel F Schreuder*
Jens R Nyengaard†
Floor Remmers‡
Joanna AE van Wijk*
Henriette A Delemarre-van de Waal‡

Department of *Pediatric Nephrology and †Pediatric Endocrinology, VU University Medical Center, Amsterdam, the Netherlands
‡Stereology and Electron Microscopy Research Laboratory and MIND Center, University of Aarhus, Aarhus, Denmark

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Corresponding author:
MF Schreuder
Department of Pediatric Nephrology
VU University Medical Center
PO Box 7057
1007 MB Amsterdam, the Netherlands
Phone: +31-20-4442419
Fax: +31-20-4442918
E-mail: mf.schreuder@vumc.nl
Abstract

A low nephron endowment may be associated with hypertension. Nephrogenesis is the process that leads to the formation of nephrons until the 36th week of gestation in man, and may be inhibited by many factors like intrauterine growth restriction and premature birth. In order to study the consequences of a low glomerular number, animal models have been developed. We describe a model of postnatal food restriction in the rat in which litter size is increased to 20 pups, which leads to growth restriction. In the rat, active nephrogenesis continues until postnatal day 8 which coincides with the growth restriction in our model. Design-based stereological methods were used to estimate glomerular number and volume. Our results show an approximately 25% lower glomerular number in rats after postnatal food restriction (30,800 glomeruli per kidney) when compared with control rats (39,600 glomeruli per kidney, p<0.001). Mean glomerular volume was increased by 35% in the growth restricted rats (p=0.006). There was a significant negative correlation between glomerular volume and glomerular number (r=-0.76, p<0.001). We conclude that postnatal food restriction in the rat leads to a low nephron endowment with compensatory enlargement. It is therefore a suitable model to study the effect of intrauterine growth restriction or prematurity on kidney development, and to study the consequences of a reduced glomerular number in later life.

Key words: nephrogenesis, glomerular number, growth restriction, stereology
Introduction

Nephrogenesis is the developmental process that leads to the formation of nephrons from the 6th week of gestation in man(20). New nephrons are formed until the 36th week of gestation, after which kidney growth and development is based on further outgrowth of existing structures(20). Interference during nephrogenesis can result in a low nephron endowment. This can be caused by many conditions, for instance intrauterine growth restriction (IUGR)(8; 12), premature birth(25), or maternal medications like ACE inhibitors(24).

The remaining nephrons will compensate for the lower number(16). A normal renal clearance is delivered by both hypertrophy and hyperfiltration (19). According to the hyperfiltration hypothesis by Brenner et al., the enlarged glomeruli are a potential threat (1; 9; 10): by reabsorbing more sodium and raising glomerular pressure, systemic blood pressure rises and albuminuria may develop. This results in sclerosis of glomeruli, culminating in a vicious circle which may continue to end-stage renal disease(9; 10). Keller et al. have shown an association between a low glomerular number and hypertension in human beings, even without evidence for glomerulosclerosis(17).

Several animal models have been used to study the effect of IUGR on the kidney, with the rat as the predominant species. Almost all models are based on manipulations during pregnancy, for instance with steroids(23), maternal food restriction(18) or uterine artery ligation(26). However, nephrogenesis in the rat continues until postnatal day 8, during which time the majority of nephrons are formed(21). Manipulations during this timeframe can therefore be expected to lead to a low nephron endowment. Maternal food restriction during lactation has indeed been shown to lead to a reduced kidney weight
(both absolute as well as relative to body weight) at the age of 9 months(3). ACE inhibition during this postnatal phase has also been shown to influence nephrogenesis(7).

Our laboratory has used a rat model of postnatal food restriction (FR) by increasing litter size in order to study the effect of “programming” on growth and development(4; 11; 14). We hypothesized that postnatal restriction of food intake influences nephrogenesis, and leads to a low glomerular number. To study this issue, we used design-based stereological techniques(22) in a rat model of postnatal FR.
Materials and methods

For all experiments approval was obtained from the Animal Welfare Committee (DEC) of the VU University Medical Center. Pregnant Wistar rats were obtained from Harlan CPB (Horst, The Netherlands) and housed in an animal room in the Clinical Animal Laboratory of the VU University Medical Center individually per plastic cage with wood chips as bedding. A 12:12-h light-dark cycle was maintained (light on at 07:00AM) in the room, at constant temperature (22 °C [SD 0.4]) and relative humidity (59% [SD 4]). Rats had free access to tap water and standard rodent chow.

Following natural birth, pups were weighed and randomly assigned to either a control litter, consisting of 10 pups, or a FR litter consisting of 20 pups, both with a 1:1 male-to-female ratio. This model has been demonstrated to lead to an equal growth restriction in each of the pups of the FR litter(14). Since there is no difference in glomerular number between sexes(26), only male rats (8 FR and 8 control) were used for this study. Animals were weaned at day 25 and males were housed two per cage.

At 75 days of age, rats were anesthetized with Nembutal (1.5ml/kg i.p., 60 mg/ml pentobarbital, Ceva Sante Animale BV, Maassluis, the Netherlands) and transcardially perfused with physiological saline followed by 250 ml 4% phosphate-buffered formaldehyde, after which either the right or left kidney was selected at random and taken out.

Glomerular number was estimated using the physical fractionator/disector technique(5; 27) as described previously(26). Briefly, the kidney was cut in half, dehydrated in graded ethanol, and embedded in glycolmethacrylate (Technovite 7100; Hereus Kulzer, Wehrheim, Germany). The kidney was cut in 20-μm-thick sections,
determining the first section sampled by a random-number table. Every 25th and adjacent section were mounted on a slide, and stained with periodic acid-Schiff and Mayer’s hematoxylin. On average, 10 section pairs were mounted per kidney. The disector method consists of a three-dimensional counting rule using pairs of parallel sections. The glomeruli were counted if they were present inside the 2D unbiased counting frame in one section (the sampling frame) but not in the adjacent section plane (the look-up section) and vice versa. On average, 124 glomeruli were counted per kidney. The coefficient of error of this technique used for counting glomeruli was estimated to be 9.3%(6).

Mean glomerular volume was calculated using the volume density of glomeruli in the kidney estimated with a random oriented point counting grid as described previously(26). Glomerular volume data were not corrected for tissue deformation, since perfusion fixation together with embedding in methacrylate prevents nearly all shrinkage. The volume of the kidney was estimated using Cavalieri’s principle(26). During stereological measurements, the observer was blinded to the group of the animal by using identification numbers.

Results are presented as mean (SD) or mean (coefficient of variation) for stereological data. Differences between groups were analyzed using an unpaired Student’s t-test. Correlation between variables was analyzed using the Pearson’s correlation coefficient. SPSS was used as statistical analysis system. A p-value of <0.05 was considered to be statistically significant.
Results

Birth weights were identical in both groups (7.8 g [SD 1.0] and 7.7 g [SD 0.9] in control and FR rats, respectively). Body weights at day 4 were not statistically different (p=0.085), but from day 7 onwards FR rats had a lower body weight than control rats (Figure 1). Body weight at sacrifice (day 75) was 370 g (SD 26) in control rats vs. 332 g (SD 28) in FR rats (p=0.012).

Kidney volume was not statistically different between the FR rats (1102 [0.087] mm$^3$) and the control rats (1166 [0.070] mm$^3$). Glomerular number (Figure 2) in the FR rats was approximately 25% lower than in the control rats (30,800 [0.093] and 39,600 [0.072] glomeruli per kidney, respectively. p<0.001). Controlling for birth weight, the partial correlation between body weight on day 10, i.e. at the end of nephrogenesis, and glomerular number was highly significant (r=0.83, p<0.001). Mean glomerular volume was increased in the FR rats (0.94 [0.19] $\cdot$ 10$^6$ µm$^3$ vs. 0.69 [0.16] $\cdot$ 10$^6$ µm$^3$ in control rats, p=0.006). Figure 3 shows the significant negative correlation between glomerular number and mean glomerular volume (r=-0.76, p<0.001).
Discussion

Our results clearly show that postnatal FR in the rat leads to a low glomerular number with glomerular enlargement. Our animal model therefore provides a simple method to induce a low nephron endowment, which facilitates further study on the consequences of a congenital renal mass reduction.

Design-based stereological methods were used to determine glomerular number and volume(22). This enables measurements without an assumption about the size, shape or orientation of the glomeruli in the kidney. The actual glomerular number is determined by nephrogenesis and glomerular loss, for instance due to glomerulosclerosis, which is associated with a low glomerular number(9). However, even at the age of 18 months, no completely sclerotic glomeruli were found in similar rats(26). Therefore, we are convinced that the low glomerular number after FR is due to a low nephron endowment, and not due to glomerular loss.

Previously, we have shown that spontaneous IUGR as well as an animal model of uteroplacental insufficiency lead to a lower glomerular number(26). Also, no difference in glomerular number between males and females was found. In the present study, birth weights were similar in both groups, which makes it unlikely that the intrauterine growth trajectory was of influence on our results. Since only male rats were studied, no definitive conclusions on the effect of FR on female rats can be made.

Premature birth in humans causes an individual to be in the extrauterine environment before completion of nephrogenesis. This results in extrauterine growth restriction in up to 97% of neonates(2), and is also associated with a low glomerular number(25). As a newborn rat also has an active nephrogenesis and our model leads to
growth restriction, postnatal FR is also a suitable model to study the consequences of prematurity on nephrogenesis. Treatment of neonatal rats with steroids, aminoglycosides, prostaglandines or diuretics can be used as a model to study the possible influence of these frequently used drugs in neonatology on human kidney development.

A recent study of Hughson et al. showed a strong inverse correlation between glomerular number and blood pressure in white adults(13), consistent with the results of Keller et al.(17). However, in African-Americans no correlation was found. It is therefore likely that different pathogenic pathways play a role in establishing hypertension in these two races. Since a low glomerular number is important in the understanding of hypertension in whites, our animal model offers an opportunity to study this field. However, one has to consider that the difference between these races may also be explained by potential confounders, as has been described for the association between birth weight and blood pressure(15). Future studies should be conducted to study the underlying mechanisms causing the low glomerular number, as well as the effect of a low nephron endowment after FR in the rat on renal function and blood pressure.

We conclude that postnatal FR in the rat leads to a low nephron endowment with glomerular enlargement. It is therefore a suitable model to study the effect of IUGR or prematurity on kidney development, and to study the consequences of a reduced glomerular number in later life.
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Reference List


Figure legends

Figure 1.
Body weights (Fig. 1A) and body weights relative to the control mean at the same age (Fig. 1B) in postnatal food restricted (FR, n=8, ●) and control (n=8, ○) rats from birth until day 75 (means with standard deviation). *, p<0.05; **, p<0.01; ***, p<0.001 FR vs. control rats at the same age.

Figure 2.
Glomerular number (Fig. 2A) and mean glomerular volume (Fig. 2B) in postnatal food restricted (FR, n=8, ●) and control (n=8, ○) rats.

Figure 3.
Scatter plot showing the distribution of mean glomerular volume by glomerular number for postnatal food restricted (FR, n=8, ●) and control (n=8, ○) rats with regression line (r=-0.76, p<0.001).
Figure 1A

A graph showing the body weight (g) of mice over age (days). The graph compares control (open circles) and FR (closed circles) conditions. Significant differences are indicated by asterisks (*, **, ***).

Figure 1A
Figure 1B
Figure 2A and 2B
Figure 3