Effects of early postnatal hypernutrition on nephron number and long-term renal function and structure in rats

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Bouhired F, Buffat C, Feuerstein J-M, Daniel L, Tsimaratos M, Oliver C, Lelièvre-Pégrior M, Simeoni U. Effects of early postnatal hypernutrition on nephron number and long-term renal function and structure in rats. Am J Physiol Renal Physiol 293: F1944–F1949, 2007. First published September 26, 2007; doi:10.1152/ajprenal.00141.2007.—Various antenatal events impair nephrogenesis in humans as well as in several animal models. The consecutive low nephron endowment may contribute to an increased risk for cardiovascular and renal diseases in adulthood. However, little knowledge is available on the influence of the postnatal environment, especially nutrition, on nephrogenesis. Moreover, the consequences of early postnatal nutrition in late adulthood are not clear. We used a model of early postnatal overfeeding (OF) induced by reduction of litter size (3 pups/litter) in rats. Systolic blood pressure (SBP; plethysmography), glomerular filtration rate (clearance of creatinine), glomerular number and volume, and glomerulosclerosis were evaluated in 22-mo-old aging offspring. Early postnatal OF was associated with increased weight gain during the suckling period (+40%, P < 0.01) and a 20% increase in glomerular number (P < 0.05). However, an increase in SBP at 12 mo by an average of 18 mmHg and an increase in proteinuria (2.6-fold) and glomerulosclerosis at 22 mo of age were observed in OF male offspring compared with controls. In conclusion, early postnatal OF in the rat enhances postnatal nephrogenesis, but elevated blood pressure and glomerulosclerosis are still observed in male adults. Factors other than glomerular number reduction are likely to contribute to the arterial hypertension induced by early postnatal OF.

Nephrogenesis; glomerular number; overfeeding; arterial blood pressure; infant; newborn; developmental origins of adult diseases

EXPERIMENTAL DATA FROM different animal species have shown that nephrogenesis can be altered by prenatal events such as intrauterine growth restriction, drug exposure, or maternal diabetes, leading to permanent nephron number deficit (4, 19). Low nephron endowment due to low birth weight is associated with arterial hypertension and/or altered renal function in adulthood in both rodents and humans and is considered an important mechanism in the developmental origins of cardiovascular disease (2, 12, 13, 16, 21, 31). Indeed, birth weight-associated inborn nephron deficit may be responsible over time for single nephron glomerular hyperfiltration, glomerular hyperperfusion, proteinuria, glomerulosclerosis, and, finally, progressive deterioration of renal function and hypertension (6).

However, the antenatally acquired nephron number deficit is considered irreversible, and few studies have addressed whether postnatal nutrition can influence nephrogenesis when it is prolonged postnatally in certain species or in circumstances such as preterm birth in humans. Postnatal alteration of nephrogenesis has been experimentally induced by early administration of renin-angiotensin system inhibitors (32) but also by postnatal undernutrition (25), showing that, postnatally, ongoing nephrogenesis may be influenced by concomitant nutrition. Nevertheless, the effects of enhanced postnatal nutrition on nephrogenesis have not been investigated.

This issue is of particular importance in light of recent studies showing that low birth weight and premature birth may predispose individuals to arterial hypertension and renal function alteration as young adults (9, 14, 15). Whereas nephrogenesis occurs until 8–10 days postnatal in rats, it is achieved at ~34–36 wk of gestation in humans. Autopsy studies in humans have shown that premature birth before such gestation duration is associated with arrested or impaired nephrogenesis, which may contribute to cardiovascular and renal disease in adulthood (24). Enhanced early nutrition of premature infants may either influence favorably the postnatal continuation of nephrogenesis or accelerate the progression toward renal insufficiency and long-term cardiovascular dysfunction (28).

We therefore designed a study to investigate the effects of early postnatal overfeeding (OF) on nephron number and long-term vascular and renal function in the rat. Furthermore, as in the majority of former studies, outcomes were examined at young adulthood; this study also included outcome assessment in aging animals.

MATERIALS AND METHODS

Animals. All procedures on animals were approved by the Institutional Animal Care and Use Committee at Université de la Méditerranée and conducted according to the American Physiological Society’s Guiding Principles in the Care and Use of Animals. Male and virgin female Sprague-Dawley rats with initial weights of 225–250 g (purchased from Charles River, l’Abresle, France) were individually housed in plastic cages. Rats had free access to tap water and were kept on standard laboratory rat chow (Safe-UAR) in a room with a 12:12-h light-dark cycle; the room was maintained at a controlled temperature of 22°C and constant humidity. Fifteen days after reception, females were mated overnight with males. The day on which sperm was seen in a vaginal smear was designated as day 1 of gestation. After birth, the weights of all pups were recorded within 6 h after delivery, and the litters were culled to 10 pups, with the largest and smallest pups removed to ensure adequate and uniform nutrient supply to the litters.
remaining pups. On day 3, pups were assigned to either control (10 pups/litter, 2 pregnant rats, n = 20) or postnatal OF groups (3 pups/litter, 4 pregnant rats, n = 12). Early postnatal OF consisted of the reduction of litter size down to three pups per litter during the suckling period. This model results in increased weight gain during the suckling period due to higher milk availability to each pup. Male-to-female ratios were the same between groups. Neonatal rats were carried by their mothers until they were weaned on day 21, when they were placed on standard rat chow (Safe-UAR) and housed three to four per cage. The offspring rats were weighed weekly during the first postnatal months and monthly thereafter until 22 mo of age (postnatal).

**Systolic blood pressure measurements in 4- and 12-mo-old animals.**

Systolic blood pressure (SBP) was determined noninvasively by the tail cuff plethysmography method (Letica 5000, Bioseb) using thermostatically warmed restrainers designed for rodents and adapted to the size of the animal. Each animal was acclimated to this procedure during 5 successive days (10 min each day) before measurements. Measurements were performed by a single operator. The mean of four to six measurements was recorded in each animal. This tail cuff method has been extensively validated and refined to reduce possible stress-related effects.

**Determination of renal function and urinary protein excretion.**

Endogenous creatinine clearance (CrCl) was determined in 2-, 4-, and 12-mo-old animals as CrCl = UCr × V/PCr − 1, where UCr and PCr are urinary and plasma creatinine concentrations, respectively, and V is urine output. Animals were individually housed for 48 h in metabolic cages; 24-h urine collection was performed gravimetrically, and a 0.5-ml blood sample was collected by incision of the tip of the tail under a brief general anesthesia (halothane, Baumont). Plasma creatinine concentration was determined by the method of Jaffé.

**Glomerular counting and renal histology analysis in aging animals.**

At 22 mo of age, rats were euthanized with pentobarbital sodium under halothane anesthesia. The left kidney was used to determine the number of glomeruli, and the right kidney was kept for histology analysis. The number of glomeruli per kidney was determined in both groups of rats as previously described (1). Briefly, kidneys were removed and weighed. Whole kidneys were incubated in 50% hydrochloric acid for 45 min at 37°C, the incubation time being dependent on kidney weight. Kidneys were rinsed with tap water and stored overnight at 4°C in a gauged flask. Following mechanical dissociation, tubules and glomeruli were suspended in water. Three 0.5-ml aliquots were taken at 4°C in a gauged flask. Following mechanical dissociation, tubules and glomeruli were suspended in water. Three 0.5-ml aliquots were taken and placed in a hemocytometer-like chamber, and the glomeruli were counted under microscope by three investigators who were unaware of the specimen origin. The three results were averaged, and then the value was used to determine the total number of glomeruli in the sample and therefore in the kidney.

The renal histology and corresponding parameters were analyzed by one investigator (L. Daniel) who had no prior knowledge of the group to which the rats belonged. One-half of the right kidney was fixed in 4% buffered formaldehyde. Kidneys were then dehydrated through graded alcohols and routinely embedded in paraffin. Transverse sections through the central portion of each kidney and 4-μm-thick sections stained with hematoxylin and eosin were obtained for light microscopic examination. In each single section of kidney, all glomeruli (i.e., superficial and juxtamedullary) sectioned through the hilum were counted and assessed for glomerular volume. More than 80 glomerular cross sections not crossing the outline of the examined field for each group and without extensive structure alterations were analyzed in each specimen. The profile of a glomerulus was captured and the perimeter of Bowman’s capsule was traced using a tablet cursor to determine the glomerular volume. Cross-sectional tuft area (GT) was calculated for each glomerulus with visible vascular pole using an image analyzer (SAMBA 2005, Alcatel, TITN Answer). Glomerular volume (GV) was then calculated assuming the glomerulus to be spherical by applying the following equation: GV = βk × (GT / k)1/3, with β being the shape coefficient for a sphere (=1.38) and k being the size distribution coefficient (=1.1) (30, 33).

Glomerular sclerosis was evaluated using Sirius red coloration to visualize fibrillar collagen. The measurement of Sirius red-stained collagen as the percentage of total glomerular surface area was thus obtained. A quantitative analysis was performed by a single examiner (L. Daniel) using the same colorimetric and light thresholds (NCSS 2004 software, Kaysville, UT). Color thresholding was applied to identify the red-stained structure. The results were reported as the mean ratio of Sirius red-stained areas to total glomerular capillary areas.

**Statistical analysis.** Data are presented as means (SD) and were evaluated for statistical significance with Statview version 5.0 software (Abacus Concepts, Berkeley, CA). A nonparametric Mann-Whitney test was used to compare the results. One-way ANOVA with Tukey-Kramer comparison test post hoc analysis was used to compare glomerular volume and glomerular sclerosis between the two groups. Two-way ANOVA, using gender and diet as independent variables, was employed to evaluate gender-related differences regarding urinary protein excretion rate, glomerular number and volume, and glomerulosclerosis. Statistical significance was defined as P < 0.05.

**RESULTS**

**Birth weight and effect of postnatal OF on postnatal growth.**

Birth weights were similar between the groups [6.47 (0.33) g and 6.45 (0.11) g in control and OF offspring, respectively]. The number of pups per litter and the duration of gestation were similar. During the suckling period, growth rate was increased in postnatally overfed offspring. This latter displayed a significantly increased body weight from day 7, persisting until 2 mo of postnatal age. OF rats remained heavier than control rats, but this difference failed to reach statistical significance thereafter (Fig. 1). At 22 mo of age, body weight was 615 (161) g in control and 699 (173) g in OF rat offspring. At 22 mo, unlike in females, body weight tended to be higher in OF males by an average of 14% compared with controls, but the difference did not reach statistical significance. During the experiment, six (controls) and four (OF) adult offspring rats died.
Effect of postnatal OF on blood pressure and renal function.

SBP values of rat offspring are shown in Figs. 2 and 3. SBP was significantly higher in OF rats from both genders, from the age of 2 mo, by an average of 10 – 15 mmHg (P < 0.05). However, at 12 mo, SBP was exclusively elevated in OF males (18 mmHg vs. control males, P < 0.05). Glomerular filtration rate (GFR) was not different between the groups in 4-mo-old [5.3 (1.1) vs. 5.4 (1.2) ml·min⁻¹·kg⁻¹ in control and OF rats, respectively] and in 12-mo-old offspring [4.8 (0.8) vs. 5.2 (1.0) ml·min⁻¹·kg⁻¹ in control and OF rats, respectively]. Yet urinary protein excretion rate was significantly increased in postnatally overfed rat male offspring (Fig. 3).

Effect of postnatal OF on renal structure and morphology in aging rats.

The kidney weights did not differ between OF and control offspring [2.14 (0.6) vs. 2.06 (0.5) g, respectively], nor did the kidney weight-to-body weight ratio. Glomerular number and mean glomerular volume are shown in Fig. 4, A and B. Global glomerular number in OF rats was 20% higher than in control rat offspring in both males [46,800 (1,250) vs. 38,467 (555) in OF and control offspring, respectively, P < 0.05] and females [44,125 (2,450) vs. 37,700 (1,450) in OF and control offspring, respectively, P < 0.05]. Mean glomerular volume was 24% lower in OF than in control offspring (P < 0.05); however, this difference concerned females [0.548 (0.4) vs. 0.817 (0.3) mm³ × 10⁻³ in OF and control offspring, respectively, P < 0.001], whereas reduction of mean glomerular volume was not significant in males [0.963 (0.35) vs. 1.13 (0.4) mm³ × 10⁻³ in OF and control offspring, respectively].
Whereas glomerulosclerosis (shown in Figs. 4C and 5) was not different between OF and control females, glomerulosclerosis was significantly higher in postnatally overfed males than in aging control male offspring \((P < 0.01)\). Table 1 shows \(P\) values obtained by ANOVA for the effects of postnatal diet and of gender on glomerular number, glomerular volume, glomerular sclerosis, urinary protein excretion, and SBP.

**DISCUSSION**

We show here that glomerular number endowment at birth can be influenced by enhanced postnatal nutrition in the rat. Early postnatal overfeeding is associated with enhanced nephrogenesis, leading to an increase in the number of nephrons and reduced glomerular volume. Such changes affect both males and females and persist despite the process of aging but do not prevent the development of arterial hypertension and progressive glomerulosclerosis. Furthermore, long-term consequences on renal function induced by early postnatal overfeeding are influenced by gender.

Several factors have been shown to alter nephron number endowment during fetal life, both in the rat and in humans. Maternal global or protein diet restriction and associated low birth weight in offspring, vitamin A and iron deficiency, maternal hyperglycemia, and fetal exposure to drugs (corticosteroids, angiotensin system inhibitors, and ciclosporine) induce a low nephron endowment during fetal life, both in the rat and in humans. Such observations may be explained by the fact that, in rodents, nephrogenesis begins in the middle gestation and continues after birth until postnatal days 8–10. It is likely that postnatal overfeeding intervened during a key window of opportunity to influence the postnatal part of nephrogenesis.

Few data sets are available on the effects of postnatal environment on nephrogenesis in the rat. Postnatal administration of the renin-angiotensin system antagonist during the first 12 days of life results in low nephron endowment and glomerular and tubular injury \((32)\). Recently, Schreuder et al. \((25)\) showed that postnatal food restriction obtained by enlarged litter size during the suckling period \((20\) vs. \(10\) pups) is associated with glomerular number reduction in 75-day-old rats.

It is noteworthy that, while postnatal overfeeding enhanced glomerular number in this study, the mean glomerular volume was similar in both groups. Because glomerular number is correlated to birth weight, it is unlikely that glomerular number at birth was different between control and overfed rats \((18, 19)\). The method of dissection, acid maceration, has been adapted for rodents, validated by numerous authors, and limited in bias, as specific investigators were unaware of the groups to which the examined kidneys belonged \((19)\). Kidney weight, which must be accurately measured, is the unique parameter taken into account to determine the characteristics of acid digestion in these studies. Interstitial matrix was not altered by the acid digestion. Although nephron number counting was performed at 22 mo in this study, it is unlikely that countings performed earlier would have provided different results. We recently measured the number of nephrons in 3- and 22-mo-old rats from control mothers in a different study: they were very similar for both periods \((34,990 \pm 490\) and \(35,230 \pm 514\), respectively) \((unpublished\ data)\). Thus our findings indicate that nephron endowment in utero is not irreversibly limited and is positively influenced by enhanced postnatal nutrition in the rat.

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Two-way ANOVA: \(P\) values for the effects of postnatal diet and gender on glomerular number (GN), glomerular volume (GV), glomerulosclerosis (GS), creatinine clearance (CrCl), urinary protein excretion rate (UrVP), and systolic blood pressure (SBP).
was reduced, suggesting that global filtration surface area remain unchanged. In such conditions, glomerular hyperfiltration to meet excretory demands due to early postnatal overfeeding could contribute to elevated blood pressure, proteinuria, and progressive glomerulosclerosis in aging overfed males.

The reasons as to why the influence of postnatal nutrition on nephron endowment is limited to the male gender are unknown. Indeed, while the magnitude of glomerular number elevation was not influenced by gender, the expected glomerular volume reduction was less in overfed males than in overfed females. A relative increase in glomerular filtration rate in overfed male offspring may have occurred. Moreover, in keeping with data available in the literature, hyperleptinemia associated with early postnatal overfeeding may influence renal functions through specific effects involving renal sympathetic hyperactivity and decreased sodium excretion, partially due to an upregulation of Na-K-ATPase (3).

The mechanisms by which early postnatal overfeeding can contribute to the promotion of nephrogenesis are unknown. A number of factors, including transcriptional and growth factors, oncogenses, and extracellular matrix, are involved in nephrogenesis (7). Increased availability of energy and nutritional substrates likely promotes postnatal nephrogenesis. The role of specific key factors that have been shown to be essential in the normal development of the nephron, such as retinol (17), remains to be investigated.

Although there are major differences among species, our findings raise the question of whether the formation of nephrons can be influenced postnatally in prematurity infants who are born before the normal achievement of nephrogenesis and are at risk for long-term alteration of cardiovascular and metabolic functions in relation to their low birth weight. In humans, nephrogenesis is usually completed by the thirty-sixth week of gestation. About 60% of the nephrons develop during the third trimester of gestation, a process that has been postulated to continue ex utero. The question is, how far should postnatal nutrition of very low birth weight infants be aimed at accelerating growth and development in a context of poor renal function due to marked kidney immaturity? Few data are available on the evolution of ex utero ongoing formation of nephrons in preterm infants. Rodriguez et al. (24) have shown in an autopsic study that glomerular endowment remains lower in preterm infants compared with term newborns; this is probably related to a defect of postnatal glomerulogenesis. Ex utero formation of nephrons in preterm infants may thus be affected by various postnatal conditions such as multiple stress events and postnatal undernutrition. Indeed, >80% of preterm infants exert a postnatally acquired growth restriction during the first week of life, due to deficits in energy and protein intake (8, 10), that could affect the ex utero ongoing nephrogenesis. Adverse postnatal environment may thus affect ex utero nephrogenesis, leading to low nephron endowment. However, extrapolation of our results to the human newborn infant remains speculative. Furthermore, factors other than congenital nephron endowment may be involved in the development of increased arterial pressure at adulthood.

This study shows that, in rats born with an appropriate birth weight, enhanced postnatal nutrition increases the nephron number but still alters renal function and structure in the long term in male adults. Altered nephrogenesis certainly plays an important role in the early origins of cardiovascular and renal diseases in adulthood (12). Low birth weight, which is associated with glomerular number deficit, is a major risk factor for systemic hypertension in adulthood (1, 27). According to Brenner et al. (6), if the glomerular number is reduced, an adaptive single nephron glomerular hyperfiltration occurs with related glomerular enlargement and glomerular hypertension. Such glomerular hemodynamic changes lead to glomerular hyperpression and glomerular injury. A vicious cycle takes place that contributes to proteinuria, glomerulosclerosis, systemic hypertension, and end-stage renal disease. Such a hypothesis may explain the association between low birth weight, low glomerular number, and systemic hypertension in humans. Epidemiological data have recently suggested that low birth weight related to premature birth is a risk factor for cardiovascular disease and hypertension in adults (9, 14, 15). Keijzer-Veen et al. (15) have shown that premature birth is associated with proteinuria and renal function deterioration in young adults born preterm.

Although early postnatal overfeeding benefits postnatal formation of nephrons in the absence of any influence of gender in our study, it is associated with high blood pressure and glomerulosclerosis in adult male offspring. High blood pressure is observed in the absence of glomerular number reduction, suggesting that mechanisms different from inborn nephron number deficit may be involved. The underlying mechanisms of such an effect, which seems gender dependent, still remain to be understood. Early postnatal overfeeding during the suckling period has been demonstrated to induce cardiovascular and metabolic disorders and obesity in adult rats (23, 34). Hyperinsulinism, hyperleptinemia, upregulation of the hypothalamo-pituitary-adrenal (HPA) axis, and other mediators and hormones derived from adipose tissue may contribute to such diseases in adulthood. Early postnatal overfeeding in rat has been shown to be associated with hyperinsulinism and insulin resistance, as soon as postnatal day 15, impairing vascular dilatation capacity through endothelial dysfunction (22, 23, 26). Hyperleptinemia is observed in adults exposed to early postnatal overfeeding (3, 20, 29). Leptin, produced by adipose tissue and the brain, affects blood pressure through upregulation of central and peripheral sympathetic nervous systems, responsible for increased vascular tone and heart rate (11). Adverse metabolic and cardiovascular effects of hyperinsulinism and hyperleptinemia potentiate each other and may be favored by an upregulation of the HPA axis. Indeed, accelerated maturation and permanent upregulation of the HPA axis, including increases in corticosterone secretion and enhanced glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase type-1 mRNA levels in visceral adipose tissue, have been observed in adult rats exposed to early postnatal overfeeding (5). However, a renal mechanism for elevated blood pressure and glomerulosclerosis observed in adult overfed males cannot be excluded in this study. Indeed, an increment in glomerular filtration rate associated with being overweight, induced by early postnatal overfeeding, may contribute to such elevated blood pressure and glomerulosclerosis in overfed male rats.

We conclude that early postnatal overfeeding in rat improves postnatal nephron number. However, enhanced nephron number is still associated with prolonged, elevated arterial pressure and glomerulosclerosis in aging male rats in this experimental
model. Whether such findings may be extrapolated to the postnatal ongoing formation of nephrons in premature human infants remains a matter of speculation. These findings suggest that a postnatal window of opportunity exists for intervention in the renal mechanisms involved in the developmental origins of hypertension. Other gender-determined mechanisms may intervene aside from renal nephron number at birth in the long-term development of arterial and renal functions.

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