Early postnatal overfeeding induces early chronic renal dysfunction in adult male rats

Farid Boubred,1,2 Laurent Daniel,3 Christophe Buffat,1 Jean-Marc Feuerstein,4 Michel Tsimaratos,4 Charles Oliver,4 Françoise Dignat-George,1 Martine Lelièvre-Pégorier,5 and Umberto Simeoni1,2

1INSERM UMR608, Faculté de Pharmacie, Université de la Méditerranée, 2Division of Neonatology, Hôpital la Conception, Assistance Publique-Hôpitaux de Marseille, and 3UPRES EA3281 and 4UPRES EA2193 Faculté de Médecine, Université de la Méditerranée, Marseille; and 5INSERM U652, Institut Biomédical des Cordeliers, Paris, France

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Boubred F, Daniel L, Buffat C, Feuerstein J, Tsimaratos M, Oliver C, Dignat-George F, Lelièvre-Pégorier M, Simeoni U. Early postnatal overfeeding induces early chronic renal dysfunction in adult male rats. Am J Physiol Renal Physiol 297: F943–F951, 2009. First published August 5, 2009; doi:10.1152/ajprenal.90704.2008. —Low birth weight is associated with an increased risk of hypertension and renal dysfunction at adulthood. Such an association has been shown to involve a reduction of nephron endowment and to be enhanced by accelerated postnatal growth in humans. However, while low-birth-weight infants often undergo catch-up growth, little is known about the long-term vascular and renal effects of accelerated postnatal growth. We surimposed early postnatal overfeeding (OF; reduction of litter size during the suckling period) to appropriate-birth-weight (NBW + OF) and intrauterine growth restriction (IUGR; IUGR + OF) pups, obtained after a maternal gestational low-protein diet. Blood pressure (systolic blood pressure; SBP) and renal function (glomerular filtration rate; GFR) were measured in young and aging offspring. Glomerulosclerosis and nephron number were determined in aging offspring (22 mo). Nephron number was reduced in both IUGR and IUGR + OF male offspring (by 24 and 26%). GFR was reduced by 40% in 12-mo-old IUGR + OF male offspring, and both NBW + OF and IUGR + OF aging male offspring had sustained hypertension (+25 mmHg) and glomerulosclerosis, while SBP and renal function were unaffected in IUGR aging offspring. Female offspring were unaffected. In conclusion, in this experimental model, early postnatal OF in the neonatal period has major long-lasting effects. Such effects are gender dependent. Reduced nephron number alone, associated with IUGR, may not be sufficient to induce long-lasting physiological alterations, and early postnatal OF acts as a “second hit.” Early postnatal OF is a suitable model with which to study the long-term effects of postnatal growth in the pathogenesis of vascular disorders and renal disease.

nephrogenesis; nephron number; catch-up growth; hypertension; glomerulosclerosis; developmental origins of adult diseases; low birth weight

Epidemiological studies across different countries have shown that low birth weight is associated with an increased risk of cardiovascular and metabolic diseases (3–5). Although with less evidence, low birth weight has been related to impaired renal function at adulthood (23, 28, 31). Moreover, it has been recently demonstrated that accelerated postnatal growth or a rapid postnatal catch-up growth during infancy in low-birth-weight infants enhances the risk of cardiovascular disease at adulthood (1, 6, 26). These findings in humans have been reproduced with various animal models of intrauterine growth restriction (IUGR), in particular in rodents. Moderate maternal gestational diet protein restriction (9 vs. 18% casein) is associated with increased blood pressure and altered renal function at adulthood (35). These observations have led to the concept of “developmental programming,” whereby prenatal and/or postnatal environmental factors at a specific time of development can cause lifelong functional and structural changes in different organs and systems.

The mechanism by which low birth weight and postnatal growth predispose to such adult diseases is unknown. It has been established that fetal growth restriction alters nephron formation and induces a permanent reduction of nephron endowment. Reduced nephron number has been postulated to result in single-nephron glomerular hyperfiltration with glomerular hypertrophy, which are responsible for glomerular injury, long-term proteinuria, glomerulosclerosis, progressive deterioration of renal function, and hypertension (10, 24, 29, 35). However, hypertension and chronic kidney disease have been observed in the absence of reduced nephron number. Obesity, with associated metabolic and cardiovascular diseases, has been responsible for chronic kidney disease (19, 38). We have recently shown that early postnatal overfeeding (OF; used herein for experimental groups only) in rats born with an appropriate birth weight improved postnatal nephrogenesis but induced long-term hypertension and impaired glomerular structure, suggesting that factors other than nephron number are involved in such a model (8). Thus it has been suggested that hypertension and chronic kidney disease have a “multi-hit” origin, of which nephron number deficit at birth constitutes the first hit (19, 37, 38).

In the present study, we aimed to determine, in aging rats, how blood pressure, renal function, and renal structure were affected by early postnatal overfeeding. We hypothesized that reduced nephron number, associated with IUGR, is not alone sufficient and that early postnatal overfeeding acts as a second hit which favors the early development of hypertension and chronic kidney disease.

MATERIALS AND METHODS

Animals and Diet

All procedures using animals were approved by the Institutional Animal Care and Use Committee of the Université de la Méditerranée and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and according the Declaration of Helsinki for experimental studies. Male and virgin female Sprague-Dawley rats (purchased from Charles Rivers, l’Abresle, France) were housed individually and were kept on standard laboratory rat chow diet 2 wk before mating. During experiments, rats had...
free access to food and tap water ad libitum in a room with a 12:12-h light-dark cycle, at a controlled temperature (22°C), and with a constant humidity. The day on which sperm was seen in a vaginal smear was designated as day 1 of pregnancy. Pregnant rats were randomly allocated to a control diet (n = 7, 22% casein, Safe-UAR) or to a moderate isocaloric low-protein diet (n = 7, 9% casein, Safe-UAR) to induce IUGR. The composition of these respective diets was as follows (% of diet: g/100 g): casein (22 vs. 9%), maize starch (23 vs. 34.5%), saccharose (38 vs. 38%), lard (3 vs. 3%), corn oil and rapeseed oil (2 vs. 2%) and vitamins (Premix vitamin 200, 1 vs. 0.5%). All diets contained the same proportion of cellulose (6%), methionine (0.3%), minerals (Premix mineral 205B), and chloride of sodium (0.2%). The low-protein diet began on the day of conception until delivery, and was proposed ad libitum. After delivery, all lactating mothers were fed standard laboratory chow whatever the diet during pregnancy. At birth, the weights of all pups were recorded within 6 h after delivery, and the litters were culled to 10 pups in all groups, with the largest and smallest pups removed to ensure an adequate and standardized nutrient supply. Litters of fewer than 10 pups were considered abnormal and were excluded from the study. On day 3, nine litters of lactating mothers exposed to the control diet or prenatally to the low-protein diet were randomly assigned to early postnatal overfeeding. Early postnatal overfeeding consisted in reducing the litter size down to three pups per litter, from postnatal day 3 and during the suckling period until day 21. This model results in increased weight gain during the suckling period in rodents and was proposed to create postnatal catch-up growth. On day 21, all the weaned offspring were supplied with the same standard laboratory rat chow ad libitum. Finally four groups of animals were investigated according to prenatal diet and to postnatal nutrition: group 1, normal birth weight (NBW): offspring of dams fed a normal diet (n = 30); group 2, IUGR: offspring of dams fed an isocaloric low-protein diet (n = 30); group 3, NBW + OF: appropriate-birth-weight offspring exposed to early postnatal overfeeding (n = 12); and group 4, IUGR + OF: offspring exposed to both a prenatal low-protein diet and early postnatal overfeeding (n = 12). Females and males were equally represented in each group. Three to four pups per litter from the NBW (3 litters), IUGR (3 litters), NBW + OF (4 litters), and IUGR + OF (4 litters) groups were randomly selected, which resulted in 10–12 offspring/group being studied.

Systolic Blood Pressure Measurements

Systolic blood pressure (SBP) was measured in 1-, 2-, 4-, and 12-mo-old animals. Blood pressure 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 acclimatized to this procedure during 5 successive days (10 min each day) before measurements. Thereafter, measurements were performed by a single operator. The mean of four to six measurements was recorded for each animal. This method has been extensively validated and refined to reduce stress-related effects.

Determination of Renal Function and Urinary Protein Excretion

Endogenous creatinine clearance (CrCl), daily urinary protein excretion rate (UprV) were determined in 4- and 12-mo-old animals as CrCl = UCr × V/ Pcr –1 and UprV = UPr × V, where Ucr and Pcr are urinary and plasma creatinine concentration, respectively, Upr is urinary protein concentration, and V is urinary output. Animals were housed individually for 48 h in metabolic cages, a 24-h collection was performed gravimetrically, and a blood sample (0.5 ml) was collected from the incision at the tip of the tail, under a brief general anesthesia (Halothane, Baumont). The blood samples were then transferred to heparinized tubes and centrifuged at 3,000 rpm for 15 min at 4°C. Creatinine, urinary creatinine, and protein concentrations were measured by a standard autoanalyzer (Synchron LX 20 autoanalyzer, Beckman Coulter). Plasma creatinine concentration was determined by the method of Jaffé.

Morphometric Measurements and Estimation of Nephron Number, Glomerular Volume, and Glomerulosclerosis in Aging Animals

At 22 mo of age, animals were anesthetized with intraperitoneal pentobarbital sodium under halothane anesthesia. Organs and perivascular adipose tissues were removed and weighted. The left kidney was rapidly harvested, weighed, and decapsulated for glomerular counting, and the right kidney was kept for histological analysis. The number of glomeruli per kidney was determined in three groups of rats as previously described (2). Briefly, 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 until placed in a hemocytometer-like chamber, and the glomeruli were counted under a 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 the kidney.

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 embedded in paraffin. Transverse sections through the central portion of each kidney and 4-μm-thick sections stained with hematoxylin α-eosin were obtained for light microscopic examination. In each single section of kidney, all glomeruli (i.e., superficial and juxamedullary) 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 glomerular volume. Cross-sectional tuft area (Gv) was calculated for each glomerulus with a visible vascular pole using image analysis software (SAMBA 2005, Alcatel, TITAN Anssware). Glomerular volume (Gv) was then calculated assuming the glomerulus to be spherical by applying the following mathematical equation as $G_v = \frac{1}{3} \frac{k}{4} \pi R^3$, where $R$ is the radius of the sphere, and $k$ is the size distribution coefficient ($k = 1.138$, 47, 54).

Glomerular sclerosis was evaluated using sirius red coloration to visualize fibrillar collagen. The measurement of Sirius red-stained areas to the percentage of total glomerular surface area was thus evaluated. A quantitative analysis was performed by a single examiner (L. Daniel) using the same colorimetric and light thresholds (NCSS 2004 software, Kaysville, UT). A color threshold 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. Statview version 5.0 software (Abacus Concepts, Berkeley, CA) was used to analyze differences among groups. Nonparametric Mann-Whitney and Kruskall-Wallis tests were used for comparisons. Repeated-measures ANOVA was used to compare SBP and postnatal growth. ANOVA with a Student-Newman-Keuls comparison test post hoc analysis was used to compare glomerular volume and glomerular sclerosis among the groups. Two-way ANOVA with the antenatal and postnatal diets as independent variables was employed to evaluate perinatal nutrition-related differences regarding SBP, renal function, glomerulosclerosis, and...
RESULTS

Birth Weight and Postnatal Growth

The birth weight of pups exposed prenatally to the low-protein diet was lower compared with pups exposed to the control diet (6.47 ± 0.64, 5.08 ± 0.9, 6.45 ± 0.1, and 5.56 ± 0.67 g in NBW, IUGR, NBW+OF, and IUGR+OF groups, respectively, P < 0.01) (Fig. 1). Litter size and sex ratio were unaffected by maternal protein restriction. During the suckling period, postnatal growth rates were different between the groups (P < 0.01). Regardless of the gender, NBW+OF pups displayed a rapid weight gain. IUGR pups exposed to early postnatal overfeeding (IUGR OF) displayed a rapid weight gain. IUGR pups exposed to early postnatal overfeeding (IUGR OF) caught up the weight of NBW pups by postnatal day 15 (body weights by postnatal day 15: 33.7 ± 5.1, 25.6 ± 5.2, 46 ± 3, and 33 ± 3 g in NBW, IUGR, NBW+OF, and IUGR+OF female offspring, respectively, P < 0.01; and 37.8 ± 1, 32.6 ± 1.6, 50 ± 1.5, and 37.1 ± 0.5 g in NBW, IUGR, NBW+OF, and IUGR+OF male offspring, respectively, P < 0.01). At 22 mo, the only observed difference was that the mean body weight in the NBW+OF group was higher than that in the IUGR group (P < 0.05). Postnatally, overfed offspring (NBW+OF and IUGR+OF) had a significant increase in central adipose tissue compared with NBW and IUGR offspring (see Table 3). As expected, males were heavier than females. During the experiment, two (NBW), two (IUGR), three (NBW+OF), and four (IUGR+OF) adult offspring died.

SBP

SBP levels are shown in Table 1 and Fig. 3A. At 1 mo of age, SBP was electively elevated in IUGR+OF offspring (P < 0.05 vs. NBW). At 2 and 4 mo of age (Table 1), SBP was significantly higher in IUGR animals and in both groups of postnatally overfed offspring than in NBW (by an average of 10–15 mmHg, P < 0.05). At the age of 12 mo (see Fig. 3A), SBP remained elevated in both postnatally overfed male offspring (NBW+OF and IUGR+OF; +25 mmHg vs. NBW, P < 0.05), while blood pressure in 12-mo-old IUGR offspring was unchanged. Postnatal overfeeding had an independent effect on SBP (Table 2), and blood pressure levels were more elevated in males (P < 0.01).

Renal Function

Glomerular filtration rate (GFR) and proteinuria (UprV) in 4- and 12-mo-old offspring are shown in Figs. 2, A and B, and 3, B and C. CrCl decreased with age in IUGR+OF male offspring, with a 40% reduction compared with NBW at the age of 12 mo. UpV was increased threefold in IUGR+OF male offspring at 4 mo (P < 0.05 vs. NBW). In NBW+OF offspring, a threefold increase in UpV appeared later at 12 mo, with unchanged CrCl. CrCl and UpV were unchanged in aging male and female IUGR offspring. As shown in Table 2, UpV in 12 mo offspring was independently associated with both antenatal and postnatal diet. Renal functions were unaffected in females.

Morphometric Measurement and Estimation of Nephron Number, Glomerular Volume, and Glomerulosclerosis in Aging 22-Mo-Old Offspring

In aging IUGR offspring, the relative kidney and heart weights were significantly decreased in IUGR+OF offspring (P < 0.05 vs. NBW) (Table 3). These differences concerned especially males (P < 0.01). Nephron number was reduced by an average of 24 and 26% in IUGR and IUGR+OF male offspring, respectively (P < 0.05). In contrast, nephron number was increased in NBW+OF male and female offspring (+20% vs. NBW P < 0.05) (Fig. 4A). No changes were observed in IUGR female offspring. Mean glomerular volume (Fig. 4B) and glomerulosclerosis (Fig. 4C) were influenced by gender (P < 0.01). NBW+OF and IUGR+OF male offspring had glomerulosclerosis (+65 and +70%, respectively, vs. NBW P < 0.01). Mean glomerular volume was significantly elevated in IUGR+OF male offspring (+75% vs. NBW, P < 0.01). Male and female NBW+OF offspring had decreased glomerular volume (~20 to ~30% in male and female NBW+OF offspring.

Table 1. Systolic blood pressure at postnatal weeks 4, 8, and 16

<table>
<thead>
<tr>
<th>Week 4</th>
<th>NBW</th>
<th>IUGR</th>
<th>NBW+OF</th>
<th>IUGR+OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 8</td>
<td>110 (7)</td>
<td>116 (8)</td>
<td>116 (7)</td>
<td>127 (4)*</td>
</tr>
<tr>
<td>Week 16</td>
<td>130 (9)</td>
<td>144 (10)*</td>
<td>140 (12)</td>
<td>145 (5)*</td>
</tr>
</tbody>
</table>

Values are means ± SD expressed as mmHg; n = 9–10. NBW, normal-birth-weight offspring; IUGR, intrauterine growth-restricted offspring; NBW+OF, appropriate-birth-weight offspring exposed to early postnatal overfeeding; IUGR+OF, intrauterine growth-restricted offspring exposed to early postnatal overfeeding. *P < 0.05 vs. NBW.

Table 2. P values for effects of antenatal and postnatal diet on SBP, CrCl, UprV, and GS in 12-mo-old offspring

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th>CrCl</th>
<th>UprV</th>
<th>GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal diet</td>
<td>0.73</td>
<td>0.24</td>
<td>0.04</td>
<td>0.009</td>
</tr>
<tr>
<td>Postnatal diet</td>
<td>0.01</td>
<td>0.38</td>
<td>0.005</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Combined effects</td>
<td>0.8</td>
<td>0.11</td>
<td>0.027</td>
<td>0.008</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; CrCl, creatinine clearance; UprV, urinary protein excretion rate; GS, glomerulosclerosis. P values are by 2-way ANOVA.
compared with NBW). No difference was observed between both groups of postnatally overfed offspring. Mean GV and glomerulosclerosis were unchanged in IUGR offspring despite the reduction in nephron number. Glomerulosclerosis was affected by early postnatal overfeeding (Table 2). Standard histology showed enlarged glomeruli, marked glomerulosclerosis, tubular distension, and interstitial inflammation in kidneys from IUGR/H11001 OF male offspring (Fig. 5).

DISCUSSION

This study provides information on how early postnatal overfeeding in the early postnatal period can affect long-term cardiovascular and renal physiology in aging rats. Early postnatal overfeeding following normal birth weight induced hypertension, proteinuria, and affected glomerular structure in aging offspring. Early postnatal overfeeding following IUGR allowed rapid postnatal catch-up growth but accelerated the development of systemic hypertension and impaired renal function in young adult offspring. Furthermore, such long-term consequences were influenced by gender, since females were protected against both prenatal and postnatal nutritional intervention.

Moreover, in aging IUGR offspring blood pressure, renal function, and renal structure were unaffected. Our findings suggest that reduced nephron number, associated with IUGR, is an important but not sufficient factor to mediate nutritionally programmed cardiovascular and renal alterations in the very long term. Early postnatal overfeeding may well constitute a second hit which favors the development of early chronic kidney disease.

Influence of a Maternal Low-Protein Diet and Postnatal Overfeeding on Nephrogenesis

It has been demonstrated in humans and in various animal species that fetal environment influences nephrogenesis, i.e., nephron endowment. It is known that IUGR is associated with reduced nephron endowment (21, 33, 35). In our study, in agreement with other reported findings, nephron number was reduced by an average of 25% in IUGR male offspring regardless of postnatal nutrition (33). Female gender is a protective factor in this experimental design (55). Interestingly, this protective factor disappears when the maternal protein diet is more restricted (casein, 6 vs. 9%) (56). We counted the glomeruli in the whole kidney, the using dissection-maceration
acid method which has been adopted for rodents, validated by numerous authors, and limited in bias as specific investigators were unaware of the group to which a specimen belonged (33, 52). We have shown previously in 20-day-old fetuses that a maternal low-protein diet reduced the nephron number by ~30% (13). The findings here show that the reduced nephron number observed at birth persists into adulthood.

The mechanisms of nephrogenesis impairment due to a maternal low-protein diet and IUGR are not clearly established. Reduction of nephron endowment may result from an early alteration of nephrogenesis. Nephrogenesis can be affected by different mechanisms, including decrease in expressivity of specific genes involved in branching morphogenesis, an increase in mesenchymal cell apoptosis, down-regulation of the intrarenal renin-angiotensin system (RAS), and an increase in fetal glucocorticoid exposure (35, 52, 53, 57). Recently, we have observed in the kidney of fetuses exposed to a maternal low-protein diet an overexpression of various genes, especially those involved in the coagulation pathway (13).

Postnatal nephrogenesis can be influenced by postnatal nutrition (8, 43). In rodents, nephrogenesis begins in the midgestation and continues after birth up to postnatal days 8–10. Postnatal nephrogenesis is impaired by postnatal food restriction and is improved by early postnatal overfeeding. In our study, while overfeeding in appropriate-birth-weight pups (NBW+OF) improved postnatal nephrogenesis, overfeeding failed to restore nephron number deficit in IUGR pups. Our findings suggest that, in the rat, the effects of early postnatal overfeeding on postnatal nephrogenesis is closely dependent on fetal environment. In another IUGR model, normal lactation could restore nephron endowment in placental restriction rats (54). A limitation in our study might be that glomeruli were counted in aging offspring, in that nephron number may be influenced by the aging process. Indeed, the velocity of nephron loss related to the aging process is unknown in our experimental groups. Also, the aim of our study was not to explore the effect of early postnatal overfeeding on postnatal nephrogenesis. Other studies are warranted to evaluate the effects and the potential mechanisms of early postnatal overfeeding on postnatal nephrogenesis.

**Influence of a Maternal Low-Protein Diet on Blood Pressure, Renal Function, and Structure**

Reduced nephron endowment is considered to be involved in the developmental origins of cardiovascular and chronic kidney diseases in adulthood (10, 11). According to Brenner et al. (10), when nephron number is reduced an adaptive single-nephron glomerular hyperfiltration with related glomerular enlargement and glomerular hypertension occur to sustain adequate renal function. Such glomerular hemodynamic changes lead to glomerular injury. A vicious cycle takes place over time, contributing to proteinuria, systemic hypertension, glomerulosclerosis, and end-stage renal disease. This renal mechanism has been confirmed in various animal models of fetal or neonatal programmed adult hypertension (35). In humans, it is well known that congenital renal agenesis is associated with an increased risk of hypertension and renal disease in adulthood (34). In addition, recent studies have supported an inverse relationship between nephron number and essential hypertension (24, 29).

In our study, in aging IUGR offspring, despite reduced nephron number, blood pressure, renal function, and glomerular structure were unchanged. These findings suggest that reduced nephron number associated with IUGR is not sufficient alone to mediate long-term nutritionally programmed cardiovascular and renal diseases. The absence of a relationship between reduced nephron number and vascular and renal diseases has been otherwise recently reported. In a study of humans, Hughson et al. (25) did not find a relationship between reduced nephron number and blood pressure. Compared with adults with congenital renal agenesis, renal transplant donors have a lower risk of hypertension and renal disease (20); a more effective compensatory adaptation in youth, related in part to increased growth velocity, has been advanced. In animal models, renal mass resection (5/6 nephrectomy) does not lead in all cases to hypertension and renal disease at adulthood (7, 18). Similar findings have been observed in different models of IUGR (22, 37). Interestingly, in a rat study, when maternal antenatal protein diet restriction (casein, 9 vs. 18%) was maintained after birth and throughout life, postnatal catch-up growth did not occur, and blood pressure and renal glomerular structure of 135-day-old male offspring was unchanged (22). Altogether, these findings suggest that the slow postnatal catch-up growth observed in our study prevents the IUGR offspring from developing hypertension and glomerulosclerosis.

In our study, blood pressure was transiently elevated in 4-mo-old IUGR offspring. Little is known about the long-term evolution of blood pressure with the aging process since most investigations have been performed until now in young adult male offspring, below the age of 12 mo (35). Blood pressure and renal function at adulthood depend on the experimental

### Table 3. Morphometric measurements and P values for effects of antenatal and postnatal diet in 22-mo-old offspring

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>Kidney</th>
<th>Heart</th>
<th>Lung</th>
<th>Liver</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBW</td>
<td>622 (162)</td>
<td>3.3 (0.1)</td>
<td>3.1 (0.1)</td>
<td>4.9 (0.2)</td>
<td>32.9 (2.80)</td>
<td>28.7 (3.3)</td>
</tr>
<tr>
<td>IUGR</td>
<td>553 (180)</td>
<td>3.3 (0.08)</td>
<td>2.9 (0.1)</td>
<td>4.2 (0.3)</td>
<td>28.1 (1.8)</td>
<td>21.8 (2.3)</td>
</tr>
<tr>
<td>NBW+OF</td>
<td>699 (173)</td>
<td>3.2 (0.7)</td>
<td>3.2 (0.5)</td>
<td>3.7 (0.7)</td>
<td>30.8 (4.3)</td>
<td>32.5 (7.8)</td>
</tr>
<tr>
<td>IUGR+OF</td>
<td>676 (233)</td>
<td>2.2 (0.1)</td>
<td>2.2 (0.1)</td>
<td>4.1 (0.3)</td>
<td>29 (1.3)</td>
<td>56.3 (3.4)</td>
</tr>
</tbody>
</table>

**P values**

- Antenatal diet: 0.47, 0.07, 0.64
- Postnatal diet: 0.06, 0.002, 0.20
- Combined effects: 0.002, 0.02, 0.29

Values for morphometric measurements are means (±SD); n = 8–9. Weight of different organs normalized to body weight (BW; mg/g body wt) in 22 mo-old NBW, IUGR, NBW+OF, and IUGR+OF offspring is shown. CAT, central adipose tissue. Also shown are P values by 2-way ANOVA for the effects of antenatal and postnatal diet.
with all rats being preconditioned to handling and tail-cuff measurements, thus limiting stress-induced tail-cuff measurements and bias within animals. However, a limitation to evidencing eventual blood pressure differences between the groups is possible in this study as telemetry was not used for blood pressure measurements.

As was the case for blood pressure, global GFR and proteinuria were unchanged in IUGR offspring. Glomerular volume was unaltered despite a reduction of nephron number in male IUGR offspring. These results suggest that in this experiment, single-nephron glomerular filtration is not dramatically increased. However, it has to be taken into consideration that the clearance of creatinine, used in our study to evaluate GFR with the advantage of allowing for repetitive measurements throughout animal life, is not the gold standard for this measurement (39). Using the clearance of inulin, the gold standard for GFR measurement, GFR per kidney weight has been shown to be decreased by 10% in 21-wk-old IUGR male offspring (57).

**Effects of Postnatal Overfeeding on Vascular, Renal Function, and Structure**

The most interesting finding of our study is that early postnatal overfeeding in the early postnatal period induced hypertension and chronic kidney disease in aging male offspring. Such functional and structural changes occurred early when overfeeding followed IUGR.

We show that overfeeding in normal-birth-eight pups induced hypertension and glomerulosclerosis in aging offspring, while postnatal nephrogenesis was improved, suggesting that factors other than nephron number are involved in the developmental programming of hypertension. Early postnatal overfeeding per se is known to induce cardiovascular and metabolic changes in adult rats, such as hypertension, enhanced central adipose tissue (which has been observed in our study), hyperleptinemia, hyperinsulinism, hyperglycemia, and upregulation of the hypothalamic-pituitary-adrenal axis (9, 40). Such hormonal changes may contribute to hypertension through various mechanisms, including impaired endothelial-dependent arterial vasodilation, central and renal nervous sympathetic hyperactivity, and upregulation of systemic and tissular RAS (36, 44). Such factors may take a part in favoring glomerulosclerosis through different mechanisms including a direct transmission of sustained systemic hypertension to glomerular capillaries and the direct deleterious effect of hyperglycemia on glomerular capillaries structure and hemodynamics (19, 38). However, it is not excluded that a certain degree of glomerular hyperfiltration associated with being overweight may occur and participate in glomerulosclerosis. Other studies are needed to investigate such mechanisms.

Early postnatal overfeeding may act as a second hit which favors the early development of chronic kidney disease in IUGR offspring. In our study, when overfeeding was superimposed to IUGR offspring, hypertension and proteinuria, a marker of glomerular injury, occurred early (1 vs. 4 mo for hypertension, and 4 vs. 12 mo for proteinuria, in IUGR+OF vs. NBW+OF, respectively), and GFR was impaired. No direct comparison of our results with data in the literature is possible since in our study early postnatal overfeeding obtained by a reduction in litter size was applied to IUGR pups at birth.

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**Fig. 4. Effect of postnatal overfeeding on renal structure and morphology in aging offspring.** Glomerular number (A), mean glomerular volume (B), and glomerulosclerosis (GS; C) in 22-mo-old NBW (open bars), IUGR (grey bars), NBW+OF (hatched bars), and IUGR+OF (black bars) offspring according to gender. GS is expressed as the mean ratio of Sirius red-stained areas to total glomerular capillary areas. Values are means ± SD; n = 7–9. *P < 0.05 vs. control. Significant differences (P < 0.05) across groups are indicated by different letters; for example, a is different from b but not different from ab, and c is different from a and b.

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The most interesting finding of our study is that early postnatal overfeeding in the early postnatal period induced hypertension and chronic kidney disease in aging male offspring. Such functional and structural changes occurred early when overfeeding followed IUGR.

We show that overfeeding in normal-birth-eight pups induced hypertension and glomerulosclerosis in aging offspring, while postnatal nephrogenesis was improved, suggesting that factors other than nephron number are involved in the developmental programming of hypertension. Early postnatal overfeeding per se is known to induce cardiovascular and metabolic changes in adult rats, such as hypertension, enhanced central adipose tissue (which has been observed in our study), hyperleptinemia, hyperinsulinism, hyperglycemia, and upregulation of the hypothalamic-pituitary-adrenal axis (9, 40). Such hormonal changes may contribute to hypertension through various mechanisms, including impaired endothelial-dependent arterial vasodilation, central and renal nervous sympathetic hyperactivity, and upregulation of systemic and tissular RAS (36, 44). Such factors may take a part in favoring glomerulosclerosis through different mechanisms including a direct transmission of sustained systemic hypertension to glomerular capillaries and the direct deleterious effect of hyperglycemia on glomerular capillaries structure and hemodynamics (19, 38). However, it is not excluded that a certain degree of glomerular hyperfiltration associated with being overweight may occur and participate in glomerulosclerosis. Other studies are needed to investigate such mechanisms.

Early postnatal overfeeding may act as a second hit which favors the early development of chronic kidney disease in IUGR offspring. In our study, when overfeeding was superimposed to IUGR offspring, hypertension and proteinuria, a marker of glomerular injury, occurred early (1 vs. 4 mo for hypertension, and 4 vs. 12 mo for proteinuria, in IUGR+OF vs. NBW+OF, respectively), and GFR was impaired. No direct comparison of our results with data in the literature is possible since in our study early postnatal overfeeding obtained by a reduction in litter size was applied to IUGR pups at birth.
contrary to other published studies. In 100- to 125-day-old IUGR offspring (obtained by a 30% reduction in global maternal diet), blood pressure, fasting insulin, and leptin levels have been shown to be amplified by a hypercaloric diet applied in weaning offspring (50). When IUGR pups were cross-fostered to a control mother, their lifespan was shortened and telomere shortening in kidneys of 13-mo-old male offspring was enhanced (27). Such enhancement in telomere shortening and hence cell senescence may result from oxidative cell damage determined by postnatal nutritional intake.

The underlying mechanism by which early postnatal overfeeding enhances, in the long term, the risk of vascular and renal diseases associated with IUGR is unknown. Such diseases may be the consequence of an enhancement of adaptive single-nephron glomerular hyperfiltration, which begins during the neonatal period. This early hemodynamic glomerular change is all the more deleterious since the immature kidney is particularly vulnerable to ischemic and glomerular damages (14). Glomerular damages, initiated during the neonatal period by early postnatal overfeeding, may evolve over time to glomerular sclerosis and chronic renal insufficiency in IUGR+OF offspring. Single-nephron glomerular hyperfiltration may result from an imbalance between reduced excretory capacity related to reduced nephron number and increased excretory demands due to early postnatal overfeeding. Early overfeeding, obtained by a reduction in litter size, induces rapid postnatal catch-up growth through an increase in maternal milk availability and thus an increase in vitamin, electrolyte, protein, and caloric intake. It is known that an increase in sodium and protein intake enhances glomerular hyperfiltration and induces systemic hypertension and glomerular injuries in adult animals with reduced nephron number (12, 18, 30, 42). Such a sequence has been shown to result from an upregulation of the RAS (15). It can be speculated that early postnatal overfeeding following IUGR enhances the single-nephron GFR through the renal RAS, which is upregulated in young IUGR offspring, and induces and initiates glomerular injuries during the neonatal period (35). Over time, early chronic kidney disease may develop through additional adverse effects, including transmitted sustained systemic hypertension to predisposed glomerular capillaries and renal and vascular consequences of metabolic and hormonal disorders associated with overfeeding (19, 38).

**Relationship to Epidemiological and Clinical Data**

Our results confirm emerging epidemiological and clinical data supporting the view that accelerated growth after birth promotes long-term cardiovascular diseases. In a longitudinal study of a Finnish cohort, Barker et al. (6, 17) showed that children who later developed coronary heart disease or hypertension were born small and that their body mass index caught up early in infancy. In a nutritional intervention study, Singhal et al. (45) showed that diastolic blood pressure was elevated in adolescents who were born prematurely and who developed a rapid weight gain within the first 15 postnatal days of life. Markers of vascular endothelial-dependent relaxation and insulin sensitivity were affected as well (46, 47). However, little is known about long-term effects of anthropometric characteristics at birth and postnatal growth on renal function in adults. Emerging knowledge is in favor of an increased risk of renal function impairment in adults born with a low birth weight, related or not to preterm birth (23, 28, 31). Indeed it has been suggested that postnatal nephrogenesis is impaired in preterm infants (41), while part of it occurs postnatally. This question is all the more important since currently a high-protein diet soon after birth is proposed to low-birth-weight infants to accelerate postnatal growth and to limit the postnatal growth restriction induced by intensive care, which is known to affect neonatal morbidity and long-term neurological performance (16). Moreover, obesity which may be influenced by a rapid weight gain during infancy, is associated with kidney disease (19, 38). Our results provide important data on how long renal function and renal structure may be influenced by early postnatal nutrition, when few epidemiological data are available on this issue. Early postnatal overfeeding following IUGR in the rat constitutes a suitable experimental model for future research on mechanisms linking perinatal growth and adult diseases, in the search for potential preventive strategies.

**Conclusion**

A maternal low-protein diet leads to IUGR associated with reduced nephron number. Female offspring are protected against such alteration of nephrogenesis. Expected long-lasting cardiovascular and renal effects are not observed in aging IUGR offspring.
Early postnatal overfeeding, which induces rapid postnatal catch-up growth, improves postnatal nephrogenesis, but induces sustained systemic hypertension and chronic kidney disease in aging male offspring. Early postnatal overfeeding following IUGR accelerates the occurrence of such early chronic disease. These findings suggest that reduced nephron number associated with IUGR is an important but not sufficient factor involved in the developmental programming of vascular and renal diseases. Other gender-determined mechanisms, including metabolic and hormonal factors, may intervene aside from nephron number reduction. “Fetal-programmed” adult vascular and renal diseases may develop with rapid postnatal catch-up growth which constitutes a second hit. Postnatal growth has to be taken into account in experimental models of fetal-programmed adult diseases. Although differences exist among species, our results support emerging data on the crucial role of rapid postnatal growth in promoting long-term cardiovascular and renal diseases. This study may provide approaches for future research on how postnatal growth may favor long-term cardiovascular and chronic kidney diseases and on how to prevent such a risk in infants and adults born with a low birth weight.

REFERENCES


8. Bremer BM, Garcia DL, Anderson S. Brenner BM. *Brenner BM.* Growth, improves postnatal nephrogenesis, but induces sustained


