Combined prenatal and postnatal protein restriction influences adult kidney structure, function, and arterial pressure

Chantal C. Hoppe,1 Roger G. Evans,2 Karen M. Moritz,1 Luise A. Cullen-McEwen,1 Sharyn M. Fitzgerald,2 John Dowling,3 and John F. Bertram1

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Hoppe CC, Evans RG, Moritz KM, Cullen-McEwen LA, Fitzgerald SM, Dowling J, Bertram JF. Combined prenatal and postnatal protein restriction influences adult kidney structure, function, and arterial pressure. Am J Physiol Regul Integr Comp Physiol 292: R462–R469, 2007. First published September 14, 2006; doi:10.1152/ajpregu.00079.2006.—The effects of prenatal protein restriction on adult renal and cardiovascular function have been studied in considerable detail. However, little is known about the effects of life-long protein restriction, a common condition in the developing world. Therefore, we determined in rats the effects of combined pre- and postnatal protein restriction on adult arterial pressure and renal function and responses to increased dietary sodium. Nephron number was also determined. Male Sprague-Dawley rats were born to mothers fed a low [8% (wt/wt), LP] or normal [20% (wt/wt), NP] isocaloric protein diet throughout pregnancy and maintained on these diets after birth. At postnatal day 135, nephron number, mean arterial pressure (MAP), and renal function were determined. A high-NaCl [8.0% (wt/wt), high-salt] diet was fed to a subset of rats from weaning. MAP was less in LP than in NP rats (120 ± 2 vs. 128 ± 2 mmHg, P < 0.05) and was not significantly altered by increased salt intake. Nephron number was 31% less in LP than in NP rats (P < 0.001). The volume of individual glomeruli was also less in LP than in NP rats, as were calculated effective renal plasma flow and glomerular filtration rate. Glomerular filtration rate, but not effective renal plasma flow, appeared to be increased by high salt intake, particularly in LP rats. In conclusion, protein restriction induced a severe nephron deficit, but MAP was lower, rather than higher, in protein-restricted than in control rats in adulthood. These findings indicate that the postnatal environment plays a key role in determining the outcomes of developmental programming.

A suboptimal in utero environment has been linked to development of adult-onset diseases, including hypertension and type 2 diabetes mellitus (4, 15). Fetal undernutrition is also associated with changes in other metabolic, endocrine, and immune functions known to play important roles in human disease (4). The evidence for these associations in humans comes mainly from studies in developed nations, in which fetal undernutrition may be followed by adequate nutrition, if not overnutrition, postnatally (25). Most studies in experimental animals have mimicked this human paradigm. For example, in rats, maternal protein restriction followed by a normal-protein diet after birth can lead to adult hypertension and/or salt-sensitive arterial pressure (21, 32, 33). Furthermore, this regimen has been associated with restricted fetal development and nephron deficit and has been extensively studied in the rat (29).

Experimental paradigms focused solely on maternal undernutrition in the prenatal/early lactational period do not mimic the human condition in much of the world, where malnutrition is not merely confined to fetal life but, rather, is a life-long condition (11, 17). The prevalence of hypertension and cardiovascular disease appears to be low under conditions of chronic undernutrition, although data bearing on this issue are limited (12–14, 18, 20, 23). In contrast, other clinical observations suggest an inverse relation between protein intake and mean arterial pressure (MAP) in adults (5, 28). Importantly, we are aware of no studies in experimental animals that have determined the impact of life-long (defined here as combined prenatal and postnatal) protein restriction on cardiovascular and/or renal function in adulthood. Therefore, in the present study, we tested the hypothesis that life-long protein restriction in rats, similar to protein restriction in utero only, results in changes in adult cardiovascular and renal function and the development of salt sensitivity of arterial pressure.

METHODS

Animals and diets. Experiments were carried out in accordance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th ed., 2004) and were approved by the Biochemistry, Anatomy, and Microbiology Animal Ethics Committee of Monash University. Pairs of adult male and female Sprague-Dawley rats were fed a normal-protein [20% (wt/wt)] or an isocaloric low-protein [8% (wt/wt)] diet from 2 wk before mating (n = 6 pairs per group; Table 1). The NaCl content of both diets was 0.26% (wt/wt). Female breeders were maintained on their given diet throughout pregnancy and lactation. Offspring were also maintained on their mother’s designated diet after weaning at 21 days. Only male offspring were used in all experiments. A subset of the offspring from normal-protein (NP) and low-protein (LP) groups was fed a high-NaCl diet [8.0% (wt/wt), 3.15% Na+ (wt/wt)] at weaning (NPHS and LPHS, respectively).

Morphometric measurements and estimation of kidney volume, glomerular number, and glomerular volume. At postnatal day 30 or 135, rats were anesthetized with pentobarbital sodium (60 mg/kg; Nembutal, Rhone Merieux, Pinkenba, QLD, Australia), and perfused retrogradely through the abdominal aorta with 10% formalin at 130 mmHg. Major organs were removed and weighed. At postnatal day 135, total number of glomeruli was estimated in five male offspring in the NP diet group and five male offspring in the LP diet group. Adult right kidneys were decapsulated and subsampled (sliced) for stereo-
logical estimation of total nephron number. Sampled slices were processed for embedding in glycolmethacrylate (Technovit 7100, Kulzer). Tissue blocks were exhaustively sectioned at 15 μm, and every 10th and 11th section (section pair) was collected.

Total number of glomeruli \( (N_{\text{glomeruli}}) \), mean glomerular volume \( (V_{\text{glomerulus}}) \), and mean renal corpuscle volume \( (V_{\text{corpuscle}}) \) were determined as previously described using unbiased stereological techniques (7).

Briefly, the areas of all sampled kidney sections, as well as complete overlying glomerular profiles by the number of points overlying \( (V_{\text{glomerulus}}) \), and mean renal corpuscle volume \( (V_{\text{corpuscle}}) \) were determined as previously described using unbiased stereological techniques (7).

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At the completion of the 24-h urine collection period, rats were prepared for conscious MAP and heart rate (HR) measurements via an indwelling tail artery catheter. The catheter was inserted under brief 1–4% (vol/vol) isoflurane anesthesia and connected to a pressure transducer (Cobe, Arvada, CO) connected to a polygraph (model 79D, Grass) and a computer equipped with an analog-to-digital converter. After 1 h of recovery, MAP and HR were measured for 1 h in the conscious and unrestrained rat. Rats were then anesthetized with pentobarbital sodium (60 mg/kg ip). A 2-ml blood sample was taken from the abdominal aorta, and rats were then perfusion fixed with 100 ml of 10% buffered formalin at 130 mmHg. Plasma was prepared by centrifugation, and plasma and urine samples were stored at −20°C. Major organs were removed and weighed.

Analytic techniques. Radioactivity in the plasma and urine samples was determined by liquid scintillation analysis (model LS 5000TA, Beckman). P-aminohippuric acid concentration was determined by a colorimetric assay as previously described (3). Sodium concentrations were determined using the Beckman-Coulter Synchron CX5 Delta.

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To validate the method by which GFR was determined using [1H]inulin, we determined creatinine clearance using the plasma and urine from the same animals (Synchronic CX5 Clinical System, Beckman). Least products linear regression analysis (19) revealed a statistically significant positive relation between creatinine and [1H]inulin clearance \( (r = 0.43, P = 0.03, n = 24) \), with no fixed bias [intercept mean (95% confidence limits) \( = -0.23 \) \( (0.81 \) to 0.36) ml/min] or proportional bias [slope \( = 0.82 \) (0.43–1.20)].

Statistics. Values are means ± SE. Biological hypotheses were tested using two-way ANOVA or Student’s t-test as appropriate with the software package SYSTAT (version 7.0, SPSS). \( P \leq 0.05 \) was taken to indicate statistical significance.

RESULTS

Body and organ weights. At postnatal day 30, the absolute weights of all organs examined, except the brain, were less in LP than in NP rats (Table 2). When expressed as a proportion of body weight, kidney and spleen weights, but not pancreas or heart weight, were less in LP than in NP rats. In contrast, corrected liver weight and brain weight were greater in LP than in NP rats. Comparable effects of protein intake on absolute organ weights were seen in adult (postnatal day 135) animals (Table 3). However, the effects of life-long protein restriction on organ weights expressed relative to body weight were different between postnatal days 30 and 135. At postnatal day 135, corrected kidney weight, but not corrected liver, spleen, pancreas, or heart weight, was less in LP than in NP rats. As was the case for postnatal day 30 rats, corrected brain weight was greater in adult LP than NP rats. Salt intake did not

<table>
<thead>
<tr>
<th>Table 1. Diet composition</th>
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<tr>
<td><strong>Diet</strong></td>
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<tr>
<td>Casein, g/kg</td>
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<tr>
<td>Canola oil, g/kg</td>
</tr>
<tr>
<td>Sucrose, g/kg</td>
</tr>
<tr>
<td>Wheat starch, g/kg</td>
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<tr>
<td>Cellulose, g/kg</td>
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<tr>
<td>nL-Methionine, g/kg</td>
</tr>
<tr>
<td>NaCl, g/kg</td>
</tr>
<tr>
<td>Protein, %</td>
</tr>
<tr>
<td>Total fat, %</td>
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<tr>
<td>Energy, MJ/kg</td>
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</table>

Semipurified diets were obtained from Specialty Feeds (Western Australia). Feed was manufactured as a 12-mm-diameter cube, vacuum packed, and stored at −20°C. Low-protein (LP) diets were rendered isocaloric by addition of sucrose/starch. NP, normal protein.

At the completion of the 24-h urine collection period, rats were prepared for conscious MAP and heart rate (HR) measurements via an indwelling tail artery catheter. The catheter was inserted under brief 1–4% (vol/vol) isoflurane anesthesia and connected to a pressure transducer (Cobe, Arvada, CO) connected to a polygraph (model 79D, Grass) and a computer equipped with an analog-to-digital converter. After 1 h of recovery, MAP and HR were measured for 1 h in the conscious and unrestrained rat. Rats were then anesthetized with pentobarbital sodium (60 mg/kg ip). A 2-ml blood sample was taken from the abdominal aorta, and rats were then perfusion fixed with 100 ml of 10% buffered formalin at 130 mmHg. Plasma was prepared by centrifugation, and plasma and urine samples were stored at −20°C. Major organs were removed and weighed.

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Male rats were exposed to NP or LP diet \(0.26\%\) (wt/wt) salt in utero and postnatally or additionally postnatally fed a high-salt \(8.0\%\) (wt/wt) salt diet. Diet significantly affect absolute organ weight in adult rats, except in the case of the kidneys: kidneys from rats fed the high-salt diet weighed more than those from rats fed the normal-salt diet (Table 3). There were no statistically significant interactions between the effects of “protein” and “salt” on body weight or absolute or corrected organ weights, indicating that the effects of protein restriction and high salt intake on these morphometric parameters were independent.

**Renal histopathology.** The kidneys of two of three NP offspring demonstrated occasional signs of tubular dilatation and slight diffuse lymphocytic and monocye infiltration in the interstitium. The kidneys of one of three LP offspring also demonstrated occasional signs of tubular dilatation. No abnormalities were observed in the kidneys of LP or NP offspring maintained on the high-salt diet.

**Nephron number and morphology.** At postnatal day 135, glomerular number was 31\% less in LP than in NP offspring: 25,257 ± 1,131 vs. 36,438 ± 1,682 (Fig. 1). Protein restriction was also associated with reduced mean glomerular volume and mean renal corpuscle volume (24\% and 30\% less, respectively) and reduced total glomerular and total renal corpuscle volume (48\% and 52\% less, respectively; Fig. 1).

**MAP and HR.** MAP and HR remained relatively stable across the 60 min of data collection, indicating complete recovery from anesthesia during the monitoring period. MAP was significantly greater in NP than in LP rats: 128 ± 2 vs. 120 ± 2 mmHg (Fig. 2A). A similar difference in MAP was observed between NPHS and LPHS rats. MAP was not significantly different between rats fed a high-salt diet and those fed a normal-salt diet, regardless of the level of protein intake. HR was similar in all four groups (Fig. 2B).

**Food and water intake and renal function.** Food intake was similar in all groups (Fig. 3). The protein composition of the diet did not significantly affect total water intake or urine flow.

### Table 2. Effects of life-long protein restriction on body and organ weights at postnatal day 30

<table>
<thead>
<tr>
<th></th>
<th>Normal Values, g</th>
<th>Normalized Values, mg/g body wt</th>
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<tbody>
<tr>
<td></td>
<td>NP</td>
<td>LP</td>
</tr>
<tr>
<td>Body</td>
<td>96 ± 7</td>
<td>49 ± 4‡</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>0.61 ± 0.04</td>
<td>0.25 ± 0.03‡</td>
</tr>
<tr>
<td>Left</td>
<td>0.58 ± 0.04</td>
<td>0.25 ± 0.03‡</td>
</tr>
<tr>
<td>Total</td>
<td>1.18 ± 0.08</td>
<td>0.51 ± 0.06‡</td>
</tr>
<tr>
<td>Liver</td>
<td>5.75 ± 0.50</td>
<td>3.79 ± 0.25‡</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.50 ± 0.04</td>
<td>0.19 ± 0.02‡</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.69 ± 0.10</td>
<td>0.30 ± 0.06‡</td>
</tr>
<tr>
<td>Heart</td>
<td>0.57 ± 0.04</td>
<td>0.32 ± 0.03‡</td>
</tr>
<tr>
<td>Brain</td>
<td>1.48 ± 0.07</td>
<td>1.37 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE \((n = 7\) NP and \(n = 8\) LP rats from 4 or 5 litters). Male rats were exposed to NP or LP diet in utero and postnatally until postnatal day 30. \(*P < 0.05; ‡P < 0.01; †P < 0.001\) (Student’s unpaired t-test).

### Table 3. Effects of life-long protein restriction and increased salt intake on body and organ weights at postnatal day 135

<table>
<thead>
<tr>
<th></th>
<th>Normal Salt</th>
<th>High Salt</th>
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<tbody>
<tr>
<td></td>
<td>NP</td>
<td>LP</td>
<td>NP</td>
<td>LP</td>
</tr>
<tr>
<td>Body</td>
<td>424 ± 20</td>
<td>341 ± 16</td>
<td>411 ± 24</td>
<td>280 ± 8</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>1.83 ± 0.09</td>
<td>1.16 ± 0.03</td>
<td>2.09 ± 0.14</td>
<td>1.28 ± 0.09</td>
</tr>
<tr>
<td>Right</td>
<td>1.92 ± 0.09</td>
<td>1.20 ± 0.05</td>
<td>2.17 ± 0.12</td>
<td>1.35 ± 0.08</td>
</tr>
<tr>
<td>Total</td>
<td>3.75 ± 0.17</td>
<td>2.36 ± 0.07</td>
<td>4.26 ± 0.25</td>
<td>2.63 ± 0.17</td>
</tr>
<tr>
<td>Liver</td>
<td>19.6 ± 1.3</td>
<td>14.6 ± 0.5</td>
<td>19.9 ± 0.7</td>
<td>14.4 ± 1.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.95 ± 0.08</td>
<td>0.80 ± 0.06</td>
<td>0.98 ± 0.10</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.58 ± 0.25</td>
<td>1.04 ± 0.15</td>
<td>1.76 ± 0.36</td>
<td>0.89 ± 0.14</td>
</tr>
<tr>
<td>Heart</td>
<td>1.55 ± 0.07</td>
<td>1.21 ± 0.07</td>
<td>1.58 ± 0.10</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>Brain</td>
<td>2.39 ± 0.10</td>
<td>2.47 ± 0.05</td>
<td>2.49 ± 0.07</td>
<td>2.41 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE \(\(n = 9\) normal rats, \(n = 7\) protein-restricted rats, \(n = 6\) normal rats fed a high-salt diet, \(n = 7\) protein-restricted rats fed a high-salt diet). Male rats were exposed to NP or LP diet [0.26\% \(\text{wt/wt}\) salt] in utero and postnatally or additionally postnatally fed a high-salt [8.0\% \(\text{wt/wt}\) salt] diet from weaning. \(P \) values were obtained from 2-way ANOVA for main effects of protein diet \(P_{protein}\) and salt intake \(P_{salt}\) and for interactions between these factors \(P_{salt*protein}\).
but these variables were increased ∼3- and 10-fold, respectively, by the high-salt diet. ANOVA failed to reveal a statistically significant effect of protein restriction on calculated GFR (Fig. 4). However, GFR, expressed in absolute terms, was significantly less in LP than in NP rats: 0.34 ± 0.06 vs. 0.80 ± 0.20 ml/min (P = 0.048, unpaired t-test; Fig. 4). When adjusted for body and kidney weight, calculated GFR was not significantly different in these two groups (P = 0.12 and P = 0.30, respectively, unpaired t-tests), inasmuch as protein-restricted rats were smaller. ANOVA revealed a significant effect of salt intake on GFR and also a significant interaction between the effects of protein and salt intake. This was due to the fact that GFR was not significantly greater in the NPHS than in the NP group (P = 0.31, unpaired t-test), yet GFR was approximately fourfold greater in the LPHS than in the LP group (P < 0.001, unpaired t-test). High salt intake significantly increased urine flow, sodium excretion, and GFR, expressed in proportion to body weight or kidney weight, in LP and NP animals, but the apparent increase in GFR was considerably greater in LPHS than in NPHS rats (Figs. 3 and 4).

Fig. 1. Effects of protein restriction on number and dimensions of glomeruli. Male rats were exposed to normal- or low-protein diet in utero and until postnatal day 135. Unbiased stereological techniques were used to determine total glomerular (nephron) number (A), mean glomerular volume (V_{glom}, B), total glomerular volume (TGV, C), mean renal corpuscle volume (V_{corp}, D), and total renal corpuscle volume (TCV, E). Values are means ± SE (n = 5). *P < 0.05; **P ≤ 0.01; ***P = 0.001 (Student’s unpaired t-test).

Fig. 2. Effects of protein restriction and increased salt intake on mean arterial pressure (MAP, A) and heart rate (HR, B) at postnatal day 135. Male rats were exposed to normal- or low-protein diet [0.26% (wt/wt) salt] in utero and postnatally or additionally fed a postnatal high-salt [8.0% (wt/wt)] diet from weaning. Values are means ± SE (n = 9 normal rats, n = 7 protein-restricted rats and protein-restricted rats fed a high-salt diet, and n = 6 normal rats fed a high-salt diet). P values were obtained from 2-way ANOVA testing for main effects of a protein diet and salt intake (P_{protein} and P_{salt}) and for interactions between these factors (P_{protein*salt}).
Protein restriction decreased absolute effective renal plasma flow (ERPF), but not ERPF normalized to total kidney or body weight. There was no statistically significant effect of salt intake on ERPF, expressed in absolute terms or in proportion to body weight or kidney weight (Fig. 5). Thus the apparent hyperfiltration in LPHS rats was due to a marked increase in filtration fraction: from 16.8 ± 4.0% in LP rats to 47.0 ± 7.2% in LPHS rats (Fig. 5).

Fig. 3. Effects of protein restriction and increased salt intake on 24-h food consumption (A), water intake (B), and urine flow (UF, C) at postnatal day 135. Male rats were fed a normal- or low-protein diet [0.26% (wt/wt) salt] in utero and postnatally or additionally postnatally fed a high-salt [8.0% (wt/wt)] diet from weaning. See Fig. 2 legend for additional information.

Fig. 4. Effects of protein restriction and increased salt intake on glomerular filtration rate (GFR) at postnatal day 135. GFR is expressed as absolute values (A) and normalized to total kidney weight (GFR/TKW, B) and body weight (GFR/BW, C). D: fractional excretion of sodium (FE_sodium). Male rats were exposed to normal- or low-protein diet [0.26% (wt/wt) salt] in utero and postnatally or additionally postnatally fed a high-salt [8.0% (wt/wt)] diet from weaning. See Fig. 2 legend for additional information.
DISCUSSION

The major novel finding from the present study was that MAP was ~8 mmHg less at postnatal day 135 in rats exposed to life-long protein restriction than in control rats fed a normal-protein diet. This observation was made in the face of a 31% deficit in nephron numbers in protein-restricted rats. Our findings of lower MAP in protein-restricted rats may be reflective of the human condition, where continued undernutrition is associated with lower arterial pressure in populations without other cardiovascular disease risk factors (18). We also found that high salt intake increased the calculated GFR-to-body weight ratio of protein-restricted rats fourfold but increased the GFR-to-body weight ratio only ~43% in rats fed a normal-protein diet. This observation raises the intriguing possibility that life-long protein restriction may markedly increase the sensitivity of GFR to the effects of a high-salt diet. Such a mechanism might help explain the devastating cardiovascular and renal disease in undernourished populations after rapid transition to increasing Western influences (2). However, there are potential pitfalls in the use of \[^3\text{H}\]inulin clearances for subacute measurement of GFR in conscious rats, so further studies are required to confirm our observations.

A new model of developmental programming: life-long protein restriction. Reduced growth occurred in protein-restricted rats, despite the fact that the LP and NP diets were isocaloric and food intake, at least when tested at postnatal day 135, was similar in all groups of rats studied. Our results from life-long protein restriction show many differences from the “prenatal-only” model of maternal protein restriction. At postnatal days 30 and 135, body and absolute organ (except brain) weights, were less in protein-restricted than in normal rats. However, when normalized to body weight, only the kidney remained smaller at both ages. Thus kidney weight remained reduced into adulthood in protein-restricted rats, whereas the weight of other organs remained, or became, relatively normal. Although it was not affected by protein restriction at either age, brain weight was significantly greater in protein-restricted than in control rats when normalized to body weight at both ages. Consistent with this finding, using a similar model of life-long protein restriction, Bennnis-Taleb et al. (6) also found “brain sparing” in protein-restricted compared with normal rats at postnatal days 3 and 110. The continued body and kidney weight deficit and brain sparing in life-long protein-restricted rats contrasts with the effects of protein restriction in utero only, since transition to normal protein intake at birth eventually normalizes body and kidney weight (8, 31, 32). Similar to observations in experimental models, there is evidence to suggest that fetal growth restriction in humans also leads to infants with whole brain weight similar to that of normal-weight infants. Although human data are limited, this information, accompanied by the fact that adult body weight is also reduced by life-long protein restriction, suggests that the probable brain sparing may persist into adulthood, if growth restriction is maintained throughout life (10). Thus, at least in this respect, our model mimics the human condition of life-long protein restriction. We chose to restrict protein intake in male and female rats before mating. Therefore, imprinting of maternal and paternal genes by protein restriction during the periconceptual period might have influenced the effects of
life-long protein restriction. Our experimental design did not allow us to investigate this issue, which merits further analysis.

Renal structure and pathology. As has been found in previous studies of prenatal protein restriction (32), adult protein-restricted rats exhibited a 31% deficit in total nephron number compared with control rats, when determined by unbiased stereology. The unbiased stereological technique that we used is the gold standard for determination of nephron number (7, 22). The absolute values for our normal rat nephron number are consistent with previous values obtained for Sprague-Dawley rats in our laboratory (7). Interestingly, glomerular hypertrophy was not observed in the present study, in contrast to other studies in which in utero protein restriction was followed by a normal-protein diet after birth (32). Indeed, the volumes of individual glomeruli and renal corpuscles were significantly less in protein-restricted than in control rats. This combined deficit in nephron number (31%) and mean glomerular volume (24%) likely underlies the 60% difference in apparent GFR between protein-restricted and control rats with normal salt intake. It has been suggested that limitation of the kidney’s ability to excrete salt stimulates glomerular hypertrophy in the presence of nephron deficit. The fact that glomerular hypertrophy did not occur in life-long protein-restricted rats indicates that postnatal protein intake has a major impact on glomerular structure in the presence of nephron deficit.

Arterial pressure. To our knowledge, this is the first report that life-long protein restriction in rats leads to lower-than-normal arterial pressure. This observation contrasts with findings of previous studies of the effects of protein restriction in utero only, which is often followed by development of hypertension (29, 32, 33). Importantly, to our knowledge, there are no reports of reduced arterial pressure in adult rats after protein restriction in the prenatal period only. Thus it seems likely that the apparent blood pressure-lowering effect of life-long protein restriction is due largely to effects mediated during the postnatal period. The mechanisms that control arterial pressure in the long term remain a matter of controversy, but the pressure natriuresis mechanism appears to play a dominant role (9).

Life-long protein restriction appears to shift the pressure natriuresis relation toward lower MAP. The precise mechanisms underlying this phenomenon are a matter of speculation at this stage but could, potentially, involve changes in structure and/or function within the kidney itself or altered function of any of the many neural and hormonal mechanisms that modulate the pressure natriuresis mechanism (9). Furthermore, although there is evidence that chronic undernutrition in humans, in the absence of other cardiovascular risk factors, is associated with a lowering of adult arterial pressure, the relation between protein intake and arterial pressure remains a matter of controversy (26, 28). Our present data indicate that prenatal (15, 32) and postnatal protein exposure are vital in determining adult arterial pressure.

We could not detect effects of increased salt intake on arterial pressure in control or life-long, protein-restricted rats in our study. In contrast, Woods et al. (33) recently demonstrated salt-sensitive adult hypertension in rats exposed to a low-protein environment in utero only. They proposed that this effect was mediated through impairment of renal development. Our present results indicate that postnatal factors probably also come into play, although their nature remains to be elucidated. Also, our experimental paradigm of increased salt intake from weaning differs considerably from the subacute increases in salt intake used by Woods et al. to investigate the chronic pressure natriuresis relation and detect salt sensitivity of arterial pressure. Nevertheless, our paradigm is physiologically relevant, inasmuch as differences in protein and salt intake between human populations separated by geography and culture are likely to persist throughout life.

Renal function. Calculated GFR was lower in protein-restricted than in control rats, at least in the absence of increased salt intake. Similar observations have been made in adult rats in which protein was restricted prenatally, but not after birth (27, 32). Thus the impact of life-long protein restriction on baseline GFR in adults is likely due, in large part, to a prenatally mediated reduction in the number of nephrons.

We found that GFR determined by [3H]inulin clearance, normalized to body weight, was increased approximately four-fold in protein-restricted rats fed a high-salt diet. In contrast, calculated GFR was increased by only ~43% after a high salt intake in protein-replete rats. This remarkable apparent effect merits some discussion of the potential pitfalls of the methodology we employed. We can be confident that our observations are not an artifact secondary to differences in urine flow between the groups of rats, since special measures were taken to maximize urine recovery (see METHODS). Windfeld et al. (30) recently demonstrated that [3H]inulin degradation can lead to underestimation of calculated GFR. This may account for the relatively low calculated GFR in our study. However, this phenomenon is unlikely to have confounded our observation of increased GFR in rats fed a high-salt diet and, in particular, the marked effect of increased salt intake on GFR in protein-restricted rats, since the impact of [3H]inulin degradation would have been consistent across all groups. To validate our method for determining GFR, we directly compared our values of [3H]inulin clearance with creatinine clearance determined from the same samples. These data were positively correlated, with no fixed or proportional bias (see METHODS). Furthermore, calculated filtration fraction was relatively normal in the rats we studied, suggesting that GFR was not grossly underestimated. Nevertheless, the GFR measurements in our present study should probably be considered only as preliminary data and interpreted with caution. There is considerable controversy regarding the validity of methods for subacute and chronic measurement of GFR in rodents, particularly for methods using [3H]inulin clearance (30). Therefore, further studies are required to confirm or deny the hypothesis that protein restriction predisposes to hyperfiltration in response to increased salt intake. If our hypothesis is correct, it might represent a compensatory mechanism that allows excretion of the chronic salt load in the presence of a nephron deficit. It could also have important implications for much of the developing world, where populations with chronic undernutrition, perhaps associated with low salt intake, are now undergoing a rapid transition to a Western lifestyle, often associated with high salt intake. Indeed, this might partly explain the high prevalence of cardiovascular and renal disease in these transitional populations (2). Given the projected epidemic of cardiovascular disease in the developing world (24), this concept merits further investigation through epidemiological studies and the use of animal models.

When expressed in absolute terms, ERPF was lower in protein-restricted than in normal rats. However, when ex-
pressed relative to total kidney weight or body weight, ERPF was not significantly affected by protein restriction. There were no significant effects of salt intake on ERPF in normal or protein-restricted rats.

**Perspectives**

Our findings indicate that, in rats, life-long protein restriction is associated with lower MAP in adulthood. This observation in rats may mimic the human condition, where chronic undernutrition and protein deprivation appear to be associated with lower arterial pressure in those populations without other cardiovascular risk factors (18, 23). In contrast, transition to a protein-replete diet at birth following in utero protein restriction is often associated with the development of hypertension in adult rats (16, 21, 29, 32, 33). The effects of “catch-up” growth have also been the subject of a number of epidemiological studies. The balance of evidence favors the association of poorer health outcomes with more rapid childhood growth, which was previously seen as desirable for low-birth-weight infants (1). For example, Zhao et al. (34) found that, regardless of birth weight, an above-average weight in adolescence is associated with an increased risk of hypertension later in life. An important implication of our present results is the key role of the postnatal environment in determining the outcomes of developmental programming. If these findings are applicable to humans, they may provide approaches for prevention of renal disease and hypertension in adulthood in the offspring of women subjected to a low-protein diet during pregnancy.

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