The effect of a low-protein diet in pregnancy on offspring renal calcium handling

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Ashton N, Al-Wasil SH, Bond H, Berry JL, Denton J, Freemont AJ. The effect of a low-protein diet in pregnancy on offspring renal calcium handling. Am J Physiol Regul Integr Comp Physiol 293: R759–R765, 2007. First published June 13, 2007; doi:10.1152/ajpregu.00523.2006.—Low birth weight humans and rats exposed to a low-protein diet in utero have reduced bone mineral content. Renal calcium loss during the period of rapid skeletal growth is associated with bone loss. Because young rats exposed to low protein display altered renal function, we tested the hypothesis that renal calcium excretion is perturbed in this model. Pregnant Wistar rats were fed ischemic diets containing either 18% (control) or 9% (low) protein throughout gestation. Using standard renal clearance techniques, Western blotting for renal calcium transport proteins, and assays for Na+K+-ATPase activity and serum calcitropic hormones, we characterized calcium handling in 4-wk-old male offspring. Histomorphometric analyses of femurs revealed a reduction in trabecular bone mass in low-protein rats. Renal calcium (control vs. low protein: 10.4 ± 2.1 vs. 27.6 ± 4.5 nmol·min⁻¹·100 g body wt⁻¹; P < 0.01) and sodium excretion were increased, but glomerular filtration rate was reduced in low-protein animals. Total plasma calcium was reduced in low-protein rats (P < 0.01), but ionized calcium, serum calcitropic hormone concentrations, and total body calcium did not differ. There was no significant change in plasma membrane Ca²⁺-ATPase pump, epithelial calcium channel, or calbindin-D₂₈K expression in low-protein rat kidneys. However, Na⁺K⁺-ATPase activity was 36% lower (P < 0.05) in low-protein rats. These data suggest that the hypercalciuria of low-protein rats arises through a reduction in passive calcium reabsorption in the proximal tubule rather than active distal tubule uptake. This may contribute to the reduction in bone mass observed in this model.

bone; kidney; programming

THE CONCEPT THAT AN ADVERSE intrauterine environment may have a long-term effect on adult health and disease arose through observations made by Barker et al. (3, 4) that low birth weight infants were more likely to develop cardiovascular disease as adults. There is now a large body of evidence that supports the view that poor maternal nutrition during pregnancy programs the fetus to develop a range of chronic diseases in adulthood. Both epidemiological studies and animal models have shown that offspring exposed to a poor maternal diet in utero are at increased risk of developing cardiovascular disease, noninsulin-dependent diabetes, and metabolic syndrome (22). It has also become apparent that development of the skeleton and the risk of osteoporosis in later life are influenced by the maternal environment (13).

A number of epidemiological studies have reported associations between poor fetal growth and decreased bone mass in adulthood. Bone mineral content (BMC) at several sites, including the lumbar spine, femoral neck, forearm, and hip, has been shown to be positively correlated with birth weight in young women in their early 20s (11) and several cohorts of older men and women aged 60–75 yr (12, 20, 45). These observations have led to the suggestion that an adverse intrauterine environment programs bone growth and hence represents a risk factor for osteoporosis (13).

Animal studies support this hypothesis. Prenatal dexamethasone exposure, which is known to induce hypertension in rat offspring (5), has been reported to have a gender-specific effect on skeletal growth (41). In male offspring, dexamethasone induced a transient increase in tibia and femur length, whereas in females there was a reduction in cortical bone thickness in the femur and an increase in periosteal and endosteal thickness. Intrauterine growth retardation, induced by uterine vessel ligation, has also been shown to influence skeletal development. Axial skeletal development was affected most, with a permanent reduction in long bone width (35). Exposure to a low-protein (LP) diet during pregnancy, a maneuver most commonly associated with the development of hypertension in the offspring (27), has been reported to reduce bone area and BMC in late adulthood (34).

Bone mass in later life is dependent on the peak bone mass achieved during skeletal growth and the subsequent rate of bone loss (23). Several longitudinal studies have shown that bone mass tracks throughout life: individuals in the upper quartile of bone mass in childhood and adolescence are likely to remain in the upper quartile in old age (16, 32). Hence, changes in bone growth resulting in a reduction in peak bone mass increase the subsequent likelihood of developing osteoporosis. Peak bone mass is influenced by both genetic and environmental factors. During fetal development, calcium accrual and the proliferation and differentiation of chondrocytes are regulated by a number of factors, including parathyroid hormone (PTH)-related peptide (25) and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] (42).

Postnatally, bone mineral accrual is enhanced by dietary calcium supplementation (29). Conversely, increased renal calcium loss can lead to a reduction in bone mineral density.
Hypercalciuria is a common feature in postmenopausal bone loss (21) and is also associated with a reduction in bone mineral density in children (17). We have shown that, in the rat, exposure to an LP diet in utero alters renal function in the offspring (37, 38). However, the effect of maternal protein restriction on renal calcium excretion has not been reported. Because bone area and BMC have been shown to be reduced in old LP rats, (34), we hypothesized that calcium homeostasis may be perturbed in this model.

Renal calcium reabsorption occurs in both the proximal tubule, via a passive paracellular pathway, and the distal nephron, via a hormone-regulated transcellular route (18). The majority of calcium is reabsorbed in the proximal tubule and the thick ascending limb (TAL), with smaller quantities being reabsorbed in the distal tubule. Passive reabsorption is linked to sodium and water transport. Active calcium reabsorption is stimulated by PTH and 1,25(OH)2D3 (18). The principal transport proteins in the distal nephron are the apical epithelial calcium channel (ECaC1; TRPV5) (24), the intracellular binding protein calbindin-D28K (8), and the basolateral plasma membrane Cyt3+−ATPase pump (PMCA) (28).

In this study, we measured renal calcium excretion in young LP rats during the period of rapid growth. We used Western blots for semi-quantitative assessment of calcium transport proteins involved in active calcium reabsorption and measured the activity of the Na+−K+−ATPase pump, which provides the driving force for passive calcium reabsorption. To determine whether hormonal control of renal calcium excretion is affected by a maternal LP diet, we quantified serum calcitropic hormone concentrations in the offspring. Finally, we sought to confirm that exposure to a LP diet in utero affects bone architecture in young animals by performing histomorphometric analysis of the femur.

METHODS

All experiments were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and received local ethical approval.

**Animals.** Wistar rats (Harlan UK, Belton, UK) were paired in individual breeding cages and held in a room at 22–24°C with a 12:12-h light-dark cycle. As soon as we confirmed mating by the presence of a sperm plug, the female’s diet was switched from standard chow (rat & mouse standard diet; Bantin & Kingman, Hull, North Humberside, UK) to one that contained either 9% protein (LP group) or 18% protein (control group). The semi-synthetic diets were formulated as described previously (38). On the day of birth, the dam’s diet reverted to standard chow; pups were weaned onto this standard diet. Male animals were studied at 4 wk of age.

**Bone morphology.** The right femur was removed from 30 4-wk-old male animals (n = 15 control animals from 7 litters and n = 15 LP animals from 7 litters). The femora were fixed immediately in 4% neutral buffered formalin for 24 h. The bones were then calcified in formic acid under radiographic control. Once decalcification was complete, the bones were carefully bisected in the coronal plane. The halves were dehydrated, embedded in paraffin wax, and sectioned at 4 μm. Two step serial sections (tissue sections taken at intervals through the tissue) were taken from the surface of each of the two bone halves and at 10 μm below the surface. Analysis further from the coronal plane is not possible because the curvature of the bone, which grossly distorts relative bone volumes, cannot be reproducibly eliminated. The four sections from each animal were stained with hematoxylin and eosin and then examined with an Olympus BX60 microscope attached to a Leica QWin image analysis system. All tissue processing techniques lead to changes in the overall size of the bone but, with careful sectioning and mounting of the tissue sections, not in the relative proportions of the soft and hard tissues in sample cohorts. Thus the following parameters (expressed as ratios of total tissue volume) were measured: cortical bone volume, trabecular bone volume, and area of primary spongiosa. Cortical bone and trabecular bone were measured in the distal half of the shaft and excluded the spongiosa. The spongiosa was defined as the actively remodeling bone extending proximally from the cartilaginous growth plate.

**Renal clearance.** Anesthetized (Intraval, 100 mg/kg body wt, thiopentone sodium BP; Link Pharmaceuticals, Horsham, West Sussex, UK) control (n = 6 from 5 litters) and LP (n = 7 from 5 litters) rats were prepared for euvolemic fluid replacement of spontaneous urine output using a servo-controlled fluid replacement system, as described previously (1). After surgery, a bolus dose of [3H]inulin (0.3 μCi) was injected via a jugular vein cannula, and servo-infusion replacement of 2.5% dextrose was initiated at a rate-matching spontaneous urine output. [3H]inulin (0.3 μCi) in 2.5% dextrose was infused continuously via a second, slow infusion pump (1 ml/h). After a 2-h equilibration period, four 15-min urine collections were made. Blood (0.3 ml) was withdrawn via a carotid artery catheter at 30 min; a similar volume of saline and clearance markers was replaced via the jugular catheter. Arterial blood pressure was measured throughout the experiment via a carotid artery catheter (PowerLab 800s; ADInstruments, Hastings, East Sussex, UK). Urine and plasma sodium and potassium concentrations were measured by flame photometry (model 480; Ciba Corning Diagnostics, Essex, UK), total calcium by atomic absorption spectrophotometry (model 3100; Perkin Elmer, Beaconsfield, Bucks, UK), and ionized calcium by an autoanalyzer (Rapidlab 865 blood-gas analyzer; Bayer, Newbury, Berkshire, UK). [3H]Inulin was determined with a 1900CA Tri-Carb liquid scintillation analyzer (Canberra Industries, Meriden, CT) beta-counter. Plasma protein concentration was determined by the Bradford method (Bio-Rad assay reagent, Bio-Rad Laboratories, Hercules, CA).

**Total body calcium.** Total body calcium was measured in a separate group of rats killed at 4 wk of age (n = 6 control rats from 6 litters; n = 6 LP rats from 6 litters). Animals were first dried to a constant body weight at 70°C and were then ashed in porcelain crucibles at 700°C for 24 h (Carbolite Furnaces, Sheffield, South Yorkshire, UK). The ash was dissolved in nitric acid, and calcium content was measured by atomic absorption spectrophotometry (model 3100; Perkin Elmer).

**Western analysis of calcium transporters and Na+−H+−exchanger 3.** Kidneys were harvested from a separate group of control (n = 5 from 5 litters) and LP (n = 5 from 5 litters) rats. Tissue was homogenized, and renal calcium transporter expression was determined according to the method of Bond et al. (7). The antisera used were mouse monoclonal anti-calbindin-D28K (1:2,500 dilution; Sigma), mouse monoclonal anti-PMCA 5F10 (1:1,500 dilution; Cambridge Bioscience, Cambridge, UK), affinity-purified rabbit polyclonal anti-ECaC1 (1:1,000 dilution; Alpha Diagnostic International, San Antonio, TX), and polyclonal rabbit anti-NHE3 [Na+−H+−exchanger 3 (NHE3), 1:300 dilution; Alpha Diagnostic International]. Negative controls were performed by omitting primary antibody. Immunoreactive species were detected with sheep anti-mouse antibody (1:1,000; Amersham) or goat anti-rabbit antibody (1:2,000; Dako) using enhanced chemiluminescence detection (Amersham). A dissected adult kidney protein sample was run on each blot to allow normalization of density data between different blots.

**Renal Na+−K+−ATPase activity.** Tissue Na+−K+−ATPase enzyme activity was measured in freshly harvested kidneys as described previously (2). Briefly, rats (n = 6 control rats from 6 litters; n = 5 LP rats from 5 litters) were anesthetized with isoflurane and killed by decapitation. The kidneys were rapidly excised, placed into ice-cold homogenization buffer, sliced, and homogenized mechanically. Protein concentration was determined by the Bradford method (Bio-Rad assay reagent).
The homogenate was mixed 1:1 with SDS (0.75 mg/ml) and then mixed with incubation solution (150 mM histidine, 640 mM NaCl, 40 mM MgCl2, 200 mM KCl). Nonspecific and Na+-K+-ATPase-specific phosphate production were determined in the presence or absence of 10 mM ouabain, respectively. After a 10-min preincubation at 37°C, 30 mM ATP was added; 5 min later, the reaction was quenched with perchloric acid. Samples were centrifuged at 1,200 g for 15 min at 2°C, after which equal volumes of supernatant and distilled water were mixed with color reagent [1 g (NH4)6Mo7O24·4H2O, 94.7 ml H2O, 3.3 ml concentrated sulfuric acid, and 4 g FeSO4·7H2O]. Absorbance of the final solution was read at 690 nm on a microplate reader (Titertek Multiskan MC, Flow Laboratories Australasia, North Ryde, Australia) with a phosphate standard curve (10–250 nM KH2PO4). Total Na+-K+-ATPase activity (nM PO4·μg protein⁻¹·h⁻¹) was calculated as the difference between inorganic phosphate liberated in the presence and absence of ouabain.

**Serum calcitropic hormone assays.** Serum was collected from a separate group of control (n = 7 from 5 litters) and LP (n = 7 from 5 litters) rats. Serum PTH concentration was determined by ELISA using a commercial assay kit (Rat intact PTH ELISA kit; Immunodiagnostic Systems, Boldon, Tyne & Wear, UK). The intra-assay and interassay coefficients of variation for the 25(OH)D3 assay were 3.0% and 4.2%, respectively; those for the 1,25(OH)2D3 assay were 7.8% and 10.5%, respectively.

**Statistical analysis.** All data are presented as means ± SE. Statistical analysis was by two-tailed independent sample t-test; analysis of the urinary calcium-to-sodium excretion rate ratios, calcium clearance rates, and fractional excretion of calcium were performed after log10 transformation as these data were not normally distributed. A correlation correction was combined with a Sidak-Holm step-down procedure to control for the family-wise error rate (39). A two-tailed partial correlation coefficient was calculated for urinary calcium and sodium excretion, controlling for mean arterial blood pressure. Significance was assumed at P ≤ 0.05 (SPSS for Windows, version 11.5.0, SPSS UK, Surrey, UK).

**RESULTS**

**Bone morphology.** Figure 1A shows a typical coronal section through the lower femur of a control and an LP rat. Total bone volume was measured in three bone compartments (shaft cortex, shaft trabecular bone, and primary spongiosia), and comparisons were made between the two groups of animals. No significant difference was found in cortical bone volume between the groups. The volume of primary spongiosia was greater in LP than in control rats (P < 0.05; Fig. 1B). By comparison, trabecular bone mass in the lower femur was reduced in the LP rats (P < 0.05; Fig. 1C).

**Renal clearance.** LP rats were smaller (P < 0.01) than age-matched control rats and had higher mean arterial blood pressure (P < 0.01; Table 1), consistent with earlier reports (27, 38). The plasma concentration of total calcium (P < 0.01) was significantly lower in LP rats, but plasma ionized calcium, sodium, potassium, and protein did not differ from levels in control rats. Total body calcium did not differ between control and LP rats (Table 1).

Glomerular filtration rate was significantly lower (P < 0.01) in LP rats, but urine flow rate was comparable with that of control rats (Table 2). In contrast, urinary calcium (P < 0.01) and sodium excretion (P < 0.05) rates were increased significantly in LP rats (Table 2). The urinary calcium-to-sodium excretion rate ratio tended to be greater in LP rats, but this did
not reach statistical significance (0.016 ± 0.003 for control vs. 0.053 ± 0.018 for LP; P > 0.05). Partial correlation analysis, controlling for mean arterial blood pressure, revealed significant positive correlations between calcium and sodium excretion in both control (r = 0.808, P < 0.001) and LP rats (r = 0.5, P < 0.05). The clearance rate (P < 0.01) and fractional excretion (P < 0.001) of calcium were significantly higher in LP rats (Table 3). Similarly, for sodium, clearance (P < 0.05) and fractional excretion (P < 0.01) rates were higher in LP rats (Table 3). In contrast, the urinary excretion rate of potassium was significantly lower in LP rats (control [n = 6] vs. LP [n = 7]; 1.4 ± 0.2 vs. 0.8 ± 0.1 μmol·min⁻¹·100 g body wt⁻¹; P < 0.01), as was the potassium clearance rate (Table 3; P < 0.01). The fractional excretion of potassium did not differ between the two groups (Table 3).

**Renal calcium transporter expression.** The polyclonal anti-ECaC1 antibody detected a single protein band at 84 kDa in kidney membrane fractions. There was no difference in the relative density of this band between control and LP rats (P > 0.05; Fig. 2A). The monoclonal anti-calbindin-D₂₈K antibody detected a single protein band at 28 kDa in the renal post-nuclear supernatant. There was no difference in the relative density of this band between control and LP rats (P > 0.05; Fig. 2B). The monoclonal anti-PMAca antibody detected a broad protein band at ~140 kDa (~133 to 147 kDa), as well as a smaller band at 92 kDa, consistent with previous observations.

**Discussion**

This study has confirmed that calcium homeostasis is perturbed in young rats after exposure to a maternal LP diet. Although total body calcium content did not differ between control and LP rats, there is evidence that long bone structure was altered in the LP group. This was associated with a marked increase in renal calcium loss at a stage of development critical to the achievement of peak bone mass. The mechanisms underlying the observed changes in bone structure are not clear. However, it is likely that the high blood pressure of the LP animals contributed to the observed hypercalciuria and natriuresis through the pressure natriuresis process.

**Renal calcium excretion is closely linked to sodium excretion** (18); thus, pressure-related increases in urinary sodium excretion are associated with hypercalciuria (10). In the proximal tubule, calcium is reabsorbed via a paracellular route, as a passive process coupled to sodium and water transport. Hence, sodium and calcium are reabsorbed in parallel in this part of the nephron. Calcium is also reabsorbed by a passive process in the TAL; however, the driving forces differ from those of the proximal tubule. In the TAL, sodium uptake via the Na⁺-K⁺-2Cl⁻ cotransporter creates an electropositive luminal, which drives paracellular calcium transport. Active, transepithelial calcium transport is seen in the distal portion of the nephron. Here, calcium crosses the apical membrane via the

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### Table 1. Body weight, mean arterial blood pressure, plasma electrolyte concentrations, and total body calcium in anesthetized, 4-wk-old male rats exposed to a control or LP diet during pregnancy

<table>
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<th>Control (n = 6)</th>
<th>LP (n = 7)</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>114.5 ± 3.6</td>
<td>90.7 ± 5.2*</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>82 ± 6</td>
<td>111 ± 5*</td>
</tr>
<tr>
<td>Plasma total Ca²⁺, mmol/l</td>
<td>2.87 ± 0.21</td>
<td>2.09 ± 0.10*</td>
</tr>
<tr>
<td>Plasma total Na⁺, mmol/l</td>
<td>1.19 ± 0.03</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>Plasma Ca²⁺, mmol/l</td>
<td>142.3 ± 1.1</td>
<td>138.0 ± 1.4</td>
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<tr>
<td>Plasma K⁺, mmol/l</td>
<td>4.16 ± 0.20</td>
<td>4.54 ± 0.40</td>
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<tr>
<td>Plasma protein, mg/ml</td>
<td>5.6 ± 0.4</td>
<td>5.9 ± 0.3</td>
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<tr>
<td>Total body calcium, mmol/g ash</td>
<td>14.1 ± 1.2</td>
<td>10.7 ± 1.4</td>
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<tr>
<td>Total body calcium, mmol/g wet wt</td>
<td>0.36 ± 0.03</td>
<td>0.30 ± 0.05</td>
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Values are means ± SE. Control, normal maternal diet; LP, low-protein maternal diet. Statistical comparisons were by independent sample t-test, adjusted by correlation correction and Sidak-Holm stepdown procedure. *P < 0.01 vs. control vs. LP.

### Table 2. GFR, urine flow rate, and urinary calcium excretion and urinary sodium excretion in anesthetized, 4-wk-old male rats exposed to either a control or LP diet during pregnancy

<table>
<thead>
<tr>
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<th>Control (n = 6)</th>
<th>LP (n = 7)</th>
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<tbody>
<tr>
<td>GFR, ml·min⁻¹·100 g body wt⁻¹</td>
<td>1.3 ± 0.9</td>
<td>0.9 ± 0.1*</td>
</tr>
<tr>
<td>Urine flow rate, ml·min⁻¹·100 g body wt⁻¹</td>
<td>38.1 ± 6.5</td>
<td>53.8 ± 6.0</td>
</tr>
<tr>
<td>Urinary Ca²⁺ excretion, mmol·min⁻¹·100 g body wt⁻¹</td>
<td>10.4 ± 2.1</td>
<td>27.6 ± 4.5*</td>
</tr>
<tr>
<td>Urinary Na⁺ excretion, mmol·min⁻¹·100 g body wt⁻¹</td>
<td>0.76 ± 0.15</td>
<td>1.55 ± 0.28†</td>
</tr>
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</table>

Values are means ± SE over 1 h of 2.5% dextrose infusion at a rate matching spontaneous urine output. GFR, glomerular filtration rate. Statistical comparisons were by independent samples t-test, adjusted by correlation correction and Sidak-Holm stepdown procedure. *P < 0.01 and †P < 0.05, control vs. LP.

### Table 3. Renal clearance and fractional excretion of calcium, sodium, and potassium in anesthetized, 4-wk-old male rats exposed to either a control or LP diet during pregnancy

<table>
<thead>
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<th>Control (n = 6)</th>
<th>LP (n = 7)</th>
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<tbody>
<tr>
<td>Ca²⁺ clearance, μl·min⁻¹·100 g body wt⁻¹</td>
<td>7.7 ± 1.5</td>
<td>20.0 ± 3.0*</td>
</tr>
<tr>
<td>Ca²⁺ fractional excretion, %</td>
<td>0.6 ± 0.1</td>
<td>2.1 ± 0.4‡</td>
</tr>
<tr>
<td>Na⁺ clearance, μl·min⁻¹·100 g body wt⁻¹</td>
<td>5.3 ± 1.0</td>
<td>11.1 ± 2.0‡</td>
</tr>
<tr>
<td>Na⁺ fractional excretion, %</td>
<td>0.41 ± 0.07</td>
<td>1.32 ± 0.23‡</td>
</tr>
<tr>
<td>K⁺ clearance, μl·min⁻¹·100 g body wt⁻¹</td>
<td>384.6 ± 4.57</td>
<td>237.2 ± 23.9*</td>
</tr>
<tr>
<td>K⁺ fractional excretion, %</td>
<td>30.1 ± 3.0</td>
<td>26.7 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE over 1 h of 2.5% dextrose infusion at a rate matching spontaneous urine output. Statistical comparisons were by independent sample t-test, adjusted by correlation correction and Sidak-Holm stepdown procedure. *P < 0.01, ‡P < 0.05, and †P < 0.001, control vs. LP.
ECaC. It binds to the vitamin D-dependent intracellular binding protein calbindin-D_{28K} (8) and is extruded by a combination of the PMCA and a Na^{+}/Ca^{2+} exchanger, the former being the dominant transport system (15). PTH stimulates this process by hyperpolarizing the membrane voltage, activating ECaC, and enhancing Na^{+}/Ca^{2+} exchanger activity. In contrast to the proximal tubule, calcium and sodium reabsorption are inversely related in the distal nephron (18).

It is unlikely that the hypercalciuria observed in the LP rats in the present study was caused by a reduction in distal tubular calcium uptake. Although it is acknowledged that immunoblots are semi-quantitative at best, it appears that expression of the distal tubular calcium transport proteins ECaC1, calbindin-D_{28K}, and PMCA did not differ between control and LP rats. Furthermore, calcium and sodium reabsorption are inversely related in the distal nephron (18), yet LP rats exhibited both hypercalciuria and a natriuresis by comparison with control animals, suggesting that the reduction in calcium reabsorption is linked to sodium excretion. Passive calcium reabsorption in the TAL parallels that of sodium (19); thus the TAL could be the potential site of altered tubular function. This seems unlikely, however, as maternal protein restriction has been reported to increase Na\textsuperscript{+} reabsorption by the proximal tubule (30). Our observation that a change in protein expression, further reducing sodium reabsorption, will result in a parallel reduction in calcium reabsorption. We cannot be certain that this is entirely due to changes in proximal tubule function, as the Na^{+}/K^{+}-ATPase pump is expressed ubiquitously by the nephron and we measured pump activity in whole kidney homogenates. However, it seems to be the most likely explanation based on the available data.

A reduction in Na^{+}/K^{+}-ATPase pump activity is not restricted to the maternal LP model of fetal programming. Similar reductions have been reported in the kidneys of rats exposed to a high-fat diet in utero (2) and in the erythrocytes of young men with low birth weight (43). The mechanisms underlying the reduction in Na^{+}/K^{+}-ATPase activity in the LP rat kidney are unknown but may be related to their elevated blood pressure. Acute hypertension results in a reduction in proximal tubule Na^{+}/K^{+}-ATPase activity (47), leading to a pressure-related natriuresis and diuresis. Furthermore, chronic exposure to hypertension has also been shown to reduce Na^{+}/K^{+}-ATPase activity in spontaneously hypertensive rats (30). Interestingly, these authors also reported that brush-border NHE3 activity was reduced by internalization, without a change in protein expression, further reducing sodium reabsorption by the proximal tubule (30). Our observation that NHE3 expression in whole kidney homogenates did not differ between control and LP rats is consistent with this concept of transporter redistribution; however, further studies are required to quantify NHE3 activity in the LP rat kidney.

Redistribution of NHE3 and reduction in Na^{+}/K^{+}-ATPase activity in the adult spontaneously hypertensive rat are thought to contribute to a resetting of the pressure natriuresis set point.

Table 4. Serum PTH, 25(OH)D_{3}, and 1,25(OH)_{2}D_{3} concentrations in 4-wk-old male rats exposed to either control or LP diet during pregnancy

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>LP (n = 7)</th>
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<tbody>
<tr>
<td>PTH, pg/ml</td>
<td>125.8±23.8</td>
<td>121.9±24.8</td>
</tr>
<tr>
<td>25(OH)D_{3}, ng/ml</td>
<td>10.8±0.8</td>
<td>9.7±0.7</td>
</tr>
<tr>
<td>1,25(OH)<em>{2}D</em>{3}, pg/ml</td>
<td>107.4±10.5</td>
<td>98.6±5.6</td>
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</table>

Values are means ± SE. PTH, parathyroid hormone; 25(OH)D_{3}, 25-hydroxyvitamin D_{3}; 1,25(OH)_{2}D_{3}, 1,25-dihydroxyvitamin D_{3}. Statistical comparisons were by independent samples t-test, adjusted by correlation correction and Sidak-Holm step-down procedure.
loss of calcium. This, in turn, may be a consequence of the high 1,25(OH)2D3 acting on the calcium-sensing receptor rather than the plasma calcium concentration (9). However, serum 1,25(OH)2D3 did not differ between LP and control rats either. Further investigation of the expression and activity of the calcium-sensing receptor in LP rats is required before these anomalies can be resolved.

Clearly, calcium homeostasis is perturbed in the LP rat. Despite the maintenance of total body calcium, bone formation was adversely affected in the young LP rat. The structure and volume of bone being formed at the growth plate were altered, leading to a marked reduction in trabecular bone, one of the key structural elements of bone. The effects of maternal protein restriction on bone structure appear to persist throughout the offspring’s life, as BMC has been reported to be lower in aged LP rats than in controls (34). The consequences for the risk of developing osteoporosis or other diseases of bone associated with aging require further investigation.

It is important to note that many of the variables described in this study were corrected for body weight, as the LP animals were 20% lighter than the age-matched control group. Such an adjustment assumes that the animals differ only in size and that body composition does not differ, which may not be the case. It is difficult to take any potential difference into account without performing an exhaustive analysis of body composition, which is beyond the scope of the present investigation. Hence, some caution must be applied when interpreting the “corrected” variables.

In conclusion, this study has shown that renal calcium excretion is perturbed in rats exposed to protein restriction in utero. LP rats also had elevated blood pressure, which, given the known effects of raised arterial pressure on renal sodium excretion, is likely to have had a major influence on the combined hypercalcuria and natriuresis seen in these animals. Assessment of transporter expression and activity linked to calcium reabsorption in the proximal and distal tubules suggests that a reduction in Na+/K+-ATPase activity underlies the loss of calcium. This, in turn, may be a consequence of the high blood pressure exhibited by the LP rat, as hypertension is known to inhibit proximal tubule Na+/K+-ATPase activity (30). However, this defect in renal transport may not be the only mechanism contributing to altered calcium homeostasis, as there appears to be a mismatch between plasma total calcium concentration and serum calcitropic hormones. Bone structure, particularly at the growth plate, is also altered in young LP rats, lending further support to the suggestion that skeletal development is programmed in utero.

REFERENCES


