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The Bone Histology Spectrum in Experimental Renal Failure: Adverse Effects of Phosphate and Parathyroid Hormone Disturbances

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Abstract Bone disease is a common disorder of bone remodeling and mineral metabolism, which affects patients with chronic kidney disease. Minor changes in the serum level of a given mineral can trigger compensatory mechanisms, making it difficult to evaluate the role of mineral disturbances in isolation. The objective of this study was to determine the isolated effects that phosphate and parathyroid hormone (PTH) have on bone tissue in rats. Male Wistar rats were subjected to parathyroidectomy and 5/6 nephrectomy or were sham-operated. Rats were fed diets in which the phosphate content was low, normal, or high. Some rats received infusion of PTH at a physiological rate, some received infusion of PTH at a supraphysiological rate, and some received infusion of vehicle only. All nephrectomized rats developed moderate renal failure. High phosphate intake decreased bone volume, and this effect was more pronounced in animals with dietary phosphate overload that received PTH infusion at a physiological rate. Phosphate overload induced hyperphosphatemia, hypocalcemia, and changes in bone microarchitecture. PTH at a supraphysiological rate minimized the phosphate-induced osteopenia. These data indicate that the management of uremia requires proper control of dietary phosphate, together with PTH adjustment, in order to ensure adequate bone remodeling.

Keywords Bone histomorphometry · Chronic kidney disease · Parathyroid hormone · Phosphate · Renal osteodystrophy

In the living body, calcium (Ca) and phosphate (P) homeostasis is maintained through a close relation among renal function, intestinal absorption, and bone tissue. When renal function is impaired, there is a progressive retention of P and increase of fibroblast growth factor (FGF-23), with calcitriol deficiency and changes in parathyroid hormone (PTH). As a result, bone abnormalities are found almost universally in patients with chronic kidney disease (CKD).

Recently, the term "chronic kidney disease-mineral bone disorder" (CKD-MBD) was introduced to describe the interacting triad of biochemical abnormalities of mineral metabolism, extraskeletal calcification, and abnormal bone present in patients with CKD [1].

In the past 10 years, the understanding of the mechanisms involved in renal bone disease has evolved significantly. However, it remains difficult to assess, in vivo, the systemic effects that an isolated mineral disturbance resulting from the loss of renal function has on bone pathology since minimal changes in the serum concentration of a specific factor rapidly trigger compensatory mechanisms aimed at reestablishing normal concentrations.

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To determine the isolated effects of P and PTH on bone tissue in uremic rats, we designed an experimental study involving parathyroidectomy (PTx) with 5/6 nephrectomy (Nx), diets with varying P content, and PTH reposition.

Materials and Methods

Experimental Protocol

A total of 55 male Wistar rats, obtained from our local breeding colony, with initial weights of 280–330 g, were used in this study. Animals were housed in individual cages in a light-controlled environment (12/12-hour light/dark cycle) at a constant temperature (25°C) and humidity (25%).

Some animals were subjected to PTx and 5/6 Nx ($n = 32$), as described below, whereas others were sham-operated ($n = 23$). All animals were subjected to implantation of an osmotic minipump for the infusion of 1–34 rat PTH (Sigma-Aldrich, St. Louis, MO), delivered at a physiological rate (0.022 μg/100 g/hour) ($n = 16$) [2] or a supraphysiological rate (0.11 μg/100 g/hour) ($n = 16$), or vehicle (2% cysteine, Sigma-Aldrich) ($n = 23$). Immediately after Nx or sham Nx, animals were fed one of three types of rodent chow (Harlan-Teklad, Indianapolis, IN). The three diets were identical except in P content, which was 0.2%, 0.7%, and 1.2%, respectively, representing low, normal, and high contents (lo-P, norm-P, and hi-P diets, respectively). All diets were equal in their content of vitamin D, calcium (0.7%), protein (24%), and calories. A pair-feeding protocol was used. Seven subgroups were created:

- Sham + norm-P (sham-operated rats fed the norm-P diet and receiving infusion of vehicle only): $n = 8$
- Sham + lo-P (sham-operated rats fed the lo-P diet and receiving infusion of vehicle only): $n = 8$
- Sham + hi-P (sham-operated rats fed the hi-P diet and receiving infusion of vehicle only): $n = 7$
- Nx + lo-P + norm-PTH (nephrectomized rats fed the lo-P diet and receiving PTH infusion at a physiological rate): $n = 8$
- Nx + hi-P + norm-PTH (nephrectomized rats fed the hi-P diet and receiving PTH infusion at a physiological rate): $n = 8$
- Nx + lo-P + hi-PTH (nephrectomized rats fed the lo-P diet and receiving PTH infusion at a supraphysiological rate): $n = 9$
- Nx + hi-P + hi-PTH (nephrectomized rats fed the hi-P diet and receiving PTH infusion at a supraphysiological rate) $n = 7$

All experimental procedures were conducted in accordance with the guidelines of the Standing Committee on Animal Research of the University of São Paulo (CAPPesq 293/01).

Surgical Procedures

The PTx procedure involved microsurgical techniques using an electrocautery. The animals, including those that were sham-operated, were allowed to recover from surgery for 7 days. Those presenting a serum concentration of ionized calcium (iCa) < 0.9 mmol/L (considered an indicator of successful PTx) were anesthetized as before and subjected to 5/6 Nx, consisting of removal of the right kidney and infarction of approximately two-thirds of the left kidney. The sham-PTx animals were also anesthetized as before and then subjected to sham Nx.

PTH Infusion

Simultaneous to the Nx procedure, an Alzet model 2mL4 osmotic minipump (Alza, Palo Alto, CA) was implanted subcutaneously, providing continuous infusion of 1–34 rat PTH (Sigma-Aldrich) or vehicle (2% cysteine, Sigma-Aldrich). On post-Nx day 28, animals were given light ether anesthesia, and the osmotic minipump (pumping lifetime 28 days) was replaced with another minipump set to the same infusion rate.

Bone Marker Administration

A fluorochrome bone marker, oxytetracycline (Terramycin), was injected (25 mg/kg ip) on days 11 and 12 as well as on days 5 and 4 before death.

Serum Sample Collection

On day 56 (after 8 weeks of PTH infusion), animals were anesthetized and killed through aortic exsanguination. Serum samples were frozen at −20°C for later biochemical evaluation.

Biochemical Evaluation

On the day of Nx, whole blood was collected by retro-orbital puncture. A model AVL-9140 autoanalyzer (AVL Scientific, Roswell, GA) was used to measure iCa levels in this blood either immediately after collection or following death (in frozen samples). Serum creatinine was determined using a colorimetric assay (modified Heinegard-Tiderström). P was determined using a different colorimetric assay (Labtest, Lagoa Santa, Brazil). Rat PTH was measured using an immunoradiometric assay kit (Immutopics, San Clemente, CA).

Bone Histomorphometry

At the end of the experimental period, the left femur of each rat was removed, dissected free of soft tissue, immersed in 70% ethanol, and processed as previously described [3]. Using a Jung K microtome (Reichert-Jung, Heidelberg, Germany), distal femurs were cut into sections of 5 and 10 μm thickness.

The 5-μm sections were stained with 0.1% toluidine blue (pH 6.4), and at least two nonconsecutive sections were examined for each sample. Static, structural, and dynamic parameters of bone formation and resorption were measured at the distal metaphyses (magnification × 250), 195 μm from the epiphyseal growth plate, in a total of 30 fields, using a semiautomatic image analyzer (Osteomeasure; Osteometrics, Atlanta, GA).

Fluorochrome-based histomorphometric indices included mineralizing surface, mineral apposition rate, surface-referent bone formation rate (BFR/BS), and mineralization lag time (MLT). Mineralizing surface was calculated as the double-labeled surface plus one-half of the single-labeled surface. Mineral apposition rate was determined by calculating the distance between the two oxytetracycline labels, divided by the time interval between the two oxytetracycline administrations, and is expressed in micrometers per day. Static histomorphometric indices included the ratio of trabecular bone volume to total bone volume (BV/TV), fibrosis volume, and the ratio of osteoid volume to total bone volume (all expressed as percentages), as well as osteoid thickness, which is expressed in micrometers. Percentage of total trabecular surface is used to express areas of eroded surface and osteoid surface. Numbers of osteoblasts (osteoblast number) and osteoclasts per bone perimeter (osteoclast number, N.Oc/B.Pm) are expressed per millimeter. Trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) are expressed in micrometers. Trabecular number (Tb.N) is expressed per millimeter.

Histomorphometric indices are reported using nomenclature recommended by the American Society of Bone and Mineral Research [4]. All histomorphometric data were obtained through blinded measurements.

Statistical Analysis

Results are presented as mean ± standard error. Biochemical parameter comparisons among the groups were made using one-way analysis of variance (ANOVA) and Newman-Keuls post hoc test. Sham groups were compared using one-way ANOVA for all parameters. A two-way ANOVA was conducted in order to understand how bone histomorphometric parameters can be affected by the following two factors: P content in the diet and PTH infusion rate. In the case of a significant interaction of P content and PTH infusion, a Bonferroni posttest was performed. When no interaction was observed, data were rearranged and grouped according to P content in the diet and PTH infusion rate and Student's *t*-test analysis was used. GraphPad (San Diego, CA) Prism software, version 4.0, was employed. $P < 0.05$ was considered statistically significant.

Results

Biochemical Findings

Table 1 shows the results of the biochemical analyses on day 56. All Nx animals (Nx + lo-P + norm-PTH,

Table 1 Biochemical data

Group	Cr (mg/dL)	P (mg/dL)	iCa (mmol/L)	PTH (pg/mL)
Sham + norm-P ($n = 8$)	0.37 ± 0.02	6.0 ± 0.7[b]	1.16 ± 0.02	54.7 ± 16.9[e]
Sham + lo-P ($n = 8$)	0.31 ± 0.01	4.4 ± 0.4[b]	1.21 ± 0.05	10.5 ± 2.9[d]
Sham + hi-P ($n = 7$)	0.45 ± 0.03	4.9 ± 0.3[b]	1.18 ± 0.03	135.9 ± 28.6[f]
Nx + lo-P + norm-PTH ($n = 8$)	0.59 ± 0.03[a]	5.6 ± 0.5	1.18 ± 0.05	114.9 ± 30.1[e]
Nx + hi-P + norm-PTH ($n = 8$)	1.09 ± 0.14[a]	14.7 ± 2.4[c]	0.61 ± 0.05[c]	86.8 ± 20.3[e]
Nx + lo-P + hi-PTH ($n = 9$)	1.02 ± 0.10[a]	5.0 ± 0.5	1.44 ± 0.07[d]	249.6 ± 41.3
Nx + hi-P + hi-PTH ($n = 7$)	0.95 ± 0.15[a]	13.0 ± 1.6[c]	0.65 ± 0.09[c]	380.0 ± 66.7

Cr creatinine, *P* phosphate, *iCa* ionized calcium, *PTH* parathyroid hormone, *norm-PTH* physiological PTH, *hi-PTH* supraphysiological PTH, *Nx* nephrectomized rat, *Sham* sham-operated, *norm-P* control phosphate diet, *lo-P* low phosphate diet, *hi-P* high phosphorus diet

[a] $P < 0.05$ vs. all sham groups

[b] $P < 0.05$ vs. Nx + hi-P groups

[c] $P < 0.05$ vs. lo-P groups

[d] $P < 0.05$ vs. all groups

[e] $P < 0.05$ vs. hi-PTH groups

[f] $P < 0.05$ vs. Sham + norm-P group

Nx + hi-P + norm-PTH, Nx + lo-P + hi-PTH, Nx + hi-P + hi-PTH rats) developed moderate renal failure. However, Nx + hi-P + norm-PTH rats presented higher serum creatinine levels than did Nx + lo-P + norm-PTH rats. Nx + hi-P + norm-PTH and Nx + hi-P + hi-PTH rats also developed hypocalcemia and hyperphosphatemia, neither of which was observed in Nx + lo-P + norm-PTH and Nx + lo-P + hi-PTH rats. The Nx + lo-P + hi-PTH group presented higher Ca levels than the other groups. Continuous infusion of 1–34 rat PTH was effective in Nx animals, and hormonal serum levels were proportional to the concentrations infused. The suppressive effect on PTH secretion was evidenced by the fact that sham-operated animals that were fed the lo-P diet presented significantly lower serum PTH levels than did those that were fed the norm-P or hi-P diet.

Histomorphometric Findings

Table 2 shows the values obtained for the static and dynamic parameters of sham groups. Animals fed the hi-P diet presented lower BV/TV and Tb.Th than the other animals. Sham + lo-P animals, however, presented higher Tb.N and Tb.Th and lower Tb.Sp, with a BV/TV similar to Sham + norm-P animals.

In Table 3, the parameters where an interaction between P and PTH was found are shown. Posttest analysis disclosed that osteoid surface was influenced by PTH infusion, whereas osteoblast perimeter was influenced by both PTH infusion and P content in the diet.

Table 4 shows the parameters where the interaction between P and PTH was not found. Student's *t*-test analysis revealed that PTH infusion at a supraphysiological rate increased BV/TV, Tb.N, and Tb.Th and decreased Tb.Sp. An increase in resorption parameters (ES/BS, N.Oc/B.Pm) as well as in BFR/BS and MLT was also observed in this group. Fibrosis was seen only in the high-PTH groups (Nx + hi-P + hi-PTH 1.36 ± 0.62%, Nx + lo-P + hi-PTH 2.34 ± 0.83%; nonsignificant).

Regarding the P content in the diet, the high-P groups presented decreased BV/TV and Tb.N and increased Tb.Sp (Fig. 1).

Table 2 Static and dynamic histomorphometric variables of trabecular bone in the distal femur in sham animals

Parameter	Sham + norm-P ($n = 8$)	Sham + lo-P ($n = 8$)	Sham + hi-P ($n = 7$)
BV/TV (%)	22.7 ± 1.7	26.7 ± 1.7	16.7 ± 4.3*
Tb.Sp (μm)	236.2 ± 22.4	161.8 ± 9.3*	274.1 ± 38.1
Tb.N (mm^{-1})	3.4 ± 0.2	4.5 ± 0.1*	3.5 ± 0.3
Tb.Th (μm)	66.5 ± 1.7	58.9 ± 6.8*	47.7 ± 1.8*
O.Th (μm)	1.6 ± 0.2	1.7 ± 0.2	2.1 ± 0.09
OS/BS (%)	3.8 ± 0.6	5.4 ± 1.3	6.7 ± 1.9
N.Ob/B.Pm (mm^{-1})	1.6 ± 0.2	2.8 ± 0.4	3.2 ± 1.2
ES/BS (%)	8.8 ± 1.4	10.3 ± 1.4	11.8 ± 2.2
N.Oc/B.Pm (mm^{-1})	1.0 ± 0.2	1.3 ± 0.2	1.4 ± 0.2
BFR/BS ($\mu m^3/\mu m^2$/years)	0.02 ± 0.002	0.023 ± 0.005	0.02 ± 0.008
MLT (days)	2.5 ± 0.5	6.2 ± 1.8	6.4 ± 1.1

BV/TV bone volume, *Tb.Sp* trabecular separation, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *O.Th* osteoid thickness, *OS/BS* osteoid surface to bone surface, *N.Ob/B.Pm* number of osteoblasts per bone perimeter, *ES/BS* eroded surface to bone surface, *N.Oc/B.Pm* number of osteoclasts per bone perimeter, *BFR/BS* bone formation rate to bone surface, *MLT* mineralization lag time, *Sham* sham-operated, *norm-P* control phosphate diet, *lo-P* low phosphate diet, *hi-P* high phosphate diet

* $P < 0.05$ vs. all groups

Table 3 Comparison of bone histomorphometric parameters of Nx animals where an interaction between P and PTH was detected

Parameter	norm-PTH		hi-PTH		*P* (two-way ANOVA)		
	lo-P	hi-P	lo-P	hi-P	Interaction	[PTH]	[P]
O.Th (μm)	2.1 ± 0.2	3.2 ± 0.7	3.4 ± 0.3	2.7 ± 0.3	0.03	NS	NS
OS/BS (%)	11.1 ± 1.7	17.0 ± 4.4	44.7 ± 2.1	29.9 ± 4.5	0.004	<0.0001	NS
N.Ob/B.Pm (mm^{-1})	4.8 ± 1.1	5.8 ± 1.9	29.8 ± 1.7	16.7 ± 3.6	0.003	<0.0001	0.01

O.Th osteoid thickness, *OS/BS* osteoid surface to bone surface, *N.Ob/B.Pm* number of osteoblast per bone perimeter, *hi-P* high phosphate diet, *lo-P* low phosphate diet, *norm-PTH* physiological PTH, *hi-PTH* supraphysiological PTH

Table 4 Analysis of the isolated effects of PTH and P on bone histomorphometric parameters of Nx animals

Parameter	PTH			P		
	norm-PTH ($n = 16$)	hi-PTH ($n = 16$)	*P*	lo-P ($n = 17$)	hi-P ($n = 15$)	*P*
BV/TV (%)	20.7 ± 1.7	33.3 ± 2.6	*	32.4 ± 2.7	20.8 ± 1.7	**
Tb.Sp (μm)	241.1 ± 29.2	140.4 ± 12.9	*	143.3 ± 13.9	244.6 ± 30.1	**
Tb.N (mm^{-1})	3.7 ± 0.2	5.2 ± 0.4	*	5.2 ± 0.4	3.6 ± 0.2	**
Tb.Th (μm)	55.1 ± 1.8	63.4 ± 2.5	*	61.1 ± 2.4	57.1 ± 2.2	NS
ES/BS (%)	13.2 ± 1.4	24.0 ± 2.2	*	19.7 ± 1.9	17.4 ± 2.7	NS
N.Oc/B.Pm (mm^{-1})	1.5 ± 0.2	2.3 ± 0.2	*	2.1 ± 0.2	1.7 ± 0.2	NS
BFR/BS ($\mu m^3/\mu m^2$/year)	0.06 ± 0.01	2.3 ± 0.2	*	1.41 ± 0.3	0.99 ± 0.3	NS
MLT (days)	8.1 ± 2.6	51.6 ± 14.7	*	30.3 ± 12.5	24.8 ± 9.3	NS

BV/TV bone volume, *Tb.Sp* trabecular separation, *Tb.N* trabecular number, *Tb.Th* trabecular thickness, *ES/BS* eroded surface to bone surface, *N.Oc/B.Pm* number of osteoclast per bone perimeter, *BFR/BS* bone formation rate to bone surface, *MLT* mineralization lag time, *hi-P* high phosphate diet, *lo-P* low phosphate diet, *norm-PTH* physiological PTH, *hi-PTH* supraphysiological PTH

* $P < 0.05$ vs. norm-PTH; ** $P < 0.01$ vs. lo-P

Discussion

The results of our study show that rats with moderate chronic renal failure present a broad spectrum of histopathologic bone alterations in response to P overload or PTH levels. Our findings also profile the adverse effects that each of these mineral disturbances can have on bone tissue.

Nx animals fed with a high-P diet presented lower serum Ca levels than did the respective controls. This hypocalcemia might have resulted from P overload, which could have decreased intestinal absorption of Ca, probably due to the formation of insoluble complexes (containing Ca and P). Under normal conditions, higher P intake increases PTH production and the excess of PTH promotes phosphaturia [5], normalizing serum P, as was observed in our sham animals.

The major finding of our study was that the animals fed a high-P diet—whether sham-operated or Nx and regardless of the PTH infusion rate—presented a decrease in bone volume, with subsequent alterations in bone microarchitecture.

Over the years, the quantity of P in the diet of individuals in various countries has consistently increased, by approximately 17% per decade [6, 7]. This is due to the use of food preservatives. In the Framingham Offspring Study, women who consumed large quantities of cola-based soft drinks presented lower bone mineral density [8]. Similar results were obtained by McGartland et al. [9], who demonstrated that bone mineral density was lower in normal adolescents whose diets were high in P and that such adolescents were more prone to fractures. Recently, Kemi et al. [10], studying young healthy women on diets including different degrees of P supplementation, demonstrated that, in parallel with increases in P intake, PTH and bone resorption markers increase, whereas ionized Ca and bone formation markers decrease. These results suggest that high P intake promotes bone loss in normal individuals [11]. Approximately 60% of patients on dialysis present hyperphosphatemia [12]. In addition to the well-known adverse effects of hyperphosphatemia, it is likely that P overload reduces the bone mass of such patients [13], contributing to the high incidence of osteoporosis among patients on dialysis [13].

In a study involving normal animals given a high-P diet, Katsumata et al. [14] demonstrated an increase in bone resorption, as determined by assessing the urinary excretion of C-terminal telopeptide of type I collagen. In another study, Huttunen et al. [15] found that bone mineral density was lower in normal animals fed a diet containing 1.2% P for 8 weeks. In accordance with our results, both groups of authors reported increases in serum PTH.

Inorganic P is an important element in the formation of mineralized bone. Its importance in the formation of hydroxyapatite is clear, but the ability of inorganic P to affect cell function and gene expression as a signaling molecule is only beginning to be understood [16, 17]. Recently, Beck et al. [18] studied osteoblastic cells treated with different P concentrations and demonstrated that P decreased collagen type-Iα2 expression (the main component of extracellular matrix) and increased the expression of proteins involved in the differentiation of osteoblasts and mineralization of extracellular matrix. These authors suggest that the regulation of these genes may represent a signaling mechanism that osteoblasts have developed to recognize the end of matrix formation and the beginning of mineralization.

In an in vitro study, Meleti et al. [19] demonstrated that P overload causes osteoblast apoptosis and that high P concentrations decrease the viability of differentiated

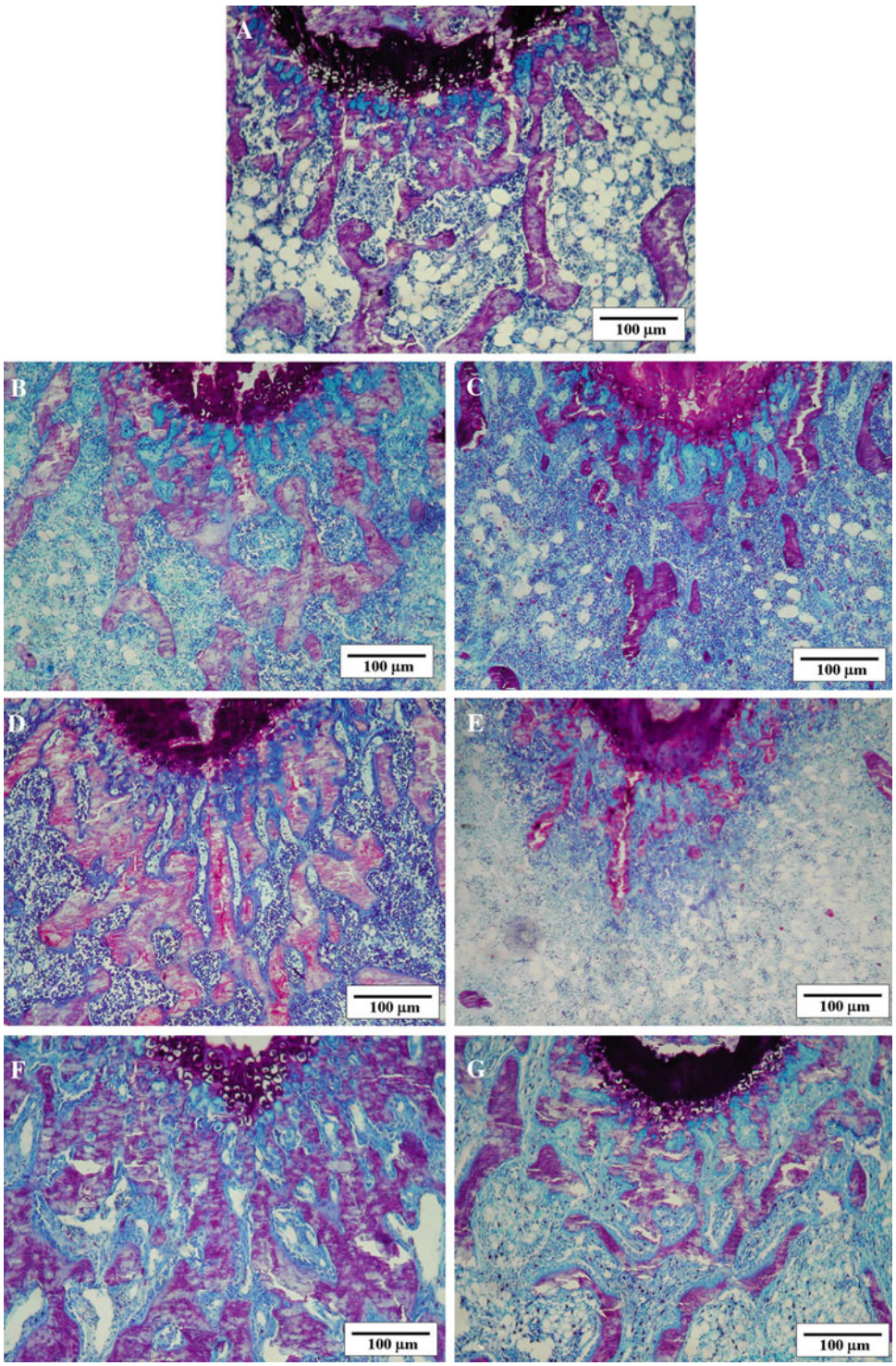

Fig. 1 Bone histologic images of animals of each group—Sham + norm-P (**a**), Sham + lo-P (**b**), Sham + hi-P (**c**), Nx + lo-P + norm-PTH (**d**), Nx + hi-P + norm-PTH (**e**), Nx + lo-P + hi-PTH (**f**), and Nx + hi-P + hi-PTH (**g**)—showing the decrease in trabecular volume of those animals with a normal PTH reposition and fed a high-P diet (magnification ×100)

osteoblasts in a time- and dose-dependent manner. In the same study, the authors successfully inhibited the apoptosis of osteoblasts using a Na–P cotransporter inhibitor, demonstrating that apoptosis took place only when P was able to enter the cell. Another group of authors, studying chondrocytes in vitro, showed that an elevated level of intracellular P is the primary indicator of chondrocyte apoptosis and that this effect depends on the extracellular concentration of calcium [20]. Similar to Meleti et al.'s findings [19], it was recently demonstrated that P overload was also able to induce human endothelial cell apoptosis through an increase in oxidative stress [21].

In the Nx animals evaluated in our study, the effects that P had on bone volume were more evident. Our results

clearly demonstrate that higher PTH concentrations are needed in order to preserve and maintain bone volume in patients with uremia. Various mechanisms have been implicated in the skeletal resistance to PTH seen in such patients: the lower number of PTH receptors in osteoblasts, the accumulation of 7–84 PTH fragments and of osteoprotegerin [22]. In the present study, the levels of serum P were higher than those reported in other studies. Such hyperphosphatemia has been shown to aggravate skeletal resistance to PTH [23]. We found that P overload was associated with hyperphosphatemia and hypocalcemia or, rather, that high PTH levels do not correct hypocalcemia. However, animals subjected to P restriction developed hypercalcemia. There are some hypotheses to explain this finding. Intestinal Ca absorption might have increased since P concentration in the intestinal lumen was lower, leading to a lower formation of Ca and P complexes [24]. Greater efflux of Ca from bone might also have occurred since skeletal resistance to PTH was lower due to the lower P load. The clinical relevance of this finding is that, in severe hyperparathyroidism, P restriction protects patients from P-related osteopenia. However, P restriction also leads to hypercalcemia, which is equally hazardous to the patient.

In the animals receiving PTH infusion at a supraphysiological rate, bone volume and bone microarchitecture were preserved. However, trabecular volume was lower in the Nx animals fed a diet rich in P than in those in which P was restricted. It is noteworthy that the Nx animals, regardless of P intake, presented bone mineralization defects, as evidenced by the fact that the accumulation of osteoid matrix and the MLT were greater than in the sham-operated animals (data not shown). These features were most accentuated in the Nx animals subjected to P restriction.

We must consider that, in our study, PTH reposition was continuous, which could have also contributed to the decrease in trabecular volume since PTH exerts a biphasic effect on osteoblastic cells. This effect depends on the concentration of PTH administered, on the duration of PTH exposure, and on the differentiation stage of these cells [25, 26]. Studies have shown that intermittent PTH administration increases the number and activity of osteoblasts and, therefore, increases bone formation. However, continuous PTH administration has a catabolic effect, increasing bone resorption rather than bone formation, thereby promoting bone loss [27]. Nevertheless, Lotinun et al. [28] showed that normal rats receiving continuous high PTH infusion (at a supraphysiological rate similar to that employed in our study) for 4 weeks presented a progressive increase in trabecular volume beginning on day 5 of infusion. The authors found that the animals developed a profile similar to hyperparathyroidism, with greater numbers of osteoblasts and osteoclasts, together with bone marrow fibrosis. In the same study, the authors concluded that high PTH increases the number of osteoblasts, recruiting lining cells and preosteoblasts as well as promoting the differentiation of bone marrow fibroblasts into osteoblasts [29].

In a previous study, we showed that rats without renal failure and subjected to continuous high PTH infusion present hyperparathyroidism, regardless of the P content of their diets, and that bone volume was greater in those rats than in those with CKD, confirming that uremia increases skeletal resistance to PTH [29].

Our study has several limitations. We did not evaluate the degree of acidosis, which has been proven to have a direct effect on bone. We did not measure the serum levels of calcitriol or FGF-23, which could be disturbed in those animals given a high-P diet. Therefore, we could not conclude if the observed effects of P on bone were direct or indirect or if they were exerted through the P effects on calcitriol and FGF-23. Despite these limitations, we could clearly demonstrate that P overload leads to a decrease in bone volume.

In conclusion, our results show that moderate renal failure accompanied by a diet high in P results in hyperphosphatemia, hypocalcemia, and changes in bone volume, all of which were minimized by the increase in serum PTH levels. The hyperparathyroidism protected the bone from the osteopenic effect of P. However, we know that hyperparathyroidism is also hazardous to the bone. These data indicate that the treatment of patients with uremia should include appropriate control of dietary P, together with PTH adjustment, in order to ensure adequate bone remodeling.

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References

1. Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G (2006) Kidney Disease: Improving Global Outcomes (KDIGO). Definition, evaluation and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 69:1945–1953
2. Berdud I, Martin-Malo A, Almaden Y, Aljama P, Rodriguez M, Felsenfeld AJ (1998) The PTH–calcium relationship during a range infused PTH doses in the parathyroidectomized rat. Calcif Tissue Int 62:457–461
3. Gouveia CH, Jorgetti V, Bianco AC (1997) Effects of thyroid hormone administration and estrogen deficiency on bone mass of female rats. J Bone Miner Res 12:2098–2107

4. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone Histomorphometry Nomenclature Committee. J Bone Miner Res 6:595–610
5. Berndt T, Kumar R (1992) The phosphatonins and the regulation of phosphate excretion. In: Seldin DW, Giebisch GH (eds) The kidney: physiology and pathophysiology. Raven Press, New York, pp 2511–2532
6. Calvo MS (1993) Dietary phosphorus, calcium metabolism, and bone. J Nutr 123:1627–1633
7. Takeda E, Sakamoto K, Yokota K, Shinohara M, Taketani Y, Morita K, Yamamoto H, Miyamoto K, Shibayama M (2002) Phosphorus supply per capita from food in Japan between 1960 and 1995. J Nutr Sci Vitaminol (Tokyo) 48:102–108
8. Tucker KL, Morita K, Qiao N, Hannan MT, Cupples LA, Kiel DP (2006) Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: the Framingham Osteoporosis Study. Am J Clin Nutr 84:936–942
9. McGartland C, Robson PJ, Murray L, Cran G, Savage MJ, Watkins D, Rooney M, Boreham C (2003) Carbonated soft drink consumption and bone mineral density in adolescence: the Northern Ireland Young Hearts Project. J Bone Miner Res 18:1563–1569
10. Kemi VE, Karkkainen MU, Lamberg-Allardt CJ (2006) High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females. Br J Nutr 96:545–552
11. Karaplis AC, Goltzman D (2000) PTH and PTHrP effects on the skeleton. Rev Endocr Metab Disord 1:331–341
12. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM (2004) Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol 15:2208–2218
13. Barreto FC, Barreto DV, Moyses RM, Neves CL, Jorgetti V, Draibe SA, Canziani ME, Carvalho AB (2006) Osteoporosis in hemodialysis patients revisited by bone histomorphometry. A new insight into an old problem. Kidney Int 69:1852–1857
14. Katsumata S, Matsuzaki H, Uehara M, Suzuki K (2006) Effects of lowering food intake by high phosphorus diet on parathyroid hormone actions and kidney mineral concentration in rats. Biosci Biotechnol Biochem 70:528–531
15. Huttunen MM, Pietila PE, Viljakainen HT, Lamberg-Allardt CJ (2005) Prolonged increase in dietary phosphate intake alters bone mineralization in adult male rats. J Nutr Biochem 17:479–484
16. Caverzasio J, Bonjour JP (1996) Characteristics and regulation of Pi transport in osteogenic cells for bone metabolism. Kidney Int 49:975–980
17. Kavanaugh MP, Kabat D (1996) Identification and characterization of a widely expressed phosphate transporter retrovirus receptor family. Kidney Int 49:959–963
18. Beck GR, Moran E, Knecht N (2003) Inorganic phosphate regulates multiple genes during osteoblast differentiation, including Nrf2. Exp Cell Res 288:288–300
19. Meleti Z, Shapiro IM, Adams CS (2000) Inorganic phosphate induces apoptosis of osteoblast-like cell in culture. Bone 27:359–366
20. Mansfield K, Teixeira CC, Adams CS, Shapiro IM (2001) Phosphate ions mediate chondrocyte apoptosis through a plasma membrane transporter mechanism. Bone 28:1–8
21. Di Marco GS, Hausberg M, Hillebrand U, Rustemeyer P, Wittkowski W, Lang D, Pavenstädt H (2008) Increased inorganic phosphate induces human endothelial cell apoptosis in vitro. Am J Physiol Renal Physiol 294:F1381–F1387
22. Iwasaki Y, Yamato H, Nii-Kono T, Fujieda A, Uchida M, Hosokawa A, Motojima M, Fukagawa M (2006) Insufficiency of PTH action on bone in uremia. Kidney Int Suppl 102:S34–S36
23. Bover J, Jara A, Trinidad P, Rodriguez M, Felsenfeld AJ (1999) Dynamics of skeletal resistance to parathyroid hormone in rat: effect of renal failure and dietary phosphorus. Bone 25:279–285
24. Brautbar N, Levine BS, Walling MW, Coburn JW (1981) Intestinal absorption of calcium: role of dietary phosphate and vitamin D. Am J Physiol 241:49–53
25. Schmitt CP, Hessing S, Oh J, Weber L, Ochlich P, Mehls O (2000) Intermittent administration of parathyroid hormone (1–37) improves growth and bone mineral density in uremic rats. Kidney Int 57:1484–1492
26. Locklin RM, Khosla S, Turner RT, Riggs BL (2003) Mediators of the biphasic responses of bone to intermittent and continuously administered parathyroid hormone. J Cell Biochem 89:180–190
27. Padagas J, Colloton M, Shalhoub V, Kostenuik P, Morony S, Munyakazi L, Guo M, Gianneschi D, Shatzen E, Geng Z, Tan HL, Dunstan C, Lacey D, Martin D (2006) The receptor activator of nuclear factor-κB ligand Inhibitor osteoprotegerin is a bone-protective agent in a rat model of chronic renal insufficiency and hyperparathyroidism. Calcif Tissue Int 78:35–44
28. Lotinun S, Sibonga JD, Turner RT (2005) Evidence that the cells responsible for marrow fibrosis in a rat model for hyperparathyroidism are preosteoblasts. Endocrinology 146:4074–4081
29. Neves KR, Graciolli FG, dos Reis LM, Graciolli RG, Neves CL, Magalhães AO, Custódio MR, Batista DG, Jorgetti V, Moysés RM (2007) Vascular calcification: contribution of parathyroid hormone in renal failure. Kidney Int 71:1262–1270