Successful Treatment of an Adynamic Bone Disorder with Bone Morphogenetic Protein-7 in a Renal Ablation Model

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Abstract. An adynamic bone disorder (ABD) is an important complication of chronic kidney disease (CKD) of unknown etiology for which there is no adequate treatment. Reported is an animal model of ablative CKD complicated by an ABD characterized by the absence of secondary hyperparathyroidism and its successful treatment with a skeletal anabolic factor, bone morphogenetic protein-7 (BMP-7). Adult mice were subjected to electrocautery of the right kidney followed by left nephrectomy. Animals were randomized into groups fed normal chow or fed low-phosphate chow supplemented with calcitriol to maintain normophosphatemia in CKD. All groups were maintained on the regimens for 12 wk. Hyperphosphatemia, secondary hyperparathyroidism, and a mild osteodystrophy developed in the CKD/chow-fed group, as expected. When dietary phosphorus was restricted and calcitriol was administered in the CKD low-phosphate/calcitriol group (ABD), Ca, PO₄, and parathyroid hormone levels were maintained normal. A significant ABD developed in the ABD group characterized by significant depressions in osteoblast number, perimeters, bone formation rates, and mineral apposition rates when compared with the sham-operated, chow-fed group. The abnormal skeletal histomorphometry was reversed by BMP-7 therapy to normal values and significantly improved from the ABD group (P < 0.05). The sham-operated low-phosphate/calcitriol–fed control group and the CKD low-phosphate/calcitriol/BMP-7 groups had reduced phosphate levels compared with the other groups (P < 0.05). ABD produced in mice with CKD in the absence of hyperparathyroidism was successfully reversed with a bone anabolic, BMP-7, associated with a reduction in plasma phosphorus.

Renal osteodystrophy is a term used to describe a heterogeneous spectrum of skeletal abnormalities found in patients with chronic kidney disease (CKD) and end-stage kidney disease (ESKD). Renal osteodystrophy ranges from states of high bone turnover to states of low bone turnover (1–3). Predominantly hyperparathyroid bone disease results in a high turnover osteodystrophy (osteitis fibrosa), and it is considered the most prevalent bone disorder in chronic kidney disease and ESKD. Low-turnover osteodystrophy reflects the lack of capacity to produce and mineralize bone matrix and has two main histologic forms: osteomalacia and the adynamic bone disorder (ABD). The ABD is an important complication of CKD associated with high rates of bone fractures (4), impaired growth (5,6), and probably vascular calcification (7,8). There has been an increase in the prevalence of ABD in patients with ESKD on dialysis (9).

The pathogenesis of the ABD is unknown. It may occur when parathyroid hormone (PTH) levels are aggressively suppressed (10). As a result, practice standards target maintaining elevated intact PTH levels (about three to five times normal) sufficient to prevent the ABD (11,12). Therapy of the ABD besides adjustment of PTH levels is not available. Because secondary hyperparathyroidism in CKD causes a distinct osteodystrophy, increasing PTH levels to increase bone remodeling cannot be considered adequate therapy for the ABD. The skeleton is resistant to the actions of PTH during CKD (13,14). Many potential mechanisms of skeletal resistance to PTH have been proposed (13,15). One, which has not been tested, is that CKD directly decreases skeletal anabolic activity. A decrease in skeletal anabolism induced by CKD would potentially produce abnormalities in phosphate homeostasis, leading to stimulation of secondary hyperparathyroidism even before sustained hyperphosphatemia developed in CKD (16). Because PTH is not a particularly good stimulant of osteoblastic function (17–21), high levels of the hormone would be required to maintain remodeling rates. These elevations in bone remodeling become dystrophic because of PTH inhibition of osteoblast collagen production (17,19) and stimulation of RANKL (22,23).

To test the hypothesis that CKD directly impairs bone remodeling in the absence of secondary hyperparathyroidism, we developed an ablative form of chronic kidney failure in the presence of normal Ca, Pi, and PTH levels. To maintain normal Ca and Pi levels to prevent PTH stimulation during kidney failure, we restricted dietary Pi and added calcitriol to (1) replace its deficiency; (2) stimulate Pi absorption; and (3)
Contribute to PTH inhibition. Because the murine species is sensitive to low Pi diet-induced osteomalacia, calcitriol was expected to prevent osteomalacia by maintaining normal Pi levels. The principle behind the approach of reducing dietary phosphorus has been effectively used in low-protein, low-PO₄ diet regimens of animals and humans in CKD and shown to prevent secondary hyperparathyroidism (24–26). The difficulty with the approach in humans is poor compliance to the dietary regimen. Lack of success with it in America and poor nutrition lead to its abandonment.

Our second hypothesis for the studies reported here was that a bone anabolic factor, bone morphogenetic protein-7 (BMP-7), useful in high turnover renal osteodystrophy (7,27), would be therapeutic in a model of the ABD. BMP-7 is an important developmental morphogen for the skeleton, kidney, and eye (28–30). BMP-7 is a regulator of osteoblast differentiation as well as having other biologic functions in the adult (7,31,32). The bone morphogenetic proteins were originally isolated from bone extracts that were capable of inducing endochondral bone formation when placed in mesenchymal derived sites (33).

In vitro, the BMPs have been demonstrated to stimulate the development of osteoblastic cells from undifferentiated precursors (34–36). BMPs signal through a transmembrane receptor complex consisting of a BMP type I and a type II receptor that have serine threonine kinase activity (31,37). The binding of ligand to the BMP receptor results in the phosphorylation of Smad proteins, translocation to the nucleus, and the transcriptional regulation of BMP responsive genes (31,38). BMP-7 is expressed in the adult kidney collecting duct. It is secreted into the bloodstream and tubular fluid, and it is excreted in the urine (39). Renal injury results in decreased BMP-7 production as shown in several models of acute and CKD (40–42). BMP-7 is an osteoblast growth and differentiation factor (43–45), and BMP-7–deficient mice have developmental defects of the skeleton, kidneys, and eyes and die shortly after birth (28–30) as a result of uremia. Thus, decreases in skeletal BMP influence could be a factor in deficient skeletal remodeling associated with CKD. The effects of an anabolic factor to reverse the ABD are supportive of the concept of skeletal anabolic deficiency produced by CKD.

Here we report an animal model of CKD causing an dynamic osteodystrophy associated with the absence of secondary hyperparathyroidism, and its successful treatment with BMP-7.

Materials and Methods

Male C57/BL6 mice were obtained from Harlan (Indianapolis, IN). BMP-7 was provided by Curis (Hopkinton, MA). Xylazine, ketamine, and calcein were obtained from Sigma-Aldrich (St. Louis, MO). The study protocol was approved by the Washington University Animal Care committee.

Induction of CKD and Treatment Protocol

Fifty 11-wk-old male C57/BL6 mice were allowed to acclimate in an animal facility for 1 wk. CKD was induced in 31 animals by the procedure previously described by Gagnon and Gallimore (46). Nine-teen animals underwent sham surgery. Stable, chronic renal injury is achieved after two surgical procedures. The mice were anesthetized by administering ketamine (150 mg/kg) and xylazine (7.4 mg/kg) intraperitoneally.

The first procedure involved electrocautery of the right kidney. A 2-cm right flank incision was made, and the right kidney was identified. The perirenal fat and adrenal gland were separated from the kidney by blunt dissection, and the kidney was exposed. The entire cortex of the right kidney was cauterized except for a 2-mm area around the hilum. The kidney was returned to the renal fossa, and the subcutaneous tissues were sutured with 3-0 silk. The skin was closed with surgical clips. The mice were fed regular mouse chow (Harlan Teklad, Madison, WI) and were allowed free access to water for 2 wk; they were then subjected to a left nephrectomy. The left kidney was exposed by the same procedure described above, the hilum was ligated with 6-0 silk, and the kidney was excised. The wound was closed as described above.

Sham surgery consisted of anesthetic, flank incision exposing the kidney, and closure of the abdominal wall. After the second surgical/sham procedure, 14-wk-old animals were randomized into five groups. The first comprised seven sham-operated mice fed regular mouse chow (0.6% phosphate, 0.8% calcium). This was the normal mouse control group. The second group comprised 11 CKD mice fed regular mouse chow. This group was expected to develop hyperphosphatemia and hyperparathyroidism and was the standard CKD control group. The third group comprised eight CKD mice fed low-phosphate chow (0.2% phosphate, 0.5% calcium) (Dyets, Bethlehem, PA) and given calcitriol (Abbott Laboratories, Abbott Park, IL) (20 ng/kg) three times a week subcutaneously. This group was expected to have normal Ca, Pi, and PTH levels. Because mice are susceptible to low Pi diet-induced osteomalacia, calcitriol was expected to contribute to maintenance of normal Pi by stimulating intestinal absorption, replacing calcitriol deficiency and contributing to inhibition of PTH secretion. Furthermore, the presence of kidney failure would diminish renal losses of Pi in the absence of increased PTH. The expected outcome was a normal serum PO₄. The fourth group comprised 12 CKD mice fed low-phosphate chow and treatment with calcitriol and BMP-7 (10 µg/kg intraperitoneally once a week). This was the therapy group. The fifth group comprised 12 sham-operated mice fed low-phosphate chow. This was the diet control group in which the presence of normal kidney function and renal Pi excretion would result in hypophosphatemia despite calcitriol therapy.

Thus, one can see that we utilized the effects of CKD to produce Pi retention to normalize and maintain serum Pi levels in our CKD low-phosphate/calcitriol group. The animals continued to receive free access to water for the duration of the study. All groups were maintained on their regimens for 12 wk until they were killed at 26 wk of age. The calcitriol dose of 20 ng/kg three times a week was chosen because this was the dose that significantly suppressed immunoreactive PTH (iPTH) levels (52.7 ± 10.2 pg/ml) to 25.7 ± 6.7 pg/ml) in a uremic rat model over 8 wk (47).

Measurements of PTH and Serum Chemistry

Blood samples were obtained at 4 and 8 wk of CKD by capillary tube aspiration of the saphenous vein, and with a different procedure (intracardiac puncture) at the time the animals were killed (12 wk CKD) and transferred to heparinized tubes. After centrifugation (400 × g for 5 min), plasma was removed, formed into aliquots, and frozen at −80°C. Intact PTH levels (performed only at the time the animals were killed because of the volume of blood required) were measured by two-site immunoradiometric assay by using a commercially avail-
able kit (Immutopics, San Clemente, CA). Blood urea nitrogen (BUN), serum calcium, and phosphorus were measured by using commercially available kits (Roche Diagnostics, Indianapolis, IN).

**Bone Histology and Histomorphometry**

Bone formation rate was determined 12 wk after nephrectomy by double fluorescence labeling. All mice received intraperitoneal calcein (20 mg/kg) 7 d before they were killed and intraperitoneal alizarin red (25 mg/kg) 2 d before they were killed. Both femurs were dissected at the time the animals were killed and placed in 70% ethanol. The specimens were implanted undecalcified in a plastic embedding kit H7000 (Energy Beam Sciences, Agawam, MA). Bones were sectioned longitudinally through the frontal plane in 5-μm sections with a JB-4 microtome (Energy Beam Sciences). Tissue was stained with Goldner trichrome stain for trabecular and cellular analysis. TRAP staining was used to identify osteoclasts and define osteoclast surfaces. Unstained 10-μm sections were used for calcein- and alizarin-labeled fluorescence analysis. Slides were examined at ×400 magnification with a Leitz microscope attached to an Osteomeasure Image Analyzer (Osteometrics, Atlanta, GA). Ten contiguous 0.0225-mm² fields of the distal femur, 150 μm proximal to the growth plate, were examined per animal. Primary, derived, and kinetic measures of bone remodeling were calculated and reported per the guidelines of the American Society of Bone and Mineral Research (48).

**Statistical Analyses**

Statistical analyses were performed by ANOVA. Differences between groups were assessed by post hoc by Dunnett’s multiple-range test and considered significant at P < 0.05. Data are expressed as mean ± SD.

## Results

### Evaluation of Renal Insufficiency

BUN levels were used to assess renal function. The renal function was stable over the first 8 wk of CKD. The level of CKD induced by electrocautery and nephrectomy could be regarded as mild to moderate because BUN levels were approximately twice normal. There was a proportional increase in the BUN levels between 8 and 12 wk in all animals receiving calcitriol. BUN levels were elevated equally in all of the CKD groups by calcitriol (Figure 1A). The levels in sham-operated low Pi/calcitriol–treated mice averaged 38.25 ± 12.91 mg/dl and in CKD mice, 73.98 ± 49.28 mg/dl (P < 0.05). BUN levels in animals with CKD treated with BMP-7 (n = 12) were not different from animals with CKD that had not received treatment (n = 19). The BUN results in the CKD chow group were, as expected, more than twofold elevated but stable from week 4 to 12. Because electrocautery removes kidney tissue fixing renal function, murine renal ablation, as compared with rodent, does not progress to end-stage CKD (49). Food consumption and weight gain were the same for each of the three CKD groups, which were less than the sham-operated groups (data not shown).

### Assessment of Parathyroid Response to CKD and Low-Phosphate/Calcitriol

As shown in Figure 1B, intact PTH levels in normal mice eating regular chow diet averaged 19 ± 11 pg/ml. Intact PTH levels were elevated only in the CKD chow-fed group, and these animals developed mild secondary hyperparathyroidism.

*Figure 1. Renal function and parathyroid hormone (PTH) levels in the groups of study animals. Blood urea nitrogen (BUN) was used as a marker of renal function. Values represent means in each group. Induction of chronic kidney disease (CKD) resulted in an increase in BUN from 20 mg/dl in the sham-operated groups to 50 mg/dl in the CKD groups. There was a late (after 8 wk) increase in BUN in the calcitriol-treated groups. This was observed in the sham-operated, low phosphorus–treated group to the same extent that it was in the CKD/low phosphorus/calcitriol–treated groups. A two-site intact PTH assay revealed an increase in PTH levels in the CKD chow-fed animals (P < 0.05). Values represent mean ± SD. PTH levels in all of the other groups were normal.*
with mean PTH levels of 45.0 ± 36.97 pg/ml. There were no differences in the levels of iPTH between the sham-operated animals fed a normal diet, the CKD animals on a low-phosphate diet provided calcitriol, the CKD animals on a low-phosphate diet plus calcitriol provided exogenous BMP-7 (adynamic plus BMP-7) and the sham-operated animals provided a low-phosphate diet and calcitriol (sham operated—low phosphate). (19.29 ± 11.28 pg/ml versus 24.50 ± 20.07 pg/ml versus 19.91 ± 11.47 pg/ml versus 17.5 ± 16.79 pg/ml, respectively) (P = NS).

Assessment of Calcium and Phosphate Metabolism

Figure 2 shows the trends of serum phosphorus, calcium, and the calcium phosphorus products in all of the study groups. All groups fed a low-phosphate diet to prevent hyperparathyroidism in CKD received calcitriol to avoid osteomalacia, to which murine strains are susceptible. Compared with the sham-operated animals fed a regular diet, CKD animals fed a regular diet developed significant hyperphosphatemia over the 12-wk period (8.07 ± 0.71 mg/dl versus 5.7 ± 1.17 mg/dl). As shown in Figure 2A, the hyperphosphatemia was prevented by a low-phosphate diet plus calcitriol in this renal ablation model, and even further reduced by the addition of BMP-7, 6.06 ± 0.66 mg/dl and 4.53 ± 1.55 mg/dl, respectively (P < 0.05). The phosphate levels in the CKD animals on a low-phosphate diet plus calcitriol receiving BMP-7 were the same as in the sham-operated animals given a low-phosphate diet plus calcitriol. As shown in figure 2B, there were no significant differences in the calcium levels at any time between the groups. The product of calcium and phosphate mirrored the phosphate results with the CKD group given a regular diet having the highest product, 72.33 ± 6.42 mg^2/dl^2, and the sham-operated group fed a low-phosphate diet the lowest product, 36.23 ± 13.17 mg^2/dl^2. The treatment with BMP-7 of the CKD ABD model decreased the product of calcium and phosphate from 53.04 ± 6.74 mg^2/dl^2 to 40.58 ± 14.08 mg^2/dl^2 (P < 0.05) (Figure 2C).

Effect of Treatment with BMP-7 on Bone Histology and Histomorphometry

Representative fields of distal metaphyseal femoral trabecular bone stained with Goldner trichrome stain in normal...
control animals, animals with CKD on a chow diet, animals with CKD/low-phosphate diet plus calcitriol, animals with CKD/low-phosphate diet plus calcitriol plus BMP-7, and normal animals on a low-phosphate diet with calcitriol, are shown in Figure 3. The CKD chow-fed animals developed a hyper-osteoidosis (Figure 3B), but they did not develop the other features of osteitis fibrosa. This is compatible with mild hyperparathyroid changes observed early in the course of renal osteodystrophy. The CKD/low phosphate/calcitriol group had very quiescent bone surfaces without osteoid or active osteoblast surfaces (Figure 3B). BMP-7 treatment restored active osteoblast surfaces in the CKD/low phosphate/calcitriol plus BMP-7 group (Figure 3D). The sham-operated (normal) animals fed a low-phosphate diet plus calcitriol had a tendency to increased osteoid surfaces.

There were no differences in bone volume, trabecular number, and trabecular separation between the various groups, despite a tendency for the adynamic group to be osteopenic (Figure 4). The animals with CKD fed regular chow had increased osteoid volume (Figure 4D). However, many of the findings of high turnover osteodystrophy were not observed in this group. For instance, in the CKD/chow group osteoblast numbers were normal (Figure 5), as were labeled surfaces (Figure 6) and bone formation rates (Figure 7). Thus, the histomorphometry was consistent with mild early hyperparathyroid induced changes. Animals with CKD on a low-phos-

Figure 3. Goldner trichrome stain of distal femur trabecular bone in normal control animals (A), animals with chronic kidney disease (CKD) on a chow diet (B), animals with CKD/low-phosphate diet plus calcitriol (C), animals with CKD/low-phosphate diet plus calcitriol plus bone morphogenetic protein-7 (BMP-7) (D), and normal animals on a low-phosphate diet with calcitriol (E) at an original magnification of ×400. CKD animals (B) demonstrated increased osteoid formation, which was absent in the CKD/low phosphate/calcitriol group (C). Osteoblast numbers were decreased in these animals (C). These histologic changes were prevented with BMP-7 treatment (D) through maintenance of active osteoblast surfaces and mineralization of the osteoid. Normal mice (sham operated) fed a low-phosphate diet and calcitriol developed early features of osteomalacia (E). Animals were 12 wk old at the time of induction of CKD and 26 wk old at the time they were killed.
phate diet and given calcitriol showed histologic features of ABD compared with the CKD group on a regular chow diet. These included normal osteoid volume (Figure 4D, yellow bar), decreased osteoblast number (Figure 5A), and perimeter (Figure 5B) without changes in osteoclast number and surfaces (Figure 5, C–E). Double-labeled surfaces were decreased in the mice with ABD (Figure 6B), as were mineralizing surfaces (Figure 6C). Bone formation rates and adjusted apposition rates were significantly diminished in the ABD (CKD/low phosphate/calcitriol) group (Figure 7).

Treatment with BMP-7 in the CKD animals with ABD resulted in a normalization of the osteoblast number and perimeter (Figure 5, A and B, blue bars), an increase in the double-labeled surfaces (Figure 6B), and a normalization in the bone formation and mineral apposition rates (Figure 7, A–C).

The sham-operated group fed a low-phosphate diet and given calcitriol had a few features of osteomalacia with a tendency to decreased bone volume and increased osteoid volume (Figure 4) and decreased osteoblast number (Figure 5A) but normal osteoblast perimeter (Figure 5B) and normal labeled surfaces (Figure 6, A and B). Bone formation rates were decreased, but mineral apposition rates, labeled surfaces, wall thickness, and activation frequency were normal (Figures 6C and 7D). Thus, in the normal animals, the osteomalacic effects of a low-phosphate diet were mostly, but incompletely, prevented by the calcitriol treatment.

Discussion

In the studies reported here, we developed a strategy to analyze skeletal modeling in a mouse model of ablative kidney failure when abnormalities in Ca, PO₄, and PTH homeostasis were avoided. The hypothesis was that in the absence of hypocalcemia, hyperphosphatemia, and elevated PTH levels, induction of kidney failure would produce abnormalities in skeletal modeling. The central focus of our strategy was to restrict PO₄ intake sufficient to maintain normal PO₄ blood levels in the presence of kidney failure. That prevention of hyperphosphatemia during kidney failure would prevent hypocalcemia and increased PTH secretion was established long ago (50,51). Application of the principle to human kidney disease has been limited by stringency of the diet and compliance to intake of PO₄ binders (52,53). In the rodent and murine species, sensitivity to the intestinal actions of calcitriol is shared with humans. So supplementation of early calcitriol deficiency would be expected to maximize intestinal PO₄ absorption in face of the low PO₄ diets. These approaches were proven successful in our study because calcium and phosphorus blood levels were maintained normal, and PTH levels were also maintained normal. The dose of calcitriol was pharmacologic, and thus direct suppression of PTH gene transcription (54,55) may have been an important component of maintaining normal PTH levels.

The skeletal outcome of our renal ablation model was severe reduction in osteoblast number, osteoblast-covered bone surfaces, bone formation rates, and mineral apposition rates. Although there was coordinate reduction in osteoclast number and osteoclast bone surfaces, these were much less than the reduction in osteoblast surfaces and insignificant, leaving an imbalance favoring bone resorption. The skeletal histomorphometry findings are those of the ABD associated with CKD.
The issue in our model is whether these findings were produced by kidney failure, calcitriol, or both. The exogenous calcitriol may have affected the histomorphometry in this study. The exact effects of calcitriol on bone are unclear. At low doses, vitamin D metabolites can prevent bone loss in models of osteopenia in rats by an antiresorptive effect, whereas at high doses they also stimulate osteoblast activity and show an anabolic effect (57). Bone histomorphometry in another in vivo study showed that 1,25(OH)2D3 alone exerts a potent proliferative effect on the osteoblasts but depresses their mineralizing capacity in a dose and time dependent manner (58). Studies have shown that vitamin D–deficient animals infused with calcium and phosphate had similar histomorphometry, bone growth, and mineralization compared with animals supplemented with vitamin D, and the mineralization defect is directly due to insufficient calcium and phosphorus in plasma at sites of mineralization (59,60). Recent studies have shown that even higher doses of calcitriol greater than were used here did not decrease bone formation in ovariectomized rats (61). In transgenic mice deficient in the 1α-hydroxylase gene (CYP27B1), a marked decrease in skeletal modeling and growth is observed corrected with a rescue diet that normalizes the serum calcium. Pharmacologic replacement of calcitriol reverses the skeletal phenotype by increasing osteoblastic activity (62). The authors conclude that there is little evidence that vitamin D plays a direct role in new bone formation, apart from its action to maintain calcium and phosphorus levels in the blood (62). Studies performed with azotemic rats suggest that 24,25(OH)2D3 promotes the maturation and mineralization of osteoid (bone formation exceeds osteoid formation), and that this metabolite has minimal effect on bone resorption (58,63).

In humans with ESKD, suppression of PTH by calcitriol administration is a common means of uncovering the ABD. However, it has not been proven that calcitriol directly decreases bone formation rates or osteoblast numbers (10,11,64). A previous study (65) has suggested that calcitriol dose-dependently exhibited inhibitory effects on osteoblastic activity. Thus, although we are unable to rule out an effect of calcitriol in producing the ABD in our animal model, the dose and the actions of 1,25(OH)2D3 in previous studies (57,58,61) suggest that it may not have been the cause of the ABD we observed.

The ABD has been produced in animals, and most theories of the ABD cite oversuppression of PTH secretion as the
ultimate cause (5,56,66,67). We have suggested that CKD decreases skeletal anabolic factors, stimulates inhibitors of osteoblast function, or both (1,2,68). In this setting, the development of hyperparathyroidism can be viewed as a failed adaptive attempt to maintain skeletal remodeling or modeling. The ABD is certainly more common when PTH levels are actively suppressed with high calcium dialysate and calcitriol (especially intravenous) therapy (64,66,67). Uremic bone is resistant to the actions of PTH (13), defined as release of calcium from the skeleton after a dose of PTH, and a certain amount of hyperparathyroidism is required to maintain bone turnover. However, PTH is a poor director of osteoblast differentiation \textit{in vitro} (17,21,69), and excess PTH ultimately leads to accumulation of fibrous cells in trabecular bone (21) and elevated rates of bone formation but excess bone resorption (\textit{i.e.} high turnover renal osteodystrophy). Thus, in CKD and ESKD, PTH is not a satisfactory anabolic factor.

The studies reported here are the first to demonstrate reversal of the ABD with a physiologic bone anabolic factor. BMI-7 therapy added bone anabolism to high turnover renal osteodystrophy secondary to excess PTH and eliminated peritrabecular fibrosis (27).

In our study, the group of animals with the highest values of serum phosphate and calcium phosphorus product was the animals with CKD fed a regular diet and with histomorphometric features and iPTH levels consistent with early mild hyperparathyroidism. The CKD animals with ABD (low-phosphate diet and calcitriol) had normal serum Pi levels that were significantly higher than those of sham-operated animals on a low-phosphate diet and calcitriol, consistent with the effect of renal ablation on phosphate homeostasis. Surprisingly, BMI-7 treatment in the CKD ABD model resulted in a reduction of phosphate levels to those of the sham-operated mice with normal renal function.

Thus, we have shown that BMI-7 therapy resulted in a decrease in both phosphate levels and the calcium phosphorus product associated with the resolution of the ABD. The hypophosphatemic action of BMI-7 was most likely the result of an increase in skeletal phosphate deposition associated with increased mineral apposition because increased renal excretion of phosphate was not demonstrated and preliminary studies demonstrate no effect of BMI-7 on renal tubular phosphate excretion.
transport (Hruska and Lederer, unpublished observations). Therefore, we favor the interpretation that increased bone formation in the BMP-7–treated animals lead to a reduction in serum phosphate compared with the untreated CKD/low phosphate/calcitriol group. In summary, in this murine renal ablation model of CKD, maintenance of normal Ca, PO₄, and PTH levels was associated with an ABD that was for the first time successfully treated with a bone anabolic factor.

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**References**


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