Low Turnover Osteodystrophy and Vascular Calcification Are Amenable to Skeletal Anabolism in an Animal Model of Chronic Kidney Disease and the Metabolic Syndrome

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LDL receptor (LDLR)–null mice fed high-fat/cholesterol diets, a model of the metabolic syndrome, have vascular calcification (VC) worsened by chronic kidney disease (CKD) and ameliorated by bone morphogenetic protein-7 (BMP-7), an efficacious agent in treating animal models of renal osteodystrophy. Here, LDLR−/− high-fat–fed mice without CKD were shown to have significant reductions in bone formation rates, associated with increased VC and hyperphosphatemia. Superimposing CKD resulted in a low turnover osteodystrophy, whereas VC worsened and hyperphosphatemia persisted. BMP-7 treatment corrected the hyperphosphatemia, corrected the osteodystrophy, and prevented VC, compatible with skeletal phosphate deposition leading to reduced plasma phosphate and removal of a major stimulus to VC. A pathologic link between abnormal bone mineralization and VC through the serum phosphorus was supported by the partial effectiveness of directly reducing the serum phosphate by a phosphate binder that had no skeletal action. Thus, in this model of the metabolic syndrome with CKD, a reduction in bone-forming potential of osteogenic cells leads to low bone turnover rates, producing hyperphosphatemia and VC, processes ameliorated by the skeletal anabolic agent BMP-7, in part through deposition of phosphate and increased bone formation.

Cardiovascular (CV) mortality in patients with chronic kidney disease (CKD) is extremely high (1,2). Conventional risk factors that are characteristic of the metabolic syndrome (3), such as hypertension, dyslipidemia, insulin resistance, and overt diabetes, are highly prevalent in CKD, but other CV risk factors with additive affects that are more specific to the uremic milieu have also been identified (4). One such is the presence of vascular calcification (VC) (5), a form of heterotopic mineralization that is predictive of CV mortality (6,7) and is both common and severe in CKD (8).

The pathogenesis of VC in CKD remains under investigation, but hyperphosphatemia is an important risk factor for both VC and CV mortality (9,10). VC and CV mortality are also associated with other abnormalities of calcium and phosphate homeostasis, including hypercalcemia, elevated calcium and phosphate ion products, vitamin D therapy, and hyperparathyroidism (9,10), and these findings suggest a link with renal osteodystrophy (ROD).

ROD is virtually ubiquitous in CKD, characterized by a spectrum of histologic abnormalities of bone that contribute to the biochemical abnormalities discussed above (11). At one end of the spectrum, osteitis fibrosa is a high-turnover state driven by secondary hyperparathyroidism, characterized by poorly differentiated osteoblast precursors manifesting a fibroblastic phenotype, and stimulating increased osteoclastic activity. This results in net bone resorption, fibrosis of the bone marrow space, and release of calcium and phosphate into the extracellular fluid (12). At the other end of the spectrum, adynamic bone disorder (ABD) is characterized by quiescent osteoblasts and osteoclasts with markedly reduced bone turnover. In this situation, reduction of the rapidly diffusible ion pool sizes of calcium and phosphorus associated with skeletal mineralization fronts reduces the body’s ability to buffer extracellular calcium and phosphate ion concentrations, causing increased postprandial fluctuations as a result (13). Thus, both forms of ROD cause abnormalities in calcium and phosphate homeostasis that are associated with VC, and it is possible to hypothesize a causative pathologic link among ROD, VC, and mortality in CKD.

We have developed an animal model of VC worsened by CKD (14). The model is partial renal ablation in the LDL receptor-deficient (LDLR−/−) mouse that is fed high-fat/cholesterol diets. This model resembles the clinical situation of CKD complicating the metabolic syndrome as the mice have obesity, hypertension, insulin resistance, and early type 2 diabetes. In these mice, CKD caused intensification of VC, which was prevented by treatment with bone morphogenetic protein-7 (BMP-7) (14).

In this study, we aimed to examine the mechanisms of VC by characterizing its relationship to ROD in CKD. We found that CKD induced low-turnover osteodystrophy in the LDL−/−
model despite the presence of hyperparathyroidism. Furthermore, CKD intensified hyperphosphatemia, which was already present in the high-fat/cholesterol–fed mice. Treatment with BMP-7 reversed the osteodystrophy, corrected the hyperphosphatemia, and prevented the VC. The role of hyperphosphatemia in the VC was investigated by using calcium carbonate as a dietary supplement to control the hyperphosphatemia. Calcium carbonate corrected the hyperphosphatemia, had no effect on the osteodystrophy, and reduced the VC. Thus, a basic mechanism of VC may reside in the control of the serum phosphate.

Materials and Methods

Animals and Diets

LDLR−/− mice of both genders in a C57/BL6 background were obtained from our colony previously reported (14). Additional C57/B6 and LDLR−/− mice were obtained from Jackson Laboratory (Bar Harbor, ME). BMP-7 was provided by Curis, Inc. (Hopkinton, MA). Xylazine, ketamine, and calcein were obtained from Sigma-Aldrich Company (St. Louis, MO). The study protocol was approved by the Washington University Animal Care committee. Mice were weaned at 3 wk to a standard diet. At 10 wk, mice either continued the standard diet or started a high-cholesterol (0.15%) diet that contained 42% calories as fat (Product No. TD88137; Harlan Teklad, Madison, WI). Mice had access to water ad libitum. Two additional groups were switched from the high-fat/cholesterol diet to the high-fat/cholesterol diet supplemented with 1% wt/wt CaCO3 after induction of CKD at 14 wk.

Induction of CKD and Treatment Protocol

LDLR−/− mice were fed standard diets from weaning until 10 wk of age, at which time some mice were placed on the high-fat/cholesterol diet that provided excess calories as fat in a high-cholesterol background. At 12 wk, CKD was induced in some mice by the procedure previously described by Gagnon and Gallimore (15) and previously reported by us (12–14). Stable, chronic renal insufficiency was achieved after two surgical procedures. Partial ablation by electrocautery of the right kidney was followed in 2 wk by a left nephrectomy. Some mice underwent sham surgery. After the surgical procedures, 14-wk-old mice were randomized into seven groups. The first was sham-operated LDLR−/− mice that were fed regular mouse diet (0.6% phosphate, 0.8% calcium; n = 7). This was the normal renal function and diet control group. The second group was sham-operated LDLR−/− mice that were fed the high-fat/cholesterol diet (n = 11). This group was expected to develop hypercholesterolemia, dyslipidemia, and atherosclerosis and was the study group for the effect of diet alone. The third group was sham-operated LDLR−/− mice that were fed the high-fat/cholesterol diet and treated with BMP-7 (10 mEq/kg intraperitoneally once a week; n = 7). This group was the control for the effect of BMP-7 in the presence of normal renal function. The fourth group was CKD mice that were fed high-fat/cholesterol diet and treated with the vehicle from weeks 14 to 28 (n = 8). This group was expected to develop elevated phosphorus levels and hyperparathyroidism. It was the untreated CKD group expected to develop VC and ROD. The fifth group was CKD mice that were fed high-fat/cholesterol diet and treated with BMP-7 (10 µg/kg intraperitoneally once a week; n = 12). This was the first therapy group. The sixth group was CKD mice that were fed high-fat/cholesterol diet supplemented with CaCO3. This was the second therapy group designed to treat hyperphosphatemia. The seventh group was CKD mice that were fed high-fat/cholesterol diet supplemented with CaCO3 and treated with BMP-7. This was the third therapy group designed to analyze combination of the two therapies. The mice continued to receive free access to water for the duration of the study. All groups were maintained on their regimens for 14 wk until they were killed (28 wks of age). The BMP-7 dose was

Table 1. BUN (ng/dl) and glucose levels and weights (g) in the various groupsa

<table>
<thead>
<tr>
<th>Groups (No.)</th>
<th>N</th>
<th>BUN (mg/dl) 14 Wk</th>
<th>BUN (mg/dl) 28 Wk</th>
<th>Glucose (mg/dl) 14 Wk</th>
<th>Glucose (mg/dl) 28 Wk</th>
<th>Weights (g) 14 Wk</th>
<th>Weights (g) 28 Wk</th>
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</thead>
<tbody>
<tr>
<td>Sham Chow (1)</td>
<td>8</td>
<td>29</td>
<td>34 ± 17</td>
<td>N/A</td>
<td>206</td>
<td>22.3</td>
<td>22.8</td>
</tr>
<tr>
<td>Sham Fat (2)</td>
<td>10</td>
<td>26</td>
<td>35</td>
<td>N/A</td>
<td>193</td>
<td>19.6</td>
<td>23.8</td>
</tr>
<tr>
<td>Sham Fat BMP-7 (3)</td>
<td>7</td>
<td>29</td>
<td>42</td>
<td>N/A</td>
<td>215</td>
<td>20.6</td>
<td>21.4</td>
</tr>
<tr>
<td>CKD Fat (4)</td>
<td>10</td>
<td>82</td>
<td>80</td>
<td>N/A</td>
<td>187</td>
<td>21.1</td>
<td>22.0</td>
</tr>
<tr>
<td>CKD Fat BMP-7 (5)</td>
<td>11</td>
<td>98</td>
<td>105</td>
<td>N/A</td>
<td>179</td>
<td>22.0</td>
<td>21.2</td>
</tr>
<tr>
<td>CKD Fat/Cal (6)</td>
<td>8</td>
<td>85</td>
<td>94</td>
<td>N/A</td>
<td>239</td>
<td>20.8</td>
<td>22.1</td>
</tr>
<tr>
<td>CKD Fat/Cal BMP-7 (7)</td>
<td>10</td>
<td>132</td>
<td>148</td>
<td>N/A</td>
<td>213</td>
<td>20.4</td>
<td>19.6</td>
</tr>
</tbody>
</table>

aBUN, blood urea nitrogen.
chosen as the low dose that was previously shown to prevent VC in these mice (14).

**Measurements of Serum Chemistries and Parathyroid Hormone**

Blood samples were obtained at 4 wk of CKD by capillary tube aspiration of the saphenous vein and with a different procedure (intracardiac puncture) at the time of killing (14 wk of CKD) and transferred to heparinized tubes. After centrifugation (400 \( \times \) g for 5 min), plasma was removed, aliquotted, and frozen at \(-80^\circ\)C. Blood urea nitrogen (BUN), serum cholesterol, glucose, calcium, and phosphorus were measured using commercially available kits (Roche Diagnostics, Indianapolis, IN). Creatinine was measured by autoanalyzer techniques in the animal facility clinical laboratory. Intact parathyroid hormone (iPTH) levels (performed only at killing because of the volume of blood required) were measured by two-site immunoradiometric assay using a commercially available kit (Immunotopics, San Clemente, CA).

**Chemical Calcification Quantification of Aortic Tissue and Histologic Analysis**

Calcium was eluted from desiccated, crushed tissue in 10% formic acid (10:1 vol/wt) for 24 h at 4°C. Assay of eluate calcium content used a Cresolphthalein complexone method (Calcium Kit; Sigma), according to the manufacturer’s instructions, and values were corrected for dry tissue weight (16).

For histology, we fixed resected specimens in formalin and then divided as previously reported (14). The heart and proximal aorta up to and including the arch were separated from the descending aorta and bisected sagittally through the aortic outflow tract. Five-micrometer-thick slices were stained with Alizarin Red and von Kossa.

**Bone Histology and Histomorphometry**

Bone formation rate was determined 14 wk after nephrectomy by double-fluorescence labeling of mineralizing surfaces. All mice received intraperitoneal Calcein (20 mg/kg) 7 and 2 d before being killed. Both femurs were dissected at the time of killing 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-\(\mu\)m sections with a JB-4 Microtome (Energy Beam Sciences). Tissue was stained with Goldner’s trichrome stain for trabecular and cellular analysis. TRAP staining was used to identify osteoclasts and define osteoclast surfaces. Giemsa and oil Red O stains were used in the determinations of fat volume. Unstained 10-\(\mu\) sections were used for Calcein labeled fluorescence analysis. Slides were examined at \(\times 400\) magnification using a Leitz microscope attached to an Osteomasure Image Analyzer (Osteometrics, Atlanta, GA). Ten contiguous 0.0225-mm\(^2\) fields of the distal femur, 150 \(\mu\)m 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 (17).

**Statistical Analyses**

Statistical analyses were performed using ANOVA. Differences between groups were assessed post hoc using Dunnett multiple range test and considered significant at \(P < 0.05\). Data are expressed as mean \(\pm\) SD.

**Results**

**Evaluation of Renal Insufficiency**

As expected, mean serum creatinine levels were at the lower limit of detection between 0.1 and 0.3 mg/dl in all of the groups.
at 28 wk (Figure 1) and at 14 wk. Because the SD of the determination is ±0.2 mg/dl, there were no significant differences in the serum creatinine between the groups of mice. Thus, BUN levels were used to assess changes in renal function in the various groups of mice. Renal function was stable during the 14-wk treatment periods (Table 1) as we have previously demonstrated (14). Electrocautery eliminates kidney tissue fixing renal function, and murine renal ablation, in distinction to rodent 5/6 nephrectomy, does not progress to end stage CKD (14). This is supported by the absence of a significant tubulointerstitial nephritis in the renal remnant of the CKD mice (data not shown). The BUN levels in sham-operated wild-type mice at 28 wk were 25 ± 10 mg/dl, and in the sham-operated LDLR−/− groups, they averaged 34 ± 17 mg/dl. The level of CKD induced by electrocautery and nephrectomy in the studies reported here could be regarded as moderate to severe, as BUN levels were approximately two to three times normal (Table 1, Figure 2A). In CKD high-fat/cholesterol–fed mice (n = 10), the mean BUN was 80 ± 20 mg/dl (P < 0.05) compared with the sham standard diet or sham fat groups. BUN levels in mice that had CKD and were treated with BMP-7 (n = 11) were 105 ± 11 mg/dl and not significantly different from mice that had CKD and had not received treatment. They were also stable from week 14 to week 28 at killing. The BUN levels of the mice that had CKD and were fed high-fat/cholesterol diet supplemented with CaCO3 were 85 ± 17 at 14 wk and 94 ± 16 at 28 wk (Table 1). BUN levels of the mice that had CKD and were fed high-fat/cholesterol diet supplemented with CaCO3 and treated with BMP-7 were 132 ± 100 at 14 wk and 148 ± 110 at 28 wk. The difference in BUN levels represent more severe renal ablation in the mice that had CKD and were treated with BMP-7. To avoid the possibility that milder renal failure would cloud the responses to CaCO3 and BMP-7, the mice that had CKD and were fed high-fat/cholesterol diet supplemented with CaCO3 and treated with BMP-7 were made moderate to severe.
especially uremic. They were stable during the 14-wk period, although they did not gain weight (Table 1).

**Assessment of Parathyroid Response to CKD**

iPTH levels in wild-type mice that were fed the regular diet averaged 19 ± 20 pg/ml. PTH levels in the LDLR−/− mice that were fed the regular diet were higher (46 ± 20; Figure 2B). There were no differences in the levels of iPTH between the sham-operated LDLR−/− mice that were fed a regular diet or a high-fat/cholesterol diet. The CKD mice that were fed a high-fat/cholesterol diet developed secondary hyperparathyroidism with mean PTH levels of 174 ± 100 pg/ml. The CKD mice that were fed a high-fat/cholesterol diet and given exogenous BMP-7 developed mean PTH levels of 162 ± 200 pg/ml, not different from the CKD mice that were fed high-fat diet and treated with vehicle. CaCO₃ supplementation normalized the PTH levels in the CKD groups that were fed high-fat/cholesterol diet supplemented with CaCO₃ (Figure 2B). Cholesterol levels were high (500 mg/dl) in regular diet–fed LDLR−/− mice (Figure 2C), and they were further massively increased by high-fat feeding. There were no differences in plasma cholesterol among the high-fat/cholesterol–fed groups. The LDLR−/− sham-operated mice were mildly hyperglycemic (normal murine blood glucose 62 to 175 mg/dl; Table 1) in the fasting state, thus insulin resistant. Glycemia was not affected.

Figure 4. Goldner’s trichrome stain of distal femur trabecular bone. Representative sections at two magnifications (×50 left, ×200 right) are shown from LDLR−/− regular diet–fed control mice (A), LDLR−/− high-fat–fed mice (B), LDLR−/− CKD high-fat–fed mice (C), and LDLR−/− CKD high-fat–fed mice that were treated with BMP-7 (D).

**Figure 4.** Goldner’s trichrome stain of distal femur trabecular bone. Representative sections at two magnifications (×50 left, ×200 right) are shown from LDLR−/− regular diet–fed control mice (A), LDLR−/− high-fat–fed mice (B), LDLR−/− CKD high-fat–fed mice (C), and LDLR−/− CKD high-fat–fed mice that were treated with BMP-7 (D). LDLR−/− regular diet–fed mice (Sham Chow) had clearly demonstrable active osteoblast surfaces in their metaphyseal trabeculae (A). High-fat feeding reduced the presence of osteoblastic trabecular surfaces (B). Addition of CKD to the high-fat–fed environment further decreased osteoblast numbers and surfaces (C). Treatment with BMP-7 in the high-fat–fed CKD environment normalized trabecular osteoblast surfaces (D).
by BMP-7 or CaCO3 supplementation. The LDLR–/– mice that were fed high-fat/cholesterol diet and subjected to sham surgery gained significantly more weight than regular diet–fed mice and became obese (Table 1). Addition of either BMP-7 or CaCO3 reversed the tendency to excess weight gain. Food consumption was the same for each of the four CKD groups, which was less than the sham-operated groups. The more azotemic mice did not gain weight.

Assessment of Calcium and Phosphate Metabolism

Figure 3 shows the trends of serum phosphorus (A), calcium (B), and the calcium phosphorus products (C) in the study groups. LDLR–/– mice that were fed a regular diet (Sham Chow) exhibited a tendency to elevated serum phosphate levels compared with wild-type mice. Upon high-fat/cholesterol feeding, this developed into frank hyperphosphatemia (14.7 ± 0.4 mg/dl; Figure 3A). The CKD mice that were fed a high-fat/cholesterol diet developed significant hyperphosphatemia (15.0 ± 2.5) during the 14-wk period, slightly greater than the sham high-fat/cholesterol–fed mice. As shown in Figure 3A, the hyperphosphatemia was reversed by BMP-7 and CaCO3 supplementation to the level of the LDLR–/– regular diet–fed, sham-operated mice. As shown in Figure 3B, high-fat/cholesterol feeding tended to decrease serum calcium levels compared with the Sham Chow group. The CKD mice that were fed high-fat/cholesterol diet supplemented with CaCO3 were hypercalcemic. The product of calcium and phosphate (Figure 3C) was highest in the LDLR–/– mice that were fed a regular diet tended to have more osteoid than wild-type counterparts, and this was reversed by high-fat feeding. The CKD mice that were fed high-fat/cholesterol diet and treated with vehicle had increased osteoid volume (2.0 ± 1.3) compared with regular diet–fed sham-operated LDLR–/– mice (0.9 ± 0.4; Figure 5B). Osteoid thickness tended to be decreased in LDLR–/– mice (Table 2) compared with wild-type mice, and CKD high-fat/cholesterol feeding tended to increase osteoid thickness, but these changes were NS (Table 2) and did not achieve the levels to be designated as osteomalacia (18). We found previously that the increase in osteoid volume was an early manifestation of renal osteodystrophy in the presence of hyperparathyroidism (13). However, the expected findings of high turnover osteodystrophy were not observed in the CKD high-fat/cholesterol–fed group. Instead, the features of the AB were observed. Osteoblast numbers and perimeters were significantly reduced in CKD high-fat–fed, vehicle-treated mice compared with regular diet–fed sham-operated mice (Figure 5, D and E), as were mineralizing surfaces (Figure 6A) and bone formation rates (Figure 6B). Bone formation rates were also significantly reduced in the sham-operated LDLR–/–, high-fat–fed, vehicle-treated mice (Figure 6B), confirming the tendency for decreased osteoblast number and perimeter shown in Figure 5, D and E, compared with the Sham Chow LDLR–/– group. Osteoblast numbers and perimeter along with bone formation rates tended to be higher in LDLR–/– regular diet–fed mice than in wild-type controls that were fed regular diet (Figures 5, D and E, and 6B). Osteoclast and numbers did not vary between the groups (Figure 5C).

The adjusted apposition rates were significantly depressed in both the LDLR–/– high-fat-fed mice and the LDLR–/– high-fat-fed CKD vehicle-treated groups (Figure 6C). Thus, the histomorphometry in the LDLR–/– high-fat-fed mice was consistent with an adynamic bone disorder worsened by CKD despite high PTH levels (Figure 2B). The LDLR–/– high-fat–fed mice had significant reductions in bone formation rates (Figure 6B) and adjusted apposition rates consistent with their exhibited tendency to reduced osteoblast perimeter (Figure 5D) and number (Figure 5E) when compared with LDLR–/– regular diet–fed mice. Treatment with BMP-7 in the CKD mice with low turnover osteodystrophy resulted in a normalization of the osteoblast number and perimeter (Figure 5, D and E), an increase in the mineralizing surfaces (Figure 6A), and a normalization of the bone formation and adjusted apposition rates (Figure 6, B and C).

Supplementation of the high-fat/cholesterol diet with CaCO3 in
CKD mice did not alter the osteoblast surface parameters, the bone formation rates, or the adjusted apposition rates when compared with the CKD high-fat/cholesterol–fed group (data not shown). Thus, the CaCO3 supplementation had no effect on the low turnover osteodystrophy of the LDLR/H11002/H11002 high-fat–fed mice with CKD. Treatment with BMP-7 corrected the low turnover osteodystrophy in CaCO3-supplemented high-fat/cholesterol–fed CKD mice by increasing osteoblast surfaces and number to normal when compared with the LDLR−/− Sham Chow–fed mice or with wild-type regular diet–fed mice (P < 0.05 versus Sham Chow–fed LDLR−/− mice). Values are mean ± SD.

Table 2. Osteoid thickness and mineral apposition rates in the various groups

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type Chow</th>
<th>Sham LDLR−/−/Chow</th>
<th>Sham LDLR−/− High Fat/Cholesterol</th>
<th>Sham Fat BMP-7a</th>
<th>CKD Fat</th>
<th>CKD Fat BMP-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoid thickness (µm)</td>
<td>1.64 ± 0.36</td>
<td>1.32 ± 0.33</td>
<td>1.33 ± 0.22</td>
<td>1.22 ± 0.30</td>
<td>1.93 ± 0.62</td>
<td>1.34 ± 0.31</td>
</tr>
<tr>
<td>Mineral apposition rate (µm/d)</td>
<td>0.67 ± 0.22</td>
<td>1.01 ± 0.24</td>
<td>0.48 ± 0.16</td>
<td>0.50 ± 0.13</td>
<td>0.54 ± 0.27</td>
<td>0.70 ± 0.21</td>
</tr>
</tbody>
</table>

*BMP-7, bone morphogenetic protein.

CKD mice did not alter the osteoblast surface parameters, the bone formation rates, or the adjusted apposition rates when compared with the CKD high-fat/cholesterol–fed group (data not shown). Thus, the CaCO3 supplementation had no effect on the low turnover osteodystrophy of the LDLR−/− high-fat–fed mice with CKD. Treatment with BMP-7 corrected the low turnover osteodystrophy in CaCO3-supplemented high-fat/cholesterol–fed CKD mice by increasing osteoblast number, bone formation rates, and the adjusted apposition rate, as it did in the CKD high-fat/cholesterol–fed BMP-7–treated group (data not shown).

Effects of CKD and Treatment with BMP-7 on Aortic Calcium Content

Aortic calcium was measured as described in the Materials and Methods section. High-fat feeding tended to increase aortic calcium levels two-fold, and CKD plus high-fat feeding increased it more than four-fold (Figure 7A). BMP-7 therapy in CKD high-fat/cholesterol–fed mice reduced aortic calcium to levels observed in the sham-operated regular diet–fed LDLR−/− mice. CaCO3 supplementation also decreased aortic calcium levels but not to the levels observed in the BMP-7–treated mice and CaCO3 supplementation plus BMP-7 therapy. We previously characterized the aortic lesions that cause the changes in calcium levels (14), and the observations made in this study confirmed our previous data. High-fat/cholesterol feeding of sham-operated LDLR−/− mice induced punctate calcium deposits in proximal aortic neointimal plaques and in the aortic media in all mice of the group (Figure 8A). These lesions were worsened by ablative CKD both in the neointima and in the medial wall (Figure 8B), accounting for the effect of CKD on aortic calcium levels (Figure 7). CaCO3 supplementation decreased the prevalence of calcifications, especially in the media (Figure 8C), but did not eliminate them from the neointimas. Calcifications remained in each of the CaCO3-supplemented CKD high-fat/cholesterol–fed mice, similar to Figure 8C. Treatment of the CaCO3-supplemented CKD high-fat/cholesterol–fed mice with BMP-7 resulted in complete disappearance of visible calcification lesions (Figure 8D), similar to the effect of BMP-7 in CKD high-fat/cholesterol–fed mice that we previously reported (14).

To analyze further the apparent inverse relationship between skeletal modeling and VC, we performed a regression analysis between the adjusted apposition rate (the estimate of the number of skeletal modeling units) and aortic calcium levels. There

Figure 5. Bone volume, osteoid volume, osteoclast number, osteoblast perimeter, and osteoblast number in metaphyseal trabeculae. There were no significant changes in bone volume between groups (A), although there was an osteopenic tendency in the LDLR−/− high-fat–fed CKD group. There was a mild hyperosteoidosis produced in the CKD high-fat–fed group (B) possibly related to the secondary hyperparathyroidism as expected (P < 0.05 versus the Sham group). There were no differences in osteoclast numbers between the groups (C). Osteoblast perimeter (D) and number (E) were significantly reduced by CKD in the LDLR−/− high-fat–fed mice, and BMP-7 therapy restored osteoblast surfaces and number to normal when compared with the LDLR−/− Sham Chow–fed mice or with wild-type regular diet–fed mice (P < 0.05 versus Sham Chow–fed LDLR−/− mice). Values are mean ± SD.
was a significant inverse multifactorial relationship between aortic calcium levels and adjusted bone apposition rates in sham-operated regular diet–fed mice, sham-operated high-fat–fed mice, and CKD high-fat–fed mice (Figure 7B).

**Discussion**

The studies reported here demonstrate that LDLR−/− mice that are fed high-fat/cholesterol diets, an animal model of the human metabolic syndrome, have significant reductions in skeletal bone forming/modeling units measured as the adjusted apposition rates in the distal femoral metaphyses. Bone formation rates were depressed, and tendencies for mineralizing surfaces, osteoblast number, and osteoblast perimeter to be reduced compared with LDLR regular diet–fed mice were observed. When ablative CKD was superimposed, these tendencies became significant reductions, and a low turnover osteodystrophy was observed. A logical interpretation is that a progressive loss of skeletal anabolism was observed going from LDLR−/− regular diet–fed mice to LDLR−/− high-fat–fed, to LDLR−/− high-fat–fed + CKD without major changes in the osteoclast compartment, the latter another hallmark of the ABD of renal failure. We demonstrated previously in animal models that CKD directly produces a loss of skeletal anabolic potential by diminishing skeletal growth factors or producing anabolic inhibitors or both (13). The surprising observations here were, first, the reduction in adjusted apposition rates stimulated by high-fat feeding and, second, the lack of response to hyperparathyroidism when CKD was induced.

The metabolic syndrome is often complicated by type 2 diabetes, and an osteodystrophy/osteoporosis is a known but poorly characterized problem associated with diabetes (19–21). However, the finding here of a reduction in bone formation and matrix apposition in this model of the metabolic syndrome before severe diabetes is a new discovery. The osteopenia- and osteoporosis-associated increase in fracture risk among humans with diabetes has not been characterized by formal bone histomorphometry in longitudinal studies. Krakuer et al. (22) performed a small bone biopsy study of eight patients with type 2 diabetes and found decreased osteoid thickness and bone formation rates (22). They concluded that osteoblast recruitment and function were markedly depressed in type 2 diabetes. These findings are in agreement with the reductions in bone formation and matrix apposition induced by high-fat feeding in the studies reported here.

Also unexpected in our studies was the advance of the osteodystrophy found in the high-fat/cholesterol–fed LDLR−/− mice to the ABD when CKD was induced despite the presence of secondary hyperparathyroidism. The skeletal histomorphometry findings in LDLR−/− high-fat–fed mice with CKD are those of the adynamic bone disorder previously reported in CKD and ESRD (23–26) except for a mild increase in osteoid,

![Figure 6. Mineralizing surfaces, bone formation rates, and adjusted apposition rates. Mineralizing surfaces (A) were significantly decreased in the CKD high-fat–fed mice. Despite the tendency for osteoblast number and perimeter to be increased in LDLR−/− regular diet–fed sham mice compared with wild-type mice (Figure 4), the mineralizing surfaces were slightly lower and further decreased by high-fat feeding. The bone formation rates (B) were significantly decreased by high-fat feeding in both the sham and CKD groups and returned to normal by BMP-7 therapy.](image-url)
which we have demonstrated to be an early response to CKD that does not progress to osteomalacia (13). In other words, the reduction of skeletal anabolism produced in this model of the metabolic syndrome rendered the skeleton unresponsive to the anabolic factor BMP-7, useful in animal models of the ABD and high turnover ROD (12,13,30), was therapeutic in the low turnover osteodystrophy observed in our animal model. BMP-7 is an important developmental morphogen for the skeleton, kidney, and eye (31–33). BMP-7 is a regulator of osteoblast differentiation as well as having other biologic functions in the adult (30,34,35). The BMP were originally isolated from bone extracts that were capable of inducing endochondral bone formation when placed in mesenchymal derived sites (36). In vitro, the BMP have been demonstrated to stimulate the development of osteoblastic cells from undifferentiated precursors (37–42). 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 (43). Renal injury results in decreased BMP-7 production as shown in several models of acute kidney disease and CKD (44–46). 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.

In our study, two groups of mice had high values of serum phosphate and calcium phosphorus products, the LDLR−/− mice that were fed high-fat/cholesterol diet and the CKD mice that were fed high-fat/cholesterol diet. Both of these groups had low turnover osteodystrophy as did the CKD high-fat/cholesterol–fed CaCO3-supplemented group. Correction of the osteodystrophy was associated with increased mineral apposition because increased renal excretion of phosphate was not demonstrated and preliminary studies demonstrate no effect of BMP-7 on renal tubular phosphate transport (Hruska and Ledderer, unpublished observations). Therefore, we favor the interpretation that increased bone formation in the BMP-7–treated mice led to a reduction in serum phosphate compared with the untreated CKD group. CaCO3 supplementation directly controlled the serum phosphorus by decreased absorption, but it did not affect the bone formation or adjusted apposition rates.

Induction of CKD led to a significant increase in aortic calcification in the studies reported here. These results were in agreement with our previous study of VC in this model (14). BMP-7 was effective in preventing the increase in VC induced by CKD, as was CaCO3 supplementation, suggesting that hyperphosphatemia was an important stimulus to VC. Thus, stimulation of skeletal mineralization by BMP-7 and reduction of the serum phosphate may have been a mechanism of decreasing vascular mineralization. The vascular smooth muscle cells that participate in mineralization are sensitive to the extracellular phosphate concentration (47–49), supporting such a con-
cept. Additional support is shown in the strong inverse relationship between skeletal adjusted apposition rates and VC in these studies, suggesting that heterotopic mineralization may be related to decreased orthotopic mineralization (50). Because BMP-7 also has direct actions on the phenotype of vascular smooth muscle cells (14,47,51), the greater benefit of BMP-7 compared with sole reduction in the serum phosphate supports the conclusion that a portion of the protective effect of BMP-7 on VC was mediated by the stimulation of bone formation and the reduction in serum phosphorus.

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