

Reversal of the Adynamic Bone Disorder and Decreased Vascular Calcification in Chronic Kidney Disease by Sevelamer Carbonate Therapy

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A model of chronic kidney disease (CKD)-induced vascular calcification (VC) that complicates the metabolic syndrome was produced. In this model, the metabolic syndrome is characterized by severe atherosclerotic plaque formation, hypertension, type 2 diabetes, obesity, and hypercholesterolemia, and CKD stimulates calcification of the neointima and tunica media of the aorta. The CKD in this model is associated with the adynamic bone disorder form of renal osteodystrophy. The VC of the model is associated with hyperphosphatemia, and control of the serum phosphorus both in this animal model and in humans has been preventive in the development of VC. This article reports studies that demonstrate reduction of established VC by the addition of sevelamer carbonate to the diets of this murine metabolic syndrome model with CKD. Sevelamer, besides normalizing the serum phosphorus, surprisingly, reversed the CKD-induced trabecular osteopenia. Sevelamer therapy increased osteoblast surfaces in the metaphyseal trabeculae of the tibia and femur. It also increased osteoid surfaces and, importantly, bone formation rates. In addition, sevelamer was found to be effective in decreasing serum cholesterol levels. These results suggest that sevelamer may have important actions in decreasing diabetic and uremic vasculopathy and that sevelamer carbonate may be capable of increasing bone formation rates that are suppressed by diabetic nephropathy.

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Progression of diabetic nephropathy (DN) generally is considered in terms of progressive loss of kidney function until end-stage kidney failure occurs and renal replacement therapy begins. However, DN is a systemic disease, and it also is fatal. Indeed, more patients with DN die before reaching the need for dialysis than accrue to modalities of renal replacement therapy (1–3). Cardiovascular mortality in patients with chronic kidney disease (CKD) is extremely high (1,4). Conventional risk factors that are characteristic of the metabolic syndrome (5), such as hypertension, dyslipidemia, insulin resistance, and overt diabetes, are highly prevalent in CKD, but other risk factors with additive effects that are more specific to the uremic milieu also have been identified (6–8). One is the presence of vascular calcification (VC) (9), a form of heterotopic mineralization that is predictive of cardiovascular mortality (10,11) and is both common and severe in CKD (12). The VC of the tunica media that is seen in CKD is similar to that observed in type 2 diabetes without DN, and when CKD is added to diabetes through DN, the cardiovascular risk is at least additive of that of CKD plus diabetes; in other words, extreme.

This study, which was performed in mice with metabolic syndrome, shows a beneficial effect of sevelamer therapy on vascular calcification, bone disease, and cholesterol levels, and is linked to a study by Gross and colleagues in this month's issue of CJASN (pp. 121–134), which analyzes the coronary calcification in renal patients that would be one target of such therapy. It describes a tendency to medial rather than intimal calcification, with an accompanying inflammatory infiltrate. Both papers contribute to better understanding and treatment of vascular calcification in renal disease.

We have developed an animal model of VC that is worsened by CKD (13). 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's complicating the metabolic syndrome, because the mice have obesity, hypertension, insulin resistance, and early type II diabetes. In these animals, CKD caused intensification of VC, which was prevented by treatment with bone morphogenetic protein-7 (BMP-7) (13). We recently demonstrated that the diabetes in this model is associated with a decrease in osteoblast surfaces and that the addition of CKD produced hyperphosphatemia and the adynamic bone disorder (ABD) despite hyperparathyroidism (14). Correction of the hyperphosphatemia diminished VC, and this may have accounted for approximately 50% of the action of BMP-7 to reverse VC (14). BMP-7 reversed hyperphosphatemia by increasing skeletal phosphate deposition through bone formation.

We recently demonstrated (15,16) that hyperphosphatemia is a novel cardiovascular risk factor in CKD through activation of

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a BMP-2–driven osteogenic program in the vasculature that leads to mineralization of atherosclerotic neointimas and the tunica media. The action of phosphorus was to induce expression of osterix, normally an osteoblast-specific transcription factor. CKD in high-fat–fed LDLR^{-/-} mice induced aortic osterix expression. Both BMP-7 and sevelamer carbonate (sevelamer) therapy reversed CKD-induced VC and osterix expression. Because a component of the action of BMP-7 was related to reversal of the adynamic bone disorder in this model, we questioned whether the same was true for sevelamer. Here, we demonstrate that sevelamer therapy reversed established VC and stimulated bone formation rates through increased skeletal osteoblast activity, demonstrating actions of sevelamer in CKD beyond control of hyperphosphatemia. We conclude that a consistent link between the skeleton and the vasculature exists in CKD that is related to divalent ion homeostasis.

Materials and Methods

Animals and Diets

LDLR^{-/-} mice of both genders in a C57Bl/6J background were purchased from Jackson Laboratory (Bar Harbor, ME) and were bred in a pathogen-free environment. Mice were weaned at 3 wk to a chow diet (1:1 mixture of Pico Lab [Purina Mills, Richmond, IN] rodent chow 20 and mouse chow 20, 6.75% calories as fat). At 10 wk, mice were continued on this chow diet or initiated on a high-cholesterol (0.15%) diet that contained 42% calories as fat (product no. TD88137; Harlan Teklad, Madison, WI), a diet that has been shown to generate atherosclerosis with VC in this genetic background. At 12 wk, CKD was induced as described next. Mice had access to water *ad libitum* and were maintained according to local and national animal care guidelines. Sevelamer carbonate was provided to us by Genzyme Corp. (Waltham, MA). Xylazine, ketamine, and tetracycline were obtained from Sigma-Aldrich Co. (St. Louis, MO). The Washington University Animal Care committee approved the study protocol.

Induction of CKD and Treatment Protocol

A two-step procedure was used to create uremia as described by Gagnon and Gallimore (17). Briefly, electrocautery was applied to the right kidney through a 2-cm flank incision at 10 wk postnatal, followed by left total nephrectomy through a similar incision 2 wk later. Control mice received sham operations in which the appropriate kidney was exposed and mobilized but not treated in any other way. Stable CKD was established after the two surgical procedures. After the surgical procedures, the 14-wk-old mice were randomized into 10 groups. The first was wild-type (WT) mice that were fed a regular diet. This was the normal renal function and diet group. The second group was LDLR^{-/-} mice that were fed regular chow. This group served as control for the diet. The third group was sham-operated LDLR^{-/-} mice that were fed a high-fat diet. This group had normal renal function. This group served as the control group to determine the effect of high-fat diet in the face of normal renal function. The fourth group was LDLR^{-/-} mice that were fed a high-fat diet and treated with 1% sevelamer. This was the first treatment group to determine whether sevelamer influenced the effects of high-fat feeding. The fifth group was LDLR^{-/-} mice that were fed a high-fat diet and treated with sevelamer 3%. The sixth group was LDLR^{-/-} mice that had CKD and were fed regular chow. This was the control group to determine the effect of CKD in the presence of a regular diet. The seventh group was LDLR^{-/-} mice that had CKD and were fed a high-fat diet and treated with vehicle. This group was expected to develop hyperphosphatemia

and VC. The eighth group was LDLR^{-/-} mice that had CKD and were fed a high-fat diet and treated with 1% sevelamer. This was the therapy group. The ninth group was LDLR^{-/-} mice that had CKD and were fed a high-fat diet and treated with sevelamer 3%. Because this was a treatment study, the 10th group served as the control group to establish baseline VC and high phosphorus at 22 wk before the initiation of therapy. Once the mice were randomized into groups, they were allowed to develop calcification from weeks 14 through 22 postnatal. Therapy was initiated at 23 wk postnatal and continued until week 28 postnatal, at which time the mice were killed. Intraperitoneal anesthesia (xylazine 13 mg/kg and ketamine 87 mg/kg) was used for all procedures. Saphenous vein blood samples were taken 1 wk after the second surgery to assess baseline postsurgical renal function. Animals were killed under anesthesia 28 wk postnatal; at that time, blood was taken by intracardiac stab, and the heart and aorta were dissected *en bloc*.

Blood Tests. Serum was analyzed on the day of blood draw for blood urea nitrogen (BUN), cholesterol, calcium, glucose, and phosphate by standard autoanalyzer laboratory methods performed by our animal facility. Mouse intact parathyroid hormone (PTH) levels were measured by an ELISA kit (Immutopics, San Clemente, CA) when the mice were killed.

Chemical Calcification Quantification. Aorta and hearts were dissected after the mice were killed, and all extraneous tissue was removed by blunt dissection under a dissecting microscope. Tissues were desiccated for 20 to 24 h at 60°C, weighed, and crushed to a powder with a pestle and mortar. Calcium was eluted in 1 N HCL for 24 h at 4°C. Calcium content of eluate was assayed using a cresolphthalein complexone method (Sigma), according to the manufacturer's instructions, and results were corrected for dry tissue weight.

Bone Histology and Histomorphometry. Bone formation was determined when the mice were killed. All mice received intraperitoneal tetracycline (5 mg/kg) 7 and 2 d before being killed. Both femurs were dissected 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 frontal plane in 5- μ m sections with a JB-4 Microtome (Energy Beam Sciences). Tissue was stained with Goldner's trichrome stain for trabecular and cellular analysis. Tartrate-resistant acidic phosphatase staining was used to identify osteoclasts and define osteoclast surfaces. Unstained 10- μ m sections were used for tetracycline-labeled fluorescence analysis.

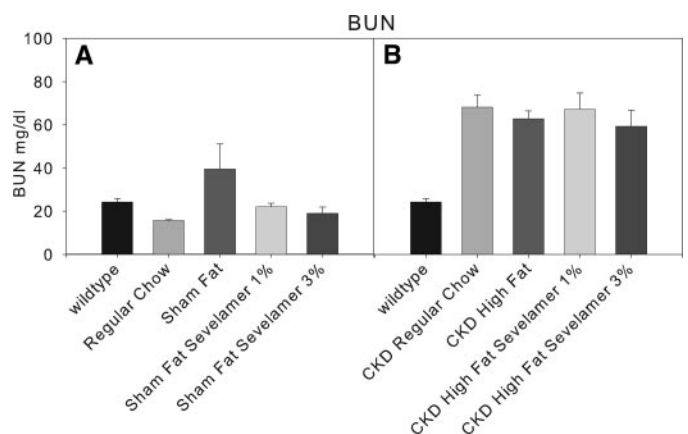


Figure 1. Blood urea nitrogen (BUN) levels in wild-type (WT) and sham-operated LDL receptor–deficient (LDLR^{-/-}) mice (A) and LDLR^{-/-} mice with chronic kidney disease (CKD) (B). Data are means \pm SEM; $n = 6$ to 10.

Table 1. Biochemical parameters group^a

Parameter	Group 1 Wild Type		Group 2 LDLR ^{-/-}		Group 3 LDLR ^{-/-}		Group 4 LDLR ^{-/-}		Group 5 LDLR ^{-/-}		Group 6 LDLR ^{-/-}		Group 7 LDLR ^{-/-}		Group 8 LDLR ^{-/-}		Group 9 LDLR ^{-/-}	
	Chow	Sham	Chow	Sham	Fat	Sham	Fat	Sham	Fat	Sham	Chow	CKD	Fat	CKD	Fat	CKD	Fat	CKD
N	4	4	5	5	5	5	6	6	5	5	5	5	7	7	5	5	7	7
Presurgical weight (g)	22.9 ± 0.7	23 ± 0.9	23.5 ± 0.6	23.5 ± 0.6	22 ± 0.8	22 ± 0.8	22 ± 0.8	22 ± 0.5	22 ± 0.5	24 ± 0.7	24 ± 0.7	21 ± 1.0	21 ± 1.0	22 ± 0.8	22 ± 0.8	20 ± 0.6	20 ± 0.6	20 ± 0.6
Weight at death (g)	26 ± 1.7	31 ± 1.9	33 ± 1.7	33 ± 1.7	29 ± 2.0	29 ± 2.0	29 ± 2.0	28 ± 2.0	28 ± 2.0	25 ± 0.9	25 ± 0.9	21 ± 0.8	21 ± 0.8	26 ± 1.6	26 ± 1.6	26 ± 1.0	26 ± 1.0	26 ± 1.0
Glucose (mg/dl)	183 ± 15	272 ± 58 ^b	364 ± 57 ^c	364 ± 57 ^c	354 ± 21 ^b	354 ± 21 ^b	354 ± 21 ^b	465 ± 68 ^c	465 ± 68 ^c	216 ± 69	216 ± 69	309 ± 19 ^b	309 ± 19 ^b	495 ± 21 ^c	495 ± 21 ^c	290 ± 55 ^b	290 ± 55 ^b	290 ± 55 ^b
PTH (pg/ml)	19 ± 20	25 ± 12	23 ± 13	23 ± 13	ND	ND	ND	ND	ND	174 ± 100 ^b	174 ± 100 ^b	444 ± 98 ^c	444 ± 98 ^c	ND	ND	10 ± 3	10 ± 3	10 ± 3

^aCKD, chronic kidney disease; LDLR^{-/-}, LDL receptor deficient; ND, not determined; PTH, parathyroid hormone.

^b*p* < 0.05 versus wild-type chow fed.

^c*p* < 0.01 versus wild type chow fed.

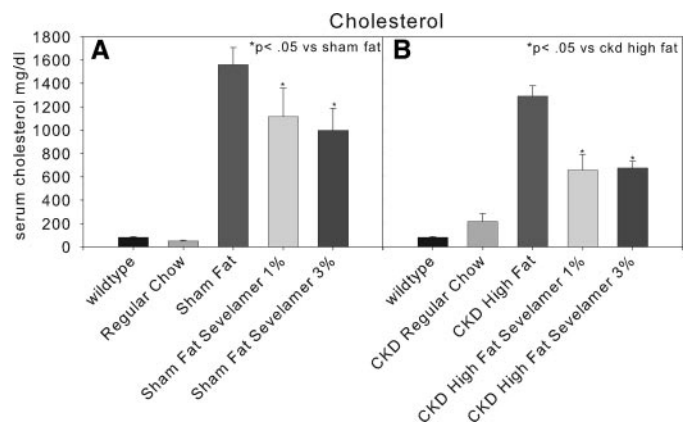


Figure 2. Effects of high-fat feeding and sevelamer on serum cholesterol (mg/dl) in WT and sham-operated LDLR^{-/-} mice (A) and LDLR^{-/-} mice with CKD (B). Data are means ± SEM; *n* = 6 to 10.

Slides were examined at ×400 magnification using 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 mouse. Primary, derived, and kinetic measures of bone remodeling were calculated and reported per guidelines of the American Society of Bone and Mineral Research (18).

Statistical Analyses

Statistical analysis was performed using ANOVA. Differences between groups were assessed *post hoc* using Dunnett multiple range test and considered significant at *P* < 0.05. Data are presented as means ± SEM. Analyses were performed using Sigma Stat statistical software (Point Richmond, CA).

Results

Evaluation of Renal Insufficiency, Diabetes, and Dyslipidemia in LDLR^{-/-} High-Fat-Fed Mice

BUN levels were used to assess changes in renal function in the various groups of mice. The BUN levels in sham-operated WT mice were 24 ± 1.6 mg/dl, and in the sham-operated LDLR^{-/-} groups, they averaged 15 ± 1.2 mg/dl with the sham-operated high-fat-fed group showing variable BUN levels (Figure 1A). In the CKD groups, the level of kidney failure that was induced by electrocautery and contralateral nephrectomy could be regarded as moderate, because BUN levels were more than two times sham-operated levels in the CKD groups (Figure 1B). Intact PTH levels in WT mice that ate a regular chow diet were 19 ± 20 pg/ml. PTH levels in the LDLR^{-/-} sham-operated mice that were fed a high-fat diet were not different among the groups or from WT mice (Table 1). The CKD mice that were on a high-fat/cholesterol diet developed secondary hyperparathyroidism with mean PTH levels of 174 ± 100 pg/ml with chow feeding and 444 ± 98 with high-fat feeding. Sevelamer therapy (3% by weight added to diet) normalized the PTH levels in CKD (Table 1).

Cholesterol levels were not high in chow-fed LDLR^{-/-} mice, but they were massively increased by high-fat feeding (1570 ± 130 mg/dl; Figure 2A). Sevelamer therapy decreased

the serum cholesterol to the 1000- to 1100-mg/dl range in sham-operated, high-fat-fed mice. The effects of sevelamer on the serum cholesterol were surprising in view of the severity of the hypercholesterolemia in the LDLR^{-/-} high-fat-fed mice. The addition of ablative CKD did not decrease significantly the serum cholesterol from the elevated level that was observed in high-fat-fed mice (Figure 2B). Addition of both sevelamer 1 and 3% to the high-fat diet of mice with CKD produced a 50% decrease in the serum cholesterol to values of 700 ± 50 and 750 ± 60 mg/dl, respectively.

The LDLR^{-/-} sham-operated mice were borderline hyperglycemic in the fasting state, and hyperglycemia was worsened by fat feeding. The LDLR^{-/-} high-fat-fed mice were insulin resistant or early diabetic (Table 1). CKD with chow feeding tended to diminish the blood sugar compared with sham-operated chow-fed LDLR^{-/-}, a widely known effect of CKD. Glycemia was not affected by sevelamer. The LDLR^{-/-} mice that were fed the high-fat/cholesterol diet and subjected to sham surgery gained more weight than chow-fed animals and became obese. Addition of CKD reversed the tendency to excess weight gain (Table 1). Food consumption and weight gain were the same for each of the four CKD groups, which were less than the sham-operated groups.

The serum phosphorus (Figure 3), calcium (Figure 4), and calcium-phosphorus products in LDLR^{-/-} mice that were fed a high-fat diet were similar to WT animals. The mice that had CKD and were fed a high-fat/cholesterol diet developed significant hyperphosphatemia during the 14-wk period that was greater than the sham high-fat/cholesterol-fed mice (Figure 3B). Compared with our previous studies, which demonstrated significant hyperphosphatemia in LDLR^{-/-} mice that were fed high-fat diets and were sham operated (14), only the LDLR^{-/-} high-fat-fed mice that had CKD and were treated with vehicle were hyperphosphatemic in this study. Sevelamer therapy produced dosage-dependent decreases in the serum phosphorus in the high-fat-fed CKD groups (Figure 3B). The serum phosphorus was normal and not significantly affected in

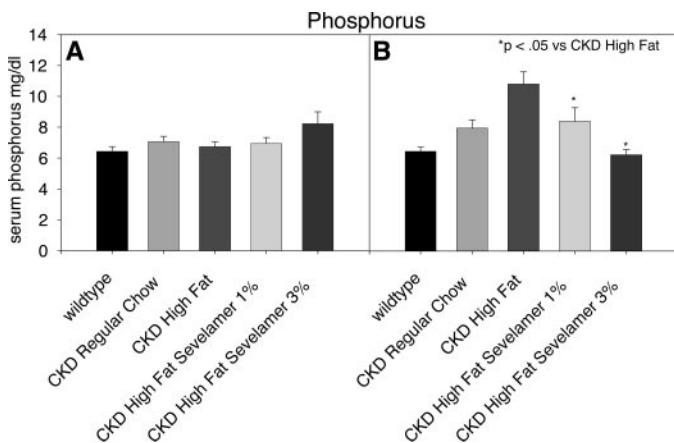


Figure 3. Effects of treatment with sevelamer in WT and sham-operated LDLR^{-/-} mice (A) and LDLR^{-/-} mice with CKD (B). LDLR^{-/-} mice with CKD (B) on the serum phosphorus (mg/dl). Data are means ± SEM; n = 6 to 10.

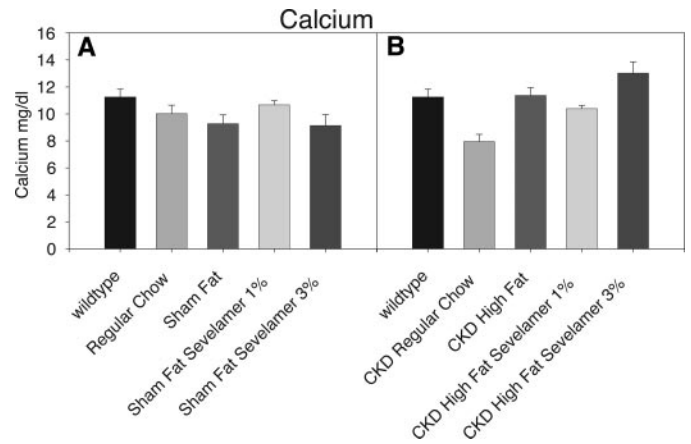


Figure 4. Effect of treatment with sevelamer on the serum calcium in the WT and sham-operated LDLR^{-/-} mice (A) and LDLR^{-/-} mice with CKD (B). Data are means ± SEM; n = 6 to 10.

the high-fat-fed, sham-operated groups that were treated with sevelamer (Figure 3A). In other words, in the presence of normal kidney function and normophosphatemia, therapy with sevelamer did not induce hypophosphatemia. As shown in Figure 4, there were no significant effects of any of the treatment groups on the serum calcium. Neither were there significant effects of CKD or high-fat feeding on the serum calcium.

Sevelamer Reverses Established VC

As shown in Figure 5, aortic calcium content was 0.18 ± 0.6 μg/mg dry weight in LDLR^{-/-} mice that were fed regular chow. High-fat feeding significantly increased vascular calcium levels in sham-operated mice compared with LDLR^{-/-} sham-operated, chow-fed mice (Figure 5A). The effect of CKD was significantly additive to the effects of high-fat feeding (Figure 5, B and A). CKD in the absence of high fat did not significantly increase VC when the

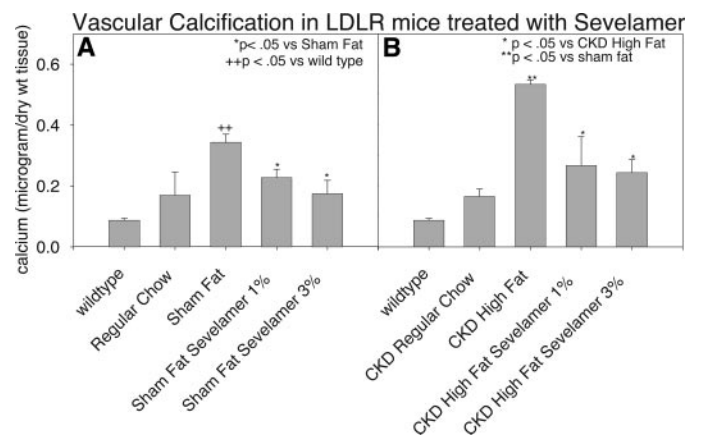


Figure 5. Reduction of established vascular calcification in WT and sham-operated LDLR^{-/-} mice (A) and LDLR^{-/-} mice with CKD (B). treated with sevelamer from 22 to 28 wk after birth. Data are means ± SEM; n = 6 to 10.

LDLR^{-/-} sham-operated, chow-fed mice were compared with the LDLR^{-/-} mice that had CKD and were fed chow. This result differs from our previous studies (13,14), and it was due to the variability in the LDLR^{-/-} sham-operated, chow-fed group, in which some mice had higher-than-expected vascular calcium levels. Both sevelamer 1 and 3% (Figure 5B) significantly reduced aortic calcium content by 50%, when compared with the LDLR high-fat-fed CKD group. Sevelamer (both 3 and 1%) also significantly decreased aortic calcium content in high-fat-fed, sham-operated animals compared with the LDLR high-fat-fed, sham-operated group.

Effect of High-Fat Feeding, CKD, and Treatment with Sevelamer on Bone Histology and Histomorphometry

To present the status of bone modeling in our study, we focused the histomorphometry measurements on mouse groups 1 (WT), 2 (LDLR^{-/-} chow fed), 3 (LDLR^{-/-} sham high-fat fed), 7 (LDLR^{-/-} CKD high-fat fed), and 8 (LDLR^{-/-} CKD high-fat fed and treated with 3% sevelamer). Trabecular bone volume was reduced in the CKD-LDLR^{-/-} high-fat-fed mice as we previously reported (14), and sevelamer therapy significantly increased bone volume to normal levels (Figure 6A). The LDLR^{-/-} chow-fed mice exhibited prominent trabecular osteoblast surfaces (Figure 6B) and normal osteoclast surfaces (Figure 6C). With high-fat feeding, the LDLR^{-/-} mice developed a reduction in trabecular osteoblast surfaces that was maximized by induction of CKD (Figure 6B). Sevelamer therapy significantly increased osteoblast surfaces in the LDLR-CKD high-fat-fed mice compared with untreated high-fat-fed mice with CKD (Figure 6B). Osteoclast surfaces (Figure 6C) were not affected significantly by any of the perturbations in the LDLR^{-/-} mice. Similar to osteoblast surfaces, osteoid volume was decreased progressively by high-fat feeding and superimposition of CKD (Figure 6D). Sevelamer therapy significantly increased osteoid volume, indicative of the increase in osteoblast activity in the LDL-CKD high-fat-fed mice (Figure 6C). Bone formation rates were decreased by high-fat feeding of the LDLR^{-/-} mice and remained reduced with induction of CKD (Figure 7A). Treatment with sevelamer significantly increased bone formation rates compared with the LDLR-CKD high-fat-fed group (Figure 7A). Mineralizing surfaces were not different in any of the LDLR^{-/-} groups compared with WT mice (Figure 7B). Adjusted apposition rates were not altered significantly between the LDLR^{-/-} groups (Figure 7C).

The CKD-LDLR^{-/-} high-fat-fed mice did not exhibit any of the features of osteitis fibrosa despite their secondary hyperparathyroidism. We previously demonstrated osteitis fibrosa that was produced by milder levels of CKD in WT C57 Bl6 mice than was produced here and that had lower PTH levels (19). The LDLR^{-/-} high-fat-fed mice are resistant to the bone remodeling effects of secondary hyperparathyroidism. This is demonstrated further in the studies reported here by the observation that sevelamer stimulated osteoblast surfaces and bone formation rates while decreasing PTH levels. The results reported in Figures 6 and 7 confirm the previous report that the

LDLR^{-/-} high-fat-fed mouse exhibits in the presence of CKD an adynamic bone disorder that is characterized by decreased osteoblast surface, decreased bone formation rates, and normal osteoclast surfaces. This is similar to the excess representation of the ABD as the form of renal osteodystrophy that is observed in human DN. Treatment with sevelamer significantly increased osteoblast surfaces and bone formation rates, ameliorating the adynamic bone disorder of the LDLR^{-/-} high-fat-fed mice with CKD.

Discussion

The studies reported here demonstrate that sevelamer, both 1 and 3%, added to high-fat/cholesterol diets of LDLR^{-/-} mice with established VC produced a reduction of the VC. The effects of sevelamer were potent and compared favorably with other agents in other studies that were able to affect VC in prevention trials (13,14). Another phosphate binder, calcium carbonate supplementation of the diet, partially prevented the development of VC in a previously reported prevention trial (14). However, there were calcifications in each of the calcium carbonate-supplemented high-fat/cholesterol fed animals with CKD in our previous study (14). Here, treatment with sevelamer resulted in significant reduction of established lesions when the 22-wk baseline calcification lesions were compared with the lesions in sevelamer-treated mice. However, because the studies are not comparable directly, whether the effects of sevelamer in this treatment trial were greater than what we observed in the previous prevention trial with CaCO₃ is not known.

We have characterized the aortic lesions that cause the changes in vascular calcium levels as apatite (the bone mineral CaPO₄ crystal) and not amorphous CaPO₄ from dystrophic calcification (13). The distribution of the calcium deposits in our model was in proximal aortic neointimal plaques and in the surrounding aortic media of high-fat/cholesterol-fed, sham-operated LDLR^{-/-} mice. These lesions were worsened by ablative CKD both in the neointima and in the medial wall, accounting for the effect of CKD on aortic calcium levels.

The actions of sevelamer to decrease the serum phosphate were dosage related, whereas its effects to reduce the serum cholesterol and VC were observed with the 1% dosage equal to the 3% dosage. This difference in dosage dependence also is compatible with actions of sevelamer on VC in addition to lowering the serum phosphorus. However, we recently established that the serum phosphorus is a direct signaling link between the skeleton and the vasculature, because in CKD, agents that stimulate bone formation and drive phosphorus into the skeleton produce a reduction in the serum phosphorus that partially accounts for their action to decrease VC (14). These data are in agreement with observational studies suggesting that hyperphosphatemia is an important risk factor for both VC and cardiovascular mortality (6,20). We have shown that phosphorus directly stimulated transcription of the osterix gene, stimulating osteoblastic differentiation of atherosclerotic vascular smooth muscle cells (15,16). These data are in agreement with studies from other laboratories that also demonstrated direct actions of phosphorus on the phenotype and

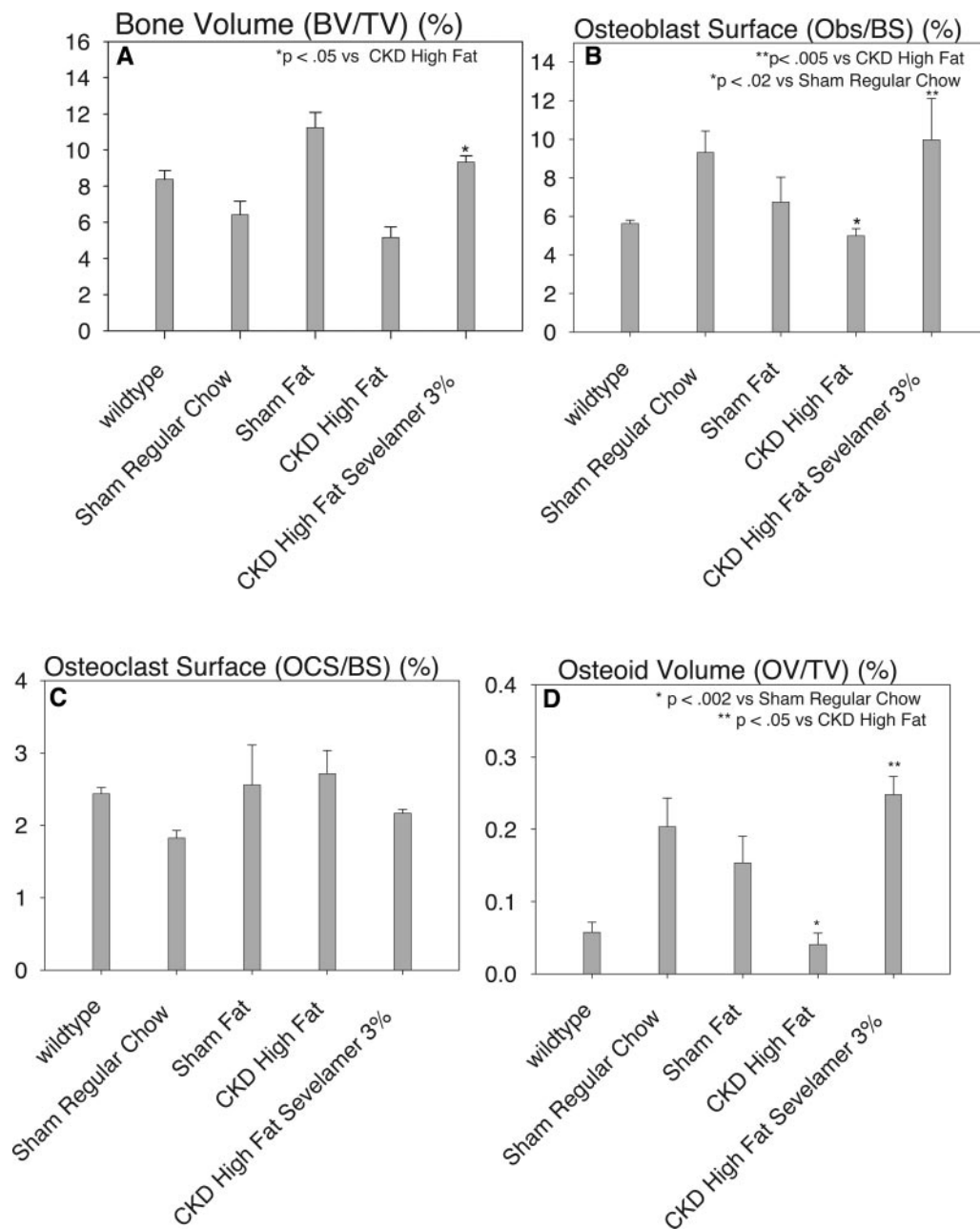


Figure 6. (A) Bone volume (BV/TV), (B) osteoblast surfaces (Obs/BS), (C) osteoclast surfaces (OCS/BS), and (D) osteoid volume (OV/TV) in LDLR^{-/-} mice that were treated with sevelamer from 22 to 28 wk after birth. Data are means ± SEM; n = 6 to 10. TV, total volume; BS, bone surface.

function of vascular smooth muscle cells (21,22). In light of these data, the control of hyperphosphatemia by sevelamer was at least a component of its mechanism of action in decreasing vascular calcium deposits in the studies reported here. However, the surprising findings of our studies that sevelamer therapy was associated reductions in cholesterol levels and with an increase in bone formation rates and metaphyseal trabecular bone volume demonstrate potential additional mechanisms of sevelamer action.

The effects sevelamer on VC were noted previously both in humans and in animal models. Chertow *et al.* (23) and Asmus

et al. (24) demonstrated that sevelamer hydrochloride treatment compared with calcium carbonate resulted in a decreased rate of increase in vascular calcium in dialysis patients. Sevelamer therapy in humans often is associated with a reduction in serum cholesterol (23,25) as observed in the studies reported here. Phan *et al.* (26) demonstrated that sevelamer hydrochloride decreased the calcification of atherosclerotic plaques during an 8-wk prevention trial in apolipoprotein E-deficient mice that were made uremic by the procedure used in our study. In the apolipoprotein E-deficient mice, CKD induced hypercholesterolemia that was not affected by sevelamer treatment, in

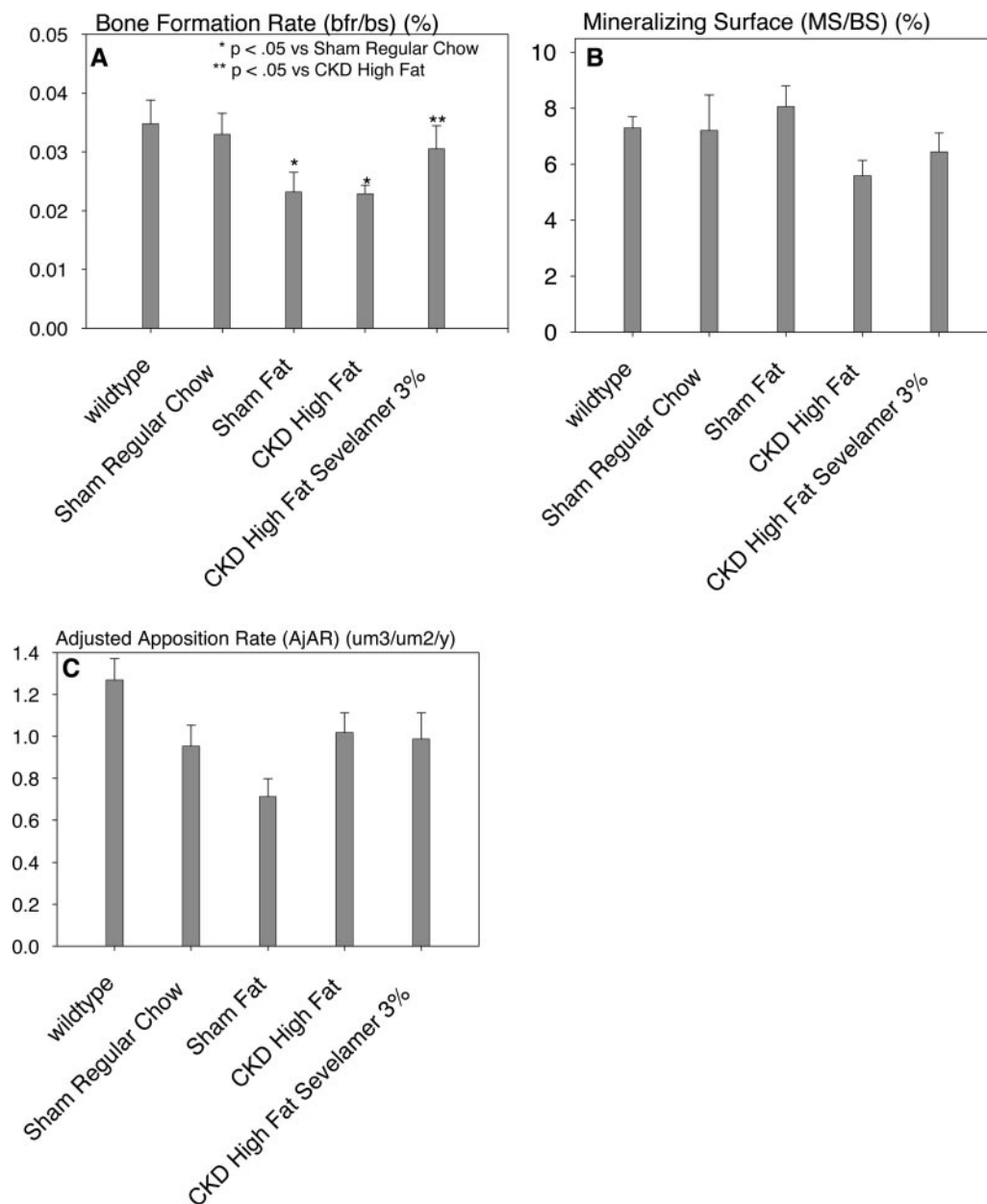


Figure 7. (A) Bone formation rates, (B) mineralizing surfaces, and (C) adjusted apposition rates in WT, sham-operated LDLR^{-/-} mice and LDLR^{-/-} mice with CKD. Data are means \pm SEM; $n = 6$ to 10. bfr, bone formation rate; MS, mineralizing surface, AjAR, adjusted apposition rate.

contrast to the results of our study (26). However, the authors demonstrated a marked reduction in atherosclerotic plaque area along with a reduction in calcification in the sevelamer-treated animals. The mechanism for the effects of sevelamer on atherosclerotic plaques was not determined. In our studies, the effects of sevelamer to correct hypercholesterolemia may have been an important component of its mechanism of action related to reduction of atherosclerotic plaques. The latter parameters were not measured in our study.

The previous studies of sevelamer hydrochloride (23,26) lend some insight to the questions of whether sevelamer carbonate, which was used in these studies, would exert greater actions on

bone modeling and skeletal anabolism and inhibition of VC than sevelamer hydrochloride. It is clear that sevelamer hydrochloride has positive actions against VC, and determination of the question of greater benefit from sevelamer carbonate requires direct comparison, which has not been performed.

The finding that sevelamer increased osteoblast number and function in terms of bone formation rate suggests that sevelamer did indeed exert additional effects besides control of hyperphosphatemia. Hyperphosphatemia control alone should not affect bone modeling rates, suggesting that the effects of sevelamer to increase orthotopic mineralization may have contributed to its actions of reducing vascular heterotopic mineralization.

Renal osteodystrophy is poorly understood in DN. It often is an adynamic bone disorder similar to the osteodystrophy in our animal model. Katsumata *et al.* (27) reported that sevelamer prevented development of renal osteodystrophy that was compatible with the increased osteoblast function and bone formation rates reported here in our studies. The mechanism of increased skeletal anabolism that is associated with sevelamer therapy is unknown, but they likely contribute to the effects of sevelamer to decrease VC.

The pathogenesis of the ABD in DN is unknown, but one hypothesis has been that the overrepresentation of the ABD in DN is due to inadequate secretion of PTH (28,29). Our studies demonstrate an interesting dissociation of the ABD from hyperparathyroidism. We demonstrate that CKD induced secondary hyperparathyroidism and the ABD, suggesting almost complete resistance to the actions of PTH on the skeleton in our model. Furthermore, sevelamer therapy stimulated osteoblast activity and bone formation despite reducing PTH levels. These results seem to disagree with the suggestion that the ABD in DN is due to low PTH levels that are induced by effects of advanced glycation end products on PTH secretion (30). They are compatible with an effect of hyperlipidemia on mesenchymal stem cell lineage commitment to adipocytes at the expense of osteoblasts, which sevelamer may have reversed.

Conclusion

The studies reported here demonstrate a reduction of established vascular calcium deposits in LDLR^{-/-} mice that has CKD and were fed a high-fat diet. The mechanisms of sevelamer action most likely included reduction of hyperphosphatemia. However, other actions of sevelamer to reduce hypercholesterolemia and to stimulate bone formation rates and alleviating the adynamic bone disorder have also may contributed to the effects of sevelamer on vascular calcium deposits.

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Disclosures

None.

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