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Skeletal levels of bisphosphonate in the setting of chronic kidney disease are independent of remodeling rate and lower with fractionated dosing

Elizabeth A. Swallow[a], Mohammad W. Aref[a], Corinne E. Metzger[a], Spencer Sacks[a], Demi R. Lehmkuhler[a], Neal Chen[b], Max A. Hammond[a], Paul R. Territo[e], Thomas L. Nickolas[f], Sharon M. Moe[b,d], Matthew R. Allen[a,b,c,d,*]

[a] Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States
[b] Department of Medicine – Division of Nephrology, Indiana University School of Medicine, Indianapolis, IN, United States
[c] Department of Biomedical Engineering, Indiana University Purdue University of Indianapolis, Indianapolis, IN, United States
[d] Roudebush Veterans Administration Medical Center, Indianapolis, IN, United States
[e] Department of Radiology, Indiana University School of Medicine, Indianapolis, IN, United States
[f] Department of Medicine, Columbia University Medical Center, New York, NY, United States

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ABSTRACT

Background: Chronic kidney disease (CKD) results in a dramatic increase in skeletal fracture risk. Bisphosphates (BP) are an effective treatment for reducing fracture risk but they are not recommended in advanced CKD. We have recently shown higher acute skeletal accumulation of fluorescently-tagged zoledronate (ZOL) in the setting of CKD but how this accumulation is retained/lost over time is unclear. Furthermore, it is unknown if alternative dosing approaches can modulate accumulation in the setting of CKD.

Methods: To address these two questions normal (NL) and Cy/+ (CKD) rats were divided into control groups (no dosing), a single dose of a fluorescent-tagged ZOL (FAM-ZOL), a single dose of non-labelled zoledronate (ZOL) or ten weekly doses of FAM-ZOL each at 1/10th the dose of the single dose group. Half of the CKD animals in each group were provided water with 3% calcium in drinking water (CKD + Ca) to suppress PTH and remodeling. At 30 or 35 weeks of age, serum, tibia, ulna, radius, vertebra, femora, and mandible were collected and subjected to assessment methods including biochemistry, dynamic histomorphometry and multi-spectral fluorescence levels (using IVIS SpectrumCT).

Results: FAM-ZOL did not significantly reduce bone remodeling in either NL or CKD animals while Ca supplementation in CKD produced remodeling levels comparable to NL. At five- and ten-weeks post-dosing, both CKD and CKD + Ca groups had higher levels of FAM-ZOL in most, but not all, skeletal sites compared to NL with no difference between the two CKD groups suggesting that the rate of remodeling did not affect skeletal retention of FAM-ZOL. Fractionating the FAM-ZOL into ten weekly doses led to 20–32% less ($p < 0.05$) accumulation/retention of compound in the vertebra, radius, and ulna compared to administration as a single dose.

Conclusions: The rate of bone turnover does not have significant effects on levels of FAM-ZOL accumulation/retention in animals with CKD. A lower dose/more frequent administration paradigm results in lower levels of accumulation/retention over time. These data provide information that could better inform the use of bisphosphonates in the setting of CKD in order to combat the dramatic increase in fracture risk.

1. Introduction

Chronic kidney disease (CKD) is characterized not only by altered kidney function, but also by dramatic alterations in bone and mineral metabolism contributing to the systemic disease known as chronic kidney disease-mineral bone disorder (CKD-MBD) [1,2]. Currently, one in ten Americans suffer from CKD and the healthcare burden associated with CKD is only expected to rise [3]. As kidney function declines, many system functions are affected including altered metabolic regulation of mineral metabolism which drives hyperparathyroidism and hyperphosphatemia [2]. These changes have established effects on the skeleton resulting in the deterioration of skeletal properties primarily in the cortical compartment with elevations in cortical porosity. Ultimately, this leads to a striking increase in fracture risk and fracture-

* Corresponding author at: Dept. of Anatomy and Cell Biology, MS 5035, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202.
E-mail address: matallen@iu.edu (M.R. Allen).

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related mortality among CKD patients [4–8].

Treatments for renal osteodystrophy are currently limited. The most common therapeutic agent used in other bone loss-related diseases, such as post-menopausal osteoporosis, are bisphosphonates, a class of anti-remodeling agents that inhibit osteoclast bone resorption. Bisphosphonates are an effective treatment for osteoporosis with a reduction in fracture risk and increase in bone mass [9,10]. Bisphosphonate use in early-stage CKD (stages 1–3) is an acceptable method of treatment according to recommendations by the Kidney Disease Improving Global Outcomes committee (KDIGO); yet their use in later stage CKD (stages 3–5) is discouraged when patients have evidence of biochemical abnormalities, such as secondary hyperparathyroidism, typical of CKD-MBD [1,11]. The concern for use of bisphosphonates among renally impaired patients come from studies utilizing rapid infusion rates and high peak concentrations of bisphosphonates (zoledronate and pamidronate) typical of preventive or therapeutic treatment of bone metastases, resulting in evidence of nephrotoxicity [12,13]. In addition to nephrotoxicity, there are concerns that bisphosphonates may accumulate in excess in the skeleton due to their dependence on the kidney to be cleared. Therefore, severe CKD cases might lead to drug overexposure and over suppression of remodeling within the skeleton in CKD patients compared to individuals with normal kidney function [14–16]. Recent work from our lab supports this concern, as rats with CKD displayed significantly higher accumulation within the skeleton after an acute dose of fluorescently-labelled zoledronate [17]. However, there is still an absence of data assessing the impact of skeletal bisphosphonate accumulation over time in conditions of varying turnover rates and with different dosing regimens [18].

The goal of this study was to measure fluorescently-tagged zoledronate accumulation and retention within the skeleton in a rodent model of progressive chronic kidney disease. We hypothesized that reduced kidney function would result in higher skeletal accumulation of bisphosphonates. We also hypothesized that skeletal accumulation of bisphosphonates would be modulated by both the frequency of bisphosphonate dosing and bone turnover levels.

2. Methods

2.1. Animals

The male Cy/+ Han:SPRD rat (referred to as CKD hereafter) derived from the colony maintained at Indiana University School of Medicine, represents an animal model of spontaneous and slowly progressing kidney disease [2]. Our previous work has shown evidence of 50% reduction in kidney function at ~20–25 weeks of age compared to normal littermates (NL) [2]. As animals age, the kidney disease progresses to the point of about 10–15% kidney function by 35 weeks. Throughout this time CKD-MBD characteristics develop with prominent hyperphosphatemia, hyperparathyroidism, vascular calcification, and skeletal deterioration [2,19–23].

2.2. Experimental design

All procedures conducted during this study were reviewed and approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee. Animals were given group designation (NL or CKD) at 10 weeks of age based on blood urea nitrogen (BUN, > 30 mg/dL designated as CKD). At 24 weeks of age, all animals began a casein-based diet (Harlan Teklad TD.04539), a method that has been shown to produce a more consistent model of CKD progression [2]. Animals were used in one of two different experiments (Fig. 1).

2.2.1. Experiment 1–30 weeks

At 25-weeks of age, NL (n = 18) and CKD animals (n = 18) were randomly divided (within genotype) into six groups (n = 6 animals each):

NL - control group (no dosing).
NL - a single subcutaneous (SQ) 180 μg/kg dose of a fluorescent-tagged ZOL (FAM-ZOL) at 25 weeks of age.
NL - a single dose of 100 μg/kg non-labelled zoledronate (ZOL) at 25 weeks of age.
CKD - control group (no zoledronic acid dosing).
CKD - a single SQ 180 μg/kg dose of a fluorescent-tagged ZOL (FAM-ZOL) at 25 weeks of age.
CKD - a single SQ 180 μg/kg dose of a fluorescent-tagged ZOL (FAM-ZOL) at 25 weeks of age with 3% calcium gluconate in drinking water (starting at 24 weeks of age (7 days before FAM-ZOL dose) and continuing throughout the experiment) to suppress bone remodeling throughout the study [21,24].

This FAM-ZOL has been previously used in our work and others to allow for assessment of accumulation within the bone [17,25]. In order to evaluate the remodeling suppression activity of FAM-ZOL animals were administered a single subcutaneous dose (180 μg/kg). The manufacture data sheet recommends dosing at 50–100 nmol/kg, yet based on the discussions with the manufacturer a dose of 255 nmol/kg (180 μg/kg) may be necessary for remodeling suppression. Thus, this dose was chosen for the current work. A group of NL animals were administered a subcutaneous single dose of non-labelled zoledronic acid (ZOL; 100 μg/kg). This dose of zoledronate has been previously shown to be effective in suppressing remodeling [21,24] and although it represents a higher molar concentration than FAM-ZOL (367 nmol/kg) we have previously shown a lower dose of ZOL (20 μg/kg; 74 nmol/kg) suppresses remodeling to a similar degree as the higher dose. Calcium water supplementation (3% calcium gluconate) was provided ad libitum in order to suppress bone turnover, which occurs in this CKD model to a similar degree as ZOL treatment [21,24].

All animals were administered a subcutaneous calcein injection (30 μg/kg) thirteen and six days before the 30-week endpoint enabling the assessment of dynamic bone formation. As previously described in our work, calcein blue was used to avoid spectral overlap with FAM-ZOL [17,26]. At 30 weeks of age animals were anaesthetized with isoflurane before euthanasia by exsanguination. Serum, tibia, ulna, radius, L3 vertebra, femora, and mandible were collected. Tissues were stored in 10% NBF for 24 h before switching to 70% EtOH for long term storage [17].

2.2.2. Experiment 2–35 weeks

At 25 weeks of age, NL and CKD animals were divided into the below groups (n = 6/gp):

NL - control group (no dosing)
NL - a single subcutaneous (SQ) 180 μg/kg dose of a fluorescent-tagged ZOL (FAM-ZOL) at 25 weeks of age.
NL - weekly SQ doses of 18 μg/kg FAM-ZOL starting at 25 weeks of age
CKD - control group (no dosing)
CKD - a single SQ 180 μg/kg dose of FAM-ZOL at 25 weeks of age
CKD - weekly SQ doses of 18 μg/kg FAM-ZOL starting at 25 weeks of age
CKD - a single SQ 180 μg/kg dose of FAM-ZOL and supplemental with 3% calcium gluconate in drinking water (starting at 24 weeks of age (7 days before FAM-ZOL dose) and continuing throughout the experiment).
CKD - weekly SQ doses of 18 μg/kg FAM-ZOL and supplemental with 3% calcium gluconate in drinking water (starting at 24 weeks of age (7 days before FAM-ZOL dose) and continuing throughout the experiment).

The animals who received a single dose of FAM-ZOL were

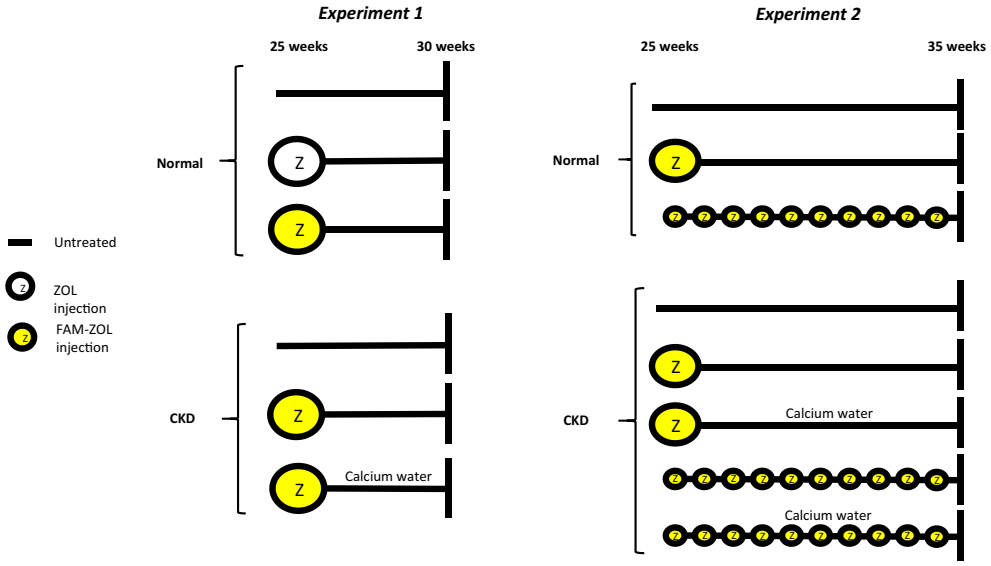

Fig. 1. Experimental design and treatments for experiment 1 (30 weeks) and 2 (35 weeks).

administered a single dose of FAM-ZOL subcutaneously (180 μg/kg) at 25 weeks of age. Similar to experiment 1, 3% calcium gluconate was supplemented to drinking water in select groups to create a low turnover CKD model [21,24]. Calcium water supplementation started at 24 weeks and continued throughout the duration of the study until euthanasia at 35 weeks of age. Some animals received ten weekly FAM-ZOL injections at 1/10th of the single FAM-ZOL dose (18 μg/kg per injection). This dose was arbitrarily determined by diving the total dose equally over the experimental period. Parallel to experiment 1, all animals received a subcutaneous calcein blue injection (30 μg/kg) thirteen and six days before euthanasia and collection of tissue at 35 weeks of age.

2.3. Biochemistries

Blood (~500 μL) was collected from the tail vein at 25-weeks of age, prior to treatment, to determine baseline biochemistries. Blood was again measured at experimental endpoint (30 or 35 weeks) prior to euthanasia. BUN (BioAssay systems #DIUR-100), calcium (Pointe Scientific, #C7503-480), and phosphorus (Pointe Scientific, #P7516-500) were analyzed using colorimetric assays. PTH was measured using an ELISA assay (Immunotopics).

2.4. Multi-spectral decomposition (MSD)

Mandible, radius, ulna, vertebra (3rd lumbar, L3), distal femur, and proximal tibia were assessed for whole bone fluorescence using reflectance epi-fluorescence imaging (IVIS SpectralCT, PerkinElmer). Our lab and others have previously used this method as an assay to measure fluorescently-tagged bisphosphonate levels in tissues [17,25,27,28]. Mandible, radius, and ulna were scanned whole whereas, vertebrae processes were removed, and distal femur and proximal tibiae were cut to 10 mm standard length sections. For each skeletal site, a bone from each animal was run on a single plate to assume uniform scan setting. While this allows comparison across animals within bone site, the optimization of settings to accommodate for the size differences among bone sites restricts the ability to compare levels across bone sites. Exposure time was approximately 1 s per sample plate using broad emission spectral excitation and emission ranging of 430-465 nm and 500-540 nm, respectively. FAM-ZOL signal was distinguished from auto fluorescence and calcein blue signals by spectrally unmixing the image series (Living Image, PerkinElmer). In all cases, bone fluorescence levels are reported as average radiant efficiency ([photons/s]/[μW/cm2]).

2.5. Histology

L3 vertebra were infiltrated and embedded with methyl methacrylate as previously described in our laboratory [29]. Vertebra were sectioned in the parasagittal plane for trabecular bone assessment. All slides were cover slipped with a Eukitt, a non-fluorescent mounting medium (Kindler, Freiburg, Germany). Sections were analyzed for dynamic histomorphometry parameters (Bioquant) from images collected by 2-photon microscopy with this method being previously described as it enabled the visualization of calcein blue [17]. Trabecular dynamics were determined from a region ~0.5 mm distal to the proximal vertebral growth plate. Mineral apposition rate (MAR. %), mineralizing surface (MS/BS, %) and bone formation rate (BFR/BS, μm3/μm2/year) were calculated using standard methods [30].

2.6. Statistics

All analyses were performed using GraphPad software. For the 30-week experiment, the goals were two-fold: first, to assess the effect of remodeling suppression NL, CKD, NL-ZOL, NL-FAM-ZOL, CKD-FAM-ZOL and CKD-Ca-FAM-ZOL were compared in a One-Way ANOVA. Second, to assess accumulation/retention of FAM-ZOL the three groups receiving FAM-ZOL (NL-FAM-ZOL, CKD-FAM-ZOL and CKD-Ca-FAM-ZOL) were compared with one-way ANOVA. For both tests, if the ANOVA $p < 0.05$, a Tukey post-hoc analysis was completed. For the 35 week experiments, primary assessment of FAM-ZOL accumulation was determined using One-Way ANOVA on those receiving single doses of compound (NL-FAM-ZOL, CKD-FAM-ZOL and CKD-Ca-FAM-ZOL). To assess the role of fractionated dosing, a Two-Way ANOVA was used for each skeletal site to study the main effects of condition (normal (NL), low (CKD), high (CKD + Ca)) and dose (single, fractionated) and then the interaction between the two. In all cases, statistical significance was determined when a priori two tailed p values were ≤ 0.05.

3. Results

At the 30-week timepoint, 5 weeks after a single dose of FAM-ZOL, there was no significant effect on trabecular bone dynamic bone remodeling parameters in either NL or CKD groups relative to the non-dosed control (Fig. 2, Table 1). In contrast, a single dose of ZOL in NL animals produced a robust suppression of MS/BS, MAR, and BFR

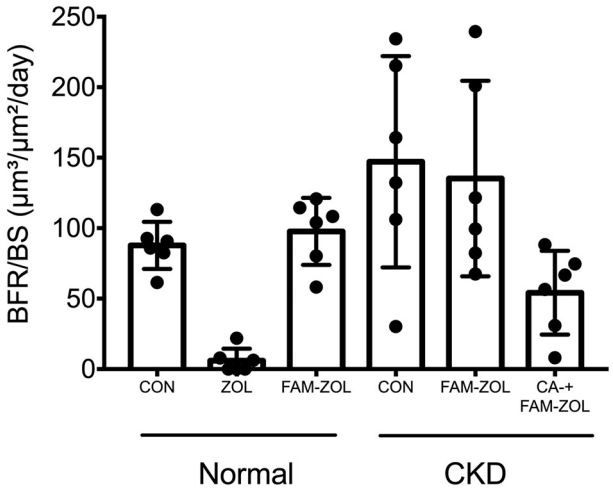

Fig. 2. Proximal tibia bone formation rate (BFR/BS) by histomorphometry analysis, 5-weeks post dosing. Data is presented as the mean and standard deviation with individual data points presented. * denotes a significant difference ($p < 0.05$) between NL control and NL ZOL. # denotes significance ($p < 0.05$) between CKD control and CKD Ca FAM-ZOL.

compared to untreated NL animals. CKD animals supplemented with calcium water also had significantly lower bone remodeling compared to CKD controls. These data demonstrate that FAM-ZOL, at this dose, does not suppress remodeling despite robust surface binding [17]. Based on this finding, results presented hereafter are considered to provide insight into FAM-ZOL accumulation/retention in the setting of high and low turnover (CKD and CKD + Ca, respectively).

The 30-week experiments (representing five weeks post-dosing) revealed that both high and low turnover CKD animals had significantly higher levels of FAM-ZOL in mandible, distal femur, and radius compared to NL (Fig. 3; Supplementary Table 1). The mandible had significantly lower FAM-ZOL levels in CKD + Ca compared to CKD. The L3 was significantly higher than NL only in low turnover (CKD + Ca) while the proximal tibia was higher only in higher turnover (CKD). The ulna did not show any significant differences.

The 35-week experiments (representing ten weeks post-dosing) revealed a similar pattern of results as was quantified at 5 weeks post-dosing (Fig. 4; Supplementary Table 1). Proximal tibia, distal femur, and mandible were significantly higher in FAM-ZOL accumulation in both high and low turnover CKD animals, relative to NL, with no difference between the two CKD groups. Levels of accumulation in L3 and the radius were significantly higher in low turnover CKD (CKD + Ca) relative to NL. The ulna did not show significant differences among groups.

There were significant main effects of dosing regimen (single dose versus fractionated dose) in the radius, ulna, and L3 (Fig. 5; Table 2). Those animals receiving a fractionated dose had significantly less accumulation/retention of drug, independent of treatment. The lone exception to this pattern was at the distal femur where only low turnover CKD animals had lower levels of accumulation with the fractionated dose compared to the full dose (Fig. 5).

Serum biochemistries, noting presence/absence of kidney disease (assessed by BUN) and hyperparathyroidism (assessed by PTH) showed expected results in groups from both experiments (Table 3).

4. Discussion

This study aimed to characterize the effects of bisphosphonate accumulation/retention in the context of progressively advancing chronic kidney disease. Our results show that a single dose of fluorescently labelled zoledronate (FAM-ZOL) is retained in the skeleton to a greater degree in rats with CKD versus those with normal kidney function irrespective of whether there is high or low turnover. Additionally, this study also demonstrates that more frequent, smaller doses of FAM-ZOL results in lower skeletal accumulation/retention regardless of disease state (NL and CKD) or level of turnover (high vs low). Alternative dosing schedules such as this could represent a path forward for dosing bisphosphonates in patients where renal safety is of a concern, although additional work on bone mechanical properties and renal function would need to be investigated first.

Zoledronate (ZOL) is a clinically relevant drug commonly used in osteoporosis to slow bone loss and reduce fracture risk. With a high affinity binding to hydroxyapatite, zoledronate is a potent suppressor of bone turnover, and when unbound to the bone surface, it is excreted unmetabolized by the kidneys [21]. Elimination through the kidney has raised concern that in the setting of reduced kidney function, higher levels of drug will be exposed to the kidney (potentially causing nephrotoxicity) and may also accumulate in the skeleton. Our lab has previously shown that the dose of zoledronate used in this study, as well as doses 5× lower, produces a robust reduction in turnover markers (BFR, MAR, and MS/BS) and thus we chose to use this specific bisphosphonate, as opposed to the numerous others, for the studies described herein [21,24]. Fluorescently labelled zoledronate (FAM-ZOL) enabled unique tracking of the compound and our previous work documents that this compound retains its high affinity binding properties illustrated by the fact that it covers the majority of surfaces (~80–100%) as assessed 24 h post dosing [17].

In agreement with our previous short-term study of increased FAM-ZOL accumulation and retention in CKD animals 24 h post-dosing [17] these results demonstrate that fluorescently-labelled zoledronate levels are significantly higher in animals with CKD compared to littermates with normal kidney function irrespective of bone remodeling rate (CKD versus CKD + Ca) (Figs. 3 and 4). Calcium water supplementation was utilized to suppress PTH levels and showed, similar to previous work [21], a robust suppression of turnover (Table 1 and Fig. 2) that was equivalent to ZOL at 5 weeks post-treatment. Assessment of accumulation in this low turnover group has clinical implications as roughly 20% of patients with stage 3–5 CKD have low bone turnover; this number increases to nearly 50% in patients undergoing dialysis [31]. Importantly, we document here that there was minimal difference in accumulation between CKD rats with high PTH/high turnover (CKD no dosing) and CKD rats with low PTH/low turnover (CKD + Ca) (Figs. 3

Table 1
Proximal tibia dynamic histomorphometry data at 5 weeks post dosing.

	NL			CKD			
	CON	ZOL	FAM-ZOL	CON	FAM-ZOL	FAM-ZOL + Ca	Overall ANOVA p value
MAR, um/day	1.02 ± 0.18	0.38 ± 0.43*	1.08 ± 0.17	1.32 ± 0.35	1.38 ± 0.45	0.87 ± 0.26	< 0.0001
MS/BS,%	23.7 ± 4.4	2.7 ± 1.9*	24.5 ± 4.08	28.5 ± 9.9	26.8 ± 8.7	16.3 ± 8.6	0.0001

Data presented as mean and standard deviation. NL-normal; CKD-chronic kidney disease; CON – no dosing; ZOL – zoledronate; FAM-ZOL – fluorescently-tagged zoledronate; CA – calcium.

* $p < 0.05$ vs NL-CON.

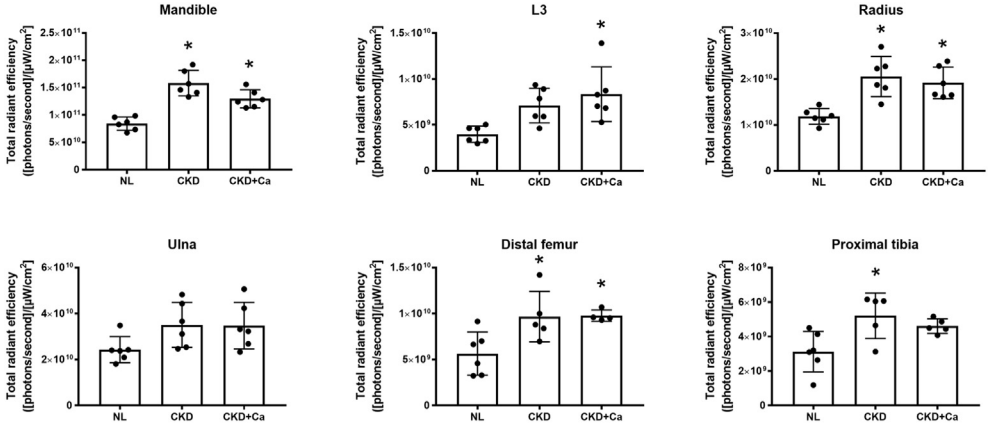

Fig. 3. Skeletal accumulation of FAM-ZOL displayed as total radiant efficiency at multiple skeletal sites 5-weeks after a single dose of FAM-ZOL. Data is presented as means and standard deviation with individual data points displayed. * denotes significance ($p < 0.05$) vs. normal control based on a one-way ANOVA.

and 4). Important in the interpretation of our results, the initial dosing of FAM-ZOL in the CKD + Ca group would have been in a state of high/moderate low turnover due to the administration and commencement of calcium water treatment simultaneously. Our previous work has shown that at the age of dosing (25 weeks old), CKD animals have 60% higher trabecular bone formation rate (BFR) compared to normal. We are unable to know the rapidity with which Ca treatment reduces bone formation rate although unpublished data from our lab show that by 7 days post Ca treatment, CKD animals have significantly higher trabecular BV/TV – a variable that further increases with longer duration Ca treatment. Thus, within the first seven days (the duration prior to BP dosing in the current work) the Ca has begun to have a biological effect. That said, we do not know the remodeling rate of these animals and so cannot know turnover levels at the time of dosing FAM-ZOL. While this could have influenced initial binding, the handling of FAM-ZOL would have occurred in a lower turnover state which we know exists for the majority of the experiment given the suppression at 30 weeks.

FAM-ZOL levels in bone were significantly higher in CKD animals at both 5- and 10-weeks post injection suggesting that there is long-term retention of the drug is not significantly dependent on turnover state (Figs. 3 and 4). Although the retention of drug in low-remodeling states is intuitive, the retention in high remodeling states seems less so. We hypothesize that in the setting of high turnover CKD, some of the FAM-ZOL is liberated from the bone but, due to reduced kidney function and high bone affinity, it is recirculated and becomes rebound rather than excreted. In support of this, a qualitative analysis of histological samples from select high-turnover CKD animals showed secondary bands of FAM-ZOL (Supplementary Fig. 1). Unfortunately, it was not possible to quantitatively differentiate levels of drug that were the result of the original dose versus that which is recirculated.

CKD rats, irrespective of turnover rate, retain higher levels of FAM-ZOL in the skeleton. Although many strategies could be employed to try and reduce accumulation, we chose to test the hypothesis that smaller, more frequent doses of FAM-ZOL would lower levels within bone. Our rationale for this approach is that, in addition to potentially lowering levels in the bone, a more frequent dose would allow a continued 'resurfacing' of bone surfaces with drug to slow resorption. This portion of the experiment provided clear evidence that irrespective of disease state, CKD or NL, the accumulation of FAM-ZOL in bone was lower with fractionated dosing (Fig. 5). In CKD animals, again bone turnover rate did not have significant effects as both high and low turnover animals had lower accumulation/retention of FAM-ZOL at multiple skeletal sites when compared to the single bolus dose (Fig. 5). Similar to the single dose, the early fractionated doses of CKD + Ca FAM-ZOL would likely have been administered prior to the effectiveness of Ca to suppress turnover yet subsequent fractionated doses, the majority of them, would have been in a low turnover state. Therefore, a greater proportion of the fractionated dose in the CKD + Ca group occurred during a lower turnover state compared to the single dose individuals.

The overall finding demonstrating a lower accumulation/retention when fractionating the bisphosphonate dose into more frequent and lower doses is beneficial for rethinking the paradigm for studying bisphosphonates in CKD patients. We speculate that smaller doses of bisphosphonates permit a greater initial clearance of bisphosphonate from the kidneys before it is able to localize to bone. Potentially more of the bisphosphonate is excreted when given in smaller and more frequent doses since the kidneys are able to handle the smaller amounts of the drug more efficiently. Alternatively, the lower accumulation in the fractionated dose could be explained by the progressive lowering of turnover over time. From a clinical standpoint, providing more frequent

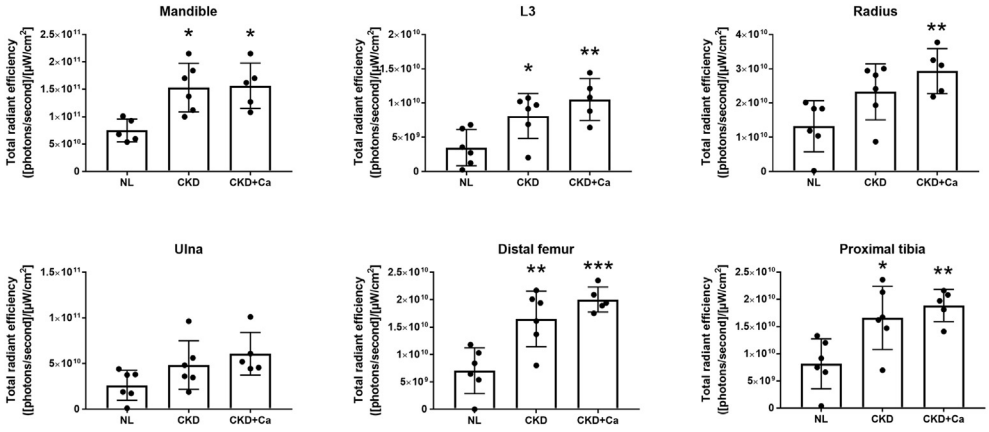

Fig. 4. Skeletal accumulation of FAM-ZOL displayed as total radiant efficiency at multiple skeletal sites 10-weeks after a single dose of FAM-ZOL. Data is presented as means and standard deviation with individual data points displayed. Significance is denoted * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. normal control based on a one-way ANOVA.

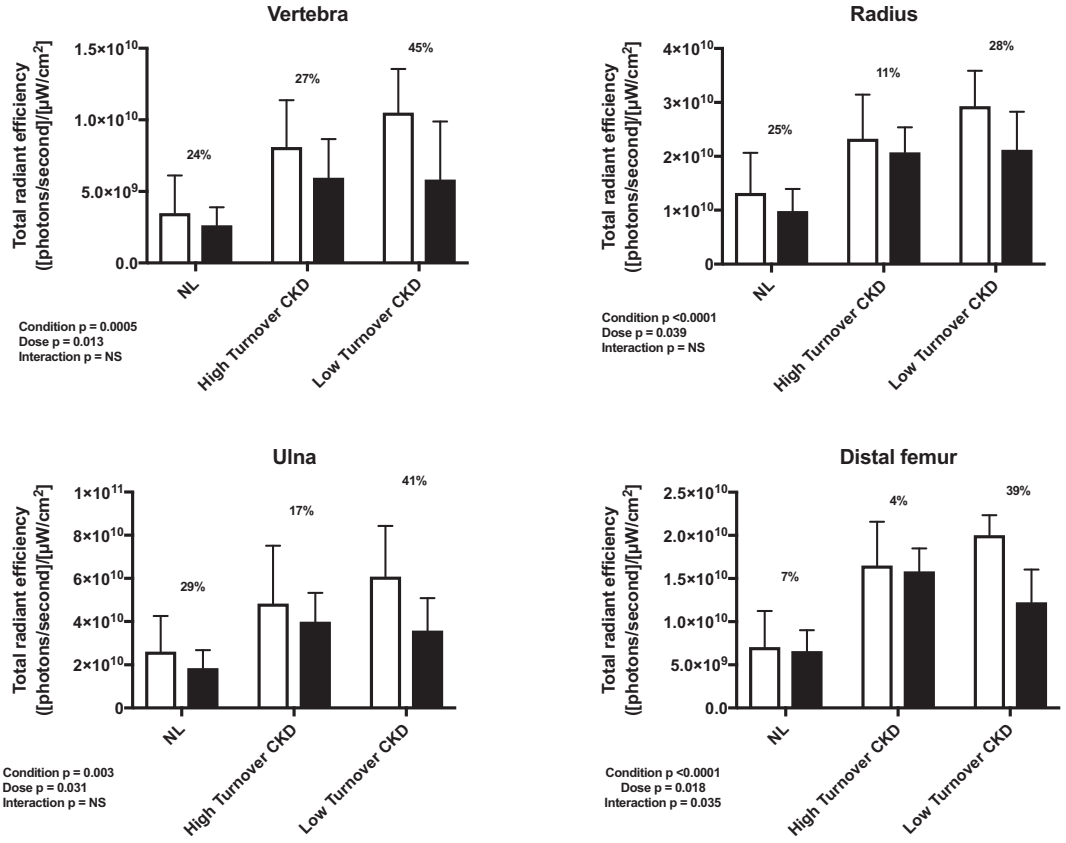

Fig. 5. Skeletal accumulation of fractionated FAM-ZOL dosing measured as total radiant efficiency by a two-way ANOVA at multiple skeletal sites. Data is presented as means and standard deviation. Clear and black bars represent single and fractionated dosing respectively. Values for interaction, condition, and disease affects are presented with significance determined when $p < 0.05$. * notes a significant difference within condition when an interaction term was significant. Percent differences comparing single vs. fractionated dosing for each treatment group across all skeletal site are displayed above the paired bar graphs.

dosing of zoledronate would seem unrealistic due to its IV administration, other bisphosphonates such as alendronate and risedronate, typically used in oral form, may be more conducive to dosing on a weekly or even monthly basis. How this alternative dosing regimen affects other aspects of the disease, such as the progression of skeletal resorption (bone porosity), reduction in mechanical properties, as well as kidney function will necessitate additional studies with active bisphosphonate.

Data regarding bisphosphonate handling (accumulation/retention) has existed for decades and is highlighted by the elegant studies using numerous doses/regimens of alendronate in rodents. These studies documented that post-dose binding occurs predominantly within the first 6 h post dose and the skeleton can be saturated for a single dose but that fractionating with repeated doses has the potential to lead to higher accumulation than single doses. There is a less robust understanding of how splitting a single dose into smaller but more frequent administrations (as done here) affects accumulation. Additionally, there is currently no data about drug retention/recycling. In vivo bound drug is thought to be released during resorption but then also freely dissociates from surfaces. The relative proportion between these two processes is not clear. This work has been undertaken almost exclusively in normal animals and thus the current work advances these findings to a disease model where the main method of drug elimination (kidney) is compromised.

There are limitations to this study which are important to note. Firstly, FAM-ZOL at this dose did not suppress bone turnover compared to zoledronate (Fig. 1 and Table 1). Despite previous work suggesting in vivo efficacy of FAM-ZOL (based on trabecular BV/TV changes), our work utilizing the gold-standard method of dynamic histomorphometry clearly shows a lack of effect on bone remodeling as compared to the native zoledronate drug. We hypothesize that the addition of a fluorescent adduct to the nitrogen side chain (R_2) changes FAM-ZOL ability to bind to the active site of the farnesyl diphosphate synthase (FPPS) enzyme which is essential in the inhibition of the FPPS pathway and subsequent osteoclast inhibition. Since FAM-ZOL compound modifications do not affect the hydroxyl (R_1) chain, regular bone and mineral binding can still occur as can be clearly noted in the current work. The dose of FAM-ZOL, chosen based on the recommendation of the manufacturer, was lower on a nmol/kg basis (255 nmol/kg) than ZOL (367 nmol/kg), yet in our previous work we have shown that this dose of ZOL (367 nmol/kg; 100 μg/kg) and a lower dose of ZOL (74 nmol/kg; 20 μg/kg) equally suppressed bone formation rate. Thus, the lower dose of FAM-ZOL is unlikely the explanation although we cannot discount that a higher dose of FAM-ZOL could have biological activity.

Despite the lack of effect, we believe the results are still noteworthy as they address FAM-ZOL accumulation/retention dynamics in the setting of high and low turnover CKD. These studies support and extend our previous short-term retention study findings showing that there is a disparity between bisphosphonate handling between CKD and normal individuals. Secondly, zoledronate was the only bisphosphonate assessed in this study, therefore, the generalizability of results to other bisphosphonates should be approached with caution. Furthermore, zoledronate and FAM-ZOL were administered subcutaneously rather than IV, as typically done in patients. However, as described previously, IV and SC dosing have been shown to exhibit comparable FAM-ZOL accumulation [17]. We can also not discount the contribution of the fluorescent conjugation to zoledronate affecting dynamics of accumulation/retention. Finally, our results assume that the low turnover state that comes from Ca water supplementation is similar to that which occurs from an active bisphosphonate and this may not be the case.

Table 2
Effects of dose fractionation on skeletal accumulation/retention of FAM-ZOL.

Condition	NL		CKD (high turnover)		CKD + Ca (low turnover)		p Value		
Dose	1 dose	10 doses	1 dose	10 doses	1 dose	10 doses	Dose	Condition	Int.
Mandible	$75.1 \times 10^9 \pm 20.8 \times 10^9$	$60.9 \times 10^9 \pm 17.0 \times 10^9$	$153 \times 10^9 \pm 44.4 \times 10^9$	$152 \times 10^9 \pm 35.7 \times 10^9$	$156 \times 10^9 \pm 41.4 \times 10^9$	$114 \times 10^9 \pm 28.3 \times 10^9$	0.1014	< 0.0001	0.3296
Proximal tibia	$8.15 \times 10^9 \pm 4.6 \times 10^9$	$6.52 \times 10^9 \pm 3.25 \times 10^9$	$16.6 \times 10^9 \pm 5.79 \times 10^9$	$15.7 \times 10^9 \pm 5.46 \times 10^9$	$18.9 \times 10^9 \pm 2.97 \times 10^9$	$14.2 \times 10^9 \pm 5.95 \times 10^9$	0.1551	< 0.0001	0.6249
Ulna	$26.1 \times 10^9 \pm 16.5 \times 10^9$	$18.4 \times 10^9 \pm 8.36 \times 10^9$	$48.4 \times 10^9 \pm 26.7 \times 10^9$	$40.0 \times 10^9 \pm 13.3 \times 10^9$	$60.8 \times 10^9 \pm 23.4 \times 10^9$	$35.8 \times 10^9 \pm 15.0 \times 10^9$	0.0307	0.003	0.4562
Radius	$13.2 \times 10^9 \pm 7.45 \times 10^9$	$9.87 \times 10^9 \pm 4.09 \times 10^9$	$23.3 \times 10^9 \pm 8.18 \times 10^9$	$20.8 \times 10^9 \pm 4.61 \times 10^9$	$29.3 \times 10^9 \pm 6.59 \times 10^9$	$21.2 \times 10^9 \pm 7.08 \times 10^9$	0.0388	< 0.0001	0.5479
L3	$3.48 \times 10^9 \pm 2.64 \times 10^9$	$2.63 \times 10^9 \pm 1.26 \times 10^9$	$8.11 \times 10^9 \pm 3.26 \times 10^9$	$5.96 \times 10^9 \pm 2.7 \times 10^9$	$10.5 \times 10^9 \pm 3.05 \times 10^9$	$5.83 \times 10^9 \pm s4.05 \times 10^9$	0.0134	0.0005	0.3082
Distal Femur	$7.06 \times 10^9 \pm 4.19 \times 10^9$	$6.59 \times 10^9 \pm 2.42 \times 10^9$	$16.5 \times 10^9 \pm 5.07 \times 10^9$	$15.8 \times 10^9 \pm 2.67 \times 10^9$	$20.0 \times 10^9 \pm 2.28 \times 10^9$	$12.3 \times 10^9 \pm 3.77 \times 10^9$ *	0.0182	< 0.0001	0.0351

Data presented as mean and standard deviation. All values are reported as: average radiant efficiency ([photons/s]/[μW/cm2]). NL-normal; CKD-chronic kidney disease; CA – calcium.
* $p < 0.05$ compared to 1 dose within condition.

Table 3
Body mass and serum biochemistries.

	Normal				CKD					
	CON	ZOL	FAM-ZOL	FAM-ZOL FRAX	CON	FAM-ZOL	FAM-ZOL FRAX	CA + FAM-ZOL	CA + FAM-ZOL FRAX	Overall ANOVA p value
30-week experiment										
Body mass, g	536 ± 25	541 ± 27	523 ± 10	–	561 ± 22	554 ± 30	–	541 ± 23	–	0.1904
Final BUN, mg/dL	19.1 ± 1.7	18.2 ± 1.8	18.4 ± 1.1	NA	39.7 ± 6.0*	37.5 ± 7.6*	NA	42.4 ± 4.9*	NA	< 0.0001
FINAL PTH, pg/ml	376 ± 298	–	379 ± 256	NA	420 ± 379	475 ± 230	NA	195 ± 159	NA	0.4900
35-week experiment										
Body mass, g	590 ± 34	NA	573 ± 44	549 ± 33	512 ± 57	569 ± 43	503 ± 60	549 ± 60	487 ± 84*	0.027
Final BUN, mg/dL	18.1 ± 1.8	NA	19.8 ± 2.5	18.9 ± 1.5	50.4 ± 8.0*	45.9 ± 6.5*	50.1 ± 8.0*	49 ± 9.4*	53.7 ± 8.7*	< 0.0001
FINAL PTH, pg/ml	123 ± 49	NA	–	–	2105 ± 340*	1403 ± 664*	1266 ± 831*	33 ± 42	49 ± 45	< 0.0001

Data presented as mean and standard deviation. NA – group was not included at that timepoint; – data not measured. NL-normal; CKD-chronic kidney disease; CON – no dosing; ZOL – zoledronate; FAM-ZOL – fluorescently-tagged zoledronate; CA – calcium.
* $p < 0.05$ vs Normal CON.

In conclusion we have documented that FAM-ZOL accumulates and is retained at significantly higher levels in the setting of CKD compared to normal and that remodeling rate is not having a significant effect on accumulation/retention in CKD. Additionally, administering FAM-ZOL with more frequent and lower doses appears to be a viable mechanism to reduce the level of drug accumulation in the skeleton in both normal and CKD settings.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.07.007.

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References

[1] M. Ketteler, G.A. Block, P. Evenepoel, M. Fukagawa, C.A. Herzog, L. McCann, S.M. Moe, R. Shroff, M.A. Tonelli, N.D. Toussaint, M.G. Vervloet, M.B. Leonard, Diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder: synopsis of the kidney disease: improving global outcomes 2017 clinical practice guideline update, Ann. Intern. Med. 168 (2018) 422–430, https://doi.org/10.7326/M17-2640.

[2] S.M. Moe, N.X. Chen, M.F. Seifert, R.M. Sinders, D. Duan, X. Chen, Y. Liang, J.S. Radcliff, K.E. White, V.H.G. Ii, A rat model of chronic kidney disease-mineral bone disorder (CKD-MBD) and the effect of dietary protein source, Kidney Int. 75 (2009) 176–184, https://doi.org/10.1038/ki.2008.456.A.

[3] S Department of Health and Human Services, Centers for Disease Control and Prevention, National Chronic Kidney Disease Fact Sheet, 2017, https://www.cdc.gov/diabetes/pubs/pdf/kidney_factsheet.pdf, (2017).

[4] H.H. Malluche, D.S. Porter, M.-C. Monier-Faugere, H. Mawad, D. Pienkowski, Differences in bone quality in low- and high-turnover renal osteodystrophy, J. Am. Soc. Nephrol. 23 (2012) 525–532, https://doi.org/10.1681/ASN.2010121253.

[5] E.M.B. McNerny, T.L. Nickolas, Bone quality in chronic kidney disease: definitions and diagnostics, Curr. Osteoporos. Rep. 15 (2017) 207–213, https://doi.org/10.1007/s11914-017-0366-z.

[6] S. Moe, T. Drüeke, J. Cunningham, W. Goodman, K. Martin, K. Olgaard, S. Ott, S. Sprague, N. Lameire, G. Eknoyan, Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO), Kidney Int. 69 (2006) 1945–1953, https://doi.org/10.1038/sj.ki.5000414.

[7] H.H. Malluche, D.S. Porter, D. Pienkowski, Evaluating bone quality in patients with chronic kidney disease, Nat. Rev. Nephrol. 9 (2013) 671–680, https://doi.org/10.1038/nrneph.2013.198.

[8] D. Nitsch, A. Mylne, P.J. Roderick, L. Smeeth, R. Hubbard, A. Fletcher, Chronic kidney disease and hip fracture-related mortality in older people in the UK, Nephrol. Dial. Transplant. 24 (2009) 1539–1544, https://doi.org/10.1093/ndt/gfn678.

[9] D.B. Burr, M.R. Allen, Bisphosphonates and PTH for preventing fractures, in: M. Silva (Ed.), Skelet. Aging Osteoporos, 5th ed., Springer, Berlin, Heidelberg, 2012.

[10] R. Eastell, J.S. Walsh, N.B. Watts, E. Siris, Bisphosphonates for Postmenopausal Osteoporosis, Bone 49 (2011) 82–88, https://doi.org/10.1016/j.bone.2011.02.011.

[11] M. Ketteler, G.A. Block, P. Evenepoel, M. Fukagawa, C.A. Herzog, L. McCann, S.M. Moe, R. Shroff, M.A. Tonelli, N.D. Toussaint, M.G. Vervloet, M.B. Leonard, Executive summary of the 2017 KDIGO chronic kidney disease–mineral and bone disorder (CKD-MBD) guideline update: what's changed and why it matters, Kidney Int. 92 (2017) 26–36, https://doi.org/10.1016/j.kint.2017.04.006.

[12] P.D. Miller, Chronic kidney disease and the skeleton, Bone Res. 2 (2014), https://doi.org/10.1038/boneres.2014.44.

[13] M.A. Perazella, G.S. Markowitz, Bisphosphonate nephrotoxicity, Kidney Int. 74 (2008) 1385–1393, https://doi.org/10.1038/ki.2008.356.

[14] A.W.Y. Shih, M.A. Weir, K.K. Clemens, Z. Yao, T. Gomes, M.M. Mamdani, D.N. Juurlink, A. Hird, A. Hodsman, C.R. Parikh, R. Wald, S.M. Cadarette, A.X. Garg, Oral bisphosphonate use in the elderly is not associated with acute kidney injury, Kidney Int. 82 (2012) 903–908, https://doi.org/10.1038/ki.2012.227.

[15] N. Toussaint, G.J. Elder, P.G. Kerr, Bisphosphonates in chronic kidney disease; balancing potential benefits and adverse effects on bone and soft tissue, Clin. J. Am. Soc. Nephrol. 4 (2009) 221–233, https://doi.org/10.2215/CJN.02550508.

[16] D. Haffner, D.C. Fischer, Can bisphosphonates play a role in the treatment of children with chronic kidney disease? Pediatr. Nephrol. 26 (2011) 2111–2119, https://doi.org/10.1007/s00467-010-1739-z.

[17] E.A. Swallow, M.W. Aref, N. Chen, I. Byiringiro, M.A. Hammond, B.P. McCarthy, P.R. Territo, M.M. Kamocka, S. Winfree, K.W. Dunn, S.M. Moe, M.R. Allen, Skeletal accumulation of fluorescently tagged zoledronate is higher in animals with early stage chronic kidney disease, Osteoporos. Int. 29 (2018) 2139–2146, https://doi.org/10.1007/s00198-018-4589-3.

[18] M.R. Allen, M.W. Aref, What animal models have taught us about the safety and efficacy of bisphosphonates in chronic kidney disease, Curr. Osteoporos. Rep. 15 (2017) 171–177, https://doi.org/10.1007/s11914-017-0361-4.

[19] S.M. Moe, N.X. Chen, C.L. Newman, J.M. Organ, M. Kneissel, I. Kramer, V.H. Gattone, M.R. Allen, Anti-sclerostin antibody treatment in a rat model of progressive renal osteodystrophy, J. Bone Miner. Res. (2015), https://doi.org/10.1002/jbmr.2372.

[20] M.R. Allen, C.L. Newman, N. Chen, M. Granke, S. Jeffry, S.M. Moe, Changes in skeletal collagen crosslinks and matrix hydration in high and low turnover chronic kidney disease, Osteoporos. Int. 26 (2015) 977–985, https://doi.org/10.1007/s00198-014-2978-9.Changes.

[21] M.R. Allen, N.X. Chen, V.H. Gattone, X. Chen, A.J. Carr, P. Leblanc, D. Brown, S.M. Moe, Skeletal effects of zoledronic acid in an animal model of chronic kidney disease, Osteoporos. Int. 24 (2013) 1471–1481, https://doi.org/10.1007/s00198-012-2103-x.

[22] C.L. Newman, S.M. Moe, N.X. Chen, M.A. Hammond, J.M. Wallace, J.S. Nyman, M.R. Allen, Cortical bone mechanical properties are altered in an animal model of progressive chronic kidney disease, PLoS One 9 (2014) 1–8, https://doi.org/10.1371/journal.pone.0099262.

[23] S.M. Moe, J.S. Radcliffe, K.E. White, V.H. Gattone, M.F. Seifert, X. Chen, B. Aldridge, N.X. Chen, The pathophysiology of early-stage chronic kidney disease-mineral bone disorder (CKD-MBD) and response to phosphate binders in the rat, J. Bone Miner. Res. 26 (2011) 2672–2681, https://doi.org/10.1002/jbmr.485.

[24] S.M. Moe, N.X. Chen, C.L. Newman, V.H. Gattone, J.M. Organ, X. Chen, M.R. Allen, A comparison of calcium to zoledronic acid for improvement of cortical bone in an animal model of CKD, J. Bone Miner. Res. 29 (2014) 902–910, https://doi.org/10.1002/jbmr.2089.

[25] A. Hokugo, S. Sun, S. Park, C.E. McKenna, I. Nishimura, Equilibrium-dependent bisphosphonate interaction with crystalline bone mineral explains anti-resorptive pharmacokinetics and prevalence of osteonecrosis of the jaw in rats, Bone 53 (2013) 59–68, https://doi.org/10.1016/j.bone.2012.11.030.

[26] C. Pautke, S. Vogt, T. Tischer, G. Wexel, H. Deppe, S. Milz, M. Schieker, A. Kolk, Polychrome labeling of bone with seven different fluorochromes: enhancing fluorochrome discrimination by spectral image analysis, Bone 37 (2005) 441–445, https://doi.org/10.1016/j.bone.2005.05.008.

[27] K.M. Kozloff, L. Quinti, S. Patntirapong, P.V. Hauschka, C.H. Tung, R. Weissleder, U. Mahmood, Non-invasive optical detection of cathepsin K-mediated fluorescence reveals osteoclast activity in vitro and in vivo, Bone 44 (2009) 190–198, https://doi.org/10.1016/j.bone.2008.10.036.

[28] K.M. Kozloff, L.I. Volakis, J.C. Marini, M.S. Caird, Near-infrared fluorescent probe traces bisphosphonate delivery and retention in vivo, J. Bone Miner. Res. (2010), https://doi.org/10.1002/jbmr.66.

[29] R.K. Fuchs, M.R. Allen, K.W. Condon, S. Reinwald, L.M. Miller, D. McClenathan, B. Keck, R.J. Phipps, D.B. Burr, Strontium ranelate does not stimulate bone formation in ovariectomized rats, Osteoporos. Int. 19 (2008) 1331–1341, https://doi.org/10.1007/s00198-008-0602-6.

[30] D.W. Dempster, J.E. Compston, M.K. Drezner, F.H. Glorieux, J.A. Kanis, H. Malluche, P.J. Meunier, S.M. Ott, R.R. Recker, A.M. Parfitt, Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR histomorphometry nomenclature committee, J. Bone Miner. Res. 28 (2013) 2–17, https://doi.org/10.1002/jbmr.1805.

[31] K.D.I.G.O. (KDIGO) C.M. Work, KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD), Kidney Int. 76 (2009), https://doi.org/10.1038/ki.2009.188 Clinical.