The Influence of Chronic Kidney Disease on the Structural and Mechanical Properties of Canine Bone

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Chronic kidney disease (CKD) is common both in human and animal patients and its prevalence substantially increases with age. The disease is irreversible and progressive in nature, and as it progresses, clinical signs become apparent and metabolic derangements worsen. One of the inevitable consequences of CKD is secondary renal hyperparathyroidism. There are many factors involved in the pathophysiology of secondary renal hyperparathyroidism including phosphorous retention, hyperphosphatemia, decreased blood ionized calcium concentration, decreased circulating 1,25-dihydroxyvitamin D (calcitriol) concentration, and increased parathyroid hormone (PTH) and fibroblast grown factor-23 (FGF-23) concentrations. The latter is produced mainly by osteocytes and osteoblasts and has been shown to play a pivotal role in mineral homeostasis in healthy individuals. It has been shown in humans that, in the very early stages of CKD, when patients are still normophosphatemic and have normal PTH concentrations, serum FGF-23 concentrations are already increased. Recently, it has been shown that FGF-23 concentration also increases in dogs with CKD as the disease progresses and is associated with mineral abnormalities such as hyperphosphatemia.

In a study of cats with spontaneous CKD, the overall prevalence of renal secondary hyperparathyroidism was 84% and was documented in 100% of cats with end-stage CKD. In dogs with CKD, hyperparathyroidism was diagnosed in 76% of the patients and was documented in 100% of dogs with advanced (stage IV) CKD based on the International Renal Interest Society classification scheme.

Parathyroid hormone promotes bone resorption by activating osteoclasts in the presence of osteoblasts. Consequently, as the concentration of PTH increases in CKD, the resorption-deposition balance is disrupted, potentially resulting in several changes such as decreased bone mineral density (BMD) and resorptive lesions, resulting in decreased bone quality and increased bone fragility. In humans, CKD is associated with decreased bone quality and increased risk for fracture. The United States Renal Data System identified a 4-fold increase in the risk of hip fractures in human dialysis patients as compared to the general population. The overall risk for pathological fractures in human CKD patients

Background: Chronic kidney disease (CKD) is common in companion animals. Secondary hyperparathyroidism is an inevitable consequence of the disease and may have deleterious effect on the bone; however, the information regarding CKD-associated bone abnormalities in companion animals is scarce.

Hypothesis/Objectives: Dogs with CKD have decreased bone quality compared to dogs without CKD.

Animals: Nine dogs diagnosed with naturally occurring CKD for at least 6 months and 9 age-matched controls.

Methods: Dogs with CKD were enrolled and compared to 9 age-, weight-, and sex-matched control dogs with no evidence of CKD. Samples were assessed using light microscopy, mechanical testing, and microcomputed tomography. Variables evaluated included microstructural features such as number, size, and density of Haversian canals, resorption cavities and osteocytic lacunae, bone mineral density, porosity and Young’s modulus.

Results: Median lacucae size was significantly smaller in the CKD group compared to the control group (P = 0.001). Resorption cavity density was higher in the CKD compared to the control group (10 [8–14] vs. 7 [4–9]/mm², respectively, P = 0.001). Overall porosity was significantly (2.3-fold) higher in the CKD compared to the control group. There was no difference in Young’s moduli between groups.

Conclusions and Clinical Importance: Naturally occurring CKD affects bone quality in dogs, but these changes are relatively mild and likely not to be manifested clinically. The duration of the disease in dogs evaluated here is short compared to cats and human patients, likely accounting for the more subtle changes seen dogs compared to other species.

Key words: biomechanics; bone; chronic kidney disease; renal failure; skeleton; structure.

Abbreviations:

| Abbreviation | Description |
|--------------|-------------|
| BMD          | bone mineral density |
| CKD          | chronic kidney disease |
| CT           | computed tomography |
| DEXA         | dual energy X-ray absorptiometry |
| QCT          | quantitative-CT |
| RI           | reference interval |

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increases by 9% with every 200 pg/mL increase in PTH concentration, and by 72% with a PTH concentration >900 pg/mL (reference range, 150–300 pg/mL). In companion animals however, information regarding bone changes associated with CKD is scarce, and their clinical relevance is questionable.

The overall mechanical performance and quality of whole bone depends on bone geometry (e.g., length, curvature, cortical thickness), properties of the material of which it is made (mostly BMD and porosity), and its micro- and nanarchitecture. Previous assessment of bone quality in human patients has focused on bone mass or BMD as predictors of fracture risk, but there is mounting evidence that decreased BMD is not the sole factor responsible for increased fracture risk. Bone microarchitecture, bone turnover rate, degree of mineralization of the bone matrix, and amount of microdamage affect bone quality and may play roles in fracture susceptibility.

Bone changes associated with secondary hyperparathyroidism in dogs and cats might be insidious and clinically silent. In a recent study, deleterious effects of naturally occurring CKD on bone quality were documented in cats both in cortical and cancellous bone, but the effect of CKD and secondary hyperparathyroidism on bone metabolism in dogs has not been studied extensively. The aim of our study was to evaluate the effect of CKD on canine bones.

Materials and Methods

Animals and Data Collection

This prospective study was approved by the institutional animal care and use committee of the Veterinary Teaching hospital of the Hebrew University. Dogs were enrolled only after their owners had signed an informed consent form and donated the animal’s body to science. Dogs considered for the study were presented to the Veterinary Teaching Hospital of the Hebrew University of Jerusalem, and either died, or were euthanized at the owner’s request, when medical management had failed. The study group consisted of 9 dogs, diagnosed with CKD for at least 6 months before death or euthanasia. Diagnosis was based on persistently increased serum creatinine concentration (>1.4 mg/dL), concurrent low urine specific gravity (<1.020), ultrasonographic changes consistent with CKD (e.g., small kidneys, hyperechoic cortices, poor corticomедullary differentiation), and was confirmed histopathologically (presence of diffuse and extensive interstitial fibrosis) at necropsy in all dogs.

The control group included 9 age-, sex- and body weight-matched dogs without any evidence of CKD (normal serum creatinine concentration, concentrated urine) that died or were euthanized in the Veterinary Teaching Hospital for reasons unrelated to diseases of the urinary system. Dogs with concurrent metabolic diseases that could potentially affect the skeleton were excluded, as were dogs that were treated for >2 weeks during the 6 months before their death with medications that could alter bone metabolism (e.g., vitamin D derivatives, corticosteroids).

Sample Collection and Preparation

Whole blood and serum samples from all dogs were collected antemortem for CBC and biochemical analysis. Tissue sample collection was performed within 12 hours of death. The right femora were carefully removed and cleaned of all soft tissue, wrapped in saline-soaked gauze, placed in a sealed plastic bag, and stored at −20°C until testing.

Light Microscopy

Thin transverse slices (400 μm thick) of the mid-diaphyseal region of all right femora were cut by a water-cooled slow-speed diamond saw. The slices then were polished by increasingly fine grit, from 320 grit to 1 μm diamond paste. Transverse cross-sections of all cortical samples were viewed by reflective light microscopy and their detailed architecture characterized by analysis of images captured by a dedicated high-resolution camera.

Image analysis was performed with public domain image processing software. Several microstructural variables were measured, such as number, size, and density of Haversian canals, resorption cavities, and osteocytic lacunae. Images underwent binarization, differentiating between “bone” (white) and “void” (black) entities. Next, all voids within the bone were measured using the ImageJ function “Analyze particles” which reports the size of each void within a selected area. Based on values of osteocytic lacunar sizes reported in the literature, and on the distribution of the sizes of voids in our specimens, voids within the bone matrix within a size range of 9–150 μm² were considered to be osteocytic lacunae, voids within a size range of 151–2000 μm² were considered to be Haversian canals, whereas voids >2000 μm² were considered resorption cavities. Voids <9 μm² were considered artifacts. Images also were visually examined by 2 of the authors (AS and NS), and categorization was altered if indicated.

Mechanical Testing

Mechanical properties of cortical bone were evaluated using 4-point bending tests performed on bone beams prepared from the cranial aspect of the mid-diaphyseal cortical region of the right femora. Beam sizes were 20 x 1.5 x 1 mm (long dimension along the bone axis).

Mechanical testing was performed with the samples immersed in saline, using a custom-built micromechanical testing device, as previously described. All samples were thawed immediately before testing for 1 hour at room temperature. The beams were placed within a saline-containing testing chamber that had a stationary anvil attached to its wall. This anvil consisted of 2 supports, which were 15 mm apart. A movable double-pronged loading anvil was attached to a load cell, which was in turn attached to a high-precision linear motor. The loading anvil had a span of 5 mm between its 2 prongs, which were centered between the 2 supports of the stationary anvil. The upper prongs were brought into contact with the tested beams at a predetermined preload (2N), the chamber was filled with physiologic saline solution at room temperature until the samples were fully immersed, and bending tests were conducted under displacement control at a rate of 140 μm/min. Force-displacement data were collected by custom-written software at 50 Hz. Load and displacement results were converted to stress and strain, respectively, based on beam theory. Stress-strain curves were used to estimate Young’s modulus of the beam material. Care was taken to minimize shear deformation at the supports by maintaining a ratio of distance between supports/beam depth of 15:1.

Microstructural Characterization by Micro-CT

All cortical beams, the right femoral diaphysis and the head of the femur were scanned by micro-CT, with the beams scanned after undergoing mechanical testing solely to assure that no major
structural defects were present. For cortical bone analysis, the measurements were performed on the femoral diaphysis (i.e., not on the cortical beams used for mechanical testing) and BMD and porosity were determined. For cancellous bone, bone volume/total volume, trabecular thickness, and trabecular number were measured.

The X-ray source was set at 50 kVp and 800 μA. A total of 450 projections were acquired over an angular range of 180°. The samples were scanned with an isotropic voxel size of 11.1 μm for the cortical bone beams and 19.6 μm for the femoral cortex and cancellous bone in the head of the femur. Integration time for all scans was 4,500 ms, and a 0.25 mm aluminum filter was used. Scans were reconstructed and analyzed using commercial software. Analyses were performed on entire beams, the mid-diaphyseal region of the femur, and cancellous bone in the head of the femur. Integration time for all scans was 4,500 ms, and a 0.25 mm aluminum filter was used. Scans were reconstructed and analyzed using commercial software. Analyses were performed on entire beams, the mid-diaphyseal region of the femur (cortical bone analysis), and cancellous bone in the femoral head (cancellous bone analysis). One-hundred and fifty slices in the mid-diaphyseal region were used for cortical analysis, and 100 slices above and 100 below the remnant of the growth plate were used for trabecular analysis. Cortical BMD was determined based on calibration with 2 phantoms of known mineral density (0.25 and 0.75 g/cm³) supplied by SkyScan®, which were scanned under exactly the same conditions as were the bone specimens.

**Statistical Analysis**

The distribution of continuous variables (normal vs. non-normal) was assessed using the Shapiro-Wilk’s test. Results are reported as mean and standard deviation or as median and range (or interquartile range) based on data distribution. Continuous variables were compared between the study and the control groups using generalized estimating equations, Student’s t-test or the Mann–Whitney U-test, respectively. Continuous variables that were measured multiple times in the same patient were compared between the study and the control group using generalized estimating equations. Sex proportion between the study group and the control group included neoplastic disease (hemangiosarcoma) (2 cases), esophageal mass, abdominal mass, megaesophagus complicated by aspiration pneumonia, head trauma (2 cases), hemotorax and hemoaobdome, and cholangiohepatitis with mucocoele.

There was no significant difference in body weight between dogs with CKD compared to controls (14.4 ± 12.13 vs. 18.2 ± 11.80 kg, respectively; P = 0.55). There was no significant difference in mean age between the study and control groups (7.3 ± 4.3 compared to 8.0 ± 4.7 years, respectively; P = 0.7). All dogs in the study group were euthanized. Seven were euthanized because of deterioration or acute decompensation of the CKD, and 2 were euthanized for other reasons (congestive heart failure and leishmaniasis).

**Clinical Pathology**

Median serum creatinine concentration in the CKD group (at time of euthanasia) was 6.3 mg/dL (range, 1.9–29.6 mg/dL) compared with a median of 0.61 mg/dL (range, 0.48–0.77 mg/dL) for the control group (reference interval [RI] 0.5–1.4 mg/dL). Median serum urea concentration in the CKD group was 418 mg/dL (range, 82–597 mg/dL; RI, 20–40 mg/dL), median serum phosphorus concentration in the CKD group was 16.4 mg/dL (range, 4.6–23.5 mg/dL; RI, 5.0–6.2 mg/dL), and median serum total calcium concentration was 11.0 mg/dL (range, 6.5–15.0; RI, 9.7–11.7 mg/dL).

**Cortical Bone Architecture**

Dogs with CKD had significantly smaller lacunae, higher density of resorption cavities and higher porosity compared to healthy controls (Table 1, Fig 1). The CKD dogs also had smaller mean Haversian canal volume in the caudal and medial aspects of the cortex. Other structural variables were not significantly different between the groups (Table 2).

Micro-CT analysis of the cortical bone in the mid-diaphyseal area of the femur identified significantly higher porosity in the CKD dogs compared to the control group (0.7%; range, 0.38–9.1% vs. 0.3%; range, 0.1–1.1%, respectively; P = 0.007, Fig 1), whereas BMD was not different between the CKD dogs and the control group (1.20 g/cm³, range, 1.07–1.35 vs. 1.21 g/cm³; range, 0.99–1.37, respectively; P = 0.82, Fig 2).

**Mechanical Properties of Cortical Bone**

The median Young’s moduli were not significantly different between the CKD dogs and the control group (17.4; range, 12.3–32.4 vs. 27.1; range, 6.9–33.8, respectively; P = 0.45).

**Architecture of Trabecular Bone of the Femoral Head**

There was no significant difference in cancellous bone analysis between the CKD dogs and the control dogs (Table 3).

**Discussion**

Our study identifies that naturally occurring CKD in dogs results in mild deterioration of cortical bone quality, expressed as an increase in density of resorption cavities and increased cortical porosity, whereas the morphology of cancellous bone is not affected. Dogs
suffering from CKD also had smaller lacunae and smaller Haversian canals in the caudal and medial aspects of the cortex. These results also may provide insight into skeletal changes occurring in humans with CKD, because the pathophysiology of the syndrome and the type of bone architecture are very similar in dogs and humans.

In humans, advanced CKD is associated with decreased bone quality, resulting in increased fracture risk, whereas fractures are uncommonly reported in dogs diagnosed with CKD. The reason for this difference was not identified. Animals may be resistant to the detrimental effects documented in the skeleton of humans with CKD, or alternatively, such effects may occur in a similar manner but be less severe, or may not become clinically apparent during the much shorter course of the disease in dogs compared to human patients. Nevertheless, with improvements in medical management of CKD and increasing availability of hemodialysis in veterinary medicine, dogs with CKD live longer and these skeletal effects may become clinically relevant.

Recently, we documented that bone quality substantially deteriorates in cats with naturally occurring CKD. Changes include a decrease in BMD, cortical cross-sectional area, cortical cross-sectional thickness, Young’s modulus, yield stress, and ultimate stress, as well as an increase in resorption cavity density. The current study demonstrates that naturally occurring CKD in dogs also results in several alterations of the architecture and morphology of the bones. One of the changes documented in our study was increased cortical porosity, which is 1 of the major determinants of bone quality, showing an inverse power relationship with bone stiffness (Young’s modulus). The increase in cortical porosity is probably due to the significantly increased density of resorption cavities, which likely results from imbalance between bone resorption and deposition in the skeleton of CKD patients. Although porosity was more than 2-fold higher in CKD dogs, the absolute porosity was still low (below 2%) in both groups, which can explain the lack of difference in the material stiffness between the study groups.

The documented increase in area density of resorption cavities in cortical bone of dogs with CKD is in agreement with findings in cats. Larger resorption cavities are expected to increase the fragility of the affected bones. The cavities influence bone more than just by their contribution to porosity, but also by decreasing the ability of bone to resist fracture propagation. It has been shown that adding cavities to bone decreases strength and stiffness more than expected from the associated changes in bone volume, and therefore, changes in density of resorption cavities may influence the ability of bone to resist fracture even when differences in porosity are small. Previous studies in a rat model demonstrated that persistently increased PTH concentration results in high bone turnover, exhibited by increased numbers of osteoclasts, increased osteoblastic activity (as measured by histomorphometry), and enhanced bone resorption. This combination might account for the small and abundant resorption cavities documented in our study, which reflects high bone turnover.

Bone mineral density is another major determinant of bone quality, and currently considered the clinical standard for prediction of fracture risk in osteoporotic patients. Bone mineral density can be measured by several methods, including area BMD using dual energy X-ray absorptiometry (DEXA), volumetric BMD by quantitative-CT (QCT), ash content, and high-resolution micro-CT analysis. As opposed to findings in cats with CKD, the present study did not demonstrate a difference in BMD between the study and control groups. It is possible that BMD is maintained initially in the cortex, while the bone becomes more porotic. Alternatively, the lack of significant difference could have been caused by small group size, higher biological variation, and shorter disease course in dogs. A decrease in BMD was reported in various studies in human CKD patients, with a wide range of values (1.3% to 17.5%). However, these studies were based on areal BMD (g/cm²), using DEXA, histomorphometry, or pQCT, whereas we measured volumetric BMD at high resolution and precision using micro-CT.

Mechanical performance of whole bones is dependent upon their geometry and the mechanical properties of the bone material. Bone material and whole-bone strength and quality can be characterized effectively by biomechanical testing and assessment of its structure and composition. Bending tests are commonly used biomechanical methods to evaluate bone properties. With these techniques, bone (whole bone or prepared
geometric bone specimens) is loaded to induce bending under displacement control, and stiffness, yield strength, and energy to fracture are measured as surrogates of whole bone (in whole bone bending tests) or material (in geometric samples testing) strength and quality. In whole-bone testing, some biomechanical variables are indicators of whole-bone properties, whereas other, secondarily calculated variables are indicators of its intrinsic material properties. The most commonly assessed property of bone material is Young's modulus, which reflects the stiffness of the material. The 2 major determinants of Young's modulus are mineral density and porosity. Cortical porosity was higher in dogs with CKD compared to controls, but in absolute values, it was <2% in most dogs. The relatively small values of porosity in both groups and the lack of difference in BMD likely account for the lack of difference in the Young's moduli between groups.

Micro-CT is used to evaluate material and morphometric properties of bone. By scanning a bone specimen with high-resolution CT (pixel size in the micron range), variables of cortical bone (such as outer and inner bone diameters, BMD, porosity), and cancellous bone (trabecular thickness, trabecular separation, bone volume/total volume) can be measured accurately in 3 dimensions. The effect of CKD on cancellous bone among different species is inconsistent. Cancellous bone was affected in cats with CKD, with a marked

Table 2. Void densities in dogs with CKD and controls.

| Area Density               | CKD median (range) | Control median (range) | P value |
|---------------------------|--------------------|------------------------|---------|
| Lacunae                   | 907 (779–1322)     | 872 (633–1059)         | 0.5     |
| Blood vessel              | 61 (42–104)        | 59 (40–105)            | 0.89    |
| Resorption cavity         | 10 (8–14)          | 7 (4–9)                | 0.001   |

Table 3. Cancellous bone morphology in dogs with CKD compared to controls.

| Parameters                  | CKD                | Control               | P value |
|-----------------------------|--------------------|-----------------------|---------|
| BV/TV (%)                   | 33 (23–44)         | 35 (15–48)            | 0.85    |
| Trabecular thickness (μm)   | 64 (48–105)        | 68 (44–104)           | 0.73    |
| Trabecular number (1/mm)    | 4.7 (3.8–14)       | 4.8 (1.5–8.2)         | 1.0     |
| Trabecular separation (μm)  | 244 (140–644)      | 266 (185–549)         | 0.70    |

Fig 1. (A) Light microscopy images of two transverse cross-sections of the femoral mid-diaphysis of dogs with CKD and control dogs. Note, increase in unfilled resorption cavities in CKD dogs compared to controls. (B) Box and whisker plot depicting the difference in porosity between CKD and control dogs. The horizontal line within the box represents the median. The box represents the interquartile range (2nd and 3rd quartiles), and the whiskers represent the range. (C) Box and whisker plot depicting the quantitative difference in resorption cavity density between CKD and control dogs. The horizontal line within the box represents the median. The box represents the interquartile range (2nd and 3rd quartiles), and the whiskers represent the range.

Fig 2. Box and whisker plot depicting the difference in bone mineral density between CKD and control dogs. The horizontal line within the box represents the median. The gray box represents the interquartile range (2nd and 3rd quartiles), and the whiskers represent the range.
decrease in trabecular thickness and lower bone volume (BV/TV). Both changes (trabecular thickness and BV/TV) negatively affect bone quality and were shown to be associated with increased risk for fracture.\(^42\) The effect of CKD on cancellous bone of rodents and humans is inconsistent and was found to have no effect or even an anabolic effect.\(^36,38,43\) We did not demonstrate differences between groups in the morphometry of cancellous bone. The inconsistency of the effect of CKD on cancellous bone may be related to species differences, variability in experimental methods, nature (naturally occurring vs induced) of the disease, its severity and its chronicity.

Some of the differences between dogs and cats can be explained by the different progression rate of the disease. In dogs, CKD tends to progress more rapidly compared to cats. Because secondary hyperparathyroidism is 1 of the early consequences of CKD, cats, like humans, may be exposed to the metabolic derangements associated with CKD for years, as opposed to dogs in which CKD often progresses over several months or a few years, and therefore, despite physiologic similarities in mineral metabolism, the effects on bone quality are less pronounced.

Because both dogs and cats show alteration in bone quality and morphology, these species should be considered as alternative models for the human disease. Bone changes associated with CKD are being extensively studied in rodent models. However, often bones are remarkably different from human bones in terms of type, architecture, structure, and biology. Most dramatically, rodent cortical bone does not remodel. Conversely, both canine and feline adult skeletons show many structural similarities to human bones, and consisting mostly of secondary osteons, remodel continuously, as do human bones.\(^36,44–46\)

Another disadvantage of studying rodent models of CKD is that the disease does not occur naturally and must be induced chemically, genetically, or surgically. Conversely, dogs and cats have a high prevalence of naturally occurring CKD. Therefore, these species are more likely to be a better model for the effects of CKD on the skeleton in humans. Reliable measurement of material bone properties also requires precise and accurate mechanical testing of carefully prepared geometric samples of cortical bone, such as beams or cubes. Such testing is difficult to achieve in rodents because of the small size of their bones, which often are tested by 3-point bending technique applied to whole bones. Such testing is hampered by various technical problems, mostly an unfavorable aspect ratio causing substantial shear at the supports, often leading to underestimation of Young's modulus.\(^47,48\) Canine and feline bones have much thicker cortices, which allows preparation of cortical bone beams and enables geometric samples of cortical bone, such as beams or cubes. Such testing is difficult to achieve in rodents and feline bones have much thicker cortices, which might be a better model for the effects of CKD on cancellous bone. The inconsistency of the effect of CKD on cancellous bone and controversy over the relationship between trabecular bone parameters and bone fracture risk.\(^49\)

In conclusion, we observed only mild deleterious effects of naturally occurring canine CKD on bone quality in dogs, including marginally increased porosity, increased bone resorption and changes in lacunae size. These changes are substantially less pronounced compared to a similar study in cats with CKD and a larger scale study is warranted to draw more definitive conclusions.

Footnotes

\(^a\) Buehler Isomet low Speed saw
\(^b\) Buehlet Minimet Polisher
\(^c\) Olympus BX-51
\(^d\) Olympus DP 71, 12 MegaPixels
\(^e\) ImageJ, NIH, v. 1.44p
\(^f\) model 31, Honeywell Sensotec, Columbus, OH
\(^g\) PI GmbH, Karlsruhe, Germany
\(^h\) LabView, National Instruments, Texas
\(^i\) Skyscan 1174 compact micro-CT scanner, Belgium
\(^j\) NRecon\(^\text{®}\) Skyscan\(^\text{®}\) software, version 1.6.1.2
\(^k\) CT analyser\(^\text{®}\) Skyscan software, version 1.9.3.2, respectively
\(^l\) SPSS 22.0 for Windows\(^\text{®}\), SPSS Inc; Chicago, IL

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.
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