Effects of zoledronate on markers of bone metabolism and subchondral bone mineral density in dogs with experimentally induced cruciate-deficient osteoarthritis

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Objective—To evaluate effects of zoledronate on markers of bone metabolism in dogs after transection of the cranial cruciate ligament (CrCL).

Animals—21 adult dogs.

Procedure—Unilateral CrCL transection was performed arthroscopically. Dogs were allocated to 3 groups (control group, low-dose zoledronate [10 µg/kg, SC, q 90 d for 12 months], and high-dose zoledronate [25 µg/kg, SC, q 90 d for 12 months]). Serum osteocalcin (OC), serum bone-specific alkaline phosphatase (BAP), and urine pyridinoline and deoxypyridinoline concentrations were measured at 0, 1, 3, 6, 9, and 12 months after surgery. Bone mineral density (BMD) was determined in the distal portion of the femur and proximal portion of the tibia via computed tomography at each time point. Data were analyzed by a repeated-measures ANOVA.

Results—Zoledronate inhibited OC in the high-dose group at 9 and 12 months and at 12 months in the low-dose group, compared with the control group. High-dose zoledronate decreased BAP concentrations 3 and 9 months after surgery. In the control group, BMD was decreased in the femoral condyle and caudal tibial plateau. Zoledronate prevented significant BMD decreases starting 1 month after transection, compared with control dogs. In the caudomedial aspect of the tibial plateau, both zoledronate groups had significant increases in BMD after 3 months, compared with control dogs.

Conclusions and Clinical Relevance—Zoledronate may reduce subchondral bone loss and effect markers of bone metabolism in dogs with experimentally induced instability of the stifle joint and subsequent development of osteoarthritis. (*Am J Vet Res* 2005;66:1487–1495)

Periarticular bone structures are altered during the development of **osteoarthritis (OA)**. In the late stages of OA, the subchondral bone plate becomes thick and sclerotic,[1] but there is also evidence suggesting an early decrease of subchondral **bone mineral density (BMD)** in osteoarthritic joints.[2–5] Both increased and decreased stiffness of the subchondral bone can alter the mechanical stresses inflicted on the overlying cartilage and result in damage.[6,7] Although there is evidence that the bone changes are not required to initiate damage in the cartilage,[8,9] others have reported[10–13] increases in bone metabolic activity prior to cartilage damage and loss. Thus, there is the possibility that the bony changes may preclude and possibly contribute to cartilage degradation.

Bisphosphonates (BPs) are a class of drugs used clinically as inhibitors of bone resorption. Increased periarticular bone resorption may allow BPs to have a possible beneficial therapeutic action in animals with OA. Assuming BPs are able to inhibit bone resorption and maintain normal BMD and subchondral bone stiffness in animals with OA, they may minimize the degradation of the overlying articular cartilage.

The exact mechanism by which BPs inhibit bone resorption is not completely understood. It is believed that BPs are adsorbed to the surface of bones and taken up by osteoclasts where they interfere with intracellular biochemical pathways. There are several types of BPs that act via various mechanisms. Bisphosphonates that are similar in structure to pyrophosphate are believed to be metabolized into nonhydrolyzable analogues of ATP, which will inhibit ATP-dependent intracellular pathways.[14,15] Nitrogen-containing BPs may inhibit the mevalonate pathway and therefore prevent synthesis of isoprenoid compounds that are required for posttranscriptional prenylation of guanosine triphospates, which will eventually lead to apoptosis of osteoclasts.[15–17] In other studies,[18–21] it has been suggested that BPs exert the aforementioned effects on osteoclasts by first affecting other cells, such as osteoblasts and osteoclast precursors. Zoledronate, a third-generation heterocyclic BP containing an imidazol ring in the R2 position, is one of the most potent antiresorptive BPs.[22–24]

In the study reported here, the effects of zoledronate on bone metabolism were evaluated during the

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development of experimentally induced OA in dogs with a transected **cranial cruciate ligament (CrCL)**. Biochemical markers of bone metabolism and BMD were measured temporally during a 1-year period. The biochemical markers evaluated were serum concentrations of **osteocalcin (OC)** and **bone-specific alkaline phosphatase (BAP)** and urine concentrations of **deoxypyridinoline (Dpd)** and **pyridinoline (Pyd)**. Our hypothesis was that zoledronate would decrease markers of bone metabolism and slow subchondral bone loss in the stifle joint following CrCL transection and protect against BMD loss seen in early OA.

Materials and Methods

Animals—Twenty-one skeletally mature male mixed-breed hound-type dogs of similar body conformation were used in the study. Dogs ranged from 11 to 24 months of age and weighed between 20 and 35 kg. Skeletal maturity was documented on the basis of radiographic evidence of closure of growth plates. Inclusion criteria included a medical history of no lameness or stifle joint disease and results within anticipated limits for a complete physical examination, CBC count, serum biochemical analyses, urinalysis, analysis of synovial fluid obtained from each stifle joint, and radiography of the stifle joints and pelvis. All procedures were approved by the University of Georgia Animal Care and Use Committee (No. 2000-10099).

Transection of the CrCL—A complete arthroscopic examination was performed to document the initial status of the articular cartilage. After the arthroscopic examination, dogs were returned to their runs and allowed to acclimate for 3 weeks prior to the beginning of the study. Each dog was allowed unrestricted activity within its run (2×6.2 m^2) for the duration of the study.

The left CrCL was arthroscopically transected in each dog. Perioperative pain was managed by administration of butorphanol tartrate (0.2 mg/kg, IM) as part of the preanesthetic medications and again at the time of endotracheal extubation. Butorphanol was administered as needed during the next 24 hours when a dog did not use the limb during ambulation immediately after recovery from anesthesia. After that time period, the dogs did not receive any medications, except for a monthly heartworm preventative.

Experimental design—Dogs were randomly allocated to 1 of 3 groups (7 dogs/group). These were a control group, low-dose group, and high-dose group. Dogs were administered injections on the day after the CrCL transection and at intervals of 90 (± 7) days for 12 months. Dogs in the control group received SC injections of an inert vehicle, whereas dogs in the low- and high-dose groups received SC injections of zoledronate (low-dose group, 10 µg/kg; high-dose group, 25 µg/kg).

All dogs were euthanatized 12 months after CrCL transection. The investigators were unaware of the treatment administered to each dog.

Sample collection—Samples of blood and urine were collected from each dog at the time of the CrCL transection (baseline) and 1, 3, 6, 9, and 12 months after transection. All samples were collected between 9 AM and 11 AM. Blood samples were collected from the jugular vein into serum separator tubes, allowed to clot, and then centrifuged to enable harvest of serum. Urine was obtained via aseptic insertion of a catheter into the urethra and bladder. A urine sample (6 to 8 mL) was obtained for analysis for each dog. Serum and urine samples were frozen at –80°C until assayed.

Serum OC concentrations—Serum concentrations of OC were measured by use of a commercially available **enzyme immunoassay (EIA)** kit[a] that has been documented to cross-react with samples obtained from dogs. The assay used monoclonal antibodies directed against the amino- and carboxy-terminal regions of intact OC. The assay was performed in accordance with the manufacturer's protocol on samples diluted 1:2. Samples were assayed in duplicate. Interassay and intra-assay variation was 10.5% and 7%, respectively. The dynamic range of the assay was between 1.5 and 50 ng/mL.

Serum BAP concentrations—Serum concentrations of BAP were measured by use of a commercially available EIA kit.[b] All samples were assayed without dilution. Interassay and intra-assay variation was 4% to 6% and 5% to 8%, respectively. The dynamic range of the assay was between 2 and 140 U/L.

Urine Dpd and Pyd concentrations—Urine concentrations of Dpd and Pyd were measured by use of commercially available EIA kits.[25,26,c,d] The competitive EIAs used monoclonal anti-Pyd and monoclonal anti-Dpd antibodies. Results for Pyd and Dpd were adjusted on the basis of urinary concentrations of creatinine. Samples were assayed primarily at a dilution of 1:40. Interassay and intra-assay variation for the Pyd assay was 3% to 11% and 6% to 10%, respectively, and corresponding variations for the Dpd assay were 4% to 8% and 3% to 5%, respectively. The dynamic range for the Pyd assay was between 15 and 750nM, whereas the dynamic range for the Dpd assay was between 3 and 30nM.

BMD analysis—Computed tomography (CT) was performed on each dog by use of a CT machine.[e] Before beginning each day of scanning, the machine was calibrated via a self-checking program and use of a water sample to establish a baseline density equal to zero. Each dog was then sedated by IM administration of a combination of medetomide (0.1 mg/kg) and butorphanol (0.2 mg/kg). Sedated dogs were positioned in dorsal recumbency with the hind limbs taped together and fully extended in a caudal direction to optimize parallel positioning.

The CT machine generated a series of transectional images (2 mm thick; 53 to 80 images/dog) of the left and right stifle joints for use in BMD analysis.[27] Sixteen 0.3- to 0.7-cm^2 regions of interest were pinpointed for analysis. Four areas of interest were measured on medial and lateral aspects of the weight-bearing surface of the femoral condyle. These regions were labeled 1 to 4 beginning with the most cranial

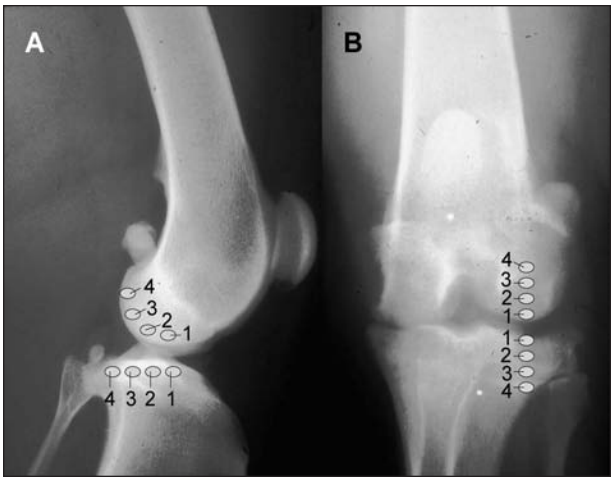

Figure 1—Lateromedial (A) and craniocaudal (B) radiographic views depicting the location of the regions of interest (circles) in the distal portion of the femur and proximal portion of the tibia that were used for bone mineral density analysis. Locations were numbered from 1 (most cranial) to 4 (most caudal) for the femur and from 1 (most medial) to 4 (most lateral) for the tibia.

and progressing to the most caudal; thus, the regions were designated M1 to M4 for the medial aspect and L1 to L4 for the lateral aspect of the femoral condyle, respectively (**Figure 1**). Similarly, 4 regions were measured on the medial and lateral aspects of the weight-bearing surface of the tibial plateau. Again, they were numbered M1 to M4 and L1 to L4, similar to the process used for the femoral condyle. Once the articular surface was identified, the investigators evaluated BMD in triplicate on the cross-sectional slice third from the articular cartilage.[27] The analysis program of the CT machine created a circular area that was manually positioned in the approximate center of the trabecular bone in the aforementioned regions (ie, medial and lateral aspects of the femoral condyle and tibial plateau). After completion of CT, medetomide was reversed by administration of atipamezole (0.1 mg/kg, IM).

Statistical analyses—An ANOVA was used to compare data between groups. Data at differing time points were modeled as repeated measurements. Normality of residuals was achieved by use of a suitable logarithmic or power transformation. To compare data with baseline values, the paired Wilcoxon test was used. All calculations were performed by use of commercial software.[28,f] Pearson correlation coefficients were also calculated between values of serum OC and BAP, urine Pyd and Dpd, serum and urine markers, and all BMD values. Significance was set at $P < 0.05$.

Results

Serum OC concentrations—Serum OC concentrations increased in the control group over time, with significant increases 9 and 12 months after CrCL transection, compared with baseline values (**Figure 2**). In the zoledronate-treated groups, no significant changes were found over time, compared with baseline values, except in the high-dose group 12 months after transection. Comparisons among groups revealed significantly lower serum OC concentrations 9 and 12 months after transection in the high-dose group and 12 months after transection in the low-dose group, compared with concentrations for the control group.

Serum BAP concentrations—Serum BAP concentrations did not change significantly over time in the control or low-dose groups. A significant decrease in serum BAP over time was detected in the high-dose group 3 and 9 months after CrCL transection, compared with baseline values (**Figure 3**). Comparisons among groups revealed no differences between the control and low-dose groups; however, a significantly lower serum concentration of BAP was found in the high-dose group 9 months after transection, compared with the value for the control group. Comparison between zoledronate-treated groups revealed a decreased amount of serum BAP in the high-dose group 3 and 9 months after transection, compared with concentrations for the low-dose group.

Urine Dpd and Pyd concentrations—Urine concentrations of Pyd were significantly decreased from the baseline value in the control group 1 and 3 months after transection. Significant decreases from baseline values were detected in the low-dose group 3, 6, 9, and 12 months and the high-dose group 6 and 9 months after transection. No significant differences in the percentage change of urine Pyd content from baseline values were detected among the groups (**Figure 4**). Examination of urine concentrations of Dpd revealed no significant changes from baseline Dpd concentrations in the control or high-dose groups, but the low-dose group had a significant decrease 12 months after transection. No significant differences in percentage change of Dpd content from baseline concentrations were detected among the groups.

BMD analysis—In the control group, BMD of the medial aspect of the femoral condyle was significantly decreased from baseline values at nearly all time points in all regions of interest (ie, M1 to M4), except for 1 month after transection for region M1 and 3 months after transection for regions M2 and M3 (**Table 1**). The low-dose group also had significant decreases from baseline values at all time points, except 1 and 12 months after transection for region M1 and 1, 9, and 12 months after transection for region M2. Similarly, the high-dose group had significant decreases from baseline values at all time points, except for 1 and 12 months after transection for region M1 and 1 month after transection for regions M3 and M4. Region M2 only had decreases 6 and 9 months after transection. Comparing BMD among groups revealed that the value for the high-dose group was significantly greater than that for the control group 1 month after transection for regions M1 and M2 and 1 and 12 months after transection for region M4. No significant differences were detected between the low- and high-dose groups.

In the lateral femoral condyle, BMD of the control group was significantly decreased from baseline values

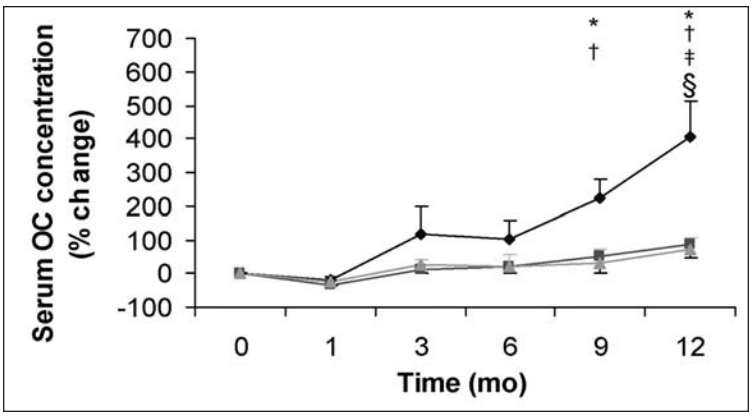

Figure 2—Mean + SD serum concentrations of osteocalcin (OC) in 3 groups of dogs (7 dogs/group) with experimentally induced osteoarthritis caused by transection of the left cranial cruciate ligament (CrCL) that received treatment with an inert vehicle (control group; black diamonds), a low dose of zoledronate (10 µg/kg; low-dose group; black squares), or a high dose of zoledronate (25 µg/kg; high-dose group; gray triangles). Injections were administered SC on the day after the CrCL transection and at intervals of 90 days for 12 months. Values reported represent the percentage change from concentrations determined on the day of transection (time 0; baseline). *Within the control group, value differs significantly ($P < 0.05$) from the baseline value. †Within a time point, value differs significantly ($P < 0.05$) between the control group and high-dose group. ‡Within a time point, value differs significantly ($P < 0.05$) between the control group and low-dose group. §Within the high-dose group, value differs significantly ($P < 0.05$) from the baseline value.

at all time points in all regions of interest (ie, L1 to L4), except for 3 and 12 months after transection for region L2 and 3 months after transection for region L3 (Table 1). Significant decreases in the low-dose group were detected at all time points > 1 month after transection for regions L1 and L4 and at all time points after transection for region L3. The low-dose group did not have significant changes from baseline values for region L2. In the high-dose group, significant decreases over time were detected at all time points > 1 month after transection for all regions, except for 12 months after transection for regions L2 and L3. Comparisons among groups revealed that BMD in the low-dose group was significantly lower than the value for the control group 1 month after transection for regions L2 and L3. The high-dose group had significantly greater BMD, compared with BMD for the control group, 1 month after transection for regions L1, L2, and L3 and 9 months after transection for regions L3 and L4. No differences were seen between the low- and high-dose groups.

Examination of the medial aspect of the tibial plateau revealed significant decreases in BMD from

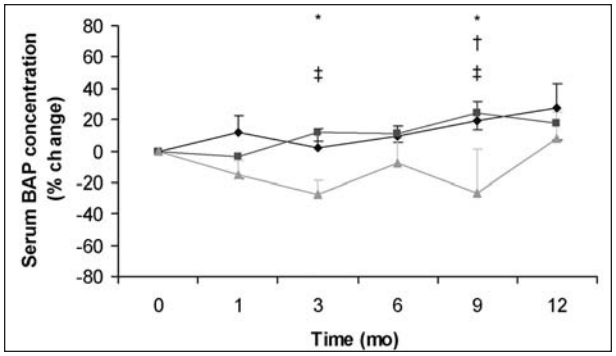

Figure 3—Mean + SD serum concentrations of bone-specific alkaline phosphatase (BAP) in 3 groups of dogs (7 dogs/group) with experimentally induced osteoarthritis caused by transection of the left CrCL that received a control treatment (black diamonds) or treatment with a low (black squares) or high (gray triangles) dose of zoledronate. Values reported represent the percentage change from concentrations determined on the day of transection (time 0; baseline). *Within the high-dose group, value differs significantly ($P < 0.05$) from the baseline value. †Within a time point, value differs significantly ($P < 0.05$) between the control group and high-dose group. ‡Within a time point, value differs significantly ($P < 0.05$) between the low-dose and high-dose groups.

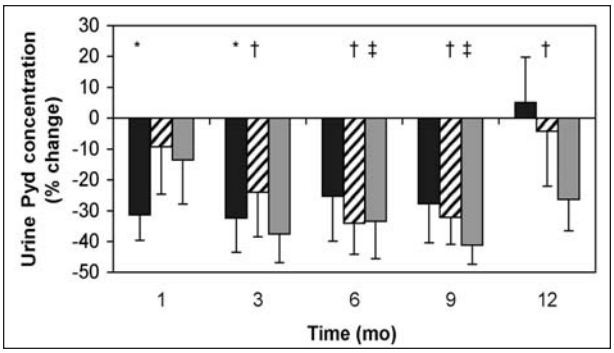

Figure 4—Mean + SD urine concentrations of pyridinoline (Pyd) in 3 groups of dogs (7 dogs/group) with experimentally induced osteoarthritis caused by transection of the left CrCL that received a control treatment (black bar) or treatment with a low (diagonal-striped bars) or high (gray bars) dose of zoledronate. Values reported represent the percentage change from concentrations determined on the day of transection (time 0; baseline). *Within the control group, value differs significantly ($P < 0.05$) from the baseline value. †Within the low-dose group, value differs significantly ($P < 0.05$) from the baseline value. ‡ Within the high-dose group, value differs significantly ($P < 0.05$) from the baseline value.

Table 1—Mean values for subchondral bone mineral density (BMD) in 8 regions of the lateral and medial femoral condyles in 3 groups of dogs (7 dogs/group) with experimentally induced osteoarthritis caused by transection of the left cranial cruciate ligament (CrCL) that received treatment with an inert vehicle (control group), a low dose of zoledronate (10 µg/kg), or a high dose of zoledronate (25 µg/kg).*

		Medial femoral condyle†				Lateral femoral condyle†			
Treatment	**Time (mo)**	**M1**	**M2**	**M3**	**M4**	**L1**	**L2**	**L3**	**L4**
Control	0	510	452	564	711	573	462	560	651
	1	438	384‡	482‡	606‡	456‡	366‡	461‡	539‡
	3	396‡	379	431	592‡	412‡	373	417	499‡
	6	380‡	345‡	411‡	561‡	415‡	362‡	437‡	498‡
	9	394‡	381‡	428‡	535‡	422‡	394‡	439‡	498‡
	12	402‡	389‡	424‡	535‡	432‡	396	473‡	527‡
Low dose	0	513	466	576	683	581	410	516	625
	1	493	467	532‡	604‡	543	408§	502‡,§	575
	3	399‡	383‡	429‡	568‡	395‡	362	427‡	497‡
	6	377‡	381‡	444‡	558‡	427‡	358	437‡	496‡
	9	406‡	406	456‡	552‡	433‡	379	442‡	494‡
	12	438	418	452‡	558‡	456‡	416	454‡	518‡
High dose	0	500	410	557	667	526	422	517	606
	1	495§	423§	516	627§	520§	418§	509§	583
	3	385‡	346	431‡	550‡	424‡	380‡	459‡	516‡
	6	366‡	342‡	434‡	521‡	394‡	364‡	426‡	475‡
	9	410‡	373‡	450‡	537‡	435‡	377‡	462‡,§	508‡,§
	12	434	400	450‡	548‡,§	446‡	394	492	536‡

Values reported are the number of Housefield units.

*Injections were administered SC on the day after the CrCL transection and at intervals of 90 days for 12 months; day of transection was designated as time 0 (baseline). †Regions were labeled 1 to 4 beginning with the most cranial and progressing to the most caudal; thus, the regions were designated M1 to M4 for the medial aspect and L1 to L4 for the lateral aspect, respectively. ‡Within a region within a treatment, value differs significantly ($P < 0.05$) from the baseline value. §Within a time point within a region, value differs significantly ($P < 0.05$) from the corresponding value for the control group.

Table 2—Mean values for subchondral BMD in 8 regions of the lateral and medial tibial plateaus in 3 groups of dogs (7 dogs/group) with experimentally induced osteoarthritis caused by transection of the left CrCL that received treatment with an inert vehicle (control group), a low dose of zoledronate (10 μg/kg), or a high dose of zoledronate (25 μg/kg).*

		Medial femoral condyle†				Lateral femoral condyle†			
Treatment	**Time (mo)**	**M1**	**M2**	**M3**	**M4**	**L1**	**L2**	**L3**	**L4**
Control	0	399	356	412	571	293	358	801	593
	1	356	302‡	327	491	259	345	608‡	556
	3	350	298	328‡	472	287	317	555‡	510
	6	311‡	326	331‡	506	262	303	522‡	500‡
	9	340	337	333‡	481‡	289	317	517‡	528‡
	12	331	343	327	521	291	311	516‡	506‡
Low dose	0	377	347	379	532	296	314	678	592
	1	356	339	354	542	273‡	355	701§	561
	3	315‡	276‡	317	572	291	286	508‡	566
	6	304‡	287‡	334	686‡,§	270	299	531‡,§	572
	9	298‡	281‡	319	668‡,§	297	316	530‡,§	557
	12	320	289‡	336	628§	308	340§	534‡,§	584
High dose	0	380	371	388	527	283	345	715	542
	1	343	323	329	531	274	312	661§	601
	3	334	336	381	594§	261	328	573	582
	6	310	295	318	653§	254	279‡	487‡,§	541
	9	305	313	311	681‡,§	255	259‡	480‡	543
	12	338	307	330	617§	275	289‡	517‡	564§

Values reported are the number of Housefield units.
See Table 1 for remainder of key.

baseline values in the control group at several time points for the regions of interest (**Table 2**). In the low-dose group, a significant decrease from baseline values was detected 3, 6, and 9 months after transection for region M1 and at all time points > 1 month after transection for region M2. The low-dose group had a significant increase above baseline values 6 and 9 months after transection for region M4, whereas the high-dose group had a significant increase above baseline values 9 months after transection for region M4. Comparison among groups revealed that the low-dose group had a significantly greater BMD, compared with BMD for the control group, at all time points > 3 months after transection for region M4. The high-dose group had a significantly higher BMD, compared with BMD for the control group, at all time points > 1 month after transection for region M4. No significant differences were detected between the high- and low-dose groups.

The lateral aspect of the tibial plateau had a significant decrease in BMD from baseline values in the control group at all time points for region L3 and at all time points > 3 months after transection for region L4 (Table 2). In the low-dose group, significant decreases from baseline values were detected 1 month after transection for region L1 and at all time points > 1 month after transection for region L3. The high-dose group had significant decreases from baseline values over time for regions L2 and L3 at all time points > 3 months after transection. Comparison among groups revealed that the control group had greater BMD 12 months after transection, compared with the low-dose group, for region L2 and 1, 6, 9, and 12 months after transection for region L3. The high-dose group had a significantly increased BMD, compared with BMD for the control group, 1 month after transection for region L3 and 12 months after transection for region L4. No differences were observed between the high- and low-dose groups.

In the contralateral nontransected stifle joints, there were various singular time points at which BMD was significantly increased or decreased from baseline values. Comparison among groups revealed that the medial aspect of the tibial plateau had significant increases in BMD for region M3 in the low- and high-dose groups 3, 6, and 9 months after transection, compared with BMD for the control group. For region M4, BMD was increased in the low-dose group at all time points > 3 months after transection and in the high-dose group 3, 9, and 12 months after transection, compared with values for the control group. Comparison among groups revealed that in the nonaffected limb, BMD was greater in the zoledronate-treated groups 9 months after transection for region M3 of the medial portion of the femoral condyle, regions L3 and L4 of the lateral aspect of the femoral condyle, and regions M3 and M4 of the medial aspect of the tibial plateau, compared with BMD for the control group.

Linear regression was performed to compare serum markers, urine markers, serum and urine markers, and BMD. No correlations were found between OC and BAP concentrations or between concentrations of serum markers (OC or BAP) and concentrations of urine markers (Dpd or Pyd). A significant positive correlation (r, 0.36) was found between Dpd and Pyd concentrations. Limited significant correlations were found between concentrations of serum markers and BMD. Concentrations of BAP were positively correlated (r, 0.20) with BMD of the left lateral tibia for region L1. Concentrations of OC were negatively correlated with BMD of the left lateral tibia for regions L3 (r, –0.23) and L4 (r, –0.19) and the left medial femur for region M3 (r, –0.21). Comparison for relationships between concentrations of urine markers and BMD revealed limited significant positive correlations. Positive significant correlation coefficients were found

between Dpd concentrations and BMD of the left femoral condyle for regions L1 (r, 0.20), L2 (r, 0.18), L3 (r, 0.24), and L4 (r, 0.30). Concentrations of Dpd also had limited positive correlation coefficients with BMD of the medial sites on the left femur for region M4 (r, 0.22) and left tibia for region M1 (r, 0.20). Significant positive correlation coefficients were found between Pyd concentrations and BMD of the left femoral condyle for regions L1 (r, 0.35), L3 (r, 0.23), and L4 (r, 0.25). Concentrations of Pyd also had limited positive correlation coefficients with BMD of the medial sites on the left femur for regions M1 (r, 0.21) and M4 (r, 0.18) and left tibia for region M1 (r, 0.19).

Discussion

In the study reported here, we examined the effects of zoledronate on serum and urine markers of bone metabolism and subchondral BMD in dogs with experimentally induced instability of a stifle joint with OA development during a 1-year period. To the authors' knowledge, this is the first report to provide a longitudinal measurement of serum and urine markers of bone metabolism and subchondral BMD for any OA development in dogs. Changes in serum OC concentrations and BMD were identified, suggesting that bone metabolism is altered by zoledronate throughout the first year of OA development and progression for this method of induced joint instability.

Specifically, increases in serum OC concentrations were identified in the control group, which is consistent with findings from another report[29] of dogs with naturally developing rupture of the CrCL. In another study[g] in which investigators used the same method for inducing OA, there were significant increases in OC concentrations 7 days after transection of the CrCL with a return to baseline values, which eventually became significant decreases from baseline values 84 and 112 days after transection. However, similar to the data reported here, dogs in that study[g] did not have significant changes from baseline values approximately 1 and 3 months after CrCL transection. Because of the design of our study, acute increases or decreases in OC concentrations in the early postoperative period were undetected, but analysis of our results suggests that increases in the bone metabolic state do not begin until > 6 months after CrCL transection. The dogs receiving zoledronate had some increases in serum OC concentrations, but these increases were muted, with significantly lower concentrations than for the control dogs > 6 months after transection, which suggested that zoledronate may have been able to decrease bone metabolism and possibly bone turnover secondary to OA in the late stages of the study reported here. Whereas OC is generally considered a marker of osteoblastic activity, the fact that it is in the bone matrix may cause an increase in serum concentrations following osteoblastic activity. Thus, the increase in serum OC concentrations in the control dogs may have been an indication of increased resorption secondary to reduced use of the limb and the effect of zoledronate to inhibit osteoclastic activity. This second possibility is supported by a lack of increase in concentrations of BAP, a marker of osteoblastic activity, in the control group.

Analysis of serum BAP concentrations revealed no significant changes after CrCL transection in control dogs. This result is similar to that in human patients in the late stages of OA progression in which serum BAP concentrations remained unchanged.[30] However, serum BAP concentrations were evaluated in dogs with OA induced by use of the same methods reported here, and investigators in that study[g] found a significant decrease from baseline values 28 days after CrCL transection with a return to baseline values approximately 126 days after transection. There also were additional decreases from baseline values at multiple time points throughout that 4-month study; it was hypothesized that those decreases were attributable to BAP diffusion into synovial fluid in response to osteophyte formation. In the study reported here, we did not focus on the acute period, but we did have similar findings at 3 months after CrCL transection. Regardless of the acute response, analysis of our results did not identify significant changes in serum BAP concentrations for up to 12 months after CrCL transection.

In contrast to serum OC concentrations, BAP concentration was not significantly altered by zoledronate treatment. Significant differences were only detected between the high-dose group and control group 3 and 9 months after CrCL transection. Evaluation of these data, along with the lack of any change in serum BAP concentration in the control dogs, suggests that serum OC concentrations may be a better biomarker for bone metabolism in OA progression than are serum BAP concentrations. Although BAP and OC are both synthesized by osteoblasts, the BAP isoenzyme is associated with early osteoblastic differentiation,[31] whereas OC is a late biomarker of osteoblastic activity associated with mineralization of bone.[32] This difference may indicate that changes in serum BAP concentrations may be more transient than serum OC concentrations, such that differences would not be found when serum BAP concentrations are evaluated at 3-month intervals, as was done in the study reported here. In addition, during OA, BAP may have more of a local effect because increases in BAP concentrations have been reported[g,h] in synovial fluid obtained from the stifle joints of dogs after CrCL transection. These increases in synovial BAP content have been associated with osteophyte formation.[g] Significant increases in synovial BAP content in CrCL-transected stifle joints were detected after treatment with zoledronate,[h] which suggests greater local osteoblastic activity in treated dogs.

Interpretation of urine concentrations of biochemical markers is less clear. Pyridinoline and Dpd are major crosslinks of mature type I, II, and III collagen in all skeletal and connective tissues of the body, except the skin.[33] Although these crosslinks are found in multiple types of collagen and various tissues, most of the total body collagen is found in bone. Pyridinoline and Dpd are released into the circulation during active bone resorption and excreted in the urine, presumably unaltered. Deoxypyridinoline has been described as the more bone-specific marker[34–36] and is significantly correlated with the amount of bone resorption in the body.[34] Therefore, the anticipated results of our study were that the pyridinium markers would increase in

the control dogs after transection and zoledronate would inhibit bone resorption in the treated dogs, which would result in fewer crosslinks in the urine. These findings were reported[35] for rabbits with induced cartilage damage after treatment with zoledronate. Analysis of results of our study revealed that urine Pyd concentrations significantly decreased from baseline values in both the high- and low-dose zoledronate groups, which was expected because of inhibition of bone resorption by zoledronate and subsequent decreases in the release of these crosslinks in the urine. Surprisingly, this decrease from baseline values was also evident in the control dogs after CrCL transection, although we anticipated an increased release of the crosslinks in the urine. Additionally, no significant changes in Pyd concentrations were detected between the low- and high-dose groups, which indicated that other factors may play a role in Pyd metabolism.

Examination of urine Dpd concentrations revealed no changes in the control and low-dose groups, but there was a significant decrease from baseline values in the high-dose group 12 months after transection. Again, no significant changes in Dpd concentrations were identified among the groups, which was unexpected because Dpd is believed to be a more specific marker of bone metabolism than is Pyd.[25,34,36] Although these results were not anticipated, they are similar to results of another study[37] in which investigators examined the use of Pyd and Dpd as markers of OA in human patients. In that study, they found that Pyd and Dpd concentrations in patients with OA did not differ from the concentrations for control groups and that Pyd and Dpd concentrations were only increased when a patient had radiographic end-stage OA. Results may differ among studies because the results reported in 1 study[34] represent acute cartilage damage, whereas the results for the aforementioned human patients[37] and the dogs reported here represent chronic OA. Analysis of results of our study suggests that Pyd and Dpd may not be adequate markers of early OA and may explain the reason that we did not detect increases in Pyd and Dpd concentrations over time in the control dogs or significant changes among groups.

Another possible explanation for the lack of significant changes among groups for the excretion of the crosslinks after treatment with zoledronate is that aminobisphosphonates may affect the ratios of free and peptide-bound crosslinks excreted in the urine.[38] A study[39] in human patients administered the aminobisphosphonate, pamidronate, for treatment of metabolic bone diseases, such as Paget's disease and osteoporosis, had a decrease in the urine concentration of peptide-bound crosslinks with no effect on the concentration of free crosslinks in the urine. Another study[40] in which investigators used neridronate for treatment of osteoporosis revealed suppression of peptide-bound Dpd concentrations with no effect on concentrations of free Dpd in the urine. In our study, we quantified crosslinks in the urine via an EIA kit that measured only free crosslinks, which may explain the reason that we did not detect significant changes in excretion of the crosslinks between the control and zoledronate-treated dogs.

It has been postulated that aminobisphosphonates may alter patterns of collagen degradation. Aminobisphosphonates activate caspase-3–like enzymes and cystine proteinases, which are important in the mechanisms of apoptosis.[39] Because BPs are concentrated in bone as a result of their high affinity for hydroxyapatite, these enzymes may affect the degradation of collagen and subsequently its products.[40]

Examination revealed that both femoral condyles and tibial plateaus of the cruciate-deficient stifle joint had a decrease in BMD over time beginning 1 month after CrCL transection, which is consistent with results of other studies[9,27,41] in which a decrease in subchondral BMD was evident in the early stages of OA. This early decrease could be attributable in part to decreased weight bearing secondary to lameness in the affected limb. In the study reported here, there was a more consistent decrease in BMD in the femoral condyle than the tibial plateau, but the tibial plateau appeared to have more of a location-specific response, with the caudal regions having more changes than the cranial regions. A varying degree of periarticular BMD loss in the knee of humans has been described,[27] but the most substantial decrease in that study was at 1 site (ie, the caudomedial femur). Given that mechanical loading can influence bone structure and mass,[42–44] these patterns of BMD loss may be explained in part by changes in contact area and stress allocation of an unstable CrCL-deficient stifle joint. In clinically normal dogs, dynamic interactions of the articular surfaces are greater on the medial side than the lateral side of the stifle joint.[45] This greater interaction of the medial articular surfaces corresponds to results of other studies[46–48] that have suggested the medial side of the stifle joint supports a greater load during typical weight bearing or following CrCL transection or partial meniscectomy. Additionally, when cartilage damage is examined in a CrCL-deficient stifle joint, the medial aspect of the femoral condyle and medial tibial plateau are the more severely affected regions.[49] Damage to the caudal horn of the medial meniscus after CrCL damage may also be a contributing factor to the increased load sharing evident caudomedially, which contributes to an increased BMD in the caudal aspect of the proximal portion of the medial tibia. Analysis of these combined data provides support for the concept that the medial compartment of the stifle joint has an increase in abnormal load sharing during cruciate instability.

Relative decreases in BMD of the cruciate-deficient stifle joint were evident in the zoledronate-treated groups, and zoledronate appeared to offer protection against a decrease in BMD in the early period after transection. In general, both the low and high doses of zoledronate appeared to maintain BMD until 3 months after CrCL transection in the femoral condyles and tibial plateaus, compared with values for control dogs, which had decreases in BMD beginning 1 month after transection. For example, the decrease in BMD in the caudomedial femur over time was not seen until 3 months after transection in the high-dose zoledronate group. One month after transection, BMD of the high-dose group was significantly greater than that for the control group. Also, although a significant decrease in

BMD over time was not achieved, many anatomic regions had an early increase in BMD for the zoledronate groups, compared with values for the control group. Again, this lack of significant differences may have been attributable to the small sample size and the possibility of a type II error. Therefore, on the basis of these results, zoledronate may slow the loss of periarticular BMD in early stages of OA. The protection from subchondral BMD loss is consistent with results of another study[h] that documented less damage to articular cartilage when dogs were administered zoledronate.

Interestingly, in a few locations of the caudomedial tibia, relative increases in BMD were detected in the zoledronate-treated dogs, and the BMD was greater than that for the control group by 6 months after transection in both the low- and high-dose groups. Similarly, in the caudomedial tibia of the nontransected stifle joints, BMD was greater than that for the control group at most of the time points. The most likely cause of this increase was an increased loading on the control (unaffected) limb. Another possible explanation is that zoledronate may exert its effects on bone metabolism at sites of active bone resorption as well as at other sites in the body. The medial tibial plateau is not the location that most commonly has changes in subchondral BMD after CrCL transection; however, it is the area in which the caudal horn of the medical meniscus is often damaged. We detected a significant decrease of BMD, compared with baseline values, for the caudomedial tibial plateau 9 months after transection in the control dogs. Although a significant decrease was only achieved at 1 time point, a pattern of a decrease in BMD was evident at a few other time points. This lack of significant differences may again be the result of the small sample size. Thus, the decrease in BMD may have been attributable to lameness caused by instability in the joint after CrCL transection and subsequent damage to the medial meniscus, or it could potentially have been a contributing factor in causing injury to the medial meniscus by altering biomechanics of the subchondral bone of the region.

Therefore, because the medial compartment of the stifle joint is an area that has increased load sharing after CrCL damage and is the site of meniscal injury, zoledronate may have the potential to be beneficial to osteoarthritic joints because it had the most substantial impact on subchondral BMD in the caudomedial tibia. Zoledronate was able to, at the least, delay the onset of subchondral BMD loss at most regions, and in the caudomedial tibia, it maintained BMD throughout the entire year of the study. Also, as mentioned previously, the most pronounced areas of articular cartilage damage in CrCL-deficient dogs are the medial femoral condyle and tibial plateau.[49] Again, in the study reported here, zoledronate altered BMD at numerous regions including the medial femoral condyle and medial tibial plateau and may have been able to alter biomechanics of the subchondral bone. Additional investigations are needed to determine the contribution of subchondral BMD changes on the pathogenesis of joints with OA.

Serum concentrations of OC and BMD are inversely proportional.[50] In the study reported here, a late increase in serum OC concentrations did not correlate with an early decrease in BMD. As mentioned previously, we may have missed early changes in serum OC concentrations because of our study design. Such changes were detected within 1 month after transection in another study.[g] Assuming this to be true, early decreases in BMD may be a reflection of the increased OC concentrations detected during the acute period following CrCL transection. Another possibility is that these localized, site-specific changes in BMD do not alter systemic serum OC concentrations, whereas later increases reflect other bony changes seen during the progression of OA. Again, the lack of early changes in serum OC concentrations may have been attributable to the small sample size.

Regardless, zoledronate treatment had an inhibitory effect on serum concentrations of this biomarker. Zoledronate may cause more of the OC to bind to the mineral phase of bone, thus increasing BMD while releasing less OC into the circulation than for the control dogs. On the basis of the results and potential problems mentioned previously for the serum BAP concentrations and urine concentrations of collagen crosslinks, it is hard to evaluate their correlation with BMD. For these reasons, the measured biomarkers may not be adequate for detection of early OA.

In the study reported here, we identified that zoledronate was able to inhibit an increase in serum concentrations of OC while slowing the loss of subchondral BMD at multiple regions in the distal portion of the femur and proximal portion of the tibia. In some cases, it caused an increase in BMD. On the basis of results of this study, BPs remain a potentially beneficial therapeutic agent for patients with OA; however, additional studies are warranted.

a. Intact human osteocalcin EIA kit, Biochemical Technologies Inc, Stoughton, Mass.
b. Metra serum BAP EIA kit, Quidel Corp, San Diego, Calif.
c. Metra DPD EIA kit, Quidel Corp, San Diego, Calif.
d. Metra PYD EIA kit, Quidel Corp, San Diego, Calif.
e. Somatom AR Star VB41A, Siemens, New York, NY.
f. SAS, version 8.2, SAS Institute Inc, Cary, NC.
g. Trumble TN, Billinghurst RC, McIlwraith CW. Bone alkaline phosphatase in synovial fluid is correlated to radiographic osteophytic changes in a canine cranial cruciate transection model of osteoarthritis (abstr), in *Proceedings*. 49th Annu Meet Orthopaed Res Soc 2003;782.
h. Dearmin M, Garcia A, Trumble T, et al. Effects of bisphosphonate treatment on bone and cartilage metabolism, radiographic, and articular cartilage changes in an experimental model of canine osteoarthritis (abstr). *Vet Surg* 2004;33:E6–E7.

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