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## Short Communication

# Serum 25-hydroxyvitamin D concentrations in dogs with osteosarcoma do not differ from those of age- and weight-matched control dogs

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## ABSTRACT

Vitamin D concentrations show an inverse correlation with incidence of certain tumors in people and dogs. Additionally, human osteosarcoma has been associated with dysregulation of vitamin D-dependent pathways. The study objective was to compare serum 25-hydroxyvitamin D<sub>2</sub> and 25-hydroxyvitamin D<sub>3</sub> in 20 dogs with osteosarcoma to age- and weight-matched control dogs. We hypothesized that dogs with osteosarcoma would have lower serum 25-hydroxyvitamin D than control dogs. The mean 25-hydroxyvitamin D<sub>3</sub> concentrations for dogs with osteosarcoma and matched-controls were 34.95 ng/mL and 33.85 ng/mL, respectively ( $P=0.784$ ). Based on these data, 25-hydroxyvitamin D insufficiency might not be important in the pathogenesis of canine osteosarcoma.

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The primary role of vitamin D is calcium homeostasis, but interest has developed in other roles and cancer risk. Epidemiologic studies by Apperly (1941) identified an inverse correlation between sun exposure and cancer risk in people. This spurred further research, which identified an association between decreased 25-hydroxyvitamin D (25[OH]D) concentrations and an increased risk of certain tumor types such as colonic, prostatic, and mammary cancers (Jacobs et al., 2016). Veterinary researchers have identified an increased overall risk of cancer, mast cell tumor, lymphoma, and malignant hemoabdomen in dogs with significantly lower 25(OH)D (Gerber et al., 2004; Mellanby, 2016; Selting et al., 2016).

The relationship between vitamin D and neoplasia prompted us to evaluate its involvement in the pathogenesis of canine osteosarcoma. Vitamin D insufficiency could contribute to oncogenesis of osteosarcomas, based on its role in several pathways utilized both in health and tumor pathogenesis (Ryan et al., 2015). Therefore, we investigated 25(OH)D<sub>2</sub>/D<sub>3</sub> concentrations, as markers of the active form of vitamin D, in dogs with osteosarcoma compared to a matched control population. We hypothesized that dogs with osteosarcoma dogs would have decreased 25(OH)D<sub>2</sub>/D<sub>3</sub> compared to age- and weight-matched control dogs.

Serum samples from twenty dogs with histopathologically confirmed osteosarcoma were collected pre-treatment (surgery and/or

**Table 1**

Age and bodyweight in dogs with osteosarcoma and control dogs.

Age	Bodyweight		
	<25 kg OSA n/control n	25–40 kg OSA n/control n	>40 kg OSA n/control n
<5 years	0/0	1/1	0/0
5–10 years	0/0	11/11	1/1
>10 years	1/1	6/6	0/0

OSA, osteosarcoma.

chemotherapy) at the North Carolina State University Veterinary Health Complex (NCSU-VHC) or through the NCSU Clinical Trials Core. All samples were acquired under an approved Institutional Animal Care and Use Committee protocol (No. 11-134-O, 19 December 2011). Serum chemistry results were within the reference range for 19/20 dogs with osteosarcoma; one dog had a slight elevation in blood urea nitrogen, but a normal creatinine concentration. Twenty age- and weight-matched control dogs (with unremarkable medical histories and physical evaluations) were paired with osteosarcoma-affected dogs after stratification into groups (Table 1), by factors that have been known to alter vitamin D metabolism (Tryfonidou et al., 2003). Serum biochemical evaluation was performed in both groups of dogs within 1 month of enrollment, and study entry requirements included serum creatinine concentration in the reference range, since renal function has also been shown to affect vitamin D (Mellanby, 2016).

Signalments of dogs with osteosarcoma included a median age of 10 years and mean age of  $9.58 \pm 2.59$  years. Most dogs were large

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breed (median weight of 29.5 kg) and breeds were as follows: Golden retriever ( $n = 5$ ), Labrador retriever ( $n = 5$ ), Rottweiler ( $n = 4$ ), Mixed breed ( $n = 3$ ), Greyhound ( $n = 1$ ), Great Dane ( $n = 1$ ), and Staffordshire terrier ( $n = 1$ ). Breeds represented in the control group included: Labrador retriever ( $n = 7$ ), Mixed breed ( $n = 3$ ), Golden retriever ( $n = 2$ ), German shorthaired pointer ( $n = 1$ ), American Staffordshire terrier ( $n = 1$ ), Australian cattle dog ( $n = 1$ ), Chow Chow ( $n = 1$ ), Border collie ( $n = 1$ ), Airedale terrier ( $n = 1$ ), and Boxer ( $n = 1$ ). All dogs were spayed or neutered.

Body condition score (BCS) was assessed using the American Animal Hospital Association 1–9 scale for dogs. BCS was available for nine of 20 osteosarcoma cases and 12 of 20 control dogs. Scoring for dogs with osteosarcoma was as follows: seven dogs scored 5/9, four dogs scored 6/9, and one dog scored 7/9. In the control group, four dogs scored 5/9, three dogs scored 7/9, two dogs scored 4/9, one dog scored 5.5/9, one dog scored 6/9, and one dog scored 8/9.

Blood samples were collected and serum aliquots were stored at  $-80^{\circ}\text{C}$  until analysis. Analysis for vitamin D as 25(OH) $\text{D}_2$  and 25(OH) $\text{D}_3$  was performed using liquid chromatography tandem mass spectrometry (LC-MS; [Supplementary Table S1](#); McLendon Clinical Laboratories, UNC Hospitals; [Singh, 2010](#)). Data were tested for normality by the D'Agostino–Pearson omnibus test. After passing normality testing, statistical analyses to compare 25(OH) $\text{D}_3$  were performed using a paired  $t$  test and an unpaired  $t$  test (Prism 7 for Mac OS X; Graphpad Software) for BCS, and  $P$  values  $< 0.05$  were considered statistically significant.

The mean 25(OH) $\text{D}_3$  concentrations for dogs with osteosarcoma and matched-controls did not differ ( $34.95 \pm 11.54$  ng/mL vs.  $33.85 \pm 10.27$  ng/mL;  $P = 0.784$ ; [Fig. 1](#)). One control dog had measurable 25(OH) $\text{D}_2$  concentrations. BCS did not differ between groups ( $P = 0.931$ ).

Limitations of this study included small sample size, lack of dietary and urinalysis information, and unmatched sex/hormone status. Based on a post-hoc power analysis (Graphpad StatMate 2.0 for Windows; GraphPad Software) with an alpha of 0.05, we would have been able to detect a difference in serum 25(OH) $\text{D}_3$  concentrations of 9.95 ng/mL with 80% power; and a difference of 11.52 ng/mL with 90% power. Dietary information would have strengthened this study, as 25(OH) $\text{D}_3$  is derived from the diet in dogs. Canine diets

vary in 25(OH) $\text{D}_3$  content and can be impacted by owner supplementation ([Sharp et al., 2015](#); [AAFCO, 2014](#)). It is also known that 25(OH) $\text{D}$  can be affected by renal function as well as sex hormone status, although all enrolled dogs were neutered ([Sharp et al., 2015](#); [Mellanby, 2016](#)).

Our findings do not exclude a role for vitamin D in both neoplastic and non-neoplastic disorders. Therefore, efforts to reach a consensus on reference intervals and define target values, and an accepted assay for the surrogate measure of vitamin D, 25(OH) $\text{D}$ , should be undertaken. While immunoassays provide increased convenience and efficiency, LC-MS offers an advantage in overall sensitivity and may warrant future efforts in validation for veterinary species ([Wallace et al., 2010](#)).

In summary, this study was unable to demonstrate a difference in serum 25(OH) $\text{D}_2/\text{D}_3$  concentrations between dogs with osteosarcoma and healthy control dogs. This suggests that mechanisms aside from vitamin D insufficiency might be responsible for the pathogenesis of osteosarcoma. However, larger controlled studies are necessary to definitively elucidate the role of 25(OH) $\text{D}_2/\text{D}_3$  and other potential drivers in canine osteosarcoma.

### Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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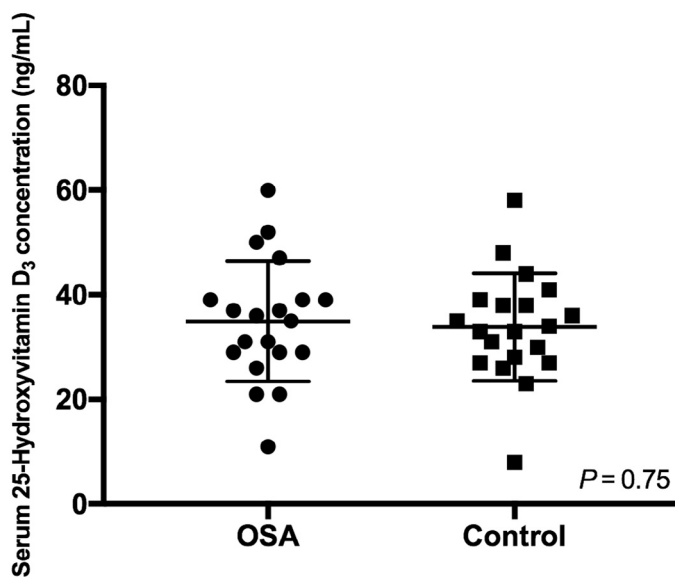
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### Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2016.10.005.

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**Fig. 1.** Serum 25-hydroxyvitamin  $\text{D}_3$  concentrations in dogs with osteosarcoma (OSA) and control dogs. Bars represent mean and standard deviation within each group.