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Serum 25-hydroxyvitamin D concentrations in dogs – correlation with health and cancer risk†

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Abstract

25-hydroxyvitamin D (25(OH)D) is important in bone health as well as many diseases including cancer. Supplementation may increase responsiveness of cancer cells to chemotherapy. Serum 25(OH)D, intact parathyroid hormone (iPTH) and canine C-reactive protein (c-CRP) were measured in healthy dogs and dogs with haemoabdomen. Regression analysis determined optimal 25(OH)D concentrations. In healthy dogs ($n = 282$), mean iPTH concentrations correlated inversely ($r^2 = 0.88$, $P < 0.001$) to 25(OH)D concentrations. Variation in both iPTH and c-CRP plateaued at 25(OH)D concentrations of 100–120 ng mL^{-1}. Haemoabdomen dogs ($n = 63$, 43 malignant and 20 benign) had 25(OH)D concentrations ranging from 19.4 to >150 ng mL^{-1}. Relative risk of cancer increased with decreasing 25(OH)D concentrations [RR = 3.9 for 25(OH)D below 40 ng mL^{-1} ($P = 0.0001$)]. Serum 25(OH)D concentrations in dogs vary widely, and are influenced by dietary VitD content. Serum vitD measurement can identify dogs for which supplementation may improve health and response to cancer therapy.

Keywords
biomarker, calcium, C-reactive protein, diet, inflammation, nutrition

Introduction

Vitamin D has become a frequent topic of research because its well-understood role in calcium metabolism has expanded to include maintenance of cellular health. Studies have found correlations between insufficient levels of vitamin D and increased risk of developing nonskeletal pathologies such as cardiovascular diseases,[1–5] hypertension,[6,7] cancer,[8–13] diabetes,[14,15] multiple sclerosis,[16] rheumatoid arthritis, [17] infectious diseases[18,19] and asthma.[20] Such diverse effects could be explained by the presence of vitamin D receptors in several organs and tissues (cardiac myocytes, vascular smooth muscle, brain, prostate, breast, colon and immune cells), through which vitamin D is believed to directly or indirectly regulate more than 2000 genes.[21] Emerging research has shown that many tissues will locally convert 25-hydroxyvitamin D [25(OH)D] to 1,25(OH)2D without influence from serum calcium.[22]

Furthermore, gene regulation by vitamin D and the impact on cellular health has been investigated in recent studies and has shown vitamin D to have an anti-inflammatory effect.[3–25] Chronic inflammation is implicated in a wide range of diseases including cancer.[26]

The optimal vitamin D concentration for immunologic and other cell microenvironment factors may be different than that required to prevent rickets. More than 30 years ago, studies

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showed people with constant exposure to sunlight had significantly higher concentrations of vitamin D than people with usual exposure.[27] Low stores of 25(OH)D have been associated with serious disease and with serum blood concentrations of biomarkers associated with calcium metabolism. This has led to a new understanding of 25(OH)D concentrations required for cellular health. Vitamin D 'sufficiency' has been defined and extensively studied by examining the inverse relationship between intact parathyroid hormone (iPTH) and vitamin D.[28–40] iPTH concentration and biological variation, or the 'scatter' about the mean iPTH value (i.e. standard deviation), both drop as 25(OH)D concentrations increase; where they 'plateau' determines the optimum concentration of 25(OH)D.

Despite the wealth of research evaluating vitamin D in humans, little has been studied in dogs. As dogs rely solely on dietary sources for vitamin D,[41] current serum concentrations of vitamin D in dogs may merely reflect supplementation levels in commercial dog food. Investigators have found associations of low 25(OH)D with canine lymphoma, cutaneous mast cell tumours, hyperparathyroidism, kidney disease, inflammatory bowel disease and heart disease[42–46]; however, to date no work has been published to define the optimum level of vitamin D in dogs.

The objective of this study was to determine the optimal serum concentrations of 25(OH)D (i.e. 'sufficiency') through the use of four biomarkers; iPTH, canine C-reactive protein (c-CRP – a measure of inflammation), calcium and phosphorous. We hypothesized that as 25(OH)D concentrations rise, iPTH and c-CRP concentrations and biological variation (scatter) will decrease. Calcium and phosphorous were measured to monitor calcium homeostasis. A secondary objective was to evaluate 25(OH)D concentrations as a risk factor for the development of cancer in dogs. Two cohorts were utilized.

Methods

Control group – apparently healthy dogs

Owners of dogs within German Shepherd and Golden Retriever breed clubs were recruited. Organized blood collection at breed club meetings and dog shows in Missouri, Minnesota, California, Massachusetts and Florida were conducted. In addition, by communicating through breed clubs, owners across the USA were encouraged to allow veterinarians to submit a blood sample if the dog was not present at a show or group blood draw. Each dog owner completed a questionnaire about diet and health history including lack of nonspecific or specific clinical signs of any disease at the time of blood collection, and any change in diet. Dogs were not examined by a veterinarian at time of enrollment and health status was established using results of health questionnaires at baseline and at least 6–12 months after enrollment. Inclusion criteria required dogs to be free of neoplasia, both benign and malignant, and other serious diseases. Dogs that died or were euthanized during the study were also excluded. The study was approved by the Clinical Studies Review Committee (Tufts). Institutional Animal Care and Use Committee approval was not required by the University of Missouri at the time of this study. Additionally each owner was required to provide signed consent.

Disease group – acute haemoabdomen dogs (this cohort was previously described)

A multicentre prospective study was performed, enrolling dogs presenting urgently to emergency or referral clinics and subsequently diagnosed with haemoabdomen and a splenic mass by means of ultrasonography, exploratory laparotomy and/or necropsy. Informed consent was obtained from all owners prior to study entry.[47] There were no breed, gender or age restrictions in eligible patients. Dogs whose owners elected euthanasia rather than therapy were eligible, as long as appropriate blood samples could be obtained prior to euthanasia and histological diagnosis of the splenic disease was obtained.

Sample collection

Blood was collected and centrifuged within 1 h of sample collection, and a minimum of 0.5 mL serum was harvested. The serum was placed in an airtight, freezer-resistant plastic tube and stored at −20 °C or colder. Tubes were coded so that the identity

of the sample was known only to the principal investigator; the laboratory was blinded.

25(OH)D assay

Serum 25(OH)D was evaluated by a commercial laboratory (Veterinary Diagnostics Institute, Simi Valley, CA, USA). The LIAISON® 25(OH)D assay (DiaSorin, Inc. Stillwater, MN, USA) is a direct, competitive chemiluminesence immunoassay (CLIA) for the quantitative determination of 25(OH)D in serum. During the first incubation, 25(OH)D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 min the tracer [25(OH)D linked to an isoluminol derivative] is added. After a second 10-min incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units and is inversely proportional to the concentration of 25(OH)D present. This assay has been validated for use in dogs with an intra- and inter-assay precision (five replicates) of 4.0 and 3.4%, respectively. Aliquots of canine samples (four samples with neat values of 81.4, 115.0, 96.7 and 66.3 ng mL^{-1}) and diluted 1:2, 1:4 and 1:8 yielded respective recovery rates of 109, 97, 95 and 110% and an r^2 of 0.99. Aliquots of canine samples (three samples with neat values of 39.0, 64.7 and 33.3 ng mL^{-1}) and spiked with a known amount of 25(OH)D3 demonstrated recovery of 114, 97 and 101%, respectively. Freeze–thaw testing for 25(OH)D stability revealed there was no statically significant difference for up to four freeze–thaw cycles; no specimen was tested beyond two freeze–thaw cycles. The method is the largest participant in DEQAS (vitamin D External Quality Assessment Scheme) surveys and has been shown to correlate to LC-MS/MS with r^2 of 0.96.[48]

iPTH assay

Serum iPTH was evaluated by a commercial laboratory (Veterinary Diagnostics Institute). The LIAISON® iPTH assay is a direct, two site, sandwich type CLIA for the quantitative determination of iPTH in serum. Affinity purified antibody to the 39–84 region of PTH is coated to the solid phase. The second affinity purified antibody is the 1–34 region is conjugated to an isoluminol derivative. During the incubation, PTH binds to the solid phase, and is subsequently bound by isoluminol conjugated antibody. After the incubation, the unbound material is removed with a wash cycle. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units and is proportional to the concentration of PTH present. This assay has been validated for use in dogs with an intra- and inter-assay precision (five replicates) of 4.8 and 6.8%, respectively. Aliquots of canine samples (three samples with neat values of 365, 380 and 387 pg mL^{-1}) and diluted 1:2, 1:4 and 1:8 yielded respective recovery rates of 113, 101 and 117% and an r^2 of 0.99. Aliquots of canine samples (three samples with neat values of 39.0, 64.7 and 33.3 ng mL^{-1}) and spiked with a known amount of iPTH demonstrated recovery of 102, 116 and 111%, respectively. Freeze–thaw testing for iPTH stability revealed there was no statically significant difference for up to three freeze–thaw cycles; no specimen was tested beyond two freeze–thaw cycles.

cCRP assay

cCRP was evaluated by a commercial laboratory (Veterinary Diagnostics Institute). The TECO® cCRP assay (TECOmedical group, Sissach, Switzerland) is a canine-specific sandwich enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of cCRP in serum. The use of this assay has been previously described.[49] The assay has intra- and inter-assay precision of 4.3 and 6.0%, respectively.

Calcium and phosphorous

Serum total calcium and phosphorous were evaluated by a commercial laboratory (Western Health Sciences Laboratory, West Hills, CA, USA). The Roche® total calcium assay (Roche Diagnostics GmbH, Germany) utilizes *o*-cresolphthalein to form a calcium complex for the quantitative measurement of calcium in serum. The Roche

phosphorous assay utilizes ammonium molybdate to form ammonium phosphomolybdate for the quantitative determination of phosphorous in serum.

Disease classifications

Dogs with malignant neoplasia were classified in the 'Cancer' group. Dogs with benign neoplasms were classified as 'benign'. All neoplasia diagnoses were obtained with haematoxylin and eosin-stained slides and reviewed by a single board-certified veterinary pathologist. In selected cases, diagnoses were refined through the use of factor VIII-related antigen (FVIII-ra) immunohistochemistry, performed according to published protocols.

Statistical analysis

Statistical analysis was performed using commercially available software (MedCalc Software, version 12.7, Belgium). Regression analysis was used to compare 25(OH)D concentrations with that of iPTH and c-CRP. Mean and standard deviation for iPTH, c-CRP, calcium and phosphorous were grouped at 10 ng mL^{-1} intervals of 25(OH)D ($IntD_{10}$) and regression analysis performed. A Kruskal–Wallis one-way analysis of variance was used to compare serum laboratory tests among cancer and noncancer dogs. A Mann–Whitney rank sum test was used to compare age and sex between cancer and noncancer groups. Relative risk was calculated between cancer and noncancer dogs at intervals of 20 ng mL^{-1} of 25(OH)D. A *P*-value of 0.05 was considered significant for all analyses.

Results and discussion

Control group – apparently healthy dogs

Study population

A total of 282 dogs met the criteria and were subsequently enrolled. Signalment information is listed in Table 1. Age ranged from 0.4 to 14 years, but most dogs were middle aged. The majority of dogs were spayed females, but distribution was relatively equal across other sex categories. There were slightly more Golden Retrievers than other breeds, which relates to the study design and case recruiting. While variation in vitamin D status may be influenced by sex, neuter status or age, the association with disease was present. Though the median age of the control group was younger than the median age of the tests groups (7 versus 10–10.5 years), the range of ages was similar and the control dogs were not all young dogs but were middle aged.

Comparison of 25(OH)D and iPTH

Serum 25(OH)D concentrations ranged from 9.5 to 249.2 ng mL^{-1} (median, Q1 and Q3; 68.9, 54.8 and 87.9 ng mL^{-1}). Serum iPTH concentrations ranged from 0.9 to 96.1 pg mL^{-1} (median, Q1 and Q3; 8.9, 6.1 and 13.7 pg mL^{-1}). There was a significant inverse relationship between serum iPTH concentration and 25(OH)D concentrations [$\text{Log}(y) = 1.04 - 0.0015x$, $r^2 = 0.02$, $P = 0.031$,

Table 1. Comparison of age, sex and breed in the control and disease groups

	Normal	Benign	HSA	Other malignancy
N	282	22	31	9
Age median (range)	7 (0.4–14)	10 (8–13)	10 (7–14)	10.5 (5–14)
Sex	67 M, 57 MN, 65 F, 92 FS and 1 NR	2 M, 11 MN, 1 F, 5 FS and 2 NR	4 M, 17 MN, 1 F, 7 FS and 1 NR	5 MN, 3 FS and 1 NR
Breed	Golden Retriever (154), GSD (119), White Shepherd (8) and Portuguese Water Dog (1)	Mixed Breed (5), Golden Retriever (8), Labrador Retriever 2, GSD (2) and others (5)	Mixed Breed (11), Labrador Retriever (6), GSD (3), Standard Poodle (2) and others (9)	Mixed Breed (4), others (4) and not recorded (1)

F, female intact; FS, female spayed; HSA, haemangiosarcoma; M, male intact; MN, male neutered; NR, not reported.
GSD = German Shepherd Dog

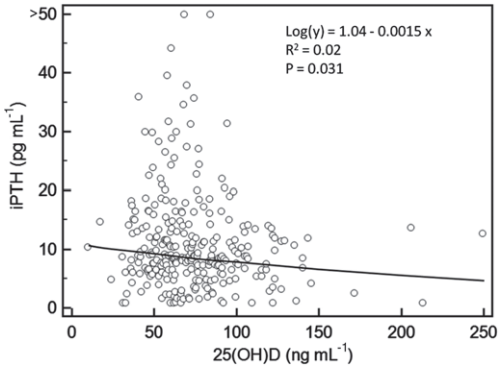

Figure 1. Scatterplot and regression of iPTH and 25(OH)D in the control group.

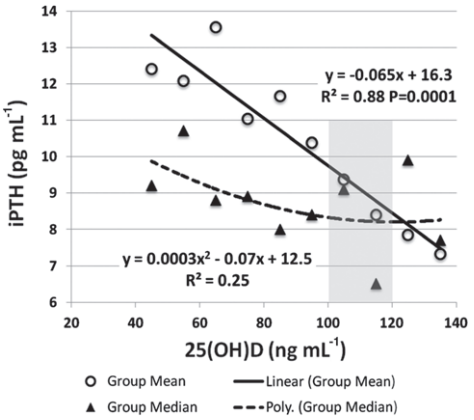

Figure 2. Mean and median iPTH at 10 ng mL^{-1} intervals of 25(OH)D in the control group.

Fig. 1]. Because of the large variability in values and unique shape to the regression analysis, it was anticipated that the r^2 value would be low and so values were also stratified into increments to better characterize this data set. When mean iPTH values at 10 ng mL^{-1} intervals of 25(OH)D were analyzed ($IntD_{10}$), the inverse relationship was significant ($y = 16.3 - 0.065x$, $r^2 = 0.88$, $P = 0.0001$) as shown in Fig. 2. Mean iPTH continued to decline as 25(OH)D concentrations increased and did not appear to plateau; however, when median iPTH values at $IntD_{10}$ were analyzed, the inverse relationship plateaued at approximately 8 pg mL^{-1} when 25(OH)D reached 100 ng mL^{-1}. The convergence of mean and median values are a reflection of a true Gaussian distribution that is obtained at 100–120 ng mL^{-1}. When mean iPTH standard deviation at $IntD_{10}$ was analyzed, a significant drop in variability occurred at 100 ng mL^{-1} of 25(OH)D ($P = 0.01$, Fig. 3).

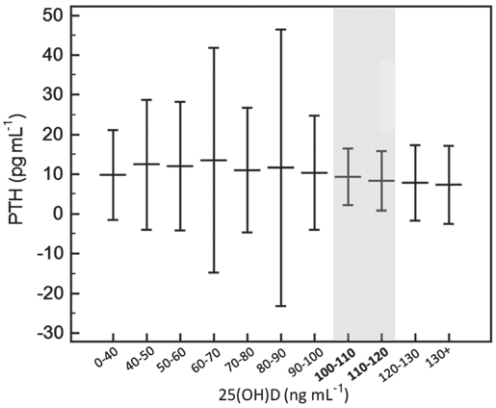

Figure 3. Mean iPTH with two standard deviation bars at 10 ng mL^{-1} intervals of 25(OH)D in the control group.

Comparison of 25(OH)D and c-CRP

Serum c-CRP concentrations ranged from 0.09 to 51.7 mg L^{-1} (median, Q1 and Q3; 1.9, 1.2 and 3.3 mg L^{-1}). There was no significant regression between serum c-CRP concentration compared with 25(OH)D concentrations ($P = 0.61$). However, c-CRP mean and standard deviation was significantly different above and below 100 ng mL^{-1} of 25(OH)D. Mean c-CRP standard deviation at $IntD_{10}$, a significant drop in variability occurred at ≥100 ng mL^{-1} of 25(OH)D ($P = 0.01$, Fig. 4). Mean c-CRP below 100 ng mL^{-1} 25(OH)D was significantly higher than above 100 ng mL^{-1} 25(OH)D, with a mean and 95% confidence interval of 2.8, 1.9–3.6 mg L^{-1} and 4.1, 3.2–5.1 mg L^{-1} c-CRP, respectively; the t test statistic was 2.1, with 165 degrees of freedom and an associated P value of 0.038 (Fig. 5). This is consistent with literature in human subjects.

Comparison of 25(OH)D and calcium

Serum calcium concentrations ranged from 3.8 to 18.1 mg dL^{-1} (median, Q1 and Q3; 11.0, 10.6 and 11.9 mg dL^{-1}). Serum calcium concentration was not correlated to 25(OH)D concentrations ($P = 0.83$). When calcium standard deviation at $IntD_{10}$ was analyzed, there was no significant

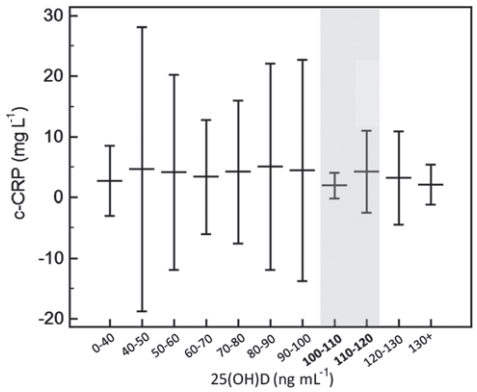

Figure 4. Mean c-CRP with two standard deviation bars at 10 ng mL^{-1} intervals of 25(OH)D in the control group.

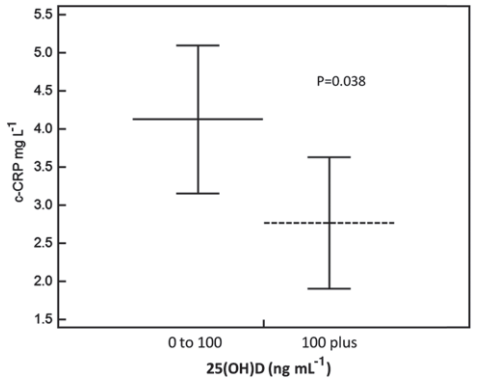

Figure 5. Mean c-CRP with 95% confidence intervals of the mean below and above 100 ng mL^{-1} 25(OH)D in the control group.

change across the range of 25(OH)D concentrations ($P = 0.83$).

Comparison of 25(OH)D and phosphorous

Serum phosphorous concentrations ranged from 2.0 to 10.5 mg dL^{-1} (median, Q1 and Q3; 4.5, 4.0 and 5.0 mg dL^{-1}). Absolute serum phosphorous concentration did not correlate to 25(OH)D concentrations ($P = 0.43$). However, when phosphorous standard deviation at $IntD_{10}$ was analyzed, there was a significant change across the range of 25(OH)D concentrations ($y = -2E-06x^3 + 0.0008x^2 - 0.0905x + 4.1887$, $r^2 = 0.91$, $P = 0.001$) resulting in a plateau at 80 ng mL^{-1} or greater of 25(OH)D (Fig. 6).

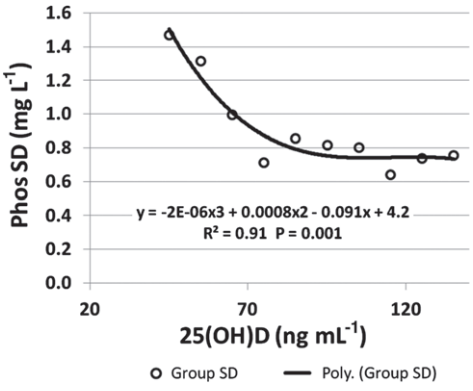

Figure 6. Phosphorous standard deviation at 10 ng mL^{-1} intervals of 25(OH)D in the control group.

Disease group – acute haemoabdomen dogs

Study population

A total of 62 dogs met the inclusion criteria. Signalment information is listed in Table 1. The control group was significantly different in both age and sex than the disease group, with median age of 7 and 10, respectively ($P < 0.001$) and a higher incidence of female spayed dogs ($P < 0.001$). Thirty-one were diagnosed with splenic hemangiosarcoma, 9 with other splenic malignancies [sarcoma (3), lymphosarcoma/lymphoma (LSA) (2), fibrohistiocytic nodules (2), round cell tumour (1) and leukemia (1)] and 22 with benign conditions [nodular lymphoid hyperplasia (8), splenic congestion (4), haematoma (3), mylelolipoma (2), haemangioma (1), necrosis (1), reactive lymphoid follicles (1) and unremarkable spleen (2)].

Cancer and 25(OH)D

Serum 25(OH)D concentrations of all cancer cases were significantly different from the control group (Fig. 7) with values ranging from 19.4 to 151 ng mL^{-1} with median, Q1, Q3 of 49.4, 39.5, 63.0 and 68.9, 54.8, 87.9, respectively ($P < 0.0001$). There was a large subset of splenic hemangiosarcomas ($n = 31$) that were significantly different from the control group (Fig. 8) with serum 25(OH)D concentrations ranging from 19.4 to 91.8 ng mL^{-1} (median, Q1 and Q3; 49.2, 38.0 and 59.6 ng mL^{-1}; $P < 0.0001$). Other cancers ($n = 9$) also differed from the control group with serum 25(OH)D concentrations ranging from 29.9 to

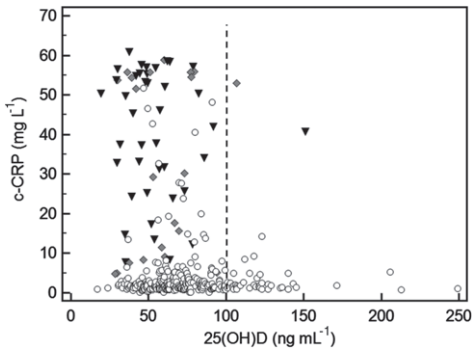

Figure 7. Scatterplot of CRP and 25(OH)D in the control and disease group (open circles are the control group, black triangles represent cancer, and shaded diamonds represent benign neoplasms). While vitamin D is variable, all dogs with disease are insufficient except for one. The normal dogs that are insufficient should still be considered to be at risk for disease.

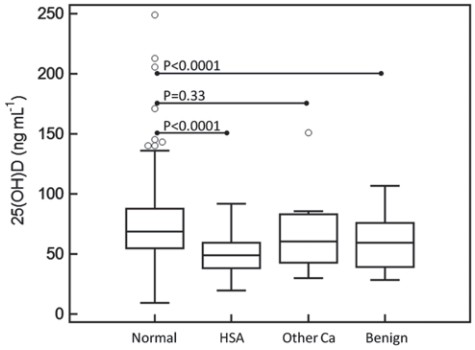

Figure 8. Box and whisker plot of 25(OH)D in the control and disease groups. Circles represent outliers.

151 ng mL^{-1} (median, Q1 and Q3; 60.5, 43.1 and 83.2 ng mL^{-1}), however, the difference was not significant ($P = 0.33$).

Benign and 25(OH)D

Serum 25(OH)D concentrations of all benign neoplasia cases were significantly different from the control group (Fig. 8) with values ranging from 28.3 to 107 ng mL^{-1} (median, Q1 and Q3; 59.5, 39.2 and 76.1 ng mL^{-1}; $P < 0.0001$).

Relative risk

Relative risk was calculated for all cancers, HSA only, and benign only, and is listed in Table 2. Risk appeared to be inversely related to 25(OH)D concentration, with a relative risk less than one for concentrations above 100 ng mL^{-1}. A possible beneficial effect was not statistically significant ($P = 0.08$) hence more data may be needed to confirm this. This study was not designed as a lifetime study in which vitamin D concentrations are monitored over time to correlate with various disease states.

Discussion

Diet is the primary source of vitamin D for dogs, as negligible amounts are produced via sunlight. We have shown in a large group of apparently healthy dogs that vitamin D stored in the form of 25(OH)D vary significantly, ranging from 9.5 to 249 ng mL^{-1}. It should be noted that dogs with insufficient vitamin D, despite a 6–12 month follow-up period, should still be considered at risk for the development of disease such as cardiac disease, inflammatory bowel disease and cancer as previously published.[42–46] Latency prior to clinical manifestation of cancer is well recognized and can vary in length. While this is not commonly discussed with regards to nonneoplastic disease, it is equally well recognized that organs compensate for dysfunction for a period of time before decompensating and developing clinical signs of disease. As the majority of these dogs eat commercial dog food diets, our data likely reflect the level of vitamin D supplementation that exists today, rather than what should be considered adequate. Similar to studies performed in human medicine, we attempted to define the level of vitamin D sufficiency by evaluating other analytes that are affected by vitamin D concentrations. This study demonstrates that iPTH and 25(OH)D have an inverse relationship. Previous studies to define sufficiency in human medicine compare vitamin D concentrations with iPTH plateau. We have shown that iPTH pleateaus at approximately 8 pg mL^{-1} when 25(OH)D concentrations reach 100 ng mL^{-1}. Furthermore, when 25(OH)D is below 100 ng mL^{-1} a normal Gaussian distribution is rejected but achieved when 25(OH)D is above 100 ng mL^{-1}. This is consistent with the convergence of mean and median iPTH

Table 2. Relative risk of the disease group compared with that of the control group

25(OH)D (ng mL^{-1})	All cancers	HSA	Benign
<40	3.9 ($P = 0.0001$)	4.1 ($P = 0.0001$)	4.5 ($P = 0.0001$)
<60	2.0 ($P < 0.0001$)	2.2 ($P < 0.0001$)	1.5 ($P = 0.111$)
<80	1.4 ($P < 0.0001$)	1.5 ($P < 0.0001$)	1.4 ($P = 0.0001$)
<100	1.1 ($P = 0.0003$)	1.5 ($P < 0.0001$)	1.1 ($P = 0.04$)
>100	0.18 ($P = 0.08$)	0.11 ($P = 0.12$)	0.32 ($P = 0.25$)

when 25(OH)D is above 100 ng mL^{-1}. As an alternate means to determine optimum levels, biological variation was examined by calculating standard deviation. As 25(OH)D concentrations increased, a sharp decline in standard deviation was observed at 100 ng mL^{-1}.

Calcium and phosphorous are also dependent upon concentrations of 25(OH)D and iPTH. Total calcium was unaffected by increasing concentrations of 25(OH)D as well as biological variation as measured by standard deviation. This does not seem surprising as the maintenance of calcium is critical and derangements of calcium homeostasis can have important consequences on health. While this study was not designed to establish an upper limit of target blood vitamin D concentrations, no dog had clinical signs of calcium excess and thus vitamin D concentrations were likely not in excess in any patient. However, when phosphorous was examined similarly, while there was a slight, insignificant, negative slope as 25(OH)D concentrations increase, a significant plateau in biological variation was achieved when 25(OH)D concentrations were at values greater than 80 ng mL^{-1} ($P = 0.001$).

Inflammation is not normally associated with calcium metabolism; however, due to vitamin D involvement in immunity, human studies have shown that increasing vitamin D has an inverse relationship with inflammation. In this study, mean c-CRP concentrations significantly decreased when 25(OH)D concentrations rose above 100 ng nL^{-1} of 25(OH)D confirming this finding in dogs. Similarly to that of iPTH, there is a definitive and significant drop in c-CRP biological variation when 25(OH)D concentrations reach 100 ng mL^{-1}.

Numerous human studies have found an association of low vitamin D stores and an increased risk for a wide variety of cancers. Recently, there are findings that low stores of 25(OH)D in dogs are associated with an increased risk with lymphoma, mast cell cancer, kidney disease, IBD and heart disease. This study included a relatively large group of dogs with splenic hemangiosarcoma and showed that relative risk decreases as 25(OH)D concentrations increase with a possible protective effect when 25(OH)D exceeds 100 ng mL^{-1}. Other cancers had a similar finding, however due to the small cohort, significance was not achieved. Benign neoplasia also had a similar risk association with 25(OH)D concentrations. In both cancer and benign disease, relative risk reached approximately one when 25(OH)D concentrations achieved 100 ng mL^{-1}. As with iPTH, c-CRP and Phos there appears to be independent confirmation that 25(OH)D concentrations of $\geq$100 ng mL^{-1} is not associated with increased relative risk of cancer, however, additional study would be needed to confirm any protective effect. Likewise, this study found associations between vitamin D status and the presence or absence of a tumour, most notably hemangiosarcoma. Cause-and-effect remain incompletely understood. It is possible that altered vitamin D is coincidentally found in populations at risk for cancer but is unrelated, or that the disease itself impacts vitamin D absorption and metabolism. More work needs to be done to refine our understanding of the role of vitamin D in cancer. The association found here and in many investigations in people and animals supports further research in this area, and provides a basis on which supplementation might be used to maintain health or improve response to treatment. These are future directions for related research.

In this study, we attempted to define 25(OH)D sufficiency through the use of multiple biomarkers. iPTH, c-CRP and phosphorous as well as relative risk of cancer suggest that 25(OH)D sufficiency is achieved at 100 ng mL^{-1}. The basic premise is

that if the major mediators of positive and negative feedback in the body are no longer stimulated, then the body is satisfied and sufficiency has been achieved. No attempts were made in this study to examine where vitamin D toxicity may occur (upper limit of adequate range). Most dogs with sufficient vitamin D had concentrations in the range of 100 – 150 ng mL^{-1}, thus toxicity most likely occurs above these levels. Therefore, in absence of a focused toxicity study, which we believe should be performed, we are suggesting that a target range of 100 – 120 ng mL^{-1} be used for establishing 25(OH)D sufficiency in dogs.

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Conflict of interest

K. A. S. is a paid consultant to VDI, and R. R. is employed by VDI.

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