



VITAMIN D
GROWING BODY OF EVIDENCE



VDI LABORATORY LLC

The Right Tests for the
Right Decisions™

📍 SIMI VALLEY, CA
🌐 WWW.VDILAB.COM
📞 805.577.6742

A growing body of evidence...



There is a growing body of evidence that low stores of vitamin D are associated with a wide range of diseases in dogs and cats:

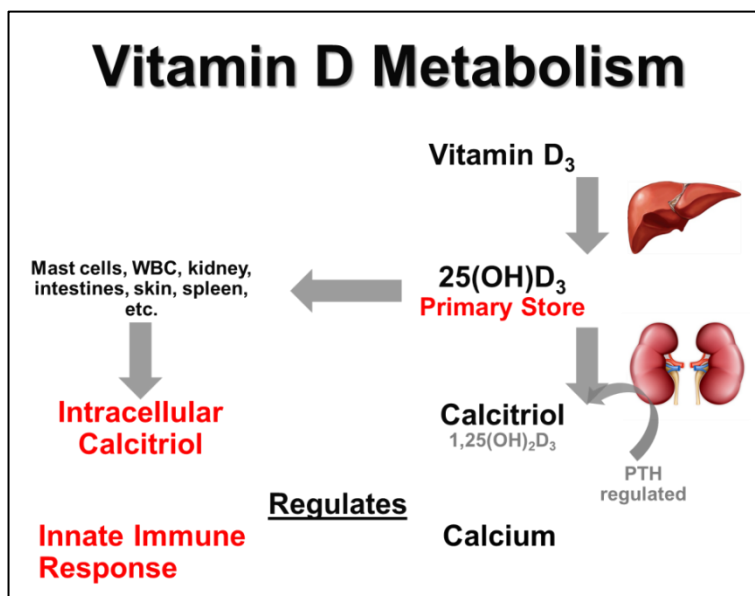
- **Cancer (2,5,8,9,11,12,13,20,26,27,56)**
- **Chronic Enteropathy (4,12,17,19,29,36,45,54)**
- **Heart disease (10,18,48)**
- **Atopic dermatitis (7,32,49,50)**
- **Kidney disease (1,2,25,26,52)**
- **Hyperparathyroidism (2)**
- **Immune mediated disease (22,31,44,50)**
- **Infection (6,9,16,28,23,40,42,53)**
- **Pancreatitis (24,33,41)**
- **Feline tooth resorption (3)**
- **Hospital Mortality (14,35)**
- **Toxicity (30,46)**
- **Mechanism of Action (8,20,36,37,38,39,43,44,47,49,51,55,56)**
- **Dietary (1315,21,30,34,46)**

Testing Information:

VDI Laboratory offers routine testing of serum 25(OH)D in dogs, cats, & horses. For more information please call 805.577.6742 or visit www.vdilab.com

Vitamin D is the precursor of the powerful steroid hormone calcitriol. Aside from its regulatory role on calcium and phosphate homeostasis, vitamin D has a strong immune-modulatory role triggered when bound with vitamin D receptor (VDR), a member of the nuclear-receptor superfamily which includes corticosteroids.

Vitamin D insufficiency has been linked to abnormal calcium management and secondary hyperparathyroidism as well as immune dysfunction. Consistent with the human literature, vitamin D insufficiency has been linked to many diseases in companion animals.



Diet is the primary source of vitamin D in the form of 25 hydroxyvitamin D (25vitD) acquired from the protein source in food. Commercial manufacturers supplement with vitamin D₃ however, in most instances, it fails to make up for insufficiencies in the commercial processing of dog and cat food.

A growing body of evidence...



Studies Conclude 25vitD Levels below 40ng/mL Result in Adverse Outcomes

Cancer

It has been reported that dogs and cats with lymphoma (2,12), mast cell tumors (5), hemangiosarcoma, carcinoma, histiocytic sarcoma, and other cancers (11,12,13) all have 25vitD values below 40 ng/mL. The relative risk of having cancer increases to almost 4x when 25vitD values are below 40 ng/mL.

Chronic Enteropathy

Disease severity and the incidence of chronic enteropathy (CE) in both cats and dogs increases substantially when 25vitD values fall below 40 ng/mL (4,12). In CE, survivors vs non-survivors can be separated when 25vitD values fall below 30ng/mL (16,19,29,36).

Heart Disease

Congestive heart disease is more prevalent in dogs with 25vitD values below 40ng/mL and there is a 2.6x increase in cardiovascular events (10). Heart remodeling in chronic valvular heart disease worsens as 25vitD values fall below 30 ng/mL (18).

Chronic Kidney Disease

Studies show acute and chronic kidney disease is more prevalent in dogs with 25vitD values below 40 ng/mL (1,2). As 25vitD values fall below 40ng/mL, creatinine increases dramatically (25).

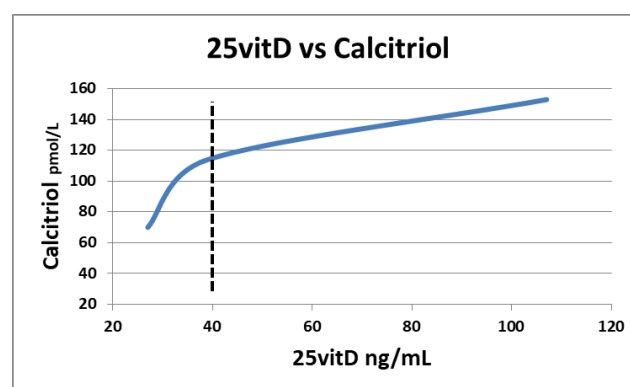
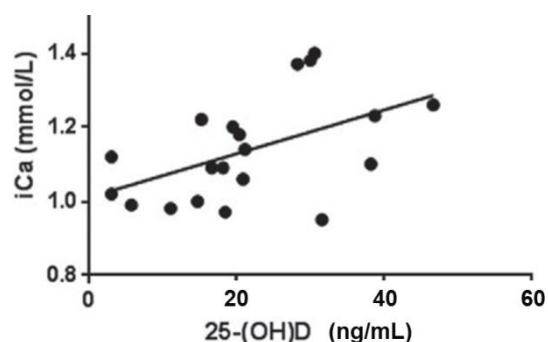
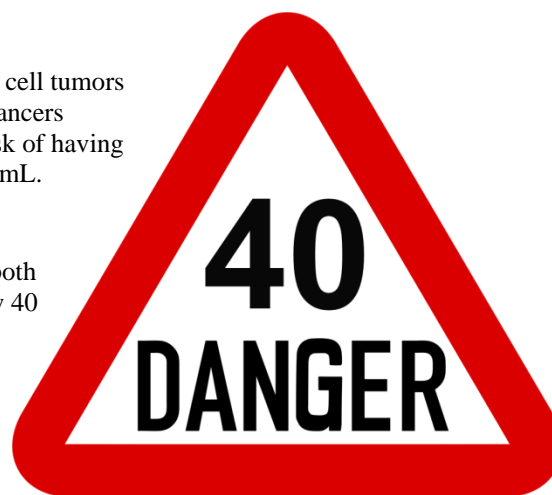
Hospital Mortality

Hospitalized cats with 25vitD levels below 40 ng/mL have an 8x higher incidence of dying (14) and hospitalized dogs with 25vitD levels below 33ng/mL have a 7x higher incidence of dying (35).

Calcitriol Drops

25vitD is the substrate for the enzymatic conversion to the active hormone calcitriol. When 25vitD values fall below 40 ng/mL, there is difficulty in maintaining proper calcitriol levels (1).

As a result, ionized calcium can fall below the reference interval (4,33). Maintaining proper ionized calcium levels is vital for muscle and nerve function.



VDI HAS CONCLUDED THAT THE BODY OF EVIDENCE SUPPORTS A HIGH RISK OF DISEASE AND/OR DEATH WHEN 25VITD LEVELS ARE BELOW 40 NG/mL AND THEREFORE DEFINES THIS AS "DEFICIENCY".

A growing body of evidence...

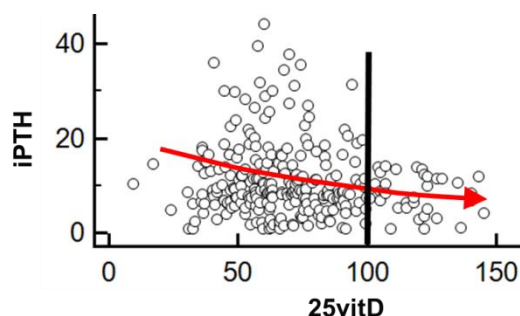


Defining Vitamin D SUFFICIENCY

25vitD sufficiency is defined not only as the proper level for the management of calcium homeostasis, but also the innate immune response. It comes from two methods; surrogate biomarkers and clinical evidence.

Surrogate Biomarkers

In a major study (13) that defined vitamin D sufficiency [modeled after a human study (i)], four surrogate biomarkers were chosen to define the proper level of 25vitD in dogs. These biomarkers were intact parathyroid hormone (iPTH), calcium, phosphorous, and C-reactive protein (CRP).



Vitamin D and PTH

Through negative feedback, the parathyroid gland senses ionized calcium levels and adjusts PTH levels to regulate the enzymatic conversion of 25vitD to calcitriol facilitating intestinal absorption of calcium. Further, PTH activates osteoclasts to increase calcium release from the bone. As 25vitD levels increase, iPTH decreases. When iPTH plateaus, 25vitD sufficiency has been attained. The study found this occurred at 100 ng/mL of 25vitD.

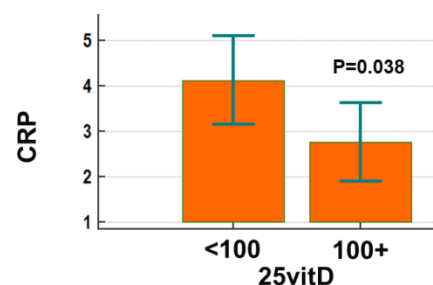


Vitamin D and Calcium/ Phosphorous

The maintenance of proper serum calcium and phosphorous is critical for proper muscle and nerve function. Calcium and phosphorous levels should be stable and variability minimal. The study found the level was reached at 100 ng/mL of 25vitD.

Vitamin D and Inflammation

Vitamin D (25vitD & calcitriol) and its receptor (VDR) are found on a variety of cells (eg, mast, WBC) and tissues (eg, skin, intestines) (36) regulating gene transcription and the innate immune response. Studies (13,37,38,39) have shown vitamin D influences the inflammatory cascade involved in the production of acute phase proteins (APP). In dogs, CRP is the major APP. The study (13) found CRP (inflammation) drops when 25vitD levels reach 100ng/mL.



Clinical Evidence

The relative risk of developing cancer drops as 25vitD levels approach 100ng/mL and becomes beneficial with values above 100 ng/mL (13).

In dogs with CKD, those whose 25vitD levels approach 100 ng/mL had the lowest creatinine levels (25).

In a vitamin D interventional study on dogs with atopic dermatitis, those with 25vitD levels within the range of 100-150 ng/mL saw significant improvement in both pruritus and CADESI scores (31).

Inflammation markers (neut ct, mono ct, IL-2, IL-6, IL-8, TNF-alpha) all significantly drop in CE dogs as 25vitD levels approach 100 ng/mL. The duodenal histopathology score also significantly improves with 25vitD levels approaching 100 ng/mL (19).

25(OH)D	Relative Risk
<40 ng/mL	3.9
<60 ng/mL	2.0
<80 ng/mL	1.4
<100 ng/mL	1.1
>100 ng/mL	0.2 (benefit)

- Hollis, B (2005), Circulating 25-Hydroxyvitamin D Levels Indicative of Vitamin D Sufficiency: Implications for Establishing a New Effective Dietary Intake Recommendation for Vitamin D. Am Soc Nut Sci

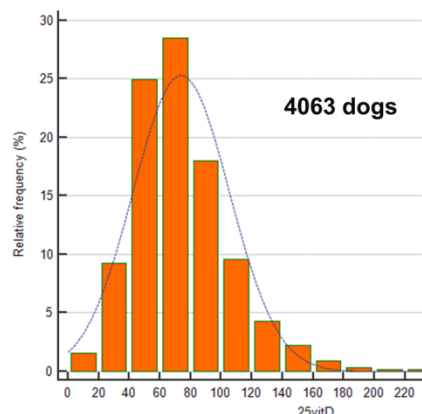
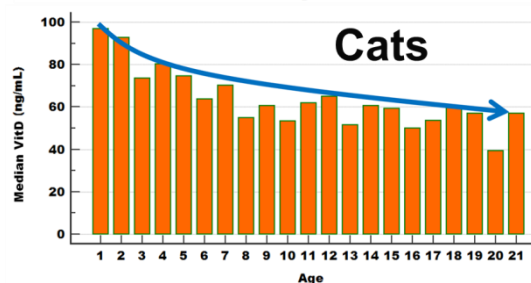
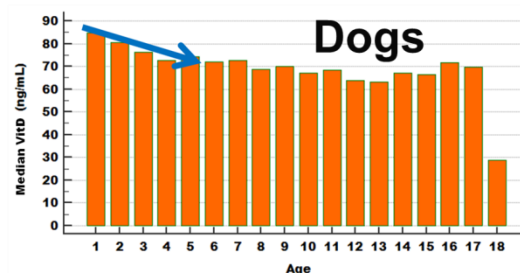
A growing body of evidence...



25vitD Status in Dogs and Cats

Diet is the primary source for vitamin D in dogs and cats whether it is D3 additives to the food or within the protein source primarily as 25vitD. Many factors affect vitamin D status which includes:

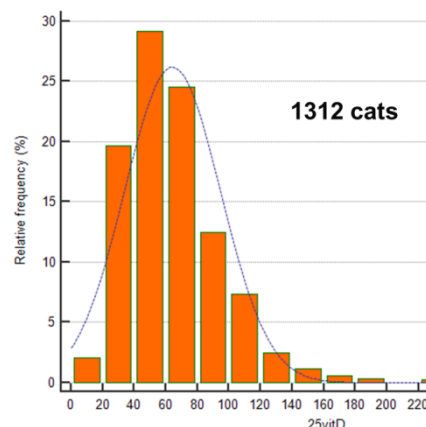
- Food
 - Certain manufacturers of food can have profound differences in 25vitD status (15).
- Age
 - As animals get older their ability to absorb vitamin D diminishes (VDI internal data).
- Neuter Status
 - Male neutered dogs have 27% less 25vitD than intact males; female spaying drops by 9% (15).
- Disease
 - Diseases affecting the GI tract have the greatest impact on vitamin D absorption (412,16,19,29,36).
- Medication
 - COX2 inhibitors (eg, meloxicam) can cause drops of up to 50% (VDI internal data).
 - Corticosteroids upregulate the consumption of vitamin D (VDI internal data).



Unsupplemented Distribution

The incidence of 25vitD deficiency, as defined as <40ng/mL, is 11% in dogs and 22% in cats.

The incidence of 25vitD sufficiency, as defined as >100ng/mL, is 21% in dogs and 14% in cats.



Testing and Treating

Vitamin D Insufficiency

Vitamin D insufficiency is easy to test and correct with inexpensive D3 supplementation. The amount of D3 required depends upon the degree of insufficiency (determined by testing), the patient's species, weight, age, intact status, and other factors. VDI has developed a D3 dosing system that is tailored to each pet.

Testing is simple and cost effective using VDI's dried serum methodology. The patient's serum is applied to a card and simply mailed by common carrier. The card easily crosses international borders without custom declarations.

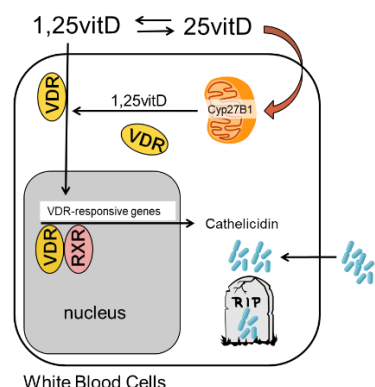
Dosing information is provided on the report with a pet-parent friendly information sheet detailing the result and D3 dosing required for their cat or dog.

Load & Go Dried Serum



Mechanism of Action

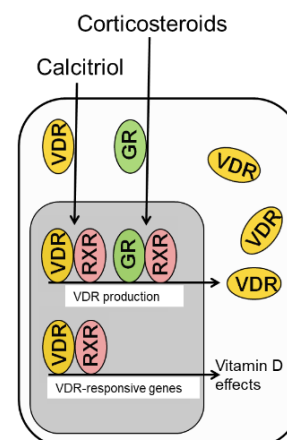
Vitamin D is a secosteroid with a powerful endocrine function. The active form of vitamin D, calcitriol (1,25vitD) influences or regulates a wide array of including calcium homeostasis and innate immune response. All forms of vitamin D (D3, 25vitD, calcitriol) are transported through the blood stream via the vitamin D binding protein (DBP). Vitamin D cellular receptors (VDR) are found throughout the body including mast cells, WBC, skin, intestines, placenta, spleen, kidney, heart, and others (36,56).



While the kidney is the primary source of calcitriol in the blood, regulated by the parathyroid gland, many cells and tissues can make intracellular calcitriol from 25vitD. White blood cells are strongly influenced by vitamin D and can convert 25vitD to calcitriol via Cyp27B1 intracellularly. Vitamin D exercises its effect on cells via VDR. VDRs are members of the superfamily of nuclear receptors for steroid hormones and act as ligand-activated transcription factors (36). In response to an infection, WBCs manufacture canine cathelicidin which acts as a natural antibiotic. Studies show calcitriol can regulate cytokine production and neutrophil viability and phagocytosis (39,44,49,51). Further, studies show immune modulating effects of calcitriol by decreasing pro-inflammatory cytokine TNF- α in both endotoxin primed and unprimed leukocytes. Calcitriol increases anti-inflammatory cytokine IL-10 in

endotoxin primed leukocytes only; calcitriol may be able to reduce the harmful effects of a pro-inflammatory state without compromising the protective effect of viable neutrophils to fight infections (37,38,39,51).

Corticosteroids are commonly used in veterinary medicine for atopic dermatitis and chronic enteropathies. Corticosteroids share the same superfamily of nuclear receptors as vitamin D known as retinoid X receptor (RXR). Corticosteroids and calcitriol work in concert to one another to exert their effects. A canine study has shown calcitriol significantly upregulates the mRNA expression of NR3C1, the glucocorticoid receptor (49). Human asthmatic studies have shown a steroid-sparing effect whereby less corticosteroids can be used to achieve the same effect when vitamin D supplementation occurs. This same steroid-sparing effect was demonstrated in dogs with atopic dermatitis. In an interventional study, canines being treated with D3 supplementation showed significant improvement ($P < 0.0001$) in both pruritus as well as the CADESI score within 8 weeks (32).



Calcitriol plays a role in cancer management by regulating ROS. Catalases are the primary enzymes for the removal of H_2O_2 and managing ROS. Catalase plays an essential role in cell defense against oxidative stress. A decrease in catalase activity is associated with diseases such as diabetes, hypertension, cancer, and even gray hair. In canine bladder transitional carcinoma cell line, calcitriol significantly increased catalase, catalase mRNA, and MAPK which alters the ROS state and induces apoptosis (20).

Apoptosis, or programmed cell death, is the cellular mechanism to remove damaged cells. When DNA damage occurs, apoptosis may be impacted allowing cancer to propagate. Calcitriol, binding to VDR, has anti-carcinogenic properties and induces apoptosis. Many cancers can attenuate the role vitamin D plays by the inhibition of VDR. In a canine study of mammary carcinomas, VDR expression in malignant tumors was 27%, benign tumors 40% and normal glands 100% (56). Vitamin D sufficiency is essential to help prevent cancer and may aid in cancer therapy in those tumors where VDR expression remains.

A growing body of evidence...

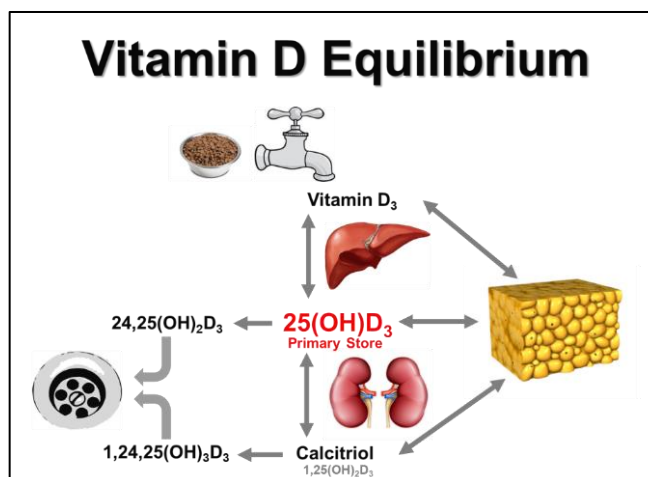


Vitamin D Supplementation

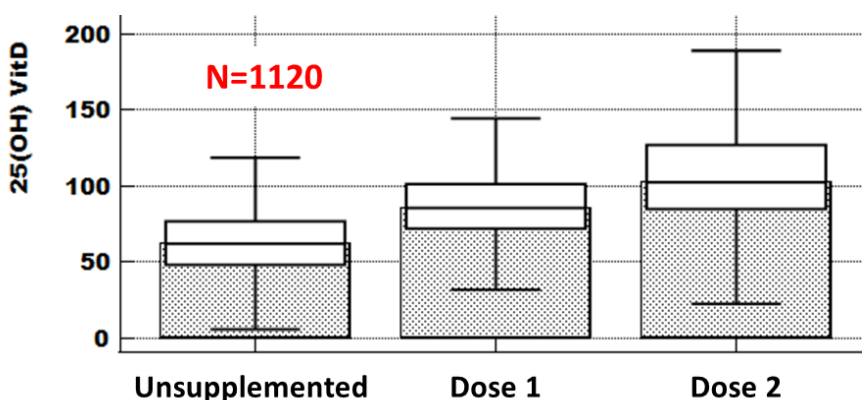
Vitamin D undergoes a series of enzymatic reactions ultimately becoming the active hormone calcitriol. However, since vitamin D is fat soluble, it is readily taken up within the adipose tissue. The adipose tissue acts as a buffer or reserve of D_3 , $25(OH)D_3$, and calcitriol.

VDI recommends supplementing with cholecalciferol or D_3 . VDI's proprietary dosing algorithm uses a series of patient specific factors including, species, weight, age, and neuter status to determine the exact dose required for the patient.

For many patients, the initial dosing recommended will achieve sufficiency however for some, especially those with GI disease or on corticosteroids, a higher second dosing level is required. Unlike antibiotics, D_3 supplementation is typically required for life and typically increases with age; **annual 25vitD testing is recommended.**



In a normal healthy dog or cat, it takes 8-10 weeks for equilibrium to establish and will need to be re-evaluated when there are changes in diet and/or D_3 supplementation.



In moderately or severely obese animals, the increased amount of adipose tissue dramatically increases vitamin D reserves and therefore a longer time is needed for a new equilibrium to develop. In these patients VDI is recommending increasing the retest interval to 4 months.

Vitamin D is routinely deactivated by an additional hydroxylation making $24,25(OH)_2D_3$ or $1,24,25(OH)_3D_3$. The half-life of $25(OH)D_3$ is about 3 weeks.

In obese patients where weight reduction is sought, a recent study (34) concluded “...**vitamin D status of dogs is not affected by obesity or loss of body fat with therapeutic weight reduction**”. Therefore, equilibrium is maintained as body mass changes.

- **Test before supplementing**
- **Wait 8-10 weeks for retesting**
- **Increase time to test to 4 months in obese patients**

1. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure

Gerber B, et al, Am J Vet Res, 2003

cohort = 64 dogs

- Mean 25vitD was significantly lower in dogs with ARF and CRF (34 and 52 ng/mL respectively) than control dogs (107 ng/mL). 1,25vitD was not significantly different.

2. Serum levels of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol in dogs with hypercalcemia

Gerber B, et al, Vet Res Commun, 2004

cohort = 60 dogs

- Median 25vitD was significantly lower in dogs with lymphoma, primary hyperparathyroidism and CRF (41, 36, and 27 ng/mL respectively) than control dogs (123 ng/mL). 1,25 VitD was not significantly different.

3. Tooth resorption and vitamin D3 status in cats fed premium dry diets

Girard N, et al, Journal Veterinary Dentistry 2010

cohort = 64 cats

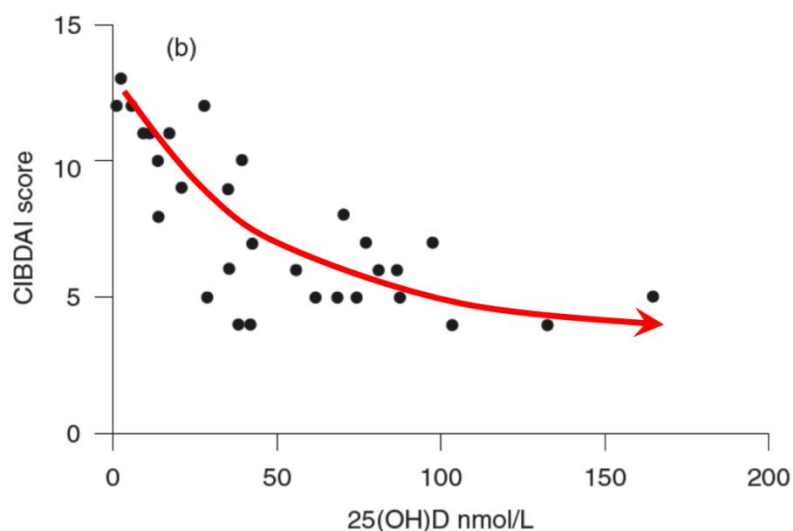
- Mean 25vitD was significantly lower in cats with significant tooth resorption (52.5 ng/mL) than healthy cats (75.1 ng/mL).

4. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminemia

Gow AG, et al, J Small Anim Pract, 2011

cohort = 118 dogs

- Median 25vitD was significantly lower in dogs with IBD and hypoalbuminemia than control dogs (median values not provided). 1,25 VitD was not significantly different.
- Canine Inflammatory Bowel Disease Activity Index (CIBDAI) score significantly declined with rising levels of 25vitD.



5. Cross-sectional study to investigate the association between vitamin D status and cutaneous mast cell tumors in Labrador retrievers

Wakshlag JJ, et al, Br J Nutr, 2011

cohort = 87 dogs

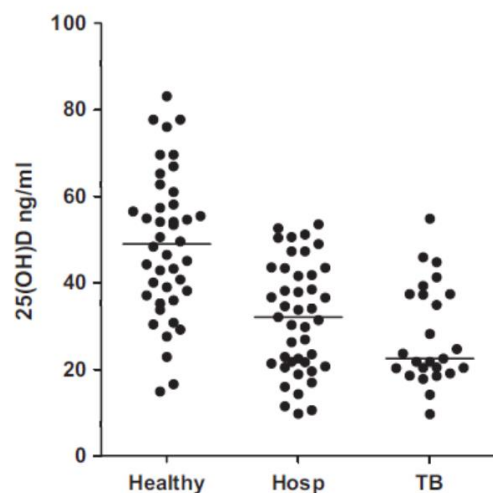
- Mean 25vitD was significantly lower in dogs with MCT (42 ng/mL) than control dogs (48 ng/mL).

6. Domesticated cats with active mycobacteria infections have low serum vitamin D (25(OH)D) concentrations

Lalor SM, et al, Transboundary and Emerging Diseases 2012

cohort = 101 cats

- Median 25vitD was significantly lower in cats with mycobacteriosis (22.2 ng/mL) than healthy cats (49.0 ng/mL).

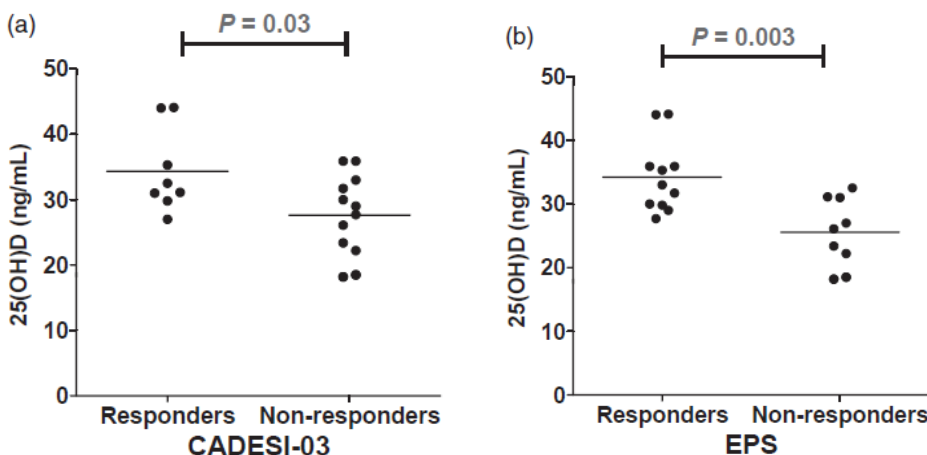


7. Prednisolone therapy for atopic dermatitis is less effective in dogs with lower pretreatment serum 25-hydroxyvitamin concentrations

Kovalik M, et al, Vet Dermatol, 2012

cohort = 20 dogs

- In dogs with atopic dermatitis being treated with prednisolone, those that exhibited improvement of their physical signs as measured by the CADESI-03 had a significantly higher 25vitD levels than those with a suboptimal response.
- 25vitD and prednisolone may have a synergistic therapeutic effect.



8. Vitamin D receptor, retinoid X receptor, Ki-67, survivin, and ezrin expression in canine osteosarcoma

Davies J, et al, Vet med Int'l, 2012

cohort = 33 dogs

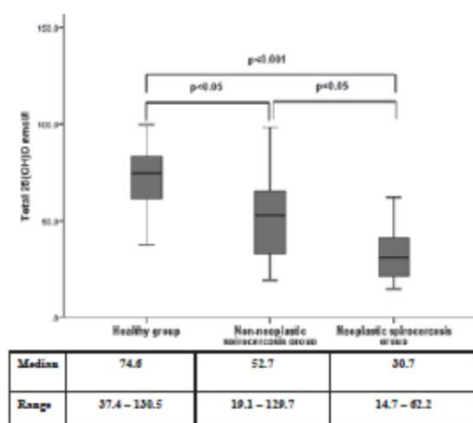
- Tissues sections of osteosarcoma were stained for the presence of the vitamin D receptor (VDR), Retinoid X Receptor (RXR), Ki-67, Survivin, and Ezrin Expression.
- 73% showed VDR expression with 27% showing 2+ positivity.
- RXR is an integral component of the RXR-VDR-VitD complex; 97% showed RXR expression.
- Ezrin is linked to metastasis in human cancers; 100% showed Ezrin expression.
- Survivin is a nuclear protein and inhibits apoptosis; 81% showed Survivin expression.
- Study supports theory that VitD administration may have a therapeutic role in canine osteosarcoma.

9. Hypovitaminosis D in dogs with spirocercosis

Rosa CT, et al, J Vet Intern Med, 2013

cohort = 75 dogs

- Median 25vitD was significantly lower in dogs with neoplastic spirocercosis (12 ng/mL) and dogs with non-neoplastic spirocercosis (21 ng/mL) than control dogs (30 ng/mL).

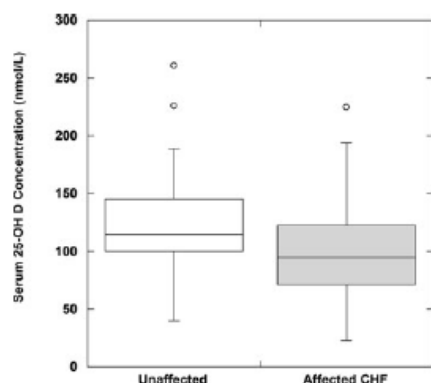


10. Relation of vitamin D status to congestive heart failure and cardiovascular events in dogs

Kraus MS, et al, J Vet Intern Med, 2013

cohort = 82 dogs

- Mean 25vitD was significantly lower in CHF dogs (40 ng/mL) than in the control group (50 ng/mL).
- There was a significant association of low 25vitD and poor outcome; those with low values had a 2.6 times greater hazard of having a cardiovascular event.



11. Low stores of 25-hydroxyvitamin D levels and its association with cancer in dogs

Husbands B, VCS presentation, 2013

cohort = 493 dogs



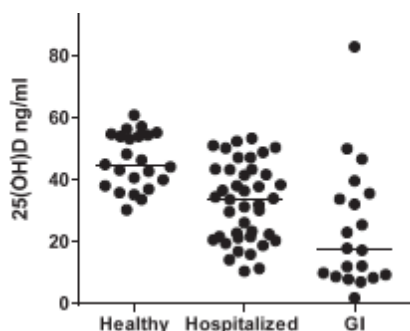
- Median 25vitD was significantly lower in the disease cohort (n=335, 313 malignant and 22 benign; 62.6 ng/mL) than the control group (67.4 ng/mL).
- Cancers that demonstrated significantly lower 25vitD levels were carcinoma (n=64), histiocytic sarcoma (n=8), hemangiosarcoma (n=10), lymphoma (n=80), and sarcoma (n=48).
- Cancers that did not show significantly lower 25vitD levels were mast cell (n=51), melanoma (n=17), osteosarcoma (n=19), and other misc cancers (n=13).
- Relative Risk of having cancer with 25vitD concentrations below 40ng/mL was 2.7 (P=0.001).

12. Cats with inflammatory bowel disease and intestinal small cell lymphoma have low serum concentrations of 25-hydroxyvitamin D

Lalor S, et al, J Vet Intern Med, 2014

cohort = 84 cats

- Median 25vitD was significantly lower in cats with IBD/ISCL (12.7 ng/mL) than in healthy cats (45.1 ng/mL) and in hospitalized cats with non-GI disease (33.8 ng/mL).



13. Circulating 25-hydroxyvitamin D levels in dogs – correlation with health and cancer risk

Selting K, et al, Vet Comp Onco, 2014

cohort = 345 dogs



- 282 healthy dogs were tested for 25vitD, PTH, CRP, tCa, and Phos to evaluate surrogate biomarkers for vitamin D sufficiency.
- Inverse relationship of 25vitD with PTH and CRP.

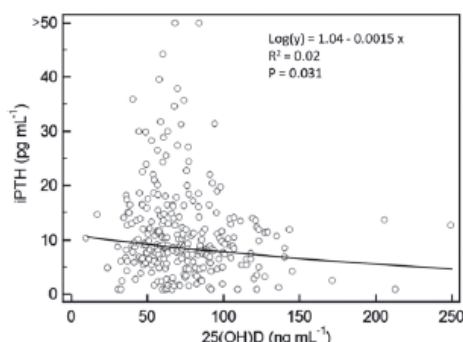


Figure 1. Scatterplot and regression of iPTH and 25(OH)D in the control group.

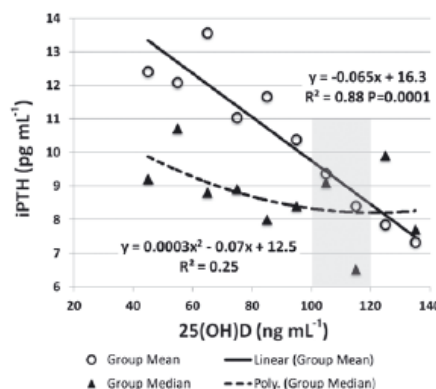
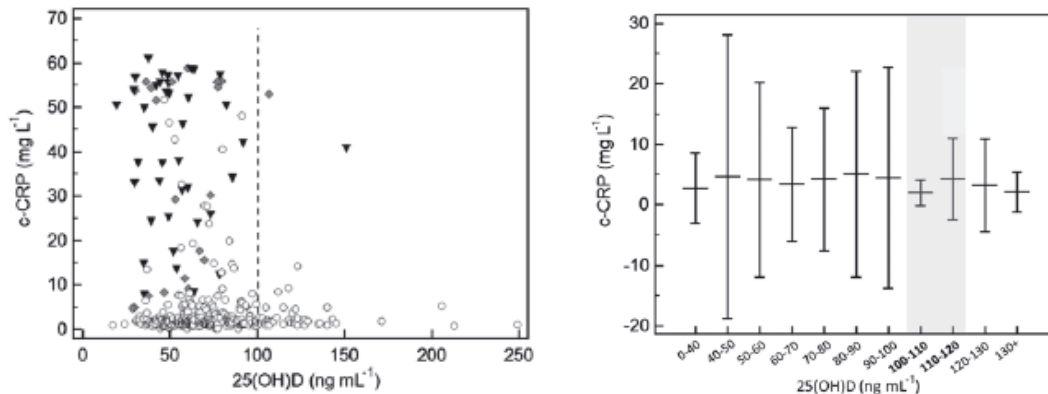


Figure 2. Mean and median iPTH at 10 ng mL⁻¹ intervals of 25(OH)D in the control group.

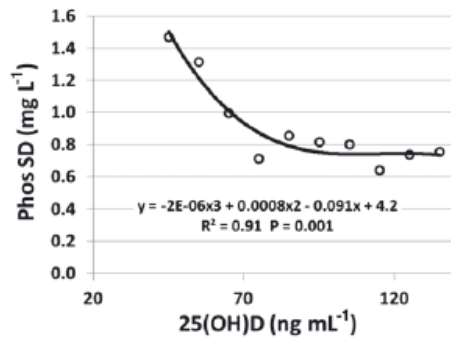
A growing body of evidence...



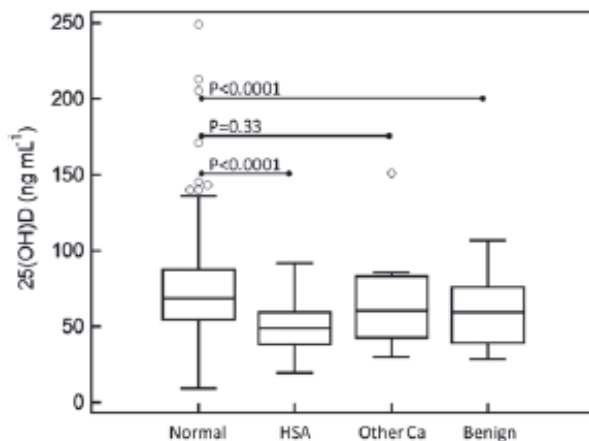
- 25vitD values above 100 ng/mL showed significantly lower levels of inflammation.



- 25vitD values over 100 ng/mL showed significantly reduced Phos levels and stability.



- Second cohort of 62 tumor bearing dogs evaluated for 25vitD.
- Median 25vitD was significantly lower in dogs with cancer of various types (49 ng/mL) than control dogs (69 ng/mL). Relative risk of cancer increased as 25vitD concentrations decreased ($P < 0.0001$).



25(OH)D	Relative Risk
<40 ng/mL	3.9
<60 ng/mL	2.0
<80 ng/mL	1.4
<100 ng/mL	1.1
>100 ng/mL	0.2 (benefit)

- Study helped to define 25vitD sufficiency to provide cellular health as 100-120 ng/mL.

A growing body of evidence...

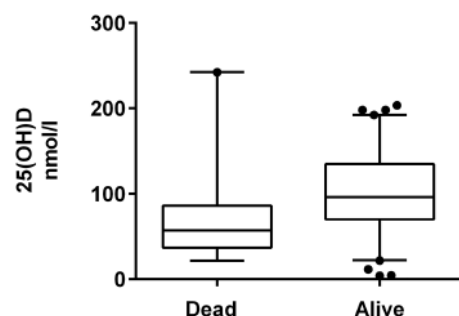


14. Vitamin D status predicts 30-day mortality in hospitalized cats

Titmarsh H, et al, PLOS, 2015

cohort = 99 cats

- 25vitD was significantly lower in hospitalized cats that died within 30 days than those that were alive after 30 days. Cats with 25vitD levels of 29 ng/mL or lower had an 8.3 times higher rate of dying than those with higher 25vitD levels ($P=0.0008$).



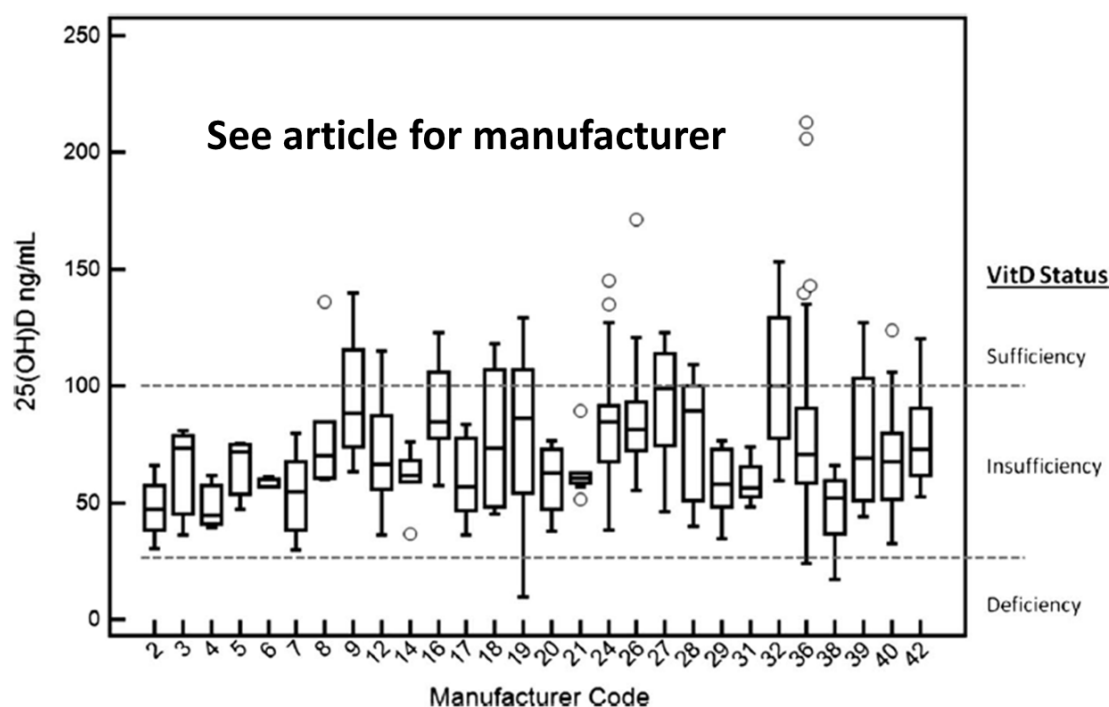
15. The effect of diet on serum 25-hydroxyvitamin D concentrations in dogs

Sharp C, et al, BMC Res Notes, 2015

cohort = 320 dogs



- 320 apparently healthy dogs on 41 different manufacturers of commercial dog food were evaluated for 25vitD levels. Overall serum 25vitD levels ranged from 9.5 – 249 ng/mL, with median, Q1, Q3 at 69.7, 54.5, 88.1 ng/mL, respectively.
- Neuter status correlated with 25vitD concentration. Median 25vitD was 9% lower in spayed compared to intact females, but 27% lower in neutered compared to intact males. Intact status, particularly males, appears to have an impact on serum 25vitD.



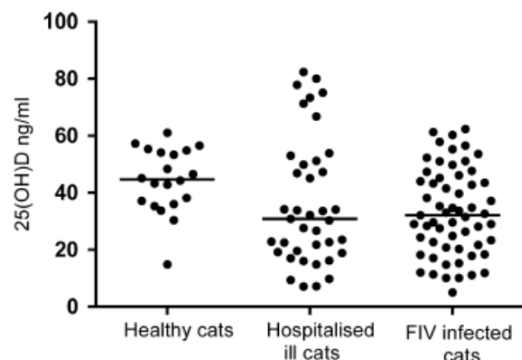
- Study demonstrates the wide variation in 25vitD levels in commercial food and that the primary source of vitamin D is from the protein source.

16. Vitamin D status in cats with feline immunodeficiency virus

Titmarsh H, et al, Vet Med Sci, 2015

cohort = 118 cats

- 25vitD was significantly lower in FIV infected cats than control cats ($P < 0.05$). FIV infected cats had a median 25vitD of 31 ng/mL and those hospitalized of 31 ng/mL.

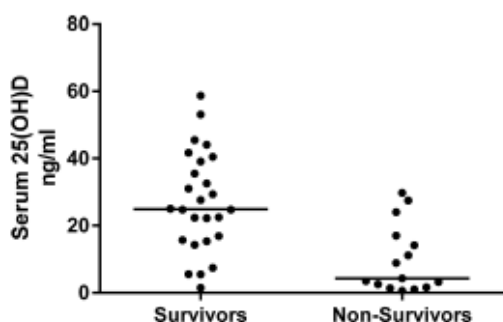


17. Association of vitamin D status and clinical outcome in dogs with chronic enteropathy

Titmarsh H, et al, J Vet Intern Med, 2015

cohort = 41 dogs

- In dogs with chronic enteropathy 25vitD was an independent predictor of mortality. The median 25vitD concentration of survivors was 25 ng/mL versus 4 ng/mL in non-survivors.

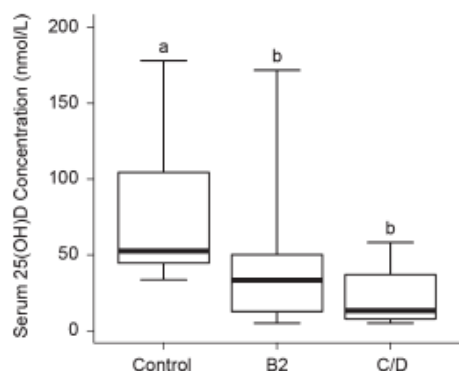


18. Vitamin D status in different stages of disease severity in dogs with chronic valvular heart disease

Osuga T, et al, J Vet Intern Med, 2015

cohort = 43 dogs

- 25vitD was significantly lower in dogs with CHVD ($P = 0.0005$)
- 25vitD was significantly lower in dogs with worsening left ventricular and atrial sizes.

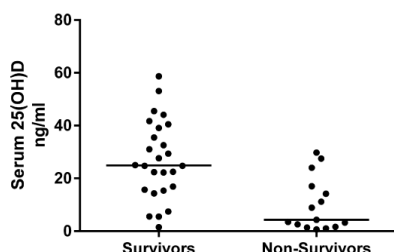


19. Low vitamin D status is associated with systemic and gastrointestinal inflammation in dogs with chronic enteropathy

Titmarsh H, et al, PLOS One, 2015

cohort = 39 dogs

- In dogs with chronic enteropathy, low stores of 25vitD was associated with higher inflammatory parameters such as neutrophil count, monocyte count, IL-2, IL-6, IL-8, and TNF-alpha. There was also a significant association with duodenal histopathology score.



20. 1,25-Dihydroxyvitamin D3 and its analogues increase catalase at the mRNA, protein and activity level in a canine transitional carcinoma cell

Middleton R, et al, Vet Comp Onco 2015

cohort = cell line

- Canine bladder transitional cell carcinoma was evaluated for the effect calcitriol (activated vitamin D) has on gene and protein expression.
- Catalase mRNA was significantly increased as well as mitogen-activated protein kinase (MAPK) signaling.
- Study supports vitamin D role in cell signaling and activation of catalase which is involved in the management of ROS.

21. Oral vitamin D supplementation at five times the recommended allowance marginally affects serum 25-hydroxyvitamin D concentrations in dogs

Young L, et al, J Nutr Sci, 2016

cohort = 46 dogs

- 72% of unsupplemented cohort was vitamin D insufficient.
- Confirming VDI guidelines, supplementation with D3 takes 8-10 weeks for effect.
- Marginal increase of 25vitD when supplementing with low dose D3. Primary store of VitD in diet comes from the protein source as 25vitD; not supplementation. 25vitD has significantly higher potency than D3.
- 24,25vitD, the inactive metabolic breakdown of 25vitD, was ~40% of total 25vitD throughout cohort – substantially higher than found in humans. Relative significance is unknown.

22. Relationship between vitamin D status and leukocytes in hospitalised cats

Titmarsh H, et al, J Feline Med Surg, 2017

cohort = 170 cats

- Hospitalized cats were tested for 25vitD and leukocytes.
- Cats with neutrophilia had a lower 25vitD level than cats with neutrophil concentrations in the upper limit of the reference interval.
- Study helps to support the relationship between 25vitD and the inflammatory response.

23. Canine Leishmaniasis progression is associated with vitamin D deficiency

Rodriguez-Cortes A, et al, Nature, 2017

cohort = 68 dogs

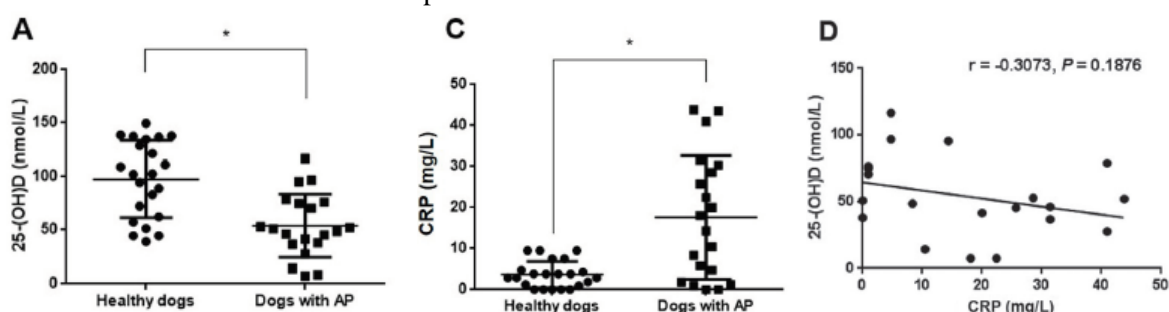
- 25vitD was significantly lower in symptomatic dogs (<20ng/mL) with leishmaniasis than asymptomatic or control dogs (P<0.001).
- 25vitD inversely correlated with leishmania specific IgG, clinicopathological score, and with parasite level in blood.

24. Serum 25-hydroxyvitamin D concentrations in dogs with suspected acute pancreatitis

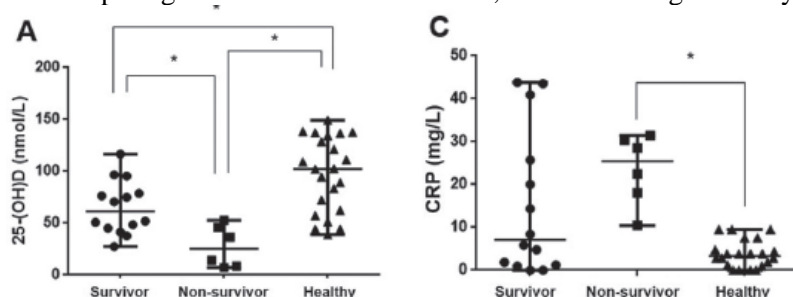
Kim D, et al, J Vet Med Sci, 2017

cohort = 42 dogs

- Dogs with acute pancreatitis were evaluated for level of 25vitD, iCa, and CRP.
- Pancreatitis dogs had significantly lower 25vitD levels than healthy control dogs; pancreatitis dogs had significantly higher CRP level than healthy control dogs; no difference in iCa.
- There was an inverse relationship between CRP and 25vitD.



- Comparing non-survivors to survivors, 25vitD was significantly lower; no difference in CRP.

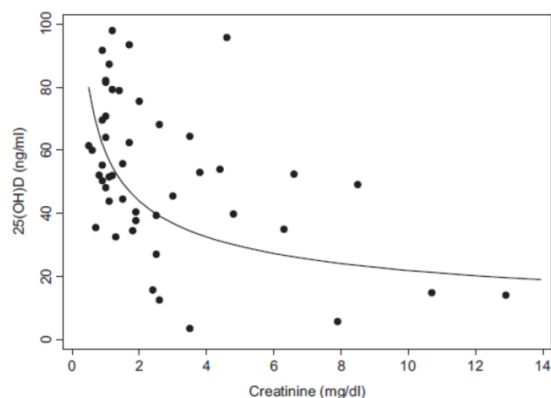


25. Association of vitamin D metabolites with parathyroid hormone, fibroblast growth factor-23, calcium, and phosphorus in dogs with various stages of chronic kidney disease

Parker V, et al, J Vet Intern Med, 2017

cohort = 47 dogs

- Low 25vitD (<50ng/mL) is found in all CKD patients (P<0.01); particularly stage 4 (<25ng/mL).
- A doubling of creatinine concentration was found with a 14% drop in 25vitD (P=0.005).
- Positive correlation was found with 25vitD, total Ca, ionized Ca, and calcitriol (P=0.02).
- 24,25vitD, the inactive metabolic breakdown of 25vitD, was ~50% of total 25vitD throughout cohort – substantially higher than found in humans. Relative significance is unknown.



26. Urinary Tamm-Horsfall protein, albumin, vitamin D-binding protein, and retinol-binding protein as early biomarkers of chronic kidney disease in dogs

Chacar F, et al, *Physiol Rpts*, 2017

cohort = 49 dogs

- Urinary albumin was only significant in dogs with stage 4 CKD.
- Vitamin D-binding protein (VDBP) was found in increasing concentrations in dogs with CKD stage 1-4 ($P < 0.05$).
- VDBP is the carrier for serum vitamin D metabolites and its loss also reduces vitamin D storage (ie, 25vitD).

27. Influence of various factors on circulating 25(OH) vitamin D concentrations in dogs with cancer and healthy dogs

Weidner N, *J Vet Intern Med*, 2017

cohort = 92 dogs

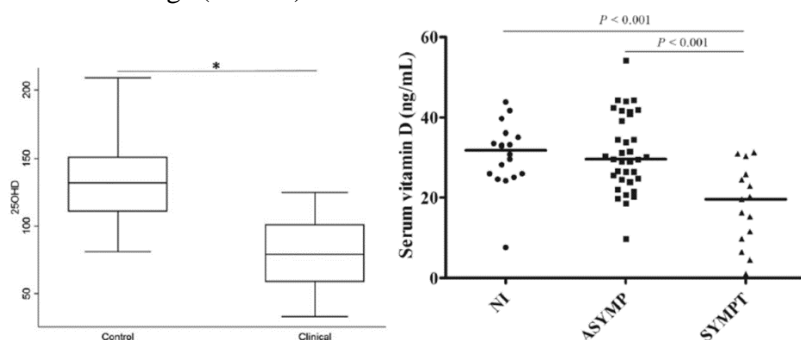
- Control group had a positive correlation between 25vitD and ionized Ca whereas cancer group had a negative correlation.
- 24,25vitD, the inactive metabolic breakdown of 25vitD, was ~50% of total 25vitD throughout cohort – substantially higher than found in humans. Relative significance is unknown.

28. 25-hydroxyvitamin D concentration in dogs with naturally acquired blastomycosis

O'Brien M, *J Vet Intern Med*, 2017

cohort = 57 dogs

- 25vitD was significantly lower in dogs with *Blastomyces dermatitidis* (median 32ng/mL) than control dogs ($P < 0.05$).

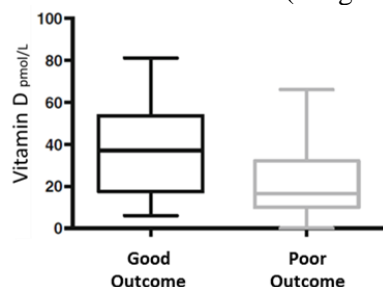


29. Hypovitaminosis D is associated with negative outcome in dogs with protein losing enteropathy: a retrospective study of 43 cases

Allenspach K, *BMC Vet Res*, 2017

cohort = 43 dogs

- 25vitD was significantly lower in PLE dogs with poor outcomes (< 10 ng/mL) than PLE dogs with favorable outcomes (15ng/mL) ($P = 0.017$).



30. Vitamin D toxicity of dietary origin in cats fed a natural complementary kitten food

Crossley V, J Feline Med and Surg Open Rpts, 2017

case study = 3 cats

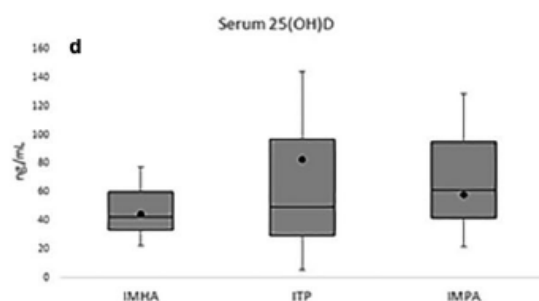
- 3 cats ingested contaminated cat food with D2/D3 content >100x of guideline. All 3 cats displayed hypercalcemia with suppressed PTH. 25vitD levels were above 400 ng/mL. Supportive care and change in diet resolved hypercalcemia within 18 days.
- Cases support 2-3 week half-life of 25vitD, toxicity when levels persist over 400 ng/mL and with suppressed PTH values.

31. Serum thymidine kinase 1, canine-c-reactive protein, haptoglobin, and vitamin d concentrations in dogs with immune-mediated hemolytic anemia, thrombocytopenia, and polyarthropathy

Grobman M, JVIM, 2017

case study = 38 dogs

- IMHA, ITP, and IMPA are immune mediated diseases for which low store of 25vitD has been associated.
- While a control group was not included in this study IMHA, ITP and IMPA were all insufficient (40 -100 ng/mL) or deficient (<40 ng/mL).
- Study support the role of vitamin D in the innate immune process.

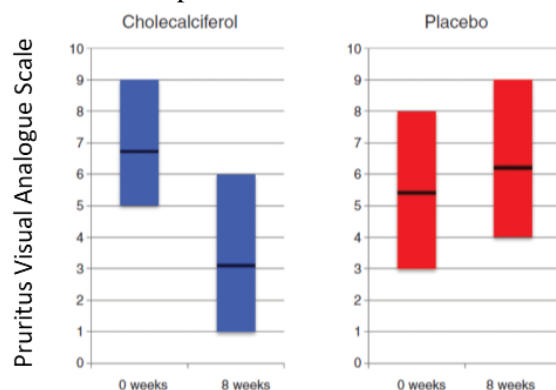


32. Vitamin D shows in vivo efficacy in a placebo-controlled, double-blinded, randomized clinical trial on canine atopic dermatitis

Klinger C, Vet Record, 2018

cohort = 23 dogs

- **True INTERVENTIONAL study evaluating cause and effect.**
- Dogs with unresolved atopic dermatitis were all insufficient with values below 50 ng/mL.
- Dogs being treated with D3 supplementation showed significant improvement ($P < 0.0001$) in both pruritus as well as the CADESI score within 8 weeks.
- Clinical improvement was within the 25vitD sufficiency range of 100-150ng/mL.

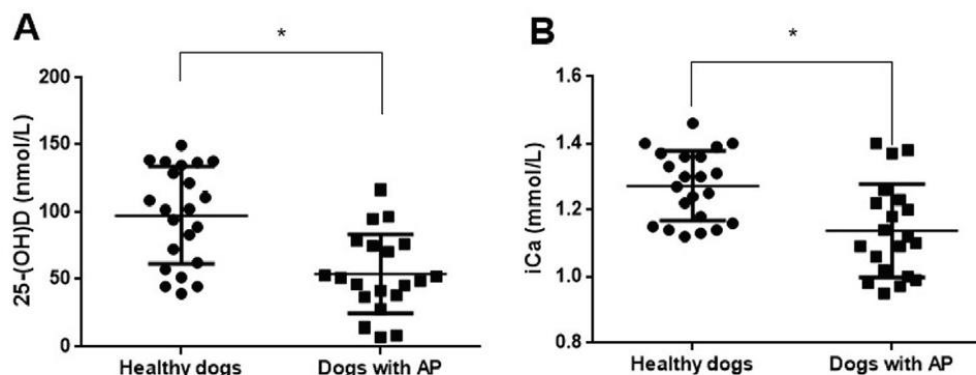


33. Serum 25-hydroxyvitamin D concentrations in dogs with suspected acute pancreatitis

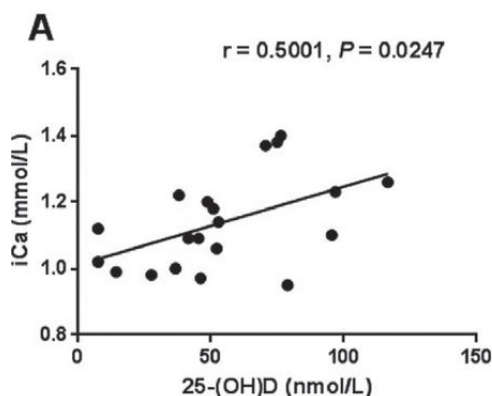
Kim D, J Vet Med Sci, 2018

cohort= 42 dogs

- 25vitD and ionized calcium was significantly lower in acute pancreatitis (AP) dogs (20 ng/mL) than control dogs (40 ng/mL) ($P<0.05$). Survivors vs non-survivors also was correlated with low stores of 25vitD (median 25 vs 10 ng/mL, respectfully) and ionized calcium ($P<0.05$).



- Ionized calcium correlated to 25vitD levels.



34. Effects of body fat mass and therapeutic weight loss on vitamin D status in privately owned adult dogs

Hookey T, J Nut Sci, 2018

cohort= 15 dogs

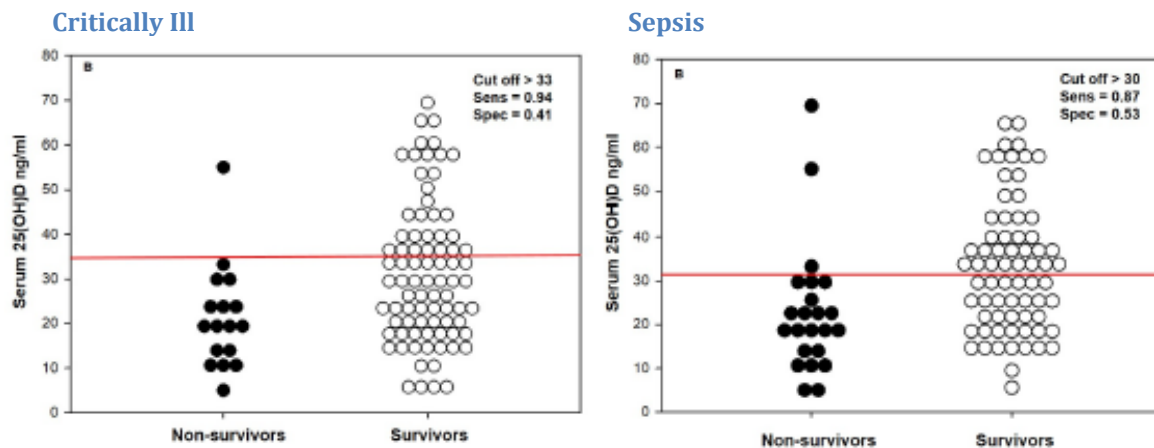
- Obese and lean dogs were compared for changes in 25vitD status when obese dogs were placed on a therapeutic weight reduction diet. No changes in 25vitD status were observed and equilibrium was maintained.

35. Serum vitamin D concentrations in hospitalized critically ill dogs

Jaffey J, PLOS, 2018

cohort= 216 dogs

- Critically ill dogs and dogs with sepsis have a significantly lower 25vitD concentrations than do healthy dogs. 25vitD concentration was an independent predictor of survival with an odds ratio of 7x.



36. Vitamin D receptor expression in dogs

Cartwright J, J Vet Int Med, 2018

cohort= 40 dogs

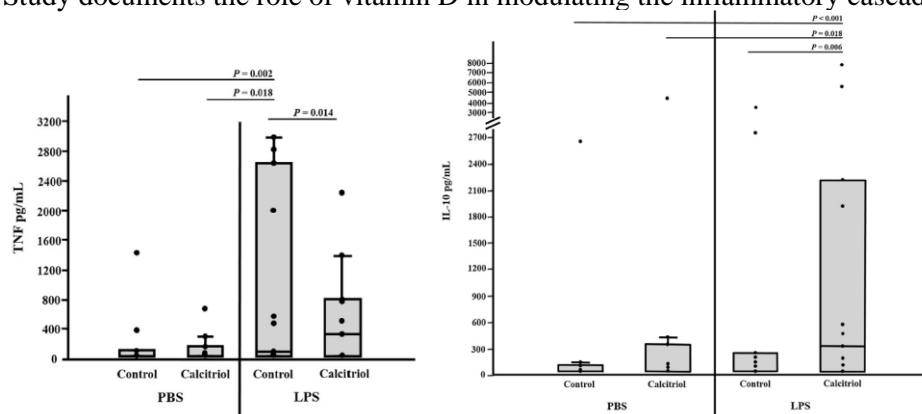
- Histochemical staining for vitamin D receptor (VDR) in non-skeletal tissues demonstrated VDR expression in intestines, kidney, skin, and spleen.
- Histochemical staining for VDR in chronic enteropathy (CE) demonstrated VDR expression within the intestines that did not decline with the presence of inflammation in contrast to humans. The strong association with survival and 25vitD in CE (15,17) as well as the presence of VDR may link the beneficial effect VitD may have in CE.

37. Effect of calcitriol on in vitro whole blood cytokine production in critically ill dogs

Jaffey J, Vet Journal, 2018

cohort= 12 dogs

- In critically ill dogs, calcitriol (activated vitamin D) significantly increased anti-inflammatory cytokine IL-10 and significantly decreased pro-inflammatory cytokine TNF- α in in-vitro whole blood.
- Study documents the role of vitamin D in modulating the inflammatory cascade.

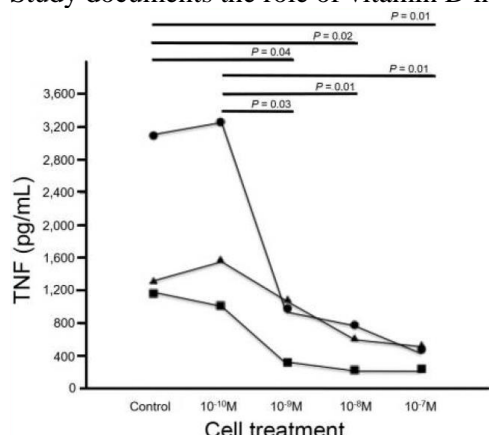


38. Effect of calcitriol on phagocytic function, toll-like receptor 4 expression, and cytokine production of canine leukocytes

Jaffey J, AJVR, 2018

cohort= 8 dogs

- In normal healthy dogs, calcitriol (activated vitamin D) significantly decreased pro-inflammatory cytokine TNF- α in a concentration dependent manner in in-vitro whole blood.
- Study documents the role of vitamin D in modulating the inflammatory cascade.



39. Effect of calcitriol on apoptosis, toll-like receptor 4 expression, and cytokine production of endotoxin-primed canine leukocytes

Jaffey J, AJVR, 2018

cohort= 6 dogs

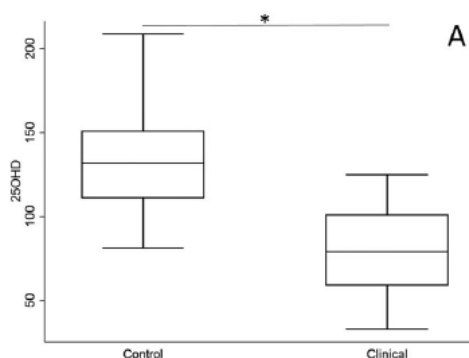
- In normal healthy dogs, calcitriol (activated vitamin D) significantly decreased pro-inflammatory cytokine TNF- α in both endotoxin primed and unprimed leukocytes, and significantly increased anti-inflammatory cytokine IL-10 in endotoxin primed leukocytes only.
- Calcitriol had no effect on leukocyte apoptosis in endotoxin primed leukocytes.
- Study supports that calcitriol can modulate the inflammatory cytokine production (which causes tissue damage in septic dogs) without affecting neutrophil viability (fortify microbial killing).

40. 25-Hydroxyvitamin D concentrations in dogs with naturally acquired blastomycosis

O'Brien M, JVIM, 2018

cohort= 57 dogs

- Dogs with blastomycosis had significantly lower median 25vitD than healthy controls (32 ng/mL vs 52 ng/mL, $P < 0.001$)
- Blastomycosis dogs had elevated iCa and suppressed PTH in response to activated macrophage generation of calcitriol. Study supports role of vitamin D in macrophage response to infection.

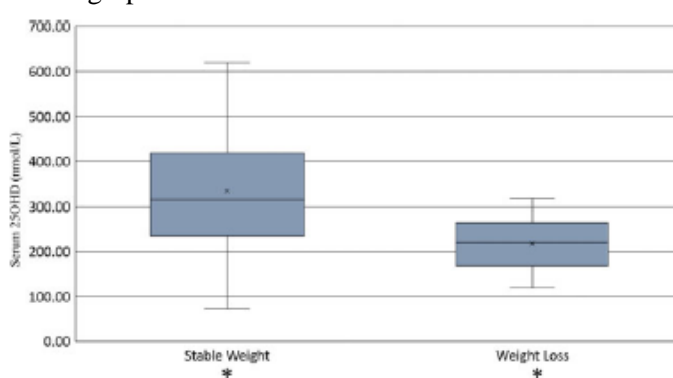
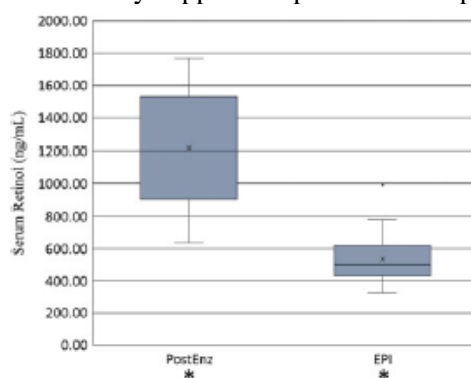


41. Serum concentrations of lipid-soluble vitamins in dogs with exocrine pancreatic insufficiency treated with pancreatic enzymes

Barko P, JVIM, 2018

cohort= 35 dogs

- EPI, a malabsorptive syndrome caused by deficient pancreatic enzymes, can be treated with pancreatic enzyme replacement therapy (PERT). PERT significantly increased VitA but did not affect 25vitD.
- EPI dogs with persistent weight loss showed significant reduction in 25vitD.
- Study supports importance of supplementing lipid soluble vitamins in EPI.

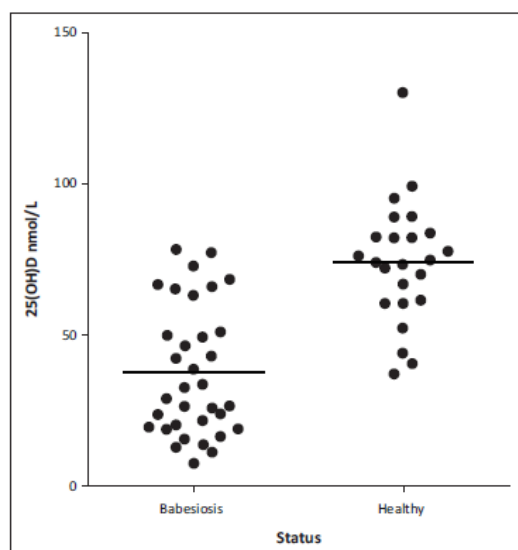


42. Vitamin D status in dogs with babesiosis

Dvir E, Onderstepoort J Vet Res, 2019

cohort= 58 dogs

- Dogs with babesiosis had significantly lower 25vitD levels than healthy controls (median 15ng/mL, 30ng/mL respectively)
- There was an inverse correlation between 25vitD levels and clinical severity score.
- Study supports the role of vitamin D in the prevention and management of infections.

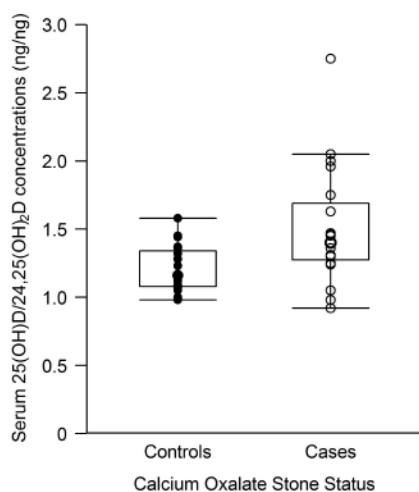


43. Vitamin D metabolism in dogs with and without hypercalciuric calcium oxalate urolithiasis

Groth E, JVIM, 2019

cohort= 38 dogs

- 25vitD is deactivated by 24-hydroxylase to produce 24,25vitD. Calcium oxalate (CaOx) urolith formation was examined for abnormalities in vitamin D metabolism
- There was no significant difference between the control group and dogs with CaOx. In a minor subset of dogs there was an increase in the 25vitD/24,25vitD ratio however further analysis is warranted.
- Study does not support vitamin D metabolic disorders for the cause of CaOx formation.

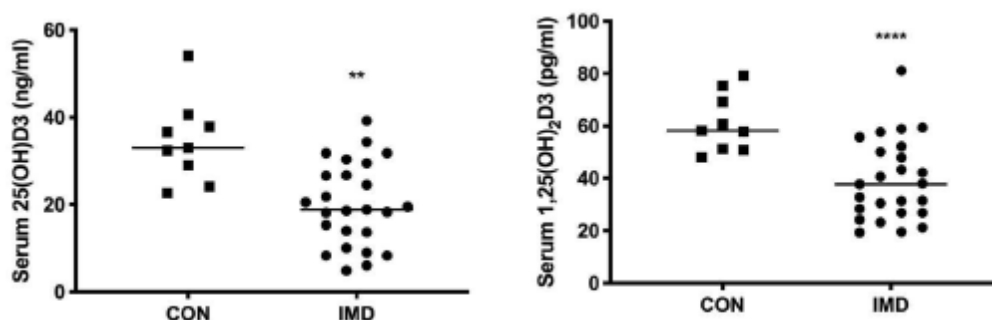


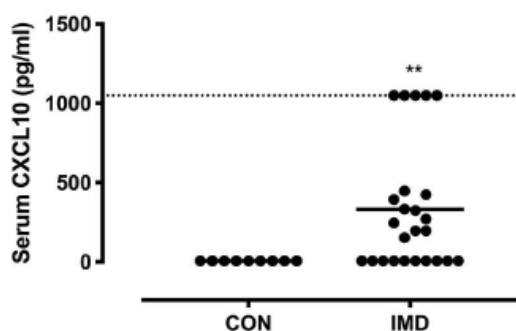
44. Serum Vitamin D Metabolites and CXCL10 Concentrations Associate with Survival in Dogs with Immune Mediated Disease

Mick P, Frontiers Vet Sci, 2019

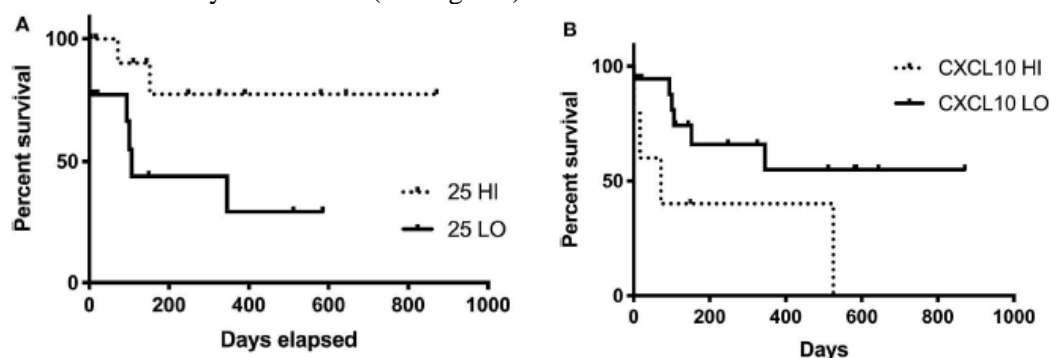
cohort= 38 dogs

- Dogs with immune mediated disease (IMD) that included IMHA, ITP, IMPA, others, were compared to control dogs for 25vitD, calcitriol, 24,25vitD, and CXCL10 (a leukocyte chemokine)
- There was a significant difference for both 25vitD and calcitriol (activated vitamin D) between control dogs and IMD dogs however there was no difference in 24,25vitD (deactivated VitD).
- IMD dogs demonstrated a significant increase in CXCL10 concentration.





- Survival time was significantly longer in IMD dogs with low 25vitD (>20 ng/mL) versus those with extremely low 25vitD (<20 ng/mL)



- Study demonstrates that vitamin D is involved in the innate immune response and that both 25vitD and CSXCL10 are prognostic biomarkers in IMD patients.

45. Comparison of clinical, clinicopathologic, and histologic variables in dogs with chronic inflammatory enteropathy and low or normal serum 25-hydroxycholecalciferol concentrations

Wennogle S, JVIM 2019

cohort= 30 dogs

- Dogs with chronic inflammatory enteropathy (CIE) were separated between low 25vitD and normal levels of 25vitD.
- Dogs with low 25vitD had higher inflammation (CRP), lower albumin, VitE, and cholesterol levels.
- 25vitD levels inversely correlated with CCECAI scores.
- Study supports 25vitD plays an important role in the management of patients with CIE.

46. Persistent Increase in Serum 25-Hydroxyvitamin D Concentration in a Dog Following Cholecalciferol Intoxication (case study)

Gerhard C, Frontiers Vet Sci, 2020

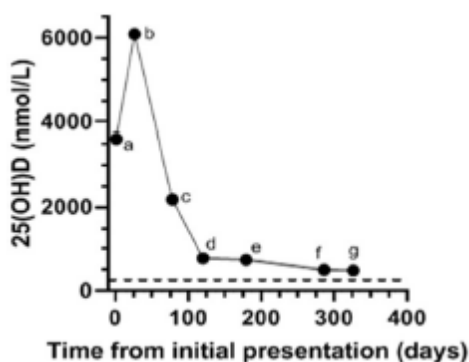
cohort= 1 dog

- Shih-Tzu mix ingested cholecalciferol (D3) containing rodenticide, managed and followed for one year.
- Within 3 days of ingestion, 25vitD levels rose to over 2400 ng/mL with hypercalcemia. Therapeutic treatment included activated charcoal, steroids, and aluminum hydroxide.
- Hypercalcemia resolved by day 8.
- High levels of 25vitD persisted for over 250 days, however fell substantially within the first 3 months.

A growing body of evidence...



- Cholecalciferol or D3 is converted to 25vitD via the liver. D3 is also stored in the adipose tissue. This study demonstrates the ability a dog can manage an extremely high dose of D3 and the time it takes to resolve.



47. Immune function and serum vitamin D in shelter dogs: A case-control study

Allison L, Vet J, 2020

cohort= 20 dogs

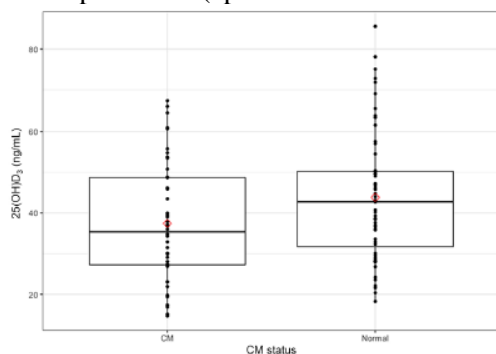
- In shelter dogs, there was a significant inverse association between serum 25vitD and LPS-stimulated leukocyte cytokine production of TNF- α . This finding supports results from previous in vitro studies in dogs (#32) that incubation of blood with calcitriol (activated vitamin D) attenuates leukocyte production of TNF- α in a concentration- dependent manner.

48. Vitamin D status in cats with cardiomyopathy

Ware W, JVIM, 2020

cohort= 100 cats

- Older cats have significantly lower 25vitD levels.
- 25vitD is an independent predictor of mortality in cats with cardiomyopathy with 40 ng/mL being an important cutoff between the two groups.
- 3-epi-25vitD (epimeric form of 25vitD) was 24% in both groups with no statistical difference.



49. Adjuvanting Allergen Extracts for Sublingual Immunotherapy: Calcitriol Downregulates CXCL8 Production in Primary Sublingual Epithelial Cells

Pelst M, Frontiers Vet Sci, 2020

cohort= 6 dogs

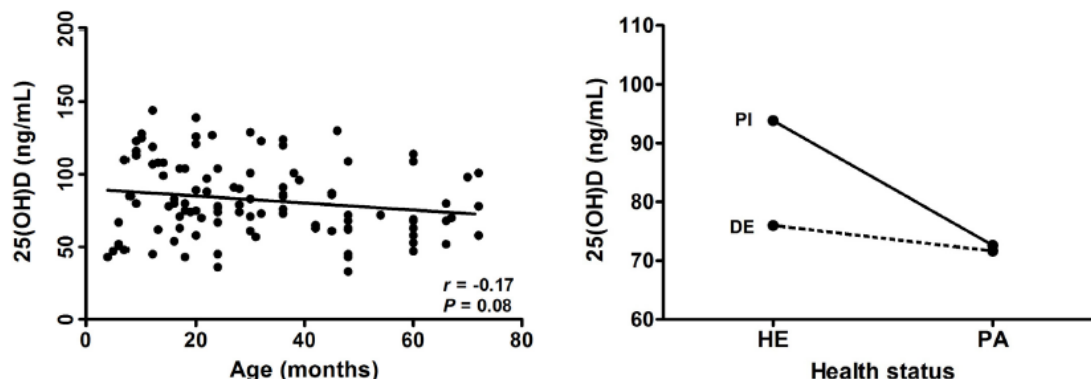
- Canine sublingual epithelial cells were used to evaluate the effects of calcitriol (activated vitamin D) on the immune response of extracts of dust mites.
- Calcitriol significantly suppressed CXCL8 (a leukocyte chemokine).
- Calcitriol significantly upregulated the mRNA expression of NR3C1, the glucocorticoid receptor.
- Study supports the immune modulating effects of calcitriol and its synergy with corticosteroids.

50. Serum 25-hydroxyvitamin D concentration in Japanese Akita dogs: A survey

Casini L, Vet Ani Sci, 2020

cohort= 103 dogs

- Japanese Akita dogs are predisposed to immune mediated diseases. Dogs were classified as healthy (HE) or pathological (PA), pigmented (PI) or depigmented (DE). Pathology included dermatitis, epilepsy, enteritis, allergy, others.
- 25vitD significantly decreased with age.
- 25vitD was significantly lower in DE and PA dogs
- Study supports immune modulating effects of 25vitD.



51. Effects of calcitriol on oxidative burst, phagocytic function, and leukocyte cytokine production in shelter dogs

Jaffey J, Canine Med Gene, 2020

cohort= 20 dogs

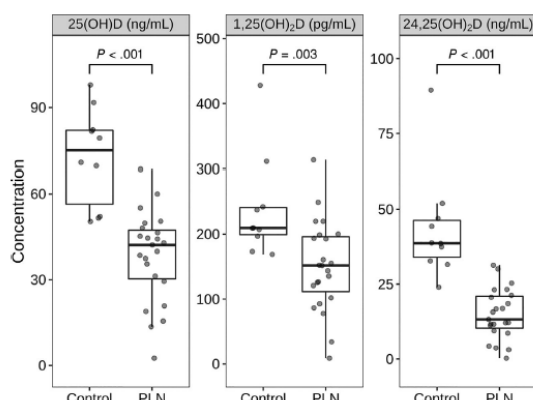
- In-vitro study of shelter dog's leukocyte production of cytokines after calcitriol stimulation.
- Calcitriol resulted in a significant decrease in leukocyte production of TNF- α and an increase in IL-10 (an inflammatory control cytokine).

52. Association between vitamin D metabolites, vitamin D binding protein, and proteinuria in dogs

Miller M, JVIM, 2020

cohort= 33 dogs

- IRIS stage 1 CKD dogs did not have significant reduction in any vitamin D metabolite.
- Dogs with persistent proteinuria (UPC>0.5) had significant reduction in 25vitD, 24,25vitD, and calcitriol (activated vitamin D),
- There was no reduction in VDBP; it is assumed VitD metabolites are lost when bound to albumin.



53. Serum 25-hydroxyvitamin D concentration and infectious respiratory disease complex in shelter dogs

Jaffey J, JVIM, 2020

cohort= 146 dogs



- Dogs with respiratory diseases had a significantly lower 25vitD level than control dogs.
- Dogs positive for canine herpesvirus had a significantly lower 25vitD level than those testing negative.
- There was no association with time of stay in the shelter with 25vitD status.
- This study supports the relationships between vitamin D and acute respiratory infections as seen in human studies.

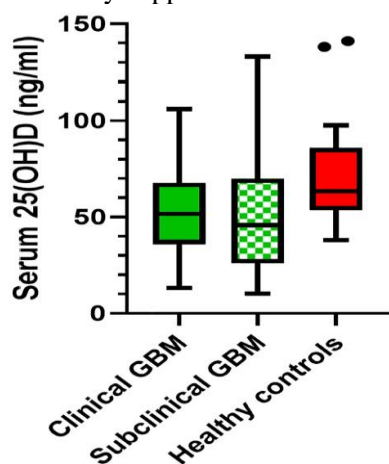
54. Serum 25-hydroxyvitamin D concentrations in dogs with gall bladder mucocele

Jaffey J, PLOS One, 2020

cohort= 95 dogs



- Gallbladder mucocele (GSM) is a common biliary disorder in dogs. Vitamin D deficiency is one of many potential causes of gallbladder hypokinesia in humans and supplementation results in complete resolution of stasis.
- Dogs with GSM had a significantly lower 25vitD level than healthy controls.
- Study supports similar findings in human literature.

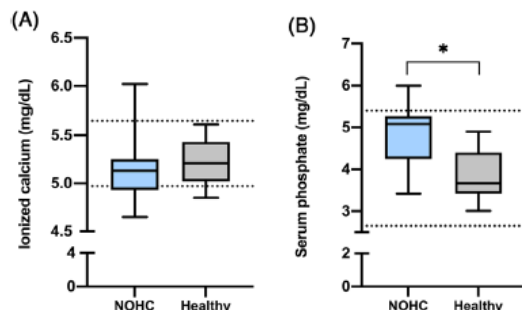


55. Calcium and phosphate homeostasis in dogs with newly diagnosed naturally occurring hypercortisolism

Corsini A, JVIM, 2021

cohort= 35 dogs

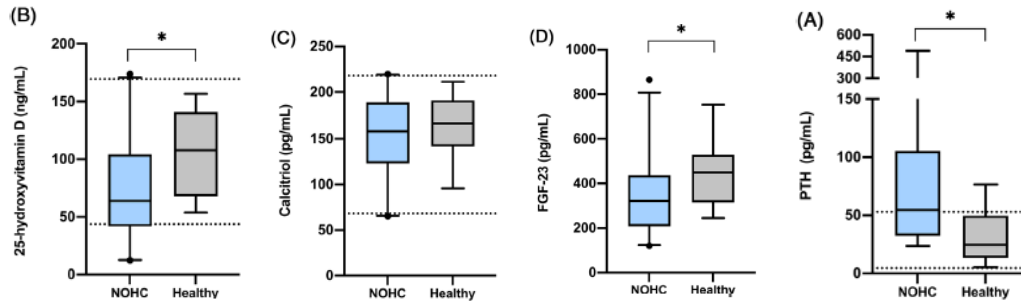
- Calcium and phosphate are tightly managed by calcitriol (activated vitamin D), PTH and fibroblast growth factor-23 (FGF-23). In naturally occurring hypercortisolism (NOHC, ie Cushing's), excessive cortisol upsets this balance.



A growing body of evidence...



- Serum 25vitD was significantly lower in NOHC than healthy controls however calcitriol did not differ. FGF-23 was also significantly lower in NOHC than healthy controls.



- Sustained hypercalciuria in NOHC dogs can cause a secondary hyperparathyroidism in response to low serum calcium via the calcium sensing receptor.
- Human studies propose an upregulation of 24-hydroxylase as the mechanism for decreased 25vitD.
- Study supports the intricate balance vitamin D has on calcium and phosphate homeostasis.

56. Vitamin D receptor (VDR) expression in different molecular subtypes of canine mammary carcinoma

Sanchez-Cespedes R, BMC Vet Res, 2021

cohort= 45 dogs

- Canine mammary carcinomas (CMC) are common in female dogs with 40-60% malignancy rate.
- The presence of VDR in the mammary gland has been documented in both human and canines.
- Calcitriol (activated vitamin D) binding to VDR has anti carcinogenic properties.
- VDR expression in malignant tumors was 27%, benign tumors 40% and normal glands 100%.
- VDR expression in mammary gland subtypes showed VDR expression to be lower in more aggressive tumors.
- Study supports the cell regulating role of vitamin D.