2019

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

14TH EDITION

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:

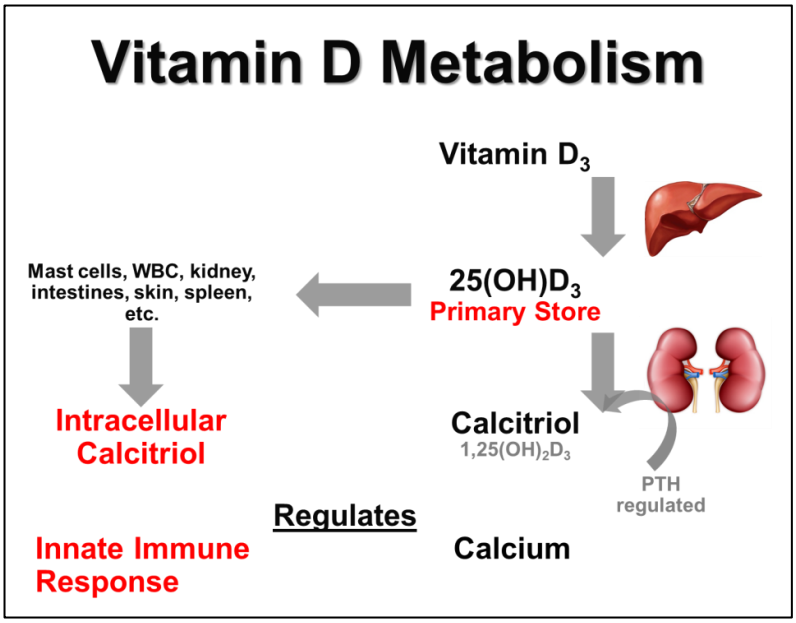
- **Cancer (2,5,8,10,11,12,22)**
- **Chronic Enteropathy (4,11,16,18,25,31)**
- **Heart disease (9,17)**
- **Atopic dermatitis (7,27)**
- **Renal disease (1,2,20,21)**
- **Hyperparathyroidism (2)**
- **Infection (6,15,23,24)**
- **Pancreatitis (28)**
- **Feline tooth resorption (3)**
- **Hospital Mortality (13,30)**
- **Toxicity (26)**
- **Mechanism of Action (32,33,34)**
- **Dietary (14,19,29)**

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 (2,11), mast cell tumors (5), hemangiosarcoma, carcinoma, histiocytic sarcoma, and other cancers (10,11,12) all have 25vitD values below 40ng/mL. The relative risk of having cancer increases to almost 4x when 25vitD values are below 40ng/mL.

Chronic Enteropathy (IBD)
Disease severity and the incidence of chronic enteropathy (CE) in both cats and dogs increases substantially when 25vitD values fall below 40ng/mL (411). In CE, survivors vs non-survivors can be separated when 25vitD values fall below 30ng/mL (15,18,25,31).

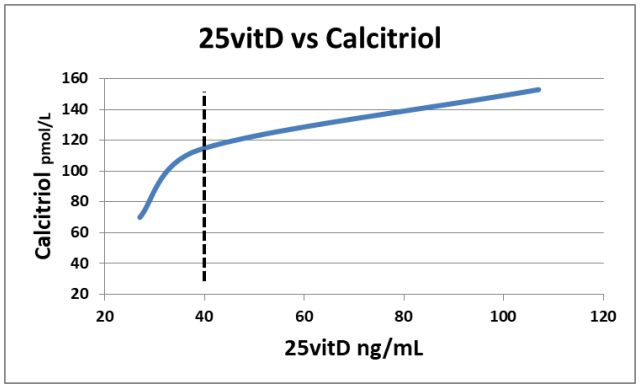
Heart Disease
Congestive heart disease is more prevalent in dogs with 25vitD values below 40ng/mL and there is a 2.6x increase in cardiovascular events (9). Heart remodeling in chronic valvular heart disease worsens as 25vitD values fall below 30 ng/mL (17).

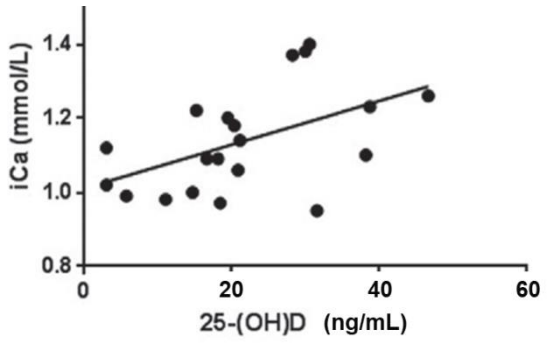
Chronic Kidney Disease
Studies show acute and chronic kidney disease is more prevalent in dogs with 25vitD values below 40ng/mL (1,2). As 25vitD values fall below 40ng/mL, creatinine increases dramatically (20).

Hospital Mortality
Hospitalized cats with 25vitD levels below 40ng/mL have an 8x higher incidence of dying (13) and hospitalized dogs with 25vitD levels below 33ng/mL have a 7x higher incidence of dying (30).

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

As a result, ionized calcium can fall below the reference interval (4,28). Maintaining proper ionized calcium levels is vital for muscle and nerve function.





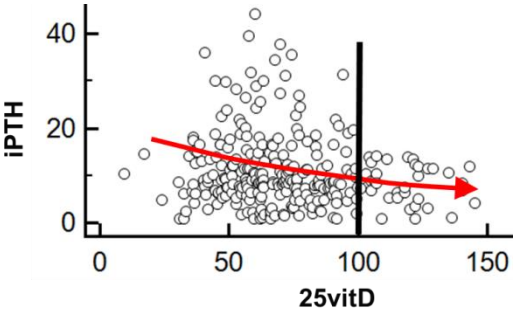
VDI HAS CONCLUDED THAT THE BODY OF EVIDENCE SUPPORTS A HIGH RISK OF DISEASE AND/OR DEATH WHEN 25VITD LEVELS ARE BELOW 40NG/ML AND THEREFORE DEFINES THIS AS "DEFICIENCY".

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 (12) 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

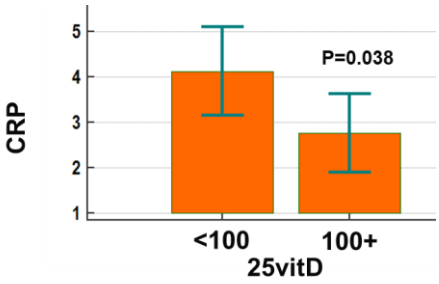
Through negative feedback, the parathyroid gland senses ionized calcium levels and adjusts PTH levels to regulate the enzymatic conversion of 25vitD to calcitriol facilitating intestinal absorption of calcium. Further, PTH activates osteoclasts to increase calcium release from the bone. As 25vitD levels increase, iPTH decreases. When iPTH plateaus, 25vitD sufficiency has been attained. The study found this occurred at 100ng/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 100ng/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) (31) regulating gene transcription and the innate immune response. Studies (12,32,33,34) 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 (12) 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 100ng/mL (12).

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)

In dogs with CKD, those whose 25vitD levels approach 100ng/mL had the lowest creatinine levels (20).

In a vitamin D interventional study on dogs with atopic dermatitis, those with 25vitD levels within the range of 100-150ng/mL saw significant improvement in both pruritus and CADESI scores (27).

Inflammation markers (neut ct, mono ct, IL-2, IL-6, IL-8, TNF-alpha) all significantly drop in CE dogs as 25vitD levels approach 100ng/mL. The duodenal histopathology score also significantly improves with 25vitD levels approaching 100ng/mL (18).

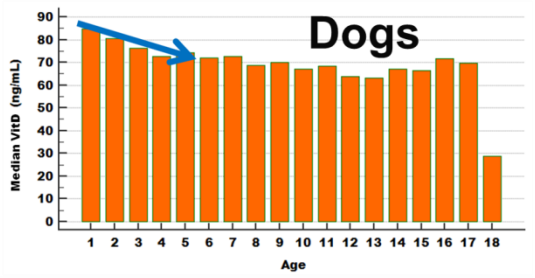
i. Hollis, B (2005), Circulating 25-Hydroxyvitamin D Levels Indicative of Vitamin D Sufficiency: Implications for Establishing a New Effective Dietary Intake Recommendation for Vitamin D. Am Soc Nut Sci

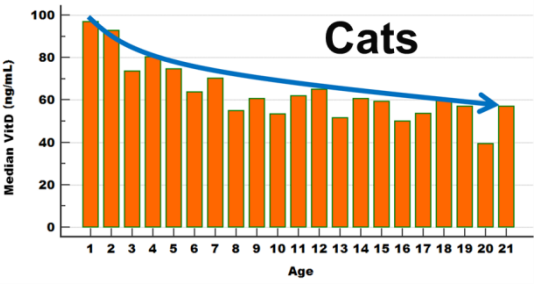
A growing body of evidence…

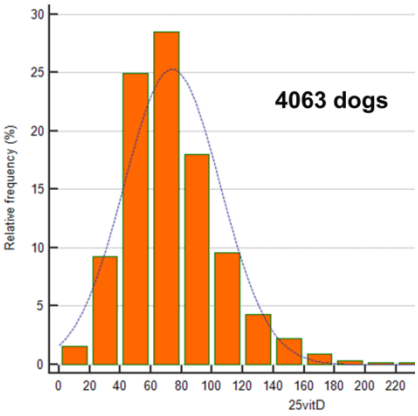
25vitD Status in Dogs and Cats

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 (14).
- 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% (14).
- Disease
  - Diseases affecting the GI tract have the greatest impact on vitamin D absorption (411,15,18,25,31).
- 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).



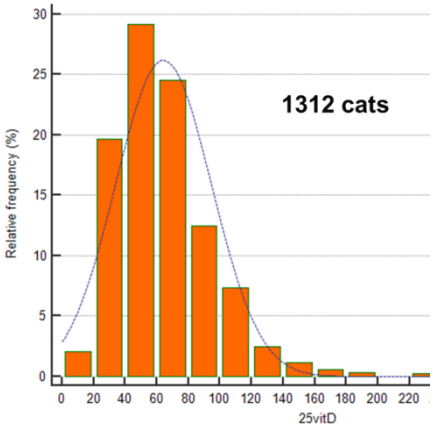




Unsupplemented Distribution

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.

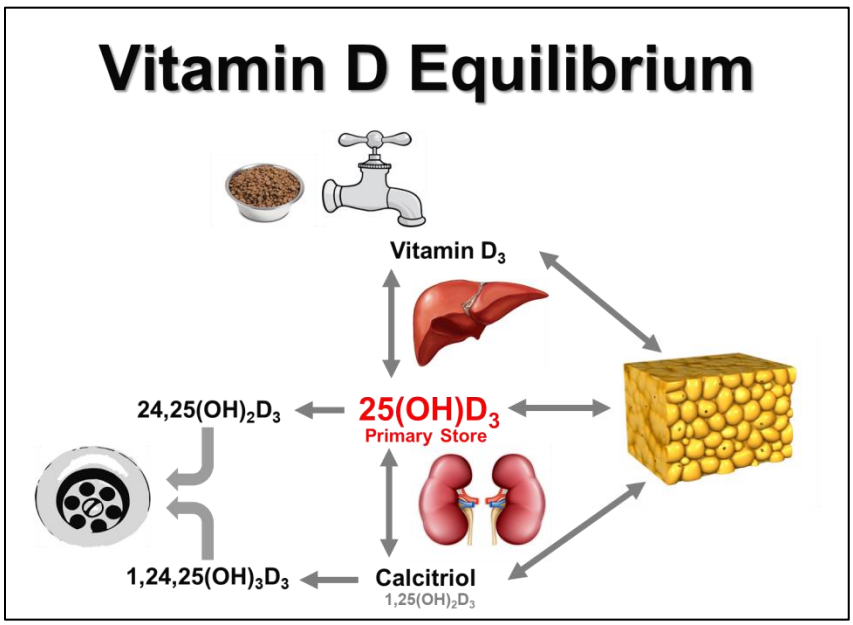
Testing is simple and cost effective using VDI's dried serum methodology. The patient's serum is applied to a card and simply mailed by common carrier. The card easily crosses international borders without custom declarations.

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

Load & Go Dried Serum

Vitamin D and Obesity

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



- **Test before supplementing**
- **Wait 8-10 weeks to test if there is a diet change or change in supplementation**
- **Increase time to test to 4 months in obese patients**

In moderately or severely obese animals, the increased amount of adipose tissue dramatically increases this reserve and therefore a longer time is needed for a new equilibrium to develop. In these patients VDI is recommending to increase 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 (29) 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.

A growing body of evidence…

1. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholacalciferol 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

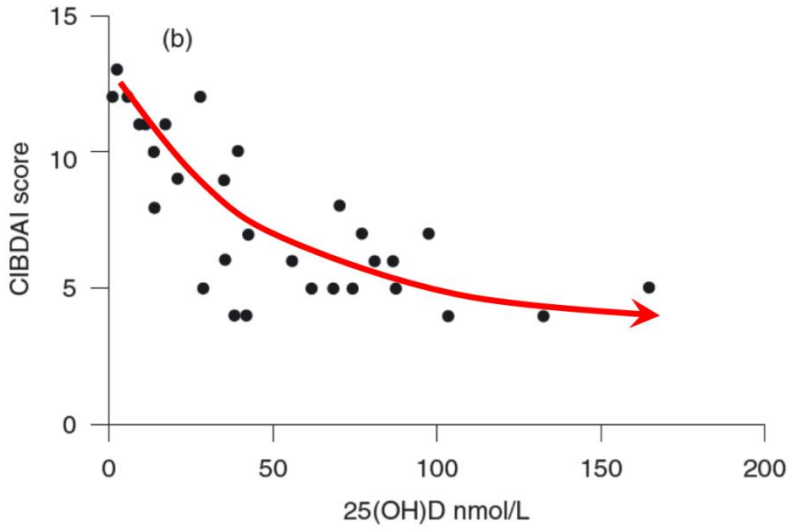
Girard N, et al, Journal Veterinary Dentistry 2010 **cohort = 64 cats**

- 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

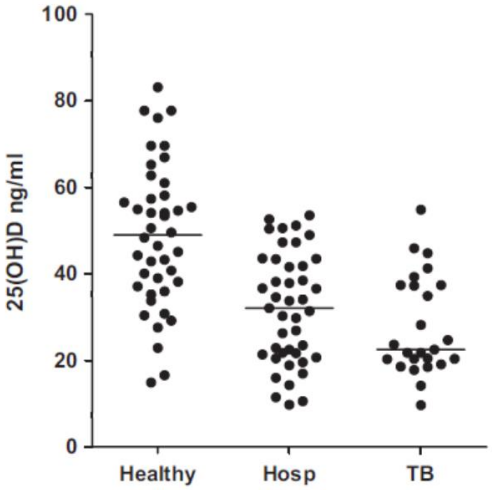
Wakshlag JJ, et al, Br J Nutr, 2011 **cohort = 87 dogs**

- 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**

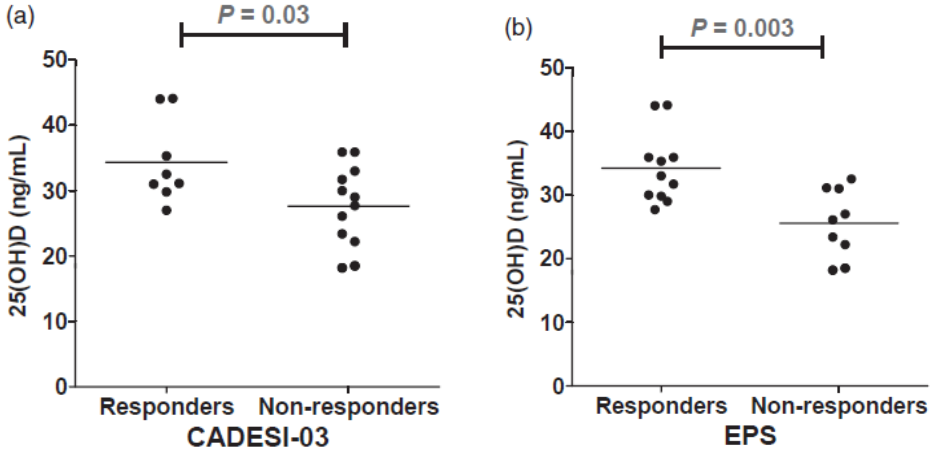
- Median 25VitD was significantly lower in cats with mycobacteriosis (22.2 ng/mL) than healthy cats (49.0 ng/mL).



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

Kovalik M, et al, Vet Dermatol, 2012 **cohort = 20 dogs**

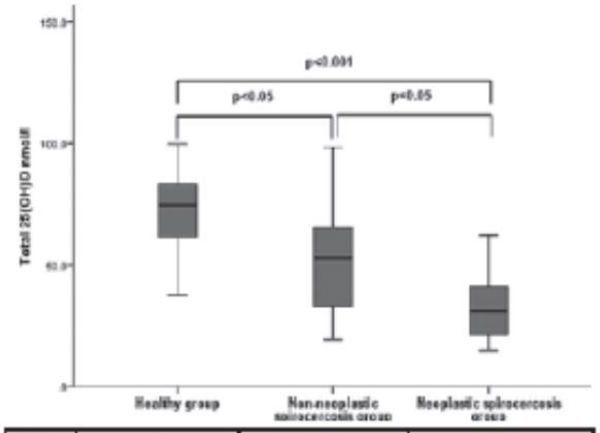
- In dogs with atopic dermatitis being treated with prednisolone, those that exhibited improvement of their physical signs as measured by the CADESI-03 had a significantly higher 25VitD levels than those with a suboptimal response.
- 25VitD and prednisolone may have a synergistic therapeutic effect.



8. 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).

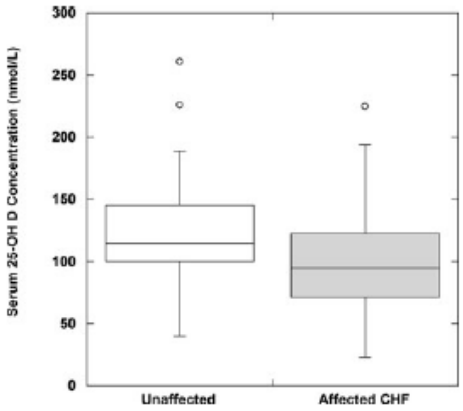


Median	74.6	52.7	30.7
Range	37.4 – 130.5	19.1 – 129.7	14.7 – 62.2

9. 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.



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

Husbands B, VCS presentation, 2013 **cohort = 335 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).

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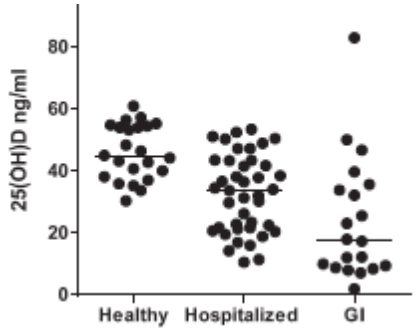
11. Cats with inflammatory bowel disease and intestinal small cell lymphoma have low serum concentrations of 25-hydroxyvitamin D

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).



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

Selting K, et al, Vet Comp Onco, 2014 **cohort = 345 dogs**

- 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).

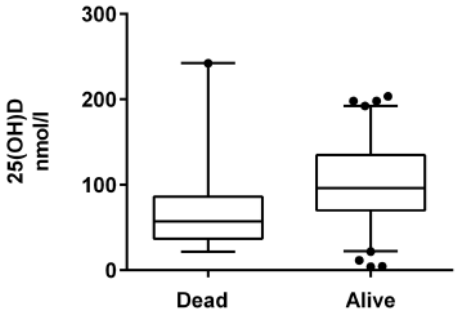
25(OH)D	Relative Risk
<40 ng/mL	3.9
<60 ng/mL	2.0
<80 ng/mL	1.4
<100 ng/mL	1.1
>100 ng/mL	0.2 (benefit)

- Inverse relationship of 25vitD with PTH and CRP.
- Sufficient 25VitD to provide cellular health is 100-120 ng/mL.

13. 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 that those with higher 25VitD levels (P=0.0008).



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

Sharp C, et al, BMC Res Notes, 2015 **cohort = 320 dogs**

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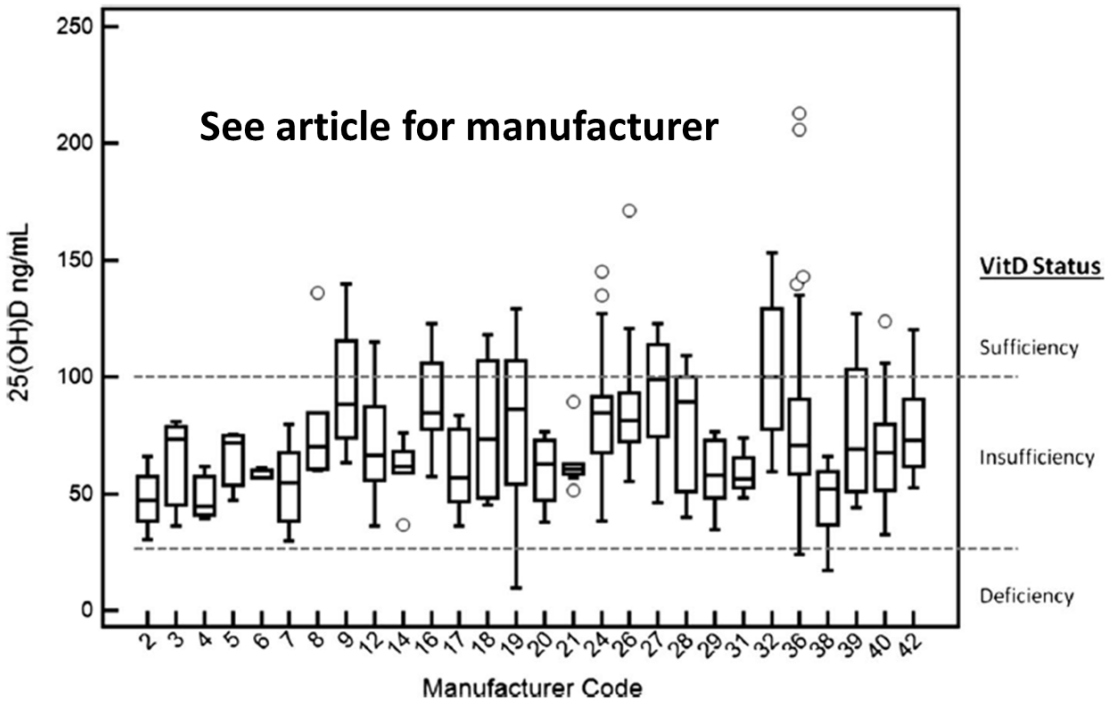
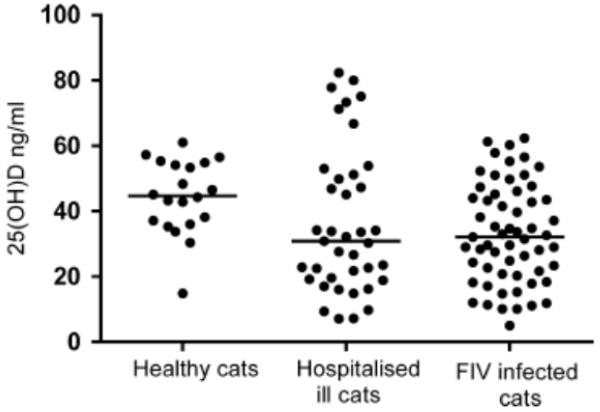
- 320 apparently healthy dogs on 41 different manufacturers of commercial dog food were evaluated for 25VitD levels. Overall serum 25VitD levels ranged from 9.5 – 249 ng/mL, with median, Q1, Q3 at 69.7, 54.5, 88.1 ng/mL, respectively.
- Neuter status correlated with 25VitD concentration. Median 25VitD 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.



15. Vitamin D status in cats with feline immunodeficiency virus

Titmarsh H, et al, Vet Med Sci, 2015 **cohort = 118 cats**

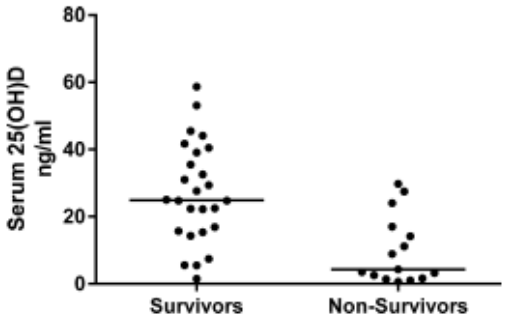
- 25vitD was significantly lower in FIV infected cats than control cats (P<0.05). FIV infected cats had a median 25vitD of 31ng/mL and those hospitalized of 31ng/mL.



16. 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**

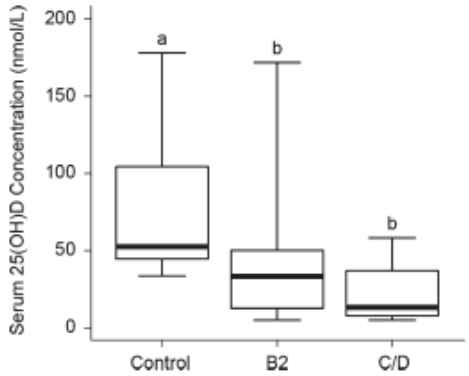
- In dogs with chronic enteropathy 25VitD was an independent predictor of mortality. The median 25VitD concentration of survivors was 25 ng/mL versus 4 ng/mL in non-survivors.



17. 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**

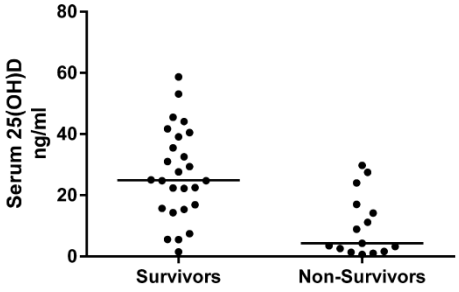
- 25vitD was significantly lower in dogs with CHVD (P=0.0005)
- 25vitD was significantly lower in dogs with worsening left ventricular and atrial sizes.



18. 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.



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

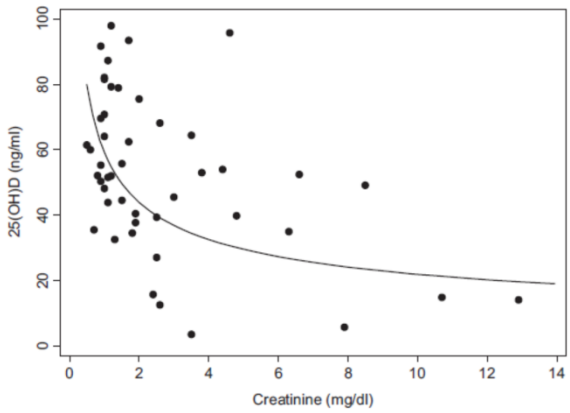
Young L, et al, J Nutr Sci, 2016 **cohort = 46 dogs**

- 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.

20. 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.



21. 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).

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

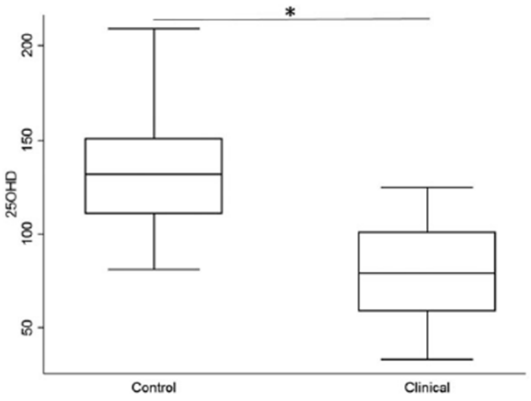
Weidner N, J Vet Intern Med, 2017 **cohort = 92 dogs**

- 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.

23. 25-hydroxyvitamin D concentration in dogs with naturally acquired blastomycosis

O'Brien M, J Vet Intern Med, 2017 **cohort = 57 dogs**

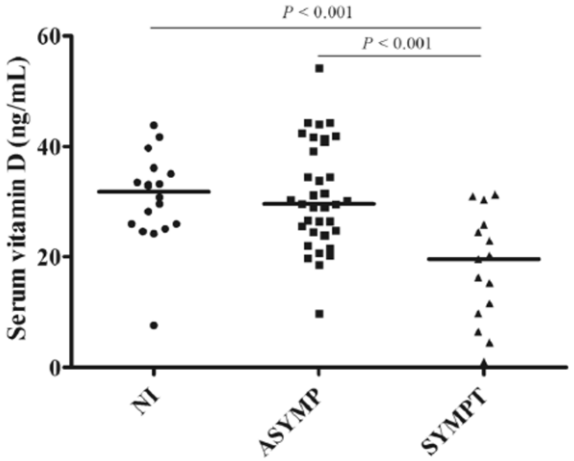
- 25vitD was significantly lower in dogs with *Blastomyces dermatitidis* (median 32ng/mL) than control dogs (P<0.05).



24. Canine Leishmaniasis progression is associated with vitamin D deficiency

Rodriguez-Cortes A, et al, Nature, 2017 **cohort = 68 dogs**

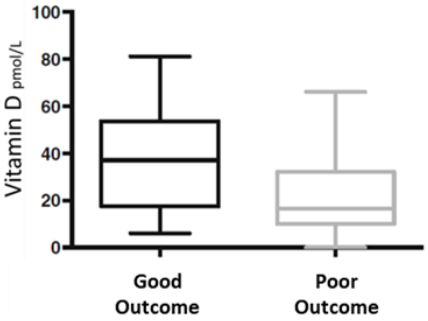
- 25vitD was significantly lower in symptomatic dogs (<20ng/mL) with leishmaniasis than asymptomatic or control dogs (P<0.001).
- 25vitD inversely correlated with leishmania specific IgG, clinicopathological score, and with parasite level in blood.



25. 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 (<10ng/mL) than PLE dogs with favorable outcomes (15ng/mL) (P=0.017).



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

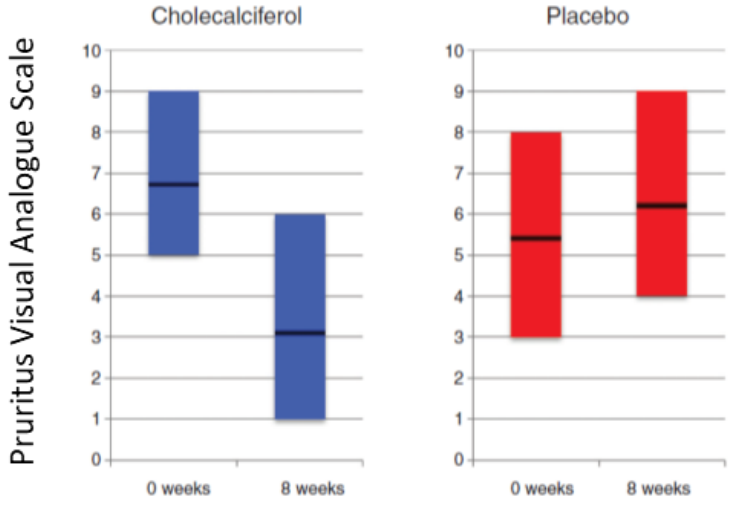
Crossley V, J Feline Med and Surg Open Rpts, 2017 **case study = 3 cats**

- 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.

27. 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**

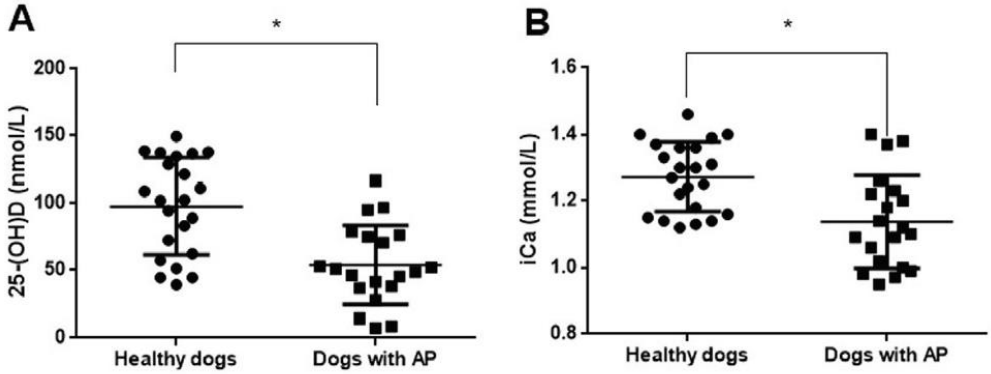
- **True INTERVENTIONAL study evaluating cause and effect.**
- Dogs with unresolved atopic dermatitis were all insufficient with values below 50 ng/mL.
- Dogs being treated with D3 supplementation showed significant improvement (P<0.0001) in both pruritus as well as the CADESI score within 8 weeks.
-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



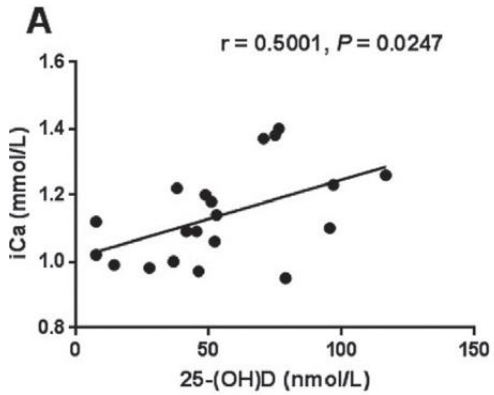
28. Serum 25-hydroxyvitamin D concentrations in dogs with suspected acute pancreatitis

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.



29. 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**

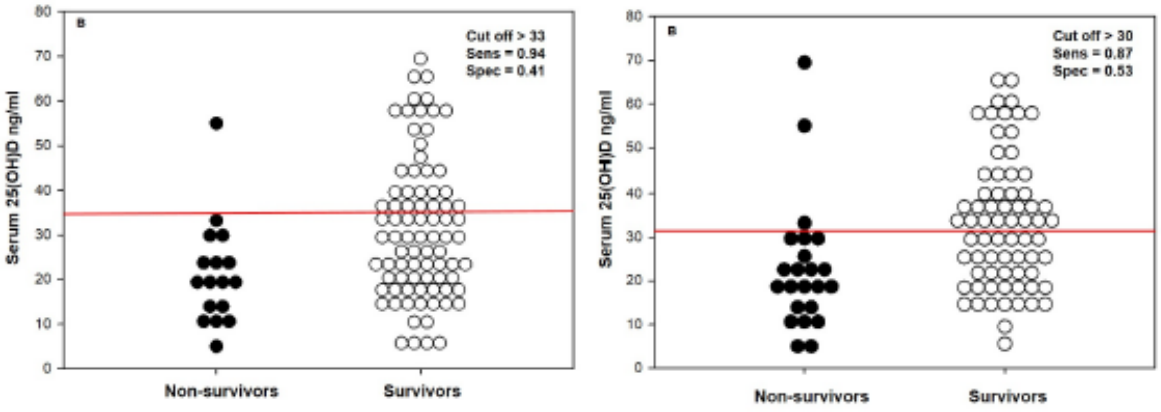
- Obese and lean dogs were compared for changes in 25VitD status when obese dogs were placed on a therapeutic weight reduction diet. No changes in 25VitD status were observed and equilibrium was maintained.

30. 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.

Critically Ill **Sepsis**



31. Vitamin D receptor expression in dogs

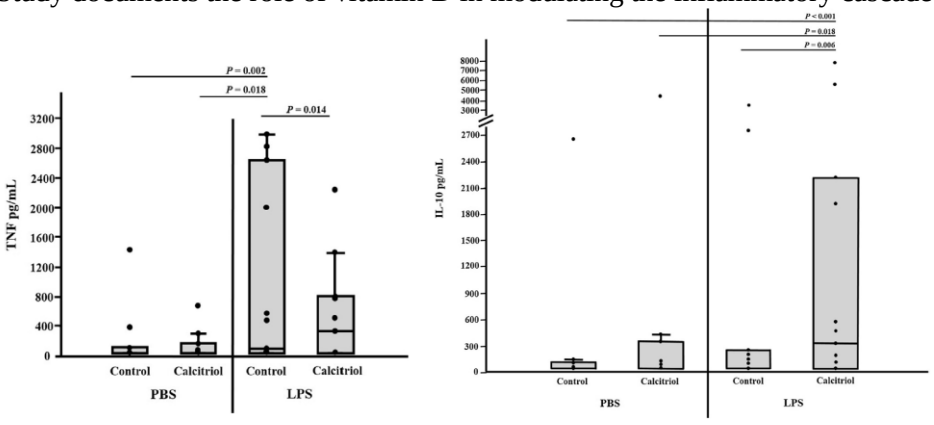
Cartwright J, J Vet Int Med, 2018 **cohort= 40 dogs**

- 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.

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

Jaffey J, Vet Journal, 2018 **cohort= 12 dogs**

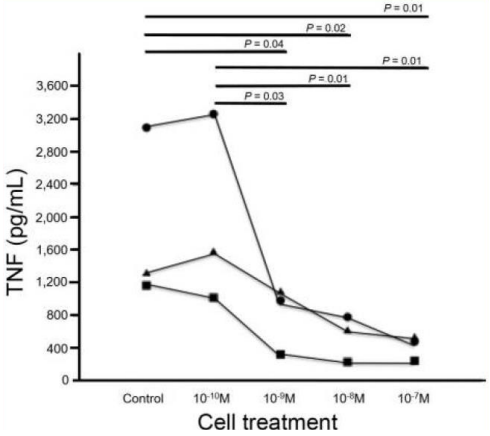
- In critically ill dogs, calcitriol (activated vitamin D) significantly increased anti-inflammatory cytokine IL-10 and significantly decreased pro-inflammatory cytokine TNF-α in in-vitro whole blood.
- Study documents the role of vitamin D in modulating the inflammatory cascade.



33. 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.



34. 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 TNK-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).