**Vitamin D: The Preventative Solution**

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**Introduction**

Vitamin D is a group of secosteroids with anti-rachitic activity. (Prevents rickets) It is similar in structure to steroid hormones such as testosterone, aldosterone, estradiol, progesterone and cortisone. In recent years, in both humans and in veterinary species, insufficient serum levels of $25(OH)D_3$ have been associated with a greater risk of a number of chronic diseases. In humans, studies have found an association of insufficient levels of vitamin D with ailments such as cardiovascular disease, hypertension, cancer, diabetes, multiple sclerosis, asthma and infectious diseases. In dogs and cats, diseases associated in studies with insufficient levels of $25(OH)D_3$ include: congestive heart failure, neoplasia, renal disease, Infectious disease, inflammatory bowel disease, and feline oral resorptive lesions (FORL).

Vitamin D receptors have been found in a wide variety of tissues, which helps to explain why insufficient vitamin D has been associated with a variety of chronic diseases. Vitamin D is believed to regulate over 2000 genes. Studies have found an increasing number of tissues where $25(OH)D_3$ is converted to its active form, calcitriol, also known as $1,25(OH)_2D_3$.

Vitamin D has an anti-inflammatory activity in the tissues where it is active. Chronic inflammation underlies many chronic conditions, including neoplasia and immune-mediated disease. It is apparent from studies measuring serum Vitamin D that serum levels that are high enough to prevent the development of rickets may still have **insufficient** vitamin D to maintain cellular health and dampen inflammatory processes.

The level of adequate vitamin D to reduce the risk of developing these diseases is termed **sufficient** serum vitamin D. Studies in humans and in animals to establish a range of sufficient serum vitamin D values have used the inverse relationship between intact parathyroid hormone (iPTH) and vitamin D. It is considered that the serum vitamin D levels are sufficient when increased levels of oral vitamin D do not cause further decreases in iPTH levels.

Selting, et al. 2014 were able to establish sufficient levels of vitamin D in the dog and cat using this approach. They found that the range of sufficient values for vitamin D for the dog and cat is 100-120 ng/mls, by measuring 4 biomarkers, including: 1) The iPTH, 2) the c-Reactive protein (c-CRP) which is a measurement of inflammation. When vitamin D levels are sufficient, inflammation should be minimized and reflected by reduced values of c-CRP. The last two biomarkers were calcium and phosphorus, as they are directly proportional to the serum levels of vitamin D and iPTH.

Vitamin D regulates absorption of calcium and phosphorus from the bowel and is involved with its mobilization from bone. In concert with calcitonin and PTH, vitamin D regulates calcium homeostasis. Vitamin D is a pro-hormone, and when it is derived from animal sources it is called cholecalciferol and converts to Vitamin $D_3$. Ergocalciferol is the plant source pro-hormone, which converts to Vitamin $D_2$. Ergocalciferol is commonly found in fungi and mushrooms. Vitamin $D_3$ is considered to be more active than Vitamin $D_2$.

Most species are able to utilize both forms, although it has been found in cats that cholecalciferol maintains Vitamin D status with greater efficiency than ergocalciferol. This has not been studied in dogs. Vitamin $D_2$ does not prevent or reverse rickets as compared to Vitamin $D_3$.

Dogs and cats, unlike humans and most other species, are unable to convert much vitamin D in their skin when exposed to ultraviolet light. Pro-vitamin $D_3$ (7-dehydrocholesterol) is not present in the skin of dogs and cats in

as high a concentration as in species that do convert the 7-dehydrocholesterol to cholecalciferol. It is thought that the enzyme 7-dehydrocholesterol $\Delta^7$-reductase found in the skin of dogs and cats is rapidly converted, creating a condition of low 7-dehydrocholesterol, thus necessitating dogs and cats receiving cholecalciferol directly from their diet in order to manufacture vitamin $D_3$.

Vitamin D is one of the fat-soluble vitamins. It becomes incorporated into the chylomicrons in the gut, assisted by the bile salts. There is no rate limitation on the amount of Vitamin D that can be absorbed, as it is via passive diffusion. 80-90% of the cholecalciferol is absorbed by the lymphatic system. Blood levels peak 12 hours post ingestion.

Cholecalciferol, when ingested orally, becomes bound to Vitamin D Binding Protein (VDBP) in the blood and then is transported to the liver where it is converted to the most prevalent form of vitamin D, known as calcidiol, or $25(OH)D_3$. It is commonly accepted that $25(OH)D_3$ levels are the best estimate of patient Vitamin D status. Vitamin D is stored in fat deposits and in the kidneys, liver, lungs, aorta and heart.

Cholecalciferol constitutes about 50% of the circulating vitamin D and calcidiol about 20%. The conversion of cholecalciferol to $25(OH)D_3$ is not very tightly regulated by negative feedback from circulating $25(OH)D_3$ and $1,25(OH)_2D_3$, but it is increased by dietary Vitamin D, and by sunlight in all species except dogs and cats. (Combs, 1998).

It takes a final hydroxylation step to activate the calcidiol ($25(OH)D_3$ into calcitriol or $1,25(OH)_2D_3$. This final activation step takes place in the target tissue where the enzyme for this conversion is present (1-α-hydroxylase), such as kidney, placenta, lung, colon, prostate and breast. Calcidiol can also be metabolized to a less active form of Vitamin $D_3$ by the enzyme 25-hydroxylase, to the inactive metabolite $24,25(OH)_2D_3$ which removes calcidiol from the pool available for conversion to its active $1,25(OH)_2D_3$ form, and transforms the molecule into a readily excretable form, calcitroic acid.

**Normal Ranges of Serum Vitamin $D_3$ in the Dog and Cat**

In a landmark study published in 2014, Selting et al. established optimal ranges for vitamin D in the dog (1). This study utilized 282 dogs that were either German Shepherds or Golden Retrievers belonging to members of their respective breed clubs.

Blood samples were collected at breed club meetings and dog shows in Missouri, Minnesota, California, Massachusetts and Florida. Dogs that were free of cancers and other serious diseases were included in this study. Dogs that died or were euthanized during the study were also excluded.

25(OH)D3 was measured in this study, as well as 4 biomarkers: canine C-reactive protein (cCRP), intact parathyroid hormone (iPTH), calcium and phosphorus. It was found that there was an inverse relationship between 25(OH)D3 levels and iPTH. iPTH levels drop as 25(OH)D3 levels increase. cCRP is an inflammation marker, and Vitamin D3 has anti-inflammatory actions.

Deficient Vitamin D is defined as the serum level of 25(OH)D3 that is not high enough to prevent the development of vitamin D deficiency as defined by the disease, rickets. Mellanby studied rickets in over 400 dogs in 1918, but there has been no definitive minimal Vitamin D requirement established to prevent the development of rickets. In part, this is because the manifestation of symptoms of rickets is dependent upon calcium and phosphorus levels in the diet, the physiological status of the dog, and its size at maturation. Growing dogs are more sensitive to a dietary deficiency of vitamin D, but mature dogs are relatively more resistant. (NRC, 2006) =25(OH)D3<<<<<<<100 ng/ml

Insufficient Vitamin D is defined as the serum level of 25(OH)D3 that is high enough to prevent the development of rickets, but which is below the levels that are defined as sufficient, based on following the inverse relationship between vitamin D and iPTH. Insufficient vitamin D levels have been found in both the human and the dog and cat to be associated with increased risk of a variety of chronic disease conditions. =25(OH)D3< 100 ng/ml

Sufficient Vitamin D is defined as the serum level of 25(OH)D3 that has an optimal effect on health, based on reaching a plateau in iPTH levels, and studies that have shown less risk for a variety of chronic disease conditions when vitamin D levels are "sufficient". =25(OH)$D_3$=100-120 ng/ml

Vitamin D is dosed based on the baseline value of 25(OH)D3 determined from testing prior to supplementation and the body weight of the patient. Since dogs and cats derive their vitamin D from their food, and there are many different levels of vitamin D found in the many different choices of food for dogs and cats, it is risky to simply supplement with vitamin D at 20 ng/kg/day without taking into account the patient's baseline vitamin D relative to their diet. If the diet changes, then the amount of vitamin D to supplement will also change.

Toxicity of Vitamin D results from serum levels much higher than the levels of sufficiency, which commonly results in hypercalcemia. The LD50 dose of 88 mg/kg has been reported. (Rumbeiha, 2013) =25(OH)D3>>>>120 ng/ml

**Diseases Associated with *Insufficient* Serum Vitamin D**

**Heart Disease:**

Kraus, et al., 2014 performed a cross-sectional study by measuring the serum 25(OH)D3 concentrations in 31 dogs with congestive heart failure (20 w/AVD and 11 w/DCM) and in 51 unaffected dogs as controls. Mean serum 25(OH)D3 concentrations were about 20% lower in CHF dogs when compared with controls (P=0.023) No difference was found in 25(OH)D3 levels in dogs with acquired valvular disease (AVD) and dilated cardiomyopathy (DCM). The authors conclude that "low serum vitamin D3 concentration might be a risk factor for development of CHF in dogs. Similar associations between low circulating serum vitamin D and CHF have been found in people."

In this same paper, Kraus describes the result of a prospective cohort study that measured the clinical outcomes of the 31 dogs from the cross-sectional study and correlated those outcomes with the measured serum 25(OH)D concentrations. They found that this group had significantly lower 25(OH)D levels when the endpoint was another clinical episode of CHF, sudden death, or adjustment of cardiac medications.

The authors conclude that this study suggests that low concentrations of 25(OH)D may be a risk factor for CHF in dogs, but that the "demonstration of an association does not prove causality." The authors note that: "Vitamin D has cardioprotective actions by suppressing the renin-angiotensin-aldosterone system, decreasing myocardial hypertrophy, inhibiting pro-inflammatory cytokines, and improving endothelial dysfunction. Additional studies are needed to determine if routine screening of all dogs with CHF for insufficient vitamin D concentrations followed by vitamin D3 supplementation will delay the onset of clinical signs or improve survival…."

**Neoplasia:**

Several studies have found an association between insufficient 25(OH)D3 levels and a variety of cancer types. Wakshlag et al., 2011 found that Labrador retrievers with cutaneous mast cell tumors (n=33) had significantly lower serum concentrations of 25(OH)D3 than 54 unaffected controls (42 ng/ml vs 48 ng/ml). The authors recommend that prospective cohort studies be performed to determine the effect of serum vitamin D concentration on the clinical outcomes in these patients.

Husbands, 2013, in a study measuring 25(OH)D3 levels in dogs with cancer as compared to control dogs without cancer, reported that median serum 25(OH)D3 concentrations were significantly lower in the cancer group. He evaluated 335 dogs with cancer (313 malignant/22 benign) and found a median 25(OH)D3 concentration of 62.6 ng/ml as compared to 67.4 ng.ml in the control group. Additionally, it was reported that cancer types with significantly lower 25(OH)D3 levels were: Carcinoma (n=64), histiocytic sarcoma (n=8), hemangiosarcoma (n=10), lymphoma (n=80) and sarcoma (n=48).

Selting, 2014 measured the serum concentrations of 25(OH)D3 in 62 dogs presenting with hemoabdomen due to neoplasia and compared them to 282 healthy dogs without neoplasia. Of the dogs with hemoabdomen, 22 dogs had benign disease, 31 had hemangiosarcoma, and 9 had other splenic malignancies. Selting reported that the relative risk of splenic neoplasia was inversely proportionate to their 25(OH)D3 concentrations. These results are summarized in this table below from Selting (1)

**Table Showing Relative Risks for Developing Cancer Relative To Measured Levels Of Serum 25(Oh)D3**

| 25(OH)D3 (ng/ml) | 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<0.000) | 1.1 (p=0.04) |
| >100 | 0.18 (p=0.08) | 0.11 (p=0.12) | 0.32 (p=0.25) |

**Inflammatory Bowel Disease:**

Gow, et al. 2011, measured serum 25(OH)D3 concentrations in healthy dogs (n=36), hospitalized dogs with non-GI complaints (n=49), dogs with inflammatory bowel disease and normoalbumenemia (n=21) and dogs with inflammatory bowel disease and hypoalbuminemia, also known as protein-losing enteropathy or PLE (n=12). Significant differences in serum 25(OH)D3 were found between the cohort with inflammatory bowel disease and hypoalbuminemia (PLE) and all of the other groups tested ($p<0.001$). The authors point out that it has been classically regarded that hypovitaminosis D was the result of the PLE. However, the authors note that accumulating evidence, including the results of this study, suggests that hypovitaminosis D itself may contribute to the pathogenesis of PLE, since Vitamin D has been shown to have a potent effect on both the innate and adaptive immune response. (Baeke, 2010; Kamen, 2010) Studies using knock-out mice that lack the membrane receptor for Vitamin D are more susceptible to experimental forms of IBD (Yu, 2008), and it has also been shown that a diet deficient in vitamin D can predispose mice to colitis (Lagishetty, 2010)

Lalor et al., 2014 in a retrospective study measured 25(OH)D3 concentrations in cats with inflammatory bowel disease (n=14) (IBD) or intestinal small cell lymphoma (n=6) (ISCL) (n=20). Healthy cats (n=23) and hospitalized cats with non-GI disease (n=41) were also measured for 25(OH)D3 as healthy and diseased control groups respectively. These researchers found that the median serum concentration of 25(OH)D3 was significantly lower in cats with IBD/ISCL than in both healthy cats and in hospitalized ill cats. This data further supports the actions that vitamin D has on the innate and adaptive immune responses: the down-regulation of the pro-inflammatory cytokines, tumor necrosis factor-α and interferon-γ; and the impairment of the ability of dendritic cells to prime T cells. (Mora, 2008). The authors comment that the results of this study are consistent with the study in dogs

cited above, and in prior studies in humans linking insufficient vitamin D levels with inflammatory bowel disease in those other species.

**Mycobacterium infections in cats:**
Spontaneous infections with tuberculosis in cats are not uncommon in the UK. The most common clinical signs seen in cats are cutaneous lesions, although systemic disease can also occur. Prior studies have indicated that vitamin D insufficiency and active tuberculosis infection are highly correlated. Emerging evidence supports the thesis that supplementation with vitamin D may enhance immunity to mycobacteria.

Lalor et al, 2011, measured serum 25(OH)D concentrations in 36 healthy cats, 41 hospitalized ill cats with no history of gastrointestinal disease or recent corticosteroid use, and 24 cats with confirmed mycobacteria infections. They found that the median serum 25(OH)D3 concentration for the cohort of healthy cats was 49.0 ng/ml, for hospitalized ill cats it was 33.8 ng/ml, and for cats with mycobacteriosis it was 22.15 ng/ml. There was a significant difference ($p<0.001$) between the healthy cats and the hospitalized ill cats, and there was a significant difference ($p<0.001$) between the healthy cats and cats with mycobacteriosis.

There was no significant difference ($p=0.46$) between the cats with mycobacteriosis and the ill hospitalized cats. In several human studies cited by the authors, there was a greater risk of human mycobacteria infections associated with lower serum vitamin D concentrations. However, the data in this study makes it unclear if the lower vitamin D concentrations found with both ill hospitalized cats and cats with mycobacteriosis was due to a difference in food intake or is a non-specific finding associated with systemic illness in cats or another cause entirely. The authors recommend further studies to better define this.

Martineau, 2011 found that supplementation with vitamin D improved human patient response to anti-microbial treatment for pulmonary mycobacteriosis. This is one of the first reports of the interventional use of vitamin D to improve immune system function.

**Renal Disease/Hyperparathyroidism:**
Gerber et al, 2003, measured the serum 25(OH)D3 and the 1,25-(OH)2D3 in 24 healthy dogs, 40 dogs with chronic kidney failure (CRF), and 10 dogs with acute renal failure (ARF). In this study, dogs with renal failure (both ARF and CRF) had significantly lower concentrations of serum 25(OH)D3 and 1,25-(OH)2D3. Dogs in the CRF group who were in acute crisis had lower values, but dogs with CRF who were not in acute crisis were not significantly different than the healthy control group values. Serum concentrations of 1,25-(OH)2D3 were not significantly different between the ARF and CRF groups. The 25(OH)D3 concentrations were lower for the CRF group versus the ARF group, but the values did not reach statistical significance ($p=0.09$).

In a follow up to their 2003 study, Gerber et al. 2004 measured the serum 25(OH)D3 concentrations and 1,25-(OH)2D3 concentrations in dogs with hypercalcemia due to lymphoma (n=12), primary hyperparathyroidism (n=5) and chronic renal failure (n=10). These values were compared to a control group of healthy normal dogs (n=24). They found no significant difference in 1,25-(OH)2D3 levels in dogs with different causes of hypercalcemia. 25(OH)D3 ranged from 160-727.5 ng/ml (median=253.8)) in dogs with lymphoma; from 165-745 ng/ml (median=227.5) in dogs with primary hyperparathyroidism; from 87.5-462.5 (median=167.5) in dogs with chronic renal failure and from 120-875 ng/ml (median = 766.3) in control dogs. 25(OH)D3 was significantly lower in dogs with hypercalcemia than in healthy controls, whereas there was no significant difference between all causes of hypercalcemia and healthy controls.

The active metabolite of 25(OH)D3, 1,25-(OH)2D3 which is commonly known as calcitriol, is manufactured in the proximal tubule of the kidney. Its interaction with parathyroid hormone (PTH) and calcium and phosphorus

maintains healthy homeostasis in the kidney and throughout the body. This occurs mostly by the genomic effects of calcitriol to block PTH synthesis in addition to calcitriol's mild calcemic and antiproliferative effect that prevents parathyroid gland hyperplasia.

The first step in the progression of renal disease is the reduction in the production of calcitriol by the proximal tubule of the kidney and the consequent increase in PTH, which, over time, will lead to renal secondary hyperparathyroidism, resulting in the clinical symptoms of azotemia commonly associated with renal disease. (de Brita Galvao, 2013)

Polzin 2005: A salutary effect of calcitriol treatment of CRF was shown in a placebo-controlled 2005 study of 37 client-owned dogs with elevated creatinine concentrations. The dose of calcitriol (2.4 ng/kg/d) was adjusted according to serial ionized calcium and PTH determinations, and ranged from 0.75 to 5.0 ng/kg/day. Dogs were randomized to receive calcitriol (n=18) or placebo (n=19). Subjects were followed for 1 year. Other than the calcitriol or placebo there was no difference in care to either group.

No significant difference in the initial creatinine concentration between the two groups was noted. By the end of the 1 year study period, it was determined that there was a significant reduction ($p<0.036$) in mortality rate in the group of dogs receiving calcitriol (28%) as compared to the placebo group (63% mortality). In dogs receiving calcitriol, the median survival time was 365 days, as compared to a median survival time of 250 days in those receiving a placebo. The authors conclude that calcitriol appears to be effective in prolonging survival in dogs with IRIS Stage 3 and 4 CKD.

Nagode et al, 2006 provide a detailed explanation of the role calcitriol plays in the dog and the cat with chronic renal disease and suggested its use as part of a comprehensive approach to the feline and canine patient with chronic renal disease. As of this writing, the evidence for the benefit of long-term calcitriol in the management of CRD in the dog has been established. (Polzin, 2005). However, although many feline practitioners employ the use of calcitriol in their patients with many reported positive outcomes, definitive, controlled studies of these benefits in the feline patient remain to be conducted. (Gunn-Moore, 2008)

**Feline Oral Resorptive Lesions (FORL)**

Girard, et al., 2010 measured serum 25(OH)D3 concentrations in healthy adult domestic cats (n=64) fed similar premium dry foods throughout their lives. Following a single complete periodontal examination, 40 out of the 64 cats were diagnosed to have 168 tooth resorptions (Type 1 n=85; Type 2 n=83). The mean serum 25(OH)D3 in cats with greater than 5 tooth resorptions (n=13) was significantly less ($p<0.05$) than those cats with no tooth resorptions. (n=24) Tooth resorption prevalence was greater in cats with lower 25(OH)D3 concentrations.

**Immune System Benefits**

Cells of the immune system have been found to contain the membrane-based vitamin D receptor (VDR) and key vitamin D metabolizing enzymes. Studies in laboratory animals, and both human epidemiologic and clinical studies support the important role that vitamin D plays in maintaining a healthy immune system balance.
In terms of specific immune system effects, Vitamin D up-regulates anti-microbial peptides which enhance the destruction of bacteria at various mucosal barrier sites. Vitamin D modulates the adaptive immune response with direct effects on T cell activation. Vitamin D also influences cell differentiation of the antigen-presenting cells (APC) and, in particular, the dendritic cells (DC). (Kamen, 2010).

Clinical studies involving immune-mediated disease in veterinary species, as of this writing, are absent from the literature, except for the single study cited earlier showing a link between inflammatory bowel disease in

vitamin D insufficiency. (Gow, 2011) But, the recent establishment of companion animal ranges of sufficient and insufficient serum vitamin D concentrations, measured by the levels of 25(OH)D3 (Selting, 2014), is helpful.

Historically, vitamin D has been used in the treatment of infections in humans. In 1849, Williams reported good results using cod liver oil (which contains both vitamin A and D) to treat over 400 patients with tuberculosis (TB). Niels Finsen received the Nobel Prize in Medicine in 1899 for his use of UV light to stimulate vitamin D production to treat lupus vulgaris, a cutaneous form of TB in over 800 humans.

Multiple studies have confirmed that human patients with systemic lupus erythematosis (SLE) suffer from vitamin D deficiency or insufficiency. Similarly, low levels of 25(OH)D3 have been measured in patients with systemic sclerosis, mixed connective tissue disease, undifferentiated connective tissue disease, Bechet's disease, anti-phospholipid antibody syndrome, and in rheumatoid arthritis. (Haroon, 2012)

The active form of vitamin D3, 1,25-(OH)2D3, also known as calcitriol, is manufactured in T and B lymphocytes and in dendritic cells from circulating 25(OH)D3. Activation of CD4+ T cells creates a 5-fold increase in Vitamin D receptors, which can turn on as many as 102 genes responsive to this activated form of vitamin D3.

**T cell effects:**
1,25-(OH)2D3 has been found to suppress T cell receptor induced T cell proliferation and alters the cytokines they produce. This produces a shift away from a TH1 phenotype toward a more TH2 "tolerogenic" profile. λ-interferon and interleukin 2 production by T cells are reduced when exposed to 1,25-(OH)2D3, both cytokines being pro-inflammatory. IL-5 and IL-10 production is increased, which is consistent with a shift to a TH2 profile. IL-4, also a TH2 cytokine can also be up-regulated by the activated form of vitamin D. Vitamin D directly inhibits TH1 cells and helps shift towards a TH2 response by inhibiting IL-12.

Organ-specific autoimmunity is modulated by TH17 cells, which are a subset of CD4+ T cells. These cells appear to play a role in maintaining inflammation, which can lead to damage of the involved tissues. It was found, using animal models for autoimmune uveitis and inflammatory bowel disease, that $1,25(OH)_2D_3$ suppresses autoimmunity and tissue damage by inhibiting this TH17 response at the level of the dendritic cell and at the level of inhibiting the TH17 cell from manufacturing the inflammatory interleukin, IL-17 through a complex process. (Daniel, 2008)

**B cell effects:**
There is a positive correlation between serum 25(OH)D3 concentrations and the ability of T regulatory cells to suppress T cell proliferation. Vitamin D has also been found to have a direct effect on B cells, and to inhibit immunoglobulin production. In human patients with systemic lupus erythematosis (SLE), exposure of peripheral blood mononuclear cells to calcitriol resulted in a significant reduction of both spontaneous polyclonal antibody production and pathogenic anti-dsDNA autoantibody production by SLE B cells. (Linker-Israeli, 2001)

**Dendritic Cell effects:**
Calcitriol leads to the development of dendritic cells with tolerogenic properties. As monocytes develop into monocyte-derived DCs, the expression of the VDR rapidly increases. Physiologic levels of 1,25(OH)2D3 inhibit the maturation of DCs and maintain an immature and tolerogenic phenotype. Activation markers such as MHC class II, CD40, CD80, and CD86 are inhibited by the calcitriol, and inhibitory molecules are up-regulated. It also down-regulates IL-12 and increases IL-10 production by DCs which overall promotes a shift from the cell-mediated TH1 phenotype to the humorally-mediated TH2 phenotype. These macrophage-derived dendritic cells that have matured in the presence of sufficient concentrations of calcitriol are less responsive to the effect of the pro-inflammatory cytokines that regulate DC migration to lymph nodes. Since the maturation of DCs can be

modulated by 1,25(OH)2D3, the vitamin D status of an individual is likely to have important immunological consequences.

**Evidence that interventional Vitamin D or sufficient serum Vitamin D creates positive clinical outcomes for autoimmune disease:**

In several animal models of autoimmune disease it was found that by administration of 1,25(OH)2D3 or a synthetic analog, the course of the disease could be prevented or improved. These diseases are: autoimmune encephalomyelitis (EAE), collagen-induced arthritis, autoimmune uveitis and lupus. Conflicting results are found in studies correlating the serum concentrations of 25(OH)$D_3$ and the incidence of rheumatoid arthritis in humans. However, other studies have found that low levels of 25(OH)D3 contribute to increased disease activity and inflammation in those patients with existing inflammatory arthritis and rheumatoid arthritis. (Munger et al., 2006)

Multiple sclerosis (MS), type 1 diabetes (T1DM), and systemic lupus erythematosis (SLE) have shown a more consistent correlation between serum levels of 25(OH)D3 and disease incidence than rheumatoid arthritis (RA). In a large study of women, it was found that vitamin D supplementation of greater than or equal to 400 IU/day were associated with a reduced risk of developing MS as compared to those individuals who received no Vitamin D supplementation. Studies have suggested that disease activity in MS patients is increased seasonally when there are lower circulating levels of 25(OH)D3.

Zipitis, 2008 showed in a meta-analysis that the risk of developing T1DM was significantly reduced in infants who were supplemented with vitamin D as compared to those who were not. This analysis also found a dose-response effect, where higher doses of vitamin D were associated with even lower risks of developing immune-mediated diabetes.

SLE cases have been found to have lower 25(OH)D3 levels than controls, suggesting that vitamin D insufficiency may be a risk factor for developing SLE. The majority of the studies have also shown an increase in SLE disease activity associated with lower levels of 25(OH)D3. Commonly SLE patients have photosensitivity, thus necessarily limiting their exposure to UV and its ability to transform pro-vitamin D in the skin to vitamin D. Oral supplementation is recommended in these patients. (Kamen, 2008)

**References:**

1. Selting KA, Sharp CR, Ringold R, Thamm DH, Backus R. Serum 25-hydroxyvitamin D concentrations in dogs – correlation with health and cancer risk. Veterinary Comp Oncol. Open access article: John Wiley & Sons, Ltd. doi:10.1111/vco.12101; 2014.
2. Kraus MS, Rassnick KM, Wakshlag JJ, Gelzer ARM, Waxman AS, Struble AM, Refsal K. Relation of Vitamin D Status to Congestive Heart Failure and Cardiovascular Events in Dogs. J Vet Intern Med 2014;28:109-115.
3. Wakshlag JJ, Rassnick KM, Malone EK, Struble AM, Vachhani P, Trump DL, Tian L. Cross-sectional study to investigate the association between vitamin D and cutaneous mast cell tumors in Labrador retrievers. Brit. J Nutr. (2011) 106; S60-S63.
4. Husbands, Brian. Low stores of 25-hydroxyvitamin D levels and its association with cancer in dogs. Veterinary Cancer Society presentation 2013.
5. Gow AG, Else R, Evans H, Berry JL, Heritage ME, Mellanby RJ. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminemia. J Sm An Pract. (2011);52:411-418.
6. Lalor S, Schwartz AM, Titmarsh H, Reed N, Tasker S, Boland L, Berry J, Gunn-Moore D, Mellanby RJ. Cats with Inflammatory Bowel Disease and Intestinal Small Cell Lymphoma Have Low Serum Concentrations of 25-Hydroxyvitamin D. J Vet Inter Med. 2014;28:351-355.

7. Yu S, Bruce D, Froicu M, Weaver V, Catorna MT. Failure of T cell homing, reduced CD4/CD8alpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. Proceed. Nat Acad Sci of USA;105:20834-20839.
8. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. (2010) Curr Opin Pharmacol. 10:482-496.
9. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. (2010) J Mol. Med.;88:441-450.
10. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyand Y, McLachlan SM, Adams JS, Hewison M. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. (2010) Endocrinology 151:2423-2432.
11. Mora JR, Iwata M, Von Adrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 2008;8:685-698.
12. Lalor SM, Mellanby RJ, Friend EJ, Bowlt KL, Berry J, Gunn-Moore D. Domesticated Cats With Active Mycobacteria Infections Have Low Serum Vitamin D (25(OH)D) Concentrations. Transboundary and Emerging Diseases; 2012;59:279-281.
13. Martineau AR, Timms PM, Bothamley Y, Hanifa K, Islam AP, et al. (2011) High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomized controlled trial. Lancet 377:242-250.
14. Gerber B, Hassig M, Reusch CE. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure. AJVR 2003;64:1161-1166.
15. Gerber B, Hauser B, Reusch CE. Serum levels of 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol in dogs with hypercalcaemia (2004) Vet Res Commun. 28(8):669-680.
16. de Brito Galvao JF, Nagode L, Schenck P, Chew DJ. Calcitriol, calcidiol, parathyroid hormone and fibroblast growth factor-23 interactions in chronic kidney disease. (2013) J Vet Emerg Crit Care. 23(2)134-162.
17. Polzin D, Ross R, Osborne C, Lulich J, Swanson L. (2005) ACVIM Abstracts, p 433.
18. Nagode LA, Chew DJ, Podell M. Benefits of Calcitriol Therapy and Serum Phosphorus Control In Dogs and Cats with Chronic Renal Failure: Both are Necessary to Prevent or Suppress Toxic Hyperparathyroidism. Vet Clinics of NA: Small Animal Practice. (Nov 1996)26(6):1293-1330.
19. Gunn-Moore D. The Diagnosis and Treatment of Renal Insufficiency in Cats. 2008 Proceedings World Veterinary Conference.
20. Girard N, Servet E, Biourge V. Tooth resorption and vitamin $D_3$ status in cats fed premium dry diets. J Vet Dent (2010) Fall:27(3):142-7.
21. Kamen DL and Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and auto-immunity.
22. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM (2008) Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. J Pharmacol Exp Ther 324(1):23–33
23. Linker-Israeli M, Elstner E, Klinenberg JR, Wallace DJ, Koeffler HP (2001) Vitamin D(3) and its synthetic analogs inhibit the spontaneous in vitro immunoglobulin production by SLE-derived PBMC. Clin Immunol 99(1):82–93
24. Haroon M and FitzGerald O. Vitamin D and its emerging role in immunopathology. Clin Rheumatol. (2012)31:199-202.
25. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A (2006) Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 296(23):2832–2838
26. Zipitis CS, Akobeng AK (2008) Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child 93(6):512–517.

27. Kamen DL, Aranow C (2008) The link between vitamin D deficiency and systemic lupus erythematosus. Curr Rheumatol Rep 10(4):273–280
28. Nutrient Requirements of Dogs and Cats. (2006) National Research Council; National Academies Press, pp 200-205.
29. Rumbeiha WK, Cholecalciferol. In: Peterson M, Talcott P, eds. Small Animal Toxicology 3rd ed. St Louis: Saunders/Elsevier; 2013 p 492.
30. Combs GF Jr, ed. The Vitamins, Fundamental Aspects in Nutrition and Health. 2nd ed. San Diego, CA: Academic Press, Inc. 1998.
31. Mellanby, E. A further demonstration of the part played by accessory food factors in the aetiology of rickets. (1918) Proc. Physiol. Soc. 5:liii-liv.