Advances in Small Animal Care 1 (2020) 265–277
ADVANCES IN SMALL ANIMAL CARE

Vitamin D in Health and Disease in Dogs and Cats

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KEYWORDS
• Calcidiol • Cholecalciferol • Calcitriol • Canine • Feline

KEY POINTS
- 25-hydroxyvitamin D is not a sensitive indicator of vitamin D status in dogs and cats.
- Food intake and food ingredient analysis are often absent in studies on vitamin D in dogs and cats.
- 1,25-dihydroxyvitamin D is the most potent vitamin D metabolite with the greatest binding affinity to the vitamin D receptor.
- Determination of other vitamin D metabolites rather than 25-hydroxyvitamin D in vitamin D studies will provide better insight in cause-effect relationships.

INTRODUCTION

Vitamin D plays an important role in several organ systems, especially in bone metabolism. However, the role of vitamin D extends well beyond bone metabolism. A low vitamin D status has been linked to different kinds of diseases, such as chronic kidney disease [1–5], chronic enteropathy [6–8], congestive heart failure [9], infectious diseases [10–15], cancer [16–18], and chronic liver disease [19]. This corresponds with recent findings that vitamin D receptors are expressed in various tissues in dogs [20]. Other reviews [21,22] of vitamin D status (mostly expressed by 25-hydroxyvitamin D [calcidiol, or 25OHD]) and its correlation with diseases in dogs and cats have already been published. However, the underlying pathophysiological mechanisms are not discussed, which is essential to determine the clinical relevance of the correlations that were found. The aim of this review was to investigate the clinical relevance of the vitamin D status for health and disease in dogs and cats, and its practical implications.

SIGNIFICANCE

Vitamin D Metabolism

Most animal species meet their vitamin D content by consuming plants (ergocalciferol), prey (cholecalciferol), or they synthesize vitamin D under the influence of sunlight (ultraviolet B light). Vitamin D is bound to vitamin D binding protein and transported to target organs. Dietary vitamin D is absorbed from the gut by protein-mediated and passive diffusion. As vitamin D is a fat-soluble vitamin, it is transported to the liver in chylomicrons [23]. Vitamin D is first metabolized in the liver by 25-hydroxylase, which is weakly regulated, and therefore, 25OHD is thought to reflect dietary vitamin D intake in dogs and cats, as they are unable to synthesize sufficient of amounts of vitamin D under the influence of sunlight and use pro-vitamin D for cholesterol synthesis instead [24,25]. 25OHD can be further metabolized into the most active metabolite 1,25-dihydroxyvitamin D (calcitriol) under the influence of 1-alpha-hydroxylase, which is predominantly present in the proximal tubules

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https://doi.org/10.1016/j.yasa.2020.07.017
2666-4518/20/

of the kidney, or 25OHD is metabolized to 24,25-dihydroxyvitamin D (24,25DHCC) by 24-hydroxylase, which is present in several tissues, or 25OHD will be stored in the liver [26]. Calcitriol formation is stimulated by parathyroid hormone (PTH), growth hormone, insulinlike growth factor-1, and inhibited by 24-hydroxylase and fibroblast growth factor 23 (FGF-23) [26]. Calcitriol is the vitamin D metabolite with the greatest binding affinity to the vitamin D receptor (VDR) [27]. 25OHD and 24,25DHCC are also able to bind to the VDR, but are 100-fold less potent compared with calcitriol [28]. Under the influence of PTH, 1-alpha hydroxylase is stimulated, and calcitriol formation is enhanced when plasma calcium level drops. The effects of calcitriol are used to restore plasma calcium level, as PTH enhances urinary phosphate excretion. Calcitriol has a negative feedback on PTH formation to prevent a perpetuating cycle. Calcitriol also stimulates 24-hydroxylase, which metabolizes calcitriol into 1,24,25-trihydroxyvitamin D (1,24,25THCC), which can be excreted by the urine. High plasma phosphorus levels stimulate FGF-23, which stimulates 24-hydroxylase to form 24,25DHCC, which stimulates mineralization of bone [29], FGF-23 also inhibits 1-alpha-hydroxylase and promotes excretion of calcitriol by enhancing 1,24,25THCC formation. On the cellular level, vitamin D actions are mediated by the VDR, a ligand-activated transcription factor that functions to control gene expression. Following ligand activation, the VDR binds directly to specific sequences located near promoters and recruits a variety of coregulatory complexes that perform the additional functions required to modify transcriptional output. Recent advances in transcriptional regulation, which permit the unbiased identification of the regulatory regions of genes, are providing new insight into how genes are regulated. The vitamin D target genes play important roles in calcium and phosphorus homeostasis, and additional targets important to these processes continue to be discovered [30].

Vitamin D Requirements

The Association of American Feed Control Officials [31], Fédération Européenne de l'Industrie des Aliments pour Animaux Familiers [32], and National Research Council [23] have determined nutritional requirements of vitamin D for dogs and cats, which are summarized in Table 1. The minimum requirement for dogs is determined based on a study demonstrating no adverse effects on bones in growing Great Dane puppies when raised on a diet with 110 IU vitamin D per 1000 kcal metabolizable energy (ME) [33]. The maximum amount for dogs is based on the same study, as adverse effects on bone were seen in puppies raised on a diet with 1000 IU vitamin D per 1000 kcal ME. Minimum requirements for cats are based on a study demonstrating no adverse effects on 25OHD plasma levels in growing kittens raised on a diet with 28 IU per 1000 kcal ME [34]. The maximum amount for cats is based on a study demonstrating no adverse effects in cats and kittens that were fed a diet with 7520 IU vitamin D per 1000 kcal ME during 18 months [35].

Vitamin D in Bone Metabolism, and Calcium and Phosphorus Homeostasis

The main function of calcitriol in bone metabolism is bone growth and remodeling. For vitamin D (ie,

TABLE 1
Nutritional Requirements of Vitamin D for Dogs and Cats

	Puppies Minimum	**Adult Dogs Minimum**	**Dogs Maximum**
AAFCO	500/125/29.9	500./125/29.9	3000/750/179
FEDIAF	552/138/33	639/159/38	2270[a]/800/191
NRC	552/136/32.5	552/136/32.5	3200/800/191
	Kittens Minimum	**Adult Cats Minimum**	**Cats Maximum**
AAFCO	280/70/16.7	280/70/16.7	30,080/7520/1798
FEDIAF	280/70/16.7	333/83.3/19.9	2270[a]/7500/1793
NRC	224/56/13.4	280/70/16.7	30,000/7500/1793

Data are expressed as amounts of vitamin D3 in IU per kg dry matter/1000 kcal metabolizable energy (ME)/MJ ME, respectively.

Abbreviations: AAFCO, Association of American Feed Control Officials; FEDIAF, Fédération Européenne de l'Industrie des Aliments pour Animaux Familiers; NRC, National Research Council.

[a] FEDIAF has defined a legal maximum for vitamin D, on dry matter basis only.

predominantly calcitriol) to be able to mineralize osteoid and cartilage, it needs calcium and phosphate. Therefore, calcitriol enhances the uptake of calcium and phosphorus from the gastrointestinal tract, the reabsorption of calcium and phosphorus from the pre-urine (filtrate) and release of calcium and phosphorus from metabolically inactive bone (ie, bone that is not frequently remodeled). Vitamin D assists PTH in maintaining plasma calcium levels, and assists FGF-23 in maintaining plasma phosphorus levels, as described previously.

Vitamin D deficiency

Vitamin D deficiency results in classic rickets. On radiographs, changes will be most apparent at epiphyseal growth plates. These changes include enlarged growth plates, hazy metaphyseal borders, ragged and cup-shaped calcification borders, thinned trabecular pattern of diaphysis, and bowed bone shafts. The changes will be most prominent in parts where growth is maximal, such as the distal growth plate of the ulna [36]. Bone pain, stiff gait, metaphyseal swelling, bowed limbs, fractures, and low serum vitamin D and calcium concentrations, are typical clinical symptoms for rickets. Symptoms of rickets are often more severe because of combined deficiencies in calcium and/or phosphorus, and aggravated by an inverse Ca:P ratio, also known as "all-meat syndrome," resulting in decreased mineralization of bone, thin cortices, greenstick fractures, and compression fractures [26].

All-meat syndrome has also been described in an adult dog, where low intake of vitamin D and calcium did not result in fractures, but instead, bone tissue was replaced by fibrous tissue, similar as in cases of renal secondary hyperparathyroidism [37].

Non–nutrition-related are the genetic types of rickets, which are also referred to as vitamin D-dependent rickets type 1 (VDDR-1) and type 2 (VDDR-2). In VDDR-1, the renal enzyme 1-alpha-hydroxylase is lacking, resulting in insufficient calcitriol production. In VDDR-2, the VDR is not responding to calcitriol due to a defective VDR (and therefore also referred to as hereditary vitamin D resistant rickets). In case of VDDR-2, high serum levels of calcitriol will coincide with low serum calcium levels. In both dogs and children with VDDR-2, alopecia is reported, but the underlying mechanism is poorly understood [38].

Vitamin D toxicity. Acute vitamin D toxicity is characterized by hypercalcemia and calcifications in bone and soft tissues. Whether these symptoms can be explained by increased levels of 25OHD, 1,25DHCC, 24,25DHCC, and/or 1,24,25THCC remains speculative. In one report, 2 dogs were diagnosed with acute vitamin D toxicity due to a commercial diet with 92.30 IU/g of vitamin D were described. Clinical findings were lethargy, polydipsia, and polyuria (due to hypercalcemia), and a stiff gait. Hypercalcemia, elevated 25OHD and calcitriol plasma concentrations, and PTH levels below the detection limit were found. The successful treatment in this case included a dietary change. Serum concentrations of calcium were within the reference range after 28 days. Serum 25OHD concentrations remained slightly elevated even after the clinical signs were gone at day 180. Serum calcitriol concentrations were within the reference range at day 150 [39].

Chronic vitamin D toxicity is not extensively studied. Previous studies demonstrated mild disturbances of endochondral ossification and irregular growth plates in puppies that were fed 135 times the recommended levels of vitamin D from 3 until 21 weeks of age. Serum concentrations of calcitonin, PTH, and all vitamin D metabolites were increased, although calcitriol was not. Instead, calcitriol serum concentrations decreased, probably due to low PTH levels or increased metabolic clearance [33].

Non–nutrition-related cases of acute vitamin D intoxication are described and related to ingestion of rodenticides. Clinical signs of vitamin D toxicosis is reported with an intake of greater than 0.5 mg (= 20,000 IU) per kg body weight in dogs and cats, but treatment is recommended from 0.1 mg (= 4000 IU) per kg body weight [40]. With suspicion of excessive intake, the author recommends to start treatment in any case, to prevent possible clinical signs. Usually, after a toxic intake of vitamin D, hyperphosphatemia, hypercalcemia and azotemia (raised blood urea nitrogen and serum creatinine levels) will develop within respectively 12, 24, and 72 hours. Other causes of hypervitaminosis that have been reported are due to treatment with the vitamin D analogues calcipotriol and tacalcitol for conditions such as psoriasis or after treatment of hypoparathyroidism [39]. Calcipotriol is a synthetic structural analogue of calcitriol; 40 to 60 μg calcipotriol per kg of body weight is reported to be the toxic dosage. Clinical signs are similar to those in rodenticide intoxication, but also soft tissue mineralization, which usually occurs within 36 hours of intoxication [41]. Fewer reports can be found on the intoxication of tacalcitol. A case report of a 21 kg dog who consumed approximately 80 μg of tacalcitol 36 to 48 hours was described. Clinical signs were mostly the same as in the calcipotriol cases, although soft tissue mineralization appeared

more severe and the lungs were filled with fluid, probably due to congestive heart failure. In this particular case, it was unclear whether the dog had ingested more tacalcitol, so whether tacalcitol intoxication is more severe compared with calcipotriol intoxication remains inconclusive [42].

Treatment of vitamin D toxicity. Treatment of vitamin D toxicity depends on the time that it is ingested, the amount that is ingested, and the severity of the clinical signs. When the toxicity is acute and consumption was within approximately 4 hours, emesis can be induced. This is only useful when clinical signs are not present, otherwise the excessive amounts of vitamin D are already taken up. Activated charcoal can be given additionally to prevent further absorption of vitamin D that was not excreted by emesis. Administration of active charcoal should be repeated every 4 to 8 hours for 1 or 2 days in case of cholecalciferol toxicity, because vitamin D recirculates through the liver and small intestine. Monitoring blood serum concentrations of calcium, phosphorus, blood urea nitrogen, and creatinine is recommended for 4 days. When clinical signs are present, immediate treatment is necessary. When clinical signs are severe or if treatment is delayed, the prognosis is guarded. Treatment of hypervitaminosis D would include the following: aggressive fluid therapy with a 0.9% saline solution, until the serum calcium concentration is back within the reference range. Fluids that contain calcium should be avoided. Decreased dietary calcium intake is prescribed to prevent further accumulation of calcium. Corticosteroids reduce vitamin D–mediated calcium absorption from the intestine, reduce bone resorption, and increase renal calcium excretion, probably due to an effect on PTH regulation. Furthermore, furosemide can be administered to promote calcium excretion by the kidneys. When the fluid therapy is not effective, treatment with bisphosphonates, such as pamidronate disodium, should be considered (1.3–2.0 mg/kg diluted in 0.9% saline, administered slowly, over 2 hours, intravenously). Bisphosphonates inhibit bone resorption through a direct effect on the osteoclast itself and by interfering hydroxyapatite crystal dissolution. Because this treatment is expensive, it is mostly applied in severe cases that do not respond well to other treatments [40].

Serum vitamin D levels

Current consensus among nutritionists is that 25OHD is not a very sensitive indicator of vitamin D status. Despite this fact, many nutrition researchers explore associations of 25OHD levels with diseases [22]. 25OHD levels are easier to measure compared with calcitriol, because calcitriol has a short half-life, and is present at much lower levels compared with 25OHD. In human medicine, especially in the western world at higher latitudes, people are vitamin D deficient in winter times because of deprivation of UVB from sunlight combined with insufficient dietary vitamin D intake. Most dogs and cats eat standardized diets largely meeting their vitamin D requirement on a daily basis in all life stages [43,44], which makes it difficult to extrapolate findings of studies in people from the western world to dogs and cats. Furthermore, there are several explanations for low 25OHD in diseased animals and people, such as low food intake, low dietary vitamin D, deprivation of UVB from sunlight (in people and several other animals than dog and cat), inadequate absorption from the gut, leakage of vitamin D in the gut (eg, when suffering from protein loosing enteropathy), reduced reabsorption from the filtrate, increased use of 25OHD for formation of calcitriol, 24,25DHCC, and or 1,24,25THCC, and increased use of vitamin D metabolites for the immune system. To be able to elucidate cause-effect relations, we need to measure all the vitamin D metabolites, which were not determined in most studies, and their results should therefore be interpreted with caution.

Another issue with interpretation of 25OHD values is variations between methodology (ie, different assays), which makes it difficult to compare results and set a normal range.

Other factors than differences in methodology can also play a role, such as the presence of epimers [45]. Serum 25OHD concentrations between 9.5 and 249.2 ng/mL in healthy dogs were demonstrated [22]. This variation has several possible explanations such as differences between breeds and sexes. An interesting finding was that intact male dogs had significantly higher serum 25OHD concentrations compared with neutered male dogs and neutered female dogs. Sexually intact female dogs had slightly higher 25OHD serum concentrations compared with neutered female dogs, but this was not significant. These findings demonstrated that either sex hormones affect 25OHD serum concentrations (and male sex hormones do so more than female sex hormones), or that gender and neutering had an effect on the amount of food eaten [46].

Although vitamin D is a fat-soluble hormone, which can be distributed and stored within adipose tissue, no significant effects of adiposity on serum 25OHD concentrations have been demonstrated [47]. When evaluating dog and cat studies on serum 25OHD concentrations, the variation in control groups is 9.5 to 249 ng/mL in dogs, and 14.9 to 83.1 ng/mL in

cats, whereas in diseased animals, serum 25OHD concentrations vary between 0 to 151 ng/mL, and 1.7 to 97.1 ng/mL, respectively [22]. In human medicine, levels of greater than 20 ng/mL are considered sufficient, but 75 to 90 ng/mL are associated with better outcome in case of disease [22]. In dogs, 100 to 120 ng/mL was suggested to be the minimum concentration to inhibit PTH secretion, which, based on the current studies, implies many dogs being vitamin D deficient according to that definition despite being fed complete and balanced diets. Unfortunately, calcitriol, 24,25DHCC, and 1,24,25THCC levels were not determined in this study, so it is difficult to draw strong conclusions [17]. Similarly, the optimal feline serum 25OHD concentrations have yet to be determined.

Associations Between Vitamin D Status and Diseases

Vitamin D and chronic kidney disease

Lower calcitriol and 25OHD concentrations were observed in dogs with acute renal failure and chronic kidney disease (CKD) [1,3]. Furthermore, significantly lower calcitriol, 25OHD, and 24,25DHCC concentrations were found in dogs with CKD IRIS (International Renal Interest Society) stages 3 and 4 [4].

As 1-alpha hydroxylase is mostly expressed in the kidney, renal disease may result in lower formation of calcitriol. In addition, loss of nephron mass increases serum phosphorus due to decreased renal excretion, which promotes FGF-23 and inhibits 1-alpha-hydroxylase activity.

An increase in PTH concentration leads to upregulation of 1-alpha-hydroxylase, which should normally increase calcitriol production, however due to progressive loss of nephron mass, the 1-alpha-hydroxylase activity remains low despite increased levels of PTH, resulting in renal secondary hyperparathyroidism. Low calcitriol, together with increased renal loss of vitamin D metabolites, can provide an explanation as to why lower vitamin D metabolite concentrations (25OHD, calcitriol and 24,25DHCC) were only observed in CKD IRIS stages 3 and 4 and not in earlier stages [4].

The endocytic receptor megalin binds to 25OHD to enter the proximal tubules in the kidney.

After binding to the megalin receptor, 25OHD can be hydroxylated to form calcitriol, or can re-enter the circulation to maintain the serum 25OHD concentration. In renal disease, decreased megalin expression contributes to lower 25OHD and calcitriol concentrations [48]. When there is a decrease in 25OHD concentration and subsequent decrease in calcitriol formation, more vitamin D metabolites will be lost via the urine due to decreased megalin expression. Additionally, decreased megalin expression is related to proteinuria.

Lower 25OHD concentration in renal disease can also be caused by decreased intake of vitamin D due to a dietary deficiency or reduced intake due to decreased appetite, vomiting, and/or diarrhea [3].

Vitamin D and gastrointestinal diseases

Lower 25OHD concentrations were found in dogs with chronic enteropathy (CE) and hypoalbuminemia, and in cats with CE or intestinal small cell lymphoma [6,7]. Furthermore, low 25OHD concentrations are linked to systemic inflammation in dogs with protein losing enteropathy, and a poor prognosis [49,50].

Decreased dietary intake because of reduced appetite or malabsorption have been suggested as contributing factors to a lower vitamin D status in dogs and cats with CE [6–8]. Inflammation of the intestinal epithelium can impair both the absorption of dietary vitamin D and reabsorption of 25OHD as a part of the enterohepatic circulation [51], however, this was not demonstrated in dogs [52].

Vitamin D signaling is important for maintenance of the intestinal mucosal barrier [53]. This barrier is essential to prevent infiltration of pathogenic microorganisms, which can evoke an immune response which can finally result in chronic inflammation. In human patients with CE, the amount of VDR in the intestinal epithelium was decreased [54], suggesting a possible correlation between gastrointestinal health and VDR expression and function. However, this was not demonstrated in dogs [20]. Vitamin D also helps to maintain a normal intestinal microbiome, as calcitriol can enhance antimicrobial activity by inducing antimicrobial peptides (AMPs) [55]. In mice, Vitamin D deficiency predisposed for colitis due to microbiome alterations [56].

Vitamin D and cardiovascular diseases

A low vitamin D status has been associated with cardiovascular diseases in humans and dogs. Significantly lower 25OHD concentrations were found in dogs with chronic valvular heart disease (CVHD) stages B2 and C/D (ACVIM Consensus Classification System for Canine Chronic Valvular Heart Disease) compared with dogs with CVHD stage B1, and in dogs with chronic heart failure (CHF) compared with healthy dogs [9,57]. Low 25OHD concentrations were also correlated to poor outcome in dogs with CHF. There are no published studies of cardiovascular diseases in relation to vitamin D in cats. In humans, lower vitamin 25OHD concentrations were associated with higher risk of cardiovascular disease [58]. For example, an

association between low 25OHD concentrations and reduced left ventricle function was found in geriatric patients [59]. This can be explained by the role of calcitriol in signal transduction.

Calcitriol can activate voltage-gated calcium channels in cardiomyocytes and improve the contractility of the myocardium [60,61]. Other cardio-protective effects of calcitriol includes inhibition of PTH and renin angiotensin aldersterone system (RAAS) activity [62], decrease in atrial natriuretic peptide expression [63] and direct, and indirect (as a consequence of PTH suppression) inhibition of hypertrophy of the myocardium [64]. Furthermore, vitamin D increases endothelial function, as serum 25OHD concentrations and flow mediated dilatation of the brachial artery were positively correlated, and therefore vitamin D could prevent the progression of coronary artery disease [65].

Vitamin D and immune function and infectious diseases

Lower 25OHD and calcitriol concentrations were found in dogs with immune-mediated diseases, such as immune-mediated thrombocytopenia, immune-mediated polyarthritis, and immune-mediated hemolytic anemia [66]. In addition, hospitalized cats with neutrophilia had lower 25OHD levels compared with hospitalized cats with normal neutrophil concentrations [67]. An in vitro study with blood of critically ill dogs showed that calcitriol has anti-inflammatory effects by suppressing the production of tumor necrosis factor alpha (a proinflammatory cytokine) and enhancing the production of interleukin (IL)-10 (an anti-inflammatory cytokine) [68].

Calcitriol enhances the antimicrobial activity by inducing AMPs and activating (the synthesis of) macrophages, supporting the first line of defense against pathogens. Furthermore, calcitriol stimulates the anti-inflammatory response by increasing Th2 response and the development of regulatory T cells, and inhibiting Th1 response, Th17 response, as well as B-cell development and differentiation [66,69–71].

A possible synergistic effect of vitamin D in prednisolone therapy in dogs with atopic dermatitis (AD) was postulated [72], as prednisolone therapy was more effective in dogs with higher serum 25OHD concentrations. A possible underlying mechanism for this finding might be found in the expression of cytokines, as overexpression of Th1 and Th2 cytokines are important in the pathogenesis of AD [73].

Various pathogenic microorganisms and parasites in relation to vitamin D status have been studied. Significantly lower 25OHD concentrations were found in cats with feline immunodeficiency virus (FIV) and mycobacteriosis compared with healthy cats and in dogs with blastomycosis, babesiosis, leishmaniasis and neoplastic spirocercosis compared with healthy dogs [10–15].

A possible explanation for the relation between lower 25OHD concentrations and bacterial infection might be a decrease in expression of cathelicidins (AMPs). Another explanation might be decreased macrophage synthesis and activation. On the contrary, increased use of 25OHD for calcitriol synthesis can be the cause of lower 25OHD levels in infectious or autoimmune diseases.

Vitamin D status in relation to viral infection was studied in humans. In patients with human immunedeficiency virus (HIV) type-1 calcitriol induced autophagocytosis, leading to inhibition of the virus in macrophages [74]. HIV closely resembles FIV and therefore a possible role for calcitriol supplementation in cats with FIV might be postulated [75].

Vitamin D in cancer and other diseases

The vitamin D status is not only linked to tumors related to bone metabolism, like osteosarcoma, but also to other tumors. Lower serum 25OHD concentrations were found in dogs with mast cell tumors (MCT) [18], and in dogs with splenic hemangiosarcoma [17]. In dogs with cancer, serum 25OHD concentrations increased with increasing serum calcium concentrations [16]. Whereas healthy dogs showed the opposite, indicating that the vitamin D metabolism is altered in patients with cancer and that serum calcium concentrations are involved in this change. These increased calcium concentrations can be caused by increased levels of PTH-related peptide, as was demonstrated in dogs with anal sac adenocarcinoma and in dogs with lymphoma. Calcitriol concentrations were increased, normal, or decreased in these dogs, but calcitriol levels decreased in these dogs after successful cancer treatment [76]. Antiproliferative effects of calcitriol in relation to tumor growth were described, including stimulation of G1/G0 cell-cycle arrest and cell death, reduction of epidermal growth factor, and suppression of invasive growth [77].

Alterations in vitamin D status also occurred in patients with liver disease. The liver is an important part of the vitamin D metabolism, as hydroxylation of cholecalciferol to form 25OHD takes place in the liver. Impaired liver function affects the vitamin D metabolism, resulting in lower serum 25OHD concentrations. Lower 25OHD concentrations were found in

cats with cholestatic liver disease, and in dogs with chronic liver disease [19,78].

In addition, lower serum 25OHD concentrations were found in dogs with acute pancreatitis [79]. In humans, it was found that CYP24A1 expression, also known as 24-hydroxylase, correlated with VDR expression in patients with chronic pancreatitis [80]. If dogs with pancreatitis have increased levels of 24,25DHCC, which is responsible for the lower 25OHD, remains speculative.

Finally, the vitamin D status has been linked to mortality. A correlation was found between low 25OHD concentrations and a poor prognosis in hospitalized dogs and cats [81–83].

A decreased vitamin D status in hospitalized patients can, however, be caused by many factors altering vitamin D metabolism, such as reduced intake and/or malabsorption, decreased immune function, or decreased liver and/or kidney function, which impairs the formation of 25OHD and calcitriol, all of which can affect prognosis.

The Role of Supplementation

It is important to understand which metabolite is most effective to administer. Recent studies have demonstrated that supplementation with vitamin D is often not effective. In humans and dogs, supplementation with 25OHD is much more effective in rising serum 25OHD concentrations compared with vitamin D. The amount of vitamin D had to be 10 times higher compared with 25OHD to obtain similar effects [84]. Most pet food ingredients contain vitamin D2 or D3, but not the other metabolites. For cats, it is important to determine whether a product contains vitamin D2 or vitamin D3, as they use D3 more effectively [85].

Vitamin D supplementation in renal disease

In renal disease, vitamin D or 25OHD supplementation is less effective compared with calcitriol, as calcitriol formation is impaired, due to reduced 1-alpha-hydroxylase activity. Calcitriol supplementation in dogs and cats with CKD has several beneficial effects. Calcitriol inhibits PTH and the renin-angiotensin-aldosterone-system [86] by suppressing the expression of the renin gene and the activation of VDR [62]. Calcitriol also decreases injury and loss of podocytes [87], possibly by increasing nephrin and Wilms tumor suppressor gene 1 (WT1) protein expression [88]. In addition, calcitriol slows down the progression of fibrosis in patients with CKD by reducing transforming growth factor-β synthesis and increasing antifibrotic factors [89]. Furthermore, calcitriol suppresses tumor necrosis factor alpha-converting enzyme, a factor involved in the mechanism of developing proteinuria, glomerulosclerosis, tubular hyperplasia, mononuclear cell infiltration, and fibrosis [90]. Finally, calcitriol therapy is associated with increased appetite, increased physical activity, and a longer life expectancy due to its inhibitory effects on PTH secretion [91].

Calcitriol therapy can be used to control renal secondary hyperparathyroidism and to slow down the progression of CKD. It is often combined with a phosphorus restricted diet to prevent hyperphosphatemia and to reduce the risk of soft tissue mineralization [89]. Calcitriol treatment should be monitored closely to prevent hypercalcemia, which is one of the consequences of vitamin D toxicosis [1,39]. Calcitriol should be supplemented when the patient is in a fasted state to prevent increased intestinal calcium and phosphorus absorption [92]. In dogs and cats, oral administration of calcitriol starts with a daily initial dose of 2.0 to 3.5 ng/kg with a maximum dose of 5.0 ng/kg [92]. An initial calcitriol dose of 2.5 to 3.5 ng/kg per day (if serum creatinine is 176–265 μmol/L) or 3.5 ng/kg per day (if serum creatinine is higher than 265 μmol/L) is described for cats [93]. No supporting evidence was given for these doses for dogs and cats.

For dogs with CKD IRIS stages 3 and 4, calcitriol therapy is recommended [92], as they have lower vitamin D levels [4], and calcitriol treatment was associated with increased survival in 37 dogs with stage 3 and 4 CKD55. Positive or negative results of calcitriol therapy in cats have yet to be demonstrated, although an experiment involving 10 cats with CKD failed to demonstrate an increase in serum PTH [2].

Vitamin D supplementation in gastrointestinal disease

The effects of 25OHD or calcitriol therapy in dogs and cats with gastrointestinal diseases have not yet been determined. In humans with Crohn's disease, a decrease in severity of symptoms was observed after oral cholecalciferol supplementation for 24 weeks [94]. A possible explanation might be reduced inflammation because of the immunomodulatory effects of calcitriol. Another study, evaluating humans with various gastrointestinal diseases, demonstrated no increase in 25OHD levels after vitamin D supplementation [55]. Calcitriol induces VDR expression in the ileum of humans and rats [95], which suggests that calcitriol could stimulate vitamin D uptake or affect the immune system in the intestinal tract.

Vitamin D supplementation in cardiovascular disease

Studies on the effects of vitamin D supplementation on the cardiovascular system in dogs and cat have not yet been performed. One case report of a cat presented with primary hypoparathyroidism and CHF demonstrated possible effects of calcitriol on the cardiovascular system. This cat received medication and calcitriol for 4 weeks. After 4 weeks, only oral calcitriol administration (twice a week) was continued. A year later, the patient had not shown symptoms of CHF nor did it need medication [96]. In humans, vitamin D supplementation resulted in a better outcome in patients with heart failure (HF), and an increased ejection fraction in geriatric patients with low vitamin D levels and HF [97,98]. In addition, oral cholecalciferol supplementation in children with CHF showed anti-inflammatory effects [99]. Vitamin D supplementation in patients with coronary artery disease has also been studied; however, cholecalciferol supplementation did not always result in significantly positive effects (such as improved endothelial function and vascular inflammation and prevention of myocardial injury) [65]. Patients with coronary artery disease supplemented with calcitriol instead of cholecalciferol demonstrated anti-inflammatory effects, but there was also an increase in severity of coronary artery disease possibly due to reduced RAAS activity [100]. Although calcitriol is negatively correlated to RAAS activity, supplementation with vitamin D, its metabolites, or analogues should not be used as a drug to control hypertension, as experimental studies failed to show beneficial effects [101].

Vitamin D supplementation in immune diseases

Calcitriol inhibits Th1 response [73], which indicates that calcitriol might have beneficial effects in patients with AD in the chronic phase of disease. In humans, possible beneficial effects of vitamin D administration in patients with immune-mediated disease have been suggested, for example, in patients with psoriasis. Topical treatment of the skin with maxacalcitol (a vitamin D3 analogue) in mice resulted in decreased inflammation of the skin, due to an increase of regulatory T cells and a decrease in IL-23 and IL-17 synthesis [102].

Vitamin D supplementation in cancer

An in vitro study observed that oral calcitriol administration improves the effects of chemotherapy in canine MCTs [103]. This might be due to the activation of VDR, as VDR expresses broadly in canine neoplastic mast cells [104]. Another possible explanation might be a decrease in receptor tyrosine kinase activity [103]. In addition, calcitriol and calcipotriol (a calcitriol analogue) showed cytotoxic effects on multidrug resistance protein-1 overexpressing cells in a study using cytotoxicity assays [105]. Impairment of the transport function of ATP-binding cassette transporters, which are related to proteins involved in multidrug resistance (P-glycoprotein, MRP1 and breast cancer resistance protein) was also observed. Another study observed synergistic effects of calcitriol and cisplatin on inhibition of proliferation of canine tumor cells in vitro [106]. This study also conducted a noncontrolled clinical trial that showed antitumor effects of intravenous calcitriol/cisplatin administration in 3 of 8 dogs with tumors that had a measurable size. In humans, a systematic review and meta-analysis concluded no evidence was found to support the use of vitamin D supplementation to decrease mortality in patients with cancer, or to decrease cancer incidence [107].

Vitamin D supplementation in liver disease

Vitamin D supplementation was found to decrease inflammation and fibrosis in cats with chronic vitamin A intoxication [108]. No evidence for effects of vitamin D supplementation on liver disease has been reported in dogs.

PRESENT RELEVANCE AND FUTURE AVENUES TO CONSIDER OR TO INVESTIGATE

Vitamin D requirements are based on a limited number of studies that were characterized by absence of disease, rather than demonstrating pathology. Differences between institutes are based on different safety margins, as all of them are referring to the same studies. More research should be done to determine true requirement intervals. Furthermore, the minimum requirement for cats is based on maintenance of 25OHD levels. As 25OHD levels are not a sensitive marker for vitamin D status, other metabolites should be included in future studies. Nutrient interactions also warrants inclusion of PTH, calcium, and phosphorus.

Food intake, amount of vitamin D, form of vitamin D (ie, D2 or D3, or metabolites) all have an effect on 25OHD levels and should therefore be measured and reported in future studies.

Several relations between low 25OHD levels and diseases have been reported, but underlying mechanisms are mostly hypothetical and need further evaluation.

Most evidence is present for calcitriol supplementation in dogs with IRIS stage 3 and 4 renal disease.

To determine whether or not enteric loss of protein-bound vitamin D is a significant factor in low serum 25OHD concentrations in CE, it is necessary to compare VDBP serum concentrations between dogs and cats with CE and a control group consisting of healthy dogs and cats. Even though the effects of vitamin D on the gastrointestinal system have been studied, it is still unknown if vitamin D has a (significant) role in the pathogenesis of CE or is just a result of CE. This requires an experimental study design, which determines if supplementation of 25OHD or calcitriol significantly improves the described effects of vitamin D in patients with CE.

Clinical trials are required to determine the use of vitamin D supplementation in dogs and cats with cardiovascular disease regarding in which form and dosage it should be administered. It is postulated that calcitriol might be more potent than cholecalciferol because calcitriol acts directly on the cardiovascular system.

It might be useful to determine if calcitriol therapy in patients with immune-mediated diseases is more potent than vitamin D3 supplementation. Calcitriol seems to enhance the immune function. However, additional studies are required to determine the differences of calcitriol effects on the immune system in humans, dogs, and cats. In addition, it has yet to be proven that vitamin D (metabolite) supplementation can have significant beneficial effects in dogs and cats with various infectious diseases.

SUMMARY

Vitamin D is an important nutrient which has a vital role in bone metabolism as well as in other vital functions. Low vitamin D intake often coincides with low calcium intake, and is characterized by bone deformities, especially during the growth period. Bent legs, retarded growth, altered locomotion, enlarged growth plates, and decreased bone mineralization are the most common clinical and radiological findings. Dogs and cats seem to be quite resistant to excessive vitamin D intake, but calcitriol supplementation can cause hypercalcemia and renal disease. Early and thorough detoxification is important to prevent permanent damage and guarded prognosis. 25OHD is often used to evaluate vitamin D status; however, this is only a rough estimation and reflects dietary intake. Other vitamin D metabolites, especially calcitriol, provide more insight in vitamin D metabolism and effects on target tissues, but are often not reported. Associations of low 25OHD levels and several diseases have been reported, but underlying mechanisms are mostly theoretic, and not well studied. Most evidence is available for effectiveness of calcitriol supplementation in dogs with IRIS stage 3 and 4 CKD, for all other disease conditions the level of evidence is preliminary.

ACKNOWLEDGMENTS

The author thanks Graciela van Schaik and Saskia van der Vaart for their valuable contributions to the manuscript, which were part of their bachelor theses.

DISCLOSURE

The author has nothing to disclose.

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