

The Role of Vitamin D in Veterinary Oncology: A Literature Review

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Abbreviations

25(OH)D	25-hydroxyvitamin D (calcifediol or calcidiol)
CDK	Cyclin dependent kinase
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
EGFR	Epidermal growth factor receptor
HIF-1	Hypoxia-inducible factor-1
IGF	Insulin-like growth factor
MMP	Matrix metalloproteinase
PTH	Parathyroid hormone
VDR	Vitamin D receptor
VEGF	Vascular endothelial growth factor

Abstract

Vitamin D, both cholecalciferol (D3) and ergocalciferol (D2), as well as the most bioactive form, calcitriol (1,25(OH)2D), are well known for calcium regulation and effects on skeletal health. Multiple mechanisms of action have been identified affecting multiple physiologic systems, including the prevention, treatment, and control of cancer. Over 2000 genes express vitamin D receptors, with influences of this hormone reaching far past skeletal health. Much research exists for the role of vitamin D in oncology, and evidence continues to be discovered for companion animals regarding the influential role vitamin D plays in veterinary health and disease. This literature review provides the current information behind the epidemiologic, anticancer, and mechanistic evidence for use of vitamin D in the treatment of cancer.

Introduction

The clinical importance of vitamin D in calcium homeostasis and in the maintenance of skeletal health has been well established throughout history, with Hippocrates describing a disease that resembled rickets as far back as 130 AD (1, 2). Various human studies have found correlations between insufficient levels of vitamin D and

increased risk of non-skeletal pathologies, such as cardiovascular disease, hypertension, cancer, diabetes, multiple sclerosis, rheumatoid arthritis, infectious disease, and asthma (3). In animals, the role of vitamin D is also important in extra-skeletal conditions, such as chronic kidney disease, inflammatory bowel disease, cardiovascular disease, infection, feline oral resorptive lesions, mortality, canine atopy, and cancer (4–11). The diverse effects of vitamin D across so many physiologic systems are in large part due to the presence of vitamin D receptors within multiple organ systems and tissues, such as B and T lymphocytes, hair follicles, muscle, adipose tissue, bone marrow, and cancer cells, regulating more than 2000 genes and widening the perceived scope of the vitamin D endocrine system (3, 12). This paper outlines the specific roles vitamin D is believed to play in the prevention and treatment of cancer and reviews the dynamic physiology and metabolism of vitamin D, along with evidence for monitoring and supplementation in veterinary oncology.

Metabolism

Vitamin D, a seco-sterol prohormone, has 2 main forms: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). The skin of most mammals can produce cholecalciferol

from the provitamin 7-dehydrocholesterol via activation with UVB light. Humans, rats, sheep, and cattle can produce cholecalciferol in the skin (13). However, this photosynthesis pathway is inefficient in dogs and cats because of the higher activity of the enzyme 7-dehydrocholesterol reductase that converts 7-dehydrocholesterol to cholesterol (14). As negligible amounts of vitamin D are produced via the skin, the primary source of vitamin D must come from the diet. Ergocalciferol is obtained by irradiation with UV light of plants or plant materials when ergosterol is converted to ergocalciferol (15). Both ergocalciferol and cholecalciferol are utilized; however, evidence in humans suggests that metabolites from ergocalciferol are much less bioavailable than those from cholecalciferol (16). In cats, ergocalciferol is less efficiently utilized than cholecalciferol (17). The exact difference in metabolism between ergocalciferol and cholecalciferol in dogs is unclear.

Following ingestion, the absorption of vitamin D via the small intestine is dependent on the presence of bile salts. As a fat-soluble hormone, vitamin D then enters the lymphatic system in association with chylomicrons and is transported primarily via the vitamin D-binding protein. It is subsequently either stored in lipid depots of adipose tissue, kidneys, liver, lungs, aorta, muscle, and heart, or it is transported to the liver for first hydroxylation via cytochrome P450 27A1 to become 25-hydroxyvitamin D (25(OH)D), also known as calcifediol or calcidiol. This is the most stable metabolite of vitamin D, with a half-life estimated to range from 10 days to 3 weeks, allowing serum concentrations of 25(OH)D to be widely accepted as an indicator of overall vitamin D status (18). The 25(OH)D then undergoes second hydroxylation by the action of 1 α -hydroxylase (cytochrome P450 27B1) to become the most biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), also known as calcitriol. Calcitriol has a half-life of 4–6 hours and blood concentrations of only 1/1000th of 25(OH)D, making serum levels of calcitriol a less useful marker of vitamin D status (19).

Primary production of calcitriol takes place in the proximal tubules of the kidneys; however, many other extra-renal tissues possess the enzyme 1 α -hydroxylase and can therefore synthesize calcitriol from 25(OH)D. The autocrine synthesis of calcitriol in various organs such as the prostate, colon, breast, and pancreas is one mechanism proposed to explain the chemopreventive properties of

vitamin D at these sites (20). Circulating concentrations of calcitriol are tightly regulated by several factors, including parathyroid hormone (PTH), calcium, phosphorus, calcitonin, and calcitriol itself (21). Calcitriol, as the most bioactive form of vitamin D, binds with the vitamin D receptor (VDR) forming a heterodimer with the retinoid X receptor and interacting with nuclear vitamin D response elements (22). These response elements are present in over 200 target genes and therefore produce a variety of biological effects.

The VDR is essential for the initiation of genomic and non-genomic signaling pathways that are induced by calcitriol and play a critical role in anti-cancer activity (23). In addition to their roles in bone health, 25(OH)D and calcitriol modulate cell growth, immune system function, and inflammatory pathways, activating genes that regulate cell proliferation, differentiation, apoptosis, invasiveness, and metastasis of cancer cells (24, 25).

Genomic Signaling Pathways of Calcitriol

Common cellular processes targeted by calcitriol include cell cycle progression, apoptosis, cellular adhesion, oxidative stress, immune system function, and steroid metabolism (26). Examples of genes that are transcriptionally activated by calcitriol include CYP24A1, encoding for 1 α -hydroxylase; BGLAP, encoding for osteocalcin and expressed in bone osteoblasts; and CDKN1A which encodes the cyclin-dependent kinase (CDK) inhibitor p21, a protein responsible for tightly regulating cell cycle progression and arrest (27). The calcitriol-mediated repression or activation of many proto-oncogenes or tumor suppressor genes has also been described in both normal and tumor tissues with increased expression of cyclin A1 in ovarian cancer cells and increased expression of cyclin D2 in breast cancer cells (28–30).

It is also important to realize that the development of cancer itself markedly influences vitamin D receptors, enzymes, and signaling pathways (31). When prostate cancer is present, there is evidence of both decreased ability of prostatic 1 α -hydroxylase to convert 25(OH)D to calcitriol as well as complete absence of the enzyme, influencing cell division and differentiation (32, 33). Certain cancer cells also demonstrate decreased expression of the VDR, with activity lost in poorly differentiated colonic tumors and in patients with increasing tumor stage

(25, 34). Levels of 1α -hydroxylase have also been found to be increased in preneoplastic lesions of colon cancer, suggesting that advanced cancer cells may have increased degradation of calcitriol (35). Even single-nucleotide polymorphisms or ethnic variations of the VDR have been linked with risks of cancer in humans, further demonstrating the intimate role of vitamin D and the VDR in cancer (31).

Non-genomic Signaling Pathways of Calcitriol

In addition to the activation of genomic pathways, calcitriol also triggers rapid, non-genomic responses that activate transmembrane signaling cascades (36). Activation of these cascades can cause rapid intestinal absorption of calcium ions (Ca^{2+}), which may subsequently activate protein kinases, regulating proliferative and antiproliferative effects. Photoprotection from UV rays is another pathway considered to be mediated by non-genomic actions of calcitriol, decreasing the DNA damage induced by sun exposure that leads to skin cancer (37).

Anticancer Mechanisms of Action

Epidemiology and Chemoprevention

Substantial epidemiological data exists to indicate that low serum vitamin D concentrations, or surrogates for vitamin D status such as geographical latitude or season, are associated with an increased risk of a variety of cancers in humans, with the strongest evidence existing for colorectal, breast, and prostate cancers. (11, 27). The first suggestion that vitamin D might influence cancer arose in 1980 when Garland and Garland proposed that the high rate of colon cancer seen in the northern U.S. as compared to the southern U.S. was due to a lower UV light-induced production of vitamin D in the skin (38). Ecological studies have since extended the “sunlight” hypothesis to 18 different types of cancer, suggesting that vitamin D deficiency may account for thousands of premature human deaths from colon, breast, ovarian, and prostate cancer every year (25, 39). Other lines of evidence also support the hypothesis that vitamin D or its metabolites have direct inhibitory effects on the development and progression of various cancers (40). Epidemiologic studies have also noted lower incidence and mortality rates for several cancers in regions with greater solar UVB exposure (41). Although ecological studies like these are the weakest forms of scientific evidence, several animal studies have provided support for these hypotheses, identifying decreased 25(OH)D levels in dogs with mast cell tumors, hemangiosarcoma, lymphoma, and

neoplastic spirocercosis, warranting further research in this area (42). Vitamin D deficiency has also been linked with poorer prognoses and outcomes in human patients with lung, colorectal, and breast cancers as well as lymphoma (43–46). Preclinical studies in mice have shown that severe vitamin D deficiency or deletion of the VDR gene increases cancer risk (47, 48). Additional studies show a reduction in tumor incidence or tumor size in animals injected with calcitriol (49, 50).

There has also been a protective relationship established between sufficient vitamin D status and lower risk of cancer in many human studies (25). It has been reported that breast and colorectal cancer incidence can be reduced by 50% with serum 25(OH)D levels measuring >32 ng/ml (51). A similar study of colorectal cancer found a 50% reduction in cancer incidence with serum 25(OH)D levels of ≥ 33 ng/ml and a 75% reduction with serum levels of 46 ng/ml (52). Similar results have been found in prostate, pancreatic, and breast cancers (53). A 4-year clinical trial with postmenopausal women also showed that an intake of 1100 IU/day of vitamin D plus 1400-1500 mg/day of calcium reduced the risk of all cancer types (54). These results provide a rationale for using vitamin D in both cancer prevention and treatment in humans. Similar epidemiological studies have not yet been expanded upon in companion animals.

Antitumor Activity

The exact mechanisms of the antitumor effects of vitamin D compounds are incompletely understood, although calcitriol, the active substrate to the VDR, has been most carefully studied both in vitro and in vivo. Plausible mechanisms for the anti-cancer activities of calcitriol are numerous, with inhibition of proliferation, induction of differentiation and apoptosis, enhanced DNA repair, antioxidant protection, anti-angiogenic and anti-metastatic actions, and immunomodulation through interference with growth factor and cytokine synthesis all well described (40, 55).

Proliferation

Anti-proliferative and pro-differentiating effects of calcitriol have been reported as early as 1981 in previous studies of malignant melanoma and myeloid leukemia cells (56, 57). Cell cycle progression and arrest in all cells are tightly regulated by proteins known as cyclins and CDKs. Activated cyclin/CDK complexes phosphorylate retinoblastoma protein, a tumor suppressor protein

associated with G1/0 arrest, the interface between non-dividing, differentiated cells (G0) and the first step in the cell cycle for replicating cells (G1). The cyclin-dependent phosphorylation of retinoblastoma protein induces the progression from G1 to S phase in both normal and malignant tissues. The expression of both cyclins and CDKs can be decreased by the administration of calcitriol, affecting progression through the cell cycle, and thus influencing cellular proliferation (58).

More upstream, CDK inhibitors regulate the activity of the cyclin/CDK complexes, and therefore progression through the cell cycle. The expression of the CDK inhibitors p19, p21, and p27 are increased after treatment with calcitriol, inducing cell cycle arrest (23). Treatment with calcitriol can also reduce phosphorylation of inhibitors such as p27, rendering the protein less prone to degradation while also upregulating expression of p21, thus affecting progression or arrest of the cell cycle (23).

Other growth regulating pathways are also influenced by calcitriol, with inhibitory effects on the epidermal growth

factor receptor (EGFR)-signaling cascade as well as on insulin-like growth factors (IGFs). The EGFRs are major contributors to a complex signaling cascade that modulates growth, signaling, differentiation, adhesion, migration, and survival of many cancer cells. These factors are targets for multiple anticancer drugs (59). Following binding with calcitriol, the VDR represses expression of EGFRs and therefore inhibits all downstream effects (60). Signaling of insulin and the IGF system also plays a key role in the development and progression of several types of cancer, and alongside chronic inflammation is an important contributor to the cancer-promoting effects of obesity (61). Binding of calcitriol to the VDR down-regulates the mitogenic IGFs while also up-regulating IGF binding proteins, the factors that control their activity (62).

Other growth factors of healthy tissue, such as transforming growth factor (TGF- β), are important in the initiation of tumor suppressor signaling cascades. Transforming growth factor is stimulated by calcitriol, thus halting the progression of malignant growth, and may play an important role in the growth-reducing action of vitamin D (63).



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Apoptosis

The ability of calcitriol to induce apoptosis, or programmed cell death, has been best demonstrated in breast and prostate cancer cell lines, but complete mechanisms have not been fully elucidated (23). Apoptosis, a mechanism innate to both normal and malignant cells, generally occurs through 2 pathways: the intrinsic (mitochondrial pathway), or the extrinsic (receptor mediated pathway) (64). Calcitriol can initiate the intrinsic pathway through decreasing expression of the anti-apoptotic factors Bcl-2 and Bcl-XL and/or by increasing the pro-apoptotic equivalents Bax and Bak (65). The cleavage of PARP-1, a family of proteins involved in the apoptosis cascade, was also shown to activate apoptosis in breast cancer cells when induced by calcitriol (23). Calcitriol was also found to increase intracellular calcium levels by depleting calcium stores in the endoplasmic reticulum and by activating calcium entry from the extracellular space. This increase, identified in breast cancer cells, was found to activate specific caspases of the intrinsic pathway, generating an apoptotic response (66). Some evidence also exists that cell death mediated by treatment with calcitriol may be due to autophagy or advanced differentiation instead of apoptosis (67, 68). Additionally, calcitriol and its analogues show evidence of enhanced cancer cell apoptosis in response to radiation and chemotherapy.

Angiogenesis and Metastasis

In vitro and in vivo experiments have shown that calcitriol can modulate angiogenesis, a key step in continued tumor growth, progression, and metastasis (23). The formation of new blood vessels is an important ability of malignant cells to guarantee their own oxygen supply, causing normally quiescent vasculature to continually sprout new vessels in order to sustain expanding neoplastic growth (69). The anti-angiogenic effects of calcitriol have been shown to inhibit the proliferation of endothelial cells as well as reduce vascular endothelial growth factor (VEGF), the most potent stimulator of new blood vessel formation (70). This activity was demonstrated in a tumor transplantation model in which mice treated with calcitriol produced tumors that were less vascularized than those found in controls (71). Additionally, in VDR knockout mice, tumors were found to have developed larger tumor vessels and volume than those in mice with VDR present (72). At the molecular level, the effects of calcitriol are believed to be mediated by decreasing the expression of hypoxia-inducible factor-1 (HIF-1), a tran-

scription factor responsible for inducing VEGF expression (73). The proangiogenic effect of cyclooxygenase-2 (COX-2) in multiple cancer cells is believed to be a result of its action to increase HIF-1 α in these tissues. Calcitriol has been found to suppress COX-2 expression and provides an indirect mechanism by which calcitriol inhibits angiogenesis, alongside direct suppressive effects of proangiogenic factors such as HIF-1 and VEGF (41, 74).

Control of angiogenesis is also important to reduce the ability of cancer cells to invade the circulatory system and metastasize to secondary sites. Calcitriol has been shown to regulate the expression of key molecules involved in invasion and metastasis, often through the interactions with components of the extracellular matrix. Proteases, such as matrix metalloproteinases (MMPs), plasminogen activators, and cathepsins degrade the extracellular matrix and consequently allow the cancer cells to invade (75). Tissue inhibitors of metalloproteinases (TIMPs) are the natural counterparts of MMPs, while cathepsins are controlled by cathepsin inhibitors (CIs). Treatment of cancer cells with calcitriol reduces the activity of MMP-9 and cathepsins while increasing the activity of TIMP-1 and CIs, resulting in inhibition of metastasis (23). Calcitriol has also been shown to be influential for tumors that show a preference for bone microenvironments as sites of metastasis, such as breast and prostate carcinomas (76). Bone resorption is crucial for metastasis in these cancer models, and administration of calcitriol and calcium has been found to decrease this metastatic ability (77).

Anti-Inflammatory Effects

Considerable evidence exists for the role of vitamin D signaling within inflammation, a process which was previously added as an enabling hallmark of cancer in 2011 (78). Inflammation contributes to the development and progression of many cancers and is recognized as a risk factor for cancer development, making it an important target for prevention and treatment. Research indicates that calcitriol has anti-inflammatory activity that likely contributes to its beneficial effects on multiple cancers (41, 79).

Prostaglandins promote carcinogenesis and cancer progression by stimulating proliferation, inhibiting apoptosis, promoting angiogenesis, and activating carcinogens (80). The enzyme responsible for PG synthesis, COX-2, is

regarded as an oncogene and an important molecular target in cancer therapy; increased expression is an indicator of more aggressive biological behavior in many cancer types (81). Calcitriol decreases the levels of biologically active PGs, causing suppression of the proliferative and angiogenic stimuli they normally provide. Combinations of calcitriol with NSAIDs cause a synergistic enhancement of the inhibition of prostate carcinoma cell proliferation, suggesting that this drug combination might be clinically relevant (82). Similar effects have been noted in breast cancer cells, with reductions in estrogen synthesis and estrogen receptor α levels reducing the mitogenic stimulus in these cell lines (83).

Calcitriol also suppresses the activation and signaling of nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), a transcription factor that regulates inflammation, immune response, and cellular proliferation and is believed to play a key role in the process leading from inflammation to carcinogenesis (41). Calcitriol directly modulates NF κ B activity in many cancer cells, leading to a decrease in the production of proinflammatory cytokines such as interleukin-6 and interleukin-8 (84, 85).

Relationships between C-reactive protein (CRP), a highly sensitive marker of inflammation often correlated with diagnosis and prognosis in various cancers, and hypovitaminosis D have been identified, with research in a population of racing sled dogs suggesting that decreases in serum concentrations of 25(OH)D correlate to concomitant increases in CRP concentrations (86).

Finally, these anti-inflammatory activities are believed to be mechanisms by which vitamin D plays a role in delaying or preventing cancer development and/or progression. These effects, along with the above-mentioned anticancer activities, are also hypothesized to be possible mechanisms of chemoprevention in various tumor types.

Concurrent Use with Anticancer Therapies

Calcitriol was first investigated in humans as a differentiating agent in myelodysplasia and acute leukemia (27). Results revealed that 20-30% of patients developed hypercalcemia when calcitriol was administered at 1.5–2.0 g daily, with subpar anticancer activity prompting investigation into combination protocols and varied dosing schedules (87, 88). The original dosing schedules were

developed for the treatment of renal osteodystrophy and osteoporosis; therefore, biologically effective and maximally tolerated doses, as well as optimal dosing schedules, have not yet been determined for maximal anticancer activity (27). In human trials, minimal hypercalcemia occurs when high-dose calcitriol is administered on an intermittent schedule; less-hypercalcemic analogues of calcitriol with modified chemical structures less prone to degradation by 1 α -hydroxylase have been developed and investigated for single agent and combination usage (41, 89). A standard phase I dose-escalation trial of calcitriol administered orally once a week in humans found that 2.8 g/kg can be safely administered without any side effects. Dose-limiting toxicity of oral calcitriol has not been observed (90).

Considerable data support the presence of synergistic activity between calcitriol and calcitriol analogues and traditional anticancer therapies, including radiation therapy. Calcitriol has been found to potentiate the anticancer activity of platinum analogues such as cisplatin and carboplatin, anthracyclines such as doxorubicin and mitoxantrone, topoisomerase inhibitors such as irinotecan and etoposide, antimetabolites such as cytosine arabinoside and gemcitabine, and taxanes such as docetaxel and paclitaxel (41). Optimal potentiation is seen when calcitriol is administered immediately before, or simultaneously with the anticancer agent, rather than afterward (91).

In an in vitro study using canine tumor cells, calcitriol and cisplatin were shown to have synergistic, antiproliferative activity in mammary carcinoma, osteosarcoma, and mastocytoma cell lines (92). In vivo results from client-owned dogs in the same study indicated that the maximum tolerated dose of intravenous calcitriol in dogs was 3.75 g/kg, and the dose-limiting toxicity was hypercalcemia. Although assessment of tumor response was not the primary goal of the in vivo study, antineoplastic activity was observed in 3 of 8 dogs. Calcitriol was administered immediately prior to cisplatin. These findings defined a phase II dosage and support the hypothesis that calcitriol has clinical activity in canine tumor models. In a follow-up study of canine mast cell tumors, in vitro treatment of mast cell lines with calcitriol exhibited synergistic, antiproliferative activity when used in combination with lomustine, vinblastine, imatinib, or toceranib (89). The concentrations required for 50% growth inhibition were 2-6 times lower when chemotherapy was used in combination with calcitriol

compared to chemotherapy alone. For the population of client owned dogs, a calcitriol analogue (DN101) was used for oral administration at a dosage of 2.25 g/kg orally once weekly for 4 treatments. This high-dose oral calcitriol induced remission in 4 of 10 dogs (1 complete remission, 3 partial remissions). Unfortunately, the majority of patients experienced toxicity (azotemia, vomiting, hypercalcemia, or anorexia), necessitating discontinuation of the trial.

In human patients with diffuse large B cell lymphoma, rituximab-mediated killing of lymphoma cells was substantially enhanced by the restoration of sufficient vitamin D levels, providing evidence that aside from high-dose intervention, even maintaining adequate vitamin D levels may be of benefit in certain cancer treatments (93).

Vitamin D compounds potentiate the antitumor effects of ionizing radiation and photodynamic therapy in preclinical models of prostate and squamous cell carcinoma, and pretreatment with calcitriol increases apoptosis in breast cancer cells undergoing ionizing radiation (94).

Additional conventional agents used in cancer treatment, such as retinoids, glucocorticoids, inhibitors of 1α -hydroxylase, and NSAIDs, exhibit synergistic relationships with calcitriol. Calcitriol, the VDR, and the retinoid X receptor interact as a heterodimer to initiate vitamin D signaling; therefore, ligands of the retinoid receptor such as vitamin A may modify calcitriol activity, with potentiation of calcitriol antitumor effects by retinoid compounds having been well described (95). Glucocorticoids have direct anticancer activity and block calcitriol-induced hypercalcemia, and synergistic antitumor effects of calcitriol and glucocorticoids have been demonstrated in human prostate cancer cell lines (96). Non-specific cytochrome p450 inhibitors such as antifungals, isoflavones such as genistein, progesterone, and specific inhibitors of 1α -hydroxylase, have been identified as enhancing the antitumor activity of calcitriol (96). As mentioned previously, PG synthesis inhibitors may potentiate the activity of calcitriol. In a single arm, open label phase II clinical study investigating the effects of the use of combination high dose calcitriol and naproxen in human patients with early recurrent prostatic carcinoma, a prolongation of serum prostate specific antigen (PSA) doubling time was identified in 75% of the patients, showing the synergistic benefits of treatment with calcitriol and an NSAID in this cancer model (82).

Monitoring and Supplementation

Historically, dogs and cats have obtained the vitamin D they need from eating the fat stores of killed prey. Around the 1900's, as dogs and cats moved to prepared pet foods, the supplementation provided in commercially available food became their primary source of vitamin D (24). This level of supplementation has been determined to be adequate to prevent rickets, but the ideal dosages and corresponding serum concentrations of vitamin D that are needed to maintain optimal health are not known (3). With human studies, there has been a movement towards categorizing vitamin D levels as *deficient*, *insufficient*, or *sufficient*, defining a serum 25(OH)D level as sufficient when a disease risk is minimized for the majority of the population (19). Varying opinions exist about the current cutoff points as well as health outcomes that should be associated with each category. This continues to be an area of debate in both human and veterinary medicine (24). Currently, sufficiency is defined in human studies as the level of 25(OH)D where PTH secretion is minimized, intestinal calcium absorption is stabilized, and/or calcium resorption from bone is minimized. The vitamin D level needed to optimize intestinal calcium absorption is 85 nmol/L (34 ng/ml), which is lower than the level needed for neuromuscular performance of 95 nmol/L (38 ng/ml) (97). However, several studies have shown that PTH levels do not plateau until serum 25(OH)D levels approach 75 nmol/L (30 ng/ml) (98). The Institute of Medicine reviewed 25(OH)D levels in relation to markers of bone health when developing dietary intake guidelines for vitamin D and concluded that 97.5% of the population was sufficient at levels ≥ 50 nmol/L (20ng/ml) (99). Current guidelines provided by the National Institute of Health cite these Institute of Medicine findings, concluding that persons are at risk of vitamin D deficiency with serum 25(OH)D concentrations of <30 nmol/L (<12 ng/ml), are potentially at risk for inadequacy with levels ranging from 30–50 nmol/L (12–20 ng/ml), are sufficient if levels are ≥ 50 nmol/L (≥ 20 ng/ml), and may experience adverse effects with serum concentrations >125 nmol/L (>50 ng/ml) (100). Additional published papers cite much higher reference ranges recommended for optimal health, noting that many cutoff points are not backed by scientific consensus and require further research to determine optimal dosing strategies.

Normal reference ranges provided by commercial laboratories for serum concentrations of 25(OH)D in animals have a wide distribution, are laboratory specific, and may

not reflect optimal levels. Michigan State University's College of Veterinary Medicine has established a reference range based on radioimmunoassay, citing 109–423 nmol/L (44–169 ng/ml) as a general indication of adequate to normal vitamin D levels, an increase from the 60–215 nmol/L (24–86 ng/ml) level listed as normal in 2014 (101). The Veterinary Diagnostic Institute, which uses chemiluminescence immunoassay, reports test results as *deficient* (<100 nmol/L [<40 ng/ml]), *insufficient* (100–248 nmol/L [40 to 99 ng/ml]), *sufficient* (250–375 nmol/L [100–150 ng/ml]), or *elevated* (>500 nmol/L [>200 ng/ml]). Selting et al (2016) used suppression of PTH secretion to define the sufficiency level of 25(OH)D in dogs representing both healthy and disease groups. Dogs in the disease group were those with an acute hemoabdomen, the majority of which were caused by hemangiosarcoma, along with other malignancies or benign changes (43 malignant, 20 benign). Additional measurements, such as CRP, calcium, and phosphorus, were also used as markers of sufficiency, without a complete explanation of the reasoning behind these choices. Variation in both PTH and CRP plateaued at 25(OH)D serum concentrations of 100–120 ng/ml, concluding that sufficiency could be defined at this level. An increased relative risk of cancer was also established with a serum 25(OH)D concentration below 40 ng/ml. Criticisms of this paper include the lack of measurement of vitamin D intake in any of the participating animals as well as lack of bloodwork, urinalysis, or medical imaging to ensure the health of enrolled animals. Further research was recommended before any consensus statements are made regarding serum 25(OH)D concentrations that define sufficiency in dogs (102). Currently, no published research exists defining similar results in cats.

The National Research Council, the Association of American Feed Control Officers (AAFCO), and the European Pet Food Industry Federation have all developed nutritional guidelines for the dietary levels of vitamin D needed to maintain health. The minimum adequate intake, minimum recommended allowance, and safe upper limit of vitamin D are readily reported through these organizations; however, they were developed with the primary endpoints of preventing rickets and investigating toxicity, mostly in young, growing animals. Serum levels of 25(OH)D were not measured. Information regarding the efficiency of different forms of vitamin D, the vitamin D requirements of adult dogs, the relationships between intake and vitamin D

status, and factors that affect these relationships are all areas of limitations to our current knowledge (102).

Supplemental vitamin D is expressed in measurements of IUs. A single IU of vitamin D can be provided by 0.025mcg of cholecalciferol. The richest natural sources of vitamin D in foodstuffs are marine fish and fish oils. The most common synthetic sources of vitamin D in pet foods are variously documented as cholecalciferol (D-activated animal sterol), vitamin D3 supplement, ergocalciferol (D-activated plant sterol), and vitamin D2 supplement. Moist foods generally contain higher amounts of vitamin D than extruded foods, and some moist foods exceeded the AAFCO maximal recommended allowance of 10,000 IU/kg for cats (17). Multiple recent recalls of canine diets due to vitamin D toxicity in 2018–2019 were widely publicized, with misformulation of vitamin premixes believed to be the underlying cause (103). Previous pet food recalls due to vitamin D toxicity were also reported in 1999, 2006, 2010, and 2016 for similar reasons. Homemade diets are most often deficient in vitamin D (24). Accepted dosing for supplemental oral cholecalciferol has not been established, and to the author's knowledge only a single paper exists describing the use of oral cholecalciferol for the treatment of pathology in companion animals. In a study of atopic dogs, patients received either placebo (palm oil), cholecalciferol at 300 IU/kg, or the VDR agonist paricalcitol orally at 0.02 μ g/kg SID for 8 weeks. The dosage was increased every 2 weeks based on the patient's blood calcium concentrations and the severity of clinical signs, from approximately 300 IU/kg to 1400 IU/kg cholecalciferol and from 0.02 μ g/kg to 0.1 μ g/kg paricalcitol by week 4. Over the study period of 8 weeks, 25(OH)D levels showed a mean increase of 80 μ g/ml (250%) in the cholecalciferol group, while in the paricalcitol group 25(OH)D concentrations increased by an average of 36 μ g/ml (105%). The placebo group showed decreased 25(OH)D levels by an average of 14 μ g/ml (–20%). The authors concluded that there was effective absorption of the supplemental cholecalciferol with preference over the VDR agonist (10). They cited a murine model of renal fibrosis as the basis of their starting dose (104). Additional research is needed to determine optimal dosing for oral cholecalciferol. To date, no clinical trials have investigated its use in veterinary oncology patients.

Summary

Vitamin D is a steroid hormone traditionally recognized as being responsible for calcium and phosphorus homeostasis

in the body; however, it is widely accepted that vitamin D also exerts extraskeletal effects, including those in relation to the prevention and treatment of cancer. Vitamin D receptors are present in over 2000 genes and various tissues throughout the body and allow for a vast array of genomic and nongenomic effects across various physiologic systems. Vitamin D can exert powerful chemopreventive, anti-inflammatory, and anti-cancer activity through various mechanisms influencing proliferation, apoptosis, angiogenesis, and metastasis, as well as synergism with conventional anticancer treatments. Definitive ranges of serum vitamin D deficiency, insufficiency, and sufficiency are not only elusive in human medicine but are even less researched in veterinary medicine and oncology, making it difficult to develop

recommendations for health and disease. A single study has made recommendations for sufficiency in dogs of serum vitamin D levels of 100-120ng/ml. Additional clinical trials are needed to confirm these findings as well as optimal dosing strategies to prevent toxicity and maintain adequate therapeutic levels. Nutritional guidelines for dietary vitamin D are readily available; however, studies are based on growing animals without correlating serum vitamin D levels. Dosing schedules for oral supplementation have not been determined, and further research will be needed to fill the gaps of limitations in our knowledge. With the recent interest and ongoing research in the area of vitamin D, the vital role of this hormone in oncology patients and beyond will no doubt be further revealed.

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