ESSAY

Beyond the skeleton: the role of vitamin D in companion animal health

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While the role of vitamin D in the maintenance of skeletal health has been well-established for many years, the discovery that many non-skeletal tissues express the vitamin D receptor stimulated renewed interest in vitamin D and its wider physiological roles. Subsequently, a vast literature has emerged over the past three decades which has linked vitamin D deficiency to the development of many human diseases including cancer, autoimmune, infectious and cardiovascular disorders. In contrast, the role vitamin D plays in the physiology of non-skeletal tissues in cats and dogs has received little attention. The situation is now starting to change with the publication of several studies that have indicated that vitamin D metabolism is deranged in numerous companion animal disorders. This article reviews the biology of vitamin D in companion animals and highlights some of the recent studies which have advanced understanding of vitamin D homeostasis in cats and dogs. Finally, the essay discusses how a "One Health" approach could further the understanding of vitamin D metabolism in mammals. Investigating vitamin D homoeostasis in companion animals offers many advantages compared to human studies in which vitamin D status is influenced by many more variables.

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VITAMIN D – AN OVERVIEW

The importance of vitamin D in calcium homoeostasis and in the maintenance of skeletal health has been known for nearly a century (Mellanby 1976, Elder & Bishop 2014). However, the subsequent discovery of the vitamin D receptor (VDR) on human leukocytes over 30 years ago raised the possibility that vitamin D may influence physiological process beyond the skeleton (Provvedini *et al.* 1983). In the proceeding three decades, a large number of studies have implicated vitamin D in the development and progression of a wide range of non-skeletal human diseases (Holick 2007). Numerous studies have also linked mutations of genes involved in the metabolism of vitamin D to the development of several human disorders (Cooper *et al.* 2011, Zhuang *et al.* 2015). There is also increasing evidence that low vitamin D status is predictive of outcome in many human diseases including all-cause mortality (Schottker *et al.* 2014). Numerous experimental studies have demonstrated that vitamin D can have a profound impact on the differentiation and phenotype of many non-skeletal cell types (Mora *et al.* 2008b, Baeke *et al.* 2010, Besusso *et al.* 2015). The discovery that vitamin D can influence a wide range of physiological processes also provides an attractive mechanistic link between the well-established relationship between latitude and disease development. For example, human diseases such as multiple sclerosis and inflammatory bowel disease have a higher prevalence in temperate regions with low exposure to vitamin D-inducing ultraviolet radiation compared to equatorial regions (Simpson *et al.* 2011, Khalili *et al.* 2012). Collectively, these studies have resulted in the widespread practice of vitamin D supplementation in humans, which has recently been estimated to be worth $600 million per annum in the USA alone (Kupferschmidt 2012).

In contrast, the non-skeletal effects of vitamin D have, until recently, received little attention in companion animals. The aim of this essay is to highlight some of the recent studies which have explored the relationship between vitamin D and non-skeletal

health outcomes in dogs and cats; the role of vitamin D on skeletal health in cats and dogs has been reviewed elsewhere (Dittmer & Thompson 2011, Cline 2012). This essay also discusses areas for future research in vitamin D metabolism in companion animals. This is important not only to ensure optimal animal health outcomes for companion animals but also because cats and dogs have the potential to be valuable models in which vitamin D biology can be probed.

Vitamin D metabolism

Dogs and cats can obtain vitamin D from ingestion of vitamin D_2 or D_3 in the diet. Vitamin D_2 is present in some plants following the conversion of ergosterol to vitamin D_2 by ultraviolet radiation. Vitamin D_3 is found in high concentrations in oily fish such as sardines and salmon, egg yolks and liver (Elder & Bishop 2014). In many mammals vitamin D_3 can also be produced in the skin from the isomerisation of 7-dehydrocholesterol by ultraviolet radiation. Humans, rats, sheep and cattle, but not cats and dogs, can produce vitamin D_3 in the skin (How *et al.* 1994a,b, 1995). The lack of vitamin D production in the skin of dogs explains the lack of seasonal variation in vitamin D status in Australian dogs (Laing *et al.* 1999). This is in marked contrast to humans, and other mammals that cutaneously produce vitamin D, which typically have a high vitamin D status in late summer and a nadir in late winter in temperate regions because of seasonal variation in ultraviolet radiation exposure (Smith & Wright 1984, Vanderschueren *et al.* 1991). Consequently, cats and dogs are reliant on dietary vitamin D.

Once vitamin D is absorbed, it preferentially binds to vitamin D-binding protein and is either stored in fat or is transported to the liver. The first hydroxylation of vitamin D takes place in the liver where 25 hydroxyvitamin D (25(OH)D) is produced. The half-life of 25(OH)D is approximately 2 weeks in humans and measurement of the serum concentration of 25(OH)D is widely used as an indicator of vitamin D status (Baeke *et al.* 2010). 25 hydroxyvitamin D then undergoes a second hydroxylation to produce 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) which is regarded as the most physiologically active vitamin D metabolite. This conversion of 25(OH)D to $1,25(OH)_2D$ is under the control of 1α-hydroxylase (CYP27B1) whose activity is regulated by parathyroid hormone, ionised calcium and $1,25(OH)_2D$ concentrations (de Brito Galvao *et al.* 2013). When ionised calcium concentrations are low, parathyroid hormone can directly stimulate renal 1α-hydroxylase production. High $1,25(OH)_2D$ concentration has a negative feedback effect, reducing activity of 1α-hydroxylase. 1,25 dihydroxyvitamin D is degraded by 24 hydroxylase (de Brito Galvao *et al.* 2013). A recent study has found that plasma concentrations of $24,25(OH)D_3$, an excretory metabolite of 25(OH)D, were higher in dogs than in humans, pigs and rodents indicating that the metabolism of 25(OH)D may be different between dogs and other species (Spoo *et al.* 2015). The nuclear VDR is the ligand-dependent transcription factor that mediates the action of its natural ligand $1,25(OH)_2D$ (Vanherwegen *et al.* 2015). The optimal serum concentration of 25(OH)D has not been precisely defined in companion animals. Vitamin D sufficiency is often defined in humans as the 25(OH)D concentration at which parathyroid hormone concentrations plateau in healthy individuals (Saliba *et al.* 2011). In healthy dogs, parathyroid hormone concentrations were found to plateau at 100 ng/mL (Selting *et al.* 2014).

The main targets for vitamin D are the intestine, bone, kidney and parathyroid glands (Elder & Bishop 2014). The principal function of vitamin D is to maintain ionised calcium and phosphate concentrations within a tight, physiologically appropriate range. In the small intestine $1,25(OH)_2D$ promotes active uptake and transcellular transport of calcium. 1,25 hydroxyvitamin D also promotes mobilisation of calcium from bones. In the kidney, $1,25(OH)_2D$ inhibits renal 1α-hydroxylase and stimulates 24-hydroxylase thereby reducing production and increasing catabolism of $1,25(OH)_2D$ (de Brito Galvao *et al.* 2013).

Vitamin D and gastrointestinal diseases

Because the gastrointestinal tract plays a key role in the maintenance of vitamin D sufficiency, numerous studies have examined the relationship between vitamin D and gastrointestinal inflammation in humans and in experimental models of inflammatory bowel disease (Lagishetty *et al.* 2010, Del Pinto *et al.* 2015). Dogs with a chronic enteropathy (CE) have lower serum concentrations of 25(OH)D than healthy dogs and hospitalised dogs with non-gastrointestinal illnesses (Mellanby *et al.* 2005a, Gow *et al.* 2011). Many of the dogs with a protein-losing enteropathy had profound hypovitaminosis D and concurrent ionised hypocalcaemia and secondary hyperparathyroidism (Gow *et al.* 2011). The severity of the clinical signs, as assessed by the canine inflammatory bowel activity index (Jergens *et al.* 2003), correlated with serum 25(OH)D concentrations (Gow *et al.* 2011). In addition, serum albumin concentrations also correlated with vitamin D status (Gow *et al.* 2011). Similarly, cats with either inflammatory bowel disease or intestinal small cell lymphoma have low serum concentrations of 25(OH)D (Lalor *et al.* 2014).

The mechanism(s) underlying the hypovitaminosis D state in companion animals with CE is unknown. Reduced dietary intake of vitamin D in dogs maybe an important cause of hypovitaminosis D especially because dogs and cats do not cutaneously produce vitamin D (How *et al.* 1994a). Because low vitamin D status in dogs with CE has been associated with decreased appetite (Gow *et al.* 2011), this may, in part, explain the low serum 25(OH)D concentrations that are frequently observed in dogs with CE. However, dogs with CE were found to have a lower 25(OH)D concentrations than hospitalised sick dogs with non-gastrointestinal disease, many of which also have reduced appetites (Gow *et al.* 2011). Consequently, the influence of appetite on vitamin D status in dogs with CE remains unclear. Other potential explanations include loss of serum vitamin D through the inflamed gastrointestinal mucosa or impaired absorption of oral vitamin D. The finding that dogs with lymphangiectasia often have ionised hypocalcaemia may result from poor intestinal absorption of fat soluble vitamin D when gastrointestinal lymphatic function is impaired (Kull *et al.* 2001). Although hypovitaminosis D in CE has traditionally been considered to be a result of intestinal disease, there is growing evidence that hypovitaminosis D may contribute to the initiation of intestinal inflammation

rather than just being a secondary consequence of the intestinal lesions. Supporting evidence for a link between hypovitaminosis D and CE comes from rodent models which have demonstrated that VDR deficient mice are more susceptible to experimental forms of inflammatory bowel disease (Froicu *et al.* 2003). Additionally, feeding mice a diet which is vitamin D depleted predisposes to the development of colitis (Lagishetty *et al.* 2010).

Vitamin D and kidney diseases

Because the active vitamin D metabolite $1,25(OH)_2D$ is produced in the kidney, numerous studies have investigated vitamin D metabolism in cats and dogs with renal disease (de Brito Galvao *et al.* 2013). Damage to renal tubules results in reduction of 1α-hydroxylase activity, which, together with increased retention of phosphorus, leads to a decline in ionised calcium and an increase in parathyroid hormone concentrations. Fibroblast growth factor-23 increases in response to the increase in phosphorus which causes a further decline in the generation of $1,25(OH)_2D$. Renal loss of vitamin D-binding protein may also be an important cause of low vitamin D status in dogs and cats with kidney disorders. Concentrations of both 25(OH)D and $1,25(OH)_2D$ were lower in dogs with acute or chronic renal failure compared to healthy control dogs (Gerber *et al.* 2003). The role of vitamin D therapy in canine and feline renal diseases remains ill-defined (Hostutler *et al.* 2006, Roudebush *et al.* 2010).

Vitamin D and cardiac diseases

Vitamin D deficiency has been associated with a range of human cardiovascular diseases including myocardial infarction and sudden cardiac death (Wang *et al.* 2008, Kendrick *et al.* 2009). Low vitamin D status is potentially relevant in the pathogenesis of cardiovascular disease because $1,25(OH)_2D$ promotes cardiac contractility and has an anti-hypertrophic effect on cardiomyocytes (Green *et al.* 2006, Weber *et al.* 2009). Mean 25(OH)D concentrations were significantly lower in dogs with congestive heart failure compared to unaffected dogs although, importantly, mean calculated vitamin D intake per kg of metabolic body weight in dogs with congestive heart failure was not significantly different from that of unaffected dogs (Kraus *et al.* 2014). Another study demonstrated that median 25(OH)D concentrations were significantly lower in dogs with chronic valvular heart disease stage B2 and C/D disease than dogs with stage B1 disease (Osuga *et al.* 2015).

Vitamin D and infectious diseases

Vitamin D deficiency has been linked to susceptibility to numerous infectious diseases in humans. For example, the role of vitamin D in the development and treatment of mycobacteria infections has been closely scrutinised not least because of the historical approach of treatment of infections with UV light exposure in solariums in the pre-antibiotic era (Coussens *et al.* 2012, Cassidy & Martineau 2014). There are now considerable data to link vitamin D and anti-mycobacteria immune responses, including the production of anti-bacterial molecules such as cathelicidins (Martineau *et al.* 2007). There is also evidence that vitamin D supplementation may improve the clinical response to treatment of mycobacterial infections (Coussens *et al.* 2012). A recent study found that cats with mycobacterial infections had lower serum 25(OH)D concentrations than healthy cats (Lalor *et al.* 2012).

Vitamin D and cancer

Low-serum 25(OH)D concentrations have been associated with an increased incidence of cancer in humans (Yin *et al.* 2013). Similarly, in dogs there has been epidemiological evidence linking vitamin D status and neoplasia. Labradors with mast cell tumours had significantly lower 25(OH)D concentrations than unaffected dogs. Importantly, the mean calculated vitamin D intake per kg body weight in Labradors with mast cell tumour was not statistically different from that of unaffected Labradors, implying that the difference in vitamin D status was not simply a result of different intake (Wakshlag *et al.* 2011). The low vitamin D status in dogs with neoplasia may be of significance since VDR is expressed on the majority of mast cell tumours (Russell *et al.* 2010). Furthermore, vitamin D metabolites have anti-proliferative effects on the growth of canine mastocytoma cells (Malone *et al.* 2010). High-dose $1,25(OH)_2D$ can even induce tumour regression, although there can also be side effects (Malone *et al.* 2010). 1,25 dihydroxyvitamin D has been shown to have in vitro anti cancerous effects on other canine cancer cell lines, with some evidence of in vivo efficacy (Kunakornsawat *et al.* 2002, Kaewsakhorn *et al.* 2005, Rassnick *et al.* 2008).

Vitamin D and inflammation

The discovery that many immune cell types express the VDR was critical in establishing that the role of vitamin D extended beyond the maintenance of skeletal health. Numerous studies have examined the relationship between the immune response and serum 25(OH)D concentrations in human patients. There is a growing body of evidence which demonstrates that vitamin D status is negatively associated with markers of inflammation, including circulating pro-inflammatory cytokines and acute phase proteins, in a number of diseases including obesity (Codoner-Franch *et al.* 2012, Bellia *et al.* 2013), inflammatory polyarthritis (Patel *et al.* 2007), diabetes mellitus (Shih *et al.* 2014), autoimmune diseases (Robinson *et al.* 2014), inflammatory bowel disease (Garg *et al.* 2013), human immunodeficiency virus (Poudel-Tandukar *et al.* 2013). Furthermore, low vitamin D status has been associated with increased markers of inflammation in healthy humans (Peterson & Heffernan 2008, De Vita *et al.* 2014, Laird *et al.* 2014).

The reasons why vitamin D status is negatively associated with inflammation is unclear. The VDR is found on most immune cells including macrophages, dendritic cells, T-lymphocytes and B-lymphocytes (Prietl *et al.* 2013). Vitamin D can promote immune tolerance by increasing regulatory T cell populations (Mellanby *et al.* 2009, Chambers *et al.* 2014), inhibiting the production of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines (Boonstra *et al.* 2001, Martineau *et al.* 2007, Khoo *et al.* 2011, Korf *et al.* 2012, Harishankar *et al.* 2014, Jeong *et al.* 2014, Nissou *et al.* 2014). Vitamin D

is also known to enhance the innate immune response to bacteria (Wang *et al*. 2004, Martineau *et al*. 2007).

In recent years, a number of studies have examined the relationship between vitamin D status and inflammation in companion animals. Serum 25(OH)D concentrations are commonly reduced in a number of inflammatory diseases in dogs including congestive heart failure (Kraus *et al*. 2014), *Spirocerca lupi* infections (Rosa *et al*. 2013), protein-losing enteropathy (Mellanby *et al*. 2005a, Gow *et al*. 2011) and renal disease (Gerber *et al*. 2003). Low vitamin D status has been negatively associated with C-reactive protein in healthy dogs (Selting *et al*. 2014). In addition, a recent study which explored the relationship between vitamin D status and inflammation in dogs with CE found a negative relationship between serum 25(OH)D concentrations and neutrophil and monocyte concentrations, serum IL-2 and IL-8 concentrations and duodenal inflammatory scores (Titmarsh *et al*. 2015c). However, other studies have reported a positive association between vitamin D and acute phase protein concentrations in racing sled dogs and racing greyhounds (Tharwat *et al*. 2014, Spoo *et al*. 2015). There is a need to further clarify whether vitamin D deficiency is a cause or consequence of inflammation in companion animals.

Importantly, inflammation can also be associated with hypercalcaemia resulting from dysregulated vitamin D metabolism (Boag *et al*. 2005, Crews *et al*. 2007). The aberrant production of $1,25(OH)_2D$ by macrophages has long been recognised as a cause of hypercalcaemia in humans with granulomatous diseases (Sharma 2000). Similarly, hypercalcaemia has been reported in dogs with several chronic inflammatory disorders. In one dog with granulomatous lymphadenitis, high serum concentrations of $1,25(OH)_2D$ and low parathyroid hormone concentrations were reported; calcium and $1,25(OH)_2D$ concentrations returned to normal following resolution of the lymphadenitis (Mellanby *et al*. 2006). Finally, vitamin D status may influence the response to anti-inflammatory treatment. A role for vitamin D in correcting unresponsiveness to glucocorticoids in humans with asthma has been postulated (Xystrakis *et al*. 2006). In dogs with atopic dermatitis the response to glucocorticoids treatment was significantly better in animals with higher serum 25(OH)D concentrations (Kovalik *et al*. 2012).

Vitamin D and mortality

Numerous studies in humans have reported a relationship between mortality and low serum 25(OH)D concentrations (Ginde *et al*. 2009, Pilz *et al*. 2012). Indeed, a recent meta-analysis which investigated the relationship between vitamin D and mortality reported "*Despite levels of 25(OH)D strongly varying with country, sex, and season, the association between 25(OH)D level and all-cause and cause-specific mortality was remarkably consistent*" (Schottker *et al*. 2014).

A recent study on all-cause mortality in cats found that cats with low serum 25(OH)D concentrations had a significant higher risk of mortality (Titmarsh *et al*. 2015b). The study evaluated the ability of a range of clinical and biochemical data including sex, age, breed, appetite, total white blood cells, packed cell volume and serum concentrations of albumin, total calcium, creatinine, sodium, potassium and 25(OH)D concentration to predict mortality by 30 days. Serum 25(OH)D concentrations were significantly lower in cats that died compared to ones that survived in a univariable analysis. However, when serum 25(OH)D concentrations was used as a linear predictor of survival within a logistic regression model, none of the variables, including 25(OH)D concentration, were significant predictors of mortality. When serum 25(OH)D concentrations were split into a categorical variables of low, medium and high vitamin D status which each contained a third of the cats in the study, cats with a 25(OH)D concentration in the lower tertile had an increased risk of mortality compared to cats in the middle tertile reference category. There was no significant difference in survival between cats in the upper and middle tertile.

A central finding in this study was not just that low vitamin D status was predictive of poor outcome but that there was not a linear relationship between vitamin D status and survival. This observation is also consistent with studies in human patients in which several studies have reported minimal benefit of having high serum 25(OH)D concentrations and a number have linked high vitamin D status to negative health outcomes (Melamed *et al*. 2008, Durup *et al*. 2012, Amrein *et al*. 2014). Low vitamin D status has also been linked with a poor clinical outcome in dogs with CE (Titmarsh *et al*. 2015a). Furthermore, dogs with congestive heart failure and low serum 25(OH)D concentrations had a significantly shorter time period to the development of clinical manifestation of heart failure or sudden death compared to dogs with congestive heart failure and higher vitamin D status (Kraus *et al*. 2014).

FUTURE DIRECTIONS

There is a rapidly expanding literature on vitamin D metabolism and a wide range of diseases in both companion animals and humans. The challenge is now to clarify whether vitamin D is causally linked to the initiation, development and outcome of numerous non-skeletal disorders or whether it is simply a marker of ill-health. Numerous studies are ongoing in healthy humans and patients with a range of diseases which aims to more precisely define the role of vitamin D in the aetiology of important causes of mortality (Kupferschmidt 2012). Furthermore, ongoing studies aim to establish whether there is a role for vitamin D supplementation in the treatment of a range of infectious, autoimmune and neoplastic conditions (Kupferschmidt 2012). Similar studies are now required in companion animals to enable the role of vitamin D in the development and treatment of important diseases of dogs and cats to be clarified. The need to avoid over-supplementation with vitamin D is clear in light of the numerous reports of hypercalcaemia caused by the inadvertent over-supplementation of companion animal diets with excessive amounts of vitamin D (Mellanby *et al*. 2005b, Wehner *et al*. 2013).

Importantly, it may be easier to clarify the role of vitamin D in health and disease in companion animals than in humans. Humans can readily modify their vitamin D status simply by altering their exposure to UV radiation either through moving

latitude or altered behaviour such as avoiding exposure to UV radiation. Since dogs and cats cannot produce vitamin D cutaneously, sunlight exposure does not influence their vitamin D status. In addition, the amounts of vitamin D in the diets of humans is more variable rather than the more standardised amounts of vitamin D added to the proprietary diets that are consumed by most companion animals. Consequently, additional research on vitamin D biology in cats and dogs could make a significant contribution to our understanding of the role vitamin D plays in non-skeletal disease in both animals and humans, particularly in establishing whether the health benefits of exposure to ultraviolet radiation are mediated by vitamin D or non-vitamin D pathways.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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