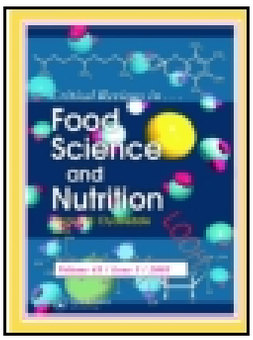

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Vitamin D in dogs

CURRENT KNOWLEDGE OF VITAMIN D IN DOGS

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Abstract

There is emerging interest in linking vitamin D status to physiological health and disease states in the dog, as evidenced by the recent increase in publications in this area. This research has most likely been spurred by the studies exploring vitamin D and disease in humans. However, there are important differences in vitamin D intake and metabolism between humans and dogs that should be accounted for. The understanding of basic vitamin D metabolism and the relationship between vitamin D intake and vitamin D status in dogs remains even more limited than current knowledge in humans. This review will summarize current knowledge of vitamin D in the dog, including metabolism and dietary recommendations. Emphasis is placed on the

limitations to current knowledge. Studies investigating links between vitamin D and disease will be discussed in light of this knowledge. Suggestions for future research, including the development of reference ranges to define blood vitamin D sufficiency,are provided.

Keywords

25(OH)D, Disease, Canine, Cholecalciferol, Ergocalciferol,Dietary Requirements,

Introduction

Vitamin D, historically well known for its role in calcium and phosphorus regulation, has become a hot and controversial topic in human medicine as evidence for the vitamin's involvement in extra-skeletal health continues to expand. Interest in vitamin D has recently translated to the veterinary world, with researchers linking vitamin D to several diseases in dogs. Although still in its infancy, this research is exciting, as evidence indicates that vitamin D may be influential in: reducing disease risk,increasing treatment efficacy and improving disease outcome, and may also have use as a biomarker of disease prognosis.

There are several main foci of vitamin D research in humans: correlation of vitamin D intake with disease (Munger et al, 2004, Annweiler et al, 2012); correlation of the marker of vitamin D status, 25(OH)D (Holick, 1995), with disease (Ananthakrishnan et al, 2012); mechanisms of action of the most biologically active metabolite, 1,25(OH)2D, in target cells/tissues (Feldman et al, 2014); and the relationship between vitamin D intake and vitamin D status (Ross et al, 2011). Each area of research is equally important to a full understanding of vitamin D's potential roles in disease pathophysiology. However, understanding the relationship between vitamin D intake and vitamin D status allows results to be translated into meaningful real world applications, such as the development of vitamin D intake recommendations by government organizations. The Institute of Medicine released a comprehensive review of vitamin D literature in 2011 (Ross et al, 2011) that highlighted the many limitations to the knowledge of the relationship between vitamin D intake and vitamin D status, including effects of factors like age, body composition and genetics. These limitations cover the knowledge of the relationship in humans only.

The same areas of vitamin D research in humans must be mirrored in dogs to effectively understand vitamin D's role in canine disease physiology. As with humans, understanding of the relationship between vitamin D intake and vitamin D status is essential to translate results into real world applications. In dogs, these real world applications include the vitamin D requirements for dog foods set by the National Research Council (NRC), the American Association of Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF). However, as "non-traditional" vitamin D research in extra-skeletal disease in dogs is still in its infancy, some areas of vitamin D research have been overlooked. In particular, the relationship between vitamin D intake and vitamin D status in adult dogs is virtually unknown. Currently, dogs receiving an AAFCO compliant dog food for adult maintenance may receive anywhere from 500-5000 IU vitamin D/kg DM (AAFCO, 2014), or 552-3200 IU vitamin D/kg DM according to FEDIAF guidelines (FEDIAF, 2014), depending on the level of vitamin D the manufacturer has chosen to include. As a result, there may be large variation in vitamin D intakes between dogs. Many manufacturers report only the amount of vitamin D added to the diet as a premix, and do not account for the endogenous vitamin D content of ingredients used. As a result, the vitamin D content of the final product reported by the manufacturer may be inaccurate. These are important considerations for researchers choosing to enroll client-owned animals as study participants.

This review will summarize current knowledge of the relationship between vitamin D intake and vitamin D status in dogs, and discuss recent studies focused on vitamin D and disease in dogs in light of this knowledge. The goal of this literature review is not to discount current work examining vitamin D and disease in dogs, but to articulate a strong argument for basic

vitamin D research in dogs, especially research focused on the relationship between vitamin D intake and vitamin D status, and to emphasize the need to include vitamin D intake as a study variable.

Metabolism

There are two forms of vitamin D. Vitamin D2, also known as ergocalciferol, is usually the form created by plants. Vitamin D3, also known as cholecalciferol, is the form created in the skin of humans during exposure to UV light (Holick et al, 2007). Humans may also ingest vitamin D through diet, which could be in the form of D2 or D3. Both forms are utilized in the body, however evidence in humans suggests that metabolites from D2 are much less potent than those from D3 (Trang et al, 1998). Vitamin D is stored predominantly in adipose tissue, but can also be found in other tissues, such as muscle (Heaney et al, 2009). After ingestion or skin production, vitamin D is transported to the liver by carrier proteins, specifically vitamin D binding protein. In the liver, Vitamin D undergoes transformation by cytochrome P450 enzymes (i.e. cytochrome P450 27A1) and becomes 25-hydroxyvitamin D (25(OH)D).

25(OH)D is one of the most stable metabolites of vitamin D, with a half-life estimated to range from 10 days to 3 weeks (Mawer et al, 1971, Vicchio et al, 1993). Circulating 25(OH)D concentrations are reflective of vitamin D obtained from the diet and skin production, and have been generally accepted as a marker of vitamin D status (Holick, 1995). 25(OH)D concentration is also the marker used for associations between vitamin D status and disease status, i.e. low concentrations of 25(OH)D being associated with increased risk of colorectal cancer in humans (Feskanich et al, 2004). 25(OH)D also serves as the precursor molecule to the most biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D).

Primary production of 1,25(OH)2D takes place in the proximal tubules of the kidneys by action of the enzyme cytochrome P450 27B1, but also occurs in many other tissues or cell types. Parathyroid hormone and the concentration of blood calcium, phosphorus and 1,25(OH)2D itself, tightly regulate renal production of 1,25(OH)2D. This regulation, combined with a short half-life of 4-6 hours (Kumar, 1986), and blood concentrations of only 1/1000th those of 25(OH)D (Holick, 2009), make 1,25(OH)2D less useful as a marker of vitamin D status (Holick, 2009). 1,25(OH)2D controls the body's calcium and phosphorus levels by increasing intestinal absorption and stimulating mobilization from bones.

Cytochrome P450 27B1 has also been found in extra-renal locations, such as skin, colorectal and pancreatic tissues, (Zehnder et al, 2001, Friedrich et al, 2006), suggesting 1,25(OH)2D production also occurs in these tissues. This local production is hypothesized to be an important regulator of cell growth (Fleet et al, 2012), which provides evidence for the proposed extra-skeletal and potentially therapeutic roles of vitamin D in target tissues.

Canine vitamin D requirements

Vitamin D is produced in the skin of most mammals when 7-dehydrocholesterol is exposed to UV light and forms pre-vitamin D (Holick et al, 1980). Pre-vitamin D undergoes thermal conversion to vitamin D3 (Holick et al, 1980). Thus, studies in humans must account for vitamin D obtained from skin production in addition to that obtained from the diet. In dogs, however, evidence suggests that UV mediated production of vitamin D is essentially insignificant (Wheatley and Sher, 1961, Griffiths and Fairney, 1988, Hazewinkel et al, 1987, How et al, 1994), meaning only dietary intake should be accounted for (Figure 1).

Wheatley and Sher (1961) found high amounts of cholesterol in lipid extracts of dog skin, but no

intermediate products of cholesterol synthesis, such as the vitamin D precursor, 7-dehydrocholesterol. The absence of 7-dehydrocholesterol formed the basis of the hypothesis that dogs have lost the ability to produce vitamin D, and rely on dietary intake of the vitamin. Building upon the work of Wheatley and Sher (1961), How et al (1994) found very low concentrations of 7-dehydrocholesterol present in dog skin. The 7-dehydrocholesterol that was present showed minimal UV mediated conversion to vitamin D, especially when compared to conversion rates in rat skin. *In vivo* studies have corroborated these findings. Researchers investigating the vitamin D status of huskies in polar latitudes observed an inverse relationship between UVB radiation from the sun and the huskies' serum 25(OH)D concentrations, instead of the expected positive association (Griffiths and Fairney, 1988).Serum 25(OH)D concentrations were more aptly explained by the huskies' dietary vitamin D intake. Hazewinkel et al (1987) fed puppies a diet containing no supplemental vitamin D. Puppies developed rickets, and this was not prevented by exposure to UVB light. These studies represent the most commonly cited evidence for limited cutaneous vitamin D production in dogs.

Further work is necessary to conclusively say that epithelial vitamin D production is insignificant in dogs. One study has shown that puppies could achieve healthy growth rates when fed a diet with no supplemental vitamin D (Kealy et al, 1991), but the basal vitamin D content of the diet is unclear. Additionally, the calcium and phosphorus levels in the diet were well above respective requirements. The vitamin D content may have been more important if levels of these minerals had been lower. The interaction between these nutrients is often overlooked, but remains important. Up-regulated activity of theenzyme7-dehydrocholestrol-Δ7-reductase, responsible for the conversion of 7-dehydrocholesterol into cholesterol, is thought to explain thelimited

epithelial vitamin D production in cats(Morris et al, 1999). This work has not yet been completed in dogs. Still, NRC (2006),AAFCO (2014), and FEDIAF (2014)have classified vitamin D as an essential dietary nutrient for dogs. Further work, such as the study done in cats (Morris et al, 1999), would help establish the extent of vitamin D production in canine skin.

Current vitamin D requirements

The NRC, AAFCO and FEDIAF have developed nutritional guidelines for the dietary level of vitamin D needed to maintain health. The minimum adequate intake, minimum recommended allowance and safe upper limit of vitamin D for the NRC (2006), AAFCO (2014) and FEDIAF (2014) can be found in Table 1.The minimum adequate intake reflects the amount required by an animal receiving a purified diet, while the minimum recommended allowance reflects the amount required by an animal receiving a typical commercial pet food (NRC, 2006, AAFCO, 2014). AAFCO and FEDIAFcompliant dog food manufacturers can choose to include any level of vitamin D within the range of the AAFCO or FEDIAF nutrient profiles.

How were vitamin D recommendations developed?

The NRC develops vitamin D recommendations by reviewing published studies that have investigated the vitamin D requirements in dogs (Zicker, 2008). The absolute minimal vitamin D requirement of puppies remains unclear as similar studies produced conflicting results (Hazewinkel et al, 1987, Kealy et al, 1991). Both studies tested if commercial dog foods, without any added vitamin D supplementation, may contain adequate vitamin D for growing dogs. Puppies were raised on either a diet with no added vitamin D and the same diet with an added 1800 IU vitamin D/kg diet (Hazewinkel et al, 1987), or a diet with no added vitamin D, and the same diet with an added 2420 IU vitamin D/kg diet (Kealy et al, 1991). Puppies fed the diet with

no added vitamin D in the first study (Hazewinkel et al, 1987) developed rickets, while puppies fed the diet with no added vitamin D in the second study (Kealy et al, 1991) showed no negative effects on skeletal health or selected serum parameters (e.g. total calcium). Unfortunately, no conclusions can be drawn about the minimum vitamin D requirements of dogs from either study, as except for the fact that no vitamin D supplement was added, the vitamin D content of the basal diets derived from the selected ingredients is unclear. The vitamin D content of the internal organs included in the diet used by Kealy et al (1991) was likely sufficient at the calcium and phosphorus concentrations used in that diet.

The minimum adequate intake suggested by the NRC (2006) (440 IU/kg DM) is somewhat supported by results from work by Tryfonidou et al (2002). Great Dane puppies were fed diets containing ~500 IU vitamin D/kg as fed, which equates to ~535 IU vitamin D/kg DM using the moisture value provided in the paper, and achieved normal growth. However, the energy density of the diets was not given, and may not have been equivalent to the energy densities of the AAFCO/NRC recommended diets. Therefore direct comparisons of the vitamin D content of the diet to AAFCO/NRC recommendations cannot be made.

Values that were 4 to 10 times the requirement were chosen as the safe upper limits in 1987 (NRC, 2006). This was adjusted in 2006, with a reference to a paper by Tryfonidou that showed impaired bone ossification with a level of vitamin D supplementation that was closer to "10 times the requirement". However, the referenced paper is not listed in the references of theNRC chapter. The diet that is referred to (~4,000 IU/kg as fed) describes one Tryfonidou et al study (2002), which showed depressed calcium absorption, but similar and healthy growth rates when compared with puppies receiving a diet containing ~500 IU/kg as fed.The impaired bone

ossification results match another Tryfonidou et al study (2003), which used a diet with a much higher vitamin D content (~54,000 IU/kg as fed). Atrue SUL, supported by clear scientific evidence for reproduction/growth and for adult maintenance, is not currently known in dogs. This knowledge, and an understanding of vitamin D metabolism in adult dogs, is urgently needed in light of the increased interest in vitamin D supplementation for canine health.

Limitations to current knowledge

The latest publication released by the NRC (2006) acknowledges that no definitive conclusions can be made on the vitamin D requirement of dogs based on the literature available for review at that time. Some of the major limitations to current knowledge are highlighted in this publication (NRC, 2006) and include: 1) Efficiency with which different forms of vitamin D may be used by the dog; 2) Knowledge of the vitamin D requirements of adult dogs; and 3) Relationship between vitamin D intake and vitamin D status, including factors that may affect this relationship.

1) Efficiency with which different forms of vitamin D may be used by the dog

Although not directly relevant to the purpose of this review paper, this limitation will be briefly discussed. There are 2 forms of vitamin D. Vitamin D2, also known as ergocalciferol, is usually created by plants. Vitamin D3, also known as cholecalciferol, is the form created in the skin of most mammals during exposure to UV light (Holick et al, 2007). Thus, vitamin D ingested through diet may be in the form of D2 or D3, depending on the ingredient source. Both forms are utilized in the human body, however evidence suggests that metabolites from D2 are much less potent than those from D3 in humans (Trang et al, 1998). Previous NRC publications (NRC, 1953, NRC,1985) have stated that vitamin D2 is used as efficiently as vitamin D3 by dogs, with reference to work by C.A. Elvehjem (Arnold and Elvehjem, 1939, Michaud and Elvehjem,

1944). However, the latest publication (NRC, 2006) states that no studies examining the differences in forms could be found in dogs, so NRC (2006) recommendations exist only for vitamin D3. This has important implications for pet food companies choosing to use vitamin D2 as the vitamin D supplement, i.e. for use in "vegan" diets. The possibility that pet food companies have conducted their own research into vitamin D2 requirements cannot be discounted, however the proprietary nature of this research results in no gain in knowledge to the scientific community. An abstract presented at the American Association for Veterinary Nutrition 2015 Symposium, confirmed the 1939 findings,showing that two adult dogs fed homemade diets supplemented with vitamin D2 maintained normal serum ionized calcium (ICa) and 25(OH)D levels (Delaney, 2015). This suggests a similar potency between D2 and D3, however larger-scale studies would be helpful to fully establish this.

2) Knowledge of the vitamin D requirements of adult dogs

Studiesexamining vitamin D requirements could only be found for puppies, sothe NRC (2006) chose to recommend the same requirements for adult maintenance as for growth and reproduction (Table 1). The same approach (Table 1) was taken by AAFCO (2014). Despite the acknowledgement that no studies of the vitamin D requirements of adult dogs could be found, the NRC (2006) states that mature dogs are relatively resistant to a dietary deficiency of vitamin D. This is dismissive to the need for basic vitamin D research in adult dogs, yet may be due to the timing of the publication (2006). Evidence for the multiple roles that vitamin D may play in an adult dog's health (Gow et al, 2011, Holowaychuk et al, 2012, Kraus et al, 2014), has since been published. As this research continues to grow, it is essential to know as much as possible about the requirements of vitamin D for every life stage, and particularly mature dogs, that have

increased riskof developing the very conditions that vitamin D has been associated with (Merlo et al, 2008, Wakshlag et al, 2011).

3) Relationship between vitamin D intake and vitamin D status, including factors that may affect this relationship

Although some studies referenced by the NRC measure both 25(OH)D concentrations and vitamin D intake (Hazewinkel et al, 1987, Tryfonidou et al, 2002), no work has been done to determine the relationship between the two. A recent study (Sharp et al, 2015) examined serum 25(OH)D concentrations of dogs fed various commercial and homemade diets. Serum 25(OH)D concentrations were significantly different among dogs fed diets from different manufacturers, and there was a large range of serum 25(OH)D in dogs receiving homemade diets. However, the vitamin D levels of each diet were not measured, so no conclusions could be drawn about the impact of vitamin D intake on serum 25(OH)D concentrations.Clinicians often measure a dog's serum 25(OH)D status, using the reference range provided by the laboratory as an indicator of the dog's health. For example, the often cited reference range for serum 25(OH)D concentration in dogs comes from the Michigan State University's Diagnostic Center for Population and Animal Health and is 60-215 nmol/L (Nachreiner et al, 2014). The use of serum 25(OH)D as a marker is now being extended to a variety of disease states in the dog, such as chronic kidney disease (Galler et al, 2012), primary hyperparathyroidism (Gerber et al, 2004), inflammatory bowel disease (Gow et al, 2011), induced endotoxemia (Holowaychuk et al, 2012), and cancer (Selting et al, 2014). However, many of these researchers (Gerber et al, 2004, Galler et al, 2012, Selting et al, 2014) failed to measure the vitamin D intake of participating animals. If the relationship between dietary vitamin D intake and serum 25(OH)D concentration is not

established, then the ability of dietary vitamin D intake to prevent or alleviate disease cannot be determined. This knowledge is essential for the development of appropriate vitamin D intake recommendations, as well as for translating the results of vitamin D – disease research into clinical applications. Potential factors to affect the relationship between vitamin D intake and serum 25(OH)D (e.g. age, body condition score, breed, genetic variation) must also be taken into consideration.

How much vitamin D does a typical dog receive?

Since AAFCO requirements range from 500-5000 IU vitamin D/kg DM, the amount of vitamin D that a dog fed an AAFCO compliant commercial diet will receive is dependent on the manufacturer's choice of inclusion level within this range. The same holds true for manufactures that follow the FEDIAF guidelines (552-3200 IU vitamin D/kg DM). As a result, dogs fed different diets (even among the same brand), may receive vastly different amounts of vitamin D (Kritikos et al, 2014, Sharp et al, 2015). Dogs receiving a homemade diet, if not formulated by a board-certified veterinary nutritionist (i.e. Diplomate of the American College of Veterinary Nutrition (ACVN) or of the European College of Veterinary and Comparative Nutrition (ECVCN)), may be fed any amount of vitamin D that the owner chooses to include or not include. However, since vitamin D is only present in a few food sources, homemade diets are often deficient according to NRC, AAFCO and FEDIAF standards (Remillard, 2008, Larsen et al, 2012, Stockman et al, 2013). Researchers must not assume that all dogs enrolled in a studyare receiving similar amounts of vitamin D, even if all dogs are receiving commercial diets, or all dogs are receiving home prepared diets. Moreover, as mentioned above, the vitamin D content of the final product reported by the manufacturer may be inaccurate.Researchers interested in

analyzing vitamin D intake should analyze feed samples for the most accurate measure of vitamin D content.

Dogs fed an AAFCO compliant commercial diet are likely receiving more dietary vitamin D than many humans, as dietary vitamin D intake, including supplement use, in humans is often reportedly lower than recommended levels (Moore et al, 2004, Calvo et al 2005). This has prompted mandatory food fortification measures in some countries, e.g. milk in Canada and the United States. However, studies suggest even this is not enough (Calvo et al, 2004, Calvo et al, 2005), especially for those with certain dietary habits, such as vegetarians or individuals with lactose intolerance. This is important to remember when comparing results from human studies and canine studies. Dogs may have a higher baseline vitamin D status as a result of higher dietary vitamin D intake, which may affect the relationship between the two, and other influential factors like parathyroid hormone (PTH) andICa.

Disease

Researchers are drawing on inspiration from human studies to investigate links between vitamin D and disease in dogs. In humans, evidence exists for a role of vitamin D in a range of health outcomes, including bone health, diabetes, cardiovascular disease, immune function and cancer(Holick, 2004, Hyppönen et al, 2001, Kamen and Tangpricha, 2010, Jenab et al, 2010).This is a novel area of research in dogs, and only a few diseases have been investigated. Focus has been placed on low vitamin D status as a potential risk factor for disease development, as a consequence of disease, and as an indicator of poor prognosis. Results may lead to improved

strategies for disease prevention and disease management. No studies linking vitamin D with survival outcomes could be found.

Bone Health

Rationale:

The latest committee formed by the Institute of Medicine in the United States chose bone health as the health outcome for development of the Dietary Reference Intakes for vitamin D (Ross et al, 2011). Although other health outcomes (e.g. cancer, cardiovascular disease) were considered, bone health was the only outcome with enough conclusiveevidence to support its use. Many of the studies referenced by the NRC (2006) for development of vitamin D intake recommendations in dogs also use bone health as a measure of adequate vitamin D intake. Vitamin D's role in bone health is well accepted, given its regulatory role in bone metabolism.

Studies:

Much of the work done on vitamin D and bone health in dogs was completed by researchers at the University of Utrecht, and has been covered in an earlier section of this review. Briefly, these studies include: the development of rickets in puppies fed a diet with no supplemental vitamin D, which resolved with feeding the same diet supplemented with vitamin D (Hazewinkel et al, 1987); normal growth rates and skeletal development in dogs fed diets containing AAFCO (2014) compliant vitamin D levels (Tryfonidou et al, 2002); and impaired endochondral ossification in puppies fed a diet containing approximately 10.8 times the maximum recommended intake set by AAFCO (2014) (Tryfonidou et al, 2003).

Chronic Kidney Disease

Rationale:

The kidneys play a central role in vitamin D metabolism as 1,25(OH)2D is produced in the proximal tubules by the enzymecytochrome P450 27B1. Chronic kidney disease (CKD) can impair this process (Al-Badr and Martin, 2008). Suboptimal 25(OH)D concentrations, the accepted marker of vitamin D status (Holick, 1995), are common in human CKD patients (Gonzalez et al, 2004, Ravani et al, 2009). Correcting this suboptimal status with supplemental vitamin D is recommended in human patients with CKD and concurrent hyperparathyroidism(Al-Badr and Martin, 2008, Holick, 2007).

Studies:

Plasma and serum 25(OH)D concentrationsare decreased in dogs with CKD when compared to healthy controls (Gerber et al, 2003, 2004, Galler et al, 2012). However, no conclusions have been reached specifically regarding vitamin D_2 or D_3 supplementation for these patients (Galler et al, 2012), however supplementation of the active form of vitamin D, 1,25(OH)2D,is used for treatment (Nagode et al, 1996, Roudebush et al, 2010). Though 1,25(OH)2D is not used as a marker of vitamin D status (Holick, 2009), measurement of 1,25(OH)2D is still useful in cases where production may be affected,as is likely with CKD, and a relationship between CKD and 1,25(OH)2D should be expected. Investigation of 1,25(OH)2D concentrations between dogs with CKD and healthy controls has yielded mixed results. Affected dogs had decreased serum 1,25(OH)2D concentrations in one study (Gerber et al, 2003) but no changes in another (Gerber et al, 2004). A relationship is more likely observed when 1,25(OH)2D concentrations arerelated to disease severity, than to the presence of disease, and this has been demonstrated inrecent work (Cortadellas et al, 2010).

Inflammatory Bowel Disease

Rationale:

Low vitamin D status is common in human patients with inflammatory bowel disease (IBD). Many hypotheses have attempted to explain this association, addressing a low vitamin D status as both a consequence of and a risk factor for IBD.

Interested readers should look to the review published by Garg et al (2012) for explanation of these hypotheses. Briefly, causes for low vitamin D status in human IBD patients include: mucosal disease or surgical resection causing malabsorption, reduced sunlight exposure, and/or reduced dietary intake. Other suggested hypotheses include: "leakage" of vitamin D through the gastrointestinal tract and reduced circulation of vitamin D metabolites (Pappa et al, 2006). Since a low vitamin D status may be observed prior to IBD diagnosis, Garg et al (2012) also summarize roles for vitamin D in IBD development and progression including: the maintenance of epithelial barrier, involvement in the innate immune response and the adaptive T-cell response, and genetic polymorphisms in vitamin D metabolism which may increase IBD risk.

Studies:

Case reports linking abnormal vitamin D metabolism to ionized hypocalcemia in dogs with protein-losing enteropathies (PLE) (Kimmel et al, 2000, Bush et al, 2001) lead researchers to investigate serum 25(OH)D in dogs with IBD, dogs with IBD andhypoalbuminemia (referred to as the PLE group), and healthy dogs (Gow et al, 2011). Only dogs with PLE had significantly decreased serum 25(OH)D concentrations when compared to dogs with IBD and healthy dogs, supporting the hypothesis that an increased loss of vitamin D through the gastrointestinal tract is responsible for lower 25(OH)D concentrations in patients with gastrointestinal disease.

Cardiovascular disease

Rationale:

Epidemiologic and mechanistic evidence support a role for vitamin D in cardiovascular disease. Current evidence indicates a decreased risk for cardiovascular disease with increasing plasma 25(OH)D (Wang et al, 2012). The shape of this relationship and the plasma 25(OH)D concentration where risk may plateau is still under debate(Wang et al, 2012, Melamed et al, 2008).Mechanisms behind this relationship have been reviewed and include improvement of insulin sensitivity, negative regulation of renin and anti-inflammatory activities (Judd and Tangpricha, 2009, Pilz et al, 2011).

Studies

Serum 25(OH)D concentrations were significantly lower in client-owned dogs with congestive heart failure (CHF) when compared to healthy dogs (Kraus et al, 2014). Researchers found no differences in vitamin D intake, when calculated per kg of metabolic body weight, between groups. Researchers also found lower serum 25(OH)D concentrations were associated withincreased risk of cardiovascular events, when events were defined as anyCHF-related medical complications, sudden death or adjustments made to cardiac medications for suspected CHF. Osuga et al (2015) investigated whether serum 25(OH)D concentrations were correlated with disease severity in client-owned dogs with chronic valvular heart disease (CVHD). Serum 25(OH)D concentrations were significantly lower in dogs with increased disease severity (Stage B2 and Stage C/D) than in dogs with stage B1 CVHD. There was a significant negative correlation between serum 25(OH)D and left atrial and ventricular size, suggesting vitamin D may be associated with the degree of cardiac remodeling.No comparisons were made to healthy dogs in this study, however median serum 25(OH)D concentration for stage B1 dogs (Osuga et

al, 2015) was much lower than those observed for healthy dogs in other studies (Wakshlag et al, 2011, Kraus et al, 2014), and falls below the often-cited Michigan State University's Diagnostic Center for Population and Animal Health's reference range of 60-215 nmol/L (Nachreiner et al, 2014). Still, these differences may be attributed to methods used for 25(OH)D measurement, as a commercially available enzyme-linked immunosorbent assay (ELISA) was used in the Osuga et al (2015) study, while radioimmunoassays were used in other studies (Wakshlag et al, 2011, Kraus et al, 2014) and by Michigan State.

Cancer

Rationale:

Studies have linked low vitamin D intake and low 25(OH)D concentrations [Holick, 1995]) to increased risk of human cancers, i.e. breast and colorectal cancer (Garland et al, 2007, Jenab et al, 2010). $1,25(OH)_2D$ shows a range of anticancer activities, such as the induction of cellular apoptosis and differentiation, inhibition of cellular proliferation, angiogenesis and metastasis, and enhancement of DNA repair (Fleet et al, 2012). The strength of the association between vitamin D and cancer seems dependent on cancer type. For instance, the World Cancer Research Fund released a meta-analysisconcluding there is suggestive evidence of a link between a low intake of foods containing vitamin D and the development of colorectal cancer (World Cancer Research Fund/American Institute for Cancer Research, 2011). The same organization released another meta-analysis concluding that the results of studies were too variable to support any relationship with vitamin Dand breast cancer risk (World Cancer Research Fund/American Institute for Cancer Research, 2010).

Studies:

The presence of vitamin D receptors has been shown in osteosarcoma and mast cell tumour tissue from dogs (Davies et al, 2012, Russel, 2010), and 1,25$(OH)_2$D induced apoptosis, differentiation, and/or reduced cell growth in canine osteosarcoma and mast cell tumour cell lines (Barroga et al, 1998, 2000, Rassnick, 2008). Selting et al (2014) reported that dogs with hemangiosarcoma had decreased vitamin D status when compared to healthy dogs. Dogs with neoplastic spirocercosis had significantly lower serum 25(OH)D concentrations compared to dogs with non-neoplastic spirocercosis and healthy dogs (Rosa et al,2013). Wakshlag et al (2011) measured vitamin D intake and serum 25(OH)D concentrations in healthy Labrador Retrievers, and those with mast cell tumours, and reported dogs with mast cell tumours had a significantly decreased serum 25(OH)D concentrations, but similar vitamin D intake when compared to healthy dogs. Two studies measured blood vitamin D metabolites in dogs with hypercalcemia (Rosol et al, 1992, Gerber et al, 2004). Dogs with lymphoma were one of the hypercalcemic groups in each study. Both studies reported high inter-individual variability of serum 1,25(OH)2D concentrations. Gerber et al (2004) included measurement of serum 25(OH)D and reported serum 25(OH)D to be significantly decreased in dogs with lymphoma when compared to healthy controls.

A recent study has explored the relationship between vitamin D intake and vitamin D status in healthy dogs and dogs with cancer, specifically lymphoma, osteosarcoma and mast cell tumours (Weidner et al, 2015). The effects of other variables (e.g. body condition score, ICa, PTH) were also investigated. Dietary vitamin D intake showed a relationship with vitamin D status, and this relationship was the same in all groups of dogs. There was also a relationship between 24,25(OH)2D and vitamin D status, which was the same in all groups. ICa and cancer

both had significant effects on vitamin D status, and interacted with each other to exert effects. Significant differences in vitamin D status between the healthy group and cancer groups were only observed at certain blood concentrations of ICa. Dietary vitamin D intake, plasma 24,25(OH)2D concentrations, plasma ICa concentrations and cancer impacted vitamin D status, further demonstrating the importance of accounting for multiple variables that may affect vitamin D status in experimental designs.

Implications for future research

These studies give researchers the beginnings of a structure on which to build future researchin this area. However, for a building to be sound, there must also be a sturdy foundation in place. A better understanding of the basic vitamin D knowledge, and its limitations, specific to dogs is essential to ensure appropriate experimental design of studies. Table 2 summarizes the aspects of vitamin D metabolism accounted for in the above studies, and illustrates that many studies do not account for the impact of vitamin D intake.Measurement of dietary vitamin D intake is important, as this contributes to the dog's vitamin D status (Hazewinkel, 1987), and because, as discussed above, vitamin D intake can be quite variable among client-owned animals. Additionally, even when vitamin D intake is measured,only one study on the relationship between vitamin D intake and blood 25(OH)D concentrations could be found (Weidner et al, 2015). This knowledge is essential for the translation of research associating blood 25(OH)D concentrations with reduction in disease risk and/or improvement in disease outcome.Without knowledge of the dietary vitamin D intake that correlates with the target blood 25(OH)D concentration, the vitamin D intake recommendations (by NRC/AAFCO/FEDIAF) and/or vitamin D supplementation recommendations (by clinicians) cannot reflect the results.

Sufficiency

The purpose of many vitamin D studies in humans is to define a blood 25(OH)D level sufficient to minimize disease risk to the majority of the population. There has been a move towards categorizing blood vitamin D levels as deficient, insufficient, and sufficient (Dawson-Hughes et al, 2005), however there are many different opinionsabout cutoff points and health outcomes that should be associated with each (Hollis, 2005, Dawson-Hughes et al, 2005,Holick et al, 2007, Canadian Pediatric Society, posted 2007, reaffirmed 2015, Ross et al, 2011, The Endocrine Society, The National Osteoporosis Foundation). Currently, most researchers define sufficiency 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 (Hollis, 2005, Dawson-Hughes et al, 2005,Canadian Pediatric Society, posted 2007, reaffirmed 2015, Ross et al, 2011).The Institute of Medicine (IOM) reviewed 25(OH)D levels in relation to markers of bone health (calcium absorption, fracture risk, osteomalacia) when developing dietary intake guidelines for vitamin D (Ross et al, 2011), and concluded that all people were sufficient at ≥ 50 nmol/L. Many researchers suggest a much higher set point, of at least 75 nmol/L, and sometimes higher (Hollis, 2005, Dawson-Hughes et al, 2005,Holick et al, 2007, Canadian Pediatric Society, posted 2007, reaffirmed 2015, The Endocrine Society, The National Osteoporosis Foundation). However, the IOMemphasizes that many cut-points are not backed by scientific consensus, and encourages further research.

Selting et al (2014) used suppression of PTH secretion to define the sufficiency level for serum 25(OH)D in dogs.Other measurements, such as the level of serum 25(OH)D where variation in phosphorus levels are minimized, were also used as markers of sufficiency, for reasons that are

not explained in the publication. Authors concluded that 25(OH)D sufficiency in dogs be defined as serum concentrations of 100-120ng/mL. If converted into nmol/L, this sufficiency range would be 115-139% of the maximum vitamin D range set by the Michigan State University's Diagnostic Center for Population and Animal Health (Nachreiner et al, 2014). The authors did not measure vitamin D intake in any of the participating animals, nor did they perform any bloodwork, urinalysis or medical imaging to ensure the health of enrolled animals. These results must be interpreted with extreme caution, and should not be used to justify supplementation to achieve blood vitamin D levels suggested by the authors. Currently, reference ranges for blood 25(OH)D are lab specific. Furthermore, reference ranges for individual labs may be quite large. For instance, the often-cited Michigan State University's Diagnostic Center for Population and Animal Health's range is 60-215 nmol/L (Nachreiner et al, 2014). Further work is necessary before any consensus statements on blood 25(OH)D concentrations that define sufficiency in dogs can be made.

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Table 1. Vitamin D requirements set by the NRC (2006), AAFCO (2014) and FEDIAF (2014) for adult dogs and dogs during growth, gestation, and lactation.

		Life Stage	Minimum Adequate Intake	Minimum Recommended Allowance	Safe Upper Limit
NRC	**DM basis[a] (IU/kg)**	All	440	552	3200
	Caloric basis (IU/1000 kcal ME)	All	110	136	800
AAFCO	**DM basis[b] (IU/kg)**	All		500	5000
	Caloric basis (IU/1000 kcal ME)	All		143	1429
FEDIAF	**DM basis[a]**	Adult[c]		639	2270[e]
		Adult[d]		552	3200[f]

	(IU/kg)	Reproduction & Early Growth (<14 weeks)		552	
		Late Growth (≥14 weeks)		500	
	Caloric basis (IU/1000 kcal ME)	Adult[c]		159	800
		Adult[d]		138	
		Reproduction & Early Growth (<14 weeks)		138	
		Late Growth (≥14 weeks)		125	

NRC = National Research Council, AAFCO = the Association of American Feed Control Officials, FEDIAF = European Pet Food Industry Federation, DM basis = Dry matter basis, IU = International Units

[a]Based on a dietary energy density of 4000 kcal ME/kg

[b]Based on a dietary energy density of 3500 kcal ME/kg

[c]Based on a dog with a daily energy intake of 95 kcal/kg$^{0.75}$

[d] Based on a dog with a daily energy intake of 110 kcal/kg$^{0.75}$

[e] Legal maximum set by European Union legislation. Legal maximums do not account for energy density, and so are given on a dry matter basis only.

[f] Nutritional maximum that should not cause any adverse effects

Table 2. Studies examining associations between vitamin D and disease in dogs. Many studies do not account for vitamin D intake. Despite measurement of both vitamin D intake and vitamin D status (25(OH)D) in some studies, only one study examined the relationship between the two variables.

Study	**Health Outcome**	**Vitamin D Intake**	**25(OH)D Status**
Hazewinkel et al, 1987	Growth/Bone health	No individual intake information **but** amount of vitamin D supplement added to test diet	X
Kealy et al, 1991	Growth/Bone health	No individual intake information **but** amount of vitamin D supplement added to test diet	X
Tryfonidou et al, 2002	Growth/Bone health	X	X
Tryfonidou et al, 2003	Growth/Bone health	X	X
Gerber et al, 2003	CKD		X
Gerber et al, 2004	CKD, lymphoma, primary hyperparathyroidism		X
Galler et al, 2012	CKD		X

Gow et al, 2011	IBD		X
Holowaychuk et al, 2012	Endotoxemia		X
Rosa et al, 2013	Spirocercosis (non-neoplastic and neoplastic)		X
Kraus et al, 2014	Congestive heart failure	X	X
Wakshlag et al, 2011	Cancer (MCT)	X	X
Selting et al, 2014	Cancer (Primarily hemangiosarcoma)		X
Spoo et al, 2015	None	X	X
Sharp et al, 2015	None		X
Osuga et al, 2015	Chronic valvular heart disease		X
Weidner et al, 2015	Cancer (LSA, OSA, MCT)	X	X

CKD = Chronic kidney disease, IBD = Inflammatory bowel disease, LSA = lymphoma, MCT = Mast cell tumour, OSA = Osteosarcoma, PTH = Parathyroid hormone, IGF-1 = Insulin-like growth factor 1, VDR = Vitamin D receptor, Vitamin D Status = Blood 25(OH)D concentration

Figure 1. Basic vitamin D metabolism in dogs

Diet is the only source of vitamin D for dogs, as production of vitamin D in dog skin is insignificant (Hazewinkel et al, 1987, How et al, 1994).

D = Vitamin D, D_2 = Vitamin D_2, D_3 = Vitamin D_3, 25(OH)D = 25-hydroxyvitamin D, $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D

