Low vitamin D status is associated with anaemia in hospitalised cats

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Abstract

Background The major physiological role of vitamin D has traditionally been considered to be the regulation of calcium homeostasis and maintenance of skeletal health. However, there is increasing evidence that vitamin D influences a wider range of physiological processes including erythropoiesis. Vitamin D (25-hydroxyvitamin D, 25(OH)D) deficiency concentrations have been associated with anaemia in human beings. In contrast, the relationship between vitamin D status and erythropoiesis has not been investigated in cats.

Methods Clinical records of cats consecutively presenting between November 2013 and February 2015 were reviewed. For each cat, data including sex, age, breed, serum albumin and creatinine concentrations, and appetite scores were extracted. A multivariable linear regression model was constructed to examine the relationship between 25(OH)D concentrations and these variables.

Results Cats with anaemia had significantly lower 25(OH)D concentrations (median 49.5 nmol/l, n=31) than cats with packed cell volume above the lower limit of the reference range (median 109.0 nmol/l, n=130) (P<0.001). A binary logistic regression found that red blood cell count and mean corpuscular volume were negatively correlated with serum 25(OH)D concentrations (P<0.001 and P=0.007, respectively).

Conclusion Vitamin D (25(OH)D) concentration is positively associated with red blood cell count and mean corpuscular volume in cats with a wide range of different illnesses.

Introduction

Classically, the main effects of vitamin D have been considered to be the maintenance of skeletal health. However, it has been demonstrated that numerous types of cells express the vitamin D receptor. Physiological effects of the active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), include regulation of cellular proliferation and differentiation, immunomodulatory and anti-inflammatory effects, regulation of secretion of hormones including insulin, and regulation of cardiovascular function. Vitamin D deficiency, which is standardly assessed by measuring serum 25-hydroxyvitamin D (25(OH)D) concentrations, is commonly observed in a variety of human diseases.2 Low 25(OH)D has repeatedly been associated with poorer prognosis in infectious, cardiac and neoplastic conditions in adult patients.3 Similarly, low vitamin D status has been reported in dogs with congestive heart failure,4 spirocercosis,5 protein losing enteropathy,6,7 renal disease,8 babesiosis5 and neoplasia.9,10 Serum 25(OH)D concentrations have been shown to be lower in cats with inflammatory bowel disease or small cell intestinal lymphoma,11 feline immunodeficiency virus infections12 and mycobacterial infections13 compared with healthy cats. Serum 25(OH)D concentrations are also lower in cats with neutropenia, indicating there may be a relationship between the immune response and vitamin D status.14 Lower serum 25(OH)D concentrations are also associated with an increased risk of all-cause mortality in hospitalised cats.15
In human medicine, low vitamin D status is associated with anaemia. Serum 25(OH)D concentrations are negatively associated with the presence of anaemia and the need to use agents to stimulate erythropoiesis within the general population. Hepcidin, the major metabolic regulator of iron metabolism, is increased when serum 25(OH)D is low, indicating the complex relationship between vitamin D and pathogenesis of anaemia. Furthermore, there is evidence that supplementing some groups of patients with anaemia with vitamin D may be therapeutically beneficial. For example, supplementing haemodialysis patients with a vitamin D analogue (alfacalcidol) improved anaemia.

Vitamin D supplementation (ergocalciferol) has improved erythropoietin response in patients with anaemia suffering from chronic kidney disease (CKD). Studies in which critical care adults received boluses of vitamin D demonstrated linear elevations in haemoglobin concentrations over time and reduced serum hepcidin concentrations, suggesting that high-dose vitamin D may improve iron metabolism and consequently aid resolution of anaemia in critical illness.

Anaemia is well recognised in hospitalised feline patients, and separate studies show that hospitalised cats have lower serum vitamin D than healthy controls. Despite the frequent observations of a positive relationship between red blood cell (RBC) count and serum 25(OH)D concentration in human medicine, the association between vitamin D status and erythropoiesis has yet to be investigated in cats.

The objective of this study was to measure serum concentrations of 25(OH)D, alongside RBC indices, in a population of hospitalised ill cats. The hypothesis of the study was that vitamin D status would be negatively correlated with RBC count in hospitalised ill cats.

Materials and methods
The clinical records of cats consecutively presenting to the Small Animal Hospital, Royal Dick School of Veterinary Studies between November 2013 and February 2015, for which archived serum samples were available, were reviewed. For each cat, data including sex, age, breed, serum albumin and creatinine concentrations, and appetite scores (as a binary value: normal or reduced) were extracted. Inclusion criteria were a full haematology profile, which was performed at the start of hospitalisation, information on appetite before admission and the presence of an archived residual serum sample, for 25(OH)D quantification. Anaemia in all cats was defined as a packed cell volume (PCV) below 24 per cent. All cats were fed commercially available diets. No cats enrolled had received darbepoetin, vitamin D supplementation, blood transfusions or cobalamin supplementation before sampling.

Haematology variables were measured using an ADVIA 2120i System with Autoslide (Siemens Medical Solutions Diagnostics, California, USA). A 100-white blood cell manual differential count was also undertaken. Creatinine and albumin were measured on an iLab650 biochemistry analyser (Diamond Diagnostics, USA). Serum was stored at −70°C before concentrations of 25(OH)D were measured, as previously described. The utilised SupraRegional Assay Service laboratory is accredited by CPA UK (CPA number 0865) and has been certified as proficient by the international Vitamin D External Quality Assurance Scheme.

Initially, the relationship between serum 25(OH)D concentrations and other variables including age, breed, sex, appetite score, RBC count, PCV, haemoglobin and mean corpuscular volume (MCV) was examined by scatter plots. As some haematology variables are highly correlated, RBC count and MCV were selected as the two most orthogonal variables in the haematology data set. A multivariable linear regression model was constructed to examine the relationship between 25(OH)D concentrations and the following measurements: RBC count, MCV, appetite score, age, sex, albumin, creatinine and breed. Covariates were removed from the initial model to minimise Akaike information criterion, a parameter penalised measure of best fit, to give a final parsimonious model.

To assess the effects of different types of diseases, cats were divided into groups dependent on the major body system affected by the primary presenting disease and end diagnosis if available. All cats had complete biochemistry, haematology and urine analysis performed. Additional diagnostics, including diagnostic imaging, were chosen independently by the clinician in charge of the case at time of presentation. Cats included in the study were subcategorised into urinary, endocrinopathy, cardiology, gastrointestinal, hepatic, respiratory, neurological, orthopaedic, dermatology or haematology disease groups. The relationship between PCV and serum 25(OH)D was assessed in these groups using a Spearman’s rank correlation test. Statistical analyses were performed using R statistical software system (R Core Team 2013). A P value of <0.05 was used to define statistical significance.

Results
A total of 161 cats were included, with four entire males, 101 neutered males, one entire female and 55 neutered females. Breeds included in the study were 105 domestic shorthairs, 14 domestic longhairs, eight Maine coons, eight Burmese, eight Bengals, four Siamese, two Oriental shorthairs, two ragdolls, and one of the following breeds: Abyssinian, Persian, British blue, Manx, Siberian, Russian blue, Egyptian mau, Norwegian forest, burmilla and Tonkinese. The median age of this cohort was 99 months (2.5–264 months). A total of nine out of the 161 cats were less than one year old.
Of the 161 cats enrolled, 38 were subgrouped as urinary, 37 as gastrointestinal, 31 as respiratory and 23 as hepatic. The remaining subgroups were less heavily represented, with 10 cats subgrouped as cardiology, six orthopaedic, five endocrine, five haematology, four neurology and two dermatology.

Figures 1 and 2 show the relationship between serum 25(OH)D concentrations and RBC count and MCV. Cats with anaemia had significantly lower 25(OH)D concentrations (median 54.8 nmol/l, n=31) than cats with PCV in the reference range (median 109.0 nmol/l, n=130) (P<0.001). The final regression model included only MCV, RBC count, albumin and sex, for which only MCV and RBC count had estimated changes in serum 25(OH)D, which were significantly different from zero (Table 1). Serum 25(OH)D concentrations were positively associated with both MCV and RBC count.

In order to examine whether the relationship between anaemia and vitamin D status was also present in disease subgroups, 25(OH)D concentrations and PCV were analysed in the four major disease subgroups. A positive correlation was seen between serum 25(OH)D and PCV in cats with renal disease (r=0.44, P=0.03), respiratory disease (r=0.70, P=0.0003) and gastrointestinal disease (r=0.49, P=0.015). The relationship between PCV and serum 25(OH)D in hepatic disease was not significant (r=0.3, P=0.26).

### Discussion

The key finding of this study is that serum 25(OH)D is positively associated with RBC count and MCV in cats with a wide range of different illnesses, and this association persists when assessing specific body systems. The effects of specific diseases on RBC and vitamin D metabolism were considered for each cat enrolled. Cats were divided into disease subgroups basic on clinical history, physical examination, primary diagnostics or final diagnosis if available. This allowed the authors to critically assess whether a positive correlation was witnessed in all disease groups. This subgrouping also facilitated additional statistical testing to ensure RBC pathologies such as immune mediated haemolytic anaemia did not confound the results. Previous studies published by the same institution have documented that lower serum 25(OH)D concentrations in cats with gastrointestinal disease correlated with albumin.11 There are a number of possible causes for this association, and one could be due to increased protein loss in protein losing enteropathy cases. Similarly the effects of renal disease on vitamin D metabolism have been widely reported.27–31 Multivariate analysis incorporated creatinine and albumin to account for plausible disease effect on 25(OH)D and RBC count. The authors found that the relationship was independent of serum creatinine and albumin concentrations, suggesting that renal disease and hypoalbuminaemia are unlikely to be confounding variables.

The nature of cat enrolment minimised selection bias. However, it resulted in the inclusion of cats that had been treated with glucocorticoid before presentation. The authors accept that there are limitations associated with the inclusion of glucocorticoids. However, it is important to note that while they know that glucocorticoids have major impact on human vitamin D physiology, notably...
with the development of osteoporosis, the effects of glucocorticoid therapy on feline vitamin D homeostasis remain poorly understood.

The findings in this feline study are consistent with numerous studies in human medicine. For example, vitamin D insufficiency has been associated with an increased risk of anaemia, particularly in association with inflammation. Vitamin D insufficiency is an independent risk factor for anaemia in illnesses and in healthy children, indicating that the relationship between vitamin D deficiency and anaemia may not be just a comorbidity in ill health. A vitamin D replete state in people is also independently associated with response to treatments, such as erythropoietin replacement in patients with end-stage renal disease, suggesting that vitamin D insufficiency could be contributing to the development of an anaemic state.

The mechanisms by which vitamin D insufficiency could cause anaemia are not fully understood in human beings and have not been investigated in cats. However, in vitro studies have shown the addition of calcitriol upregulates the proliferation of progenitor cells by increasing the sensitivity of erythrocytes precursors to erythropoietin. Vitamin D insufficiency may also result in anaemia, as calcitriol influences the intestinal absorption of folate and iron. Additionally, calcitriol negatively regulates the transcription of genes encoding for hepcidin. Increases in serum hepcidin result in iron sequestration by macrophages and hepatocytes and decreased iron absorption in the gastrointestinal tract, therefore reducing iron available for erythropoiesis. Increased hepcidin concentrations are reported in people with inflammation and CKD. It has been shown that supplementing people with vitamin D reduces circulating hepcidin concentrations, although this has not always been a universal finding. Increased serum hepcidin has now been associated with reduced total iron binding capacity and haematocrit in cats suffering from CKD. Considering these findings, reductions in serum 25(OH)D concentrations may increase hepcidin concentration in sick cats, increasing the risk of anaemia, and this concept requires investigation.

A common cause of anaemia in cats and human beings is anaemia of chronic disease. Anaemia of chronic disease occurs in a number of different conditions in human beings as a result of systemic inflammation. Serum 25(OH)D concentrations have been reported to be reduced in a number inflammatory diseases in human beings, including polyarthritis, diabetes mellitus, autoimmune diseases and inflammatory bowel disease. Furthermore it has been suggested that serum 25(OH)D is a negative acute phase reactant and that increases in inflammatory cytokines cause a decrease in circulating 25(OH)D concentrations. Therefore, the association between serum 25(OH)D concentration and anaemia may be due to the concurrent effects of inflammation on vitamin D homeostasis and iron metabolism. However, evidence also suggests that vitamin D can attenuate inflammation. It would therefore be interesting to assess if cats sufficient in vitamin D have reduced systemic inflammation and are therefore less likely to have ineffective erythropoiesis.

Cats are dependent on dietary sources of vitamin D in the form of both cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂); therefore, appetite was assessed for each cat enrolled. It may be hypothesised that ill, anaemic cats may have reduced serum vitamin D concentrations as a consequence of reduced appetite. However, the results of the regression analysis show no clear relationship between vitamin D and appetite score. The assessment of appetite as normal or reduced was based on each owner’s perception of their cats’ food intake. There will invariably be some differences in how owners assess appetite, which is difficult to quantify and limits the authors’ ability to fully assess this variable. Furthermore, all but one cat were fed an exclusive commercial cat food. One cat was fed a cooked prawn diet but had ad libitum access to commercial cat food. Commercial cat food is typically supplemented within recommended amounts of vitamin D₃ and vitamin D₂ via use of animal and plant products, respectively (FEDIAF European Pet Food Industry Federation, 2014). Therefore, differences in dietary vitamin D intake are unlikely to contribute to the relationship between anaemia and reduced serum 25(OH)D concentrations in this population of cats. However, it is beyond the ability of this study to assess the effect of storage, individual dietary supplements and food preparation of dietary concentrations of vitamin D. Consequently, the fact that the cats did not all consume the same diet is a limitation of the present study.

In conclusion, serum 25(OH)D concentrations are negatively associated with anaemia in hospitalised cats. Although causation cannot be inferred from these results, the findings of this study indicate that low vitamin D could have a role in anaemia and that supplementation could prove beneficial for anaemia resolution. This study’s main findings clearly indicate a requirement to investigate this further.

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**Competing interests** None declared.

**Ethics approval** Informed consent for the use of residual blood samples for research was obtained for each cat. Ethical approval was obtained from the University of Edinburgh’s Veterinary Ethical Review Committee.

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