



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Low vitamin D status is associated with systemic and gastrointestinal inflammation in dogs with a chronic enteropathy

Citation for published version:

Titmarsh, HF, Gow, AG, Kilpatrick, S, Cartwright, JA, Milne, EM, Philbey, AW, Berry, J, Handel, I & Mellanby, RJ 2015, 'Low vitamin D status is associated with systemic and gastrointestinal inflammation in dogs with a chronic enteropathy', *PLoS ONE*, vol. 10, no. 9, e0137377.
<https://doi.org/10.1371/journal.pone.0137377>

Digital Object Identifier (DOI):

[10.1371/journal.pone.0137377](https://doi.org/10.1371/journal.pone.0137377)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

PLoS ONE

Publisher Rights Statement:

Copyright: © 2015 Titmarsh et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



RESEARCH ARTICLE

Low Vitamin D Status Is Associated with Systemic and Gastrointestinal Inflammation in Dogs with a Chronic Enteropathy

Helen F. Titmarsh¹, Adam G. Gow¹, Scott Kilpatrick¹, Jennifer A. Cartwright¹, Elspeth M. Milne¹, Adrian W. Philbey¹, Jacqueline Berry², Ian Handel¹, Richard J. Mellanby¹*

1 Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Roslin, Midlothian, EH25 9RG, United Kingdom, **2** Vitamin D Research Laboratory, Endocrinology and Diabetes, Institute of Human Development, University of Manchester, Manchester Academic Health Science Centre, Manchester Royal Infirmary, Manchester, M13 9WL, United Kingdom

* Richard.Mellanby@ed.ac.uk



Abstract

Introduction

Vitamin D deficiency, as assessed by serum concentrations of 25 hydroxyvitamin D (25(OH)D), has been linked to the development of over-zealous and inappropriate inflammation in humans. However, the relationship between vitamin D status and inflammation in dogs is ill-defined. Chronic enteropathies (CE) are frequently diagnosed in client owned dogs, have a wide range of serum 25(OH)D concentrations, and represent a spontaneous model in which to probe the relationship between vitamin D and inflammation. The hypothesis of this study was that vitamin D status would be negatively associated with systemic and gastrointestinal inflammation in dogs with a CE. The aim of this study was to examine the relationship between serum 25(OH)D concentrations and markers of systemic and gastrointestinal inflammation in a cohort of dogs with CE.

Methods and Materials

Serum 25(OH)D concentrations, together with neutrophil, monocyte, eosinophil and lymphocyte counts, duodenal histopathology scores, serum IL-2, IL-6, IL-8 and TNF α concentrations and were measured in 39 dogs with histologically confirmed CE. A linear regression model examined the relationship between serum 25(OH)D status and measures of inflammation.

Results

Serum 25(OH)D concentrations were negatively associated with neutrophil and monocyte counts, duodenal histopathology scores and serum IL-2 and IL-8 concentrations. Dogs with low serum 25(OH)D concentrations typically had an inflammatory signature characterised by high monocyte and neutrophil numbers together with low lymphocyte numbers. There is

OPEN ACCESS

Citation: Titmarsh HF, Gow AG, Kilpatrick S, Cartwright JA, Milne EM, Philbey AW, et al. (2015) Low Vitamin D Status Is Associated with Systemic and Gastrointestinal Inflammation in Dogs with a Chronic Enteropathy. *PLoS ONE* 10(9): e0137377. doi:10.1371/journal.pone.0137377

Editor: Andrzej T Slominski, University of Alabama at Birmingham, UNITED STATES

Received: July 17, 2015

Accepted: August 17, 2015

Published: September 2, 2015

Copyright: © 2015 Titmarsh et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: Richard Mellanby is supported by a Wellcome Trust Intermediate Clinical Fellowship.

Competing Interests: The authors have declared that no competing interests exist.

a need to establish whether low vitamin D status is a cause or consequence of inflammation.

Introduction

The traditional functions of vitamin D relate to its role in the maintenance of calcium homeostasis and bone metabolism. In recent decades, many cell types have been shown to express the vitamin D receptor, and the physiological roles of vitamin D have been shown to extend beyond skeletal metabolism [1, 2]. Consequently, numerous studies have examined the relationship between the immune response and vitamin D status as assessed by serum 25 hydroxy-vitamin D (25(OH)D) concentrations in human patients. There is a growing body of evidence 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 [3, 4], inflammatory polyarthritis [5], diabetes mellitus [6], autoimmune diseases [7], inflammatory bowel disease [8, 9], and human immunodeficiency virus [10]. Furthermore, low vitamin D status has been associated with increased markers of inflammation in healthy humans [11–13].

The reasons why vitamin D status is negatively associated with inflammation is unclear. The vitamin D receptor (VDR) is found on most immune cells including macrophages, dendritic cells, T-lymphocytes and B-lymphocytes [14]. Vitamin D can promote immune tolerance by increasing T-regulatory cell populations [15], inhibiting the production of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines [16–22]. Vitamin D is also known to enhance the innate immune response to bacteria, by increasing the production of anti-microbial peptides such as cathelicidin [18, 23].

The relationship between vitamin D status and inflammation is poorly understood in dogs. Serum 25(OH)D concentrations are commonly reduced in a number of inflammatory diseases in dogs including congestive heart failure [24], *Spirocerca lupi* infections [25], protein losing enteropathy [26, 27] and renal disease [28]. Low vitamin D status has been negatively associated with C-reactive protein (CRP) in dogs with haemoabdomen [29]. However, other studies have reported a positive association between vitamin D and CRP concentrations in racing sled dogs [30]. Consequently, further work is needed to clarify the relationship between vitamin D and inflammation in dogs.

Dogs with chronic enteropathies (CE) represent a spontaneous model in which to explore the relationship between vitamin D status and inflammation. We have previously demonstrated the dogs with CE can have a range of vitamin D concentrations, ranging from profound deficiency to sufficiency [26, 31]. However, the relationship between 25(OH)D concentrations and inflammation in dogs with CE is poorly understood. A number of markers of systemic inflammation, including serum cytokines and leukocyte profiles can be measured in dogs [32]. In addition, the WSAVA gastrointestinal histopathology scoring system for endoscopic biopsies [33] provides a standardized means of assessing the extent of inflammation within gastrointestinal biopsies from dogs with a CE.

The hypothesis of this study was that vitamin D status would be negatively associated with local and systemic markers of inflammation in dogs with a CE. The objectives were to investigate the relationship between serum concentrations of 25(OH)D and blood neutrophil, lymphocyte, monocyte and eosinophil numbers, duodenal histopathology inflammation scores, and serum cytokine concentrations in dogs with histologically confirmed CE.

Material and Methods

The records of dogs referred to the Hospital for Small Animals, Royal (Dick) School of Veterinary Studies for investigation of chronic gastrointestinal disease of more than three weeks in duration were retrospectively reviewed. Inclusion criteria including the following clinical signs, common reported in dogs with CE: vomiting, diarrhoea, increased borborygmi, abdominal pain, increased or decreased appetite and weight loss and also that the dogs had histopathological evidence of inflammation within the small or large intestine. Furthermore, there were no clinically significant abnormalities detected on hematology, biochemistry or abdominal ultrasonography indicative of non-gastrointestinal diseases for any of the dogs enrolled. In addition, the faeces of all dogs were negative for both helminth and *Giardia* infections.

Haematology variables, which included total white blood cell count, mature neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils, total red blood cell counts, packed cell volume, haemoglobin, mean cell volume, mean cell haemoglobin concentration and platelet number were measured on ADVIA(r) 2120i System with Autoslide (Siemens Medical Solutions Diagnostics Ltd California, USA). At least a 100-white blood cell manual differential count was undertaken to establish the concentrations of neutrophils, monocytes, lymphocytes, eosinophils and basophils. Blood smears were evaluated under the direct supervision of a Board-certified veterinary clinical pathologist in every case. Biochemistry variables were measured on an ILab650 biochemistry analyser, (Diamond Diagnostics, USA). The area of intestinal tract which was biopsied (duodenum or duodenum and colon) was at the discretion of the primary clinician managing the case and based on presenting clinical signs. A diagnosis of CE was made if there was histological evidence of intestinal inflammation and no underlying aetiology was identified by on abdominal ultrasound, faecal samples or blood tests.

Serum samples retained for 25(OH)D measurement were frozen after being used for routine biochemical analysis. They were stored at -70°C before being sent to the laboratory for analysis on dry ice. Serum concentrations of 25(OH)D were measured as previously described in detail [34, 35]. Samples were extracted using acetonitrile and applied to C18 Silica Sep-paks. Separation of metabolites was by straight phase high performance liquid chromatography (HPLC) (Waters Associates, Milford, MA, USA) using a Hewlett-Packard Zorbax-Sil Column (Hichrom, Reading, UK) eluted with hexane:propan-2-ol:methanol (92:4:4). Serum 25(OH)D₂ and 25(OH)D₃ were measured separately by application to a second Zorbax-Sil Column eluted with hexane:propan-2-ol (98:2) and quantified by ultraviolet absorbance at 265 nm and corrected for recovery (sensitivity 5 nmol/L, intra- and inter-assay coefficients of variation 3.0% and 4.2%, respectively) [36]. This Supraregional Assay Service laboratory is accredited by CPA UK (CPA number 0865) and has been certified as proficient by the international Vitamin D Quality Assurance Scheme (DEQAS). Total 25(OH)D was defined as the sum of 25(OH)D₂ and 25(OH)D₃.

Canine pro-inflammatory cytokines (IL-2, IL-6, IL-8 and TNF- α) were measured from serum samples using a multiplex electrochemiluminescence immunoassay system (Meso Scale Discovery; MSD) as previously described [32]. Assay diluent (25 μL) was added to all wells, plates sealed and incubated for 30 min at room temperature on an orbital shaker (600 rpm). Samples and standards, diluted in assay diluent, were added at 25 μL per well. Plates were again sealed and incubated for a further 2 hours at room temperature with shaking. At the end of the incubation period, wells were washed three times with 200 μL phosphate-buffered saline (PBS), supplemented with 0.05% Tween 20 (Sigma-Aldrich) for 30 s, then discarded. Detection antibody was added at 25 μL per well, plates sealed and incubated for a further 1 h at room temperature with shaking. Plates were washed three times and 150 μL of MSD Read Buffer added to

each well, then electrochemiluminescence measured using the MSD Sector Imager 2400 plate reader.

A single veterinary pathologist (AP) reviewed the histopathological samples. The degree of inflammation present was assessed using a qualitative scoring system (WSAVA Standards for the Diagnosis of Gastrointestinal Inflammation in Endoscopic Biopsy Samples) [33]. Parameters which are assessed using this system include the following: histological changes (villous stunting, epithelial injury, crypt distension, lacteal dilatation, and mucosal fibrosis) and inflammatory infiltrates (intra-epithelial lymphocytes, lamina propria lymphocytes and plasma cells, lamina propria eosinophils, and lamina propria neutrophils). Changes were graded as normal (0), mild (1), moderate (2) or severe (3). The sums of all these parameters were added together to determine an intestine inflammatory score which ranged from 0 (normal) to 30 (very severe).

In order to investigate the relationship between multiple inflammatory markers and serum 25(OH)D concentrations, individual variables were examined using histograms and parameters which were not normally distributed (Anderson-Darling test) were transformed. Log₁₀ transformation was undertaken except for square root transformation of eosinophil numbers since some eosinophil numbers were zero. The relationship between age, sex and 25(OH)D was examined using scatter plots and linear regression model. The relationship between 25(OH)D and inflammatory parameters was investigated using linear regression models. Age and sex was included in initial models in order to examine for any confounding effects. Final models were selected by removing potential confounders if their removal did not worsen model fit as assessed by the Akaike information criterion (AIC, a penalty parameter penalised measure of model fit). As the study wanted to examine whether 25(OH)D concentrations correlated to an inflammatory signature, medoid based partitioning was used to assign haematology parameters to three clusters. Three clusters was chosen on the basis of a consensus of results from 30 cluster count algorithms [37]. These clusters were identified independently of 25(OH)D concentrations. A Kruskal Wallis test was used to determine if there was difference in 25(OH)D concentrations between the three clusters [38]. Tukey and Kramer posthoc test for pairwise comparisons were then used to identify which clusters differ. Data was collected using Excel and analysed using R statistical software system [39]. A p value of <0.05 was used to define statistical significance.

The University of Edinburgh's Veterinary Ethical Review Committee approved the study. Informed consent for the storage and subsequent use of residual clinical blood samples for research purposes was obtained at admission for each dog enrolled.

Results

Thirty-nine dogs with a diagnosis of chronic enteropathies were enrolled. Breeds enrolled included; Boxer (9), cross breed (5), Labrador (2), Lurcher (2), Springer Spaniel (2), Cavalier King Charles Spaniel (2), Staffordshire Bull Terrier (2), and one of each of the following breeds; Irish Setter, Italian Greyhound, Toy Poodle, Shar-pei, Yorkshire Terrier, Pyrenees Mountain Dog, Hungarian Vizsla, Greyhound, German Short Haired Pointer, West Highland White Terrier, Cocker Spaniel, Dogue de Bordeaux, Shetland Sheep dog, Border Collie and a Border Terrier. The age range of dogs included was 6 to 136 months (median 65 months). Haematology, biochemistry, faecal parasitology and abdominal ultrasonography did not reveal any significant clinical abnormalities in any of the 39 dogs. Sixteen dogs underwent upper gastrointestinal endoscopy and 23 dogs had both upper and lower gastrointestinal endoscopy. Thirty-two dogs had duodenal biopsy samples which were available for histological review by a veterinary pathologist who was blinded to the clinical history and vitamin D status of the dogs. Only 32

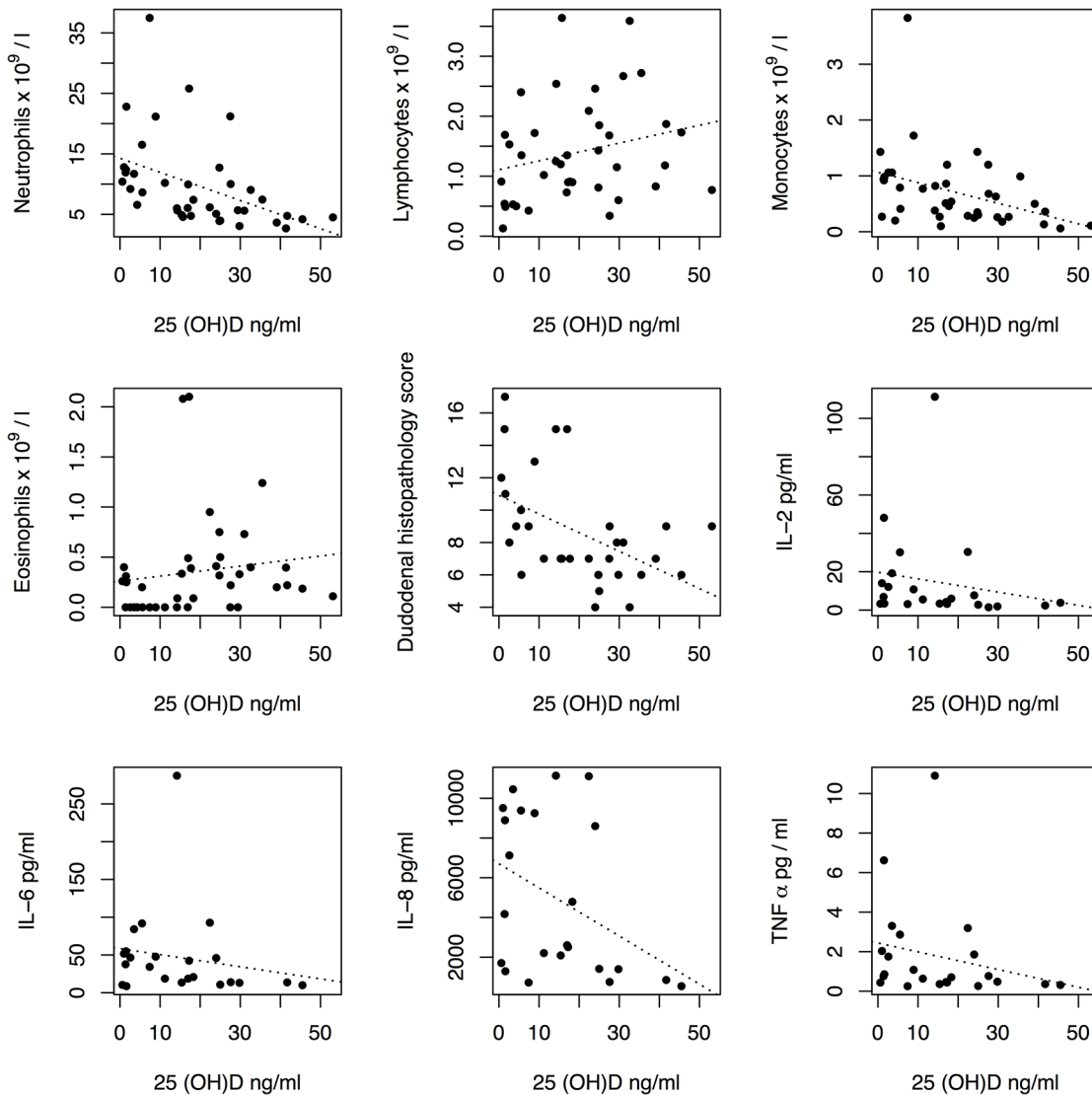


Fig 1. Relationship between haematology, serum cytokines and duodenal inflammatory score and serum 25(OH)D concentrations.

doi:10.1371/journal.pone.0137377.g001

samples were available for review as the remainder could not be retrieved from archived stores. Cytokine analysis was available in 23 cases as archived serum samples were not available for all cases.

Age and sex were not predictive of serum 25(OH)D concentrations in a linear regression model. The relationships between serum 25(OH)D concentrations and haematology, duodenal inflammation score and serum cytokine results are shown in Fig 1. Neutrophil and monocyte counts, duodenal histopathology score and serum IL-2 and IL-8 concentrations were negatively associated with 25(OH)D concentrations (Table 1). Three clusters were identified in the haematology data (Figs 2 and 3). There was a significant difference in 25(OH)D concentrations between the three clusters ($p = 0.009$). Post-test analysis revealed a significant difference between clusters 2 and 3 ($p = 0.006$).

Table 1. Results of logistic regression model of inflammatory parameters on serum 25(OH)D concentrations.

Variable	Co-efficient (SE)	P value
Neutrophil number (log10)	-0.011 (0.003)	<0.001 *
Monocyte number (log10)	-0.016 (0.004)	<0.001 *
Eosinophil number (sqrt)	0.008 (0.004)	0.09
Lymphocyte number (log10)	0.005 (0.004)	0.16
Duodenal histopathology score (log10)	-0.012 (0.004)	0.006 *
IL-2 (log10)	-0.015 (0.007)	0.048 *
IL-6 (log10)	-0.011 (0.006)	0.09
IL-8 (log10)	-0.018 (0.006)	0.01 *
TNF- α (log10)	-0.014 (0.007)	0.06

*denotes statistical significance P<0.05

doi:10.1371/journal.pone.0137377.t001

Discussion

The central finding of this study is that serum 25(OH)D concentrations negatively correlate with systemic markers of inflammation including neutrophil and monocyte counts, serum IL-2 and IL-8 concentrations and local inflammation as measured by duodenal histopathology scores. Linear regression and medoid based partitioning analysis demonstrated that dogs with low vitamin D status had an inflammatory signature consisting of high neutrophil and monocyte counts and low lymphocyte numbers. A typical stress and/or inflammatory leukogram in dogs consist of neutrophilia, monocytosis and lymphopenia. These changes provide evidence of an association between reduced serum 25(OH)D concentrations and systemic inflammation in this population of dogs.

The results of this study also show that IL-2 and IL-8 concentrations are increased in the serum of dogs with CE and associated with lower 25(OH)D concentrations. Interleukin-2 acts on activated lymphocytes resulting in their expansion, and differentiation into cytotoxic lymphocytes, natural killer cells and T helper cells [40]. Interleukin-2 is under direct transcriptional regulation by 1,25(OH)₂D and can decrease IL-2 production [41]. Culture of T lymphocytes with vitamin D from patients with systemic lupus erythematosus decreased production of IL-2 [42]. Similarly, vitamin D supplementation has been associated with decrease in circulating IL-2 concentrations in children with atopy [43]. In addition, increased IL-2 concentrations have been reported in vitamin D insufficient adults [44]. Serum IL-8 concentrations were also increased in this population of dogs with CE and lower 25(OH)D concentrations. In human IBD, IL-8 is an important inflammatory mediator, playing a role in the initiation and maintenance of IBD by recruiting neutrophils into the inflamed gastrointestinal tract [45]. An increase in serum IL-8 concentrations have also been reported in human patients with IBD [46]. Vitamin D has been shown to exert anti-inflammatory effects by decreasing IL-8 production in intestinal cell cultures [47]. Our results also indicate that low serum vitamin D concentrations are associated with more severe inflammation as assessed by duodenal histopathology scores. This provides evidence of an association between intestinal inflammation and reduced vitamin D concentrations.

It is unclear if the low 25(OH)D concentrations observed in inflammatory diseases are causally related to inflammation or if lower vitamin D status occur as a consequence of the inflammatory process itself. Serum 25(OH)D concentrations have been shown to decrease following elective surgical procedures in human patients, coupled with increases in inflammatory markers such as CRP, suggesting vitamin D is a negative acute phase reactant [48]. Serum 25(OH)D

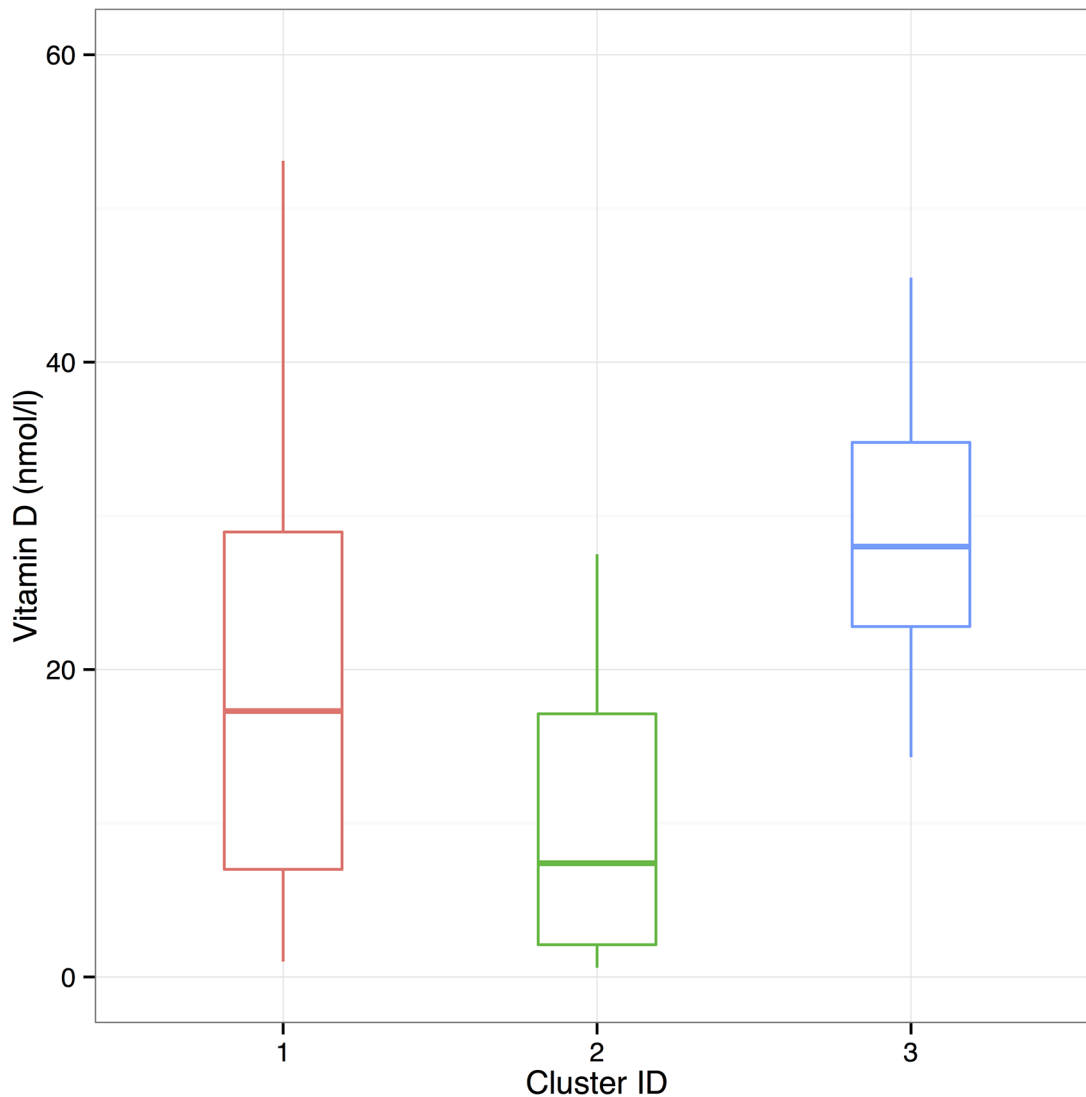


Fig 2. Box and whisker plot of serum 25(OH)D concentrations by cluster. The box extends from 25% to 75% percentile with the median and the whiskers extend to limits of the data.

doi:10.1371/journal.pone.0137377.g002

concentrations also fall rapidly in other inflammatory conditions such as acute pancreatitis [49]. Increases in pro-inflammatory cytokines have been proposed to be the cause of decreases in serum 25(OH)D in acute inflammation. For example, changes in serum 25(OH)D over several weeks corresponded with increases in serum pro-inflammatory cytokines, including TNF- α , IFN- γ , IL-1 β , GM-CSF and IL-6 concentrations, in patients undergoing knee arthroplasty [50]. However, not all studies looking at illness events associated with acute inflammation have demonstrated decreases in serum 25(OH)D concentrations [51, 52]. In addition, changes in vitamin D status following inflammation may relate to changes in vitamin D binding proteins. For example, serum vitamin D binding protein decreases in the face of acute inflammation [53].

Numerous studies have investigated the potential benefit of vitamin D supplementation in a range of inflammatory diseases. In ill patients, vitamin D administration reduces some markers of inflammation in early chronic kidney disease [54], end-stage renal disease [55], congestive

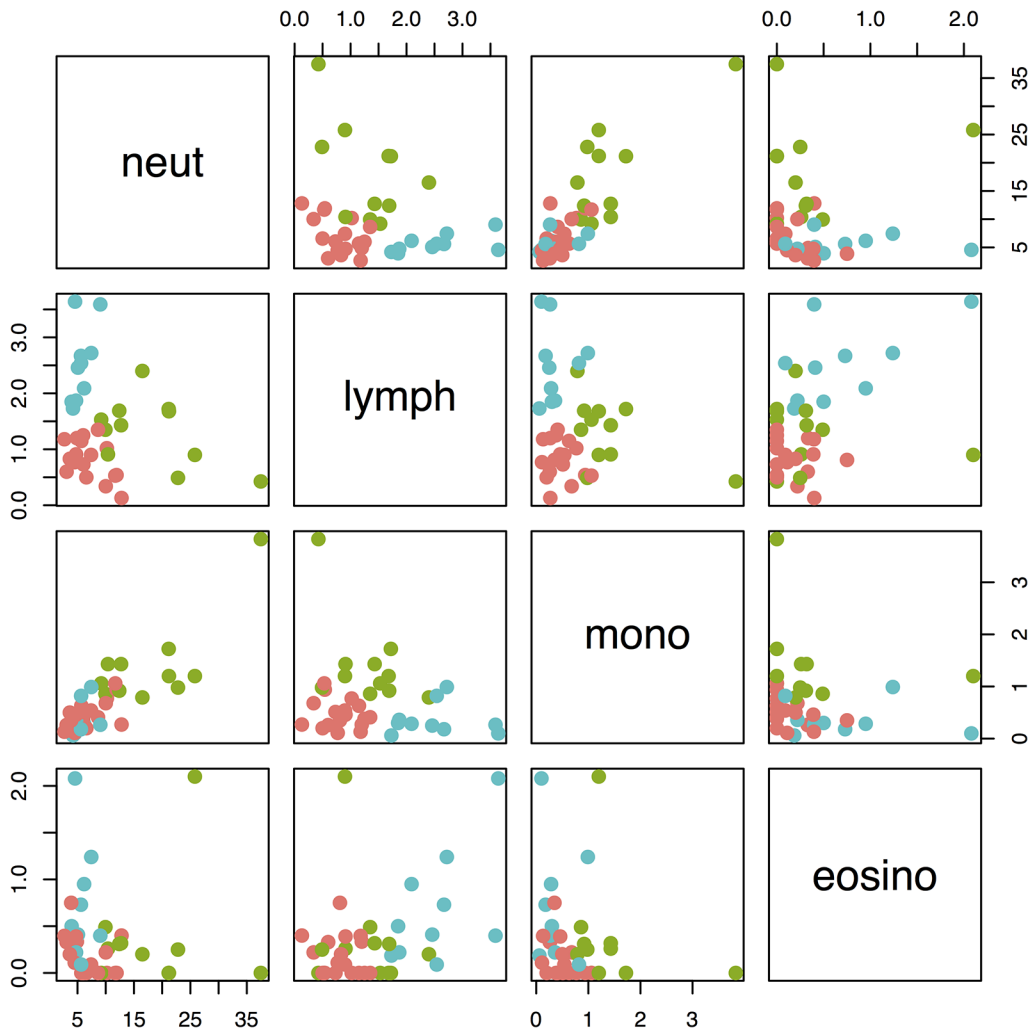


Fig 3. Pair-wise scatter plot of number of neutrophils (neut), monocytes (mono), eosinophils (eosino) and lymphocytes (lymph) labelled by cluster (red cluster 1, green cluster 2, blue cluster 3). Values on x and y axis denote cell concentrations $\times 10^9/l$.

doi:10.1371/journal.pone.0137377.g003

heart failure [56], systemic lupus [57] and colorectal adenoma [58]. In addition markers of inflammation decreased in elderly women with vitamin D insufficiency in response to supplementation with a mega-dose of vitamin D3 [59]. However, other studies investigating the effects of vitamin D supplementation on markers of inflammation have failed to demonstrate *in-vivo* anti-inflammatory effects. For example, high dose vitamin D treatment did not result in a decrease in inflammatory markers in people with low vitamin D status diagnosed with: pre-diabetes and type 1 diabetes [6, 60], hypertension [61] and urticaria [62]. Furthermore, increases in pro-inflammatory cytokines were documented in patients with osteoporosis after vitamin D supplements were administered [63]. There are many potential explanations for why the results of different studies reported differing effects of vitamin D supplementation on markers of inflammation. These include differences in the duration for which vitamin D supplementation was given, the type of vitamin D used for supplementation and the effects of various diseases. In one meta-analysis it appears that the benefits of vitamin D in reducing inflammatory markers is dependent on the disease state studied and initial, pre-treatment 25 (OH)D concentrations [64]. The benefits of vitamin D supplementation appear to be most

evident in markedly inflammatory diseases where serum 25(OH)D concentrations are initially low.

Although hypovitaminosis D in CE has traditionally been considered to be the result of intestinal disease, there is growing evidence that hypovitaminosis D may contribute to the initiation of intestinal inflammation. Intestinal epithelial vitamin D receptor is important in the regulation of mucosal inflammation by maintaining the integrity of the mucosal barrier [65], suggesting hypovitaminosis D may also perpetuate inflammation. However, the expression of VDR is influenced by mucosal inflammation and is down-regulated by mucosal pro-inflammatory cytokines [65, 66]. Studies in experimental models of inflammatory bowel disease have demonstrated that vitamin D can have an anti-inflammatory effect. For example, in a murine model of IBD, 1,25(OH)₂D was shown to inhibit a number of genes involved in regulating TNF- α production and signaling [67]. Furthermore, VDR knockout mice produce significantly higher cytokine concentrations in response to chemically induced gastrointestinal inflammation than wild type mice [68]. These findings suggest that vitamin D is important in regulating gastrointestinal inflammation. However, it is not clear whether these findings can be extrapolated directly to patients with IBD. For example, CRP, ESR, TNF- α , IL-17, IL-10 and vascular endothelial growth factor concentrations did not change following vitamin D₃ supplementation in people with IBD [69]. Therefore, further work is needed to determine the role of vitamin D in the treatment of inflammatory bowel disease in people and dogs.

There are some limitations to this study. The retrospective nature of this study made it impossible to standardise the drugs and dietary treatments the dogs received prior to referral. In addition, serum cytokine concentrations and gastrointestinal histology slides were not available from all dogs.

Conclusion

In summary, this study has shown that serum 25(OH)D concentrations are inversely associated with markers of systemic inflammation in dogs with CE, and with severity of inflammatory changes seen in histopathology samples. Further studies are required to assess whether low vitamin D status is a cause or a consequence of inflammation.

Acknowledgments

The authors would like to acknowledge all the clinicians, nurses and owners of the dogs who contributed towards the study. Richard Mellanby is supported by a Wellcome Trust Intermediate Clinical Fellowship. Patients were recruited from, and investigated at, the Hospital for Small Animals at the Royal (Dick) School of Veterinary Studies, The University of Edinburgh. Serum 25 hydroxyvitamin D measurement was performed at the Vitamin D research laboratory at the University of Manchester.

Author Contributions

Conceived and designed the experiments: HT AG RM IH. Performed the experiments: HT SK JC AG RM IH JB EM AP. Analyzed the data: HT RM IH AP AG. Contributed reagents/materials/analysis tools: HT SK JC AG RM IH JB EM AP. Wrote the paper: HT SK JC AG RM IH JB EM AP.

References

1. The Nonskeletal Effects of Vitamin D: An Endocrine Society Scientific Statement. *Endocrine Reviews*. 2012; 33(3):456–92. doi: [10.1210/er.2012-1000](https://doi.org/10.1210/er.2012-1000) PMID: [22596255](https://pubmed.ncbi.nlm.nih.gov/22596255/).

2. Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. *Journal of Cellular Biochemistry*. 2003; 88(4):695–705. PMID: [12577303](#)
3. Codoner-Franch P, Tavarez-Alonso S, Simo-Jorda R, Laporta-Martin P, Carratala-Calvo A, Alonso-Iglesias E. Vitamin D status is linked to biomarkers of oxidative stress, inflammation, and endothelial activation in obese children. *The Journal of Pediatrics*. 2012; 161(5):848–54. Epub 2012/06/09. doi: [10.1016/j.jpeds.2012.04.046](#) PMID: [22677566](#).
4. Bellia A, Garcovich C, D'Adamo M, Lombardo M, Tesauro M, Donadel G, et al. Serum 25-hydroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. *Internal and Emergency Medicine*. 2013; 8(1):33–40. Epub 2011/03/26. doi: [10.1007/s11739-011-0559-x](#) PMID: [21437585](#).
5. Patel S, Farragher T, Berry J, Bunn D, Silman A, Symmons D. Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. *Arthritis and Rheumatology*. 2007; 56(7):2143–9. Epub 2007/06/30. doi: [10.1002/art.22722](#) PMID: [17599737](#).
6. Shih EM, Mittelman S, Pitukcheewanont P, Azen CG, Monzavi R. Effects of vitamin D repletion on glycemic control and inflammatory cytokines in adolescents with type 1 diabetes. *Pediatr Diabetes*. 2014. Epub 2014/12/20. doi: [10.1111/pedi.12238](#) PMID: [25524404](#).
7. Robinson AB, Tangpricha V, Yow E, Gurion R, McComsey GA, Schanberg LE. Vitamin D deficiency is common and associated with increased C-reactive protein in children and young adults with lupus: an Atherosclerosis Prevention in Pediatric Lupus Erythematosus substudy. *Lupus Science and Medicine*. 2014; 1(1):e000011. Epub 2014/11/15. doi: [10.1136/lupus-2014-000011](#) PMID: [25396060](#); PubMed Central PMCID: PMC4225734.
8. Raftery T, Merrick M, Healy M, Mahmud N, O'Morain C, Smith S, et al. Vitamin D Status Is Associated with Intestinal Inflammation as Measured by Fecal Calprotectin in Crohn's Disease in Clinical Remission. *Dig Dis Sci*. 2015. Epub 2015/03/12. doi: [10.1007/s10620-015-3620-1](#) PMID: [25757449](#).
9. Garg M, Rosella O, Lubel JS, Gibson PR. Association of Circulating Vitamin D Concentrations with Intestinal but Not Systemic Inflammation in Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*. 2013; 19(12):2634–43. doi: [10.1097/01.MIB.0000436957.77533.b2](#) PMID: [00054725-201311000-00014](#).
10. Poudel-Tandukar K, Poudel KC, Jimba M, Kobayashi J, Johnson CA, Palmer PH. Serum 25-hydroxyvitamin d levels and C-reactive protein in persons with human immunodeficiency virus infection. *AIDS Research And Human Retroviruses*. 2013; 29(3):528–34. Epub 2012/09/26. doi: [10.1089/aid.2012.0120](#) PMID: [23003113](#); PubMed Central PMCID: PMC3581068.
11. De Vita F, Lauretani F, Bauer J, Bautmans I, Shardell M, Cherubini A, et al. Relationship between vitamin D and inflammatory markers in older individuals. *Age (Dordr)*. 2014; 36(4):9694. Epub 2014/08/05. doi: [10.1007/s11357-014-9694-4](#) PMID: [25086618](#); PubMed Central PMCID: PMC4150893.
12. Laird E, McNulty H, Ward M, Hoey L, McSorley E, Wallace JM, et al. Vitamin D deficiency is associated with inflammation in older Irish adults. *The Journal of Clinical Endocrinology and Metabolism* 2014; 99(5):1807–15. Epub 2014/03/13. doi: [10.1210/jc.2013-3507](#) PMID: [24606079](#).
13. Peterson CA, Heffernan ME. Serum tumor necrosis factor- α concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *Journal of Inflammation* 2008; 5:10. Epub 2008/07/26. doi: [10.1186/1476-9255-5-10](#) PMID: [18652680](#); PubMed Central PMCID: PMC2503979.
14. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. *Nutrients*. 2013; 5(7):2502–21. Epub 2013/07/17. doi: [10.3390/nu5072502](#) PMID: [23857223](#); PubMed Central PMCID: PMC3738984.
15. Chambers ES, Suwannasaen D, Mann EH, Urry Z, Richards DF, Lertmengkolchai G, et al. 1 α ,25-dihydroxyvitamin D3 in combination with transforming growth factor- β increases the frequency of Foxp3(+) regulatory T cells through preferential expansion and usage of interleukin-2. *Immunology*. 2014; 143(1):52–60. Epub 2014/03/29. doi: [10.1111/imm.12289](#) PMID: [24673126](#); PubMed Central PMCID: PMC4137955.
16. Khoo A-L, Chai LYA, Koenen HJPM, Oosting M, Steinmeyer A, Zuegel U, et al. Vitamin D3 down-regulates proinflammatory cytokine response to Mycobacterium tuberculosis through pattern recognition receptors while inducing protective cathelicidin production. *Cytokine*. 2011; 55(2):294–300. <http://dx.doi.org/10.1016/j.cyto.2011.04.016>. doi: [10.1016/j.cyto.2011.04.016](#) PMID: [21592820](#)
17. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1 α ,25-Dihydroxyvitamin D3 Has a Direct Effect on Naive CD4+ T Cells to Enhance the Development of Th2 Cells. *The Journal of Immunology*. 2001; 167(9):4974–80. doi: [10.4049/jimmunol.167.9.4974](#) PMID: [11673504](#)
18. Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, Skolimowska K, et al. IFN- γ - and TNF-Independent Vitamin D-Inducible Human Suppression of Mycobacteria: The Role of Cathelicidin

- LL-37. *The Journal of Immunology*. 2007; 178(11):7190–8. doi: [10.4049/jimmunol.178.11.7190](https://doi.org/10.4049/jimmunol.178.11.7190) PMID: [17513768](https://pubmed.ncbi.nlm.nih.gov/17513768/)
19. Harishankar M, Afsal K, Banurekha VV, Meenakshi N, Selvaraj P. 1,25-Dihydroxy vitamin D3 downregulates pro-inflammatory cytokine response in pulmonary tuberculosis. *International Immunopharmacology*. 2014; 23(1):148–52. Epub 2014/09/10. doi: [10.1016/j.intimp.2014.08.021](https://doi.org/10.1016/j.intimp.2014.08.021) PMID: [25194676](https://pubmed.ncbi.nlm.nih.gov/25194676/).
 20. Jeong MS, Kim JY, Lee HI, Seo SJ. Calcitriol May Down-Regulate mRNA Over-Expression of Toll-Like Receptor-2 and -4, LL-37 and Proinflammatory Cytokines in Cultured Human Keratinocytes. *Ann Dermatol*. 2014; 26(3):296–302. Epub 2014/06/27. doi: [10.5021/ad.2014.26.3.296](https://doi.org/10.5021/ad.2014.26.3.296) PubMed Central PMCID: [PMC4069638](https://pubmed.ncbi.nlm.nih.gov/PMC4069638/). PMID: [24966627](https://pubmed.ncbi.nlm.nih.gov/24966627/)
 21. Nissou MF, Guttin A, Zenga C, Berger F, Issartel JP, Wion D. Additional clues for a protective role of vitamin D in neurodegenerative diseases: 1,25-dihydroxyvitamin D3 triggers an anti-inflammatory response in brain pericytes. *J Alzheimers Dis*. 2014; 42(3):789–99. Epub 2014/06/18. doi: [10.3233/jad-140411](https://doi.org/10.3233/jad-140411) PMID: [24934545](https://pubmed.ncbi.nlm.nih.gov/24934545/).
 22. Korf H, Wenes M, Stijlemans B, Takiishi T, Robert S, Miani M, et al. 1,25-Dihydroxyvitamin D3 curtails the inflammatory and T cell stimulatory capacity of macrophages through an IL-10-dependent mechanism. *Immunobiology*. 2012; 217(12):1292–300. Epub 2012/09/05. doi: [10.1016/j.imbio.2012.07.018](https://doi.org/10.1016/j.imbio.2012.07.018) PMID: [22944250](https://pubmed.ncbi.nlm.nih.gov/22944250/).
 23. Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting Edge: 1,25-Dihydroxyvitamin D3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression. *The Journal of Immunology*. 2004; 173(5):2909–12. doi: [10.4049/jimmunol.173.5.2909](https://doi.org/10.4049/jimmunol.173.5.2909) PMID: [15322146](https://pubmed.ncbi.nlm.nih.gov/15322146/)
 24. Kraus MS, Rassnick KM, Wakshlag JJ, Gelzer ARM, Waxman AS, Struble AM, et al. Relation of Vitamin D Status to Congestive Heart Failure and Cardiovascular Events in Dogs. *Journal of Veterinary Internal Medicine*. 2014; 28(1):109–15. doi: [10.1111/jvim.12239](https://doi.org/10.1111/jvim.12239) PMID: [24205918](https://pubmed.ncbi.nlm.nih.gov/24205918/)
 25. Rosa CT, Schoeman JP, Berry JL, Mellanby RJ, Dvir E. Hypovitaminosis D in Dogs with Spirocercosis. *Journal of Veterinary Internal Medicine*. 2013; 27(5):1159–64. doi: [10.1111/jvim.12161](https://doi.org/10.1111/jvim.12161) PMID: [23952621](https://pubmed.ncbi.nlm.nih.gov/23952621/)
 26. Gow AG, Else R, Evans H, Berry JL, Herrtage ME, Mellanby RJ. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. *Journal of Small Animal Practice*. 2011; 52(8):411–8. Epub 2011/07/30. doi: [10.1111/j.1748-5827.2011.01082.x](https://doi.org/10.1111/j.1748-5827.2011.01082.x) PMID: [21797872](https://pubmed.ncbi.nlm.nih.gov/21797872/).
 27. Mellanby R, Mellor P, Roulois A, Baines E, Mee A, Berry J, et al. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. *Journal of small animal practice*. 2005; 46(7):345–51. PMID: [16035452](https://pubmed.ncbi.nlm.nih.gov/16035452/)
 28. Gerber B, Hassig M, Reusch CE. Serum concentrations of 1,25-dihydroxycholecalciferol and 25-hydroxycholecalciferol in clinically normal dogs and dogs with acute and chronic renal failure. *American Journal of Veterinary Research*. 2003; 64(9):1161–6. doi: [10.2460/ajvr.2003.64.1161](https://doi.org/10.2460/ajvr.2003.64.1161) PMID: [WOS:000185038700015](https://pubmed.ncbi.nlm.nih.gov/1300185038700015/).
 29. Selting KA, Sharp CR, Ringold R, Thamm DH, Backus R. Serum 25-hydroxyvitamin D concentrations in dogs—correlation with health and cancer risk. *Veterinary and Comparative Oncology*. 2014:n/a-n/a. doi: [10.1111/vco.12101](https://doi.org/10.1111/vco.12101)
 30. Spoo JW, Downey RL, Griffiths C, Horst RJ, Levine CB, Childs RM, et al. Plasma Vitamin D Metabolites and C-Reactive Protein in Stage-Stop Racing Endurance Sled Dogs. *Journal of Veterinary Internal Medicine*. 2015; 29(2):519–25. doi: [10.1111/jvim.12546](https://doi.org/10.1111/jvim.12546) PMID: [25818207](https://pubmed.ncbi.nlm.nih.gov/25818207/)
 31. Mellanby RJ, Mellor PJ, Roulois A, Baines EA, Mee AP, Berry JL, et al. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. *Journal of Small Animal Practice*. 2005; 46(7):345–51. Epub 2005/07/23. PMID: [16035452](https://pubmed.ncbi.nlm.nih.gov/16035452/).
 32. Kilpatrick S, Gow AG, Foale RD, Tappin SW, Carruthers H, Reed N, et al. Plasma cytokine concentrations in dogs with a congenital portosystemic shunt. *The Veterinary Journal*. 2014; 200(1):197–9. Epub 2014/02/13. doi: [10.1016/j.tvjl.2014.01.007](https://doi.org/10.1016/j.tvjl.2014.01.007) PMID: [24518641](https://pubmed.ncbi.nlm.nih.gov/24518641/).
 33. Day M, Bilzer T, Mansell J, Wilcock B, Hall E, Jergens A, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. *Journal of Comparative Pathology*. 2008; 138:S1–S43. doi: [10.1016/j.jcpa.2008.01.001](https://doi.org/10.1016/j.jcpa.2008.01.001) PMID: [18336828](https://pubmed.ncbi.nlm.nih.gov/18336828/)
 34. Mawer EB, Hann J, Berry JL, Davies M. Vitamin D metabolism in patients intoxicated with ergocalciferol. *Clinical Science*. 1985; 68(2):135–41. PMID: [3871380](https://pubmed.ncbi.nlm.nih.gov/3871380/)
 35. Mawer EB, Berry JL, Cundall JP, Still PE, White A. A sensitive radioimmunoassay using a monoclonal antibody that is equipotent for ercalcitriol and calcitriol (1, 25-dihydroxy vitamin D 2 and D 3). *Clinica Chimica Acta*. 1990; 190(3):199–209.
 36. Berry J, Martin J, Mawer E. 25-Hydroxyvitamin D assay kits: speed at the cost of accuracy. *Vitamin D Endocrine System: structural, biological, genetic and clinical aspects*. 2000:797–800.

37. Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K. Cluster Analysis Basics and Extensions. R package version 201 (2015).
38. Charrad M, Ghazzali N, Boiteau V, Niknafs A. NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*. 2014; 61(6):1–36.
39. Team RC. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2014.
40. Swain SL. Lymphokines and the immune response: the central role of interleukin-2. *Current Opinion in Immunology*. 1991; 3(3):304–10. [http://dx.doi.org/10.1016/0952-7915\(91\)90028-Y](http://dx.doi.org/10.1016/0952-7915(91)90028-Y). PMID: [1910607](#)
41. Matilainen JM, Räsänen A, Gynther P, Väisänen S. The genes encoding cytokines IL-2, IL-10 and IL-12B are primary $1\alpha,25(\text{OH})_2\text{D}_3$ target genes. *The Journal of Steroid Biochemistry and Molecular Biology*. 2010; 121(1–2):142–5. <http://dx.doi.org/10.1016/j.jsbmb.2010.03.020>. doi: [10.1016/j.jsbmb.2010.03.020](#) PMID: [20236616](#)
42. Czifra G, Toth B, Kovacs I, Biro T, Griger Z, Barath S, et al. The in vitro treatment with vitamin D3 is ineffective on the expression of PKC isoenzymes, but decreases further the impaired production of IL-2 in the T lymphocytes of SLE patients. *Rheumatology International*. 2014; 34(5):717–20. Epub 2013/04/25. doi: [10.1007/s00296-013-2751-y](#) PMID: [23612780](#).
43. Di Filippo P, Scaparrotta A, Rapino D, Cingolani A, Attanasi M, Petrosino MI, et al. Vitamin D Supplementation Modulates the Immune System and Improves Atopic Dermatitis in Children. *Int Arch Allergy Immunol*. 2015; 166(2):91–6. Epub 2015/03/21. doi: [10.1159/000371350](#) PMID: [25791938](#).
44. Barker T, Martins TB, Hill HR, Kjeldsberg CR, Dixon BM, Schneider ED, et al. Circulating pro-inflammatory cytokines are elevated and peak power output correlates with 25-hydroxyvitamin D in vitamin D insufficient adults. *European journal of applied physiology*. 2013; 113(6):1523–34. Epub 2013/01/08. doi: [10.1007/s00421-012-2582-7](#) PMID: [23292315](#).
45. Mazzucchelli L, Hauser C, Zraggen K, Wagner H, Hess M, Laissue JA, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. *The American journal of pathology*. 1994; 144(5):997. PMID: [8178948](#)
46. Szkaradkiewicz A, Marciniak R, Chudzicka-Strugała I, Wasilewska A, Drews M, Majewski P, et al. Proinflammatory cytokines and IL-10 in inflammatory bowel disease and colorectal cancer patients. *Archivum immunologiae et therapeuticae experimentalis*. 2009; 57(4):291–4. doi: [10.1007/s00005-009-0031-z](#) PMID: [19578817](#)
47. Hidaka M, Wakabayashi I, Takeda Y, Fukuzawa K. Vitamin D3 derivatives increase soluble CD14 release through ERK1/2 activation and decrease IL-8 production in intestinal epithelial cells. *European Journal of Pharmacology*. 2013; 721(1–3):305–12. <http://dx.doi.org/10.1016/j.ejphar.2013.09.014>. doi: [10.1016/j.ejphar.2013.09.014](#) PMID: [24060240](#)
48. Waldron JL, Ashby HL, Cornes MP, Bechervaise J, Razavi C, Thomas OL, et al. Vitamin D: a negative acute phase reactant. *Journal of Clinical Pathology*. 2013; 66(7):620–2. doi: [10.1136/jclinpath-2012-201301](#) PMID: [23454726](#)
49. Bang UC, Novovic S, Andersen AM, Fenger M, Hansen MB, Jensen J-EB. Variations in Serum 25-Hydroxyvitamin D during Acute Pancreatitis: An Exploratory Longitudinal Study. *Endocrine Research*. 2011; 36(4):135–41. doi: [10.3109/07435800.2011.554937](#) PMID: [21973232](#).
50. Henriksen VT, Rogers VE, Rasmussen GL, Trawick RH, Momberger NG, Aguirre D, et al. Pro-inflammatory cytokines mediate the decrease in serum 25(OH)D concentrations after total knee arthroplasty? *Med Hypotheses*. 2014; 82(2):134–7. Epub 2013/12/18. doi: [10.1016/j.mehy.2013.11.020](#) PMID: [24332533](#).
51. Barth JH, Field HP, Mather AN, Plein S. Serum 25 hydroxy-vitamin D does not exhibit an acute phase reaction after acute myocardial infarction. *Ann Clin Biochem*. 2012; 49(Pt 4):399–401. Epub 2012/05/01. doi: [10.1258/acb.2011.011195](#). PMID: [22543926](#). doi: [10.1258/acb.2011.011195](#)
52. Friis H, Range N, Pedersen ML, Molgaard C, Changalucha J, Krarup H, et al. Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. *J Nutr*. 2008; 138(12):2474–80. Epub 2008/11/22. doi: [10.3945/jn.108.094979](#) PMID: [19022975](#).
53. Reid D, Toole BJ, Knox S, Talwar D, Harten J, O'Reilly DSJ, et al. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. *The American Journal of Clinical Nutrition*. 2011; 93(5):1006–11. doi: [10.3945/ajcn.110.008490](#). PMID: [21411617](#)
54. Alvarez JA, Zughaier SM, Law J, Hao L, Wasse H, Ziegler TR, et al. Effects of high-dose cholecalciferol on serum markers of inflammation and immunity in patients with early chronic kidney disease. *European journal of clinical nutrition*. 2013; 67(3):264–9. Epub 2013/01/31. doi: [10.1038/ejcn.2012.217](#) PMID: [23361158](#); PubMed Central PMCID: [PMCPMC3824962](#).

55. Stubbs JR, Idiculla A, Slusser J, Menard R, Quarles LD. Cholecalciferol Supplementation Alters Calcitriol-Responsive Monocyte Proteins and Decreases Inflammatory Cytokines in ESRD. *Journal of the American Society of Nephrology*. 2010; 21(2):353–61. doi: [10.1681/asn.2009040451](https://doi.org/10.1681/asn.2009040451) PMID: [20007751](https://pubmed.ncbi.nlm.nih.gov/20007751/)
56. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *The American Journal of Clinical Nutrition*. 2006; 83(4):754–9. PMID: [16600924](https://pubmed.ncbi.nlm.nih.gov/16600924/)
57. Abou-Raya A, Abou-Raya S, Helmii M. The effect of vitamin D supplementation on inflammatory and hemostatic markers and disease activity in patients with systemic lupus erythematosus: a randomized placebo-controlled trial. *The Journal of rheumatology*. 2013; 40(3):265–72. Epub 2012/12/04. doi: [10.3899/jrheum.111594](https://doi.org/10.3899/jrheum.111594) PMID: [23204220](https://pubmed.ncbi.nlm.nih.gov/23204220/).
58. Hopkins MH, Owen J, Ahearn T, Fedirko V, Flanders WD, Jones DP, et al. Effects of Supplemental Vitamin D and Calcium on Biomarkers of Inflammation in Colorectal Adenoma Patients: A Randomized, Controlled Clinical Trial. *Cancer Prevention Research*. 2011; 4(10):1645–54. doi: [10.1158/1940-6207.capr-11-0105](https://doi.org/10.1158/1940-6207.capr-11-0105) PMID: [21724580](https://pubmed.ncbi.nlm.nih.gov/21724580/)
59. Cavalcante IG, Silva AS, Costa MJ, Persuhn DC, Issa CI, de Luna Freire TL, et al. Effect of vitamin D3 supplementation and influence of Bsm1 polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency: Vitamin D3 megadose reduces inflammatory markers. *Experimental gerontology*. 2015. Epub 2015/04/02. doi: [10.1016/j.exger.2015.03.011](https://doi.org/10.1016/j.exger.2015.03.011) PMID: [25827670](https://pubmed.ncbi.nlm.nih.gov/25827670/).
60. Sinha-Hikim I, Duran P, Shen R, Lee M, Friedman TC, Davidson MB. Effect of long term vitamin d supplementation on biomarkers of inflammation in latino and african-american subjects with pre-diabetes and hypovitaminosis d. *Hormone and Metabolic Research*. 2015; 47(4):280–3. Epub 2014/07/11. doi: [10.1055/s-0034-1383652](https://doi.org/10.1055/s-0034-1383652) PMID: [25011019](https://pubmed.ncbi.nlm.nih.gov/25011019/).
61. Carlson N, Mah R, Aburto M, Peters MJ, Dupper MV, Chen LH. Hypovitaminosis D correction and high-sensitivity C-reactive protein levels in hypertensive adults. *Perm J*. 2013; 17(4):19–21. Epub 2013/12/24. doi: [10.7812/tpj/13-007](https://doi.org/10.7812/tpj/13-007) PMID: [24361015](https://pubmed.ncbi.nlm.nih.gov/24361015/); PubMed Central PMCID: [PMCPMC3854803](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC3854803/).
62. Grzanka A, Machura E, Mazur B, Misiolek M, Jochem J, Kasperski J, et al. Relationship between vitamin D status and the inflammatory state in patients with chronic spontaneous urticaria. *Journal of Inflammation* 2014; 11(1):2. Epub 2014/02/04. doi: [10.1186/1476-9255-11-2](https://doi.org/10.1186/1476-9255-11-2) PMID: [24484740](https://pubmed.ncbi.nlm.nih.gov/24484740/); PubMed Central PMCID: [PMCPMC3913618](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC3913618/).
63. Karim Y, Turner C, Dalton N, Roplekar R, Sankaralingam A, Ewang M, et al. The relationship between pro-resorptive inflammatory cytokines and the effect of high dose vitamin D supplementation on their circulating concentrations. *International Immunopharmacology*. 2013; 17(3):693–7. <http://dx.doi.org/10.1016/j.intimp.2013.08.010> doi: [10.1016/j.intimp.2013.08.010](https://doi.org/10.1016/j.intimp.2013.08.010) PMID: [24007780](https://pubmed.ncbi.nlm.nih.gov/24007780/)
64. Cannell JJ, Grant WB, Holick MF. Vitamin D and inflammation. *Dermato-Endocrinology*. 2014; 6(1):e983401. doi: [10.4161/19381980.2014.983401](https://doi.org/10.4161/19381980.2014.983401)
65. Li YC, Chen Y, Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. *The Journal of Steroid Biochemistry and Molecular Biology* 2015; 148:179–83. Epub 2015/01/21. doi: [10.1016/j.jsbmb.2015.01.011](https://doi.org/10.1016/j.jsbmb.2015.01.011) PMID: [25603468](https://pubmed.ncbi.nlm.nih.gov/25603468/); PubMed Central PMCID: [PMCPMC4361385](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC4361385/).
66. Chen Y, Du J, Zhang Z, Liu T, Shi Y, Ge X, et al. MicroRNA-346 mediates tumor necrosis factor alpha-induced downregulation of gut epithelial vitamin D receptor in inflammatory bowel diseases. *Inflammatory Bowel Diseases*. 2014; 20(11):1910–8. Epub 2014/09/06. doi: [10.1097/mib.0000000000000158](https://doi.org/10.1097/mib.0000000000000158) PMID: [25192497](https://pubmed.ncbi.nlm.nih.gov/25192497/).
67. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol*. 2005; 35(1):217–24. Epub 2004/12/14. doi: [10.1002/eji.200425491](https://doi.org/10.1002/eji.200425491) PMID: [15593122](https://pubmed.ncbi.nlm.nih.gov/15593122/).
68. Froicu M, Cantorna MT. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC immunology*. 2007; 8:5. Epub 2007/04/03. doi: [10.1186/1471-2172-8-5](https://doi.org/10.1186/1471-2172-8-5) PMID: [17397543](https://pubmed.ncbi.nlm.nih.gov/17397543/); PubMed Central PMCID: [PMCPmc1852118](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC1852118/).
69. Yang L, Weaver V, Smith JP, Bingaman S, Hartman TJ, Cantorna MT. Therapeutic effect of vitamin d supplementation in a pilot study of Crohn's patients. *Clin Transl Gastroenterol*. 2013; 4:e33. Epub 2013/04/19. doi: [10.1038/ctg.2013.1](https://doi.org/10.1038/ctg.2013.1) PMID: [23594800](https://pubmed.ncbi.nlm.nih.gov/23594800/); PubMed Central PMCID: [PMCPMC3636524](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC3636524/).