Relationship between vitamin D status and clinical outcomes in dogs with a cranial cruciate ligament rupture

Relationship between vitamin D status and clinical outcomes in dogs with a cranial cruciate ligament rupture

Dylan N. Clements [a], John M. Ryan [a], Ian G. Handel [a], Adam G. Gow [a], Susan Campbell [a], Emma Hurst [a,b] and Richard J Mellanby [a*]

[a] Royal (Dick) School of Veterinary Studies and The Roslin Institute,

The University of Edinburgh,

Hospital for Small Animals,

Easter Bush Veterinary Centre,

Roslin, Midlothian,

EH25 9RG,

United Kingdom.

[b] Mass Spectrometry Core

Edinburgh Clinical Research Facility

Queen's Medical Research Institute

The University of Edinburgh

Little France Crescent

Edinburgh

Scotland

EH16 4TJ

*Corresponding author Richard.Mellanby@ed.ac.uk

Key words : cranial cruciate ligament rupture, dog, vitamin D, 25 hydroxyvitamin D

Abstract

Cranial cruciate ligament rupture (CCLR) is one of the most common orthopaedic disorders diagnosed in dogs yet the factors which influence postoperative clinical outcomes are poorly understood. Low vitamin D status has been linked to poorer clinical outcomes in human patients undergoing elective orthopaedic surgery. The aim of this study was to examine the relationship between pre-operative vitamin D status, as defined by serum 25 hydroxyvitamin D (25(OH)D) concentrations, and initial disease severity and clinical outcomes in dogs undergoing surgical treatment for a CCLR .

Serum 25(OH)D concentrations were measured in 44 dogs with a CCLR on the day before surgery. C-reactive protein concentrations were measured at a median time of 1 day post-surgery and the patient's clinical and radiographic response to CCLR surgical treatment was assessed at a median timepoint of 60 days post-surgery. Serum 25(OH)D concentrations in dogs with a CCLR was not significantly different to a population of healthy dogs (median 74.1 nmol/L and 88.40 nmol/L, respectively). There was no significant correlation between pre-operative serum 25(OH)D concentrations and length of pre-diagnosis clinical signs, pre-operative lameness scores or day 1 post-operative CRP concentrations. Thirty nine of the 44 dogs were re-examined at a median 60 days post-surgery. There was no relationship between the day 60 lameness scores and pre-operative serum 25(OH)D concentrations.

In summary, we discovered that the vitamin D status of dogs with a CCLR was not significantly lower than healthy dogs and pre-operative serum 25(OH)D concentrations were not correlated to either pre-surgical disease severity or post-operative clinical outcomes.

Introduction

Cranial cruciate ligament rupture (CCLR) is a one of the most common causes of hind limb lameness and osteoarthritis in dogs (Taylor-Brown et al., 2015). Female, neutered large breed dogs have been reported to be predisposed and the underlying aetiology of the disease remains poorly understood (Comerford et al., 2011; Taylor-Brown et al., 2015). Cranial cruciate ligament rupture is typically managed by surgical treatments with a wide range of approaches reported including extracapsular, intracapsular and osteotomy techniques (Molsa et al., 2014). Whilst the response to treatment is typically good, the factors involved in suboptimal clinical outcomes post-surgery include high body weight (Casale and McCarthy, 2009; Coletti et al., 2014), German Shepherd breed (Coletti et al., 2014), a tibial plaeau angle greater than 30° (Coletti et al., 2014) and young age at time of surgery (Casale and McCarthy, 2009). A better understanding of the factors involved in suboptimal clinical outcomes such as mobility, exercise tolerance and surgical healing following surgery in dogs with a CCLR would help guide the development of novel approaches to improve treatment outcomes.

Vitamin D has historically being linked to the development and maintenance of skeletal health and the regulation of calcium homeostasis (Mellanby, 2016). However, low vitamin D status is increasingly linked to non-skeletal canine disorders driven, in part, by a growing appreciation that the vitamin D receptor is widely expressed on canine tissues (Cartwright et al., 2018). For example, hypovitaminosis D has been reported in dogs with infectious (Dvir et al., 2019; Rosa et al., 2013), neoplastic (Wakshlag et al., 2011) and inflammatory conditions (Gow et al., 2011). Furthermore, low serum concentrations of 25 hydroxyvitamin D, the vitamin D metabolite which is most widely used to assess vitamin D status, have been linked to a pro-inflammatory immune response in dogs and cats (Titmarsh et al., 2016; Titmarsh et al., 2015c) and increased mortality rates in companion animals (Allenspach et al., 2017; Titmarsh et al., 2015a; Titmarsh et al., 2015b). There is a growing evidence base that vitamin D can influence the evolution of an immune response both in experimental models of disease and in *ex-vivo* studies of canine leukocytes fuelling interest in the

wider health benefits of vitamin D in dogs (Besusso et al., 2015; Jaffey et al., 2018a, b; Saul et al., 2019).

Despite the growing interest in the role vitamin D may play in skeletal and non-skeletal health in companion animals, little work has been undertaken to explore the relationship between vitamin D status and orthopaedic disease severity and response to treatment. This contrasts to the situation in human orthopaedics where the relationship between circulating 25(OH)D concentrations and orthopaedic disorders have been extensively explored (Iglar and Hogan, 2015; Zhang et al., 2018). Human patients undergoing elective knee arthroplasty are often vitamin D insufficient or deficient (Reid et al., 2011; Waldron et al., 2013) and lower 25(OH)D concentrations have been associated with more severe pre-operative clinical signs (Maniar et al., 2016). In addition, low pre-operative vitamin D status has been linked to poorer clinical outcomes in patients undergoing elective knee surgery (Shin et al., 2017). The relationship between vitamin D status and orthopaedic disorders was emphasised in a recent meta-analysis of 12 studies which examined the relationship between pre-operative 25(OH)D concentrations and clinical presentation and outcomes in patients undergoing knee or hip surgery. This meta-analysis found that pre-operative Harris Hip Scores and Knee Society Score, which assess hip and knee pain and function respectively, revealed greater disease burden in patients with low vitamin D status compared to patients with a normal vitamin D status (Zhang et al., 2018). In addition, post-operative Harris Hip Score was significantly lower in the patients which were hypovitaminosis D pre-operative than in the euvitaminosis D group (Zhang et al., 2018). In light of the evidence that vitamin D status is linked to presentation and outcome in human elective knee surgery and the paucity of research in this area in veterinary patients, the aim of this study was to investigate the relationship between pre-operative serum 25(OH)D concentrations and presenting clinical signs and subsequent post-operative clinical outcomes.

Methods

All dogs diagnosed with a CCLR at the Hospital for Small Animals, University of Edinburgh between June 2015 and December 2017 were considered eligible for inclusion in the study. Entry criteria included a diagnosis of CCLR as confirmed by direct visual inspection of the ruptured ligament during exploratory arthrotomy, a pre-operative measurement of serum 25(OH)D, a one day post-surgery CRP measurement and a pre-operative lameness assessment. The onset of lameness and use of non-steroidal anti-inflammatory drugs (NSAID) were ascertained from the clinical history at presentation. The lameness of each dog was visually assessed preoperative and ascribed a lameness score using a numerical rating scale (Impellizeri et al., 2000). A radiographic osteophytosis score was ascribed from the pre-operative radiographs from all cases using a subjective grading scheme (Rayward et al., 2004). All cases were invited to be re-evaluated 60 days following surgery, for re-assessment of lameness and radiographic follow up. The requirement for continued (>2 week) NSAID analgesia was also recorded. The healing of the osteotomy was graded radiographically at re-evaluation on day 60 using an osteotomy healing classification (Conkling et al., 2010). All radiographic assessments were undertaken by a single European diplomat in small animal surgery (DC), and all lameness assessments were undertaken by a European diplomat in small animal surgery. The surgical treatment method undertaken was decided upon by the attending clinician and was either a tibial plateau levelling osteotomy (TPLO) or cranial closing wedge osteotomy (CCWO).

Blood samples collected pre-operative and at one day post-surgery were placed into plain blood collection tubes which were refrigerated immediately after collection. The samples were then centrifuged and serum frozen at -80˚C until analysis within 3 hours of collection. C reactive protein concentrations were measured using an Au480 Chemistry Analyser (Beckman Coulter (UK) Ltd, High Wycombe, UK) using a species-specific immunoturbidimetric assay. Serum concentrations of total $25(OH)D_2$ and $25(OH)D_3$ were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) by the Vitamin D Animal Laboratory (VitDAL) which has been certified as proficient by the international Vitamin D Quality Assurance Scheme (DEQAS) as previously described (Hurst et al.,

2020b). The results are presented as total 25(OH)D which is defined as the sum of 25(OH)D_2 and 25(OH)D_3.

A power calculation revealed 40 patients were required in the healthy and CCLR arms to have an 80% chance of detecting, as significant at $p<0.05$, a 20nmol/l change in 25(OH)D concentrations between the healthy group (mean 25(OH)D conc 89.1 nmol/l, standard deviation 32.0) and the CCLR group (Hurst et al., 2020a). The serum 25(OH)D concentrations between healthy dogs and dogs with CCLR were compared using a Mann Whitney U test. A control population of healthy control dogs was used from a previously reported study which includes details of their signalment (Hurst et al., 2020a). This population of dogs were recruited in the same hospital over a similar timeframe as the CCLR dogs and had 25(OH)D measured in the same laboratory using the same LC-MS/MS equipment. The presence of a correlation between pre-operative vitamin D concentrations and clinical variable was assessed using a Spearman rank correlation test. Differences in serum 25(OH)D concentrations between dogs with various defined pre- or post-operative clinical assessment scores were compared using either a Mann-Whitney U test or a Kruskall Wallis test. Significance was set at $p<0.05$.

The study was approved by the University of Edinburgh Veterinary Ethics Research Committee and the University of Edinburgh Animal Welfare and Ethics Review Board (project license number 7007937, date approved 30th December 2016).

Results

Forty four dogs were enrolled on the study. The median age of the dogs was 7.1 years and ranged from 2.1 years to 12.9 years. There were 20 neutered females, 18 neutered males, three entire females and three entire males. The cohort consisted of sixteen Labradors, ten Crossbreed dogs, four Cocker spaniels, four golden retrievers, two Staffordshire Bull terriers, one Border terrier, one border collie, one Bulldog, one Beagle, one Boxer, one English springer spaniel, one chow and one Dogue de Bordeaux. The median weight of the dogs was 29.3kg (range 14.0kg to 64.4kg). Twenty four dogs had a rupture of the right and twenty dogs had a rupture of the left cranial cruciate ligament. The median duration of clinical signs was 8 weeks (range 2 weeks to 52 weeks). A TPLO surgical procedure was undertaken in 31 dogs and a CCWO was performed in 13 dogs.

The median serum 25(OH)D concentration the day before surgery was 74.1 nmol/L (range 43.5 – 149.8 nmol/L) which was not significantly different from the median serum 25(OH)D concentration we previously reported in 117 healthy control dogs (88.4 nmol/L, p=0.10) (figure 1) (Hurst et al., 2020a). None of the dogs with a CCLR had a pre-operative serum 25(OH)D concentration outside the reference range of 39.5 – 172.7 nmol/L. There was no correlation between pre-operative 25(OH)D concentration and duration of pre-diagnosis clinical signs (r=0.08, p=0.61) or pre-operative lameness scores (r=-0.13, p=0.42). Nine dogs were not receiving NSAID treatment at time of presentation. There was no significant difference in 25(OH)D concentrations between dogs which were receiving NSAID treatment at time of diagnosis compared to dogs which were not administered NSAIDs (p=0.23). There was no significant difference between 25(OH)D concentrations and pre-operative osteophytosis scores (p=0.94).

There was no correlation between pre-operative serum 25(OH)D concentration and median 1 day post-operative CRP concentrations (r=0.14, p=0.37). Thirty nine of the 44 dogs were re-examined at a median 60 days after surgery. The radiographic healing was assessed to be 1 in 6 dogs, 2 in 7 dogs,

3 in 14 dogs and 4 in 12 dogs. There was no difference in the pre-operative 25(OH)D concentrations in the 4 radiographic healing score subgroups (p=0.55). There was no difference in pre-operative 25(OH)D concentrations in dogs which were administered NSAIDs at day 60 (n=10) compared to dogs which did not require NSAIDs treatment (n=39) (p=0.86). The lameness scores declined in 36 of the 39 dogs by day 60 post-surgery with one dog's lameness score remaining unchanged and the lameness scores of two dogs slightly increased. There was no relationship between the day 60 post-surgery lameness scores and pre-operative 25(OH)D concentrations (p=0.85). Finally, there was no relationship between 25(OH)D concentrations and the extent of change in lameness scores from pre-operative to day 60 assessments (p=0.97).

Discussion

This study has revealed that dogs with a CCLR did not have a significantly lower vitamin D status than healthy dogs, with no dogs diagnosed with a CCLR having a pre-operative serum 25(OH)D concentration outside the reference range. In addition, pre-operative vitamin D status was not correlated to immediate post-operative inflammation, as assessed by CRP concentrations, or to longer term post-operative clinical outcomes. Whilst we cannot discount the clear possibility that our study was under powered to detect relatively modest impacts on vitamin D status and CCLR surgical outcomes, our study provides no evidence that pre-operative vitamin D status has a dramatic impact on shaping the clinical outcomes in dogs undergoing surgical treatment of CCLR.

In contrast, several human studies of elective knee surgery have shown that patients often are pre-operatively vitamin D deplete (Bogunovic et al., 2010; Reid et al., 2011; Waldron et al., 2013; Zhang et al., 2018). This may relate to the differential importance of the source of vitamin D in dogs compared to humans as dogs are entirely reliant on oral sources of vitamin D whereas vitamin D can be obtained from the diet or be cutaneously produced in humans (Hurst et al., 2020b; Mellanby, 2016). Since the appetite of dogs with a CCLR is often normal, the amount of vitamin D consumed in their diet is unlikely to be different from healthy dogs. Humans with chronic knee disease and reduced ambulatory capacity may not be able to access outside areas as readily as healthy individuals, thereby reducing UVB exposure, leading to lower vitamin D production in the skin (Elder and Bishop, 2014). As dogs do not produce vitamin D cutaneously, variation in UVB exposure following a CCLR injury will not influence their vitamin D status.

Another potential reason why the serum 25(OH)D were not significantly different from healthy dogs is the lack of systemic inflammation which is typically present pre-operatively in dogs with a chronic CCLR injury (Lofqvist et al., 2018). Systemic inflammation is frequently negatively correlated to vitamin D status in both veterinary and human patients (Mellanby, 2016). For example, in dogs with

a protein losing enteropathy serum 25(OH)D concentrations negatively correlated with neutrophil, monocyte and plasma cytokines (Titmarsh et al., 2015c). Further support for the role inflammation plays in lowering vitamin D status comes from human orthopaedic studies were serum 25(OH)D concentrations often decline following elective surgery following the induction of a strong systemic inflammatory response (Reid et al., 2011; Waldron et al., 2013). In dogs with a CCLR, pre-operative C-reactive protein concentrations, a widely used measure of systemic inflammation, are typically low which indicates that systemic inflammation, which is present in many diseases where hypovitaminosis D has been reported (Dvir et al., 2019; Gow et al., 2011; Rosa et al., 2013), is unlikely to be present or manipulating vitamin D status in dogs with a CCLR (Lofqvist et al., 2018).

We found no evidence that pre-operative vitamin D status was linked to the extent of post-operative inflammatory response as assessed by CRP concentrations. There is growing interest in the concept that a low vitamin D status may predispose to the development of inflammation based mainly on the findings of negative associations between pro-inflammatory biomarkers and circulating 25(OH)D concentrations in cross-sectional human studies (de Oliveira et al., 2017; Peterson and Heffernan, 2008). However, these observations could be explained by reverse causation and no relationship between vitamin D status and inflammation has been reported in human orthopaedic studies. In addition, studies in humans following knee arthroplasty found the extent of changes in 25(OH)D concentrations immediately after surgery did not correlate to post-operative CRP concentrations (Reid et al., 2011; Waldron et al., 2013). These observations suggest that vitamin D status may only have a limited impact on modifying post-operative inflammation which is consistent with systematic reviews reporting that vitamin D supplementation has a modest impact on circulating biomarkers of inflammation (Mazidi, 2018).

We found no evidence that vitamin D status is associated with any of the pre-operative disease severity scores or post-operative clinical outcomes that we measured. As we used a wide range of

measures including clinical and radiographic assessment of disease severity and clinical improvement, we can be confident that in our cohort there was no association between low vitamin D status and increased pre-operative disease severity or adverse clinical outcomes. However, clinical assessments were subjective, and so may lack the sensitivity to detect subtleties in outcome which would be verbally communicated by human patients, resulting in a more crude outcome grading system in dogs with a CCLR. A limitation of this study was that we only included 44 dogs with CCLR but the failure to detect any relationship between 25(OH)D concentration and any clinical outcome measures suggests that vitamin D status is unlikely to play an important part in shaping clinical disease severity or outcomes in dogs with a CCLR. This contrasts with human patients undergoing elective orthopaedic surgery where low vitamin D status has been linked to lower pre-operative functional performance (Maniar et al., 2016), longer post-operative hospitalisation stays (Maier et al., 2016), adverse post-operative functional outcomes (Shin et al., 2017) and reduced post-surgery muscle strength recovery (Barker, 2011). The difference may be related to the relative greater importance vitamin D plays in ameliorating clinical signs in orthopaedic diseases and supporting recovery in humans compared to dogs. It may also reflect that all the dogs with a CCLR in our study had a serum 25(OH)D concentration within the reference range with no dogs having a 25(OH)D serum concentration below 40nmol/l and only 4 out of 44 dogs (9%) having a serum 25(OH)D concentration below 50nmol/l. This is much lower proportion than is widely reported in human patients with chronic orthopaedic disorders (Zhang et al., 2018). This observation may be important since clinical trials of vitamin D supplementation in other human conditions have shown that the patients which benefit the most are the individuals with vitamin D deficiency at point of randomisation (Jolliffe et al., 2017; Martineau et al., 2017). As hypovitaminosis D is more commonly observed in humans with orthopaedic disorders compared to dogs, it may be more feasible to probe the relationship between vitamin D status and clinical outcomes in humans than in dogs.

Conclusion

In summary, our study has indicated that dogs with a CCLR do not have significantly lower vitamin D status than healthy dogs. In addition, serum 25(OH)D concentrations did not correlate to pre-operative clinical signs or post-operative clinical outcomes. Further studies are required to explore the factors behind suboptimal post-surgery CCLR clinical outcomes.

Conflict of interest

The 25(OH)D measurements were performed in the corresponding author's laboratory which offers a 25(OH)D assay to external parties on a not-for-profit basis.

Acknowledgements

The authors acknowledge the support of all the staff and owners involved in the care of the dogs reported in the study.

Figure legends

Figure 1 : Serum 25(OH)D concentrations in healthy dogs (Hurst et al., 2020a) and dogs diagnosed with a CCLR (CCLR).

References

Allenspach, K., Rizzo, J., Jergens, A.E., Chang, Y.M., 2017. Hypovitaminosis D is associated with negative outcome in dogs with protein losing enteropathy: a retrospective study of 43 cases. BMC Vet Res 13, 96.

Barker, T., Martins, T.B, Hill, H.R., Kjeldberg, C.R., Trawick, R.H., Weaver, L.K. and Traber, M.G. , 2011. Low vitamin D impairs strength recovery after anterior cruciate ligament surgery. Journal of Evidence-Based Complementary & Alternative Medicine 16(3), 201-209.

Besusso, D., Saul, L., Leech, M.D., O'Connor, R.A., MacDonald, A.S., Anderton, S.M., Mellanby, R.J., 2015. 1,25-Dihydroxyvitamin D3-Conditioned CD11c+ Dendritic Cells are Effective Initiators of CNS Autoimmune Disease. Front Immunol 6, 575.

Bogunovic, L., Kim, A.D., Beamer, B.S., Nguyen, J., Lane, J.M., 2010. Hypovitaminosis D in patients scheduled to undergo orthopaedic surgery: a single-center analysis. J Bone Joint Surg Am 92, 2300-2304.

Cartwright, J.A., Gow, A.G., Milne, E., Drummond, D., Smith, S., Handel, I., Mellanby, R.J., 2018. Vitamin D Receptor Expression in Dogs. J Vet Intern Med 32, 764-774.

Casale, S.A., McCarthy, R.J., 2009. Complications associated with lateral fabellotibial suture surgery for cranial cruciate ligament injury in dogs: 363 cases (1997-2005). J Am Vet Med Assoc 234, 229-235.

Coletti, T.J., Anderson, M., Gorse, M.J., Madsen, R., 2014. Complications associated with tibial plateau leveling osteotomy: a retrospective of 1519 procedures. Can Vet J 55, 249-254.

Comerford, E.J., Smith, K., Hayashi, K., 2011. Update on the aetiopathogenesis of canine cranial cruciate ligament disease. Vet Comp Orthop Traumatol 24, 91-98.

Conkling, A.L., Fagin, B., Daye, R.M., 2010. Comparison of tibial plateau angle changes after tibial plateau leveling osteotomy fixation with conventional or locking screw technology. Vet Surg 39, 475-481.

de Oliveira, C., Biddulph, J.P., Hirani, V., Schneider, I.J.C., 2017. Vitamin D and inflammatory markers: cross-sectional analyses using data from the English Longitudinal Study of Ageing (ELSA). J Nutr Sci 6, e1.

Dvir, E., Rosa, C., Handel, I., Mellanby, R.J., Schoeman, J.P., 2019. Vitamin D status in dogs with babesiosis. Onderstepoort J Vet Res 86, e1-e5.

Elder, C.J., Bishop, N.J., 2014. Rickets. Lancet 383, 1665-1676.

Gow, A.G., Else, R., Evans, H., Berry, J.L., Herrtage, M.E., Mellanby, R.J., 2011. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. J Small Anim Pract 52, 411-418.

Hurst, E.A., Homer, N.Z., Denham, S.G., MacFarlane, E., Campbell, S., Boswinkel, M., Mellanby, R.J., 2020a. Development and application of a LC-MS/MS assay for simultaneous analysis of 25-

hydroxyvitamin-D and 3-epi-25-hydroxyvitamin-D metabolites in canine serum. J Steroid Biochem Mol Biol 199, 105598.
Hurst, E.A., Homer, N.Z., Gow, A.G., Clements, D.N., Evans, H., Gaylor, D., Campbell, S., Handel, I., Mellanby, R.J., 2020b. Vitamin D status is seasonally stable in northern European dogs. Vet Clin Pathol.
Iglar, P.J., Hogan, K.J., 2015. Vitamin D status and surgical outcomes: a systematic review. Patient Saf Surg 9, 14.
Impellizeri, J.A., Tetrick, M.A., Muir, P., 2000. Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. J Am Vet Med Assoc 216, 1089-1091.
Jaffey, J.A., Amorim, J., DeClue, A.E., 2018a. Effects of calcitriol on apoptosis, toll-like receptor 4 expression, and cytokine production of endotoxin-primed canine leukocytes. Am J Vet Res 79, 1071-1078.
Jaffey, J.A., Amorim, J., DeClue, A.E., 2018b. Effects of calcitriol on phagocytic function, toll-like receptor 4 expression, and cytokine production of canine leukocytes. Am J Vet Res 79, 1064-1070.
Jolliffe, D.A., Greenberg, L., Hooper, R.L., Griffiths, C.J., Camargo, C.A., Jr., Kerley, C.P., Jensen, M.E., Mauger, D., Stelmach, I., Urashima, M., Martineau, A.R., 2017. Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data. Lancet Respir Med 5, 881-890.
Lofqvist, K., Kjelgaard-Hansen, M., Nielsen, M.B.M., 2018. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau leveling osteotomy in dogs. Acta Vet Scand 60, 30.
Maier, G.S., Maus, U., Lazovic, D., Horas, K., Roth, K.E., Kurth, A.A., 2016. Is there an association between low serum 25-OH-D levels and the length of hospital stay in orthopaedic patients after arthroplasty? J Orthop Traumatol 17, 297-302.
Maniar, R.N., Patil, A.M., Maniar, A.R., Gangaraju, B., Singh, J., 2016. Effect of Preoperative Vitamin D Levels on Functional Performance after Total Knee Arthroplasty. Clin Orthop Surg 8, 153-156.
Martineau, A.R., Jolliffe, D.A., Hooper, R.L., Greenberg, L., Aloia, J.F., Bergman, P., Dubnov-Raz, G., Esposito, S., Ganmaa, D., Ginde, A.A., Goodall, E.C., Grant, C.C., Griffiths, C.J., Janssens, W., Laaksi, I., Manaseki-Holland, S., Mauger, D., Murdoch, D.R., Neale, R., Rees, J.R., Simpson, S., Jr., Stelmach, I., Kumar, G.T., Urashima, M., Camargo, C.A., Jr., 2017. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 356, i6583.
Mazidi, M., Rezaie, P. and Vatanparast, H. , 2018. Impact of vitamin D supplementation on C-reactive protein; a systematic review and meta-analysis of randomized controlled trials. BMC Nutr 4, 1.
Mellanby, R.J., 2016. Beyond the skeleton: the role of vitamin D in companion animal health. J Small Anim Pract 57, 175-180.
Molsa, S.H., Hyytiainen, H.K., Hielm-Bjorkman, A.K., Laitinen-Vapaavuori, O.M., 2014. Long-term functional outcome after surgical repair of cranial cruciate ligament disease in dogs. BMC Vet Res 10, 266.
Peterson, C.A., Heffernan, M.E., 2008. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond) 5, 10.
Rayward, R.M., Thomson, D.G., Davies, J.V., Innes, J.F., Whitelock, R.G., 2004. Progression of osteoarthritis following TPLO surgery: a prospective radiographic study of 40 dogs. J Small Anim Pract 45, 92-97.
Reid, D., Toole, B.J., Knox, S., Talwar, D., Harten, J., O'Reilly, D.S., Blackwell, S., Kinsella, J., McMillan, D.C., Wallace, A.M., 2011. The relation between acute changes in the systemic inflammatory response and plasma 25-hydroxyvitamin D concentrations after elective knee arthroplasty. Am J Clin Nutr 93, 1006-1011.
Rosa, C.T., Schoeman, J.P., Berry, J.L., Mellanby, R.J., Dvir, E., 2013. Hypovitaminosis d in dogs with spirocercosis. J Vet Intern Med 27, 1159-1164.

Saul, L., Mair, I., Ivens, A., Brown, P., Samuel, K., Campbell, J.D.M., Soong, D.Y., Kamenjarin, N., Mellanby, R.J., 2019. 1,25-Dihydroxyvitamin D3 Restrains CD4(+) T Cell Priming Ability of CD11c(+) Dendritic Cells by Upregulating Expression of CD31. Front Immunol 10, 600.
Shin, K.Y., Park, K.K., Moon, S.H., Yang, I.H., Choi, H.J., Lee, W.S., 2017. Vitamin D deficiency adversely affects early post-operative functional outcomes after total knee arthroplasty. Knee Surg Sports Traumatol Arthrosc 25, 3424-3430.
Taylor-Brown, F.E., Meeson, R.L., Brodbelt, D.C., Church, D.B., McGreevy, P.D., Thomson, P.C., O'Neill, D.G., 2015. Epidemiology of Cranial Cruciate Ligament Disease Diagnosis in Dogs Attending Primary-Care Veterinary Practices in England. Vet Surg 44, 777-783.
Titmarsh, H., Gow, A.G., Kilpatrick, S., Sinclair, J., Hill, T., Milne, E., Philbey, A., Berry, J., Handel, I., Mellanby, R.J., 2015a. Association of Vitamin D Status and Clinical Outcome in Dogs with a Chronic Enteropathy. J Vet Intern Med.
Titmarsh, H., Kilpatrick, S., Sinclair, J., Boag, A., Bode, E.F., Lalor, S.M., Gaylor, D., Berry, J., Bommer, N.X., Gunn-Moore, D., Reed, N., Handel, I., Mellanby, R.J., 2015b. Vitamin D status predicts 30 day mortality in hospitalised cats. PLoS One 10, e0125997.
Titmarsh, H.F., Cartwright, J.A., Kilpatrick, S., Gaylor, D., Milne, E.M., Berry, J.L., Bommer, N.X., Gunn-Moore, D., Reed, N., Handel, I., Mellanby, R.J., 2016. Relationship between vitamin D status and leukocytes in hospitalised cats. J Feline Med Surg.
Titmarsh, H.F., Gow, A.G., Kilpatrick, S., Cartwright, J.A., Milne, E.M., Philbey, A.W., Berry, J., Handel, I., Mellanby, R.J., 2015c. Low Vitamin D Status Is Associated with Systemic and Gastrointestinal Inflammation in Dogs with a Chronic Enteropathy. PLoS One 10, e0137377.
Wakshlag, J.J., Rassnick, K.M., Malone, E.K., Struble, A.M., Vachhani, P., Trump, D.L., Tian, L., 2011. Cross-sectional study to investigate the association between vitamin D status and cutaneous mast cell tumours in Labrador retrievers. Br J Nutr 106 Suppl 1, S60-63.
Waldron, J.L., Ashby, H.L., Cornes, M.P., Bechervaise, J., Razavi, C., Thomas, O.L., Chugh, S., Deshpande, S., Ford, C., Gama, R., 2013. Vitamin D: a negative acute phase reactant. J Clin Pathol 66, 620-622.
Zhang, H., Zhu, X.H., Dong, W., Wang, Q.M., 2018. Vitamin D Status and Patient Outcomes after Knee or Hip Surgery: A Meta-Analysis. Ann Nutr Metab 73, 121-130.