RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2025; 39 (2): 90 - 96
http://www.fusabil.org

Serum Iron and Vitamin D Levels During Experimentally Induced Sterile Inflammation in Rabbits *

Mehmet ÇALIŞKAN [1, a]
Sümeyye BABACAN [1, b]

[1] Fırat University,
Faculty of Veterinary Medicine,
Department of Internal Medicine,
Elazığ, TÜRKİYE

[a] ORCİD: 0000-0002-8843-8394
[b] ORCİD: 0000-0001-8007-8315

Vitamin D deficiency is frequently reported in inflammatory diseases. However, the causal relationship between vitamin D and inflammation has not yet been fully elucidated. The aim of this study was to investigate the changes in vitamin D levels during the course of sterile inflammation. Twenty rabbits were randomly divided into two groups: The turpentine oil group (TOG, n=12) and the control group (CG, n=8). Sterile inflammation was induced in the TOG by turpentine oil injection, while the CG received saline. Blood samples were collected before injection (0 h) and at 6, 12, 24, and 72 hours post-injection, and serum levels of 25-hydroxyvitamin D (25-D) and 1.25-dihydroxyvitamin D (1.25-D) were measured. To confirm inflammation, body temperature, white blood cell (WBC) count, and serum iron levels were monitored. In the TOG, body temperature increased significantly from 12 hours onward, and WBC count decreased from 6 hours. Serum iron levels declined at 6 hours, remained low at 12 and 24 hours, and returned to CG levels at 72 hours. Between-group comparisons showed that 25-D levels in the TOG decreased at 72 hours, while no significant difference was observed in 1.25-D levels. In within-group comparisons, 25-D levels in the TOG were lower at 72 hours compared to baseline, whereas 1.25-D levels increased at 12 and 72 hours. These results indicate that sterile inflammation decreases 25-D and increases 1.25-D levels, suggesting that inflammation may play a role in vitamin D deficiency observed in many inflammatory diseases.

Key Words: *Rabbit, turpentine oil, sterile inflammation, 25-hydroxyvitamin D, 1.25-dihydroxyvitamin D*

Tavşanlarda Deneysel Olarak Oluşturulan Steril İnflamasyon Sürecinde Serum Demir ve D Vitamini Düzeyleri

İnflamatuvar hastalıklarda D vitamini düşüşleri yaygın olarak bildirilmektedir. Ancak, vitamin D ile inflamasyon arasındaki sebep-sonuç ilişkisi tam olarak açıklanamamıştır. Bu çalışmanın amacı, steril bir inflamasyon süreci boyunca D vitamini seviyelerindeki değişiklikleri belirlemektir. Çalışmada yirmi adet tavşan rastgele iki gruba ayrılmıştır: turpentin oil grubu (TOG, n=12) ve kontrol grubu (CG, n=8). Steril inflamasyon oluşturmak amacıyla TOG'a turpentin oil, CG'ye ise tuzlu su enjeksiyonu uygulanmıştır. Kan örnekleri enjeksiyon öncesi (0. saat) ve enjeksiyondan 6, 12, 24 ve 72 saat sonra toplanmış ve 25-hidroksivitamin D (25-D) ve 1,25-dihidroksivitamin D (1,25-D) seviyeleri ölçülmüştür. Ayrıca inflamasyonun doğrulanması için vücut sıcaklığı, WBC sayısı ve serum demir düzeyleri de izlenmiştir. Vücut sıcaklığı TOG'da 12. saatten itiberen CG'ye göre anlamlı olarak artmış. WBC ise 6. saatten itibaren azalmıştır. Serum demir düzeyleri TOG'da 6. saatte düşmüş, 12. ve 24. saatlerde düşük kalmış ve 72. saatte CG seviyelerine ulaşmıştır. Gruplar arası karşılaştırmalarda 25-D seviyelerinin TOG'da, 72. saatte CG'ye göre azaldığı, 1,25-D seviyelerinde ise bir fark gözlemlenmiştir. Grup içi karşılaştırmalarda ise, 0. saate kıyasla 25-D seviyelerinin TOG'da 72. saatte azaldığı; 1,25-D seviyelerinin ise 12. ve 72. saatlerde arttığı belirlenmiştir. Veriler, steril inflamasyonun 25-D seviyelerini düşürüp 1,25-D seviyelerini artırdığını göstermektedir. Bu durum, inflamasyonun birçok inflamatuvar hastalıkta görülen D vitamini düşüklüğünde rolü olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: *Tavşan, turpentine oil, steril inflamasyon, 25-hidroksivitamin D, 1,25-dihidroksivitamin D*

Received : 25.03.2025
Accepted : 02.06.2025

Correspondence

Mehmet ÇALIŞKAN
Fırat University,
Faculty of Veterinary Medicine,
Department of Internal Medicine,
Elazığ – TÜRKİYE

mcaliskan@firat.edu.tr

Introduction

Inflammation refers to a complex set of defensive responses by the immune system against internal or external factors perceived as threats (1, 2). While inflammation is essential for safeguarding the body and promoting tissue regeneration, excessive or prolonged inflammation can lead to adverse effects (1-4). The process of inflammation is initiated by chemical signals released by host cells following any injurious stimulus, whether infectious or non-infectious (sterile) in origin (1, 4). Unlike pathogen induced inflammation, several non-infectious conditions including ischemia-reperfusion injury, traumas that cause tissue necrosis, some toxins, burn wounds and some tumors can cause sterile inflammation (3, 5). Sterile inflammation plays a significant role in the pathophysiology of numerous diseases, including but not limited to cardiovascular diseases, autoimmune disorders, certain cancers, rheumatoid arthritis, diabetes, and Alzheimer's disease (4, 6, 7).

Vitamin D is well-known for its involvement in maintaining calcium balance, but it has also many crucial roles in regulating various functions of the immune system (8, 9).

* This study was financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK) ARDEB 1002, Grant no: 122O614.

The term of vitamin D encompasses a group of steroid-like molecules and can be obtained through dietary intake or synthesized in the skin, making it both a vitamin and a hormone (10). The synthesis of vitamin D in the skin involves several steps: 7-dehydrocholesterol is converted to pre-vitamin D and then cholecalciferol (vitamin D3) through exposure to ultraviolet light. In the liver, vitamin D3 is transformed into 25-hydroxyvitamin D [calcidiol (25-D)] by the enzyme cytochrome P450 2R1 [25-hydroxylase (CYP2R1)] (11). Further conversion of 25-D to 1.25-dihydroxyvitamin D [calcitriol (1.25-D)], the biologically active form of vitamin D, occurs mainly in the proximal renal tubules through the action of cytochrome P450 27B1 [25(OH)D3-1α-hydroxylase (CYP27B1)] (12).

For many years, the kidney was believed to be the sole organ expressing CYP27B1; however, recent findings indicate that various cells and tissues, including epithelial cells, bone and cartilage cells, brain, placenta, liver, and macrophages, also possess CYP27B1 and can produce 1.25-D (12, 13). Additionally, it was initially suggested that 1.25-D synthesized locally outside the kidneys exerts autocrine or paracrine effects and thus does not significantly alter circulating levels of 1.25-D (13). However, it was later stated that extrarenally produced 1.25-D, especially from macrophages, could substantially increase circulating levels of 1.25-D and lead to hypercalcemia in conditions such as sarcoidosis and Crohn's disease (14). Recently, Dabak et al. (15) demonstrated that antigenic stimulation caused a significant increase in serum 1.25-D in rats that undergone bilateral nefrectomy.

In many extraskeletal diseases accompanied by inflammation, lower serum 25-D levels in diseased subjects have been reported than in the healthy groups (16-19). However, since these are generally clinical studies, it is not possible to know the exact levels of vitamin D metabolites before the onset of the disease, and it is also unclear how much of the circulating 25-D is used for 1.25-D synthesis by immune cells during the course of these diseases. Consequently, it is not exactly known whether the decreased 25-D level reported in these studies is a causative factor and/or a result of the disease (16-19). Experimental studies are needed to obtain descriptive data about this uncertainity encountered in the clinical researches. The objective of the present study was to investigate the changes in circulating 25-D and 1.25-D levels during the course of an experimental sterile inflammation in rabbits. Turpentine oil (TO), commonly used to induce sterile inflammation in various animals including rabbits, was employed (20-23).

Materials and Methods

Research and Publication Ethics: The study protocol received approval from the Ethics Committee for Experimental Animals at Fırat University on July 6, 2022 (2022/12), and all methods were conducted in compliance with the relevant guidelines.

Animal Procedures: Twenty female New Zealand White rabbits, aged between 4 and 6 months, were obtained from the Fırat University Experimental Research Center. Throughout the study, the rabbits were maintained under a 12-hour artificial light/dark cycle and provided with standard laboratory chow (Elazığ Feed Industry Inc., Elazığ, Türkiye). and unlimited access to water. The rabbits were randomly divided into two groups: the turpentine oil group (TOG), comprising 12 rabbits, and the control group (CG), consisting of 8 rabbits.

Inflammation Protocol: To create sterile inflammation, 5 mL of TO (Sigma-Aldrich, Germany) was divided into equal doses and injected subcutaneously into four different sites of the rabbits in TOG. Similarly, each rabbit in the CG was injected subcutaneously with 5 mL isotonic 0.9% NaCl into the same injection sites. Body temperature, total leukocyte (WBC) count, and serum iron levels were evaulated to confirm the inflammation.

Blood Sampling: Blood samples were taken fromthe marginal ear veins of all the rabbits by using 22G catheter (Mediflon, India) before (0th hour) and at 6th, 12th, 24th and 72nd hours after the TO or isotonic 0.9% NaCl injections. In each sampling, 0.5 mL and 6 mL blood samples were collected into the tubes containing EDTA (BD Vacutainer, UK) and containing gel and clot activator (BD Vacutainer, UK), respectively.

Clinical, Hematological and Biochemical Analysis: Body temperatures of rabbits in both groups were measured rectally just before the each blood sampling. WBC was manually determined by a protocol using a Thoma slide (24). The samples were spun in a centrifuge (Hettich, Germany) at 7000 rpm for 5 minutes following a clotting period of about 1 hour at room temperature. Serum samples were taken into eppendorf tubes and stored at -20°C until the measurements were done. Iron concentrations were measured with an autoanalyzer (Siemens Advia Centaur XPT, USA) using the colorimetric method. 25-D and 1.25-D levels were measured by the Liquid Chromatography-Mass Spectrophotometry/Mass Spectrophotometry (LC-MS/MS) method (Sciex 5500 Qtrap).

Statistical Analysis: Statistical analysis was performed with SPSS 22 (Statistical Package for the Social Sciences for Windows, SPSS Inc., Chicago, IL, USA) program. Normal distribution of the data was assessed using the Shapiro-Wilk test. Since all the data did not show normal distrubution, non-parametric tests were used. Group comparisons were performed using the Mann-Whitney U test, while pairwise comparisons within groups over time were conducted using the Wilcoxon test. The significance threshold was set at $p<0.05$.

Results

The Results of Clinical and Hematological Examinations: Body temperatures of the rabbits and statistical significance of the values are presented in Figure 1. In the TOG, body temperature exhibited significant increases at 12, 24, and 72 hours post-injection compared to the CG.

WBC counts of the rabbits in all sampling times and results of statistical analyzis are given Figure 2. A notable reduction in WBC count was observed in the TOG at 6, 12, 24, and 72 hours post-injection in comparison to the CG.

Serum iron levels during the study and statistical analysis results are given in Figure 3. The results indicate that iron levels decreased in the TOG compared to the CG at the 6th, 12th and 24th hours. At the 72nd hour iron levels increased again in the TOG and there was not statistical difference between the groups.

The results of Serum 25-D and 1.25-D Analysis: Serum 25-D levels and results of statistical analysis are given in Figure 4. The results indicate that 25-D decreased in the TOG compared to CG at the 72nd hour (p=0.025). Furthermore, while there were no significant changes observed in the CG, a decrease in 25-D levels was noted within the TOG at the 72nd hour compared to the baseline (0th hour) levels (p=0.034).

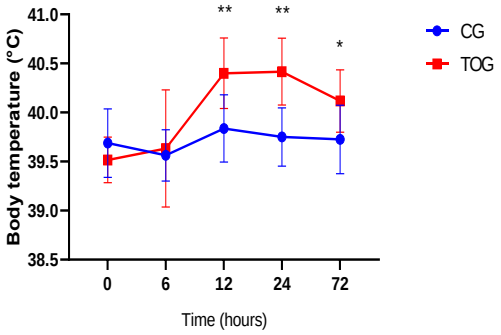

Figure 1. Mean body temperatures of the rabbits in the control group (CG) and turpentine oil group (TOG) during the experiment. Statistically significant difference between the groups are marked. *: p<0.05, **: p<0.01

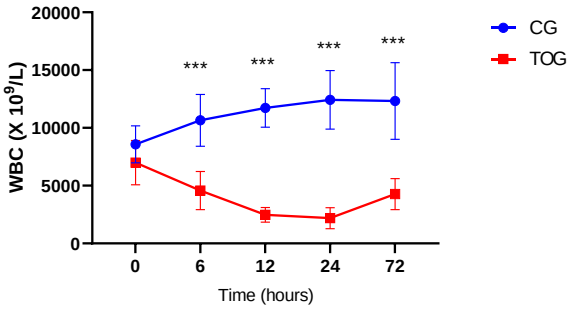

Figure. 2. Mean WBC counts of the rabbits in the control group (CG) and turpentine oil group (TOG) during the experiment. Statistically significant difference between the groups are marked. ***:p<0.001.

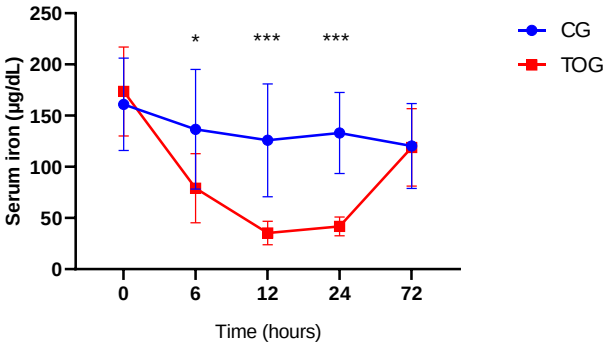

Figure 3. Mean serum iron status of the the rabbits in the control group (CG) and turpentine oil group (TOG) during the experiment. Statistically significant difference between the groups are marked. *: p<0.05, ***:p<0.001.

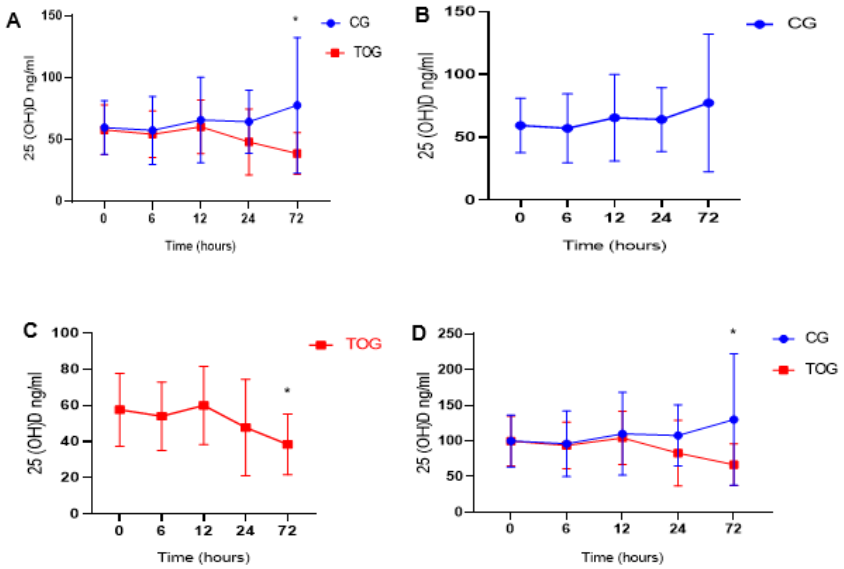

Figure 4. Mean 25-D levels of the rabbits in the control group (CG) and turpentine oil group (TOG) during the experiment. A:Intergroup comparision between the CG and TOG. *: statistical difference between the groups (p<0.05) B: Intragroup change comparing to the 0th hour in the CG. C: Intragroup change comparing to the 0th hour in the TOG. *: statistical difference compared to the 0th hour (p<0.05). D: Percentage change compared to the 0th hour . *: statistical difference between the groups (p<005).

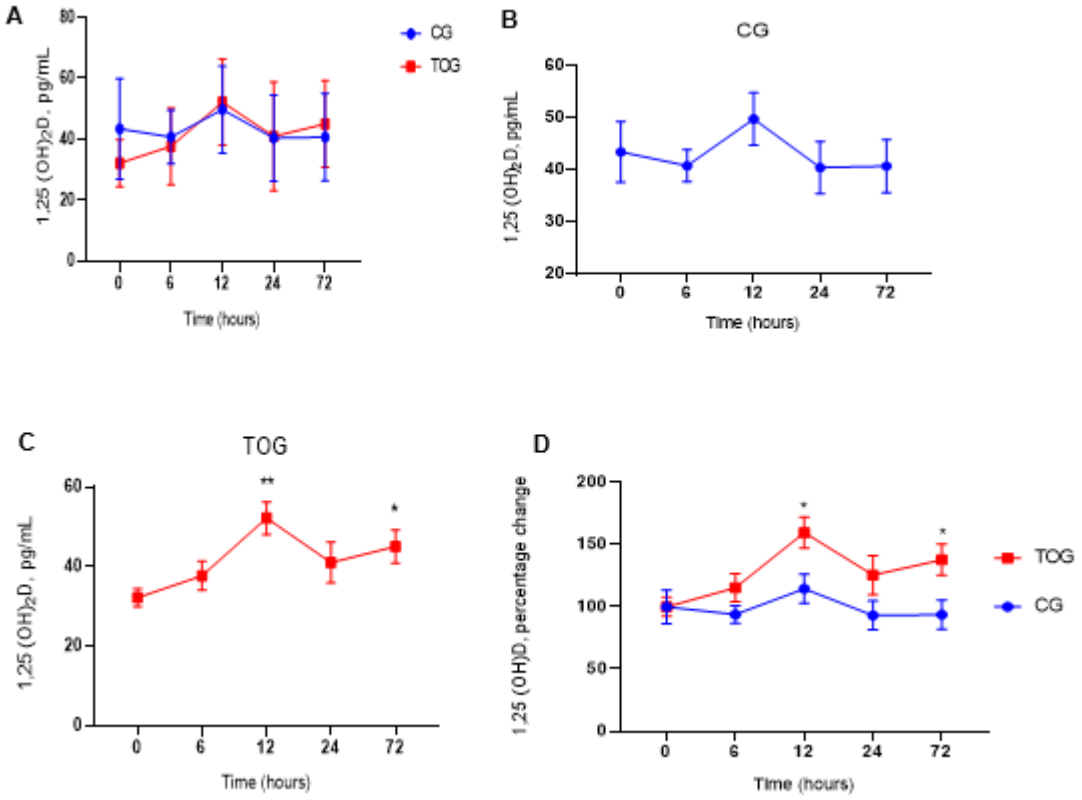

Figure 5. Mean 1.25-D levels of the rabbits in the control group (CG) and turpentine oil group (TOG) during the experiment. Statistically significant difference (p<0.05) between the groups are marked. A: Intergroup comparisıon between CG and TOG. B: Intragroup change comparing to the 0th hour in the CG. C: Intragroup change comparing to the 0th hour in the TOG. *: statistical difference compared to the 0th hour (p<0.05). **: statistical difference compared to the 0th hour (p<0.01). D: Percentage change compared to the 0th hour . *: statistical difference between groups (p<0.05).

Serum 1.25-D levels and corresponding statistical analysis results are presented in Figure 5. The findings indicate no significant difference in 1.25-D levels between the groups throughout the study. However, within the TOG, 1.25-D levels exhibited an increase at the 12th (p=0.0068) and 72nd hours (p=0.0161) compared to the baseline (0th hour), while no significant changes were observed in the CG.

Discussion

Sterile inflammation is an important part of the pathogenesis of numerous clinical and subclinical disease conditions (4, 6, 7). Within these inflammatory disorders, serum 25-D levels are frequently observed to be lower compared to healthy individuals (16-19). While clinical trials offer the opportunity to compare vitamin D levels between patients and healthy controls, they do not ascertain whether the observed disparity precedes or results from the disease. Numerous factors such as dietary habits, disease stage, comorbidities, prior treatments, etc., often confound clinical studies, making precise detection challenging. Furthermore, determining the pre-disease vitamin D status of patients is frequently unattainable (16, 17, 19). The present study was designed to evaluate the effects of an experimental sterile inflammation induced by TO injection on serum vitamin D levels.

The quantity of TO administered per animal to evoke sterile inflammation in rabbits typically ranges from 3 mL to 6 mL (22, 23, 25). In the current study, 5 mL of TO was administered, and systemic inflammation was confirmed through changes in body temperature, WBC count and serum iron levels.

Body temperature values showed significant increases at 12., 24. and 72. hours in the TOG compared to the CG, as reported in the literature (26, 27). In the TOG, WBC counts exhibited a decrease at 6, 12, 24, and 72 hours, while there was no significant change observed in the throughout the experiment. This contrasts with the findings of Gossett et al. (27), who reported an increase in WBC counts following turpentine oil injection in dogs, contrary to the observations in the present study. Similarly, Colditz et al. (26) reported an increase in WBC counts in sheep, both of which differ from the results observed in the present study. As both leukopenia and leukocytosis are recognized as signs of inflammation (28), the observed changes in WBC counts in the present study confirm the presence of systemic inflammation. Discrepancies in WBC values between the current investigation and other reports may be attributed

to variations in species, dosage, or route of administration.

Reference value of serum iron for rabbits is reported between 97-292 µg/dL (29). While mean serum iron levels in the CG were within the reference during the experiment, in the TOG the values were below the reference at the 6th, 12th and 24th hours of the experiment. The findings indicate that a solitary TO injection results in a precise decrease in serum iron levels, at least for the first 24 hours. However, by the 72nd hour following the injection, these levels rebounded and returned to those observed in the CG. In a study conducted on Pony horses, sterile inflammation was induced by a single intramuscular 5 mL TO injection. In this study it was reported that serum iron levels decreased significantly 24 h after the injection, started to increase at 72nd hour and reached to the controls' level at day 6 after the injection (30). In another study performed on rats, 5 mL/kg TO was injected intramuscularly. In this study serum iron reduction reached to the minimum at 24th hour and increased at 36th hour (31). The variances in the time taken to decrease and to return to the normal levels between the studies could be attributed to differences in the species or injection method.

Vitamin D exerts its effects by binding to vitamin D receptor (VDR) in target cells. Notably, the affinity of 1.25-D to VDR is significantly higher, approximately 100-200 times greater than that of 25-hydroxyvitamin D (25-D). Moreover, 1,25-D is recognized as the most active form of vitamin D (32). However, despite the potency of 1,25-D, serum 25-D levels are utilized to assess individuals' vitamin D sufficiency due to several reasons: i) Many imbalances in vitamin D metabolism are correlated with concentrations of 25-D rather than 1.25-D; ii) The half-life of 1.25-D is relatively short, ranging from 4 to 15 hours, compared to the substantially longer half-life of 25-D, which lasts from 20 to 24 days; iii) The circulating concentration of 1.25-D is approximately 1/1000th of that of 25-D; iiii) Serum 25-D levels are indicative of the total amount of vitamin D acquired from dietary sources and synthesized in the skin (32, 33).

In the present study serum 25-D and 1.25-D levels were measured by LC-MS/MS method, which is considered as gold standard for measurement of vitamin D levels (34). Mäkitaipale et al. (35) has been reported that mean serum 25-D levels in healthy rabbits are 25,9 ng/mL and values below 17 ng/mL indicate vitamin D deficiency. In the current study, none of the rabbits exhibited vitamin D deficiency, as the mean serum 25-D levels for both the TOG and CG rabbits at the start of the experiment (0th hour) were 59.5 ng/mL and 57.6 ng/mL, respectively. The study revealed a significant decrease in serum 25-D levels in the TOG at the 72nd hour compared to the CG. Additionally, when changes within the groups over time were examined, a significant decrease was observed in the TOG at the 72nd hour compared to those of 0th hour, whereas no difference was noted in the CG. This decline may be attributed to the utilization of 25-D as a substrate in the heightened synthesis of 1.25-D in the TOG.

As reported by Titmarsh et al. (18) and Vieira et al. (36), serum 25-D levels commonly decrease in various inflammatory conditions. However, clinical studies are influenced by numerous factors such as patient demographics, diet, disease stage, comorbidities, prior treatments, and the inability to ascertain pre-disease vitamin D levels in most cases, rendering it challenging to discern whether the decrease in serum 25-D levels is the cause or consequence of the disease (16, 33). Therefore, to investigate the relationship between sterile inflammation and vitamin D levels experimentally can help mitigate these uncertainties observed in clinical studies. The data obtained from this study demonstrate that sterile inflammation leads to a significant decrease in circulating 25-D levels. This finding suggests that sterile inflammation plays a causal role in vitamin D deficiency, a common occurrence in sterile inflammatory diseases.

Although no statistical difference was detected between the TOG and CG in terms of 1.25-D levels throughout the experiment, intra-group statistical analyses revealed a significant increase within the TOG at the 12th and 72nd hours compared to the 0th hour. It has been reported that serum 1.25-D levels increase in many sterile and non-sterile inflammatory diseases (37-39). It is known that immune system cells can synthesize sufficient 1.25-D to elevate its circulating levels in certain conditions, facilitated by the CYP27B1 enzyme they contain (37-39). Therefore, it is likely that the observed increase in 1.25-D levels in the TOG is attributable to its synthesis in immune system cells activated by inflammation.

In conclusions, vitamin D deficiency or insufficiency is frequently observed in inflammatory conditions, yet due to the inherent limitations of clinical research, the causal relationship remains incompletely understood. However, in the current experimental study, it was demonstrated that sterile inflammation induces a decrease in serum 25-D levels and an increase in 1.25-D levels. Based on these findings, it can be inferred that vitamin D deficiency or insufficiency is at least partially, if not entirely, a consequence of inflammation.

References

1. Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. Nature Immunology 2017; 18: 826-831.

2. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the lifespan. Nature Medicine 2019; 25: 1822-1832.

3. Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nature Reviews Immunology 2010; 10: 827-837.

4. Bennett JM, Reeves G, Billman GE, Sturmberg JP. Inflammation-Nature's Way to Efficiently Respond to All Types of Challenges: Implications for Understanding and

Managing "the Epidemic" of Chronic Diseases. Front Med (Lausanne). 2018; 5: 316.

5. Shen H, Kreisel D, Goldstein DR. Processes of sterile inflammation. The Journal of Immunology 2013; 191: 2857-2863.

6. Tasneem S, Liu B, Choudhary MI, Wang W. Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. Pharmacological Research 2018; 139: 126-140.

7. Slavich GM. Understanding inflammation, its regulation, and relevance for health: A top scientific and publimc priority. Brain Behav Immun 2015; 45: 13-14.

8. Sassi F, Tamone C, D'Amelio P. Vitamin D: Nutrient, Hormone, and Immunomodulator. Nutrients 2018; 10: 1656.

9. Fletcher J, Bishop EL, Harrison SR, Autoimmune disease and interconnections with vitamin D. Endocr Connect 2022; 11: e210554.

10. Fleet JC. The role of vitamin D in the endocrinology controlling calcium homeostasis. Mol Cell Endocrinol 2017; 15: 453: 36-45.

11. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. Physiology Review 2016; 96: 365-408.

12. Bikle DD. Extrarenal synthesis of 1,25-dihydroxyvitamin D and its health implications. Clinical Reviews in Bone and Mineral Metabolism 2009; 7: 114-125.

13. Hermann M, Farrell CL, Pusceddu I, Fabregat-Cebello N, Cavalier E. Assessment of vitamin D status-a changing landscape. Clin Chem Lab Med 2017; 55: 3-26.

14. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, molecular mecanism of action, and pleiotropic effects. Physiol Rev 2015; 96: 365-408.

15. Dabak M, Dabak DO, Kuloglu T,et al. Increased circulatory extrarenal 1α,25-Dihydroxyvitamin D3 in bilaterally nephrectomised rats. Endocrine, Metabolic & Immune Disorders-Drug Targets 2020; 20: 1523-1530.

16. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: A systematic review. Lancet Diabetes & Endocrionology 2014; 2: 76-89.

17. Bizzaro G, Antico A, Fortunato A, Bizzaro N. Vitamin D and autoimmune diseases: Is vitamin D receptor (VDR) polymorphism the culprint? The Israel Medical Association Journal 2017; 19: 438-443.

18. Titmarsh HF, Lalor SM, Tasker S, et al. Vitamin D status in cats with feline immunodeficiency virus. Veterinary Medicine and Sience 2015; 1: 72-78.

19. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: The infection connection. Inflamm Res 2014; 63: 803-819.

20. Kuribayashi T, Seita T, Momotani E,et al. Elimination half-lives of acute phase proteins in rats and beagle dogs during acute inflammation. Inflammation 2015; 38: 1401-1405.

21. Chikazawa S, Nakazawa T, Hori Y, et al. Chance in serum ferritin concentration in eperimentally induced anemia of chronic inflammation in dogs. The Jornal of Veterinary Medical Science 2013; 75: 1419-1426.

22. Kushner I, Feldman G. Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. Journal of Experimental Medicine 1978; 148: 466-477.

23. Iovu MO, Héroux L, Vérges J, et al. Effect of chondroitin sulphate on turpentine-induced down-regulation of CYP1A2 and CYP3A6. Carbohydrate Research 2012; 355: 63-68.

24. Weiser G. Laboratory technology for veterinary medicine. In: Thrall MA, Weiser G, Allison RW, Campbell TW, editors. Veterinary Hematology and Clinical Chemistry Oxford: Wiley Blackwall, 2012: 8-12.

25. Bleau AM, Levitchi MC, Maurice H, du Souich P. Cytocrome P450 inactivation by serum from humans with a viral infection and serum from rabbits with a turpentine-induced inflammation: The role of cytokines British Journal of Pharmacology 2000; 130: 1777-1784.

26. Colditz IG, Paul DR, Hervault G, Aubriot D, Lee C. Development of a lameness model in sheep for assessing efficiacy of analgesics. Australian Veterinary Journal 2011; 89: 297-304.

27. Gossett KA, MacWilliams PS, Cleghorn B. Sequential morphological and quantitative changes in blood and bone marrow neutrophils in dogs with acute inflammation. Canadian Journal of Comperative Medicine 1985; 49: 291-297.

28. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016; 315: 762-774.

29. Jones RT. Normal values for some biochemical constituents in rabbits. Lab Anim 1975; 9: 143-147.

30. Smith JE, Cipriano JE. Inflammation-induced changes in serum iron analytes and ceruloplasmin of Shetland ponies. Vet Pathol 1987; 24: 354-356.

31. Naz N, Malik IA, Sheikh N, et al. Ferroportin-1 is a 'nuclear'-negative acute-phase protein in rat liver: A comparison with other iron-transport proteins. Lab Invest. 2012; 92: 842-856.

32. de Brito Galvao JF, Nagode LA, Schenk PA, Chew DJ. Calcitriol, calcidiol, parathyroid hormone, and fibroblast growth factor-23 interactions in chronic kidney disease. J Vet Emerg Crit Care 2013; 23: 134-162.

33. Adams JS, Rafison B, Witzel S, et al. Regulation of the extrarenal CYP27B1-hydroxylase. J Steroid Biochem Mol Biol 2014; 144: 22-27.

34. Heureux N. Vitamin D testing-Where are we and what is on the horizon? Advances in Clinical Chemistry 2017; 78: 59-101.

35. Mäkitaipale J, Sankari S, Sievänen H, Laitinen-Vapaavuori O. The relationship between serum 25-hydroxyvitamin D and parathyroid hormone concentration in assessing

vitamin D deficiency in pet rabbits. BMC Vet Res 2020; 16: 403.

36. Vieira IH, Rodrigues D, Paiva I. Vitamin D and autoimmune thyroid disease-cause, consequence, or a vicious cycle? Nutrients 2020; 1-18.

37. Dabak M, Dabak DO, Karapinar T, Bulut H. Vitamin D status in cattle with malignant cattharral fever. The Journal of Veterinary Medicine Science 2012; 74: 125-128.

38. Blaney GP, Albert PJ, Proal AD. Vitamin D metabolites as clinical markers in autoimmune and chronic disease. Annals of the New York Academy of Sciences 2009; 1173: 384-390.

39. Rhodes SG, Terry LA, Hope J, Hewinson RG, Vordermeier HM. 1,25-dihydroxyvitamin D3 and development of tuberculosis in cattle. Clinical and diagnostic laboratory immunology 2003; 10: 1129-1135.