Vitamin D Status in Different Stages of Disease Severity in Dogs with Chronic Valvular Heart Disease

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Background: In humans with heart disease, vitamin D deficiency is associated with disease progression and a poor prognosis. A recent study showed that serum 25-hydroxyvitamin D [25(OH)D] concentration, the hallmark of vitamin D status, was lower in dogs with heart failure than in normal dogs, and a low concentration was associated with poor outcome in dogs with heart failure.

Objectives: To elucidate the vitamin D status of dogs with chronic valvular heart disease (CVHD) at different stages of disease severity.

Animals: Forty-three client-owned dogs with CVHD.

Methods: In this cross-sectional study, dogs were divided into 3 groups (14 dogs in Stage B1, 17 dogs in Stage B2, and 12 dogs in Stage C/D) according to ACVIM guidelines. Dogs underwent clinical examination including echocardiography. Serum 25(OH)D concentrations were measured in each dog.

Results: Serum 25(OH)D concentration was significantly lower in Stage B2 (median, 33.2 nmol/L; range, 4.9–171.7 nmol/L) and C/D (13.1 nmol/L; 4.9–58.1 nmol/L) than in Stage B1 (52.5 nmol/L; 33.5–178.0 nmol/L) and was not significantly different between Stage B2 and Stage C/D. Among clinical variables, there were significant negative correlations between 25(OH)D concentration and both left atrial-to-aortic root ratio and left ventricular end-diastolic diameter normalized for body weight.

Conclusions and Clinical Importance: These results indicate that vitamin D status is associated with the degree of cardiac remodeling, and the serum 25(OH)D concentration begins to decrease before the onset of heart failure in dogs with CVHD.

Key words: 25-Hydroxyvitamin D; Cardiac remodeling; Dog; Echocardiography.

Recent evidence suggests that vitamin D plays a role in the pathophysiology of heart disease. Studies in experimental animals have indicated that calcitriol, the most active vitamin D metabolite, promotes cardiac contractility by binding to vitamin D receptors on cardiomyocytes and affecting intracellular calcium handling within the cells. In addition, calcitriol regulates cardiac remodeling by exerting antihypertrophic effects on cardiomyocytes and modulating myocardial extracellular matrix turnover.

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This study was performed at the Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.

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In humans, epidemiological evidence indicates that vitamin D deficiency is an adverse risk factor in patients with cardiovascular disease. Vitamin D deficiency has a high prevalence in patients with heart failure and low serum concentrations of 25-hydroxyvitamin D [25(OH) D], a hallmark of vitamin D status, are associated with cardiac dysfunction and remodeling, severe heart failure symptoms, and poor prognosis. In addition, observational retrospective studies indicate that vitamin D supplementation is associated with a better prognosis in patients with heart failure and vitamin D deficiency. Furthermore, randomized double-blind controlled trials have shown that vitamin D supplementation improves left ventricular systolic function in patients with heart failure and vitamin D deficiency.
In veterinary medicine, a recent study showed that the serum 25(OH)D concentration is lower in dogs with congestive heart failure caused by chronic valvular heart disease (CVHD) or dilated cardiomyopathy than in normal dogs, and that the low concentration is associated with poorer outcome in dogs with heart failure. In that study, however, asymptomatic CVHD dogs were not enrolled, and therefore it is still unknown when vitamin D status changes in various stages of disease severity. In addition, the association of vitamin D status with cardiac remodeling and function remains to be elucidated.

Therefore, the overall aim of our cross-sectional study was to elucidate vitamin D status in different stages of disease severity in dogs with CVHD. Specific aims were to: (1) determine the association between vitamin D status and disease stage, and (2) examine correlations between vitamin D status and echocardiographic parameters of cardiac remodeling and function as well as hemodynamics in dogs with CVHD.

Materials and Methods

Study Animals

The study population included retrospectively (between October 2010 and July 2012, 17 dogs) and prospectively (between August 2012 and November 2013, 26 dogs) recruited client-owned dogs with CVHD from the Veterinary Teaching Hospital of the Graduate School of Veterinary Medicine, Hokkaido University. Informed owner consent was obtained. Each dog was included only once in the study. Some of the enrolled dogs were part of another retrospective longitudinal study of naturally occurring CVHD in which associations among echocardiographic parameters and survival times in dogs were evaluated.

Dogs were included in this study: (1) if they had been diagnosed with CVHD, and (2) echocardiographic examinations and serum sample collection had been performed on the same day. Diagnostic criteria for CVHD were the combination of the presence of mitral valve prolapse, any degree of mitral valve leaflet thickening by 2-dimensional echocardiography, and identification of any degree of mitral valve regurgitation by color Doppler examination, with or without mitral valve thickening. Dogs were excluded if they had congenital heart disease, dilated cardiomyopathy, or concurrent disorders known to be associated with vitamin D metabolism, including hepatic insufficiency, protein-losing nephropathy or enteropathy, endocrine disorders, systemic hypertension, or clinically relevant systemic disease. Dogs with renal insufficiency that developed after the initiation of cardiovascular medications were not excluded.

Owner interviews and physical examinations were performed to provide the following data: age, sex, body weight, body condition score (BCS, on a scale of 1–5), and medical history.

Echocardiography

Echocardiographic examinations were performed using commercially available ultrasonographic equipment with a 3- to 7-MHz sector probe and continuous ECG recording. No dogs were sedated for echocardiographic tests. All data were stored digitally and analyzed off-line by 1 observer. The mean of 3 cardiac cycles was calculated for all variables. From a right parasternal short-axis view, M-mode variables of the left ventricle including left ventricular end-diastolic and systolic diameters were obtained and left ventricular fractional shortening (LV-FS) was calculated. Left ventricular end-diastolic diameter was normalized for body weight (LVEDD) using the following formula: left ventricular end-diastolic diameter/(body weight [kg])0.294. Left ventricular end-systolic diameter was normalized for body weight (LVESD) using the following formula: left ventricular end-systolic diameter/ (body weight [kg])0.315. Left atrial-to-aortic root ratio (LA/Ao) was obtained from the 2-D right parasternal short-axis view. Pulsed-wave Doppler echocardiography was used to measure transmural flow velocity from the left apical 4-chamber view. Peak velocities of the early diastolic transmural flow wave (E-wave) and late diastolic transmural flow wave (A-wave) were measured, and the ratio of the peak velocity of the E-wave to the peak velocity of the A-wave was calculated. Tissue Doppler imaging velocities of myocardial motion were recorded from the left apical 4-chamber view with the sample volume positioned at the septal mitral annulus. The peak velocities of the early diastolic wave (E-wave) and systolic wave (S-wave) of myocardial velocity were measured, and the ratio of the peak velocity of the E-wave to peak velocity of the E-wave was calculated.

Parameters of the left atrial function were determined as previously reported. Briefly, 2-D cine loops from an apical 4-chamber view were obtained with an ECG trace (lead II) recorded simultaneously to be analyzed with off-line software based on 2-D speckle tracking echocardiography. A frame corresponding to the peak R wave on ECG was selected and the endocardium of the left atrium was manually traced in that frame. The area of the left atrium then was automatically calculated using software throughout the cardiac cycle to derive a time-left atrial area curve. Left atrial fractional area changes (LA-FAC) were calculated from the obtained curve as follows:

\[
\text{Total LA-FAC} = 100 \times \frac{\text{LAAmax} - \text{LAAmin}}{\text{LAAmax}}
\]

\[
\text{Passive LA-FAC} = 100 \times \frac{\text{LAAmax} - \text{LAAp}}{\text{LAAmax}}
\]

\[
\text{Active LA-FAC} = 100 \times \frac{\text{LAAp} - \text{LAAmin}}{\text{LAAp}}
\]

where LAAmax, LAAp, and LAAmin represent the maximum left atrial area at ventricular end-systole, the least atrial area at the onset of the P wave on ECG, and the minimum left atrial area at ventricular end-diastole, respectively. Total, passive, and active LA-FAC are indicators of left atrial reservoir, conduit, and booster pump functions, respectively.

Measurement of Plasma Biochemistry Variables and Serum 25-Hydroxyvitamin D [25(OH)D] Concentration

Fasted blood samples were obtained from each dog. Blood samples were placed in heparinized tubes and centrifuged (3,000 g, 5 minutes, room temperature). Blood urea nitrogen (reference interval, 9.2–29.2 mg/dL), plasma creatinine (reference interval, 0.4–1.4 mg/dL), inorganic phosphorus (reference interval, 1.9–5.0 mg/dL), and total calcium (reference interval, 9.3–12.1 mg/dL) concentrations were determined using an automated analyzer.

In addition, serum was harvested and stored at –20°C until analysis of 25(OH)D. Measurements of 25(OH)D were performed 1–30 months after the time of sampling. Previous studies reported that 25(OH)D is stable for 3–24 years when stored at –25 to –20°C.
Serum concentrations of 25(OH)D were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s recommendations. Serum 25(OH)D concentrations were determined using a standard curve generated with the calibrators provided with the kit. The manufacturer reported the percentage cross-reactivity with related compounds as follows: vitamin D3 (0.53%) or D2 (0.30%), 25-hydroxyvitamin D3 (100%) or D2 (81.5%), 1,25-dihydroxyvitamin D3 (467%) or D2 (91.5%), 24,25-dihydroxyvitamin D3 (5.9%), 1α-hydroxyvitamin D3 (0.52%), or D2 (0.58%). However, the observed cross-reactivity to 1,25-dihydroxyvitamin D3 or D2 is not of major concern to the overall reported results of the assay because the circulating concentrations of this metabolite are approximately 1,000-fold lower than those of 25(OH)D. The intra- and interassay coefficients of variation reported by manufacturer were <3.4, and <15.8%, respectively. The biological sensitivity of the assay was 4.9 nmol/L. Serum concentrations below the sensitivity level were reported as 4.9 nmol/L for statistical analysis in this study.

**Clinical Classification of Dogs**

In each dog, based on clinical signs, echocardiographic examinations, and thoracic radiography, CVHD was classified as Stage B1, B2, C, or D according to the American College of Veterinary Internal Medicine (ACVIM) consensus statement. Stage B1 included dogs without clinical signs of CVHD and echocardiographic evidence of cardiomegaly (ie, LA/Ao < 1.6 and LVEDDDN < 1.85). In the present study, Stage B1 dogs were regarded as hospital controls (control group dogs) because: (1) it would be difficult to recruit demographically matched healthy controls for Stage B2 to D dogs considering their demographics and the prevalence of CVHD, and (2) Stage B1 dogs were deemed healthy except for having hemodynamically inconsequential mitral regurgitation and were expected to be relatively well-matched for demographics with Stage B2 to D dogs. Stage B2 included dogs with echocardiographic evidence of cardiomegaly (ie, LA/Ao > 1.6 or LVEDDDN > 1.85), but without clinical signs caused by CVHD (group B2 dogs). Stages C and D included dogs with clinical and radiographical signs of left-sided heart failure. Stage C and D dogs were combined in 1 group (group C/D dogs) because of small sample size.

**Statistical Analysis**

Statistical analysis was performed by use of commercially available software. Normal distribution of the data was confirmed by means of a Shapiro-Wilk test. For parametric data, the overall difference among groups (control, B2, and C/D dogs) was determined by one-way ANOVA, and then post hoc multiple comparisons were made using Tukey’s test. For nonparametric data, the overall difference among groups was determined using the Kruskal-Wallis test, and then post hoc multiple comparisons were made using the Steel-Dwass test. Spearman’s rank-correlation test was used to investigate the presence of a correlation between variables. The level of significance was set at \( P < .05 \).

**Results**

**Clinical Dogs**

A total of 43 dogs with CVHD, including 14 in the control group (Stage B1), 17 in Stage B2, and 12 in Stage C/D were recruited (Table 1). The most commonly represented breed was Chihuahua (n = 8), followed by Shih tzu (n = 6), Cavalier King Charles Spaniel (n = 4), miniature Dachshund (n = 4), mixed breed (n = 4), Beagle (n = 2), Maltese (n = 2), and Pomeranian (n = 2). In addition, 8 other small- and medium-sized breeds with 1 dog each were enrolled. All dogs in the control group except for 2 dogs receiving angiotensin-converting enzyme inhibitors (ACEI) were not treated with cardiac medications (Table 1). Some Stage B2 dogs were treated with ACEI. The majority of Stage C/D dogs were treated with ACEI, pimobendan, and diuretics (loop diuretics, spironolactone, or both).

**Table 1. Demographic variables and medical history among disease stages in dogs with chronic valvular heart disease.**

| Variable | Control Dogs (Stage B1, n = 14) | Stage B2 Dogs (n = 17) | Stage C/D Dogs (n = 12) | ANOVA or Kruskal-Wallis | Overall P-Value |
|----------|-------------------------------|------------------------|-------------------------|-------------------------|----------------|
| Age (years) | 9.5 (4–14)b | 11 (5–15)b | 12.5 (8–15)b | A | .017 |
| Weight (kg) | 6.0 (2.2–10.4)a | 7.0 (2.2–23.6)a | 4.1 (2.0–11.9)a | K | .14 |
| BCS <4 (no. of each BCS) | 9 (2 = 2; 7 = 3) | 10 (3 = 2; 7 = 3) | 10 (4 = 2; 6 = 3) | .42 |
| ≥4 | 5 (5 = 4) | 7 (5 = 4; 2 = 5) | 2 (2 = 4) | .10 |
| Sex | Male (no. of intact) | 6 (2) | 14 (4) | 6 (3) | .3 |
| Female | 8 (5) | 3 (2) | 6 (3) | .3 |
| Administered drugs* | ACEI | 2 | 7 | 8 |
| Pimobendan | 0 | 0 | 0 |
| Loop diuretics | 0 | 0 | 0 |
| Spironolactone | 0 | 2 | 3 |
| Digoxin | 0 | 0 | 2 |
| Nitrates | 0 | 0 | 2 |
| Beta-blocker | 0 | 0 | 1 |

A, ANOVA; ACEI, Angiotensin-converting enzyme inhibitors; BCS, Body condition score; K, Kruskal-Wallis test.

Continuous data are expressed as the median (range). Values with different superscript letters indicate significant differences among groups.

*Several dogs were administered more than 1 medication.
**Differences in Study Variables among Groups**

In all except 2 dogs, serum 25(OH) D concentrations were detectable (>4.9 nmol/L). Median serum 25(OH) concentrations were significantly lower in groups B2 and C/D than in controls (control versus stage B2, \( P = .0093 \); control versus stage C/D, \( P = .0015 \), Table 2, Fig 1). In addition, serum 25(OH)D concentrations were not significantly different between Stage B2 and C/D dogs.

Age was significantly different between the control group and Stage C/D (\( P = .016 \), Table 1). No significant differences were observed among the groups in the body weight, BCS, or sex distribution.

Blood urea nitrogen concentrations were significantly higher in group B2 than in the control group (\( P = .0077 \)), but not significantly different between group C/D and each of the control or group B2 (Table 2). No significant differences in plasma creatinine, inorganic phosphorus, and total calcium concentrations were observed among the groups.

The LVEDDDN values increased significantly with disease severity (control versus group B2, \( P = .0013 \); control versus group C/D, \( P = .0001 \); group B2 versus group C/D, \( P = .029 \)), whereas LVEESDN was not significantly different among the groups (Table 2). The passive LA-FAC values were significantly different between the control and group B2 (group C/D versus control, \( P = .016 \)). The LA-FAC values increased significantly with disease severity (control versus group B2, \( P < .0001 \); control versus group C/D, \( P < .0001 \); group B2 versus group C/D, \( P = .0015 \); Table 2).

Transmitral flow velocities could be determined in all except 1 dog (because of fusion of the mitral E- and A-waves because of high heart rate). The E-wave velocities were higher in group C/D compared with the control group and group B2 (group C/D versus control, \( P < .0001 \); group C/D versus group B2, \( P = .0001 \)) as well as E/A ratios (group C/D versus control, \( P < .0001 \); group C/D versus group B2, \( P = .0001 \); Table 2). The A-wave velocities were not significantly different among the groups. The E/E’ ratios could be determined in all except 2 dogs (1 dog, fusion of mitral E- and A-waves because of high heart rate; 1 dog, missing data). The E/E’ ratios were higher in group C/D when compared to the control group (\( P = .014 \)), but not significantly different between group B2 and each of the control or group C/D (Table 2).

The LA-FAC values could be calculated in all except 3 dogs (2 dogs, fusion of time-area curves in early and late diastolic phases because of high heart rate; 1 dog, poor image quality). There were no significant differences in total LA-FAC among the groups (Table 2). The passive LA-FAC values were significantly higher in group C/D compared with the control group (\( P = .0055 \)). The active LA-FAC values decreased significantly with disease severity (control versus group B2, \( P = .031 \); control versus group C/D, \( P = .0010 \); group B2 versus group C/D, \( P = .0058 \)).

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**Table 2.** Serum 25-hydroxyvitamin D [25(OH)D] concentrations and echocardiographic parameters among disease stages in dogs with chronic valvular heart disease.

| Variable                        | Control Dogs (Stage B1, \( n = 14 \)) | Stage B2 Dogs (\( n = 17 \)) | Stage C/D Dogs (\( n = 12 \)) | ANOVA or Kruskal-Wallis | Overall \( P \)-Value |
|---------------------------------|--------------------------------------|-------------------------------|-------------------------------|-------------------------|---------------------|
| Serum 25(OH)D (nmol/L)          | 54.4 (33.5–178)*                     | 35.8 (49–127)b                | 13.1 (49–581)                 | K                       | .0005               |
| BUN (mg/dL)                     | 15.8 (8.5–37.0)*                     | 26.2 (12.4–54.2)b             | 24.3 (5.8–123)p,b             | K                       | .0067               |
| Plasma creatinine (mg/dL)       | 0.5 (0.2–1.0)b                       | 0.6 (0.2–1.5)b                | 0.6 (0.3–2.4)                 | K                       | .23                 |
| Plasma iP (mg/dL)               | 3.3 (2.5–5.3)c                       | 3.8 (1.9–6.0)c                | 3.5 (2.1–6.4)c                | A                       | .57                 |
| Plasma iCa (mg/dL)              | 10.8 (9.5–12.4)a                     | 10.8 (9.2–12.2)c              | 10.3 (8.1–12.2)c              | A                       | .21                 |
| LVEDDDN (15.0 (12.3–18.0))     | 17.5 (14.1–22.3)                     | 20.1 (15.6–25.9)              | \(< .0001\)                   | A                       | .30                 |
| LVEESDN (8.2 (7.3–10.0))       | 9.3 (5.6–12.4)c                      | 9.5 (5.5–13.3)c               | \(< .0001\)                   | A                       | .023                |
| LV-FS (%)                       | 44.5 (31.0–51.1)b                    | 47.1 (27.0–69.5)              | 50.7 (44.8–65.4)              | A                       | \<.0001             |
| LA/Ao                           | 1.37 (1.09–1.54)c                    | 1.90 (1.45–3.06)c             | 2.49 (1.92–3.74)              | K                       | \<.0001             |
| Transmitral flow (\( n = 42\))*| 0.71 (0.51–1.03)b                    | 0.74 (0.53–1.30)b             | 1.38 (1.06–1.79)              | K                       | \<.0001             |
| E-wave (m/s)                    | 0.67 (0.44–0.99)c                    | 0.87 (0.52–1.04)c             | 0.75 (0.43–1.26)              | A                       | .097                |
| A-wave (m/s)                    | 1.09 (0.61–1.61)c                    | 1.00 (0.60–1.09)              | 1.96 (1.26–3.12)              | A                       | \<.0001             |
| S-wave (cm/s, \( n = 42\))*    | 6.9 (5.0–12.2)                       | 8.9 (4.5–12.4)                | 9.2 (5.4–20.2)                | A                       | .045                |
| E/E’ (\( n = 41\))*            | 12.3 (6.5–18.2)c                     | 14.3 (6.4–23.9)               | 17.1 (12.6–34.3)              | A                       | .014                |
| LA-FAC (\( n = 40\))*          | 46.1 (37.4–58.0)                     | 45.1 (37.1–61.9)              | 41.0 (20.7–57.8)              | A                       | .37                 |
| Total (%)                       | 18.1 (10.6–32.8)b                    | 24.8 (10.8–46.5)              | 28.5 (15.5–41.7)              | A                       | .0076               |
| Passive (%)                     | 31.3 (26.9–47.5)c                    | 28.4 (20.2–42.2)              | 17.6 (6.1–32.2)               | K                       | \<.0001             |

A, ANOVA; BUN, blood urea nitrogen; iP, inorganic phosphorus; K, Kruskal-Wallis test; LA/Ao, left atrial-to-aortic root ratio; LA-FAC, left atrial fractional area change; LVEDDDN, left ventricular end-diastolic diameter normalized for body weight; LVEDSDN, left ventricular end-systolic diameter normalized for body weight; LV-FS, left ventricular fractional shortening; iCa, total calcium.

Continuous data are expressed as the median (range). Values with different superscript letters indicate significant differences among groups.

*These variables were not recorded in all dogs.
The negative correlations between serum 25(OH)D concentration and indices of left-sided filling pressures (eg, the E-wave velocity and the E/E' ratio) in these populations may be explained in several ways. Firstly, increases in these indices may have been caused by volume overload associated with mitral regurgitation. Secondly, these indices may have been increased in association with deterioration of left ventricular intrinsic diastolic properties. Experimental studies have shown that vitamin D exerts a protective effect against left ventricular diastolic dysfunction by inhibiting myocardial fibrosis and promoting cardiac relaxation through modulation of myocardial calcium handling. Previous studies have shown the presence of myocardial fibrosis and altered myocardial calcium handling in dogs with CVHD and the contribution of vitamin D to such myocardial changes still needs to be clarified.

Our study did not clarify associations between serum 25(OH)D concentrations and echocardiographic findings of left ventricular systolic dysfunction. Previous experimental studies have shown that cardiac contractility is enhanced by vitamin D through modulation of intracellular calcium metabolism. Furthermore, clinical trials in humans have shown the protective effects of vitamin D on left ventricular systolic function of heart failure patients with vitamin D deficiency improves with supplementation of vitamin D. The lack of association between vitamin D status and left ventricular systolic dysfunction in our study may be explained by the difficulty of evaluating left ventricular systolic function in dogs with CVHD because of the effect of volume loading on the left ventricle.

Our study had several limitations. Firstly, it was a cross-sectional study, and therefore our findings do not prove causality between vitamin D status in each of various stages of disease and cardiac remodeling. Secondly, vitamin D metabolism was not investigated, and the mechanisms involved in the decrease in serum 25(OH)D concentrations (eg, decreased intake, hepatic synthetic dysfunction, renal loss) were not determined. In particular, the vitamin D intake in enrolled dogs was not determined, although the calculated vitamin D intake in dogs with CVHD was not significantly different from that of healthy dogs in a previous study. Thirdly, the definition and prevalence of vitamin D deficiency in dogs with CVHD remain unclear. In humans, vitamin D deficiency generally is defined as a serum 25(OH)D concentration of <50 nmol/L. Serum concentrations below this concentration are associated with an increase in serum...
parathyroid hormone concentrations, which were not evaluated in the present study.\(^1\)\(^{23}\) Fourthly, invasive gold standard tests of cardiac function, such as left ventricular \(dP/dt\) and \(\tau\), were not determined in the present study. Fifthly, sample sizes were small. Sixthly, the presence of cardiomegaly was not evaluated based on values of left ventricular and atrial volumes. This may have led to the misclassification of enrolled dogs into Stage B1 or B2.\(^{24}\)\(^{25}\)

In conclusion, the findings of this study suggest that the vitamin D status of dogs with CVHD worsens before the onset of heart failure and is correlated with the degree of cardiac remodeling. Additional studies are needed to define deficient concentrations of serum 25 (OH)D in dogs and establish the clinical efficacy of vitamin D supplementation for the prevention of disease progression of dogs with CVHD.

**Footnotes**

| Footnote | Reference |
|----------|-----------|
| a | HI VISION Preirus, Hitachi Aloka Medical Ltd., Tokyo, Japan |
| b | EUP-SS2, Hitachi Aloka Medical Ltd., Tokyo, Japan |
| c | Left Atrial Tracking, Hitachi Aloka Medical Ltd., Tokyo, Japan |
| d | FUJI DRI-CHEM 7000V, Fujifilm Corp., Tokyo, Japan |
| e | 25(OH) Vitamin D ELISA Kit, Enzo Life Sciences Inc., Farmingdale, NY |
| f | JMP, version 11.0, SAS Institute Inc., Cary, NC |

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**Conflict of Interest Declaration:** Authors disclose no conflict of interest.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

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