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Bone Resorption in Dogs with Calcium Oxalate Urolithiasis and Idiopathic Hypercalciuria

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Abstract

People with calcium oxalate (CaOx) urolithiasis and idiopathic hypercalciuria (IH) often have evidence of increased bone resorption, but bone turnover has not previously been investigated in dogs with these conditions. The aim of this study was to determine whether a marker of bone resorption, β -crosslaps, differs between dogs with CaOx urolithiasis and IH compared to controls. This retrospective, cross-sectional study used a canine specific ELISA to measure β -crosslaps concentrations in stored frozen serum samples from 20 dogs with CaOx urolithiasis and IH and 20 breed-, sex-, and age-matched stone-free controls (18 Miniature Schnauzers, 14 Bichons Frise, and 8 Shih Tzus). Dogs with CaOx urolithiasis and IH had lower β -crosslaps concentrations relative to controls ($P = 0.0043$), and β -crosslaps had a moderate negative correlation with urinary calcium-to-creatinine ratios ($r = -0.44$, $P = 0.0044$). Miniature Schnauzers had lower β -crosslaps concentrations than the other two breeds ($P = 0.0035$). The ELISA had acceptable intra-assay precision, but concentrations decreased when samples were repeatedly assayed over time. Assay recovery rates were also below acceptance criteria. In conclusion, Miniature Schnauzers, Bichons Frise, and Shih Tzus with CaOx urolithiasis and IH have evidence of decreased bone resorption compared to stone-free controls. This suggests that other causes of IH, such as intestinal hyperabsorption of calcium, underlie risk for CaOx urolithiasis in these breeds. Results should be confirmed in larger populations and with other β -crosslaps assays and additional biomarkers of bone turnover. The stability of canine serum β -crosslaps after freeze-thaw cycles and storage at various temperatures requires investigation.

Graphical Abstract

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Keywords

bone resorption; urolithiasis; calcium oxalate; hypercalciuria; dogs

Urolithiasis is a common clinical problem in dogs, and CaOx uroliths represent 40-50% of the canine submissions to veterinary urolith centers in North America (Houston and Moore, 2009; Low et al., 2010; Osborne et al., 2009). Patient characteristics affecting CaOx urolithiasis risk, such as breed, age, and sex, are well established (Houston and Moore, 2009; Lekcharoensuk et al., 2000; Low et al., 2010; Lulich et al., 2013). However, the specific metabolic factors influencing canine CaOx stone formation remain elusive.

In human medicine, hypercalciuria (increased urinary calcium excretion) is the most common metabolic disturbance identified in CaOx stone formation, and the majority of cases are idiopathic (Worcester and Coe, 2008). Proposed mechanisms for IH include absorptive, resorptive, and renal leak hypercalciuria (Moe and Bonny, 2005). Absorptive hypercalciuria occurs from excessive gastrointestinal absorption of calcium, resorptive hypercalciuria is due to increased bone resorption, and renal leak hypercalciuria occurs from defective renal retention of calcium from the ultrafiltrate. Idiopathic hypercalciuria is associated with CaOx urolithiasis in Miniature Schnauzer, Bichon Frise, and Shih Tzu dogs (Furrow et al., 2015; Lulich et al., 1991). Stone formers of these breeds also have significantly higher blood ionized calcium concentrations than breed-, sex-, and age-matched stone-free control dogs, though overt hypercalcemia is rare (Furrow et al., 2015). The high-normal blood calcium suggests that the IH is absorptive and/or resorptive in origin; pure renal leak is unlikely, as it would be expected to reduce the blood calcium concentration.

People typically fall into multiple mechanistic categories of IH, and evidence for bone resorption is common. Patients with nephrolithiasis and IH have decreased bone mineral density leading to osteopenia, osteoporosis, and fragility bone fractures (Arrabal-Polo et al., 2014; Tasca et al., 2009). Markers of bone turnover, including bone degradation proteins (e.g. β -crosslaps), bone formation proteins (e.g. osteocalcin), and cytokines that regulate bone resorption (e.g. IL-1, IL-6, and TNF- α), are altered in patients with calcium-containing nephrolithiasis and IH (Arrabal-Polo et al., 2011; Arrabal-Polo et al., 2012; Arrabal-Polo et al., 2013; Arrabal-Polo et al., 2014; Tasca et al., 2009).

Measurement of serum, plasma, or urine β -crosslaps is a major method of documenting increased bone resorption, and β -crosslaps has been repeatedly documented to be increased

in human patients with nephrolithiasis and IH (Arrabal-Polo et al., 2011; Arrabal-Polo et al., 2012; Arrabal-Polo et al., 2013; Arrabal-Polo et al., 2014). This biomarker is a product of type I collagen breakdown by osteoclasts. Serum β -crosslaps has been used as a marker of bone turnover in dogs (Beli et al., 2010; Beli et al., 2012); however, work on bone resorptive markers has not been previously performed in dogs with CaOx urolithiasis and IH.

The aims of this study were to use serum β -crosslaps to evaluate bone resorption in three breeds that have been shown to have IH, the Miniature Schnauzer, Bichon Frise, and Shih Tzu, and to test for an association of serum β -crosslap concentrations with CaOx urolithiasis and IH in these breeds. We hypothesized that the dogs with CaOx urolithiasis and IH would have elevated serum β -crosslaps concentrations, indicating increased bone resorption, relative to a group of breed-, sex-, and age-matched stone-free controls. This work will help to address whether bone resorption plays a major role in the pathophysiology of canine CaOx urolithiasis and IH.

Materials and Methods

Samples and Study Population

The study had a retrospective, cross-sectional design. Stored (-80°C) serum samples were available from dogs that had participated in studies on CaOx urolithiasis at the Veterinary Medical Center at the University of Minnesota between February 2011 and January 2015 (Furrow et al., 2015; Furrow et al., 2017). Written informed consent was obtained from owners at the time of sample collection, and the collection protocol was approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol #1509-33019A). Samples from Miniature Schnauzer, Bichon Frise, and Shih Tzu breeds were selected for the study, as these breeds have been reported to have IH associated with their risk for CaOx urolithiasis (Furrow et al., 2015). Dogs were classified as cases or controls at the time of their study visit. Cases were defined as dogs with a history of uroliths composed of CaOx ($>70\%$ of the central core), as determined by standard stone analysis at the Minnesota Urolith Center (polarizing light microscopy and infrared spectroscopy). Urine was obtained on the day of study participation for measurement of a fasting urinary calcium-to-creatinine ratio (UCa/Cr, mg/mg), and cases selected for the present study had IH, as defined by UCa/Cr >0.05 , the upper end of the 95% confidence interval reported in stone-free dogs (Furrow et al., 2015). Controls had no history of CaOx uroliths. Screening abdominal radiographs and UCa/Cr were performed the day of study participation, and controls selected for the present study had no radiopaque uroliths and UCa/Cr ≤ 0.05 . All dogs had blood ionized calcium (iCa) and/or serum total calcium concentrations within laboratory reference ranges; serum creatinine concentrations were also within the laboratory reference range (≤ 1.6 mg/dL). The blood iCa were determined as previously reported (Furrow et al., 2015), and, where available, this data was included in the analyses as described below. Body weight, current medications, and nutritional information were obtained from medical records. Dogs were excluded if they had received hydrochlorothiazide or potassium citrate within 24 hours of sample collection or if they had a clinical diagnosis of a disease that alters bone turnover or urinary calcium excretion (e.g.

kidney disease, hyperparathyroidism, hyperadrenocorticism, osteolytic disease, or granulomatous disease). The primary diet fed was classified as urinary A (Prescription Diet® canine u/d®, Hill's Pet Nutrition Inc.), urinary B (Royal Canin Veterinary Diet® canine urinary SO®, Waltham Centre for Pet Nutrition), or other (diets not formulated for the prevention of stone disease).

Stored samples were available from 61 dogs (24 cases and 37 controls). Forty of these samples (20 cases and 20 controls) were selected to match breed, sex, and age (± 2 years) across case and control groups in the study. The remaining samples (4 cases and 17 controls) were excluded because they would create an imbalance in number, breed, sex, and/or age between case and control groups.

β -Crosslaps Assay

The serum samples were obtained on the day of study participation and immediately stored at -80°C for up to 4 years prior to analysis. While human serum β -crosslaps is reported to be stable for years at this temperature (Qvist et al., 2004), the storage time was recorded for each sample and statistically analyzed for an effect on β -crosslaps concentration as described below. To minimize possible effects of diurnal variations and feeding, all samples selected for the study were collected between 8 am and 2 pm after food was withheld from the dog for 12-18 hours (Liesegang et al., 1999). The concentration of β -crosslap in the serum samples was measured by use of commercial canine-specific ELISA assay (Canine Beta-Crosslaps ELISA Kit, MyBioSource, Inc.) according to the manufacturer's instructions. Absorbance was measured with an ELx808 Absorbance Microplate Reader using Gen5 software version 2.00.18 (BioTek Instruments, Inc.). The study samples were run in duplicate on a single assay (96-well plate), along with six standards, ranging in concentration from 0.25 – 8 ng/mL, and a purchased control (normal canine serum, Lot #19-141-020312, ImmunoReagents, Inc.). The average of each sample's replicates was used in the data analysis. Assay linearity was determined by measuring β -crosslaps concentration in the purchased control after spiking with the highest standard (8.0 ng/mL) then diluting to 50% and 25% (using sample diluent). Each spiked or diluted sample was tested 6-8 times in two assays. Recovery was calculated by spiking the purchased control with 4 ng/mL and 8 ng/mL standards and testing each 4-6 times in 2 assays. The purchased control was used to determine the intra-assay CV over two assays, using repeated measurements ($n = 20$ in one assay and $n = 16$ in a second assay). The purchased control and serum from two study participants (one case and one control) were used for inter-assay CV determination over four assays (days 0, 2, 3 and 7). Intra-assay and inter-assay CVs were calculated by dividing the SD by the mean for each sample. The results of these validation steps were compared with the University of Minnesota Veterinary Diagnostic Laboratory's standard acceptance criteria: linearity and recovery levels of 80-120% of expected values, intra-assay CV $< 15\%$, and inter-assay CV $< 20\%$.

Statistical Analysis

The distribution of the data was visually inspected with Q-Q plots to determine if transformations or nonparametric tests were necessary. Age, blood iCa, and sample storage time were normally distributed; these data are reported as mean $\pm$ standard deviation (SD)

and were compared between cases and controls with a Student's *t*-test. UCa/Cr and serum β -crosslaps concentrations were not normally distributed; these data are reported as median (range) and were compared between cases and controls with a Wilcoxon rank sum test. The serum β -crosslaps data was log-transformed to achieve a more normal distribution, and a multivariable regression analysis was performed on the log-transformed values with the following variables as predictors: stone status (case versus control), breed, sex (male versus female), age (years), body weight (kg), diet (urinary A, urinary B, or other), and sample storage time (years). The significance of each predictor was assessed using ANCOVA. For ease of interpretation, estimates of the regression coefficients and 95% confidence intervals were back-transformed to their original scale for reporting and therefore represent estimates for the multiplicative (factor) change in β -crosslaps rather than the additive change. Simple linear regressions were used to test the correlation of log-transformed serum β -crosslaps with log-transformed UCa/Cr and blood iCa (untransformed). All analyses were performed using R software for statistical computing (<http://www.R-project.org/>), and a $P < 0.05$ was considered significant.

Results

The study included 40 samples from 18 Miniature Schnauzers, 14 Bichons Frise, and 8 Shih Tzus with an equal number of CaOx urolithiasis cases and stone-free controls per breed, as per the sample selection criteria. Characteristics of the study population are presented in Table 1. Males outnumbered females (1.5:1) in the overall population, but sex was evenly distributed across cases and controls. All dogs were neutered with the exception of one intact male Shih Tzu control. The mean age of the study population did not differ significantly between cases and controls ($P = 0.79$). All dogs were less than 12 kg (mean = 7.8 ± 2.0 kg), and body weight did not differ between cases and controls ($P = 0.96$). As expected, the case group had a significantly higher median UCa/Cr compared with the control group ($P < 0.001$). Blood iCa was available for 37 of the 40 dogs and was significantly higher in cases compared to controls ($P < 0.001$). See Table 1 for further breakdown of case and control characteristics within each breed. Over half of the cases (14/20, 70%) had experienced recurrence of their CaOx urolithiasis. Nine of the 20 case dogs (45%) were being fed therapeutic stone-prevention diets (5 urinary A and 4 urinary B). The remaining case and control dogs were being fed a variety of commercial diets. Three dogs were receiving medications, including tramadol for pain associated with a urethral obstruction, cyclosporine 0.2% ophthalmic solution for keratoconjunctivitis sicca, and tetracycline and niacinamide for lupoid onychodystrophy.

Assay linearity ranged from 64 – 134%, and recovery rates ranged from 51 – 82%, both of which were outside of the acceptance criteria. The assay had good intra-assay precision with 14% CV (acceptance standard $<15\%$ CV). However, the inter-assay precision was high at 53% CV (acceptance standard $<20\%$ CV). On further review, a trend was noted with decreasing concentrations over time in the two study participant samples (one case and one control) that were used for the inter-assay precision analysis (Figure 1). Unlike the purchased control, which was aliquoted after receipt and reconstitution (from a lyophilized state), these two samples were single aliquots and underwent two freeze-thaw cycles on days

0 and 2 (after which they were aliquoted for the final two assays). The validation samples were stored at -20°C between assays (days 0 – 7).

In contrast to the validation samples, the study participant samples used for the study were stored at -80°C prior to the assay and run together in duplicate on a single ELISA plate. The length of serum storage at -80°C prior to the assay was not different between cases and controls (average of 2.9 years for both, $P=0.91$). The median serum β -crosslaps concentration was significantly lower in cases than controls with medians of 0.43 ng/mL (range, 0.28 – 1.60 ng/mL) and 0.66 ng/mL (range, 0.36 – 2.50 ng/mL), respectively ($P=0.0043$, Figure 2). Results of the multivariable regression are shown in Table 2. In addition to the significant relationship between stone status and serum β -crosslaps concentration, the regression revealed an effect of breed with lower concentrations in Miniature Schnauzers compared to the other two breeds (Figure 3). Age, sex, weight, diet, and storage time did not have a significant impact on serum β -crosslaps concentration. A moderate negative correlation ($r=-0.44$, $P=0.0044$) was found between serum β -crosslaps concentration and UCa/Cr (Figure 4). The correlation between serum β -crosslaps concentration and blood iCa was weak and non-significant ($r=-0.18$, $P=0.28$).

Discussion

This study demonstrates that a marker of bone resorption, serum β -crosslaps, is decreased in Miniature Schnauzer, Bichon Frise, and Shih Tzu dogs with a history of CaOx urolithiasis and IH relative to breed-, sex-, and age-matched stone-free controls. Furthermore, there was a moderate negative correlation between the degree of hypercalciuria as measured by spot fasting UCa/Cr and the magnitude of the serum β -crosslaps concentration. These results refute our initial hypothesis that markers of bone resorption are increased in dogs with CaOx urolithiasis and IH. The data instead suggest that dogs with CaOx urolithiasis and IH have reduced bone resorption, which may be a compensatory response to a disturbance in calcium homeostasis within the body.

A previous study on Miniature Schnauzers suggested that increased intestinal absorption of calcium was responsible for IH and CaOx urolithiasis risk within this breed (Lulich et al, 1991). This hypothesis was based on the finding of increased severity of hypercalciuria in fed versus fasted states. Increased intestinal absorption of calcium could result in a compensatory decrease in bone resorption. Serum samples from 29 of the 40 dogs in this study were subsequently included on a study of vitamin D metabolism (Groth et al, forthcoming). A subset of dogs with CaOx urolithiasis and IH had evidence for decreased deactivation of 1,25-dihydroxyvitamin D, which is a potential mechanism for absorptive hypercalciuria. Further testing using radiolabeled calcium could be performed to evaluate the role of intestinal hyperabsorption in dogs with CaOx urolithiasis and IH (Worcester and Coe, 2008). As previously discussed, renal leak hypercalciuria is considered less likely the source of IH, given that affected dogs often have high-normal blood iCa. Renal leak is also not supported by the present data, as bone resorption would be expected to increase in response to urinary loss. It is important to consider that the results of this study may not be applicable to all breeds. The mechanism of CaOx urolithiasis and IH may differ in breeds not studied here, and bone resorption or renal leak phenotypes may exist in the canine

population. Additionally, while the cases in this study had significantly lower β -crosslaps concentrations than controls, there was overlap between groups, and two cases had concentrations greater than the median for controls of their breed. Thus, even within a breed, the extent of bone turnover may not be the same for all dogs with CaOx urolithiasis and IH.

In this study population, there was an effect of breed on the concentration of serum β -crosslaps. Specifically, Miniature Schnauzers had lower concentrations than the other two breeds. A previous study on another serum biomarker of collagen breakdown, carboxyterminal telopeptide of type-I collagen, found no difference between Pomeranians and Irish Wolfhounds (Breur et al., 2004), and the results for both breeds in that study were also within the range reported for Beagles (Allen et al., 1998). However, the influence of breed on β -crosslaps may be different from other bone resorption biomarkers. In fact, one study reported a significant effect of body weight on canine β -crosslaps with lower serum concentrations in dogs >25 kg compared with lower weight groups (Beli et al., 2010). All of the dogs in this study were <12 kg, and there was no significant effect of weight on serum β -crosslaps concentration in the multivariable regression. Another possibility is that Miniature Schnauzer serum has a biological difference from the other breeds that affects recovery of β -crosslaps. This breed has a high prevalence of hyperlipidemia (Xenoulis et al., 2007). Lipemia is not reported to cause interference with crosslaps detection in human serum (Rosenquist et al., 1998), but interfering substances have not been investigated in dogs.

A previous study indicated that female dogs have higher concentrations of serum β -crosslaps than male dogs (Beli et al., 2012). Male-to-female ratio was matched between cases and controls to account for this potential difference in bone turnover rates between sexes, and sex was considered a covariate in the regression model. In our population, there was no significant effect of sex on serum β -crosslaps concentration. The absence of an influence of sex may be related to reproductive status. All but one dog in this study were neutered, whereas the study that identified a difference between sexes included only reproductively intact dogs.

To our knowledge, this is the first study reporting use of a canine specific ELISA β -crosslaps assay. The previous two studies on serum β -crosslaps in dogs used a human ELISA assay, and they did not report validation assessments (Beli et al., 2010; Beli et al., 2012). The linearity of the canine specific ELISA was poor, which confirms the manufacturer's statement that the samples should not be diluted for this assay. Recovery rates were also below the acceptable range, suggesting that there may be binding components in canine serum that reduce the β -crosslaps detector response. The potential source of interference is unknown; common serum components (e.g. hemoglobin, bilirubin, lipids, ascorbic acid) do not interfere with detection of crosslaps in human serum (Rosenquist et al., 1998). The canine specific ELISA intra-assay precision was acceptable. However, the inter-assay CV was high. The high variability appeared to be caused by decreasing β -crosslaps concentration over time rather than random variation. This indicates an issue with stability of the β -crosslaps molecule rather than the assay. Human β -crosslaps has been reported to be stable at -20°C or -80°C for years (Qvist et al., 2004) and room temperature (20°C) for days (Rosenquist et al., 1998), and there is no significant effect of multiple freeze thaw

cycles (Rosenquist et al., 1998). However, β -crosslaps will degrade in serum stored at higher temperatures; the $T_{1/2}$ is 4 days at 37 °C (Qvist et al., 2004). Two of the samples used in the validation assays underwent freeze-thaw cycles, and all three validation samples were stored at –20 °C between assays. It is unknown whether canine β -crosslaps differs from human and is less stable under these conditions or if there was another undetermined factor on the assay dates that affected stability. Since the samples used in the present study were all run on a single plate with the same handling and storage conditions, the suboptimal recovery and inter-assay CV do not affect interpretation of case-control comparisons. Until more is understood about canine β -crosslaps recovery and stability, future studies should similarly aim to minimize variation in sample handling and storage and only compare results within an assay, not across studies.

The most clinically relevant consequence of IH in dogs is the morbidity associated with CaOx urolithiasis. In contrast to people, there are no reports of documented bone demineralization in dogs with CaOx urolithiasis. The results of this study support an absence of a bone resorption phenotype in canine CaOx urolithiasis and IH. The biochemical differences between species have clinical implications and require different treatment strategies. For example, bisphosphonates inhibit osteoclastic bone resorption and are used in people with IH and bone demineralization (Arrabal-Polo, et al., 2014), but they would be contraindicated in dogs with IH based on the findings of this study.

In conclusion, the present study found evidence for decreased bone resorption in Miniature Schnauzer, Bichons Frise, and Shih Tzu with CaOx urolithiasis and IH. Measurement of β -crosslaps in larger populations and further investigation of bone turnover biomarkers, such as osteocalcin, are warranted to confirm these findings. Evaluation of the other possible causes of IH, such as intestinal hyperabsorption, will also help to uncover the mechanism underlying canine CaOx urolithiasis and IH.

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Abbreviations

CaOx	calcium oxalate
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
IH	idiopathic hypercalciuria
iCa	ionized calcium
UCa/Cr	urinary calcium-to-creatinine ratio
ANCOVA	analysis of covariance

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Figure 1 –.

Inter-assay variation in canine serum β -crosslaps (ng/mL) for a dog with a history of calcium oxalate urolithiasis (case) and a stone-free dog (control) used in assay validation. The samples underwent freeze-thaw cycles after assays performed on day 0 and 2. A trend is present with decreased β -crosslaps concentrations with each assay.

Figure 2 -

Box and whisker plots of serum β -crosslaps (ng/mL) in dogs with a history of calcium oxalate urolithiasis (cases) and matched stone-free dogs (controls). The boxes represent the interquartile range (25th–75th percentile), the horizontal line within the boxes represents the median and the whisker bars extend to 1.5 times the interquartile range. Dots represent individual dog measurements; those that fall above or below the whisker bars are outliers. The difference between cases and controls was significant, $P = 0.0043$.

Figure 3 -

Box and whisker plots of serum β -crosslaps (ng/mL) in dogs with a history of calcium oxalate urolithiasis (cases) and stone-free dogs (controls) for three different breeds. The boxes represent the interquartile range (25th–75th percentile), the horizontal line within the boxes represents the median and the whisker bars extend to 1.5 times the interquartile range. Dots represent individual dog measurements; those that fall above or below the whisker bars are outliers. Miniature Schnauzers had significantly lower serum β -crosslaps concentrations than the other two breeds, $P < 0.001$.

Figure 4 –.

Correlation of serum beta-crosslaps (logβ-crosslaps) and urinary calcium-to-creatinine ratio (logUCa/Cr); $r = -0.44$ and $P = 0.0044$. Data are plotted on a base 10 logarithmic scale.

Table 1.

Patient characteristics, fasting spot urinary calcium-to-creatinine ratio (UCa/Cr), blood ionized calcium concentrations in dogs with a history of calcium oxalate stones (cases) and matched stone-free dogs (controls).

Breed	N	Age (years)	Sex (M:F)	UCa/Cr (mg/mg)	Blood iCa (mg/dL)
Miniature Schnauzer					
Cases	9	10.9 ± 2.0	5:4	0.10 (0.05 – 0.28) [‡]	5.5 ± 0.2 [‡]
Controls	9	9.8 ± 1.4	5:4	0.02 (0.01 – 0.03)	5.2 ± 0.1
Bichon Frise					
Cases	7	10.0 ± 1.8	5:2	0.10 (0.05 – 0.29) [‡]	5.5 ± 0.1 [*]
Controls	7	10.0 ± 1.6	5:2	0.02 (0.01 – 0.05)	5.2 ± 0.3
Shih Tzu					
Cases	4	9.0 ± 0.8	2:2	0.13 (0.12 – 0.14) [*]	5.6 ± 0.4
Controls	4	10.5 ± 2.4	2:2	0.02 (0.01 – 0.03)	5.4 ± 0.1
Overall					
Cases	20	10.1 ± 1.6	12:8	0.12 (0.05 – 0.29) [‡]	5.5 ± 0.2 [‡]
Controls	20	10.2 ± 1.9	12:8	0.02 (0.01 – 0.05)	5.3 ± 0.2

Age and blood iCa are reported as average ± SD, and UCa/Cr is reported as median (range). Significant differences between cases and controls are represented with ^{*} $P < 0.05$, [†] $P < 0.01$, and [‡] $P < 0.001$.

Table 2.

Multivariable regression for effects of calcium oxalate (CaOx) stone status, breed, sex, age, weight, diet, and sample storage time (at -80°C) on serum β -crosslaps. For categorical variables with 2 degrees of freedom, the status corresponding to the estimate is in parentheses. For continuous variables, the predictor unit is in parentheses.

Parameter	Estimate of the Coefficient*	95% CI		P value
		Lower	Upper	
CaOx status (case)	0.62	0.44	0.88	0.0092
Breed				0.0044
Miniature Schnauzer	Referent		NA	NA
Bichon Frise	1.51	1.04	2.19	0.032
Shih Tzu	2.20	1.41	3.44	0.0011
Sex (male)	0.92	0.68	1.25	0.57
Age (year)	1.03	0.94	1.13	0.48
Weight (kg)	1.00	0.92	1.09	0.98
Diet				0.21
Other	Referent		NA	NA
Urinary A	0.80	0.45	1.42	0.44
Urinary B	1.37	0.76	2.48	0.28
Sample storage time (year)	1.00	0.85	1.18	0.97

CI = Confidence interval. Model coefficient of determination = 0.57. Significant *P* values (<0.05) are bolded.

* The regression was performed with log-transformed β -crosslaps as the outcome. For ease of interpretation, the regression coefficients and 95% CI have been back-transformed to the original scale and represent estimates for the factor change in β -crosslaps per status change or 1-unit increase in the predictor.