Relationship of serum total calcium to serum albumin in dogs, cats, horses and cattle

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Abstract

A retrospective study was performed in order to assess the relationship between serum calcium and serum albumin concentrations in domestic animals. Results of 9041 canine, 1564 feline, 2917 equine, and 613 bovine serum samples from hospitalized patients were examined by regression analysis. Subpopulations of cases with concurrent elevations in creatinine or that were less than six months of age were evaluated separately. Statistically significant linear relationships between calcium and albumin concentrations were established for each species ($p < 0.05$). The coefficients of determination (r^2) were 0.169 for dogs, 0.294 for cats, 0.222 for horses, and 0.032 for cattle. The correlation coefficients (r) computed were: dogs = 0.411, cats = 0.543, horses = 0.471, cattle = 0.182. Neither increases in creatinine concentration nor juvenile age appreciably influenced the relationship between calcium and albumin concentrations. Interspecies variation was marked, and a strong correlation between calcium and albumin concentrations was not established in any species.

Résumé

Le rapport entre le calcium sérique total et l'albumine sérique chez le chien, le chat, le cheval et la vache

Une étude rétrospective a été effectuée pour évaluer le rapport entre les taux de calcium sérique total et d'albumine chez les animaux domestiques. Une analyse statistique par régression linéaire a été effectuée sur des échantillons de sérum d'animaux hospitalisés comprenant 9041 canins, 1564 félins, 2917 équins et 613 bovins. Les animaux dont le taux de créatinine était élevé ou ceux âgés de moins de six mois ont été évalués séparément en sous-groupes. Le coefficient de corrélation (r^2) ($p < 0,05$) a été établi pour chaque espèce : 0,169 pour les canins, 0,294 pour les félins, 0,222 pour les équins et 0,032 pour les bovins. Le coefficient de corrélation calculé était respectivement : canins : 0,411; félins : 0,543; équins : 0,471 et bovins : 0,182. Les résultats indiquent qu'un taux élevé en créatinine ou le jeune âge de l'animal n'influencent pas de façon appréciable le rapport entre les taux de calcium et d'albumine. Les variations interespèces étaient importantes et une forte corrélation entre les taux de calcium et d'albumine n'a pu être établie pour plusieurs espèces.

(Traduit par Dr Thérèse Lanthier)

Can Vet J 1993; 34: 360–364.

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Introduction

Serum calcium concentration is commonly assessed during routine biochemical evaluation of veterinary patients. Total serum calcium consists of two major fractions, ionized and protein-bound. A small portion of serum calcium is complexed with anions, such as citrate and phosphate. During homeostasis, the amount of ionized calcium, which is the physiologically active fraction, is tightly regulated through the interactions of parathyroid hormone, calcitonin, and 1,25-dihydrocholecalciferol (vitamin D_3). Derangements in the amount of ionized calcium are clinically important and associated with specific disease processes (1,2). However, determination of the ionized calcium concentration requires specialized equipment, such as ion-selective electrodes as well as strict collection conditions not practical for use in most veterinary laboratories (3,4). In addition, serum pH influences the amount of calcium that is present in the ionized form, and studies that have not been adjusted for this parameter are likely to be of questionable validity (5). As approximately half of the total serum calcium is bound to proteins, changes in serum albumin concentration may result in a change in the concentration of total serum calcium, though this does not necessarily reflect a change in the portion of calcium available for physiological processes. Formulae have been calculated based on regression analysis of albumin and calcium concentrations in order to "correct" for apparent hypocalcemia that occurs concurrently with hypoalbuminemia or hypoproteinemia (6,7). These equations are intended to allow for adjustment of the total calcium concentration, and indirectly of the ionized calcium fraction, over varying concentrations of albumin, assuming a significant linear relationship between these two variables.

The validity of established correction formulae has been tested in relatively small populations of dogs and cats (6,8). The purpose of the study reported here was to discern whether the previously published relationship between calcium and albumin could be applied to a large population of dogs and cats, and whether a similar association exists in horses and cattle.

Materials and methods

Sample population

A retrospective analysis was performed on 9041 canine, 1564 feline, 2917 equine, and 613 bovine serum samples submitted for biochemical evaluation between October 1, 1987, and May 26, 1992, from the Veterinary Teaching Hospital of the University of Guelph. All available data entries from feline, equine, and bovine patients were included in the analysis. However, 36 dogs with calcium concentrations exceeding 4 mmol/L and with concurrent albumin values within the reference range (22 g/L to 35 g/L) were excluded

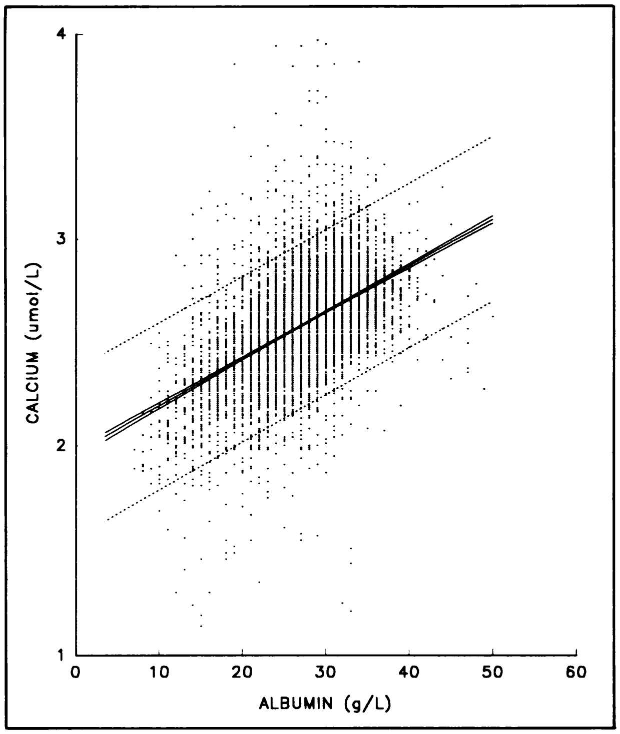

Figure 1. Scatter-plot, least-squares regression line, 95% confidence intervals (— — —), and 95% prediction intervals (•••) of serum calcium and albumin concentrations in dogs ($n = 9005$, $r^2 = 0.169$, $r = 0.411$, $p < 0.05$).

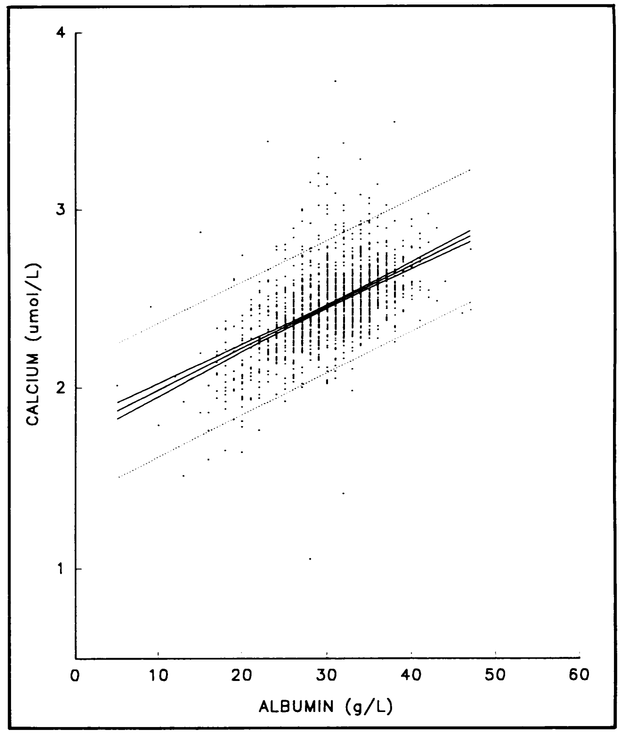

Figure 2. Scatter-plot, least-squares regression line, 95% confidence intervals (— — —), and 95% prediction intervals (•••) of serum calcium and albumin concentrations in cats ($n = 1564$, $r^2 = 0.294$, $r = 0.543$, $p < 0.05$).

from the analysis. Hypercalcemia in these cases was determined to be associated with either a paraneoplastic syndrome, due to lymphosarcoma (n = 26), apocrine adenocarcinoma of the anal sac (n = 2), osteosarcoma (n = 2), pheochromocytoma (n = 1); or with renal failure (n = 2); primary hyperparathyroidism (n = 2); or systemic granulomatous disease (n = 1).

Subpopulations of each species with elevated creatinine concentrations (canine ≥145 μmol/L, feline ≥180 μmol/L, equine ≥165 μmol/L, bovine ≥168 μmol/L) were evaluated separately. Similarly, subsets of juvenile canine and bovine cases (age ≤6 months) were individually examined.

Calcium and albumin concentration determinations
All biochemical values were determined using an automated serum analyzer (Coulter DACOS, Coulter Electronics Inc., Hialeah, Florida, USA) that quantitates calcium by a cresolphthalein complexone method, and albumin by bromcresol green complex formation. Representative quality control indices for a six-month period showed a coefficient of variation of 3.5 % for calcium and 3.3 % for albumin.

Statistical analysis
The relationship between serum calcium and albumin concentrations was assessed by linear regression analysis (Northwest Analytical Inc., Portland, Oregon, USA). Scatter diagrams were plotted, and the least-squares lines, 95% confidence intervals, and 95% prediction intervals were computed (SIGMA PLOT, Jandel Scientific, San Rafael, California, USA). The regression equations were evaluated by calculating the coefficient of determination (r^2), and by testing the null hypothesis of no linear relationship between the two variables. An F distribution was constructed from the sample variances and tested for significance (9). The strength of the linear relationship was assessed by obtaining the correlation coefficient (r), and testing whether it was different from zero. T-tests were performed to evaluate whether the slopes of the regression lines were significantly different from zero. Differences were considered significant if $p < 0.05$.

Results

Relationship of calcium and albumin concentrations
A positive linear relationship between calcium and albumin was found in all species (Figures 1 - 4). The statistical parameters of the relationships are summarized in Table 1. Results of analysis of variance indicated that there was a significant linear relationship between the two variables for each species, and that the two variables were correlated ($p < 0.05$). In all instances, the correlation coefficients and slopes were significantly different from zero.

Subgroups
Within the total canine sample population, 732 dogs (8.13%) had increased creatinine concentrations, and 121 dogs (1.34%) had calcium and creatinine concentrations that were concurrently increased. Dogs less than six months of age constituted 3.78% (n = 340) of the sample population, and 56 of them had elevated serum calcium concentrations. Within the total canine sample population, the percentage of juvenile dogs with increased calcium concentrations was, therefore, 0.62%.

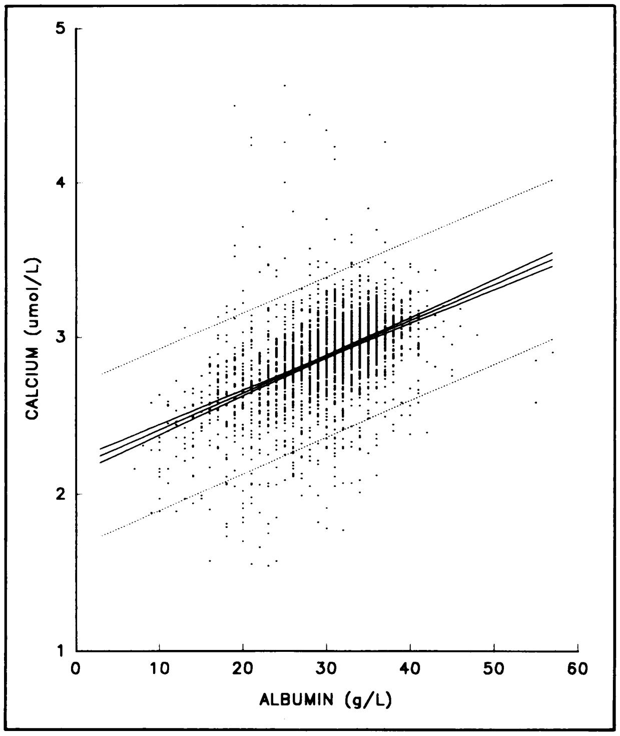

Figure 3. Scatter-plot, least-squares regression line, 95% confidence intervals (– – –), and 95% prediction intervals (•••) of serum calcium and albumin concentrations in horses ($n = 2917$, $r^2 = 0.222$, $r = 0.471$, $p < 0.05$).

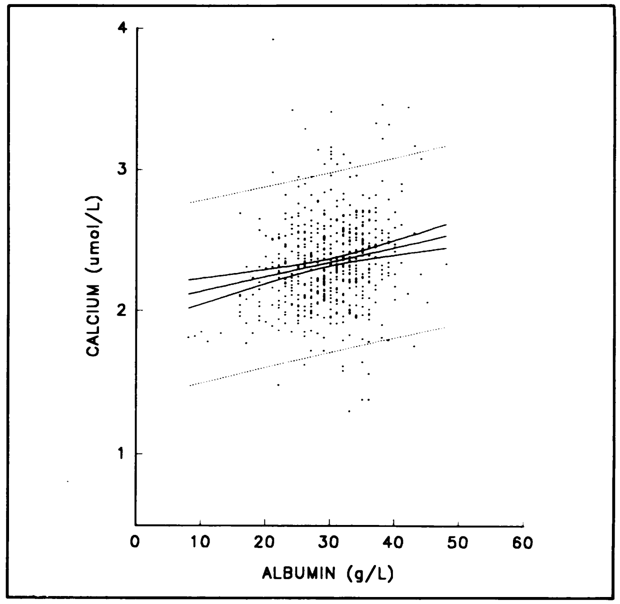

Figure 4. Scatter-plot, least-squares regression line, 95% confidence intervals (– – –), and 95% prediction intervals (•••) of serum calcium and albumin concentrations in cattle ($n = 613$, $r^2 = 0.032$, $r = 0.182$, $p < 0.05$).

Among 1564 cats evaluated, 321 (20.52%) had creatinine concentrations above the reference range. However, only 17 cats (1.09%) had concurrently elevated calcium and creatinine concentrations.

In the equine sample population, 603 animals (20.67%) had elevated creatinine values, and 28 (0.96% of $n = 2917$) had concurrent abnormally high serum calcium concentrations.

Six hundred and thirteen bovine samples were evaluated: in 135 (22.02%), the creatinine concentration was elevated, and in 37 (6.04%), both the creatinine and calcium concentrations were increased. Regression analysis of calcium concentration on creatinine concentration resulted in $r^2 = 0.006$, and $r = -0.081$. Within the total bovine sample population, 234 (38.17%) animals were less than six months of age. Forty-seven (7.67% of $n = 613$) cattle were of juvenile age and had concurrently increased calcium concentrations. Evaluation of the relationship between age and serum calcium concentration yielded $r^2 = 0.032$, and $r = -0.180$.

Table 1. Summary of regression analyses of serum calcium and albumin concentrations for dogs, cats, horses, and cattle

Species	n	r	r^2	F-test[a]
Canine	9005	0.411	0.169	1824.376
Feline	1564	0.543	0.294	651.867
Equine	2917	0.471	0.222	831.788
Bovine	613	0.182	0.032	20.949

[a] All values were significant at $p < 0.05$

Discussion

Hypocalcemia and hypercalcemia are frequently detected on biochemical profiles from domestic animals. However, clinical signs attributable to abnormal concentrations of ionized calcium are not commonly recognized (10). Presumably, most of these measured changes are due to temporary alterations in protein binding of calcium, as may occur with prolonged venous occlusion, different postures, and unusual compositions of the protein component of serum (11), and do not reflect variations in the ionized compartment. Albeit, true aberrations in ionized calcium that are of great clinical importance do occur and may not be revealed by the total calcium concentration. The goal of this study was to examine the relationship of serum calcium and albumin concentrations in large populations of four domestic animal species. Eight canine cases with a diagnosis of primary disturbance of calcium homeostasis were included in the analysis, as their exclusion did not appreciably affect the statistical analysis. Diagnoses of hyperparathyroidism or hypoparathyroidism were not recorded for cats, horses or cattle.

Our results in dogs partially agree with a previous study of 209 dogs regarding the relationship of calcium and albumin concentrations ($r = 0.575$, $r^2 = 0.33$) (6). The correlation coefficient obtained in our analysis ($r = 0.411$) indicates a moderately strong linear relationship between the two variables examined, and compares favorably with the previously published results. However, the closeness of fit of the regression equation is poor, and only approximately 17% of the change in calcium concentration ($r^2 = 0.169$) can be attributed to variations in albumin concentrations (Table 1). Lack of a greater interdependence, therefore, questions the ubiquitous use of correction formulae to adjust total calcium values for hypoalbuminemic canine patients. In vitro studies have failed to demonstrate that a finite amount of calcium binds to albumin at physiological concentrations of these analytes (12). Moreover, many different albumin sites capable of binding calcium with varying affinities have been identified, and it was noted in a study of human patients that albumin-bound calcium varied inversely with the absolute albumin concentration (13). Such phenomena may also occur in dogs, and until ionized and protein-bound fractions have been determined over a wide range of albumin concentrations, adjustments based on a linear correlation are likely to lead to erroneous interpretations of normocalcemia, particularly in hypoalbuminemic patients.

In the population of dogs examined, animals with creatinine concentrations exceeding 145 μmol/L were assessed separately in order to eliminate renal failure as a factor potentially contributing to hypercalcemia. However, as only 1.34% of the total sample population had concurrently high values of calcium and creatinine, and since the regression analysis did not differ appreciably when these populations were eliminated, dogs with this biochemical abnormality were not excluded from the overall analysis. Similarly, juvenile age was not found to result in frequent hypercalcemia, so dogs less than six months of age were included in the analysis.

Malignancy is the most common cause of hypercalcemia in dogs (7), and the elevation reflects a true increase in all calcium fractions (14). Lymphosarcomas are the neoplasms most frequently associated with hypercalcemia as they may elaborate a parathyroid hormone-like circulating factor that stimulates calcium resorption from bone and by proximal tubules via activation of adenylate cyclase (14). As dogs with lymphosarcoma are commonly normoalbuminemic, unless renal failure with proteinuria secondary to persistent hypercalcemia has occurred, no presumed relationship exists between the two variables; therefore, such cases were excluded from this analysis.

The data obtained from cats in this study had a correlation coefficient of 0.543, indicating that there was a relatively strong linear relationship between the serum calcium and albumin concentrations. This finding corresponds to a recent report where the authors found an r-value ($r = 0.42$) similar to ours; however, their coefficient of determination was markedly lower than in our analysis ($r^2 = 0.18$ vs. $r^2 = 0.29$) (8). Although our computations indicated that approximately 29% of the variation in calcium could be explained by a given value of albumin, we feel this is insufficient justification for the routine use of adjustment formulae. The lack of a stronger correlation of albumin and calcium concentrations in cats may be attributable to differences in calcium-protein interactions in the cat in contrast to humans.

Assessment of a subpopulation of cats with increased serum creatinine and calcium concentrations resulted in a group of only 17 animals, or 1.03% of the total sample population; therefore, these animals were included in the overall analysis. Only two cats with markedly increased creatinine concentrations (> 400 μmol/L) were hypercalcemic; thus, renal failure in cats apparently rarely leads to hypercalcemia.

Regression analysis of the 2917 equine serum samples demonstrated a positive linear relationship between albumin and calcium concentrations with $r^2 = 0.222$. Therefore, for any given albumin value, there is still a relatively large amount of variation to be expected in the calcium concentration that is, as yet, unexplained. The correlation coefficient computed in this study is markedly higher than that previously reported in a sample of 39 horses; this may partly be attributed to the large disparity in sample size (15). The reported percentage of ionized calcium is greater in horses than in humans (5,15), suggesting that anionic serum components, such as citrate, phosphate, lactate, and bicarbonate, may have a more vital role in calcium-binding in horses. Therefore, the importance of albumin appears to be relatively decreased in this species.

It was suggested in a report on chronic renal failure in horses with profound hypercalcemia and concurrent edema and hypoalbuminemia that the equine kidney has great importance for calciuresis during homeostasis, and that decreased renal clearance may lead to an accumulation of calcium (16). However, our results indicate that concurrence of hypercalcemia and hypercreatininemia is an isolated phenomenon (28 of 603 cases with elevated creatinine), and that no significant linear relationship exists between the above analytes ($r^2 = 8.27 \times 10^{-7}$, $r = -0.001$). The majority of horses examined in our study had mild elevations in creatinine (< 300 μmol/L), which were likely due to decreased circulatory volume. Correspondingly, horses with marked increases in creatinine concentration were not reliably found to have increased serum calcium concentrations. Therefore, all horses with increased creatinine concentrations were included in the analysis.

The relationship of serum albumin to calcium concentrations was found to be poorest in cattle. Although a statistically significant relationship was found, the coefficient of determination was very small ($r^2 = 0.032$). A recent study involving 141, clinically normal, Holstein cows determined that ionized calcium was consistently approximately 50% of total calcium and that both values were highly correlated ($r = 0.80$) (2). When ionized calcium was pH-adjusted, the r-value was 0.90; however, with both determinations, the time between collection and analysis was important (2). Although the albumin concentration was not assessed concurrently, the excellent correlation found between ionized and total calcium could be extended to 85 cows afflicted with various pathological conditions (2). Combining our results with those of the above study, it might be concluded that ionized and total calcium concentrations in cattle are relatively independent of albumin-binding, while other factors, such as concurrent acid-base or electrolyte abnormalities, are likely of greater importance. Hypomagnesemia, for example, reduced the rate of calcium mobilization in cattle, while increases in pH resulted in decreased ionized calcium concentrations (17). Therefore, the total serum calcium concentration alone may be sufficient to allow for diagnosis of clinically important changes in ionized calcium concentration.

In summary, while correction formulae may aid calcium interpretations in some instances, the lack of a strong association in the sample populations examined here precludes their use for adjustments of calcium concentration based solely on albumin concentration. Variations in the strength of relationship between the variables were found among the species assessed; the apparent greatest association existed in cats, and the least in cattle. However, the overall binding characteristics of calcium to albumin or other proteins are likely too complex to be predictably characterized by a simple linear regression analysis.

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