Study of the Effect of Total Serum Protein and Albumin Concentrations on Canine Fructosamine Concentration

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ABSTRACT

The relationship among serum fructosamine concentration and total serum protein and albumin concentrations were evaluated in healthy and sick dogs (diabetics and dogs with insulinoma were not included). Fructosamine was determined using a commercial colorimetric nitroblue tetrazolium method applied to the Technicon RA-500 (Bayer). Serum fructosamine concentration was not correlated to total protein in normoproteinemic (r = 0.03) and hyperproteinemic dogs (r = 0.29), but there was a high correlation (r = 0.73) in hypoproteinemic dogs. Similar comparison between serum fructosamine and albumin concentrations showed middle correlation (r = 0.49) in normoalbuminemic dogs and high degree of correlation (r = 0.67) in hypoalbuminemic dogs. These results showed the importance of recognizing serum glucose concentration as well as total serum protein and albumin concentrations in the assay of canine serum fructosamine concentration.

RÉSUMÉ

La relation entre la concentration sérique de fructosamine et les concentrations sériques totale de protéine et d'albumine a été évaluée chez des chiens en santé et malades (les chiens diabétiques et ceux ayant un insulinome furent exclus de l'étude). Le niveau de fructosamine fut déterminé à l'aide d'un appareil Technicon RA-500 (Bayer) par une méthode colorimétrique utilisant le nitrobleu de tétrazolium. Aucune corrélation n'a été détectée entre la concentration sérique de fructosamine et le taux de protéines totales chez les chiens avec un taux normal (r = 0,03) ou élevé (r = 0,29) de protéines, alors qu'une forte corrélation (r = 0,73) était observée chez les chiens hypoprotéinémiques. Des comparaisons semblables entre la concentration sérique de fructosamine et les concentrations d'albumine ont démontré une forte corrélation (r = 0,67) seulement chez les chiens ayant de faibles taux sériques d'albumine. Ces résultats démontrent l'importance des concentrations sériques de glucose, des protéines totales et de l'albumine lors de la détermination des concentration sériques de fructosamine.

(Traduit par le docteur Serge Messier)

INTRODUCTION

The term fructosamine refers to a nonenzymatic chemical reaction between a glucose molecule and a free amino group, typically the epsilon-amino group of the lysine residues on proteins. The fructosamine concentration depends on the serum protein concentration and its composition, and on the average serum glucose concentration over the circulatory lifetime of the serum proteins. The serum fructosamine concentration will increase with prolonged hyperglycemia, but will fall with prolonged hypoglycemia or an increased protein turnover (1,2). Albumin is the most abundant serum protein and it is glycated more rapidly than the other serum proteins. Thus, glycated albumin accounts for about 80% of the glycated serum proteins (3,4). Therefore, because the average life of albumin in dogs is 8 d, the serum fructosamine concentration can be used to evaluate the mean glucose concentration over the preceding 1 to 2 wk (5,6).

The relationship between serum fructosamine and glucose concentrations is well-known (5–9). The studies about the effect of total serum protein and albumin concentrations on serum fructosamine concentration showed an interaction among these parameters. These results suggested that fructosamine concentration should be corrected for the total serum protein and albumin concentrations (6,10,11). Nevertheless, the use of this correction factor is still a matter for debate. Some authors consider that correction of fructosamine concentration on protein was inappropriate in diabetic patients (12), in patients with hypo- or hyperthyroidism (13) and in normoproteinemic patients (14). On the other hand, the correlation between corrected fructosamine and serum glucose concentration in diabetic patients was higher (15). The significance of the effects of total serum protein and albumin concentration on canine serum fructosamine has been a matter for debate by many authors (6,16,17).

The objective of the study was to determine the influence of serum total protein and albumin concentrations on fructosamine concentration in canine serum samples.

MATERIAL AND METHODS

SERUM SAMPLES

Blood samples were obtained from 207 dogs (96 healthy dogs and 111 sick dogs) of different ages, sexes and breeds from the Small Animal

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TABLE I. Serum fructosamine concentrations in dogs with different total serum protein levels

Fructosamine (μmol/L)	*n*	$x \pm 2SD$
Group 1[a]	27	238.38 ± 106.20
Group 2	107	289.20 ± 87.04[b]
Group 3	9	221.55 ± 135.00

[a] Group 1: serum total proteins < 55 g/L
Group 2: serum total proteins = 55-77 g/L
Group 3: serum total proteins ≥ 77 g/L
[b] Significant differences between Group 1 and Group 2 and between Group 2 and Group 3 ($P < 0.01$)

Internal Medicine Department at the Veterinary Faculty of the University of Zaragoza. In order to study the effect of total serum protein on fructosamine concentration, dogs were distributed in 3 groups according to the total protein concentration: Group 1 (n = 27): total serum protein < 55 g/L, Group 2 (n = 107): total serum protein = 55-77 g/L, Group 3 (n = 9): total serum protein ≥ 77 g/L.

In the same way, the effect of serum albumin on fructosamine concentration was studied in 2 groups of animals: Group 1 (n = 26): albumin < 30 g/L, Group 2 (n = 113): albumin ≥ 30 g/L.

SAMPLE COLLECTION

Blood samples were obtained from the jugular or cephalic veins. Blood without anticoagulant was centrifugated. Serum was then aspirated and frozen at −20°C before the determination of total protein, albumin and fructosamine concentrations.

ASSAY PROCEDURES

Serum fructosamine concentration was determined using a commercial colorimetric nitroblue tetrazolium (NBT) method (Fructosamine MRP3, 1054686, Boehringer Mannheim) applied to the Technicon RA-500 analyzer (Bayer). Each specimen was analyzed with the use of normal (Precinorm Fructosamine, 1098985, Boehringer Mannheim) and pathological control samples (Precipath Fructosamine, 1174118, Boehringer Mannheim).

Total protein (Total Protein Reagent T01-1301, Bayer) and albumin (Albumin Reagent T01-1377, Bayer) as well as fructosamine were applied to the automatic analyzer (Technicon RA-500, Bayer).

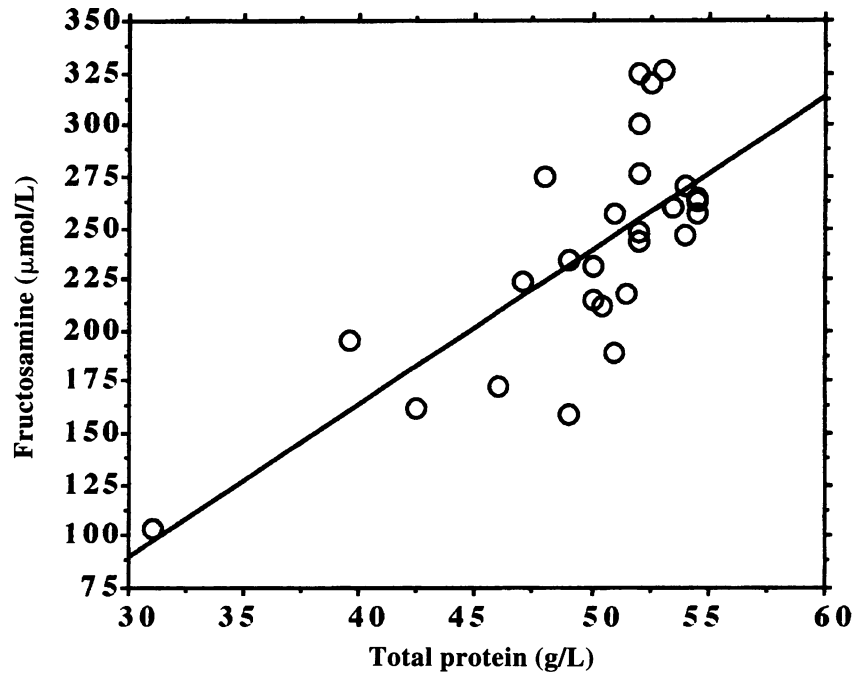

Figure 1. Regression of serum fructosamine and total serum protein concentrations in hypoproteinemic dogs (r = 0.73); $y = 74.87; x - 134.55$; $r^2 = 0.54$.

STATISTICAL METHODS

The fructosamine, total protein and albumin concentration values were evaluated for approximate normality of distribution. The reference intervals were calculated as the mean value ± 2 standard deviation ($x \pm 2SD$). Linear regression and correlation analysis (StatView) were used to determine the linear relationship between total serum protein/fructosamine and albumin/fructosamine concentrations.

RESULTS

Table I shows the mean serum fructosamine concentration in each of the 3 groups of animals with different levels of total serum protein (diabetics and dogs with insulinoma were not included). The highest fructosamine concentration (289.20 ± 87.04 μmol/L) corresponds to those animals with normal total protein levels. This fructosamine concentration is very similar to the previously established reference value (282.60 ± 69.44 μmol/L) (18). Nevertheless, serum fructosamine concentration decreased in hypo- and hyperproteinemic dogs (238.38 ± 106.20 μmol/L; 221.55 ± 135.00 μmol/L, respectively). Significant differences ($P < 0.01$) were observed between Groups 1 and 2 and Groups 2 and 3 (Table I).

In order to study the correlation between total serum protein and fructosamine concentration all the results as well as each group of animals were analyzed by use of linear regression. No correlation (r = 0.11) between either parameters was found for all the results. However, the study of each group showed a different correlation according to the total serum protein concentration. In this way, no correlation (r = 0.03) between total protein and fructosamine concentrations was found in Group 2 (normal total protein concentration). Little correlation (r = 0.29) was obtained in hyperproteinemic dogs (Group 3), and this correlation was higher (r = 0.73) when total protein levels decreased (Figure 1).

In the same way, serum fructosamine concentration was obtained according to the albumin concentration (Table II). Serum fructosamine concentration was lower in hypoalbuminemic dogs (Group 1) (202.50 ± 103.14 μmol/L) than in normoalbuminemic dogs (Group 2) (290.78 ± 75.12 μmol/L). Significant differences ($P < 0.01$) were observed.

The study of both groups of animals together showed a high degree of correlation (r = 0.76) between

TABLE II. Serum fructosamine concentration in dogs with different serum albumin levels

Fructosamine (μmol/L)	*n*	$x \pm 2SD$
Group 1[a]	26	202.50 ± 103.14[b]
Group 2	113	290.78 ± 75.12

[a] Group 1: serum albumin < 30 g/L
Group 2: serum albumin ≥ 30 g/L
[b] Significant difference between Group 1 and Group 2 ($P < 0.01$)

fructosamine and serum albumin concentrations. When fructosamine concentration was compared only in normoalbuminemic dogs medium correlation (r = 0.49) was obtained. But when fructosamine concentration was compared for hypoalbuminemic samples alone, a high degree of correlation (r = 0.67) was observed (Figure 2).

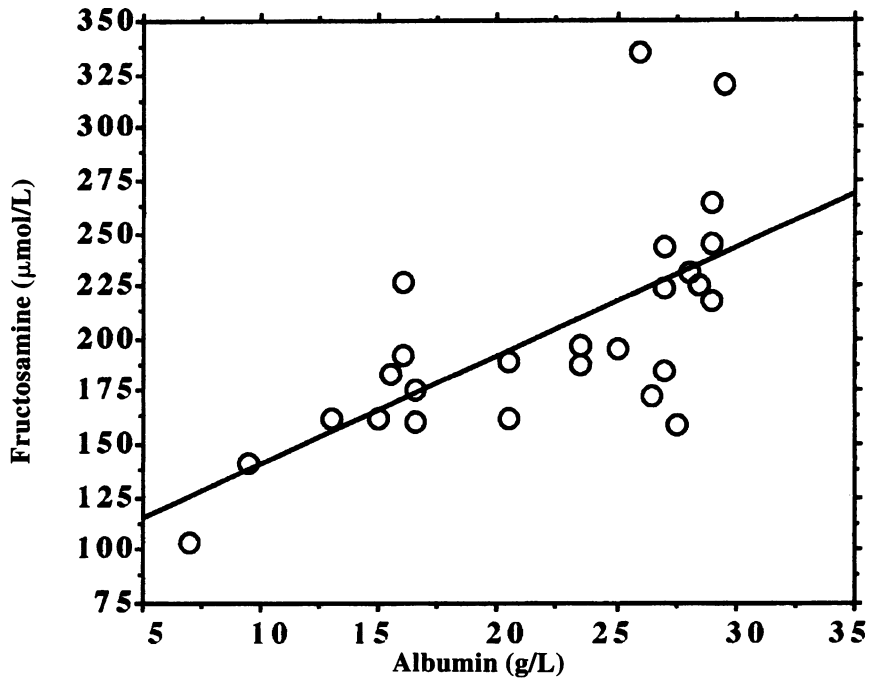

Figure 2. Regression of serum fructosamine and albumin concentrations in hypoalbuminemic dogs (r = 0.67); $y = 51.2; x + 89.6$; $r^2 = 0.44$.

DISCUSSION

There are a small number of studies about the relationship between serum fructosamine and serum protein concentrations in veterinary medicine. That is why there are no other facts that corroborate our results on the different levels of fructosamine according to the total serum protein concentrations. In a study including healthy, diabetic, and hypo- and hyperproteinemic dogs, serum fructosamine was not correlated to total serum protein (r = 0.24) (6). This result was similar to ours (r = 0.11), when all the animals were included. Similar results were described in cats (r = 0.20) by Lutz et al (19). The correlation between fructosamine and total protein concentrations in hypoproteinemic dogs (r = 0.73) was higher than that reported by Thoresen and Bredal (r = 0.47) (17).

Our results are the opposite to those reported in human serum samples. Desjarlais et al (20) reported a highly significant correlation (r = 0.81) between serum fructosamine and total serum protein. The explanation for this difference may lie in differences in amino acid composition and the sequence of canine proteins compared with human proteins (10). The human sample results suggested the efficiency of correcting the serum fructosamine concentration only when the serum total protein concentration were outside the reference intervals (14,21–23).

With regard to the application of any correction factor in veterinary science, the authors opinions were divergent. The studies on canine serum samples showed that the correction of the serum fructosamine concentration may not be necessary because of the low correlation with total serum protein concentration (6). Moreover, diabetic dogs had high uncorrected and corrected serum fructosamine concentrations, and no significant differences were observed between them (16).

In relation to the studies on cats, although no correlation was found between fructosamine and total protein in healthy cats, those parameters were correlated in sick cats. Thus, abnormal total protein concentration must be considered when interpreting fructosamine concentration (19).

As we have previously commented, the highest correlation between serum fructosamine and total protein concentration (r = 0.73) were found in hypoproteinemic dogs (Group 1). It could be useful, in these cases, to use a correction factor. Nevertheless, in our study, hypoproteinemic dogs had mainly urinary diseases but normal glycemia. Thus, if we know the clinical, biochemical and pathological findings we could confirm that the decrease on fructosamine concentration was not doubted to be an abnormality of carbohydrate metabolism. In these cases it will not be necessary to use any correction factor.

This study shows a decrease of serum fructosamine concentration in hyperproteinemic dogs. However, a little correlation (r = 0.29) between protein and fructosamine concentrations was found. This result can be explained because most of these dogs had a high hypoalbuminemia. Thus, the high hypoalbuminemia can be responsible for the lowest fructosamine concentration in dogs with hyperproteinemia (Table I).

With regard to the relationship between fructosamine and serum albumin concentrations, our results were very similar to those reported by Kawamoto et al (6) in canine serum samples. When fructosamine concentration was compared in all the animals, a moderately high correlation (r = 0.62) was obtained. This correlation was slightly lower than ours (r = 0.76). As well as in our study, Kawamoto et al (6) and Thoresen and Bredal (17) compared fructosamine concentrations for normoalbuminemic and hypoalbuminemic dogs. Little correlation (r = 0.17, 6) (r = 0.20, 17) was obtained in normoalbuminemic

dogs, lower than that observed in our study (r = 0.49). On the contrary, the correlation between fructosamine and albumin concentrations in hypoalbuminemic dogs was high (r = 0.71, 6) (r = 0.66, 17), and similar to our result (r = 0.69).

Kawamoto et al (6) warranted normalization of fructosamine because of the high correlation observed between serum fructosamine concentration and hypoalbuminemia. Jensen (16) proposed using correction factors only when the serum albumin concentration was outside the reference intervals. Thus, it could be useful to adjust fructosamine concentration to the lower limit of the albumin reference range only when albumin concentration was under this limit (6).

The albumin correlation to fructosamine concentration in hypoalbuminemic human sera (r = 0.74) was as high as in canine serum samples. Many authors recommend the use of a correction factor in those patients with a high decrease in serum albumin levels (14,21–23).

In conclusion, canine serum fructosamine concentration was found to be highly correlated to serum total protein in hypoproteinemic dogs and to serum albumin concentration in hypoalbuminemic dogs. This study suggests the importance of knowing serum protein and albumin concentrations in the assay of canine serum fructosamine concentration. A small variation on this parameters can significantly interfere with fructosamine concentration.

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