

Fructosamine and glycated hemoglobin in the assessment of glycaemic control in dogs

Araceli LOSTE*, M. Carmen MARCA

Department of Animal Pathology, Veterinary Faculty, University of Zaragoza,
Miguel Servet 177, 50013 Zaragoza, Spain

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Abstract – Fructosamine and glycated hemoglobin (HbA_{1c}) concentrations were measured simultaneously in 222 dogs (96 healthy and 126 sick dogs). The dogs were divided into 3 groups according to the glucose concentration: hypo, hyper and euglycaemic dogs. Serum fructosamine concentrations were measured by the reduction test with nitroblue tetrazolium. A turbidimetric inhibition immunoassay and specific polyclonal antibodies were used to evaluate glycated hemoglobin concentrations. A significant correlation was found between glucose concentration and either fructosamine ($r = 0.63, p < 0.0001$) or glycated hemoglobin ($r = 0.82, p < 0.0001$). The correlation was higher in hyperglycaemic dogs for fructosamine ($r = 0.80, p < 0.0001$) and in hypoglycaemic dogs for glycated hemoglobin ($r = 0.91, p < 0.005$). We found a significant correlation between serum fructosamine and glycated hemoglobin ($r = 0.65, p < 0.0001$) when all the dogs were studied. A significant correlation was observed between serum fructosamine and glycated hemoglobin only in hyperglycaemic dogs ($r = 0.82, p < 0.0003$). Thus, fructosamine and HbA_{1c} may be considered for use in screening tests for diabetes mellitus in dogs and clinical tests for monitoring control and evaluation of the diabetic animal's response to treatment. The choice of the analytical assay depends on the characteristic and analytical opportunities of the laboratory, as well as the number of serum samples to be analysed.

fructosamine / glycated hemoglobin / glycaemic control / dog

Résumé – Utilisation de la fructosamine et de l'hémoglobine glycosylée dans l'évaluation de la glycémie des chiens. Les concentrations de fructosamine et d'hémoglobine glycosylée (HbA_{1c}) ont été évaluées chez 222 chiens (96 sains et 126 malades). Les chiens ont été répartis en 3 groupes : chiens hypo, hyper et normoglycémiques. La concentration de fructosamine a été déterminée par réduction du bleu de nitrotétrazolium. Le dosage de l'hémoglobine glycosylée a été fait selon une technique immunologique. Les auteurs ont trouvé une corrélation significative entre la concentration en glucose et la fructosamine ($r = 0,63; p < 0,0001$) d'une part, et entre la concentration en glucose et l'hémoglobine glycosylée ($r = 0,82; p < 0,0001$) d'autre part. La corrélation était plus forte chez les chiens hyperglycémiques (fructosamine $r = 0,80; p < 0,0001$) et hypoglycémiques (hémoglobine glycosylée

* Correspondence and reprints

Tel.: (34) 976 76 15 76; fax: (34) 976 76 16 12; e-mail: aloste@posta.unizar.es

$r = 0,91$; $p < 0,005$). La fructosamine était significativement corrélée à l'hémoglobine glycosylée ($r = 0,65$; $p < 0,0001$) chez l'ensemble chiens. Une corrélation significative entre l'hémoglobine glycosylée et la fructosamine a été observée uniquement chez les chiens hyperglycémiques ($r = 0,82$; $p < 0,0003$). La mesure des concentrations en fructosamine et en HbA_{1c} peut être considérée comme test de dépistage de diabète sucré chez le chien et comme test clinique utile pour le contrôle et l'évaluation de la réponse de l'animal diabétique au traitement. Le choix de l'un ou l'autre test analytique dépend des possibilités du laboratoire, ainsi que du nombre d'échantillons sanguins à analyser.

fructosamine / hémoglobine glycosylée / contrôle de la glycémie / chien

1. INTRODUCTION

The evaluation of carbohydrate metabolism has been enhanced through the development of methods for monitoring long-term glycaemic conditions, such as fructosamine and glycated hemoglobin (HbA_{1c}) measurements. Fructosamine and HbA_{1c} are both blood proteins that are widely used to monitor glycaemia in human patients, especially in the diagnosis and monitoring of diabetes mellitus.

Serum fructosamine is formed through non-enzymatic irreversible reactions between glucose and serum proteins. Fructosamine concentration directly depends on the blood protein concentrations and their composition [12, 17, 18] and on the plasma glucose concentration. In dogs, because the average life of albumin is 8.2 days, the fructosamine concentration reflects glycaemic status over the previous 1 to 3 weeks [8, 10].

Glycated hemoglobin is the product of a slow, nonenzymatic and irreversible process and it is directly related to serum glucose concentration and erythrocyte lifespan (120-day-lifespan). Hemoglobin A_{1c} is the most important glycated fraction of the hemoglobin molecule [19, 20]. With a normal turnover of erythrocytes, HbA_{1c} provides an accurate index of the average glucose concentration over the preceding 2 to 3 months [3, 5].

Although it is known that prolonged hypo and hyperglycaemia modify serum fructosamine and HbA_{1c} concentrations, there is little information on the correlation

between both parameters and their diagnostic usefulness [1, 14, 15]. Thus, the purpose of the present study was to analyse the correlations between fructosamine/glucose and HbA_{1c}/glucose concentrations in hypo, hyper and euglycaemic dogs, and to evaluate the correlation between fructosamine and HbA_{1c} measurements in canine blood samples.

2. MATERIALS AND METHODS

2.1. Animals

The study population consisted of 222 dogs of different ages, sex and breeds from the Small Animal Internal Medicine Department at the Veterinary Faculty of Zaragoza (Spain). The dogs were divided into the following 3 groups.

The first group included dogs with insulinoma ($n = 5$). The diagnosis of insulinoma was confirmed on the basis of history, physical examination findings, serum glucose and insulin concentrations, and the good response to treatment. Post mortem studies corroborated the diagnosis of insulinoma in all of these dogs.

The second group ($n = 205$) included either healthy dogs (history, physical examination and results of routine clinicopathologic examinations: cell blood count, serum biochemical analysis and complete urine analysis were normal) or dogs with different diseases (gastrointestinal, respiratory, parasitic, etc.).

The third group included dogs with diabetes mellitus ($n = 12$). In these dogs, diabetes was newly identified or was considered as having a poor response to treatment.

Sequential glucose evaluations were performed on all the animals during one month. Only those with persistent low, normal or high glycaemic level were included in the following final experimental groups:

- group 1 ($n = 7$) (hypoglycaemic dogs):
glucose < 3.3 mM;
- group 2 ($n = 190$) (euglycaemic dogs):
glucose = 3.3 – 6.6 mM;
- group 3 ($n = 25$) (hyperglycaemic dogs):
glucose ≥ 6.6 mM.

The number of animals per group in the Tables I and II is not coincident because both fructosamine and glycated hemoglobin concentrations could not be analysed in all the animals.

2.2. Sample collection

The dogs were fasted for 12 hours before the collection of each blood sample. Blood samples were collected from the jugular vein and were placed in EDTA-tubes and tubes without anticoagulant. Serum was obtained by centrifugation ($2400 g$, 10 min). Blood and serum samples were divided into several portions and stored at -20 °C until their analysis.

2.3. Analytical procedures

Fructosamine was measured by a reduction test with nitroblue tetrazolium (Fructosamine MRP3, Ref. 1054686, Boehringer Mannheim, Barcelona, Spain) on an automatic analyser (Technicon RA-500, Bayer, Barcelona, Spain), using controls supplied

Table I. Fructosamine concentrations (mean \pm SD) in dogs with different levels of glycaemia.

Groups	Number of animals	Fructosamine ($\mu\text{mol}\cdot\text{L}^{-1}$)
Hypoglycaemic dogs Glucose < 3.3 mmol·L ⁻¹	7	$215.2 \pm 41.0^{***}$
Euglycaemic dogs Glucose = 3.3 – 6.6 mmol·L ⁻¹	125	$276.0 \pm 52.2^{***}$
Hyperglycaemic dogs Glucose ≥ 6.6 mmol·L ⁻¹	24	$350.1 \pm 110.48^{***}$

*** $p < 0.0006$.

Table II. Levels of glycated hemoglobin (expressed as HbA_{1c}/Tot. Hb) (mean \pm SD) in dogs with different levels of glycaemia.

Groups	Number of animals	HbA _{1c} (% of total Hb)
Hypoglycaemic dogs Glucose < 3.3 mmol·L ⁻¹	7	$1.0 \pm 0.4^{***}$
Euglycaemic dogs Glucose = 3.3 – 6.6 mmol·L ⁻¹	100	$1.4 \pm 0.3^{***}$
Hyperglycaemic dogs Glucose ≥ 6.6 mmol·L ⁻¹	16	$3.4 \pm 2.4^{***}$

*** $p < 0.0004$.

by the manufacturer. This analytical test was validated in dogs [7, 11].

Glycated hemoglobin was evaluated by a commercial immunological in vitro assay (Tinaquant[®] Hemoglobin A1c II, Ref. 1488414, Boehringer Mannheim, Barcelona, Spain) applied to the same autoanalyser. The instrument calculates the HbA1c/total Hb concentration ratio and expresses HbA1c results as a fraction of total Hb. Haemolysates were prepared by adding 10 μ L of blood to 500 μ L of a hemolysing reagent (Ref. 1488457, Boehringer Mannheim, Barcelona, Spain). Marca and Loste [14] validated this test in dogs. In each analysis, normal and pathological commercial controls (Precinorm[®] HbA1c, Ref. 1488422 and Precipath[®] HbA1c, Ref. 148849, Boehringer Mannheim, Barcelona, Spain) were included.

The serum glucose concentration was quantified by the glucose oxidase method (Glucinet[®] T01-1492, Bayer, Barcelona, Spain).

2.4. Statistical analysis

Differences in fructosamine and glycated hemoglobin (% of total Hb) among the

3 groups of dogs were calculated by a Kruskal-Wallis non-parametric analysis. Linear regression and correlation analysis (StatView) were used to determine the linear relationship between fructosamine/glucose, glycated hemoglobin/glucose and fructosamine/glycated hemoglobin.

3. RESULTS

Table I shows the average serum fructosamine concentration in each of the 3 groups of dogs with different glucose concentrations. Statistically significant differences between groups were observed.

In order to study the correlation between serum fructosamine and glucose concentrations, the 3 groups of dogs were analysed by use of linear regression. A significant correlation ($r = 0.63$, $p < 0.0001$) was found when individual data for all the 222 dogs were analysed. However, the correlation between fructosamine and glucose concentrations varied in each one of the groups. So, no significant correlation was found in euglycaemic and hypoglycaemic dogs. On the contrary, a significant correlation

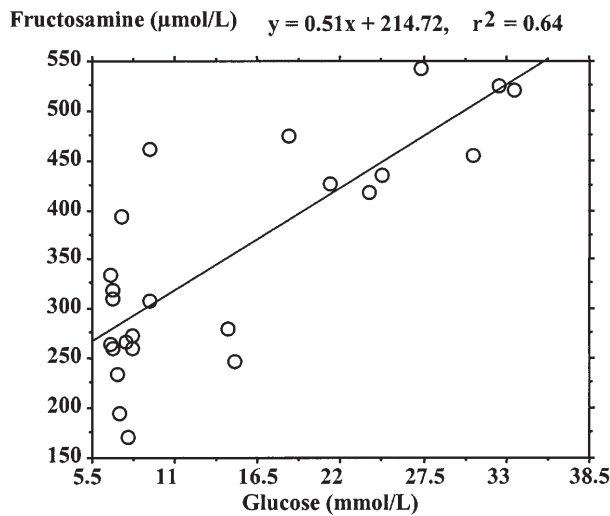


Figure 1. Linear regression of serum fructosamine and glucose concentrations in hyperglycaemic dogs ($r = 0.80$, $p < 0.0001$).

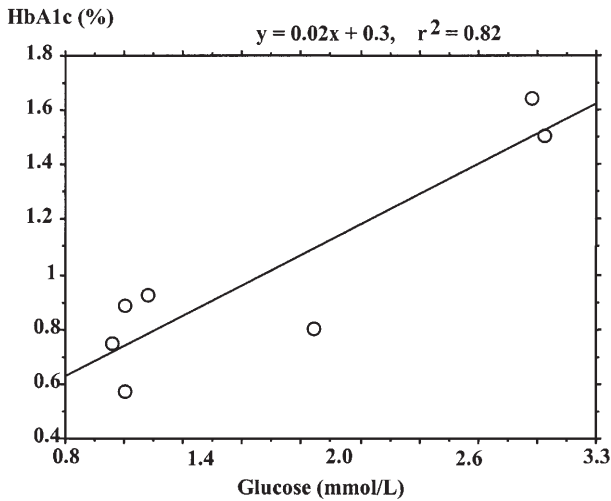


Figure 2. Linear regression of HbA1c and glucose concentrations in hypoglycaemic dogs ($r = 0.91, p < 0.005$).

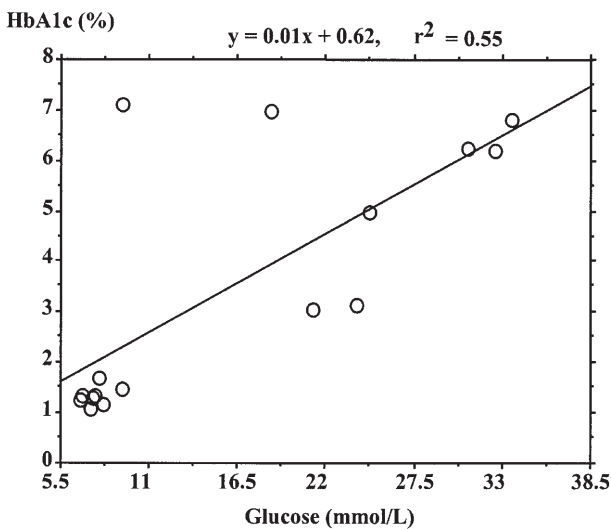


Figure 3. Linear regression of HbA1c and glucose concentrations in hyperglycaemic dogs ($r = 0.74, p < 0.001$).

($r = 0.80, p < 0.0001$) was found in hyperglycaemic dogs (Fig. 1).

In the same way, the average levels of glycated hemoglobin concentration (% of total Hb) was obtained for the 3 groups of dogs. As with fructosamine, statistically significant differences between groups were observed (Tab. II).

A significant correlation ($r = 0.82, p < 0.0001$) between glycated hemoglobin and glucose concentrations was obtained from all the dogs ($n = 222$). This correlation was high in those animals with glucose metabolism disturbances, either hypoglycaemic (group 1) ($r = 0.91, p < 0.005$) (Fig. 2) or hyperglycaemic dogs ($r = 0.74,$

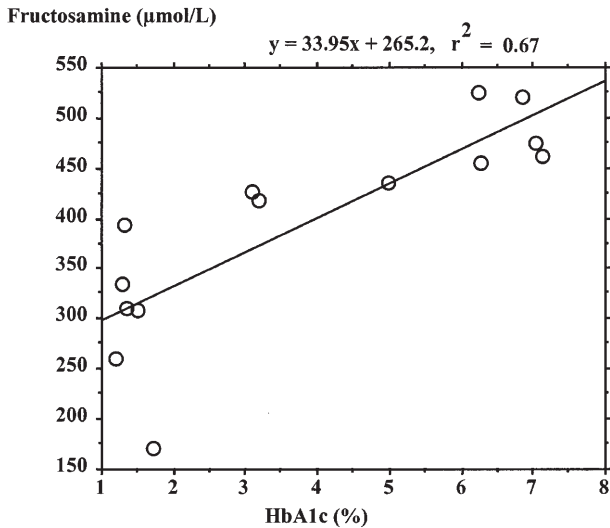


Figure 4. Linear regression of serum fructosamine and HbA1c concentrations in hyperglycaemic dogs ($r = 0.82$, $p < 0.0003$).

$p < 0.001$) (Fig. 3). No correlation however was found in euglycaemic dogs.

A significant correlation ($r = 0.65$, $p < 0.0001$) was found between serum fructosamine and glycated hemoglobin in the 222 dogs. When blood glucose concentration was considered, no correlation was found in either euglycaemic nor hypoglycaemic dogs, but the correlation was highly significant in hyperglycaemic sera ($r = 0.82$, $p < 0.0003$) (Fig. 4).

4. DISCUSSION

This study showed that fructosamine concentrations increased in parallel with glucose levels in all dogs. The differences between the three groups were confirmed using statistical analysis. The significant correlation between fructosamine and glucose concentrations in hyperglycaemic dogs suggests that fructosamine analysis is a useful test for the diagnosis of diabetes mellitus. Moreover, after the beginning of the treatment, it is necessary to monitor the responses of the dogs. Thus, fructosamine

levels is a useful parameter for the control of treated diabetic dogs.

As we have previously described, it could be useful to use protein correction of fructosamine in hypoproteinemic dogs [12]. Nevertheless, most of these animals with urinary diseases had normal glucose levels. Thus, if we know the clinical, biochemical and pathological findings we could confirm that the decrease in fructosamine concentration was not due to an abnormality of carbohydrate metabolism. In these cases it would not be necessary to use any correction factor.

With respect to albumin, it could be useful to adjust fructosamine concentration to the lower limit of the albumin concentration only when albumin concentration was under this limit [10].

As with fructosamine, HbA1c concentrations were related to glucose concentrations in the 3 groups of dogs. A high correlation between HbA1c and glucose concentrations was found in both hyper and hypoglycaemic dogs. The results suggest that the glycated hemoglobin test is more

sensitive in detecting chronic low blood glucose concentrations than fructosamine.

In veterinary science, there are few studies on the correlation between these parameters. A recent study [20] showed a high correlation ($r = 0.67$) between blood HbA1c and glucose concentrations in healthy and diabetic dogs using m-aminophenylboronic acid (PBA) chromatography. Although the correlation was lower than in our results ($r = 0.82$), these authors did not include hypoglycaemic dogs.

Most studies have used blood feline samples. A high correlation between fructosamine and glucose ($r = 0.70$) was found in cats (including cats with transient hyperglycaemia, healthy and diabetic cats). This correlation was even higher when stress hyperglycaemic cats were excluded. When cats with stress hyperglycaemia were analysed alone, no correlation was found [2]. A high correlation between fructosamine and glucose concentrations ($r = 0.90$) was obtained when both healthy and sick cats were included [9]. The study of healthy and sick cats with transient hyperglycaemia showed a lower correlation in healthy cats than in sick cats and no correlation was found after an injection of glucose solution [13].

In our study, a significant correlation between fructosamine or HbA1c and glucose concentrations appeared in hyperglycaemic dogs. These results were similar to those reported in human medicine [4, 16].

Veterinary reports concerning the relationship between serum fructosamine and glycated hemoglobin concentrations are scarce, especially those concerning dogs. At the present time we only know of one study in cats, in which a poor correlation between fructosamine and HbA1c ($r = 0.34$) was found. The reason for this poor correlation is unknown, but it may be due to an increased sensitivity of feline fructosamine compared to HbA1c to temporary fluctuations in blood glucose [1].

Serum fructosamine and glycated hemoglobin are measured in human sera to assess long-term glycaemic control. Both analytical assays are valuable for the diagnosis of diabetes mellitus in dogs and cats. Serum fructosamine assays are still used, and can be measured quickly, easily and economically [8, 10, 11]. On the contrary, glycated hemoglobin has not been used for the routine assessment of glycaemic control in animals [3, 5, 6, 14, 15].

Our results showed that either fructosamine or glycated hemoglobin assays are able to detect chronic changes of blood glucose concentrations in dogs. Both have a high correlation with glycaemia, mainly in hyperglycaemic dogs. Thus, fructosamine and HbA1c may be considered as screening tests for diabetes mellitus in dogs and as useful clinical tests for monitoring control and evaluating the diabetic animal's response to treatment. Furthermore, there was a high correlation between serum fructosamine and glycated hemoglobin concentrations. The main difference between both parameters is that glycated hemoglobin concentration reflects the average serum glucose concentration over a longer period of time (2–3 months) than does fructosamine (1–3 weeks). The choice of one or another analytical assay depends on the characteristics and analytical opportunities of the laboratory, as well as on the number of serum samples to be analysed.

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