Fructosamine concentrations in hyperglycemic cats

Thomas A. Lutz, Jacqueline S. Rand, Elizabeth Ryan

Abstract

The aims of this study were 1) to establish a reference range for fructosamine in cats using a commercial fructosamine kit; 2) to demonstrate that the fructosamine concentration is not increased by transient hyperglycemia of 90 min duration, simulating hyperglycemia of acute stress; and 3) to determine what percentage of blood samples submitted to a commercial laboratory from 95 sick cats had evidence of persistent hyperglycemia based on an elevated fructosamine concentration.

Reference intervals for the serum fructosamine concentration were established in healthy, normoglycemic cats using a second generation kit designed for the measurement of the fructosamine concentration in humans. Transient hyperglycemia of 90 min duration was induced by IV glucose injection in healthy cats. Multisourced blood samples that were submitted to a commercial veterinary laboratory either as fluoride oxalated plasma or serum were used to determine the percentage of hyperglycemic cats having persistent hyperglycemia.

The reference interval for the serum fructosamine concentration was 249 to 406 µmol/L. Transient hyperglycemia of 90 min duration did not increase the fructosamine concentration and there was no correlation between fructosamine and blood glucose. In contrast, the fructosamine concentration was correlated with the glucose concentration in sick hyper- and normoglycemic cats.

It is concluded that the fructosamine concentration is a useful marker for the detection of persistent hyperglycemia and its differentiation from transient stress hyperglycemia. Fructosamine determinations should be considered when blood glucose is 12 to 20 mmol/L and only a single blood sample is available for analysis.

Résumé

Concentration de fructosamine chez les chats hyperglycémiques

Les buts de cette étude étaient de déterminer les valeurs habituelles de fructosamine chez les chats à l'aide d'une trousse commerciale, de démontrer qu'une hyperglycémie transitoire d'une durée de 90 minutes, simulant une hyperglycémie reliée au stress, n'influence pas la concentration de fructosamine et de déterminer quel pourcentage des échantillons sanguins prélevés sur 95 chats malades démontrait une hyperglycémie persistante. Les valeurs sériques normales du fructosamine ont été déterminées chez des chats normoglycémiques, en bonne santé, à l'aide d'une trousse de deuxième génération conçue pour mesurer la concentration de fructosamine chez l'homme. Une injection de glucose par voie intraveineuse a été administrée pour provoquer une hyperglycémie transitoire d'une durée de 90 minutes. Le pourcentage des chats hyperglycémiques présentant une hyperglycémie persistante a été déterminé à partir d'un échantillonnage de plasmas de fluorure d'oxalates ou de séra provenant de diverses sources soumises à un laboratoire vétérinaire commercial.

Les valeurs usuelles de fructosamine variaient entre 249 et 406 µmol/L. Les données indiquent que lors d'une hyperglycémie transitoire d'une durée de 90 minutes, la concentration de fructosamine n'est pas augmentée et qu'il n'existe pas de corrélation entre le fructosamine et le glucose sanguin. Toutefois, la concentration du fructosamine était corrélée à la concentration du glucose chez les chats malades hyper et normoglycémiques. Les auteurs concluent que la concentration du fructosamine est un indice valable pour déceler une hyperglycémie persistante et pour la différencier d'une hyperglycémie transitoire reliée au stress. La valeur du fructosamine devrait être évaluée lorsque le glucose sanguin se situe entre 12 et 20 mmol/L et qu'un seul échantillon sanguin est disponible pour analyse.

(Traduit par Docteure Thérèse Lanthier)

Can Vet J 1995; 36: 155–159

Introduction

Acute stress associated with blood sampling can produce marked hyperglycemia in cats, so diagnosis of diabetes mellitus based on a single blood sample is often difficult (1). Fructosamines (FRs) are measured in human diabetics to assess long-term glycemic control (2); they have also been reported as being useful in identifying persistently hyperglycemic cats (3–5).

Fructosamines are formed through nonenzymatic irreversible reactions between glucose and serum proteins, and their concentration directly depends on the plasma glucose concentration. Because of the half-lives of serum proteins and the rate of glycation, the FR concentration in humans correlates with the mean glucose concentration over the previous 2 to 3 wk (2,6). As FRs are expressed as an absolute concentration, their concentration also depends on the plasma total protein concentration (7–10).

Reports of the usefulness in cats of the commercially available, first generation FR assay are conflicting (3–5). In 1991, second generation kits were released (Hoffmann-La Roche, Basel, Switzerland; Boehringer Mannheim,

Companion Animal Medicine and Surgery, School of Veterinary Science, University of Queensland, St. Lucia, 4072, Queensland, Brisbane, Australia (Lutz, Rand); Veterinary Pathology Services, Manila Street, East Brisbane, Queensland, Australia (Ryan). Present address of Thomas A. Lutz: Institute of Veterinary Physiology, University of Zuerich, Winterthurerstrasse 260, CH 8057 Zuerich, Switzerland.

Reprints not available.

T.A. Lutz was supported by an Overseas Postgraduate Research Scholarship and a University of Queensland Postgraduate Research Scholarship. Part of the work was presented at the 11th Forum of the American College of Veterinary Internal Medicine (May 20–23 1993) in Washington D.C., USA.

Mannheim, Federal Republic of Germany). These were not affected by many of the substances that caused interference using the first generation kit.

The aims of this study were 1) to establish a reference range for FR in cats using a second generation kit (Fructosamine Test Plus, Hoffmann-La Roche); 2) to demonstrate that FR is not increased by transient hyperglycemia of 90 min duration, simulating hyperglycemia of acute stress; and 3) to determine what percentage of blood samples submitted to a commercial laboratory from 95 sick cats had evidence of persistent hyperglycemia, based on an elevated FR concentration.

Materials and methods

Reference values for FR were established in 17 clinically healthy cats (Group 1-a) that had fasting normoglycemia (plasma glucose concentration 3.3 to 6.9 mmol/L; x = 5.5 s_x = 0.2 mmol/L) and a normal glucose tolerance test result ($T_{1/2}$ glucose < 80 min following 1g/kg glucose, IV). Transient hyperglycemia was induced in these cats (Group 1-b) by the IV injection of a 50% glucose solution (1 g/kg body weight) (Glucose for injection, Astra Pharmaceuticals Proprietary, Ltd., North Ryde, New South Wales, Australia). Fructosamine, glucose, and total protein concentrations were measured in samples collected preinjection and 90 min later. Glucose injection and blood collection were made via catheters placed under thiopentone anesthesia (2.5 % solution, IV) 24 h before blood sampling. Fructosamine was measured in plasma samples with ethylenediaminetetraacetic acid (EDTA).

Multisourced blood samples submitted to a commercial veterinary laboratory (Veterinary Pathology Services, East Brisbane, Queensland, Australia) were used in the study. Fructosamine, glucose, and total protein concentrations were measured in 95 randomly selected sick cats (Group 2), of which 41 were hyperglycemic and 17 were considered likely diabetics. Cats were defined as likely diabetic if blood glucose was >20 mmol/L in one sample (10 cats), or between 12 and 20 mmol/L in two samples taken several days apart (7 cats; 1 mo apart in 1 cat), or >6.9 mmol/L after insulin therapy (6 cats). Samples from 33 cats were submitted in tubes containing sodium fluoride/sodium oxalate, and from 62 as serum samples. Only the samples submitted as fluoride oxalated plasma were considered for exact statistical analysis, because the glucose concentration measured in serum may not reflect the true value due to glucose metabolism by erythrocytes prior to separation of cellular components.

Fructosamine was measured on an automatic analyser device (Olympus Reply Analyser, Olympus, Sydney, New South Wales, Australia), using controls supplied by the manufacturer. Because there is good agreement between FR values measured in EDTA or heparinized plasma and in serum (11), EDTA, fluoride oxalated plasma, and serum samples were used in the study.

Glucose and total protein concentrations were also measured in fluoride oxalated plasma or in serum, using an automated analyser. Analysis for FR and total protein was performed on either fresh or frozen samples stored for up to 4 wk at −20°C. Glucose analysis was performed on fresh samples.

Mean values are presented as mean ± standard error of the mean ($x \pm s_x$). For statistical evaluation of the results, the different groups were compared using the *t*-test. Spearman Rank correlation coefficients were calculated or linear regression analysis was used to correlate glucose, total protein, and FR levels. *P* values <0.05 were considered to be statistically significant.

Table 1. Reference values from 17 normal cats for fructosamine (FR) concentrations and FR concentrations after short-term hyperglycemia

	Reference values			90 min values[a]		
Cat	Glc[b]	FR[c]	TP[d]	Glc	FR	TP
1	4.8	309	67	11.7	295	56
2	5.7	340	68	16.7	291	61
3	6.6	354	68	9.6	323	57
4	5.2	343	79	16.8	333	67
5	5.8	406	74	23.6	315	68
6	6.2	301	67	12.1	263	66
7	3.9	323	68	17.7	281	64
8	6.1	295	72	15.9	288	66
9	6.8	357	61	8.3	289	61
10	5.1	406	61	23.0	306	60
11	4.5	306	57	9.4	255	63
12	4.5	254	57	9.5	245	52
13	5.5	294	67	12.5	247	63
14	6.1	347	—[e]	10.4	310	66
15	6.1	299	69	8.1	295	70
16	6.4	363	66	14.3	309	63
17	4.8	249	67	4.8	273	—
x	5.5	326	67	13.2	292	62
s_x	0.2	11	1	1.3	8	1

[a]90 min after the injection of 1 g glucose/kg body weight
[b]Glucose concentration in mmol/L
[c]Fructosamine concentration in µmol/L
[d]Concentration of total protein in g/L
[e]Missing value

Results

The observed range for FR concentrations in clinically healthy cats with fasting normoglycemia and normal glucose tolerance (Group 1-a, n = 17) was 249 to 406 µmol/L (x = 326, s_x = 11 µmol/L; Tables 1, 2). Glucose and FR were significantly correlated (r = 0.46, P = 0.02), but there was no significant correlation between FR and total protein (r = 0.20, P = 0.45).

Blood glucose levels in all but one cat 90 min after glucose injection (Group 1-b) were still above 6.9 mmol/L, and significantly different from those in group 1-a (P<0.001). Packed cell volumes (data not shown) and total protein and FR concentrations were all lower in group 1-b than in group 1-a, as a result of hemodilution (Table 1). When FR was corrected for hemodilution using total protein (TP) [$FR_{correct} = TP_{0\ min}/TP_{90\ min} \times FR_{90\ min}$], FR concentrations in group 1-b were not different from those in group 1-a (Table 2) and were not significantly correlated with glucose concentrations ($r = 0.16$, $P = 0.44$).

In group 2, 41 of the 95 sick cats had hyperglycemia. Only the 33 samples from sick cats submitted in fluoride oxalate were considered for statistical evaluation. Twenty-four of the 33 samples were hyperglycemic (Tables 2 and 3). In 20 of these 24 cats (approximately 80%), FR concentrations were above the reference

Table 2. Fructosamine concentrations in reference, short-term hyperglycemic, and hyperglycemic sick cats

	(n)	Glucose[a] (mmol/L)	Fructosamine (μmol/L)
Reference cats	17	5.5 ± 0.2	326 ± 11 (249–406)
Reference cats 90 min after inducing hyperglycemia	17	13.2 ± 1.3	316 ± 10[b]
Hyperglycemic sick cats	24		
normal FR	4	11.6 ± 2.3	351 ± 17
elevated FR	20	18.4 ± 1.7	606 ± 31
likely diabetic[c]	17	22.1 ± 2.2	630 ± 51

[a]All glucose concentrations measured in fluoride oxalated plasma
[b]Fructosamine concentration corrected for haemodilution (see text for details)
[c]Likely diabetes mellitus defined as one blood sample above 20 mmol/L glucose, 2 samples taken 1 mo apart between 12 and 20 mmol/L, or glucose concentration >6.9 mmol/L after insulin

Table 3. Distribution of samples with normal and elevated fructosamine (FR) concentrations in three glucose ranges for 33 sick cats

Glucose[a] (mmol/L)	total number (n)	FR normal (≤406 μmol/L) (n)	FR elevated (>406 μmol/L) (n)
<3.3	1	1	0
3.3–6.9	8	6	2
>6.9	24	4	20
7.0–11.9	6	2	4
12–20	12	2	10
>20	6	0	6
likely diabetic[b]	17	2	15

[a]All glucose concentrations measured in fluoride oxalated plasma
[b]Likely diabetes mellitus defined as one blood sample above 20 mmol/L glucose, 2 samples taken 1 mo apart between 12 and 20 mmol/L, or glucose concentration >6.9 mmol/L after insulin

range; in the other 4, they were normal. In 2 cats, the FR concentrations were slightly above our normal range (444 μmol/L and 447 μmol/L) despite normoglycemia, and the total protein concentrations were normal. Fourteen of 18 cats (approximately 75%) with hyperglycemia below 20 mmol/L had elevated FR concentrations. Of 12 cats with hyperglycemia in the range that could be caused by either acute stress or diabetes mellitus (glucose 12 to 20 mmol/L), 10 (83%) had FR concentrations above normal. Fructosamine and glucose were correlated ($r_s = 0.62$, $P<0.001$) in this group.

Group 2 contained 17 cats that were defined as likely diabetic (Tables 2 and 3). Of these, 15 had elevated FR values (425 to 955 μmol/L). All cats with a blood glucose concentration above 20 mmol/L also had elevated fructosamine concentrations. Two likely diabetic cats had FR values within the normal range: one was an insulin-treated diabetic that was considered clinically well controlled despite elevated blood glucose; the other was polyuric and azotemic with glucose levels of 12 to 20 mmol/L in 2 samples taken 1 mo apart, and 15 mo later was clinically well and had regained the weight lost during the previous illness, without specific diabetes treatment. The mean FR value for cats defined as likely diabetic was 630 ± 51 μmol/L, and the FR values for the group were significantly different ($P<0.001$) from the reference cats (Group 1-a).

Of the 62 sick cats from which samples were submitted as unseparated serum, 17 had hyperglycemia and 7 (41%) of these had elevated FR values. In 7 cats, the FR concentrations were elevated despite the fact that the cats were apparently normo- or hypoglycemic. In 6 of these cats, the FR concentrations were markedly elevated (>450 μmol/L).

Although no correlation was found between FR and total protein concentrations in the clinically healthy,

normoproteinemic cats comprising group 1-a or 1-b, FR and total protein were correlated ($r = 0.45$, $P<0.001$) in the 95 sick cats (Group 2), of which 10 had total protein levels outside the normal range for the laboratory (51 to 85 g/L). Six of these 10 cats had FR concentrations above the normal range, which were still elevated if corrected for a mean normal total protein concentration (68 g/L).

Discussion

The upper limit of our reference range for FR was higher than previously reported for healthy cats (228 to 341 μmol/L; x = 281, s_x = 31 μmol/L) (4), and 2 of our reference cats had FR values (405, 406 μmol/L) that fell just within the previously reported range for diabetic cats (400–501 μmol/L) (4). Genetic or age differences in the normal cat population, small sample size, or interlaboratory differences may account for the higher upper limit. Although our range was established using EDTA plasma, good agreement had been reported between values obtained in EDTA or heparinized plasma, and in serum (11).

Because the FR concentrations were not increased by experimentally induced hyperglycemia of 90 min duration, transient stress-induced hyperglycemia of 1 to 2 h (e.g., stress caused by blood sampling) would not be expected to cause increased FR values. In humans, FR has been shown to increase after less than 1 wk of persistent hyperglycemia (6), and the FR value reflects the median glucose over a period of 2 to 3 wk (2,6). Similar studies in cats have not been reported, but in vitro incubation of feline blood with glucose at 22 mmol/L found that FR was increased after 100 h (4a).

Our results suggest that FR measurement is a useful diagnostic test for commercial laboratories and that if diagnosis is based on blood glucose measurement alone, a significant number of subclinical or mildly clinical feline diabetics will go undiagnosed. Using FR measurements, we found that approximately 83% of multisourced feline blood samples with moderate hyperglycemia (glucose 12 to 20 mmol/L) that were submitted to a commercial laboratory as fluoride oxalated plasma had increased FR values consistent with subclinical or mildly clinical diabetes mellitus. Cats with hyperglycemia and normal FR may have had transient stress-induced hyperglycemia or persistent hyperglycemia of insufficient duration or severity to cause an elevation of FR (Link and Rand, unpublished; 4a). All cats with blood glucose >20 mmol/L had elevated FR, but 3 of 9 cats with blood glucose concentrations of 15 to 20 mmol/L had a normal FR concentration. Therefore, if blood glucose is >20 mmol/L, FR measurement is probably unnecessary and diabetes is the likely cause of the hyperglycemia. However, when the glucose concentration is 12 to 20 mmol/L and only a single blood sample and no urinalysis is available, FR concentration should be measured to identify diabetic cats. Our finding that, except in transiently hyperglycemic cats, FR was correlated with blood glucose is consistent with initial reports that FR is a useful indicator of glycemic control in treated diabetic cats (3,4).

Although submission of blood samples treated with fluoride oxalate for glucose measurement was recommended by the laboratory, two-thirds of feline blood samples were submitted by practitioners as whole blood (unseparated serum). Hypoglycemia was more frequent in the serum samples than when fluoride oxalate had been used, probably reflecting glucose metabolism by erythrocytes. Because the measured glucose concentration in unseparated serum samples may not reflect the true value, FR determination should be considered when blood is submitted to a commercial laboratory as unseparated serum. This is underlined by the finding of a significant proportion of serum samples with markedly elevated FR concentrations despite subnormal, normal, or slightly elevated measured glucose concentrations.

The higher proportion of samples treated with fluoride oxalate with elevated FR concentration compared to unseparated serum samples probably reflects a bias by veterinarians for sample submission using fluoride oxalate when diabetes mellitus is suspected. Elevated FR concentration with normoglycemia was only found twice in the fluoride oxalate group, and in both cases FR was only mildly elevated above the upper limit of normal (<450 μmol/L). Based on these findings, the diagnosis of diabetes mellitus should be considered equivocal when FR is between 407 and 450 μmol/L. Of the 17 hyperglycemic cats defined as likely diabetics, only 2 had a FR concentration <406 mmol/L. One of these was a clinically well-controlled, insulin treated diabetic, and the other was hyperglycemic on 2 samples taken 1 mo apart, and although it met our definition of likely diabetic, it may have had two episodes of acute stress-induced hyperglycemia. When urine is not readily available for glucose measurement, FR measurement may be a more reliable and cheaper method of identifying persistent hyperglycemia than collection of a second blood sample, especially when a second visit to the veterinarian is required.

Although no correlation was found between FR and total protein in healthy cats, FR and total protein were correlated in sick cats with a variety of diseases and a wide range of total protein concentrations. Thus, it would appear that within the normal range of total protein concentration, total protein does not seem to markedly affect FR values. However, when total protein concentration is outside the normal range, its effect on FR must be considered when interpreting the measured FR value. This agrees with previously published results in humans, which indicated that correction of FR concentration for total protein concentration was only necessary when total protein or albumin concentration was markedly abnormal (7–10).

The usefulness of FR measurement in cats has been questioned (5). However, the investigators used a first generation FR assay kit and the results cannot be compared to those found in our study using a substantially modified second generation kit (12). Although FR levels measured with first and second generation kits have been correlated, absolute values were not comparable (2,12,13). Akol *et al* (5) found that FR concentration in poorly-controlled diabetics overlapped with values from normal cats. In contrast, only 2 cats defined as likely diabetic in our study had a normal FR concentration. One may have had two episodes of acute stress hyperglycemia and not persistent hyperglycemia of diabetes mellitus, and the other was a well-controlled diabetic. However, Akol *et al* (5) used a first generation kit, which probably

explains the discrepancy in results between the two studies. Other studies using the second generation kit have found that FR values allow a differentiation between healthy and diabetic cats (this study; 3, 4, 4a).

In conclusion, we recommend the measurement of FR for detection of persistent hyperglycemia in cats. As blood glucose concentrations above 20 mmol/L are mostly caused by chronic hyperglycemia, the determination of FR is considered a good screening test for diabetes when the blood glucose concentration is between 12 and 20 mmol/L, or if blood is not submitted in fluoride oxalate. Correction of FR values for total protein concentration is only necessary when total protein concentration is markedly abnormal. Determination of FR is easy and fast to perform and can be done with various sample materials (serum and EDTA or heparinized plasma), which can be stored for up to 2 d at 2 to 8°C, or 2 mo at −20°C (Hoffmann-La Roche, unpublished data).

Acknowledgments

We thank Veterinary Pathology Services, Manila Street, East Brisbane, Queensland, Australia, for their support, and Roche Diagnostic Systems, Brisbane, Queensland, Australia, for the supply of kits. CVJ

References

1. Feldman EC, Nelson RW. Feline diabetes mellitus. In: Kirk RW, ed. Current Veterinary Therapy — Small Animal Practice. Vol 9. Philadelphia: WB Saunders, 1986: 1000–1005.
2. Johnson RN, Metcalf PA, Baker JR. Fructosamine: A new approach to the estimation of serum glycosylprotein. An index of diabetic control. Clin Chim Acta 1982; 127: 87–95.
3. Staudacher G. Fruktosamine als neues wertvolles Kriterium für die Einstellung diabetischer Tiere sowie ihre photometrische Bestimmung. Tieraerztl Prax 1990; 18: 441–446.
4. Reusch C, Liehs M. Evaluation of fructosamine as a new marker for long-term metabolic control in diabetic dogs and cats. Proc 9th Forum Am Coll Vet Int Med 1991: 881 (abstract).

4a. Reusch C, Hoyer-Ott M. Glycated hemoglobin and fructosamine: Studies of in-vitro glycolisation. Proc 12th Forum Am Coll Vet Int Med 1994: 993 (abstract).

5. Akol KG, Waddle JR, Wilding P. Glycated hemoglobin and fructosamine in diabetic and nondiabetic cats. J Am Anim Hosp Assoc 1992; 28: 227–231.
6. Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. Br Med J 1984; 288: 1484–1486.
7. Lamb E, Mainwaring-Burton R, Dawnay A. Effect of protein concentration on the formation of glycated albumin and fructosamine. Clin Chem 1991; 37: 2138–2139.
8. Kunika K, Itakura M, Yamashita K. Correction of fructosamine value for serum albumin and globulin concentrations. Diabetes Res Clin Pract 1991; 13: 37–44.
9. Saeed BO, Elwali NM, Atabani GS, Elmahdi EMA, Mukhtar E. The use of plasma fructosamine in the assessment of diabetic control. Trop Geogr Med 1991: 51–54.
10. Das BS, Satpathy SK, Mohanty S, Bose TK. Influence of serum albumin & total protein on fructosamine measurement. Ind J Med Res 1992; 96: 60–64.
11. Lehmann P. Investigation of interfering factors. Workshop Report on Fructosamine from Boehringer Mannheim, Federal Republic of Germany, 1989: 39–46.
12. Cefalu WT, Bell-Farrow AD, Petty M, Izlar C, Smith JA. Clinical validation of a second-generation fructosamine assay. Clin Chem 1991; 37: 1252–1256.
13. Baker JR, Metcalf PA, Johnson RN, Newman D, Rietz P. Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. Clin Chem 1985; 31: 1550–1554.