Evaluation of two point-of-care analysers for measurement of fructosamine or haemoglobin A1c in dogs

Measurement of glycosylated proteins such as fructosamine and haemoglobin A1c (HbA1c) can be used to assess glycaemic control in canine diabetic patients. Two point-of-care analysers, designed for human diabetics, were evaluated for use in dogs. Blood samples were collected from 50 normoglycaemic dogs, 100 diabetic patients and five dogs with insulinoma and tested using the In Charge fructosamine meter and the Haemaquant/Glycosal HbA1c meter. Readings were obtained in all cases except for 21 of 50 diabetics, which were above the upper limit of the In Charge meter. Diabetic dogs had higher fructosamine and HbA1c concentrations compared to controls. However, there was poor agreement between the In Charge meter readings and serum fructosamine concentrations, suggesting that there are problems associated with the use of this device in dogs. HbA1c concentrations showed a high degree of correlation with glycosylated haemoglobin measured at an external laboratory, suggesting that the Haemaquant/Glycosal meter warrants further evaluation for veterinary use.

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INTRODUCTION

Diabetes mellitus is a common endocrinopathy seen in dogs in general practice (Marmor and others 1982, Doxey and others 1985). Relative or absolute insulin deficiency results in persistent hyperglycaemia, glucosuria and clinical signs including weight loss, polyuria and polydipsia. Although insulin therapy can be successfully used to manage these cases, suboptimal glycaemic control can lead to secondary complications such as cataract formation and ketoacidosis (Nelson 2000).

Frequent blood glucose testing, often up to six times daily, can allow human diabetic patients to achieve good glycaemic control and minimise complications. Additionally, regular measurement of glycosylated blood proteins such as fructosamine and glycosylated haemoglobin is used for longer term monitoring of treatment. These parameters are not affected by transient changes in blood glucose and reflect the average glucose concentration over the preceding weeks (Koening and others 1976, Johnson and others 1982).

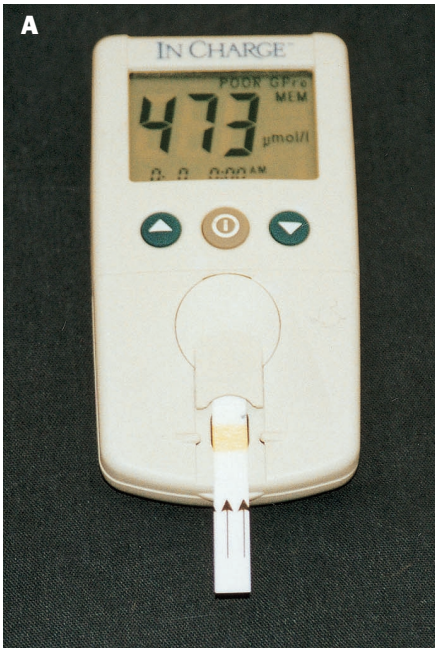
Repeated blood glucose testing is not a practical option for the owners of most diabetic dogs. Therefore, assessment of glycaemic stability over a period of weeks to months using a single blood sample offers a valuable means by which the veterinarian can monitor these cases.

Fructosamine

Fructosamine is a term used to describe plasma proteins (eg, albumin) which have undergone non-enzymatic, irreversible glycosylation in proportion to their surrounding glucose concentration (Jensen 1992, 1995). In dogs, the serum fructosamine concentration is related to the average blood glucose concentration over the previous one to two weeks (Kawamoto and others 1992), and in vitro studies suggest that persistent hyperglycaemia is required for four days before an increase in fructosamine concentration is seen (Jensen 1995).

Glycosylated haemoglobin and haemoglobin A1c

In 1971, Trivelli and colleagues described differences in haemoglobin fractions in human patients with diabetes mellitus compared to non-diabetics (Trivelli and others 1971). The term glycosylated haemoglobin is used to describe any type of haemoglobin that has gradually and irreversibly become chemically bound to glucose, which occurs in proportion to the concentration of glucose in the surrounding medium (Bunn and others 1978). Haemoglobin A1c (HbA1c) is a fraction of haemoglobin in which the β-chain has been glycosylated at the terminal valine residue. Measurement of HbA1c is



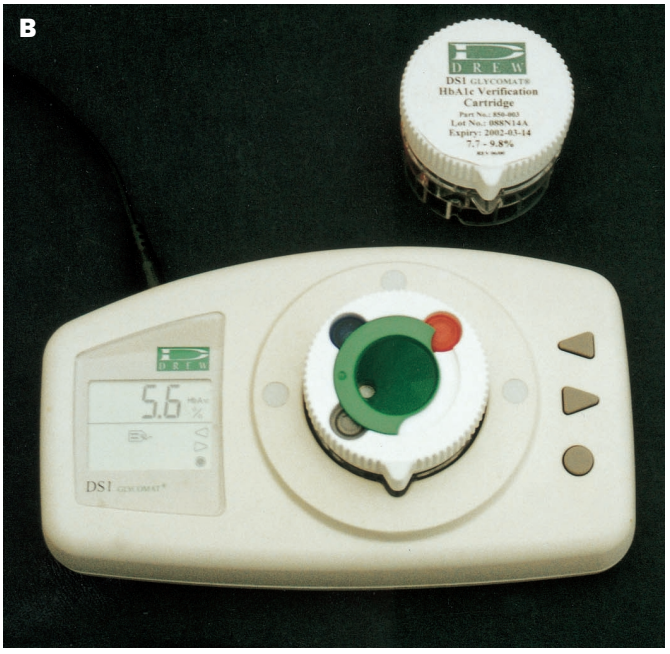


FIG 1. Point-of-care analysers used in the study. (A) In Charge glucose/fructosamine meter (LXN Corporation). (B) Haemaquant device and Glycosal cartridge (Provalis)

regarded as the best indicator of glycaemic control in diabetic humans (Koening and others 1976).

Elevated HbA1c concentrations have been demonstrated in diabetic dogs (Wood and Smith 1980). Further studies demonstrated that HbA1c concentrations increase within two weeks of the onset of persistent hyperglycaemia (Smith and others 1982), and that glycosylated haemoglobin reflects the average blood glucose concentration over the preceding two to three months (Nelson 2000). It follows that any change in glycaemic control will be reflected in fructosamine concentrations before HbA1c concentrations are affected.

Following the successful introduction of fructosamine and HbA1c assays for human diabetics, the value of these tests for the diagnosis and monitoring of canine diabetic patients has also been demonstrated (Wood and Smith 1980, Mahaffey and Cornelius 1982, Reusch and others 1993, Jensen 1995). Several commercial veterinary laboratories now offer fructosamine assays, but there is currently no commercially available assay for canine glycosylated haemoglobin in the UK.

Two point-of-care analysers have recently become available for measuring fructosamine (In Charge; LXN Corporation) or HbA1c (Haemaquant/Glycosal; Provalis) in human blood. These have the advantage of being able to provide an objective assessment of glycaemic control during the diabetic patient's visit to the clinic. If these devices were suitable for use in veterinary practices, the authors believe this would encourage more frequent testing and hence improved monitoring of canine diabetic patients.

MATERIALS AND METHODS

Animals

EDTA blood samples, taken for diagnostic purposes, were available from 14 colony beagles (University of Cambridge) and 36 normoglycaemic dogs referred to the Queen's Veterinary School Hospital (QVSH), University of Cambridge. These control animals were confirmed as normoglycaemic (range 3·3 to 5·6 mmol/litre) using a commercial glucometer (Glucometer Esprit; Bayer). Dogs receiving exogenous glucocorticoids were not included in the study.

Serum (0·5 to 1 ml) and EDTA blood (0·5 ml) samples were collected for diagnostic or monitoring purposes from 100 diabetic dogs. The majority of samples were submitted by veterinary practitioners in response to a letter placed in the veterinary press (Davison and others 2001). Diabetes mellitus had been diagnosed prior to sample submission on the basis of a combination of clinical signs, elevated blood glucose and persistent glucosuria. Information was obtained for each animal regarding age, breed, sex, bodyweight, insulin therapy and length of time since diagnosis of diabetes.

Serum and EDTA blood samples were additionally available from five dogs with beta cell neoplasia (insulinoma) referred to the QVSH. Insulinoma was confirmed by histopathology. Blood glucose was also assessed in these samples within two hours of blood collection.

Fructosamine measurement

Fructosamine was measured in 30 control and 50 diabetic blood samples using the In Charge fructosamine meter (Fig 1A) and glucoprotein test strips (LXN Corporation), according to the manufacturers' instructions. A 20 µl sample of EDTA blood was used in each case and an automated digital reading of fructosamine concentration was provided by the meter within 80 seconds. Prior to the use of the In Charge meter, two standard reference solutions, supplied with the meter, were tested and found to be within the indicated range. Serum fructosamine was also measured by external laboratories in all diabetic animals (Idexx Laboratories,

Table 1. Summary of results for control dogs, diabetics and insulinoma patients

Group	Number of dogs	Sex	Age (years)		Packed cell volume (%)		Plasma total protein (g/dl)		Fructosamine In Charge (µmol/litre)		Fructosamine Laboratory* (µmol/litre)		Haemoglobin A1c (%)	
			Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Median	Range	Median	Range	Median
Beagles	14	14 FE	0·5	0·5±0	37-46	40·5±2·9	48-56	51·8±3·4	170-358	222·5	ND	ND	2·5-3·6	2·7
Control patients	36	18 FN 11 MN 4 ME 3 FE	1·2-17	7·1±3·7	24-66	45·7±9·2	34-110	65·5±14·2	154-543 (n=16)	292 (n=16)	ND	ND	2·1-3·7	2·8
Diabetic patients	100	46 FN 33 MN 9 ME 2 FE	0·5-16	9·8±2·89	3 -64	48±7·6	48-118	73·6±14·3	369->700 (n=50)	682 (n=50)	240-831[a] (n=30) 309-1778[b] (n=70)	509[a] (n=30) 758·2[b] (n=70)	2·5-7·0	4·4
Insulinoma patients	5	3 F 2 M	8-10	8·6±0·9	35-54	41·4±8·1	57-77	68·2±8·5	184-477	281	167-209[c]	180[c]	2·0-3·0	2·5

F Female, M Male, E Entire, N Neutered, ND Not determined
*External laboratories: [a]Idexx Laboratories (reference range 187-386 µmol/litre)
[b]Vetlab Services (reference range <300 µmol/litre)
[c]Cambridge Specialist Laboratories (reference range 258-343 µmol/litre)

Wetherby; Vetlab Services, Southwater) and in dogs with insulinoma (Cambridge Specialist Laboratory Services, Cambridge).

Haemoglobin A1c measurement

HbA1c was measured in all dogs using the Haemaquant device and disposable Glycosal test cartridges (Provalis) (Fig 1B), according to the manufacturers' instructions. A 7·5 µl sample of EDTA blood was analysed in each case, with the result available in less than 10 minutes. Prior to each use of the Haemaquant meter, a standard reference cartridge was tested. HbA1c was measured in selected samples (n=15) using the Haemaquant meter and compared to glycosylated haemoglobin concentrations measured by boronate affinity chromatography in a hospital clinical pathology laboratory (Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne). These samples were selected to represent the range of HbA1c readings obtained using the Haemaquant meter.

Statistical analysis

Statistical analysis was carried out using SPSS v10.0 or Microsoft Excel 97 for Windows. Linear regression was used to analyse the relationship between packed cell volume (PCV) and HbA1c and between plasma total protein (TP) and fructosamine. The Mann-Whitney U test was used to compare differences in HbA1c, fructosamine, TP and PCV between diabetic, insulinoma and control populations. The British Standards Institute (BSI) reproducibility coefficient was calculated to determine agreement between the methods of fructosamine measurement. Spearman's rank correlation coefficient was calculated to analyse the relationship between the HbA1c concentration measured using the Haemaquant/Glycosal meter and the glycosylated haemoglobin concentration measured by the hospital laboratory.

RESULTS

Control, diabetic and insulinoma populations

The beagle group (n=14) consisted of six-month-old entire female beagles. The control patient population (n=36) consisted of six Labrador retrievers, five terriers, four spaniels, four collies and 17 dogs of other breeds including rottweilers, boxers and cross-breeds. The average age of this group was 7·1 years and 20 of the 36 dogs were females.

The diabetic group (n=100) consisted of 26 terriers, 18 collies, 16 Labrador retrievers or their crosses, and many other breeds including eight cross-breeds, four samoyeds and three dachshunds. The average age of this group was 9·8 years, and 55 dogs were female (Table 1). The majority of the diabetic dogs had been treated with

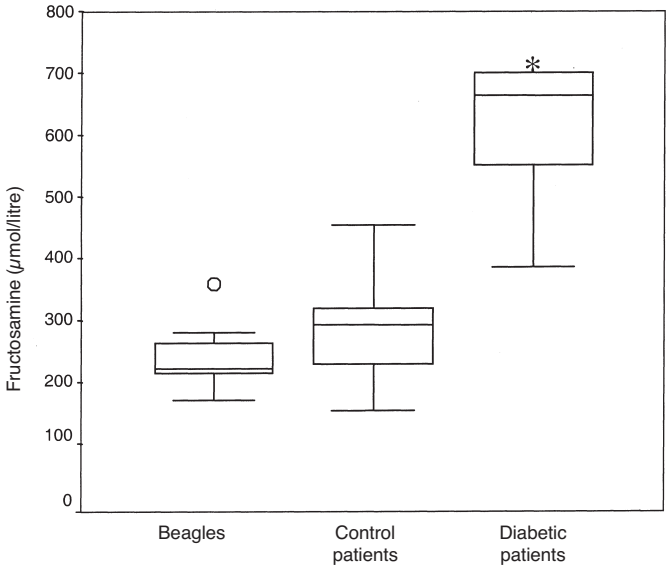


FIG 2. Box and whisker plot demonstrating whole blood fructosamine concentrations in colony beagles (n=14), normoglycaemic patients (n=16) and diabetic dogs (n=50). A 20 µl sample of EDTA blood was analysed using the In Charge analyser and glucoprotein test strips. The maximum measurable fructosamine concentration using this device was 700 µmol/litre (*). The box represents the interquartile range which contains 50 per cent of the values. The whiskers extend to the highest and lowest values, excluding outliers, which are marked as open circles. The line across the box indicates the median

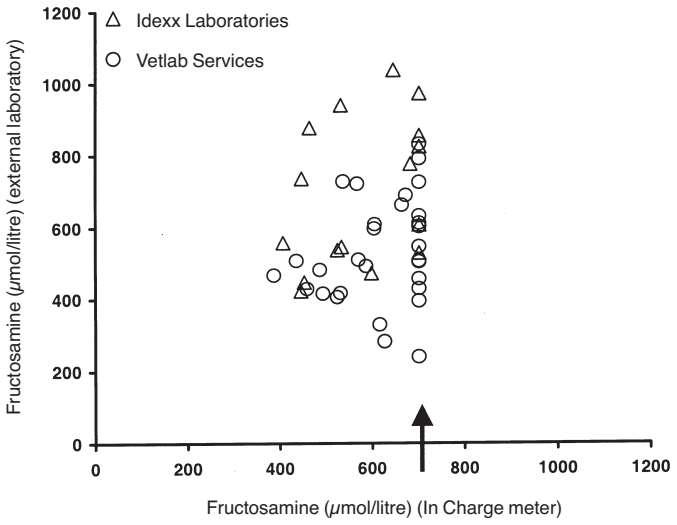


FIG 3. Fructosamine values obtained using the In Charge analyser compared to those measured by commercial laboratories. A 20 µl sample of EDTA blood was analysed using the In Charge glycoprotein analyser (arrow indicates upper limit of detection) and 100 µl serum from the same dog was submitted to commercial laboratories (Idexx Laboratories or Vetlab Services) for measurement of serum fructosamine

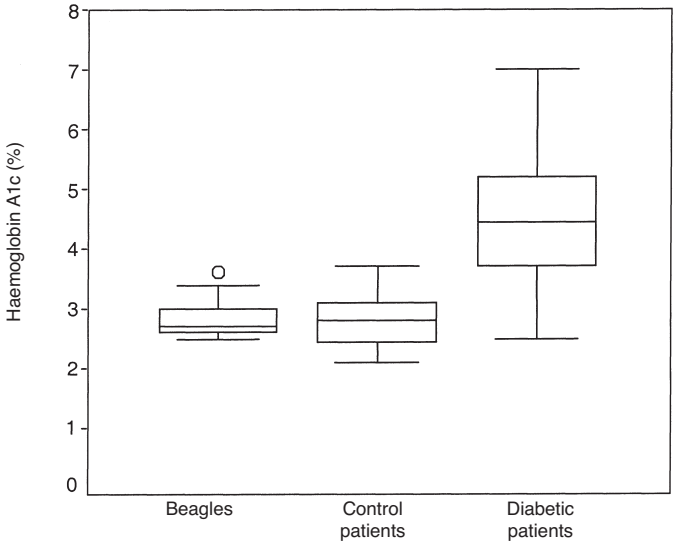


FIG 4. Box and whisker plot demonstrating whole blood HbA1c concentrations in colony beagles (n=14), normoglycaemic patients (n=36) and diabetic dogs (n=100). A 7·5 µl sample of EDTA blood was analysed in each case using the Haemaquant/Glycosal HbA1c meter. The box represents the interquartile range which contains 50 per cent of the values. The whiskers extend to the highest and lowest values, excluding outliers, which are marked as open circles. The line across the box indicates the median

insulin (95 of 100) for a mean of 13·4 months (range two weeks to 93 months). Seventy-seven dogs were receiving bovine lente insulin (Insuvet Lente; Schering-Plough) and 18 were receiving other insulin types including porcine insulin (six dogs) (Caninsulin; Intervet), protamine zinc insulin (11 dogs) (Insuvet PZI; Schering-Plough) and isophane insulin (one dog). The insulin dose per injection ranged from 0·3 to 4·4 iu/kg (mean 1·3 iu/kg).

The insulinoma group consisted of five dogs (three female, two male) – one Labrador retriever, one golden retriever, one Cavalier King Charles spaniel, one English spaniel and one springer spaniel. The average age of the group was 8·6 years (range eight to 10 years).

Measurement of fructosamine in diabetic patients

The In Charge meter provided a quantitative measurement of fructosamine concentration in 14 of 14 beagles, 16 of 16 patient controls and 29 of 50 diabetics (Fig 2). The median (and range) for fructosamine concentration using this meter was 222·5 (170 to 358) µmol/litre in the beagles and 292 (154 to 453) µmol/litre in the control patients. The difference between these two groups was statistically significant ($P<0·05$). Fructosamine concentrations measured using the In Charge meter were significantly higher in the diabetic patients compared to either the beagles ($P<0·05$) or the control patients ($P<0·05$) (Table 1).

Blood samples from 50 diabetic dogs were assessed using the In Charge meter and also submitted to external laboratories for serum fructosamine measurement (Idexx n=30, Vetlab n=20). Twenty-one of the 50 diabetic samples were reported to be above the limit of detection by the In Charge meter, indicating a fructosamine concentration of >700 µmol/litre (assigned the value 701 µmol/litre for graphical display purposes) (Fig 3). In the 29 samples where a quantitative value was obtained, the agreement with external laboratory results was poor (BSI reproducibility coefficient = 267·8 [Idexx], 384·5 [Vetlab]).

A significantly lower plasma TP concentration was recorded in beagles compared to patient controls ($P<0·05$). TP was significantly higher in the diabetics compared to either control group ($P<0·05$) (Table 1). Fructosamine concentrations in diabetic dogs were not influenced by plasma TP when measured by the In Charge meter ($r^2=0·08$, $P>0·05$), Idexx ($r^2=<0·01$, $P>0·05$) or Vetlab ($r^2=<0·01$, $P>0·05$). Similarly, there was no significant correlation between plasma TP and fructosamine concentration in the control population ($r^2=0·04$, $P>0·05$).

Measurement of HbA1c in diabetic patients

A reference cartridge (7·7 to 9·8 per cent HbA1c) was provided with the Haemaquant meter and was tested before each batch of samples, giving a between-run coefficient of variation of 0·5 per cent (n=55). Five repeated measurements using three samples, representing high (6·0 per cent), medium (4·8 per cent) and low (3·1 per cent) values of the observed range yielded an average within-run coefficient of variation of 4 per cent, within acceptable limits for veterinary clinical use (Hooghuis and others 1994). There was no significant difference in HbA1c concentrations between normoglycaemic patients and colony beagles ($P=0·85$) (Table 1). The median (and range) for the control dogs was 2·8 per cent (2·1 to 3·7 per cent), while that for the diabetics was significantly higher at 4·4 per cent (2·5 to 7·0 per cent) ($P<0·001$) (Fig 4). There was no correlation between PCV and HbA1c in diabetics, control patients or beagles ($r^2=0·03$, 0·02 and 0·06, respectively; $P>0·05$ in all cases).

Table 2. Fructosamine values reported in other studies

Study	Number of controls	Control mean (µmol/litre)	Control reference range* (µmol/litre)	Number of diabetics	Diabetic mean (µmol/litre)	Diabetic range (µmol/litre)
Present study using In Charge fructosamine meter	14 colony beagles, 16 control patients	235·8 291·8	146·0-325·6 182·2-471·4	50	682 (median)	369->701
Reusch and others (1993)	48	312 (median)	249-374	14 untreated, 8 well controlled, 7 moderately controlled, 10 poorly controlled	476 251 422 476	325-834 216-474 295-528 382-745
Coppo and Coppo (1997)	89	275·0	192·6-357·4	3	550·7	433-663

*Reference ranges in these studies calculated as mean ± 2 standard deviations

Total glycosylated haemoglobin was measured in selected samples (n=15) using boronate affinity chromatography. Glycosylated haemoglobin concentrations showed a high degree of correlation with the HbA1c values measured with the Haemaquant meter (r^2=0·86, P<0·01).

Measurement of fructosamine and HbA1c in insulinoma patients

The average blood glucose in this group was 2·3 mmol/litre (range 1·5 to 4·0 mmol/litre) with four of five insulinoma patients demonstrating blood glucose concentrations below the normal reference range (3·3 to 6·0 mmol/litre). There was no significant difference in fructosamine concentrations between the control dogs and insulinoma patients when measured using the In Charge meter. However, serum fructosamine concentrations in all five insulinoma patients were below the reference range established by the external laboratory (Table 1). HbA1c concentrations in the insulinoma patients were lower than those obtained for beagles or control patients. However, this did not reach statistical significance (P=0·06).

DISCUSSION

The dogs recruited for the current study included normoglycaemic patients referred to the QVSH, University of Cambridge, a group of colony beagles, dogs diagnosed with diabetes mellitus and dogs with insulinoma. Labrador retrievers and terriers predominated in the diabetic population, which also contained more females than males, as would be expected from previous studies (Berkow and Ricketts 1965, Doxey and others 1985). The average age of the diabetic population was higher than that of the control group, consistent with reports that diabetes is more common in dogs over seven years of age (Marmor and others 1982). Since collection of control samples relied on excess blood being available from patients undergoing diagnostic procedures, it was not possible to age and sex match these cases with the diabetic patients.

In addition to hospital patients, it was decided to include samples from healthy dogs. Blood samples from colony beagles were available, although a previous study had demonstrated differences in HbA1c concentrations in colony animals compared to normoglycaemic pet dogs (Mahaffey and Cornelius 1982). The beagle population were all six months old at the time of sampling, and their juvenile status could account for their low PCV (Weiser 1995) and plasma TP (Werner and others 1994) compared to other groups. The insulinoma group was made up of retrievers and spaniels, previously reported to be at an increased risk of beta cell neoplasia (Leifer and others 1986, Dunn and others 1993). The average age of this group was 8·6 years, which is consistent with previous studies (Leifer and others 1986, Dunn and others 1993).

Fructosamine analysis

The In Charge meter was able to assay whole blood fructosamine concentrations for all of the control dogs and 29 of 50 diabetic blood samples tested (Table 1). It was simple to use and the manufacturers report a precision of 10 per cent for human EDTA blood. However, fructosamine concentrations could not be quantified in 21 of 50 diabetic dogs since they were above the upper limit of detection (700 µmol/litre) and registered 'HI' on the meter.

The fructosamine values reported for the control dogs in the present study are similar to those reported previously, although a wider range was recorded by the In Charge meter compared to studies where other methods were used (Table 2). Serum fructosamine concentrations measured by the external laboratories in diabetic dogs (Table 1) were higher in the current study than those previously reported, reaching 831 µmol/litre (Idexx Laboratories) and 1218 µmol/litre (Vetlab Services). This could be related to differences in the method of fructosamine measurement between laboratories or could reflect poor glycaemic control in the diabetic cases recruited for this study.

It is interesting to note that in the study carried out by Kawamoto and others (1992), fructosamine concentrations obtained from colony animals were lower than those for normoglycaemic hospital patients. This finding is reflected in the present study and, although sample numbers are small, it is possible to speculate that a mild chronic elevation in blood glucose associated with illness, while not enough to cause measurable hyperglycaemia, could contribute to the elevation in fructosamine concentrations in 'unhealthy' control animals.

Table 3. Glycosylated haemoglobin values reported in other studies

Study	Number of controls	HbA1 or HbA1c	Mean HbA1c (%) controls	Reference range HbA1c (%)	Number of diabetics	Mean HbA1c (%) diabetics	Range of HbA1c (%) diabetics
Present study using Haemaquant HbA1c meter	36 control patients, 14 colony beagles	HbA1c	2·8 2·9	2·0-3·6 2·3-3·5	100	4·5	2·5-7·0
Wood and Smith (1980)	7	HbA1c	2·95	ND	7	4·97	3·82-7·36
Easley (1986)	44	HbA1	7·1	4·9-9·3	5 untreated	12·5	10·1-15·4
Mahaffey and Cornelius (1982)	40 control patients, 16 colony dogs	HbA1	6·43 5·62	4·43-8·43 4·26-6·98	16	9·63	6·24-13·33
Elliott and others (1997)	63	HbA1	3·3	1·7-4·9	23 untreated, 43 poorly controlled, 31 well controlled	8·7 7·3 5·7	6·0-15·5 2·8-14·1 2·6-11·6
Haberer and Reusch (1998)	50	HbA1	3·0 (median)	2·4-3·4	21	6·1	4·5-8·6

ND Not determined

One disadvantage of the In Charge meter was that it was not possible to quantify fructosamine concentrations in 21 of 50 diabetic samples tested, since these were reported to be above 700 µmol/litre, the upper limit of detection for this device. Testing of these samples at external laboratories, however, did not confirm these values, since only seven of 21 samples were reported to be in excess of 700 µmol/litre fructosamine. Furthermore, of the diabetic samples which had serum fructosamine concentrations above 700 µmol/litre, only eight of 15 gave a similar reading with the In Charge meter. Excluding readings above the limit of detection, comparison of fructosamine concentrations recorded by the In Charge meter with those measured by external laboratories also demonstrated a lack of agreement.

There are several possible explanations for inaccuracies in measurement of fructosamine in canine blood by the In Charge meter. It is possible that the colorimetric assay and detection procedure does not give accurate measurements of canine fructosamine when calibrated for human fructosamine. This could be related to the fact that human and canine albumin, the major blood protein glycosylated during hyperglycaemia, share only 80 per cent amino acid identity (Genbank database accession numbers AJ_133489.1 [canine albumin] and XM_031322.1 [human albumin]). Additionally, although EDTA blood samples can be tested after storage, the meter was designed as a 'point-of-care' device for analysis of fresh human capillary blood. Several of the diabetic samples sent by post were partially haemolysed on arrival. Free haemoglobin in such samples could interfere with the optical measurements of the chemical reaction on the strip, resulting in a false 'HI' reading. Several samples were lipaemic, which could also have interfered with the assay (Reusch and Harberer 2001). Poor repeatability could partly explain the unsatisfactory results obtained with this instrument. Calculation of within- and between-run coefficients of variation using the In Charge meter with canine samples would help to determine if this was the case, but this was not investigated in the current study.

Since fructosamine is a plasma protein, the authors investigated whether the plasma TP concentration had any influence on fructosamine values. This was not found to be a significant factor in diabetics or controls, regardless of the method of fructosamine measurement. This is consistent with a previous study (Kawamoto and others 1992) where fructosamine correlated with albumin concentrations only in hypoalbuminaemic dogs, and not in dogs with normal plasma protein concentrations.

In dogs with beta cell neoplasia, lower fructosamine concentrations have been reported in association with persistent hypoglycaemia (Loste and others 2001). The In Charge meter was not able to distinguish between control patients and insulinoma patients (P=0·45). However, low serum fructosamine values were reported by the commercial laboratory in all five patients. Thus, there was poor agreement between fructosamine values at high concentrations in diabetic samples, comparing the In Charge meter with external laboratories, and results from insulinoma patients also cast doubt as to the accuracy of this meter at low fructosamine concentrations. It is possible that there would be agreement between the In Charge meter and laboratory fructosamine concentrations within the normal fructosamine range; however, these values were not compared in normoglycaemic control dogs.

Haemoglobin A1c analysis

The Haemaquant/Glycosal meter employs boronate affinity chromatography to separate glycosylated from non-glycosylated haemoglobin. These are then measured photometrically and the result is automatically converted into an HbA1c value using an internal algorithm. HbA and HbA1c have been measured in normoglycaemic and diabetic dogs by many different methods including commercial glycohaemoglobin kits (Mahaffey and Cornelius 1982), ion-exchange microchromatography and thiobarbituric acid colorimetry (Hooghuis and others 1994), turbidimetric inhibition assay (Marca and Loste 2000) and column cation exchange (Delack and Stogdale 1983). Thus, the reference ranges for glycosylated haemoglobin or HbA1c vary according to the assay and laboratory, but the mean and range of HbA1c concentrations obtained using the Haemaquant/Glycosal meter are similar to previous reports (Table 3).

In contrast to a previous study, the colony beagle population had similar HbA1c concentrations to non-diabetic hospital patients (Mahaffey and Cornelius 1982). There was a significant difference in HbA1c in diabetics compared to control

dogs ($P<0.001$), although there was a degree of overlap between the measured ranges in controls and diabetics. It has been shown that PCV can influence glycosylated haemoglobin concentrations in anaemic dogs (Elliott and others 1997), but there were no anaemic patients in the current study and no correlation was found between PCV and HbA1c.

One of the limitations of this study was that it was not possible to compare HbA1c meter readings with those obtained using a standard laboratory technique. The use of high performance liquid chromatography, employed to measure human HbA1c, did not yield meaningful results, suggesting that while the amino acid sequences of human and canine HbA1c are similar, their physicochemical properties are different. It was possible to compare total glycosylated haemoglobin with the HbA1c values obtained using the Haemaquant device in 15 patients and a high degree of correlation was seen.

The algorithm used by the Haemaquant meter to convert total glycosylated haemoglobin to HbA1c has been derived using human blood samples and might not be applicable to canine samples. Further work is required to calculate an accurate algorithm for canine samples and, until this has been done, it might be more appropriate to report canine results obtained using the Haemaquant meter as glycosylated haemoglobin rather than HbA1c.

Conclusions

The In Charge fructosamine meter was simple to use but overall the results were unreliable. Although the meter was able to distinguish the control population from the diabetic population, agreement with external laboratories was poor. The In Charge meter had the additional disadvantage that over 40 per cent of the diabetic samples were reported to be above the measurable range.

The Haemaquant/Glycosal HbA1c meter was simple to use and provided a reading in all cases. Although further studies are required to fully evaluate this device, the Haemaquant/Glycosal HbA1c meter has a potential veterinary use, allowing clinicians to monitor HbA1c concentrations in canine diabetics. This could be performed 'in house' and would provide a rapid assessment of glycaemic control during routine consultations.

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References

Berkow, J. W. & Ricketts, R. L. (1965) Spontaneous diabetes mellitus in dogs. *Journal of the American Veterinary Medical Association* **146**, 1101-1105

Bunn, H. F., Gabbay, K. H. & Gallop, P. M. (1978) The glycosylation of hemoglobin: relevance to diabetes mellitus. *Science* **200**, 21-27

Coppo, J. A. & Coppo, N. B. (1997) Serum fructosamine: a reference interval for a heterogeneous canine population. *Veterinary Research Communications* **21**, 471-476

Davison, L. J., Catchpole, B., Ristic, J. & Herrtage, M. E. (2001) Research into canine diabetes mellitus. *Veterinary Record* **148**, 352

Delack, J. B. & Stogdale, L. (1983) Glycosylated hemoglobin measurement in dogs and cats: implications for its utility in diagnostic monitoring. *Canadian V eterinary Journal* **24**, 308-311

Doxey, D. L., Milne, E. M. & Mackenzie, C. P. (1985) Canine diabetes: a retrospective survey. *Journal of Small Animal Practice* **26**, 555-561

Dunn, J. K., Bostock, D. E., Herrtage, M. E., Jackson, K. F. & Walker, M. J. (1993) Insulin-secreting tumours of the canine pancreas: clinical and pathological features of 11 cases. *Journal of Small Animal Practice* **34**, 325-331

Easley, J. R. (1986) Glycosylated hemoglobin in dogs: precision, stability and diagnostic utility. *Veterinary Clinical Pathology* **15**, 12-15

Elliott, D. A., Nelson, R. W., Feldman, E. C. & Neal, L. A. (1997) Glycosylated hemoglobin concentrations in the blood of healthy dogs and dogs with naturally developing diabetes mellitus, pancreatic β cell neoplasia, hyperadrenocorticism and anaemia. *Journal of the American Veterinary Medical Association* **211**, 723-727

Haberer, B. & Reusch, C. E. (1998) Glycated haemoglobin in various pathological conditions: investigations based on a new, fully automated method. *Journal of Small Animal Practice* **39**, 510-517

Hooghuis, H., Rodrigues, M. & Castano, M. (1994) Ion-exchange microchromatography and thiobarbituric acid colorimetry for the measurement of canine glycated hemoglobins. *Veterinary Clinical Pathology* **23**, 110-116

Jensen, A. L. (1992) Serum fructosamine in canine diabetes mellitus – an initial study. *Veterinary Research Communications* **16**, 1-9

Jensen, A. L. (1995) Glycated blood proteins in canine diabetes mellitus. *Veterinary Record* **137**, 401-405

Johnson, R. N., Metcalf, P. A. & Baker, J. R. (1982) Fructosamine – a new approach to the estimation of serum glycosylprotein, an indication of diabetic control. *Clinical Chemistry Acta* **127**, 87-95

Kawamoto, M., Kaneko, J. J., Heusner, A. A., Feldman, E. C. & Koizumi, N. (1992) Relation of fructosamine to serum protein, albumin and glucose concentrations in healthy and diabetic dogs. *American Journal of Veterinary Research* **53**, 851-855

Koening, R. J., Peterson, C. M., Jones, R. L., Saudek, C., Lehrman, M. & Cerami, A. (1976) Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *New England Journal of Medicine* **295**, 417-420

Leifer, C. E., Peterson, M. E. & Matus, R. E. (1986) Insulin-secreting tumour: diagnosis and medical and surgical management in 55 dogs. *Journal of the American Veterinary Medical Association* **188**, 60-64

Loste, A., Marca, M. C., Perez, M. & Unzueta, A. (2001) Clinical value of fructosamine measurements in non-healthy dogs. *Veterinary Research Communications* **25**, 109-115

Mahaffey E. A. & Cornelius, L. M. (1982) Glycosylated hemoglobin in diabetic and non-diabetic dogs. *Journal of the American Veterinary Medical Association* **180**, 635-637

Marca, M. C. & Loste, A. (2000) Glycosylated hemoglobin assay of canine blood samples. *Journal of Small Animal Practice* **41**, 189-192

Marmor, M., Willeberg, P., Glickman, L. T., Priester, W. A., Cypess, R. H. & Hurvitz, A. I. (1982) Epizootiologic patterns of diabetes mellitus in dogs. *American Journal of Veterinary Research* **43**, 465-470

Nelson, R. W. (2000) Diabetes mellitus. In: Textbook of Veterinary Internal Medicine, 5th edn. Eds S. J. Ettinger and E. C. Feldman. W. B. Saunders, Philadelphia. pp 1438-1460

Reusch, C. E. & Harberer, B. (2001) Evaluation of f ructosamine in dogs and cats with hypo- or hyperproteinaemia, azotaemia, hyperlipidaemia and hyperbilirubinaemia. *Veterinary Record* **148**, 370-376

Reusch, C. E., Leihs, M. R., Hoyer, M. & Vochezer, R. (1993) Fructosamine: a new parameter for diagnosis and metabolic control in diabetic dogs and cats. *Journal of Veterinary Internal Medicine* **7**, 177-182

Smith, J. E., Wood, P. A. & Moore, K. (1982) Evaluation of a colorimetric method for canine glycosylated hemoglobin. *American Journal of Veterinary Research* **43**, 700-701

Trivelli, L. A., Ranney, H. N. & Lai, H. T. (1971) Hemoglobin components in patients with diabetes mellitus. *New England Journal of Medicine* **284**, 353-357

Weiser, M. G. (1995) Erythrocyte responses and disorders. In: Textbook of Veterinary Internal Medicine, 4th edn. Eds S. J. Ettinger and E. C. Feldman. W. B. Saunders, Philadelphia. pp 1873-1874

Werner, L. L., Turnwald, G. H. & Barta, O. (1994) Immunologic and plasma protein disorders. In: Small Animal Clinical Diagnosis by Laboratory Methods, 2nd edn. Eds M. D. Willard, H. Tvedten and G. H. Turnwald. W. B. Saunders, Philadelphia. pp 253-254

Wood, P. A. & Smith, J. E. (1980) Glycosylated hemoglobin and canine diabetes mellitus. *Journal of the American Veterinary Medical Association* **176**, 1267-1268