Evaluation of day-to-day variability of serial blood glucose concentration curves in diabetic dogs

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Objective—To evaluate day-to-day variability of serial blood glucose concentration curves in dogs with diabetes mellitus.

Design—Prospective clinical study.

Animals—10 dogs with diabetes mellitus.

Procedure—Paired 12-hour serial blood glucose concentration curves performed during 2 consecutive days were obtained on 3 occasions from each dog. Dogs received the same dose of insulin and meal every 12 hours on both days. For each pair of curves, comparison was made between the results of days 1 and 2.

Results—Mean absolute difference (without regard to sign) between days 1 and 2 for each parameter was significantly > 0, disproving the hypothesis that there is minimal day-to-day variability of serial blood glucose concentration curves when insulin dose and meals are kept constant. Coefficient of variation of the absolute difference between days 1 and 2 for each parameter ranged from 68 to 103%. Evaluation of the paired curves led to an opposite recommendation for adjustment of the insulin dose on day 2, compared with day 1, on 27% of occasions. Disparity between dosage recommendations was more pronounced when glucose concentration nadir was < 180 mg/dL (10 mmol/L) on 1 or both days. In this subset of 20 paired curves, an opposite recommendation for dosage adjustment was made on 40% of occasions.

Conclusions and Clinical Relevance—There is large day-to-day variation in parameters of serial blood glucose concentration curves in diabetic dogs. Day-to-day variability of serial blood glucose concentration curves has important clinical implications, particularly in dogs with good glycemic control. (*J Am Vet Med Assoc* 2003;222:317–321)

Diabetes mellitus is a common endocrine disease of dogs characterized by an absolute or relative deficiency of insulin.[1] Lifelong daily SC insulin administration is required for all affected dogs. Regular reappraisal of this treatment is vital if optimal control of the disease is to be achieved. The current recommendation is that adjustment of the insulin dose should be based on history and physical examination findings in addition to results of serial blood glucose measurements after the dog's usual insulin injection and meal.[1-9] When interpreting the curves, clinicians typically assume that the major influences on blood glucose concentration are the type of insulin used, dose of insulin injected, composition of meals, and timing of meals. If these factors are kept constant, it is expected that the serial blood glucose concentration curve for an individual dog will be similar on different days. The purposes of the study reported here were to test the hypothesis that there is minimal day-to-day variability of serial blood glucose concentration curves in diabetic dogs when insulin and meal factors are kept constant and determine the clinical implications if significant day-to-day variability was found.

Materials and Methods

Ten client-owned dogs (3 male, 7 female; median body weight, 20.5 kg [45 lb]; range, 15 to 36 kg [33 to 79 lb]) with naturally occurring diabetes mellitus were included in this study. Each had received insulin for a variable duration (median, 13.5 weeks; range, 3 to 156 weeks), and no attempt was made to ensure optimal glycemic control of diabetes before the dogs entered the study. All were managed as outpatients and received SC administration of porcine lente insulin every 12 hours. Insulin dose differed among dogs. All were fed the same nutritionally balanced canned dog food divided into 2 equal-sized meals each day, which were fed at the time of insulin injection. On each occasion, the dogs consumed the entire meal within 30 minutes of being fed. All dogs had 3 test periods with at least 2 weeks between each test period. Dogs were admitted to the University of Queensland Veterinary Teaching Hospital on the morning of the first day and kept hospitalized for the duration of the test period. An IV catheter was placed in a cephalic vein to facilitate repeated blood sampling.[10] Blood glucose concentration was measured with a portable glucose meter[a] (intra- and interassay coefficients of variation, < 3.4%). This meter has high correlation (r^2 = 0.9788) with the hexokinase reference method of measuring blood glucose concentration[b] and gives clinically reliable results (92.6% of results lead to correct clinical decisions within the reference, hyperglycemic, or hypoglycemic ranges, and the remaining 7.4% of results lead to benign or no treatment errors).[c]

During each test period, paired 12-hour serial blood glucose curves were obtained on 2 consecutive days. The dog's usual meal and insulin dose were administered at 8 AM and 8 PM, and injections were given in approximately the same anatomic location. Blood glucose measurement was performed every 2 hours for 12 hours beginning immediately prior to the morning insulin injection and meal. If adjustment of the insulin dose or meal size was required, this adjustment was made at the conclusion of the visit, and at least 2 weeks was allowed for the dog to become accustomed to the changes before reassessment. Parameters recorded for each serial blood glucose curve were blood glucose concentrations obtained just before the 8 AM and 8 PM insulin injections, maximum and minimum (nadir) blood glucose concentrations, time from administration of insulin injection to

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Table 1—Parameters of paired 12-hour serial blood glucose concentration curves obtained on 3 occasions from 10 dogs with diabetes mellitus

Parameter	Overall mean	Mean absolute difference between days 1 and 2	SD of absolute difference	Coefficient of variation (%)	95% Confidence interval
Minimum blood glucose (mg/dL)	187	63	43	68	47–79
Mean blood glucose (mg/dL)	283	54	41	78	38–70
J-index	137	43	36	82	30–56
Difference between morning preinsulin blood glucose and nadir (mg/dL)	148	115	99	86	79–151
Morning preinsulin blood glucose (mg/dL)	335	115	101	88	77–153
Time from insulin injection to nadir (h)	7	3.3	2.9	90	2.2–4.4
SD blood glucose (mg/dL)	77	29	29	97	18–40
Evening preinsulin blood glucose (mg/dL)	310	101	99	98	63–139
Maximum blood glucose (mg/dL)	398	68	68	101	43–94
Area under the curve (h•mg/dL)	3,267	776	803	103	476–1,076

nadir, difference between 8 AM and nadir blood glucose concentrations, area under the blood glucose concentration curve, mean blood glucose concentration during 12 hours, SD of the blood glucose measurements, and the J-index, which arithmetically combines the mean and SD into a single value.[11]

To test the hypothesis that there is minimal day-to-day variability of serial blood glucose curves in diabetic dogs when insulin and meal factors are kept constant, the absolute difference (without regard to sign) between days 1 and 2 was calculated for each parameter. The mean of the absolute difference for each parameter was then calculated for the 30 sets of paired curves. A paired *t*-test was used to assess whether the means of the absolute differences were significantly different from zero. To identify significant sources of variation in the paired curves, a balanced ANOVA was performed with the day effect regarded as fixed and the dog and visit effects as random. That is, each dog and each visit were considered to have a random effect on the data, whereas days 1 and 2 were considered a fixed effect. Results were considered significant at $P \leq 0.05$.

To examine the clinical implications of any day-to-day variability of the serial blood glucose curves, a theoretical recommendation for adjustment of the dog's insulin dose was based on the results of each curve. The recommendations were that the insulin dose should be increased, left unchanged, or decreased. A recommendation to increase the insulin dose was made if the nadir was > 145 mg/dL (8 mmol/L) and the blood glucose measurements obtained at 8 AM and 8 PM were both > 180 mg/dL (10 mmol/L). A recommendation to make no change in the insulin dose was made if the nadir was 90 to 145 mg/dL (5 to 8 mmol/L) and the blood glucose measurements at 8 AM and 8 PM were both > 180 mg/dL (10 mmol/L). A recommendation to decrease the dose was made either if the nadir was < 90 mg/dL (5 mmol/L) or at least 1 of the blood glucose measurements at 8 AM or 8 PM was < 180 mg/dL (10 mmol/L). Comparison was made between the clinical recommendations for the entire set of paired serial blood glucose curves and a subset of paired curves with a nadir < 180 mg/dL (10 mmol/L) on 1 or both days, which were assumed to represent dogs with better glycemic control.

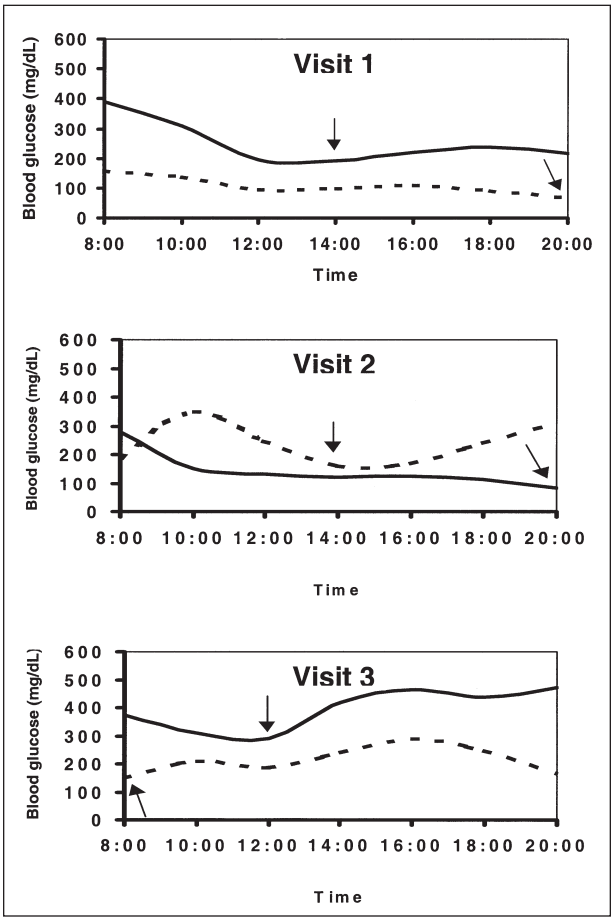

Figure 1—Paired 12-hour blood glucose concentration curves obtained during 3 hospital visits from a dog with diabetes mellitus. The dog's usual meal and insulin dose were administered at 8 AM and 8 PM. For each curve within a pair, insulin dose, meal size, and composition were consistent. Blood glucose measurement was performed every 2 hours for 12 hours beginning immediately prior to the morning insulin injection. For each 2-day hospital visit, solid lines represent data from day 1 and dashed lines represent data from day 2. Arrows depict nadir.

Results

The absolute difference between values obtained on consecutive days would approximate zero if the hypothesis were true that there was minimal day-to-day variability of serial blood glucose curves when insulin dose and meals of diabetic dogs were kept constant. For each parameter, the mean of the absolute difference in values between days 1 and 2 was significantly different from zero and the SD was almost as high as the mean, indicating a large day-to-day variability (**Table 1**). This was reflected in the high coefficients of variation, which ranged from 68% for the minimum blood glucose concentration to 103% for the area under the blood glucose concentration curve. Considerable variability was also seen in the shapes of the 12-hour blood glucose curves obtained on consecutive days (**Fig 1**).

Analysis of variance identified several significant sources of variation. There was a significant difference among dogs for the evening preinsulin blood glucose concentration ($P < 0.001$), maximum blood glucose concentration ($P = 0.042$), nadir ($P = 0.007$), mean ($P = 0.008$) and SD ($P = 0.043$) of the blood glucose concentrations during 12 hours, and the J-index ($P = 0.042$). No significant difference among dogs was found for the morning preinsulin blood glucose concentration, the time from insulin injection to nadir, the difference between the morning blood glucose concentration and the nadir concentration, or for the area under the blood glucose concentration curve.

Some dogs had significantly lower preinsulin blood glucose concentrations on the first morning of their visits, whereas other dogs had significantly lower preinsulin blood glucose concentrations on the second morning of their visits. For individual dogs, this resulted in a significant day effect on the difference between the first blood glucose concentration at 8 AM and the nadir. That is, in some dogs there was a significantly different glucose-lowering effect of the insulin dose on day 1, compared with day 2.

Evaluation of the 30 sets of paired 12-hour curves led to an opposite theoretical recommendation for adjustment of the dog's insulin dose on day 2, compared with day 1, on 27% of occasions. For 17% of the curves, a different but not opposite recommendation resulted. The same recommendation for dosage adjustment on both days was made in only 57% of the paired curves. The disparity between the dosage recommendations resulting from the paired 12-hour curves was more pronounced when the nadir was < 180 mg/dL (10 mmol/L) on 1 or both days. In this subset of 20 paired 12-hour curves, an opposite recommendation for dosage adjustment was made on 40% of occasions, a different but not opposite recommendation resulted 25% of the time, and the same recommendation for both days occurred in only 35% of the sets of paired curves.

Discussion

The large day-to-day variability of serial blood glucose curves seen in the diabetic dogs in this study is also reported in human diabetics. Day-to-day variability of glycemic indices in human diabetics[12,13] has been associated with daily fluctuations in the postprandial glycemic response to a standard meal,[14] variable sensitivity to insulin,[15] and variation in the rate of absorption of insulin from the SC injection site, particularly if different anatomic regions are used.[16] Additional factors that are relevant in humans are the level of diabetic instability,[17-19] the amount of residual β-cell function,[20,21] and inherent error when measuring insulin doses in a syringe.[22] All of these factors could be expected to influence blood glucose concentrations in diabetic dogs.

A significant difference among dogs was found for many of the parameters derived from the blood glucose curves. This suggests that serial blood glucose measurement is a useful test for identifying differences in glycemic control among dogs. This information is particularly relevant for research projects that compare the glycemic response of groups of diabetic dogs by use of the results of serial blood glucose testing. The parameters that were significantly different among dogs and likely to be most reliable for distinguishing levels of glycemic control among dogs are the preinsulin blood glucose concentration obtained at the end of the 12-hour curve (8 PM), and the maximum, minimum, mean, and SD of the blood glucose concentrations during the 12 hours. The preinsulin blood glucose concentration obtained at the beginning of the curve, the difference between this blood glucose concentration and the nadir, the time to nadir, and the area under the blood glucose concentration curve all failed to identify any differences among dogs in this study. Consequently, these parameters may be less useful for comparing the glycemic responses of groups of diabetic dogs.

The only variable in the design of this study was where the dog spent the 24 hours prior to the first blood sample of the 12-hour curve. On the first day, the dogs were brought to the hospital by their owners and admitted just before the 8 AM sample. On the second morning, the dogs had already been in the hospital for 24 hours when the 8 AM sample was collected. This variable appears to have had a significant effect on the morning preinsulin blood glucose concentration. However, the effect was found to vary among dogs, with certain dogs having a significantly lower blood glucose concentration following morning admission and other dogs having a significantly lower concentration after overnight hospitalization. Interestingly, the effects on 8 AM blood glucose concentration for the paired blood glucose curves did not appear to substantially affect the nadir concentration or the time to nadir of the curves. It was the difference between the morning preinsulin blood glucose concentration and the nadir, that is, the glucose lowering effect of the insulin dose, that was significantly different on consecutive days in certain dogs.

Although the serial blood glucose curve appears to be a useful test for distinguishing levels of glycemic control in a group of diabetic dogs, it seems to be an unreliable clinical tool for evaluation of insulin dose in individual diabetic dogs. There was marked disparity between the theoretical recommendations for dose adjustment based on the curves obtained on consecutive days, particularly in dogs with lower minimum blood glucose concentrations, which represented those with better glycemic control. On the basis of the results of this study, it would be expected that attempts to adjust a diabetic dog's insulin dose on the basis of serial blood glucose assessment alone might prove unreliable. It would seem advisable to always consider additional indicators of glycemic control when appraising the insulin dose.

The 2 primary aims of insulin administration in diabetic dogs are resolution of clinical signs and avoidance of insulin-induced hypoglycemia.[9] This can be achieved by administration of the lowest dose of insulin that will allow a diabetic dog to be active and alert, maintain optimal body condition, and have no polyuria, polydipsia, or ketonuria. Reliance on history, physical examination, and change in body weight is

effective for assessing control of glycemia in diabetic dogs.[8] Measurement of fructosamine or glycosylated hemoglobin is an additional way of assessing glycemic control,[8,23-26,d,e] although there is little evidence that this is better than monitoring clinical signs. Once it has been determined that there is a need for adjustment of insulin dose, clinicians still usually perform a serial blood glucose curve to determine the direction of adjustment.

The principle reason for performing a serial blood glucose curve in a diabetic dog is to evaluate whether the insulin dose can be increased without risk of inducing hypoglycemia. Although assessment of clinical signs is valuable for identifying dogs with poor glycemic control, it is often not effective for identifying dogs at risk of clinical hypoglycemia.[27] Regular dipstick monitoring for urine glucose may help reduce the risk, because persistent negative results may indicate subclinical hypoglycemia.[9] The morning and evening preinsulin blood glucose concentrations and the blood glucose nadir are likely to give the best indication of the risk of hypoglycemia.

The preinsulin blood glucose values provide an indication of the blood glucose concentration when the dog is due for an insulin injection at home. This is important because hypoglycemia is more likely if the effects of the previous insulin injection overlap with the next insulin dose, producing an additive effect. If 1 or both of the preinsulin blood glucose concentrations are < 180 mg/dL (10 mmol/L), considerable residual effect of the previous insulin injection may be present after 12 hours and, if the current insulin dose is continued, the dog may be at risk for hypoglycemia. In this scenario, it would be prudent to recommend a reduced dose.

The nadir blood glucose concentration gives an indication of the maximal blood glucose response to the current dose of insulin and can occur at any point in the blood glucose curve, including just prior to the next dose of insulin. As there is large day-to-day variation of the time between insulin administration and the blood glucose nadir, it is important to always perform serial measurements to ensure that the nadir is not missed. Predicting the timing of a diabetic dog's nadir on the basis of a previous serial blood glucose curve and obtaining a single sample at that time is unlikely to give a reliable result. The guidelines used in this study to determine a theoretical recommendation for adjustment of the dog's insulin dose were based on published clinical recommendations for diabetic dogs.[9] A nadir < 90 mg/dL (5 mmol/L) signifies that there may be risk for hypoglycemia if the current insulin dose is continued. A nadir of 90 to 145 mg/dL (5 to 8 mmol/L) with an absence of clinical signs of either hyperglycemia or hypoglycemia indicates that the dog is likely to have optimal glycemic control. In this study, the insulin dose was theoretically increased if the nadir was > 145 mg/dL (8 mmol/L) and the preinsulin blood glucose concentrations were both > 180 mg/dL (10 mmol/L). However, to minimize the risk of inducing hypoglycemia, it is recommended that the insulin dose should be increased only if the dog's clinical signs are also consistent with chronic hyperglycemia. If instead the dog's clinical signs indicate good glycemic control, it would be advisable to be more conservative and keep the dose the same or use only a minimal increase of no more than 1 unit. Because insulin-induced hypoglycemia is potentially life threatening, it is prudent to always err on the side of caution when basing adjustments of insulin dose on serial blood glucose measurements in diabetic dogs.

For clinicians treating individual diabetic dogs, the most useful parameters of a serial blood glucose curve are the preinsulin and minimum blood glucose concentrations. This is because the principle reason for performing a serial blood glucose curve in a diabetic dog is to evaluate whether the insulin dose can be increased without risk of inducing hypoglycemia. It is advisable to always consider additional indicators of glycemic control, such as changes in the dog's water intake, body weight, and urine glucose concentrations, when appraising insulin dose. The large day-to-day variability of the curves and the serious sequelae that may result from insulin overdose justify the need for a conservative approach to dosage recommendation. The clinical implications of the large day-to-day variability of serial blood glucose curves particularly apply to dogs with good glycemic control.

[a]Accutrend, Roche Diagnostics, Basel, Switzerland.
[b]Cobas Mira analyzer, Roche Diagnostics, Basel, Switzerland.
[c]Bell MA, Labuc RH, Mills JN. Murdoch University, Perth, Australia: Personal communication, 2002.
[d]Graham PA, Nash AS. The critical evaluation of tests for monitoring glycemic control in canine diabetes mellitus (abstr). *J Vet Intern Med* 1997;11:123.
[e]Briggs C, Nelson R, Feldman E, et al. Serum fructosamine for monitoring glycemic control of diabetic dogs (abstr). *J Vet Intern Med* 1998;12:211.

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