Comparison of Glucose Fluctuations between Day- and Night-Time Measured Using a Continuous Glucose Monitoring System in Diabetic Dogs

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(Received 13 March 2012/Accepted 27 August 2012/Published online in J-STAGE 10 September 2012)

NOTE Internal Medicine

Abstract. Monitoring of blood glucose concentration is important to evaluate the diabetic status of dogs. Continuous glucose monitoring systems (CGMS) have been applied in veterinary medicine for glucose monitoring in diabetic dogs. The purpose of the study was to evaluate the daily glucose profiles obtained with CGMS and compare glucose fluctuations between day- and night-time in diabetic dogs. Five diabetic dogs were used in this study and were treated with either NPH insulin or insulin detemir. For data analyses, day-time was defined as 9:00 am–9:00 pm and night-time as 9:00 pm–9:00 am. Using glucose profiles, we determined the mean glucose concentrations (1- and 12-hr intervals), and times spent in hyperglycemia (>200 mg/dl) or hypoglycemia (<60 mg/dl). None of the parameters differed significantly between day-time and night-time in dogs treated with NPH insulin or insulin detemir. In conclusion, this study confirmed, using CGMS, that there are no differences in glucose fluctuations between day- and night-time, in diabetic dogs on a similar feeding regimen and insulin administration.

Keywords: continuous glucose monitoring system, diabetes mellitus, insulin.

Insulin-dependent diabetes is the predominant type of diabetes in dogs, and insulin injections are commonly used for long-term glycemic control [8, 11, 15]. Repeated sampling every 1–2 hr over a 12–24-hr period is necessary to assess the effectiveness of insulin and its duration of action, as well as to identify glucose troughs and peaks [5]. However, repeated venipuncture can be stressful and painful for the patient and, there is a risk that a significant blood glucose peak or nadir will fall between two sampling times and will not be recorded. Moreover, this frequency and mode of sampling are not always compatible with the patient’s daily activities, especially at night [13].

A continuous glucose monitoring system (CGMS), Medtronic MiniMed CGMS Gold (Medtronic Inc., Tokyo, Japan), was recently approved in Japan to evaluate daily glucose profiles in human patients with diabetes. The CGMS device can provide estimated blood glucose values every 5 min for 3 days [7, 9]. Interstitial glucose concentrations measured by CGMS are closely correlated with plasma glucose concentrations [12]. Furthermore, several CGMS devices have been validated in veterinary settings and are used to assist the treatment of diabetes [4, 22].

Nocturnal hypoglycemia often occurs in insulin-treated human patients and, although blood glucose levels are often low during sleep, they are seldom routinely measured.

Almost 50% of all episodes of severe hypoglycemia occur at night during sleep. To our knowledge, however, no studies have conducted glucose monitoring during the night in diabetic dogs. Moreover, the differences, if any, in glucose variations between day-time and night-time have not been reported in diabetic dogs. Therefore, the aim of this study was to determine glucose profiles during the day-time and night-time using CGMS in diabetic dogs. We also tested two insulin preparations that are commonly used in diabetic dogs, neutral protamine Hagedorn (NPH) insulin and insulin detemir [2, 8, 14, 16] to investigate their effects on glucose profiles. NPH insulin is an intermediate-acting preparation, whereas insulin detemir is a long-lasting preparation in canines [14, 16]. Moreover, NPH insulin has an approximate time of onset of 1 hr and a 12-hr duration of action period with a peak at 4–6 hr in normal dogs. Insulin detemir is a long-acting, human synthetic basal insulin analogue. Its duration of action is over 24 hr, with a peak at 8–10 hr in normal dogs.

Three dogs with streptozotocin-induced diabetes [14] and two dogs with juvenile-onset diabetes were used in this study (Table 1). All of the dogs had confirmed diabetes mellitus for over 3 years, with diabetes diagnosed based on clinical signs (polyuria and polydipsia), persistent fasting hyperglycemia (>250 mg/dl) and glycosuria. Before use in our study, the dogs were being treated with twice daily insulin detemir injections (8:10am and 8:10pm) to maintain serum glycated albumin at 13–17% [17, 18] while housed in our laboratory. This study was approved by the Nippon Veterinary and Life Science University Animal Research Committee. All dogs were fed a commercial diet (Select Protein, Royal canine Japan, Tokyo, Japan) twice daily (8:00am and...
8:00 pm). Calorie intake was set at $\frac{1}{2} \times (1.6 \text{ to } 2.0) \times$ resting energy requirement (body weight$^{0.75} \times 70$) at each meal. The dogs were housed in individual cages and provided with water ad libitum. The animal room was maintained at 24 ± 2°C and 55 ± 10% relative humidity, with a 12-hr light/dark cycle (lights on from 9:00 am to 9:00 pm). The living conditions remained constant throughout the study.

The CGMS (Medtronic MiniMed CGMS Gold) was placed as follows. First, hair was shaved from a 10-cm$^2$ patch of skin on the rear of the neck, and the area was cleaned with alcohol. The sensor consists of a glucose oxidase-based platinum electrode that is introduced into the subcutaneous space using a needle stylet provided with the CGMS. The stylet was removed after implanting the electrode. The sensor was adhered to the skin with adhesive tape. After a 5-min ‘wetting period’ to establish contact between the sensor and the interstitial fluid, the cable was connected to the sensor, and the monitor was activated. Enzyme-mediated oxidation of glucose in the interstitial fluid generates an electrical current that is carried by the cable to the monitor. After the wetting period, the sensor was immobilized with adhesive bandage. The sensor can be left in place for up to 3 days, and is connected to a pager-sized monitor that is worn by the dogs, similar to Holter ECG monitors (Fig. 1). CGMS measures blood glucose every 10 sec, and records mean values every 5 min. Therefore, 288 measurements are recorded daily to determine diurnal blood glucose variation. During CGMS monitoring, blood glucose levels were checked with an SMBG (self-monitoring of blood glucose) device (ACCU-CHEK Aviva, Roche Diagnostics K.K., Tokyo, Japan) at least four times each day. The glucometer used in this study was previously validated for veterinary use [21]. After monitoring for 3 days, the data were downloaded to a personal computer to analyze glucose profiles and glucose excursion parameters using MiniMed Solutions software. The analysis was limited to data obtained from the middle 48 hr of recording to avoid bias caused by inserting and removing the CGMS or inadequate stability of the monitoring system.

This study was carried out in two phases, with each phase lasting up to 7 days. In the first phase (Days 1–7), the dogs received one of two doses of NPH insulin (0.45 or 0.7 IU/kg) as a single injection twice daily at a 12-hr interval (at 9:00 am and 9:00 pm) (Table 1). It was previously reported that the normal recommended dose of NPH insulin for diabetic dogs ranges from 0.4–0.7 IU/kg twice daily [14]. CGMS was conducted at Days 5–7. In the second phase (Days 8–14), the dogs received one of two doses of insulin detemir (0.2 and 0.34 IU/kg) at the same times (Table 1). CGMS was conducted at Days 12–14. In both phases, the dogs continued the dietary regimen used before the study.

Data obtained were analyzed the entire recording period, during the night (9:00 pm–9:00 am) on days 5, 6, 12 and 13 and during the day (9:00 am–9:00 pm) on days 6, 7, 13 and 14, giving two sets of data for each of the five dogs corresponding to the first and second phases. Using these profiles, we determined the mean glucose concentration (1- and 12-hr intervals), times spent in hyperglycemia >200 mg/dl (time>200 mg/dl) and hypoglycemia <60 mg/dl (time<60 mg/dl), and minimum and maximum glucose concentrations.

Before proceeding, we needed to determine the optimal doses of NPH insulin and insulin detemir for each dog. Therefore, we conducted a pre-clinical study before the present study. The pre-clinical study lasted for 10 days in which the dogs were treated with both types of insulin for 5 days. Blood samples were obtained every 2 hr to determine the appropriate insulin doses. The dose of insulin preparation was determined to be ideal, if it met the following conditions: (1) did not induce clinical hypoglycemia, and (2) maintained blood glucose concentrations at 40–400 mg/dl, since the CGMS was capable of measuring this range of glucose concentrations. Therefore, the optimal dose of each insulin preparation is in accordance with the dose required for optimal glycemic control in individual dogs.

For statistical analysis, values are expressed as means ±
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SD. Statistical significance was determined by Mann-Whitney U test or two-way repeated measures ANOVA, when appropriate (GraphPad Prism analysis software, GraphPad Software Inc., San Diego, CA, U.S.A.). The significance level was set at \( P<0.05 \).

No problems were encountered in placing the sensor in any of the dogs. The CGMS device and monitoring were tolerated by the dogs, and the devices were worn for up to 3 days by each dog. None of the dogs showed any signs of inflammation or discomfort at the sensor site. Furthermore, we observed no abnormal behaviors, such as rolling, biting at, or rubbing the site of sensor placement, or chewing.

No significant changes in mean glucose concentrations (1-hr interval), at different time points, were observed between day-time and night-time using both NPH insulin and insulin detemir (Two-way repeated measures ANOVA) (Fig. 2). In terms of night-time glucose fluctuations using NPH insulin, the mean glucose concentration decreased from 160 to 136 mg/dl at 10–11 hr, increased to 221 mg/dl at 1–2 hr, decreased to 147 mg/dl at 6–7 hr, and increased to 167 mg/dl at 8–9 hr. In terms of day-time glucose fluctuations using NPH insulin, the mean glucose concentration increased from 175 to 214 mg/dl at 12–1 hr, decreased to 132 mg/dl at 5–6 hr and increased to 154 mg/dl by 8–9 hr. Regarding night-time glucose fluctuations with insulin detemir, the mean glucose concentration increased from 175 to 214 mg/dl at 12–1 hr, gradually decreased to 132 mg/dl at 5–6 hr, and increased to 154 mg/dl by 8–9 hr. For day-time glucose fluctuations with insulin detemir, the mean glucose concentration increased from 233 to 259 mg/dl at 12–1 hr, gradually decreased to 170 mg/dl at 4–5 hr and increased to 186 mg/dl by 8–9 hr. There were no significant differences in mean glucose concentrations (12-hr interval), time>200 mg/dl, time<60 mg/dl, or the minimum and maximum glucose concentrations between day-time and night-time using both NPH insulin and insulin detemir (Mann-Whitney U test) (Table 2). When using NPH insulin, the mean glucose concentrations during the day and night were 168 ± 41 and 176 ± 34 mg/dl, respectively. Overall, 33 ± 24 and 37 ± 22% of the recording time in the day and night, respectively, were spent in hyperglycemia, compared with 6.2 ± 9.9 and 3.5 ± 4.9% in hypoglycemia. The maximum glucose concentrations were 285 ± 57 and 274 ± 62 mg/dl, and the minimum glucose concentrations were 78 ± 43 and 69 ± 35 mg/dl, respectively. When using insulin detemir, the mean glucose concentrations during the day and night were 210 ± 61 and 199 ± 51 mg/dl, respectively. Overall, 57 ± 31 and 52 ± 34% of the recording time in the day and night, respectively, were spent in hyperglycemia, while 4.6 ± 9.0 and 2.8 ± 6.1% of the recording time were spent in hypoglycemia. The maximum glucose concentrations were 328 ± 68 and 312 ± 52 mg/dl, and the minimum glucose concentrations were 102 ± 69 and 115 ± 67 mg/dl, respectively.

To our knowledge, no veterinary studies have focused on nocturnal hypoglycemia, because the monitoring and detection of night-time hypoglycemia are difficult. The use of

![Fig. 2. Comparison of mean ± SD glucose concentrations (1-hr interval) measured by CGMS between day-time and night-time after injection of NPH insulin (A) and insulin detemir (B).](image)

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<th>Table 2. Comparison of glycemic parameters recorded during the day- and night-time with each insulin preparation</th>
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Data were over 2 days after treatment with each insulin preparation in each dog \( (n=5) \).
CGMS can overcome this limitation, and can be used to determine blood glucose profile throughout the day, including overnight without waking the dogs. Therefore, we evaluated daily glucose fluctuations in dogs using CGMS. Interestingly, we found no differences in glucose profiles between the day-time and night-time with NPH insulin and insulin detemir in this study. Similarly, no statistically significant differences in the duration of hypoglycemia or area under the hypoglycemia curves were found between the day and night in human patients with type 1 diabetes [20]. Nevertheless, nocturnal hypoglycemia is a major concern for patients with type 1 diabetes. Amin et al. [1] reported that hypoglycemia was more common at night compared with during the day. In the Diabetes Control and Complications Trial, more than half of all episodes of severe hypoglycemia occurred during the night [6]. In human patients with diabetes, the risk of nocturnal hypoglycemia is associated with various factors, including age, insulin dose, insulin regimen and body weight [1]. Furthermore, the blood levels of injected insulin may be affected by exercise, site of injection, temperature, and day-to-day intra-individual variation in the rate of insulin absorption, which may vary by up to 50% [3]. In the present study, the diabetic dogs were maintained under controlled living conditions (cage rest), including room temperature, humidity and light/dark cycle, precluding differences in environmental factors. Circadian hormone secretory patterns might also affect glucose fluctuations in diabetic dogs. However, cortisol and growth hormone, which strongly influence glucose fluctuations, did not show significant circadian secretory fluctuations [10, 19]. As such, environmental factors and circadian hormone secretion weakly, if at all, affected daily glucose fluctuations in the present study. Furthermore, all of the dogs were injected with the same doses of insulin and were fed the same amount of food twice daily at a 12-hr interval (9:00 am and 9:00 pm). Therefore, daily glucose fluctuations might be lower in dogs than in humans who regularly eat three meals each day and inject insulin three or four times each day.

The insulin preparations NPH insulin and insulin detemir, were used to compare glucose fluctuations between day-time and night-time. However, we cannot compare these two insulin preparations, because their doses (i.e., number of insulin units) were not equivalent. The mean glucose concentrations after administration of NPH insulin were maintained at 130–220 mg/dl in the day-time and night-time, and no peaks or troughs were apparent. The mean glucose concentrations after administration of insulin detemir gradually increased at 12–1 hr and then gradually decreased at 4–6 hr without apparent peaks or troughs. Neither insulin preparation resulted in apparent peaks or troughs, and the mean glucose concentration was maintained at 100–300 mg/dl, which represents good glycemic control [16]. Therefore, the doses of both insulin preparations adjusted in the pre-clinical study were considered to be appropriate.

Interstitial glucose concentrations measured by CGMS are closely correlated with whole-blood glucose concentrations, and are sensitive to abrupt changes in blood glucose concentrations in human subjects. In clinical medicine, CGMS is used to generate the maximum amount of information regarding the direction, extent, durability and frequency of glucose fluctuations throughout the day. Subcutaneous insertion of the sensor and wearing of the device were well tolerated by all of the dogs. The system is able to detect rapid changes in glucose concentrations and provides accurate measurements compared with measurement of venous blood glucose concentrations, with little or no discomfort to the dogs during sensor insertion or observation. In this study, the CGMS, worn similar to a Holter ECG monitor, was able to monitor the glucose concentration without apparent difficulties for 3 days. Furthermore, the increased frequency and simplicity of data collection made possible using CGMS have marked benefits for veterinary medicine.

A CGMS allows the clinician to identify hypoglycemic and hyperglycemic events. Hypoglycemia is the most serious condition in insulin-treated diabetic humans and dogs, and should always be avoided [6]. Hypoglycemia associated with blood glucose <60 mg/dl was observed after treatment with both NPH insulin and insulin detemir. Notably, we did not observe any episodes of clinical hypoglycemia with blood glucose concentrations <35 mg/dl. This may represent a limitation of our study, as the Somogyi phenomenon can occur when blood glucose drops to <60 mg/dl. In this scenario, the body tries to correct the hypoglycemia by releasing counterregulatory hormones, such as epinephrine and glucagon, and the blood glucose concentration quickly increases, which might affect the daily glucose fluctuations. Another limitation of our study is the low statistical power because of the small number of diabetic dogs used in this preliminary study. Consequently, because of the large inter-individual variability and the small sample size, it is not possible to fully assess the true differences in glucose fluctuations between day-time and night-time. Also, the experimental period of each phase (1 week) might be short, since diabetic dogs usually require several weeks to achieve stable glycemic control. In the future, we need further studies, extend the experimental period and evaluate glycemic control marker to understand detailed effects of insulin preparations.

In conclusion, we found no significant differences in glucose fluctuation between day-time and night-time in a group of diabetic dogs. However, we conducted the study using dogs at our animal facility, where the environmental conditions (i.e., room temperature, humidity and light/dark cycle), diet composition, and frequency and timing of meals and insulin injection were kept constant throughout the study. Therefore, it is important to repeat this study in diabetic dogs housed at home rather than in a laboratory setting.

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Pract. 36: 1087–1105. [Medline] [CrossRef]