

# Evaluation of long-term glucose homeostasis in lean and obese cats by use of continuous glucose monitoring

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**Objective**—To evaluate intraday and interday variations in glucose concentrations in cats and to test the utility of a continuous glucose monitoring system (CGMS).

**Animals**—6 lean and 8 long-term (> 5 years) obese cats.

**Procedures**—Blood glucose concentrations were measured during the course of 156 hours by use of a laboratory hexokinase-based reference method and a handheld glucometer. Interstitial glucose concentrations were evaluated with a CGMS.

**Results**—Paired measures of glucose concentrations obtained with the CGMS typically were marginally higher than concentrations for the reference method and less biased than concentrations obtained with the glucometer. This was partially confirmed by the concordance correlation coefficients of the concentration for the CGMS or glucometer versus the concentration for the reference method, although the correlation coefficients were not significantly different. Mean  $\pm$  SD area under the curve for the glucose concentration (AUCG) did not differ significantly between lean ( $14.0 \pm 0.5$  g/dL•h) and obese ( $15.2 \pm 0.5$  g/dL•h) cats during the 156-hour period, but one of the obese cats had a much higher AUCG. Within-day glucose variability was small in both lean and obese cats.

**Conclusions and Clinical Relevance**—Glucose homeostasis was maintained, even in long-term obese cats, and intraday glucose fluctuations were small. One obese cat might have been classified as prediabetic on the basis of the AUCG, which was approximately 25% higher than that of the other obese and lean cats. The CGMS can be useful in the evaluation of long-term effects of drugs or diet on glucose homeostasis in cats. (*Am J Vet Res* 2012;73:1100–1106)

Obese cats are at higher risk of developing diabetes mellitus. Even severely obese cats have fasting blood glucose concentrations within the reference range, but they have evidence of glucose intolerance when challenged with glucose during an IV glucose tolerance test.<sup>1–5</sup> However, it is not known whether glucose concentrations in obese cats differ from those in lean cats throughout the course of a day during their regular activities, including consuming food.

Because many cats are easily stressed and may have stress hyperglycemia when samples are obtained for mea-

ABBREVIATIONS	
AUCG	Area under the curve for the glucose concentration
CGMS	Continuous glucose monitoring system
CI	Confidence interval

surement of blood glucose concentrations, a noninvasive method for monitoring glucose concentrations would be optimal. Continuous glucose monitoring systems are used frequently in humans with diabetes and provide data about patterns of glucose concentrations. They also have been used in prediabetic children and adults who are at risk of developing diabetes.<sup>6,7</sup> In cats, a CGMS has been used to monitor diabetic client-owned animals.<sup>8–10</sup>

Several CGMSs are commercially available. A newer model consists of a disposable glucose sensor containing a small flexible electrode that measures the current produced by glucose in the interstitial fluid. It is placed into the interstitial space and is directly attached to a transmitter placed on the skin. The transmitter sends the data wirelessly to a monitor. Data are recorded every 5 minutes and can be downloaded to a computer for analysis.

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The purpose of the study reported here was to evaluate and compare glucose concentrations between lean and obese cats during a 156-hour period during their regular activities. We also compared blood glucose concentrations obtained via the CGMS with those obtained via a handheld glucometer and a laboratory hexokinase-based reference method.

## Materials and Methods

**Animals**—Eight (4 female and 4 male) obese adult purpose-bred neutered cats (mean  $\pm$  SD body weight,  $7.3 \pm 0.9$  kg; range, 5.9 to 8.5 kg) and 6 (3 female and 3 male) lean adult purpose-bred neutered cats (mean  $\pm$  SD body weight,  $3.3 \pm 0.2$  kg; range, 2.9 to 3.7 kg) that were part of a research colony were used in the present study. Mean  $\pm$  SD age of the lean cats was  $10.2 \pm 1.9$  years, and that of the obese cats was  $10.5 \pm 1.2$  years. The obese cats had all been obese for  $> 5$  years. Obesity was induced by allowing the cats ad libitum access to food, whereas food intake of the lean cats was restricted.

Cats were maintained at the University of Illinois College of Veterinary Medicine Animal Care Facility under standard colony conditions, which included a cycle of 12 hours of light and 12 hours of darkness. Room temperature was maintained at  $21^\circ\text{C}$ . Cats were housed separately in cages and were allowed free access to water. Cats were fed a commercial diet<sup>a</sup> once daily and had been fed this diet for several months before the start of the present study. All lean cats ate their food within a 15-minute period, whereas obese cats ate their food throughout the day. Food intake of each cat was measured once daily; cats were weighed once weekly, and food intake was adjusted to maintain the current body weight.

Cats were assessed as healthy, apart from the obesity, on the basis of results of physical examination and clinical laboratory data. None of the cats were excluded because of abnormal clinical laboratory results. All cats were provided social interactions daily and were accustomed to daily handling. The study reported here was approved by the University of Illinois Institutional Animal Care and Use Committee and conducted in accordance with established guidelines.<sup>11,12</sup>

**Procedures**—A catheter<sup>b</sup> was placed into a jugular vein of each cat 15 to 18 hours before the beginning of the testing period for use in collection of blood samples. Catheter patency was maintained by injection of 0.5 mL of 0.38% sterile citrate flush solution<sup>c</sup> (citric acid and trisodium salt dehydrate) every 8 to 14 hours. All blood samples were collected through the jugular catheter into tubes containing EDTA. The blood was placed on ice immediately and centrifuged at  $405 \times g$  for 5 minutes. Plasma was harvested and stored at  $-80^\circ\text{C}$  until assayed.

The sensor of a CGMS<sup>d</sup> was inserted into the interstitium of each cat at a location caudal to the scapulae. The skin in the area was shaved and then wiped several times with swabs containing 70% alcohol. The sensor was inserted through the skin; it was secured to the skin with a cover provided by the manufacturer and

elastic tape.<sup>e</sup> Day of sensor insertion was designated as day 1.

Blood samples (0.2 mL/sample) for the calibration of the CGMS were collected at least twice daily from days 1 to 7; samples were collected approximately 12 hours apart. Glucose concentrations were measured beginning 2 hours after the CGMS was inserted and calibrated (time 0 [first baseline]) and at 1 (second baseline), 6, 12, 21, 24, 30, 36, 45, 48, 54, 60, 69, 72, 78, 84, 93, 96, 102, 108, 117, 120, 126, 132, 141, 144, 150, and 156 hours; concentrations were measured with a glucometer<sup>f</sup> and a colorimetric glucose oxidase method<sup>g</sup> that served as the reference method. Interstitial glucose concentrations were monitored with the CGMS. After 156 hours, the CGMS was removed. When a sensor became nonfunctional, it was removed and a new sensor was placed. Data were recorded by means of a wireless system. The cats were fed at 3 hours after insertion of the CGMS sensor and every 24 hours thereafter.

**Analysis of data**—The data comprised repeated measures for each cat. Each set of data (cat and method) was fitted via least squares linear regression to identify and remove outliers. An ANCOVA (time, method, and the time by method interaction) was performed by means of mixed linear regression, with cat and intercept as random variables.<sup>h</sup> The AUCG, which was defined mathematically as the integral under the continuous concentration curve, was estimated as the sum of all the trapezoids and triangles bounded by the time-versus-concentration curve. Values of  $P < 0.05$  were considered significant.

Agreement among methods for measurement of glucose concentrations was evaluated visually with Bland-Altman plots<sup>13,i</sup> and numerically with the Lin concordance correlation coefficient.<sup>14,15</sup> Bland-Altman plots consist of scattergrams of the differences of paired measures plotted against the mean of the 2 measures or the value for a criterion-referenced standard. Variability is detected with respect to the degree of systematic (bias) and random (heteroschedastic) deviation between the 2 methods. The Lin concordance correlation coefficient is an index of agreement between paired measures that accounts, within a context of best-line fitting, for precision (ie, related to random deviation assessed via the Pearson correlation coefficient) and accuracy (ie, systematic deviation of the best-fit line from the identity line). Bland-Altman plots and related statistical calculations, including the Lin concordance correlation coefficients, were generated with statistical software.<sup>16,i</sup>

Daily between- and within-cat fluctuations in glucose concentrations were assessed via linear mixed-effects modeling by use of a statistical package available in the statistical software.<sup>17,j</sup> The analysis accounted for the natural hierarchic grouping of the data determined by repeated measurements available for the 2 groups of cats (lean and obese), for each cat, for days 1 to 7, and for 0, 6, 12, and 21 hours of each day relative to the first baseline sample obtained at time 0. For simplicity, the second baseline sample at 1 hour was considered a replicate of the first sample. For each measurement method, all available glucose concentrations measured in the 28 samples collected from each cat were used for

comparison between lean and obese cats, among time points during the day, and among days. Selection of the best model (ie, the best choice of regression covariates and random terms at different grouping hierarchies) was based on the Akaike information criterion and the likelihood ratio test.

## Results

The least squares mean  $\pm$  SD glucose concentration for all cats during the 156-hour period measured with the reference method was  $94 \pm 2$  mg/dL, which was similar to that measured with the CGMS ( $96 \pm 2$  mg/dL) but significantly ( $P < 0.001$ ) lower than that measured with the glucometer ( $101 \pm 2$  mg/dL). All pairs of glucose concentrations measured at the same time point with the criterion-referenced standard method and the CGMS or glucometer were compared via Bland-Altman plots (Figures 1 and 2) and the Lin concordance correlation coefficient. There was no bias in the

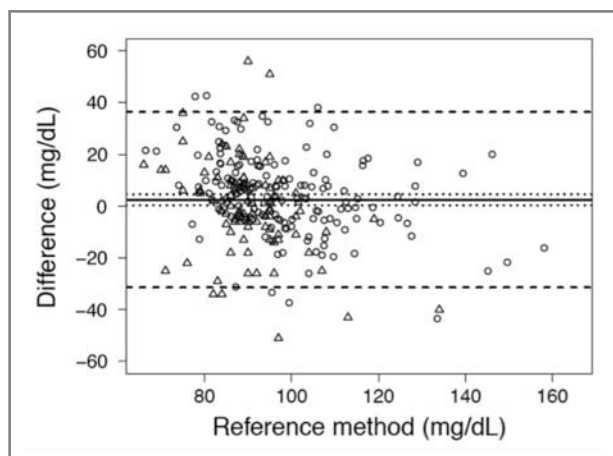


Figure 1—Bland-Altman plot of paired glucose concentrations during a 156-hour period in 6 lean (triangles) and 8 obese (circles) cats measured with the CGMS and the hexokinase-based reference method. Calculations were based on all pooled data points for obese and lean cats. The mean difference (solid line) and 95% CI of the mean difference (dotted lines) between methods and 95% limits of agreement (dashed lines) are indicated.

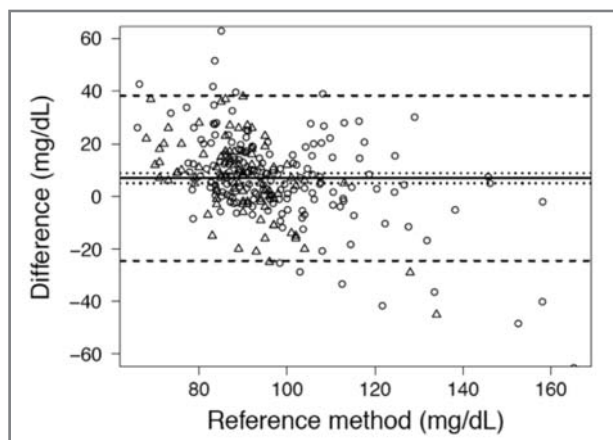


Figure 2—Bland-Altman plot of paired glucose concentrations during a 156-hour period in 6 lean (triangles) and 8 obese (circles) cats measured with a glucometer and the hexokinase-based reference method. Calculations were based on all pooled data points for obese and lean cats. See Figure 1 for remainder of key.

pattern of glucose concentrations associated with body condition between lean and obese cats. The CGMS-derived glucose concentrations typically were slightly but significantly higher than those measured with the reference method (mean difference, 2.29 mg/dL; 95% CI, 0.36 to 4.63mg/dL), as determined by use of *t* tests. Approximately 95% of the CGMS-based glucose con-

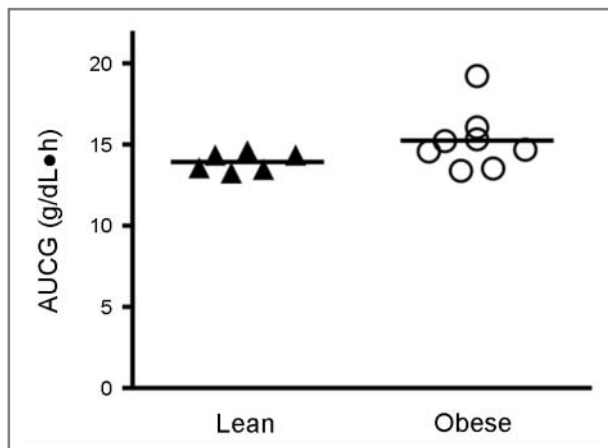


Figure 3—The AUCG determined by use of glucose concentrations measured with the reference method in 6 lean and 8 obese cats during a 156-hour period. Each symbol represents the result for 1 cat; the mean for each group is indicated (solid line). The SE for both the lean and obese cats was  $\pm 0.5$  g/dL·h.

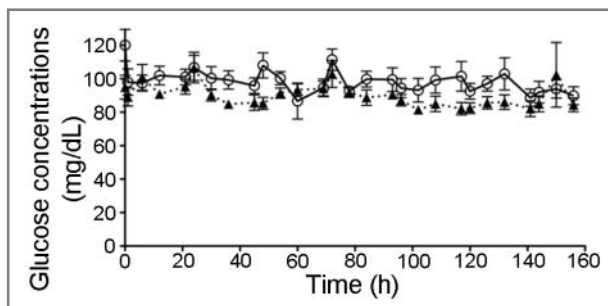


Figure 4—Mean  $\pm$  SD glucose concentrations determined with the reference method for 6 lean (triangles) and 7 obese (circles) cats during a 156-hour period (results for 1 obese cat were excluded because it was a statistical outlier). Glucose concentrations were measured beginning 2 hours after a CGMS was inserted and calibrated (time 0). The cats were fed every 24 hours beginning at 3 hours.

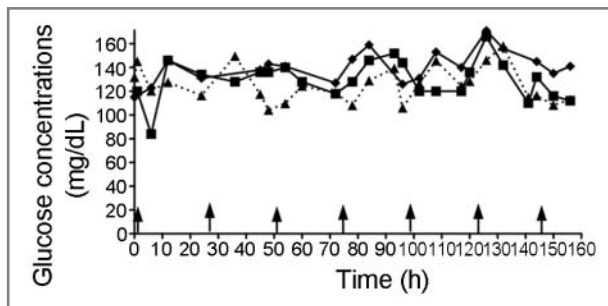


Figure 5—Glucose concentrations determined with the reference method (triangles), glucometer (diamonds), and CGMS (squares) in 1 obese cat during a 156-hour period; this cat had the highest AUCG and was considered a statistical outlier. The cats were fed every 24 hours (arrows) beginning at 3 hours. See Figure 4 for remainder of key.

centrations were between 31.4 mg/dL lower and 36.4 mg/dL higher than the corresponding concentrations for the reference method (the 95% agreement interval of the CGMS and reference method was defined as the mean difference  $\pm$  1.96 times the SD of the difference). No particular pattern of differences associated with measured concentrations or body condition (lean or obese) of the cats could be identified.

In contrast, glucose concentrations measured with the glucometer typically were 6.9 mg/dL higher than

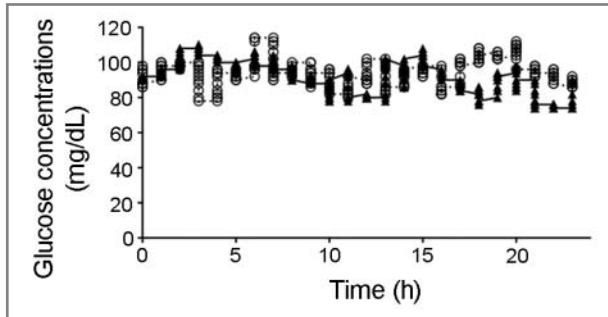


Figure 6—Glucose concentrations recorded at 5-minute intervals with a CGMS in 1 representative lean cat (triangles) and 1 representative obese cat (circles) during a 24-hour period. Time 0 represents midnight.

the corresponding concentrations for the reference method; 95% of the differences were between 38.4 mg/dL higher and 24.5 mg/dL lower than the corresponding concentrations for the reference method. Moreover, the glucometer apparently overestimated low glucose concentrations and underestimated high glucose concentrations, compared with concentrations determined via the reference method.

The discrepancies between concentrations measured with the CGMS and glucometer as depicted in the Bland-Altman plots were partially supported by the Lin concordance correlation coefficient between the reference method and the 2 other methods; the correlation coefficient was slightly higher for the CGMS ( $\rho_c$ , 0.469; 95% CI, 0.372 to 0.556) than for the glucometer ( $\rho_c$ , 0.441; 95% CI, 0.348 to 0.525). The concordance correlation coefficient between the CGMS and glucometer was even slightly higher ( $\rho_c$ , 0.495; 95% CI, 0.390 to 0.587).

The mean  $\pm$  SD AUCG measured with the reference method during the 156-hour period was 14.0  $\pm$  0.5 g/dL•h in the 6 lean cats and 15.2  $\pm$  0.5 g/dL•h in the 8 obese cats (Figure 3). These values did not differ significantly. One obese cat had an AUCG of 19.2 g/dL•h and was considered a statistical outlier. Graphs were created of the glucose concentrations measured

Table 1—Estimates of fixed-effects variables and the SDs of random effects of glucose concentrations obtained in 6 lean and 8 obese cats during a 156-hour period with a hexokinase-based reference method, glucometer, and CGMS.

Method	Variable	Value	95% CI	P value*
Reference method	Intercept	101.368	95.35, 107.39	—
	6 h	-3.897	-7.12, -0.68	0.019
	12 h	-3.109	-6.34, 0.12	0.061
	21 h	-5.346	-8.80, -1.89	0.003
	Lean	-9.451	-19.13, 0.22	0.056
	SD (cat)	7.614	4.94, 11.73	—
	SD (cat/d)	5.628	3.98, 7.96	—
	SD (residuals)	11.667	10.73, 12.68	—
	Glucometer	Intercept	103.032	95.34, 110.72
6 h		1.924	-1.77, 5.61	0.309
12 h		3.651	-0.15, 7.46	0.062
21 h		5.143	1.25, 9.03	0.011
Lean		-8.910	-21.14, 3.30	0.142
SD (cat)		10.033	6.76, 14.88	—
SD (cat/d)		2.697	0.94, 7.77	—
SD (residuals)		10.566	9.56, 11.68	—
CGMS		Intercept	103.642	95.52, 111.77
	6 h	-2.612	-6.54, 1.32	0.195
	12 h	-2.891	-6.91, 1.13	0.162
	21 h	-5.591	-9.89, -1.29	0.012
	Lean	-12.314	-25.48, 0.85	0.067
	SD (cat)	10.428	6.86, 15.84	—
	SD (cat/d)	6.755	4.76, 9.59	—
	SD (residuals)	11.485	10.33, 12.76	—

Glucose concentrations were measured beginning 2 hours after a CGMS was inserted and calibrated (time 0). The intercept represents the expected population mean glucose concentration at hour 0 in obese cats. The coefficients for 6, 12, and 21 hours represent the expected deviation from baseline glucose concentrations during a day at each of those time points. Lean represents the expected variation of baseline glucose concentration in lean cats. Although this coefficient did not differ significantly from zero according to the 95% CI, it was included in the model on the basis of the Akaike criterion of parsimonious increase in the number of model parameters to improve the model fit. The SD for cat and SD for cat per day quantify the SD for between-cat and SD for between-days-within-cat of the intercept, respectively. The SD for residuals represented the SD of the final model prediction error, which accounted for individual random effects.

\*Values were considered significant at  $P < 0.05$ .

— = Not applicable.

with the reference method for the 6 lean and 7 obese cats (excluding the aforementioned outlier with the high AUCG; **Figure 4**) and for the obese cat that was a statistical outlier (**Figure 5**). Glucose concentrations measured with the CGMS during a 24-hour period in a representative lean and representative obese cat were also plotted (**Figure 6**).

The sensors of the CGMS had to be replaced more frequently in lean cats than in obese cats because of failure of the sensor to remain in the interstitial tissue, biofouling (ie, the adhesion of proteins or other biological matter on the sensor surface or sometimes impregnation of the sensor that renders it nonfunctional),<sup>18</sup> or both. Therefore, data for all lean cats obtained with the CGMS were available only for the first 102 hours (**Figure 2**). After that time, it became too difficult and cost prohibitive to continue replacing sensors in some of the lean cats that were extremely active.

The model that best represented daily fluctuations in glucose concentrations for all 3 measurement methods described glucose concentrations in terms of variations from the daily baseline at hour 0 in obese cats (intercept), with constant regression coefficients (fixed effects) associated with the categorical variables hour of day and body condition (lean vs obese) and with random effects associated with the categorical variables cat and day. The estimates of fixed-effects variables and the SDs of random effects were obtained for the 3 methods of measuring glucose concentrations (**Table 1**). The intercept represented the expected population mean glucose concentration at hour 0 in obese cats.

The coefficients for 6, 12, and 21 hours were the expected deviation from baseline glucose concentrations during a day at each of those time points, and greater agreement was seen between the CGMS and reference method than between the glucometer and reference method. Glucose concentrations obtained via the reference method and the CGMS were significantly lower at 21 hours but were higher than concentrations obtained with the glucometer at that same time.

Lean was the coefficient that represented the expected variation of basal glucose in lean cats, compared with that of obese cats. Although this coefficient was not significantly different from zero according to the usual 95% CI, it was included in the model on the basis of the Akaike criterion of parsimonious increase in the number of model parameters to improve the model fit.

The SD for cat and SD for cat per day quantify the SD for between-cat and SD for between-days-within-cat of the intercept, respectively. They were both larger than the within-day SD. The SD for residuals represented the SD of the final model prediction error, taking into account individual random effects.

A power calculation was performed. Estimates reported for the reference method of glucose measurement, based on the assumption of a mean difference in baseline glucose concentration of 9.45 mg/dL between lean and obese cats, SD of baseline glucose of 7.61 mg/dL between cats and 5.6 mg/dL between days within a cat, and a negligible glucose measurement error, and accounting for the fact that baseline measurements were repeated 7 times in each cat, yielded a 2-sample *t* test power calculation with a 1-sided alternative (mean number of cats in the 2 groups, 7) of 0.8413.

## Discussion

In obese humans, an increase in fasting and postprandial blood glucose concentrations are early indicators of a deterioration of blood glucose control, and strict recommendations have been set for the criteria to identify and treat people at risk of becoming diabetic. Postprandial glucose concentrations depend on the quantity of glucose ingested with the meal and hepatic glucose production, which are modulated by factors such as rate of gastric emptying, absorption from the intestinal tract, and glucose transport to the liver and peripheral organs. In humans, postprandial hyperglycemia appears early after a meal in those patients who eventually develop diabetes mellitus.<sup>19</sup> Therefore, regulation of prandial glucose concentrations is an important goal for obese human patients.

In the study reported here, obese cats, even after having been severely obese for several years, were still able to maintain glucose homeostasis under normal feeding conditions and had blood glucose concentrations that were not different from those in lean cats when monitored during a 7-day period. In obese cats, fasting blood glucose concentrations have been reported to remain within the reference range despite peripheral insulin resistance.<sup>1-5</sup> In another study<sup>20</sup> conducted by our research group, we recently reported that this observation may be attributable to suppression of hepatic glucose production. However, we did not determine in that study<sup>20</sup> the amount of variation in plasma glucose concentrations in obese and lean cats during the course of several days nor whether hyperglycemia could be detected after the intake of a meal. Recently, however, we found that long-term obese cats also regulated postprandial hepatic glucose production.<sup>21</sup>

In the present study, the CGMS performed better than did the glucometer. On the basis of the linear mixed effects analysis, the variable estimates determined from the glucose concentrations (measured with the CGMS) were consistently closer to those determined via the reference method, compared with estimates obtained from concentrations measured with the glucometer, although the 95% agreement limits were broad. In particular, glucose concentrations at 21 hours predicted a substantial reduction from baseline concentrations for estimates based on the glucose concentrations determined with the reference method and CGMS, but predicted a substantial increase for the estimates based on concentrations determined with the glucometer. In contrast, the predicted reduction from the baseline concentration in lean cats was consistent among the measurement methods.

The within-day variations of glucose concentrations were small, compared with between- and within-cat variability and with prediction residuals that represented measurement error and physiologic fluctuations. Therefore, it can be concluded that although the predicted intraday variations in glucose concentrations were significant, their estimates were small when compared with interday variations in glucose concentrations and with measurement errors and may not be noticed when monitoring glucose concentrations in individual cats by use of a sparse number of blood samples.

Analysis of the data suggested that the CGMS method would be clinically more useful than would a glucometer for measurement of glucose concentrations, especially when considering blood samples were needed only twice daily for calibration. There was a lack of substantial bias between the CGMS and reference method, and glucose measurements were recorded every 5 minutes; therefore, a large number of data points were available for analysis. Because a single baseline glucose concentration is not a good indicator of glucose homeostasis in cats, the CGMS appeared to be a suitable method to provide information about daily glucose control, as has been reported in diabetic cats.<sup>8-10</sup>

Analysis of the data suggested that the CGMS may also be useful when examining effects of diets or drugs on glucose control during several days and might be preferable to multiple venipunctures. However, the CGMS was a work-intensive method because the wireless signal transmitted to the monitor needed to be closely observed. A weak signal required immediate attention and may have been attributable to the fact a cat had moved too far away from the monitor, the sensor was no longer placed correctly in the subcutaneous tissue, or there was biofouling of the sensor.<sup>18</sup> Biofouling and failure of the sensor to remain in place were frequent problems in lean cats. We postulate that this may have been caused by the much higher amount of physical activity of the lean cats, compared with that of the obese cats, which resulted in shifting of the sensor or accumulation of organic material as a result of minor tissue damage. Anecdotally, lean humans also have a greater rate of sensor failure than do obese humans.

One of the obese female cats had a much higher AUCG than did the other obese cats. Clearly, this cat should be monitored carefully, and a strict weight loss regimen should be implemented to prevent development of overt diabetes. In another study<sup>4</sup> conducted by our research group, we found that weight loss normalizes insulin sensitivity and other metabolic abnormalities associated with obesity in cats. The increased blood glucose concentration in the obese cat of the present study may have been a result of failure of the liver to maintain hepatic insulin sensitivity or a failure of beta cells to maintain the increased insulin secretion seen in nondiabetic obese cats.<sup>22</sup>

It could be argued that a lack of differences in glucose concentrations between lean and obese cats was not evident because of differences in food intake (ie, lean cats ate their food during a 15-minute period, whereas obese cats ate their food throughout the day) and that a difference might have been detected if the obese cats had eaten their daily ration within the same time period as the lean cats. However, we wanted to evaluate whether obese cats were hyperglycemic during their daily routine, which typically does not involve rapid ingestion of a large amount of food. Despite the small number of cats included in the present study, results suggested that there were no differences between obese and lean cats with regard to the within-day variations at 6, 12, and 21 hours from baseline concentrations. Results also suggested that there may have been differences between obese and lean cats with regard to the baseline glucose concentration; however, we did

not detect significant differences in baseline glucose concentrations, probably because of the small number of cats in the present study and the low power of the study.

For the study reported here, we did not detect differences in glucose concentrations between lean and obese cats during a 156-hour period of routine daily activity. Similarly, we did not detect differences in daily variations in glucose concentrations between lean and obese cats. The CGMS appears to be a valuable tool for use in monitoring glucose concentration of cats during several days and can be useful in the study of long-term glucose homeostasis.

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- a. Pro Plan Adult Dry Cat Food, Nestle Purina Co, St Louis, Mo.
  - b. BD Intracath, Becton-Dickinson, Sandy, Utah.
  - c. Sigma Co, St Louis, Mo.
  - d. Guardian system, Medtronic Minimed, Northridge, Calif.
  - e. Elastikon, Johnson & Johnson, Skillman, NJ.
  - f. Wavesense Presto, AgaMatrix Inc, Salem, NH.
  - g. Genzyme Diagnostics PEI Inc, Charlottetown, PE, Canada.
  - h. JMP 8, SAS Institute Inc, Cary, NC.
  - i. Software R, R Development Core Team, Vienna, Austria.
  - j. nlme, R package, version 3.1, R Development Core Team, Vienna, Austria.
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