Received: 10 July 2020 | Accepted: 15 January 2021

DOI: 10.1111/jvim.16046

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Assessment of postprandial hyperglycemia and circadian fluctuation of glucose concentrations in diabetic dogs using a flash glucose monitoring system

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Funding information
Gift from Ms. Catharine Adler

Abstract

Background: Postprandial hyperglycemia (PPH) and circadian glucose concentration fluctuations recorded in the home environment of dogs with naturally occurring diabetes mellitus (DM) have not been reported.

Objectives: To determine if a flash glucose monitoring system (FGMS; FreeStyle Libre) can detect PPH and circadian fluctuations in glucose concentrations in dogs with variably controlled DM.

Animals: Fourteen client-owned dogs with DM.

Methods: Prospective observational study. Interstitial glucose (IG) concentrations measured by the FGMS during a 13-day study period were analyzed.

Results: A total of 17, 446 FGMS IG concentrations were analyzed. For all dogs analyzed together, median IG concentration measured within 30 (288 mg/dL), 60 (286 mg/dL), 90 (285 mg/dL), and 120 (285 mg/dL) minutes of meals was each significantly higher than the median IG concentration at all other times (260 mg/dL, 259 mg/dL, 258 mg/dL, and 257 mg/dL, respectively; range, 40-500 mg/dL; $P < .001$ for each). Median night-time IG concentration measured from all dogs on 3,547 samples recorded between 1:00 am and 6:00 am (268 mg/dL; range, 40-500 mg/dL) was significantly higher than median IG measured on 13, 899 samples at all other time points (259 mg/dL; range, 40-500 mg/dL; $P < .001$).

Conclusions and Clinical Importance: The FGMS can be used for future studies of PPH and circadian fluctuations of glucose concentrations in dogs with DM in their home environment.

KEYWORDS
canine, daytime, diabetes mellitus, FreeStyle Libre, interstitial glucose, night-time

1 | INTRODUCTION

The management of diabetes mellitus (DM) in dogs is rapidly changing as continuous glucose monitoring devices become more accessible.[1-3] The use of these devices in humans with DM has markedly enhanced

Abbreviations: ABA, automated biochemistry analyzer; DM, diabetes mellitus; FGMS, flash glucose monitoring system; IG, interstitial glucose; NPH, neutral protamine Hagedorn insulin; POCG, point-of-care blood glucometer; PPH, postprandial hyperglycemia; TP, total protein.

the ability to evaluate postprandial hyperglycemia (PPH) and circadian fluctuations in glucose concentrations.[4-6] In humans with DM, PPH is associated with an increased risk of cardiovascular complications, retinopathy, and glomerulopathy as well as decreased overall glycemic control and quality of life.[7] Additionally, nocturnal hypoglycemia and early morning hyperglycemia occur in diabetic humans at a prevalence ranging from 12.6% to 68% and 3% to 55%, respectively.[8-11] Documentation of PPH and changes in circadian glucose concentrations in humans by the use of continuous glucose monitoring devices allows for interventions and preventative strategies that improve diabetic control as well as patient confidence and compliance.[6,12-14]

Postprandial hyperglycemia also has been documented in dogs.[15-18] Although some of the comorbidities associated with PPH in humans are also known to develop in dogs with DM, the association of these comorbidities with PPH has not been studied in dogs.[19-21] The study of PPH and circadian glucose concentration fluctuations in dogs has been hampered by lack of an easy and reliable method to identify glucose concentration changes over an extended time period and in the dog's home environment.

Continuous glucose monitoring devices have been used in small animals since 2003, but their use was limited historically by bulky devices that required daily calibration with blood glucose concentration measurements.[22-25] The use of a newer flash glucose monitoring system (FGMS; FreeStyle Libre, Abbott, Alameda, California) has become more common in small animal practice because of its small size, easy application, affordable price, factory calibration, and user-friendly interface.[1,2] However, the use of the FGMS to analyze PPH and circadian fluctuations in glucose concentrations in dogs with naturally occurring DM has not been reported. A better understanding of postprandial and circadian fluctuations in glucose concentrations could lead to improved treatment of dogs with DM. The FGMS previously has been validated in healthy diabetic dogs without comorbidities, as well as in dogs hospitalized with diabetic ketoacidosis.[1,2] However, the device has not been fully validated in outpatient diabetic dogs with comorbidities that are receiving concurrent medications other than insulin. Concurrent illnesses are common in diabetic dogs and potentially could interfere with application, tolerability, and accuracy of the FGMS device both directly and indirectly.[26-28]

Our study had several aims: first, to assess postprandial and circadian fluctuations in glucose concentrations in diabetic dogs using the FGMS; second, to further validate this device in a broader population of diabetic dogs using a new sensor application method; and third, to assess adverse events associated with the device in this population of dogs.

2 | MATERIALS AND METHODS

2.1 | Study population

Client-owned dogs with DM that were examined at a university teaching hospital between April and October 2019 were prospectively enrolled in an observational validation study. To be included, dogs with DM had to weigh >3 kg, be assessed as <5% dehydrated, be fed twice a day, and have a body condition score ≥ 3/9.[29] Owners also had to agree to bring the dogs into the clinic for three 10-hour outpatient visits within a 13-day period. Exclusion criteria included anemia (PCV < 35%) and recent exposure to drugs that can interfere with FGMS readings (acetaminophen, dopamine, icodextrin, salicylates, and ascorbic acid).[30] Additionally, dogs could not undergo anesthesia, radiography, computed tomography, or magnetic resonance imaging for the 13-day study period, because the FGMS sensor can be affected by these imaging modalities.[30] There were no restrictions on insulin type, dosage, or frequency, and the treatment of each dog was left to the discretion of the attending clinician. Dogs with concurrent diseases and on various other medications were included. The University Privately Owned Animal Protocol Committee and the Institutional Animal Care and Use Committee approved the study and informed consent form, and owners signed the written informed consent form before enrollment of their dogs.

2.2 | Data collection

On the day of enrollment (day 0), a 14-day FGMS sensor was placed in a standardized fashion by 1 of the 2 authors (E. K. S. or R. S. H.). An approximately 5 × 5 cm square of skin in the area of the dorsal neck was clipped and cleaned using dilute chlorhexidine followed by alcohol, and the area was allowed to dry fully for at least 1 minute before placement of the sensor. The sensor was placed according to the manufacturer directions, with the addition of 3 to 4 drops of tissue glue on the adherent side of the sensor before placement.[31] In addition, the sensor applicator was held firmly in place for 30 seconds after deployment of the sensor. No other adhesives or bandages were used, but some owners elected to place a shirt on their dog to prevent scratching of the sensor. Dogs were fed and received their insulin and any other medications at home, before arrival at the hospital.

For each dog on day 0, the first blood sample was drawn after a 1-hour calibration period. A maximum of 2 mL was obtained from a peripheral or jugular vein for measurement of PCV, total protein (TP) concentration, point-of-care blood glucometer reading (POCG; Accu-Chek Performa, Roche Diagnostics Corp, Indianapolis, Indiana), and blood glucose concentration measurement on the automated biochemistry analyzer (ABA; Vitros 4600 Chemistry System, Ortho-Clinical Diagnostics, Rochester, New York). Subsequent blood samples were drawn at 2-hour intervals for a total of 5 samples per day per dog, and only blood glucose concentration, measured on the POCG and ABA, was recorded in these subsequent samples. Interstitial glucose (IG) concentration was measured by scanning the FGMS sensor using the dog's designated reader within 1 minute of the blood draws. The POCG measurements were performed using 1 drop of whole blood immediately after venipuncture on a single study designated POCG; ABA blood glucose measurements were performed on serum. For ABA glucose concentration measurements, blood samples were submitted immediately to the clinical pathology laboratory and centrifuged within 15 minutes of collection. The ABA utilized in the study

measures serum glucose concentration using a colorimetric glucose oxidase method. The serum for each sample also was analyzed for evidence of hemolysis, lipemia, and icterus by trained laboratory technicians and subjectively graded as none, mild, moderate, or marked.

The sensor was left in place for the 13-day study period, and owners were instructed to scan the sensor with the reader a minimum of 3 times per day at 8-hour intervals, because the sensor can store data for a maximum of 8 hours. Each dog returned for 2 subsequent visits at approximately 1-week intervals. For these visits, the same procedure was followed as outlined above for the day of enrollment, but no calibration period was necessary because the sensor was already in place. At each of the visits, owners filled out a standardized questionnaire including their dog's clinical signs, medications, times of meals and insulin administration, and any concerns they had noted about the FGMS sensor. At the end of the final visit day, the sensor was removed by 1 of the study authors.

2.3 | Flash glucose monitoring system data analysis

The FGMS sensor measures IG concentration every minute and stores an average of these concentrations every 15 minutes. Data can be stored in the sensor for up to 8 hours and are transferred to the reader whenever the sensor is scanned by the reader. The FGMS reader can store data for up to 90 days. Data from the reader can be uploaded to an online platform (LibreView, Abbott, Chicago, Illinois), which can be accessed by clinicians and caregivers. The web site generates various graphic reports, and the data also can be downloaded as an Excel spreadsheet that includes all recorded glucose concentrations as well as times of meals and insulin administration, if they are recorded on the reader. Because owners of dogs in the current study inconsistently recorded meal and insulin times on the reader, these times were added based on those reported on the standardized questionnaires completed at each of the 3 in-person visits. Meal and insulin administration time points were added only if an IG concentration measurement was recorded within 30 minutes of the time of the meal and insulin administration. Duplicate entries were deleted. This FGMS data set reports IG concentrations of ≤40 mg/dL as 40 mg/dL and IG concentrations of ≥500 mg/dL as 500 mg/dL.

For analysis of PPH, median IG concentration measured within 30, 60, 90, and 120 minutes after meals were each compared to the median of all other IG concentrations recorded during the study period. For analysis of circadian fluctuations, median night-time IG concentrations recorded from 1:00 am until 6:00 am were compared to the median IG concentrations recorded at all other times during the study period.

2.4 | Validation data analysis

All samples were analyzed together and in subgroups based on the ABA glucose concentration. Hypoglycemia, normoglycemia, hyperglycemia, and pronounced hyperglycemia were defined as an ABA-measured blood glucose concentration of <65 mg/dL, 65 to 112 mg/dL, >112 mg/dL, and >250 mg/dL, respectively. The detection limits of the sensor are 40 to 500 mg/dL, and 20 readings outside of the detection limits were excluded from the validation analysis. Three additional erroneous sensor readings also were excluded. For hemolysis, lipemia, and icterus, samples graded as "none" or "mild" were grouped together, and those graded as "moderate" or "marked" were grouped together.

2.5 | Sample size calculation

Three sample size calculations were performed for the validation study. Two-sided paired *t* tests were used to determine the number of samples required to detect a difference of at least 15 mg/dL between blood glucose concentration measured by the ABA and IG concentration measured by the FGMS. The calculation for normoglycemic samples was made on the basis of the mean ± SD glucose concentration (117 ± 25 mg/dL) used to establish the reference interval for the ABA. The calculation for hyperglycemic samples was made using a mean of 250 ± 25 mg/dL and the calculation for hypoglycemic samples was made using a mean of 65 ± 25 mg/dL. It was assumed that the SD of the glucose concentrations determined by the FGMS would be similar to that of the glucose concentrations determined by the ABA. Additional assumptions included a power of 0.8, type I error rate of 0.05, and a ratio of 1 between the ABA and FGMS sample sizes. The calculation resulted in a required sample size of 45 paired ABA and FGMS measurements for each of the 3 categories of glucose concentration for a total of 135 measurements. Each dog contributed 15 glucose measurements by providing 5 blood glucose and IG concentration measurements per day on 3 separate days over the 13-day study period. Therefore, 9 dogs were to be enrolled for a sample size of 135 blood and IG concentration measurements. However, in a previous study, approximately 30% of sensors were reported to fall out unintentionally before the end of the study, and therefore 13 dogs were required to account for case attrition.[1] Sample collection continued beyond enrollment of 13 dogs until the end of the allocated research time.

2.6 | Statistical analysis

Variables were assessed for normality visually and using the skewness/kurtosis tests for normality. Interstitial glucose concentrations were not normally distributed. Therefore, the 2-sample Wilcoxon rank sum (Mann-Whitney) test was used for comparison of postprandial IG concentrations to all other IG concentrations and for comparison of night-time IG concentrations to all other IG concentrations in all dogs combined and in each individual dog.

The ABA was considered the gold standard, and FGMS and POCG glucose concentrations were compared to ABA concentrations using the Lin's concordance correlation coefficient test. Bland-Altman plots were generated to visually evaluate the correlations. The Lin's concordance correlation coefficient (ρ_c) was calculated and compared to a coefficient of

0 (no correlation). The effects of ABA, PCV, TP, hemolysis, lipemia, and icterus on FGMS glucose concentrations were evaluated using a multivariable linear regression model. Most variables, including ABA and POCG glucose concentrations, were not normally distributed and therefore are reported as median (range) or count and percentage.

A mixed effects model also was fitted to the data to account for glucose variability among dogs. Interstitial glucose concentration was modeled as a function of blood glucose concentration as follows:

$$IG = \beta_0 + \beta_{1*}(\text{ABA measured glucose}) + \text{random effect on dog} + \text{error term},$$

with $\beta_0 + \beta_{1*}$ (ABA measured glucose) serving as the fixed effect of the model, the random effect occurring at the dog level, and an error term which is a function of undetermined factors such as within-dog variability, exercise, or meal time. For the purpose of comparison, POCG glucose measurements were modeled similarly as a function of blood glucose concentration as follows:

$$\text{POCG glucose measurements} = \beta_0 + \beta_1 * (\text{ABA measured glucose}) + \text{random effect on dog} + \text{error term}$$

The normality of the residuals of the generalized linear models was confirmed graphically by plotting kernel density estimates and standardized normal probability (P-P) plots. An identity covariance structure was applied to the mixed effects models.

P values <.05 were considered significant for all comparisons. All statistical analyses were performed using a statistical software package (Stata, version 14.0 for Mac; Stata Corp, College Station, Texas).

3 | RESULTS

3.1 | Clinical findings

Fourteen individual diabetic dogs were enrolled, 2 of which were enrolled in the study twice, for a total of 16 sensor placements. Ten dogs were castrated males, 3 were spayed females, and 1 was an intact male. Breeds included 4 mixed breed dogs, and 1 each of the following: Bichon Frise, Chihuahua, German Shorthaired Pointer, Jack Russell Terrier, Labrador Retriever, Maltese, Miniature Pinscher, Miniature Poodle, Pug, and West Highland White Terrier. The median age was 10.5 years (range, 2-14 years), and the median weight at the time of enrollment was 8.1 kg (range, 4.1-28 kg).

All dogs received insulin injections twice daily, with a median dose of 5 units (range, 2-29 units) at the time of enrollment. No dogs were newly diagnosed diabetics nor were any in a diabetic crisis (diabetic ketoacidosis or hyperosmolar hyperglycemic state) throughout the study period. The insulin administered most commonly was neutral protamine Hagedorn insulin (NPH, Novolin N, Novo Nordisk, Bagsvaerd, Denmark) given to 7 of 14 dogs (50%). The diet fed most commonly was w/d (Hill's, Topeka, Kansas) fed to 6 of 14 dogs (43%). Diets and insulin types administered to study dog are reported in Table S1.

The majority of dogs (11/14, 78.6%) had concurrent illnesses, with most of these dogs (7/11, 63.6%) having >1 concurrent disease. Concurrent diseases included cataracts (8), liver or biliary tract disease (3), cardiac disease (3), hyperadrenocorticism (2), pancreatitis (2), hypothyroidism (2), clinically relevant dental disease (2), and 1 each of the following: urinary tract infection, dermatologic disease, gastrointestinal disease, tracheal collapse, and carbapenem-resistant *Escherichia coli* colonization. One additional dog had a prior history of hyperadrenocorticism that had been treated by adrenalectomy. Dogs were treated with a variety of noninsulin medications throughout the study period, including topical/ocular medications (6), trilostane (2; Vetoryl, Dechra, Overland Park, Kansas), ursodeoxycholic acid (2; compounded formulation), levothyroxine (2; Soloxine, Virbac AH, Inc, Fort Worth, Texas), and 1 each of the following: metronidazole (Flagyl, G.D. Searle & company, Chicago, Illinois), amoxicillin (AmoxiTabs, Zoetis, Kalamazoo, Michigan), doxycycline (Vibramycin, Pfizer Labs, New York, New York), meloxicam (Metacam, Boehringer Ingelheim Vetmedica, Inc, St. Joseph, Missouri), omeprazole (Prilosec, AstraZeneca Pharmaceuticals, Wilmington, Delaware), maropitant (Cerenia, Zoetis), pimobendan (Vetmedin, Boehringer Ingelheim Vetmedica, Inc), trazodone (Desyrel, Teva Pharmaceuticals, Sellersville, Pennsylvania), cetirizine (Zyrtec, Pfizer Labs), and fish oil and vitamin E supplementation. In 1 dog, ocular medications included prednisolone acetate (Pred Forte, Allergan, Irvine, California) given 4 to 6 times daily.

On all 3 in-hospital study days, all dogs were assessed to be subjectively well hydrated on examination. The median PCVs on days 0, 7, and 13 were 44.5% (range, 35%-54%), 42% (range, 39%-55%), and 44% (range, 39%-47%), respectively. The median TP concentrations on days 0, 7, and 13 were 7.6 g/dL (range, 6.4-9.2 g/dL), 7.4 g/dL (range, 6.4-8 g/dL), and 7.8 g/dL (range, 6.9-8.3 g/dL), respectively.

3.2 | Postprandial hyperglycemia and circadian fluctuations

Online data were available for 11/14 dogs, and these data were utilized to analyze glucose concentration fluctuations over the 24-hour period. A total of 17, 446 FGMS IG concentrations were analyzed for PPH and circadian fluctuations. When data from all 11 dogs were analyzed together, median IG concentrations measured within 30, 60, 90, and 120 minutes of meals were each significantly higher than the median IG concentration at all other times ($P < .001$, Table 1). When data were analyzed separately for each dog, most dogs (8/11, 73%) had significantly higher postprandial IG concentrations than the median IG concentration at all other times. Median IG concentration measured within 30, 60, 90, and 120 minutes of meals were significantly higher than the median IG concentration at all other times in 6 of 11 (55%), 7 of 11 (64%), 7 of 11 (64%), and 7 of 11 (64%) dogs, respectively (Table S2).

Median night-time IG concentration measured on 3,547 samples recorded between 1:00 am and 6:00 am in all 11 dogs combined was 268 mg/dL (range, 40-500 mg/dL), which was significantly higher than the median day-time glucose concentration of 259 mg/dL (range, 40-500 mg/dL) measured on 13, 899 samples at all other time points ($P < .001$). Median night-time and day-time IG concentrations

TABLE 1 Comparison of postprandial interstitial glucose concentrations to interstitial glucose concentrations measured at all other time points, in all 11 dogs combined

Time	Number of postprandial samples	Median postprandial IG (range) (mg/dL)	Number of samples at all other times	Median IG at all other times (range) (mg/dL)	*P* value
Within 30 min of meal	1,033	288 (40-500)	16, 413	260 (40-500)	<.001
Within 60 min of meal	1,715	286 (40-500)	15, 731	259 (40-500)	<.001
Within 90 min of meal	2,361	285 (40-500)	15, 085	258 (40-500)	<.001
Within 120 min of meal	3,015	285 (40-500)	14, 431	257 (40-500)	<.001

Note: All interstitial glucose concentrations were measured by the flash glucose monitoring system.
Abbreviation: IG, interstitial glucose.

TABLE 2 Descriptive statistics of glucose concentrations by method of measurement (analyzer) and by glucose concentration subgroup

Type of sample	Analyzer	Number of samples	Mean ± SD (mg/dL)	Median (range) (mg/dL)
All samples	FGMS	158	274 ± 121	257.5 (50-494)
	POCG	189	258 ± 117	251 (37-577)
	ABA	184	281 ± 122	261.5 (45-575)
Samples with hyperglycemia	FGMS	146	288 ± 114	272.5 (50-494)
	POCG	176	272 ± 109	265.5 (100-577)
	ABA	171	295 ± 114	293 (113-575)
Samples with pronounced hyperglycemia	FGMS	73	382 ± 72	379 (246-494)
	POCG	101	348 ± 77	344 (175-577)
	ABA	96	378 ± 79	373.5 (251-575)
Samples with normoglycemia	FGMS	10	96 ± 20	97 (61-125)
	POCG	10	80 ± 10	79 (69-94)
	ABA	10	98 ± 10	103.5 (85-108)
Samples with hypoglycemia	FGMS	2	84.5 ± 13	84.5 (75-94)
	POCG	3	51 ± 12	56 (37-59)
	ABA	3	54 ± 9	55 (45-63)

Note: Hyperglycemia, pronounced hyperglycemia, normoglycemia, and hypoglycemia were defined based on automated biochemistry blood glucose concentrations >112 mg/dL, >250 mg/dL, >65 and <112 mg/dL, and <65 mg/dL, respectively.
Abbreviations: ABA, automated biochemistry analyzer measurement (serum); FGMS, flash glucose monitoring system (interstitial glucose); POCG, point of care glucometer measurement (whole blood).

TABLE 3 Lin's concordance correlation analyses of the difference between the gold standard automated biochemistry analyzer serum glucose concentration and interstitial glucose concentration measured by the flash glucose monitoring system or blood glucose concentration measured by the point of care glucometer

Type of sample	Analyzer	ρ_c (95% confidence interval)	Bias correction factor	Mean difference ± SD (95% limits of agreement) (mg/dL)
All samples	FGMS	0.93 (0.91-0.95)	0.98	17.2 ± 39.0 (−59.3 to 93.7)
	POCG	0.96 (0.95-0.97)	0.98	−22.9 ± 24.1 (−70.0 to 24.3)
Samples with hyperglycemia	FGMS	0.91 (0.89-0.94)	0.97	18.4 ± 40.0 (−60.1 to 96.8)
	POCG	0.95 (0.94-0.97)	0.98	−23.5 ± 24.8 (−72.0 to 25.1)
Samples with pronounced hyperglycemia	FGMS	0.75 (0.66-0.84)	0.88	30.2 ± 37.6 (−43.5 to 103.9)
	POCG	0.88 (0.83-0.92)	0.95	−25.8 ± 29.5 (−83.5 to 31.9)

Abbreviations: ρ_c, Pearson's correlation coefficient; FGMS, flash glucose monitoring system (interstitial glucose); POCG, point of care glucometer measurement (whole blood).

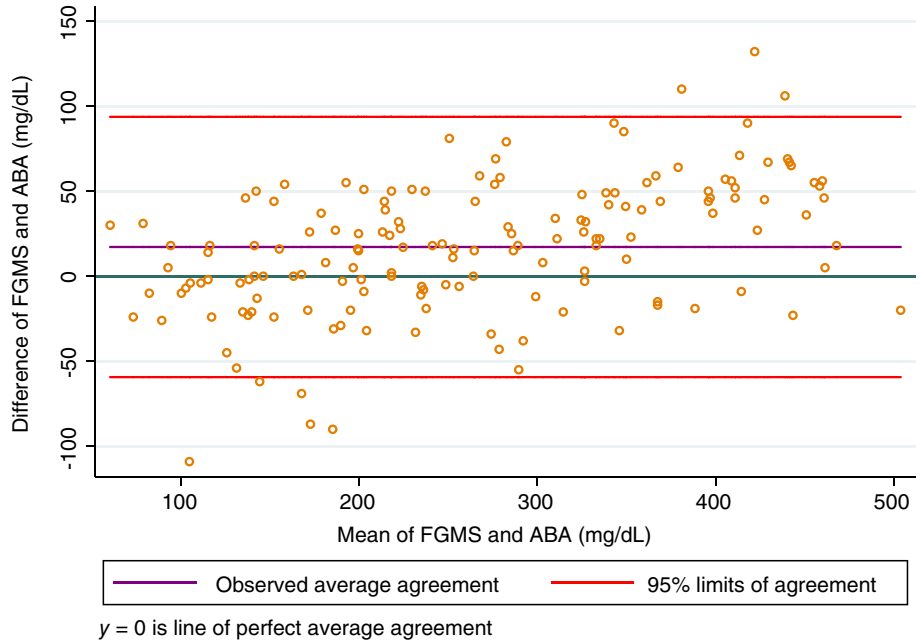

FIGURE 1 Bland-Altman plot of the difference between the gold standard automated biochemistry analyzer serum glucose concentration and interstitial glucose concentration measured by the flash glucose monitoring system in all samples. For each plot, the green line represents a mean difference of 0 between the glucose concentrations being compared, the purple line represents the mean difference between the glucose concentrations being compared, and the red lines represent the 95% limits of agreement (mean difference ± 1.96 SD). The closer the purple line is to the green line, the better the agreement between the 2 glucose concentrations being compared

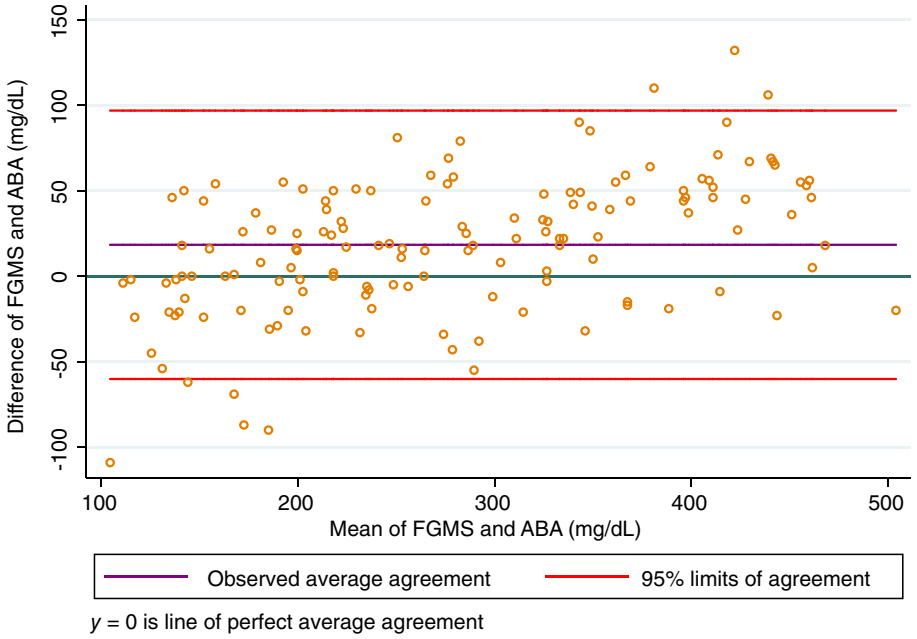

FIGURE 2 Bland-Altman plot of the difference between the gold standard automated biochemistry analyzer serum glucose concentration and interstitial glucose concentration measured by the flash glucose monitoring system in hyperglycemic samples (automated biochemistry blood glucose greater than 112 mg/dL). See Figure 1 for key

measured in each dog individually are reported in Table S3. When data were analyzed separately for each dog, approximately half of the dogs (6 of 11, 55%) had significantly higher median IG concentration measured at night-time compared to daytime (Table S3).

3.3 | Validation data

Descriptive statistics for 158 paired glucose concentrations measured by the FGMS, POCG, and ABA are reported in Table 2. These measurements were obtained from 16 sensor placements in 14 dogs. Results of the Lin's concordance correlation analyses are reported in Table 3. Bland-Altman limits-of-agreement plots demonstrating these correlations are presented in Figures 1 to 3. Lin's concordance correlation analysis for the normoglycemic and hypoglycemic groups was not possible because of small sample size in these subgroups.

Results of the mixed effect models are reported in Table 4 and indicate that a significant positive correlation existed between the FGMS and ABA glucose concentrations as well as between POCG and ABA glucose measurements ($P < .001$ for both). In these models, it is expected that for every 1 mg/dL increase in ABA-measured blood glucose concentration there is an increase of 1.2 mg/dL on the FGMS-measured IG concentration and an increase of 0.95 mg/dL on the POCG-measured blood glucose concentration. Both mixed effect models were significantly

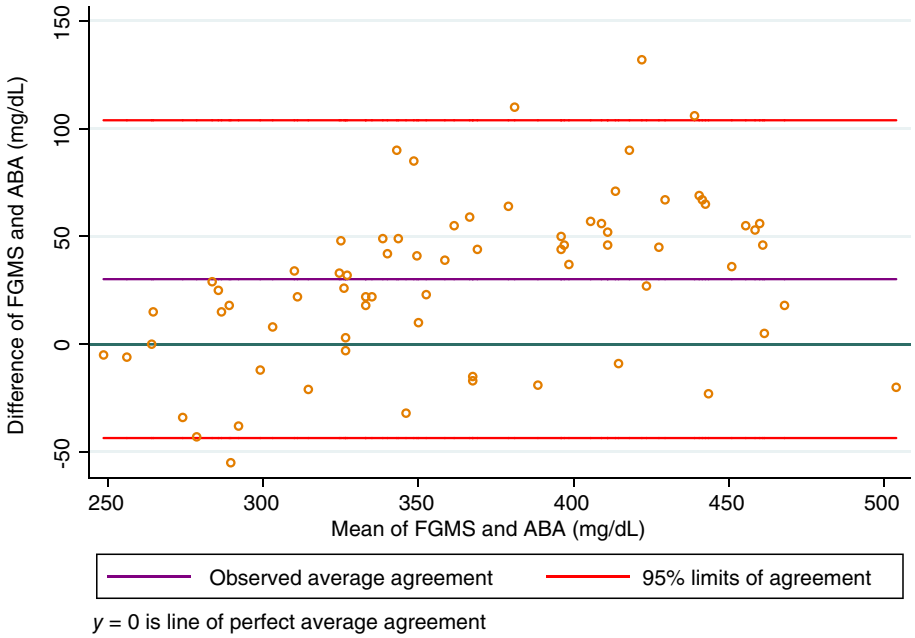

FIGURE 3 Bland-Altman plot of the difference between the gold standard automated biochemistry analyzer serum glucose concentration and interstitial glucose concentration measured by the flash glucose monitoring system in samples with pronounced hyperglycemia (automated biochemistry blood glucose greater than 250 mg/dL). See Figure 1 for key

TABLE 4 Results of mixed effects models modeled as: Interstitial glucose = $\beta_0 + \beta_{1^*}$ (ABA measured blood glucose) + random effect on dog + error term and Point-of-care glucometer measurements = $\beta_0 + \beta_{1^*}$ (ABA measured blood glucose) + random effect on dog + error term

Outcome variable (number of observations)	Mixed model term	Estimate	SE	*Z* value	*P* value
Interstitial glucose measured by the flash glucose monitoring system (157)	β_1 coefficient	1.16	.029	40.44	<.001
	SD of random effects at dog level	24.62	5.65	N/A	N/A
	SD of error term	29.60	1.75	N/A	N/A
Blood glucose measured by the point-of-care glucometer (184)	β_1 coefficient	0.95	0.01	63.04	<.001
	SD of random effects at dog level	12.08	3.15	N/A	N/A
	SD of error term	20.52	1.12	N/A	N/A

Abbreviation: ABA, automated biochemistry analyzer measurement (serum).

different from an ordinary linear regression model, indicating that the random effects were significant in both models.

The multivariable linear regression model identified a significant positive correlation between the ABA and FGMS glucose concentrations ($P < .001$) and a significant negative correlation between TP and FGMS IG concentrations ($P = .02$). The coefficient for the ABA variable was 1.1 and the coefficient for the TP variable was −10.4. Therefore, for every 1 mg/dL increase in ABA blood glucose concentration there is an expected increase of 1.1 mg/dL in the FGMS-measured IG concentration, and for every 1 g/dL increase in TP, there is an expected decrease of 10.4 mg/dL in the FGMS-measured IG concentration. No significant correlations were found between FGMS IG concentrations and PCV, hemolysis, icterus, or lipemia.

3.4 | Adverse events

In all dogs, the sensor was placed on the dorsal neck in a standardized fashion as described. In 15/16 sensor placements (93.8%), sensor placement went smoothly with no complications; in 1 dog, the filament within the sensor bent and the sensor stopped working within a few hours of placement, and a new sensor was placed without complication. In 7/16 sensor placements (43.8%), the initial sensor fell off, was removed by the dog, or stopped working before the end of the 13-day study period. All 6 dogs for which the sensor failed prematurely weighed <10 kg; 1 of these dogs was enrolled in the study twice, and the sensor failed during both study periods. None of the 5 dogs that weighed >10 kg had sensor failures, and 1 of these dogs was enrolled in the study twice. Only 3 of 9 dogs weighing <10 kg completed the study without sensor failure. Owners elected to place a shirt on 3 individual dogs, all weighing <10 kg, that were enrolled for 4 sensor placements; 2 of these dogs had no sensor failures and the 3rd dog had a premature sensor failure during both enrollment periods. No owners noted any change in their pet's activity level while the sensor was in place or any complaints directly related to the sensor.

4 | DISCUSSION

Postprandial and nocturnal hyperglycemia recorded over a 13-day period in the home environment of dogs with naturally occurring DM are reported here for the first time. Additionally, the FGMS was

successfully validated using glue rather than bandaging material for sensor application and in a population of diabetic dogs with concurrent diseases.

Postprandial hyperglycemia previously has been examined in dogs with experimentally induced DM, naturally occurring DM evaluated in the hospital, and in obese and healthy dogs without DM.[15-18,32-35,36] The overarching goal of evaluating PPH is to study interventions that could decrease it and improve overall glycemic control. An effective summary of these studies is limited by the fact that they are small, have involved a variety of diets and diet acclimation periods, and measurements of PPH often have spanned only a single day. Methods of PPH quantification also have varied with changes in glucose concentration being reported in absolute values, SD from normal, or area under the curve. However, significant decreases in PPH have been reported in healthy dogs fed high fiber or low digestible carbohydrate diets, and glycemic control also was improved in diabetic dogs fed a diet high in insoluble fiber.[32,34,36,37] The addition of lispro insulin to NPH in diabetic dogs fed a diet high in insoluble fiber also decreases PPH.[17]

Although postprandial IG concentrations were significantly higher than IG concentrations measured at all other time points in all dogs combined, the magnitude of increase in glucose concentrations detected during PPH in our analysis was not clinically relevant (Table 1). However, in some individual dogs, the magnitude of increase in glucose concentrations detected during PPH could be clinically relevant (Table S2). The lack of clinical relevance of PPH in the analysis of all dogs combined may have been influenced by dogs in which no significant changes in postprandial glucose concentrations occurred and by dogs in which decreases in postprandial glucose concentrations occurred. Nonetheless, results of our study indicate that the FGMS detects PPH in diabetic dogs in their home environment, thereby offering an easy, reliable, cost-effective, and noninvasive method for monitoring PPH in future studies designed to investigate interventions to decrease PPH. Our study was not designed to investigate the effect of diet or insulin type on PPH, and treatment regimens therefore were not standardized or compared. Remarkably, detection of PPH was possible despite the fact that the study included dogs with numerous concurrent disorders, receiving various medications, eating a number of different diets, and exhibiting variable glycemic control over time and compared to each other. It is possible that with standardization of some of these variables, PPH detected by the FGMS would be clinically relevant.

Night-time IG concentrations were significantly higher than all other IG concentrations when data from all dogs were analyzed together, although this statistical difference also was not clinically relevant. When data were analyzed separately for each dog, it became apparent that a small number of dogs could have a clinically important increase in IG concentration during night-time. The time frame of 1:00 am to 6:00 am was chosen because it was assumed that most pet owners and dogs would be asleep during this time. This finding is in contrast with the commonly diagnosed nocturnal hypoglycemia documented in humans with type 1 diabetes.[8,9] Some dogs with DM may experience night-time hyperglycemia because of lack of activity during that time frame, whereas humans experience nocturnal hypoglycemia because of intensive insulin regimens including administration of intermediate-acting insulin 3 times per day or combination treatment using rapidly acting insulin along with intermediate or long-acting insulin products.[7,9] Three dogs had a significantly lower median IG concentration at night-time, indicating individual variability in circadian fluctuations of blood glucose concentration that could depend on various factors such as concurrent illness, diet, insulin type, timing of insulin administration, and exercise. Only 1 previous study in the veterinary literature has examined night-time glucose concentrations in dogs using a different continuous glucose monitoring device, and this study found no significant differences in day- and night-time glucose concentrations.[38] However, this study analyzed data from only 5 dogs over 7 days and could have been under-powered to detect a significant difference.[38]

Our study also further validated the use of the FreeStyle Libre FGMS in a population of stable diabetic dogs with concurrent illnesses. Because the FGMS measures IG concentration rather than blood glucose concentration, perfect correlation is not expected because a lag period for equilibration of blood glucose and IG concentrations has been documented previously in dogs.[22,39] The device used in our study has been previously validated in diabetic dogs without concurrent illnesses as well as in hospitalized dogs with diabetic ketoacidosis, but the dogs in the latter study wore the sensor in the hospital only and concurrent illnesses and medications were not noted.[1,2] Furthermore, in the study validating the FGMS in dogs with diabetic ketoacidosis, the POCG was used as the reference standard, whereas an ABA was used in our study and the original study of healthy diabetic dogs.[1,2] Similar to the previous validation study, our study found that the FGMS recorded higher glucose concentrations than the ABA on average, whereas the POCG recorded lower glucose concentrations than the ABA on average, and thus using the POCG as a reference standard could underestimate the accuracy of the FGMS.[1,2] The degrees of correlation between the FGMS and ABA for all analyzed samples were similar in our study and the previous study of diabetic dogs with no concurrent illness.[1] However, the previous study reported a range of the median difference between IG concentration measured by the FGMS and ABA of −385 to 309 mg/dL, whereas this difference in our study was much smaller (Table 3).[1] Potential reasons for this difference include outliers in the previous study, the use of a different method of application, the use of a different ABA, or differences in dog populations.

Although both mixed effect models effectively predicted the dependent variable, the between-dog SD and error term SD were larger in the model predicting IG concentration than in the model predicting POCG measurements. This could indicate a larger variation of between and within-dog observations in the model in which the dependent variable is IG concentration compared to the model in which the dependent variable is POCG measurements.

The linear regression model showed that PCV, lipemia, hemolysis, and icterus did not correlate with IG concentrations, although no dogs in the study were icteric or anemic. An unexpected finding was the correlation between increased TP concentration and decreased IG concentration. According to the manufacturer, dehydration can cause inaccuracies in the FGMS readings.[40] An increase in TP may be a

marker for subclinical dehydration, because the hydration status of the study dogs was subjectively assessed as normal at each of the visits. Additional research is needed to confirm this finding and explore its importance further.

Although the FGMS generally was well tolerated by most dogs in the study, the sensor needed to be replaced because of displacement or dysfunction in many dogs. Our study was not designed to detect a statistical difference based on body size, but all premature sensor failures occurred in dogs weighing <10 kg. Although the FGMS can be useful in some small dogs, a higher risk of sensor failure could be present, and owners should be warned of this possibility. Future studies directly comparing sensor functionality with different application methods are needed to determine the optimal location and technique for sensor placement.

Our study had several limitations. First, sample size calculations did not account for between and within-dog variability or for between-day variability, nor did they account for a possible lack of equilibrium between IG and blood glucose concentrations. The assumption that the SD of glucose concentrations was the same in samples with normoglycemia as in samples with hyperglycemia or hypoglycemia also contributed to inaccuracies in the sample size calculations. The times of meals and insulin administration were noted based on normal routines indicated by owners on a questionnaire, because owners did not regularly input this information into the FGMS reader. This could have resulted in recall bias and inaccuracies in meal-time determination. There were also several lapses in IG recordings because of sensor errors and owner compliance problems. Additionally, dogs were fed a variety of diets that could have affected PPH differently. Finally, dogs in our study generally had poorly controlled DM, such that the number of samples with normoglycemia and hypoglycemia was small, and the accuracy of the FGMS in these glucose concentration ranges was not validated. Other studies also have not been powered to evaluate the accuracy of the FGMS in hypoglycemic samples, but a recent experimental study found that the same FGMS device had limited agreement with an ABA during hypoglycemia in dogs with experimentally induced rapidly changing glucose concentrations (Patterson C, Evaluation of a Flash Glucose Monitoring System in Dogs with Rapidly Changing Glucose Concentrations [abstract]).[1,2]

In conclusion, the FGMS is suitable for detection of postprandial and night-time hyperglycemia occurring in variably regulated diabetic dogs. Future research into modifications of diabetic management that could mitigate these glucose concentration fluctuations is warranted and can be performed using the FGMS. The FGMS is a valid and useful tool for future studies of glucose concentration fluctuations in diabetic dogs, but it should be used with caution in dogs weighing <10 kg.

ACKNOWLEDGMENT

Funding for this study was provided by a gift from Ms. Catharine Adler.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC of the University of Pennsylvania.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Shea EK, Hess RS. Assessment of postprandial hyperglycemia and circadian fluctuation of glucose concentrations in diabetic dogs using a flash glucose monitoring system. *J Vet Intern Med*. 2021;35:843–852. https://doi.org/10.1111/jvim.16046