Journal of the American Association for Laboratory Animal Science
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Vol 59, No 4
July 2020
Pages 430–437

Assessment of a Noninvasive Chronic Glucose Monitoring System in Euglycemic and Diabetic Swine (*Sus scrofa*)

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Models of type-I diabetes are well-characterized and commonly used in the preclinical evaluation of drugs and medical devices. The diabetic minipig is an excellent example of a translational model. However, chronic glucose monitoring in this species can be challenging; frequent blood sampling can be technically difficult and poorly tolerated in conscious swine. Skin-patch continuous blood glucose monitors are FDA-approved for human use and offer a potential refinement to cage-side blood collection. However, this modality has not been evaluated in pigs. In this study, young adult male STZ-induced diabetic Yucatan minipigs (*n* = 4) and healthy York pigs (*n* = 4) were implanted with a 14-d skin-patch continuous glucose monitor. Readings from continuous glucose monitors were time-matched to whole blood samples, with glucose measurements performed using point-of-care blood glucose monitors, serum chemistry or both. The aims of the study were to assess if a continuous glucose monitoring system could accurately detect glucose levels in swine, and to compare the readings to both point-of-care glucometers and serum chemistry results. We hypothesized that a continuous glucose monitoring system would accurately detect glucose levels in swine in comparison with a validated analyzer and could serve as an animal welfare refinement for studies of diabetes. We found that the continuous glucose monitor used in this study provided an adequate adjunct for clinical management in the stable diabetic pig and a minimally invasive and inexpensive option for colony maintenance of chronically diabetic swine.

Abbreviations: BG, blood glucose; POCM, point-of-care-monitor; CGM, continuous glucose monitor; CHEM, serum chemistry

DOI: 10.30802/AALAS-JAALAS-19-000140

As of 2014, an estimated 422 million people globally were living with diabetes mellitus.[43] Type 1 diabetes is an autoimmune disease that destroys the insulin producing β cells of the pancreas[3,4] while type 2 diabetes is due to cellular resistance to insulin, which is induced by obesity, reductions in both insulin activity and secretion, and elevated endogenous glucose.[40] The incidence of type 1 diabetes is increasing annually in both Europe and the United States[31,32] and with that, the demand for effective therapy and disease management. In addition, the chronic systemic effects of hyperglycemia from all diabetic states contribute to rising healthcare costs and diminished quality of life.[1] In preclinical research, animal models of diabetes are well-established and commonly used to evaluate the safety and efficacy of diabetes-related therapies.

Diabetes can be induced in both rodent and non-rodent species through pharmacologic or surgical interventions. In non-rodent models, type 1 diabetes is typically induced chemically, through pancreatectomy, or a combination of both.[21] At our institution, we use a Type I diabetic swine model, chemically induced with streptozotocin [2-deoxy-2-(3-(methyl-3-nitrosoureido)-d-glucopyranose] (STZ). STZ is a DNA alkylating agent, synthesized from *Streptomycetes achromogenes*, and demonstrates highly selective toxicity to pancreatic β cells.[28] When administered at a dose of 200 mg/kg[17] intravenously, rapid and irreversible destruction of endogenous insulin production occurs. The resulting β cell depletion and insulin dependency produces a stable diabetic state and permits the evaluation of glycemic management therapies and chronic sequelae to hyperglycemic aberration. However, some side effects can complicate this model, and animals require regular monitoring and exogenous insulin supplementation.[17]

The diabetic minipig is an excellent translational model, ; however, chronic blood glucose (BG) monitoring can pose a challenge as frequent blood sampling can be technically difficult and poorly tolerated in conscious swine. Historically, we have acquired ear-prick blood samples and used a veterinary blood glucose point-of-care monitoring system (POCM) to assess blood glucose levels. Although swine are gregarious and social by nature, chronic studies that require multiple daily BG readings via ear pricks followed by subcutaneous injections of insulin can be aversive. Given the rapid and significant impact of stress on blood glucose levels, excessive excitement can be reflected as hyperglycemic artifacts, confounding data collection and insulin dosing.[29] Positive-reinforcement, training techniques, and gentle handling of swine can improve animal comfort and decrease stress. However, additional refinement could optimize animal welfare and accurate study results. Surgical implantation of continuous glucose monitors and glucose telemetry has been previously validated,[14,26,36] however, surgical manipulations may be undesirable or overly invasive for some study designs.

Recently, several FDA-approved minimally invasive continuous glucose monitoring systems (CGM) have become available

Received: 23 Sep 2019. Revision requested: 18 Oct 2019. Accepted: 07 Nov 2019.
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for human diabetic patients. These systems are characterized by a "skin patch" design, with a small transmitter unit that adheres to the skin and covers a thin filament that extends into the subcutis. The sensor is placed at home by the patient with a corresponding applicator device, which painlessly inserts the filament through the skin. Once in place, the sensor continuously reads interstitial glucose levels and communicates with a handheld reader or compatible smartphone. In some designs, the sensor and reader are in continuous communication. In others, the system operates as a "flash" monitor, with communication established when the sensor is scanned. Once scanned, up to 8 h of data and trends are available for review and are stored within the device. These products are designed to be kept in place for 7 to 14 d, without interfering with typical human lifestyle activities like showering or exercise. In humans, the lower abdomen or the posterior aspect of the upper arm provides an ideal application site, with relatively thin skin, limited tension, and suitable subcutaneous tissue deposition. In manufacturer studies, these devices have demonstrated comparable precision and accuracy to the standard finger-stick capillary blood glucose monitors in Type I and II diabetes.[9,15] Taken together, the features of the CGM offer an attractive potential refinement to cage-side blood sampling; however, this modality has not been evaluated in pigs.

We sought to determine if a CGM system could accurately measure glucose levels in swine. We compared CGM readings to a validated point-of-care glucometers and reference analyzer glucose serum chemistry (CHEM) in both euglycemic and diabetic pigs. We hypothesized that a CGM system would accurately detect glucose levels in swine in comparison with a validated analyzer and could be a refinement for diabetic studies in swine. As an ancillary goal, we also evaluated the accuracy of a point-of-care veterinary glucometer in comparison with the reference glucose analyzer.

Materials and Methods

Animals. Purpose-bred castrated male 4 to 6 mo old naive American Yorkshire pigs (n = 4) and STZ-induced diabetic Yucatan minipigs (n = 4) were acquired (York, Animal Biotech Industries; Yucatan, Sinclair Bioresources) and acclimated for at least 48 h prior to experimental use as per institutional standard operating procedures. Healthy pigs and diabetic pigs were fed a standard diet (Laboratory Mini-Pig Grower Diet, Lab Diet). Pigs were individually housed in 16.25ft^2 elevated caging with grated flooring and rubber mats. All pigs were provided with daily socialization and were in the standard enrichment program. All pigs were housed and maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, the Guide)[19] in an AAALAC-accredited facility. All procedures were approved by the Columbia University IACUC and followed applicable governmental policies and regulations. After completion of all study-driven events, pigs were euthanized with Euthasol (100 mg/kg IV) in accordance with the protocol and AVMA guidelines.[27]

Glycemic Regulation. Insulin administration (Humulin-N, subcutaneously twice daily, range 0 to 6 IU) was titrated to target nonketotic chronic hyperglycemia (300 to 400 mg/dL) in the diabetic cohort. Insulin dose was based on cageside ear prick sampling, using a validated portable blood glucose monitor (POCM A). Blood glucose was measured prior to morning or afternoon feedings. Healthy pigs exhibited normal blood glucose levels; no glycemic manipulations were performed on these animals.

All pigs were instrumented with the FreeStyle Libre (Abbott Labs) CBGM sensor, placed posterior to the base of the ear in conscious free-moving pigs or under light sedation with Tiletamine–zolazepam (5 mg/kg IM, Zoetis). If possible, placement was performed under scheduled sedation to ensure security of sensor placement for this particular study, although it should be noted that sensors may be placed in awake animals. The skin site was clipped of hair and cleansed with alcohol to remove external debris, and the sensor was placed using the manufacturer's provided application device. Prior to placement of the sensor, the Yorkshire pigs were also instrumented with a 5 French indwelling central line in the external jugular vein for a subsequent study (Groshong NXT PICC, BD). For central line placement, each pig was sedated with tiletamine–zolazepam (5 mg/kg IM, Zoetis), orotracheally intubated under propofol (2 to 5 mg/kg IV to effect), and maintained under inhalant general anesthesia (isoflurane 1% to 3%, delivered in 100% oxygen). The pig was placed in dorsal recumbency and once adequately anesthetized, the ventral neck was aseptically prepped. Ultrasound-guided percutaneous access was acquired in the right or left external jugular and the central line was placed by modified Seldinger technique. After confirming proper placement within the superior vena cava, the pig was repositioned into lateral recumbency and the dorsal interscapular region was aseptically prepped. Using a trocar, the line was tunneled to exit at the interscapular region and secured with 2-0 silk anchoring sutures. Pigs were fitted with a mesh jacket (Lomir Biomedical) and the externalized line was placed within the dorsal jacket pocket. Prior to anesthetic recovery, pigs received buprenorphine (0.02 mg/kg IM) and carprofen (4 mg/kg SQ). In the postoperative period, pigs received carprofen 4 mg/kg PO SID for 2 d after line placement.

For reference serum chemistry analysis, whole venous blood was collected (1.5 mL, n = 43 samples). Serum chemistry analysis was performed inhouse by trained personnel using a calibrated Heska Element DC. In the healthy cohort, whole blood was sampled using indwelling central venous access in acclimated, free-moving pigs. Diabetic pigs were lightly sedated with Telazol (5mg/kg IM) and blood was collected via an auricular vein.

Peripheral blood glucose measurements were collected via cage-side ear prick sampling for daily assessments over a 30-d period and insulin determinations. In addition, all whole blood collected for chemistry was measured on glucometers (POCM A: AccuCheck Aviva, Roche; POCM B: AlphaTRAK-2, Zoetis). POCM A has been validated for use in humans while POCM B has been validated for veterinary use. At each ear prick or venous blood collection, data were collected from the CBM device using the manufacturer's handheld monitor (n = 45 samples).

Statistical Analysis. All blood glucose values are expressed as mean ± SD. Linear correlation and Bland-Altman analyses were performed for all datasets, with Bland-Altman plots generated by plotting the average of the test and reference blood glucose values against the difference between the measurements.[8] Surveillance error grid (SEG) analysis was performed as an aggregated risk assessment, aimed at estimating the risk of glycemic interventions based on blood glucose measurements with a known degree of error. SEG analysis is similar to the classic Clarke and Parkes error grid, with modifications to incorporate finer gradations of risk for inaccurate blood glucose measurements.[22] This provides a clinically relevant interpretation of measurement errors, as the risk of blood glucose over- or underestimation changes in nonlinear fashion with reference

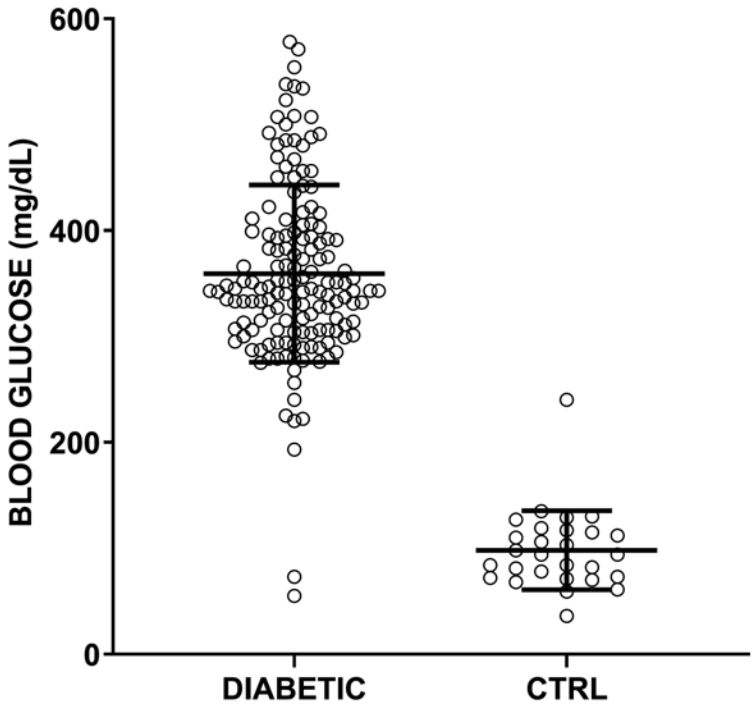

Figure 1. Individual blood glucose measurements for all subjects, assessed via POCM A (n = 179 samples; n = 151 from diabetic cohort, n = 28 from healthy cohort).

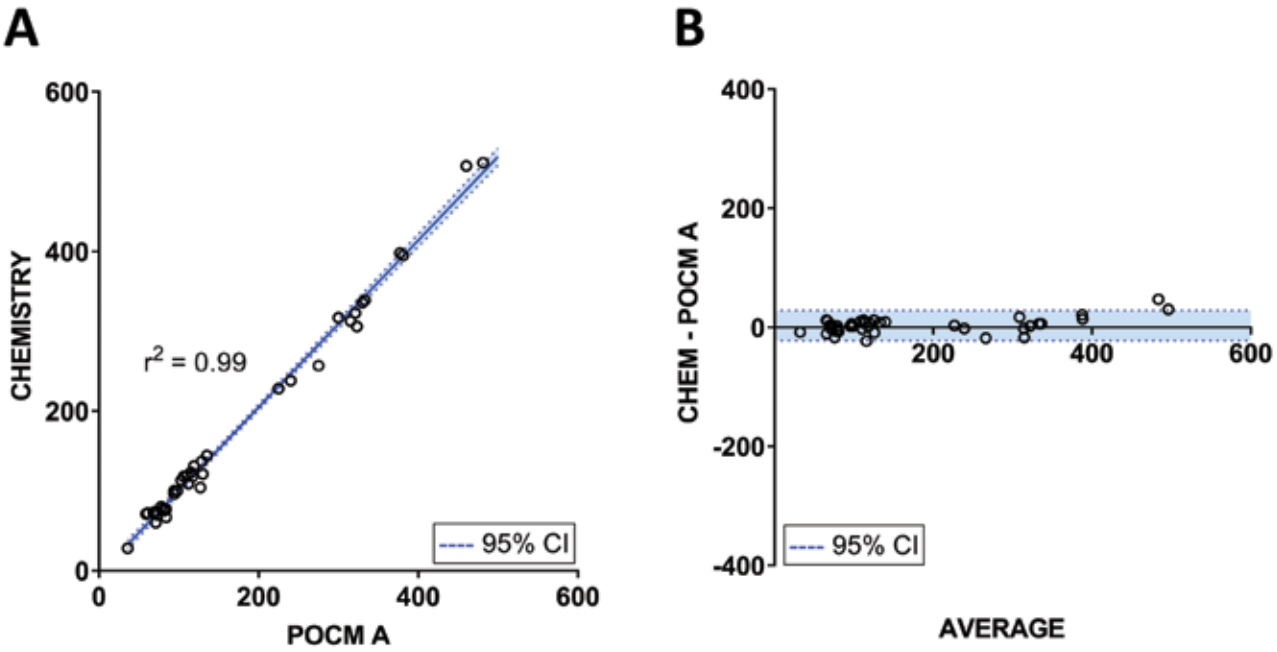

Figure 2. Comparison of point-of-care monitor A and chemistry analyzer blood glucose measurement (mg/dL). Linear correlation (left) and Bland-Altman plot (right). Bland-Altman is plotted as difference over average (chemistry analyzer value - point-of-care monitor A over the average of both values).

blood glucose and is difficult to estimate by standard statistical analyses. Plots were generated using the SEG software.[25] Device validation was defined based on ISO performance standards for glucometers (ISO 15197:2013), with 95% of values within 15% for reference values equal to or greater than 100 mg/dL, and within 15 mg/dL for reference values below 100 mg/dL.[20]

Results

Diabetic pigs exhibited persistent hyperglycemia, while healthy pigs exhibited normal blood glucose levels (Figure 1, 359 ± 83 compared with 98 ± 37 mg/dL by POCM A, respectively; range 41 to 536 mg/dL, n = 179 samples).

In 40 samples, time-matched chemistry analysis was performed for validation of the POCM A, with good linear

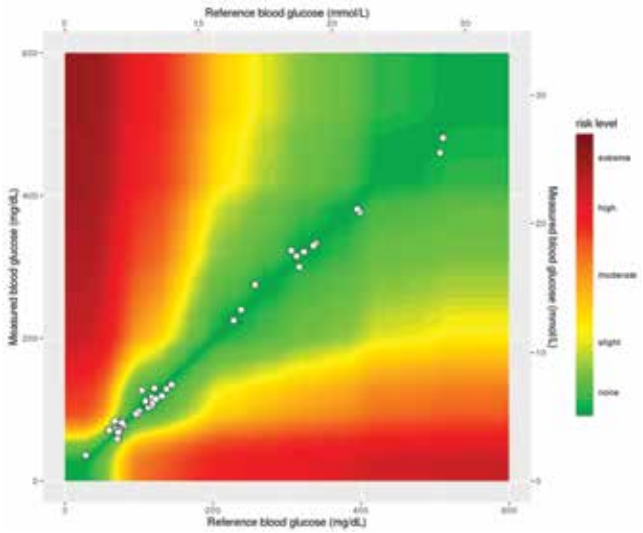

Figure 3. Color-coded surveillance error grid analysis of point-of-care monitor A blood glucose measurements (y-axis), with chemistry values as the reference (x-axis).

Table 1. Blood glucose analyzer distributions across surveillance error grid risk categories, compared against the reference analyzer

	POCM A		CBGM		POCM B	
SEG Risk Category	*n*	%	*n*	%	*n*	%
None	35	87.5	40	88.9	23	54.8
Slight, Lower	4	10	3	6.7	17	40.5
Slight, Higher	1	2.5	2	4.4	1	2.4
Moderate, Lower	0	0	0	0	1	2.4
Moderate, Higher	0	0	0	0	0	0
Severe, Lower	0	0	0	0	0	0
Severe, Higher	0	0	0	0	0	0
Extreme	0	0	0	0	0	0

Table 2. Blood glucose measurement distributions according to International Organization for Standardization ranges.

	POCM A		CBGM		POCM B	
ISO Range	*n*	%	*n*	%	*n*	%
≤5% or 5 mg/dL	17	42.5	12	26.7	3	7.1
>5% to 10% or 5–10 mg/dL	17	42.5	10	22.2	5	12
>10% to 15% or 10–15 mg/dL	4	10	7	15.6	3	7.1
>15% to 20% or 15–20 mg/dL	1	2.5	5	11.1	7	16.7
>20% or 20 mg/dL	1	2.5	11	24.4	24	57.1
% within ≤15% or 15 mg/dL	95		64.6		26.2	

ISO range = difference between the test blood glucose analyzer (POCM A, CBGM, POCM B) and the reference analyzer as percent of REF for REF > 100 mg/dL and in mg/dL for REF <= 100 mg/dL

correlation (Figure 2, $r^2 = 0.99$). Using chemistry results as reference values, surveillance error grid analysis indicated no clinical risk (87.5%) or slight risk (12.5%) for POCM A comparison errors (Figure 3, Table 1). POCM A measurements met ISO performance standards for glucometer validation (Table 2), and was used as a reference analyzer for CBGM evaluation, alongside chemistry analysis and whenever time-matched corresponding chemistry analysis was not available (Figure 4).

Time-matched CGM demonstrated good linear correlation with both reference methods (Figure 5, $r^2 = 0.92$ POCM A, $n = 42$ samples; $r^2 = 0.96$ CHEM, $n = 17$ samples). The slope difference between CGM compared with POCM A and CGM compared with CHEM was insignificant ($P > 0.05$). For Bland-Altman plots and SEG analysis, CGM was compared with pooled CHEM and POCM samples ($n = 45$, REF). CHEM was used when available ($n = 17$ samples) and POCM A for remaining samples ($n = 28$). Surveillance error grid analysis indicated no clinical risk (89%) or slight risk (11%) for all CGM comparison errors using this pooled dataset (Figure 6, Table 1). However, only 64% of CGM measurements met ISO performance standards for glucometer validation (Table 2). On Bland-Altman plots, the CGM demonstrated a greater bias than did the validated POCM A (Figure 5).

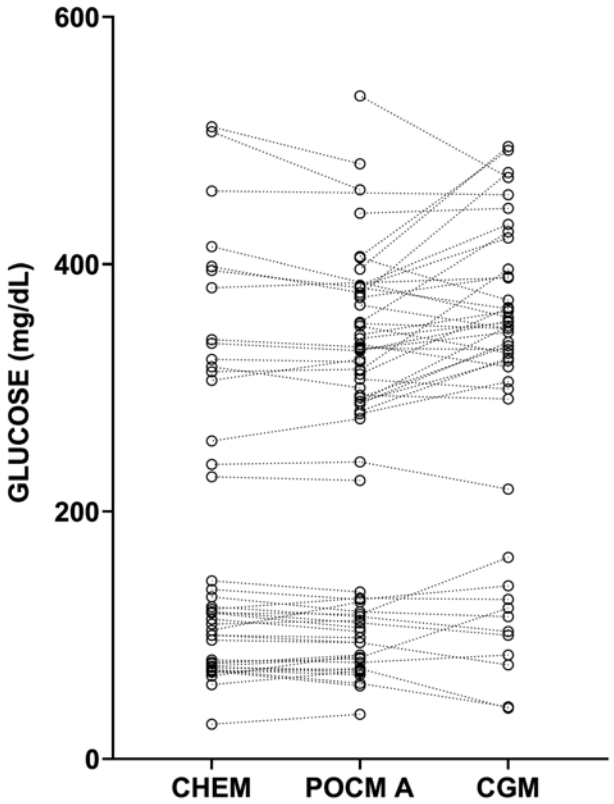

Figure 4. Time-matched glucose measurements, assessed via chemistry ($n = 43$), point-of-care monitor A ($n = 68$), and/or continuous blood glucose monitor ($n = 45$).

The POCM B glucometer also showed a linear correlation with the chemistry reference (Figure 7, $r^2 = 0.93$, $n = 43$ samples), with a greater bias demonstrated on Bland-Altman plots compared with the POCM A or CGM. On SEG analysis (Figure 8), 55% of POCM B measurement errors indicated no clinical risk against the reference value, and 45% indicated slight or moderate clinical risk (Table 1). Only 26% of measurements were compliant with ISO standards (Table 2).

Discussion

Our results indicate that the noninvasive skin patch CGM can contribute to diabetic management with little to no clinical risk. However, the accuracy of the CGM is not superior to the existing point-of-care monitors in the current study design and fails to achieve validation according to ISO standards for glucometers. Therefore, the GCM is not a suitable substitute for data collection. Only POCM A met ISO validation standards under our conditions. POCM B exhibited a bias toward overestimation of blood glucose as reflected on Bland-Altman plots. This would

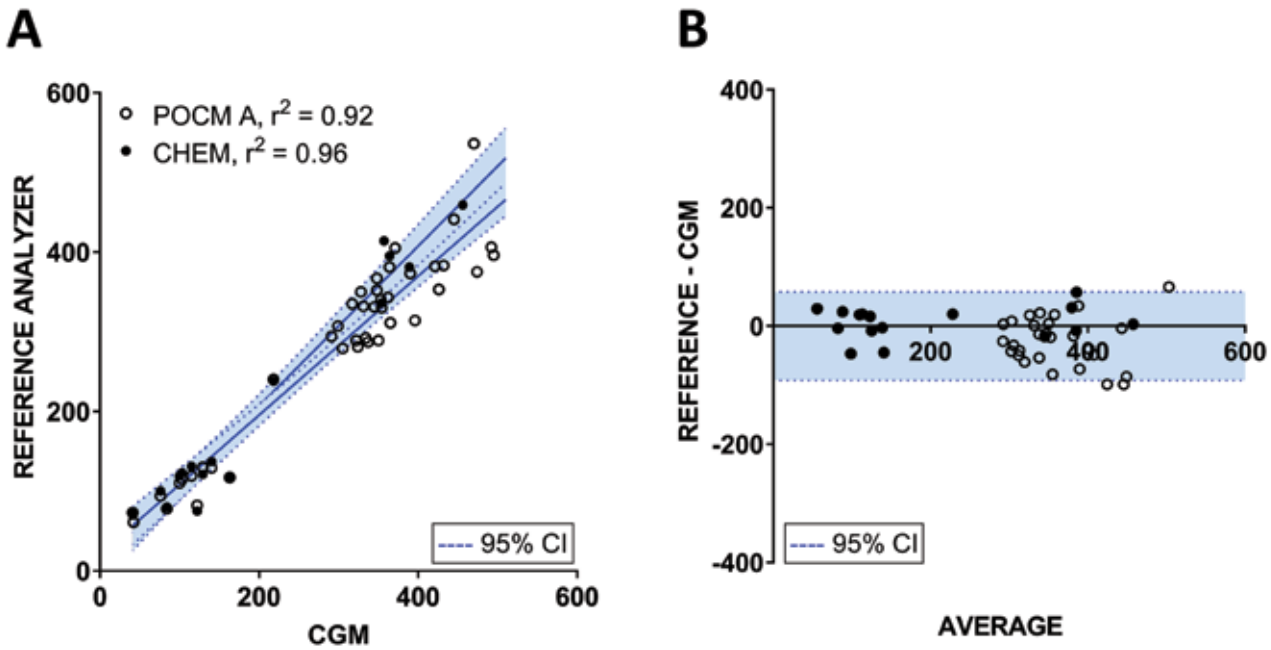

Figure 5. Comparison of continuous glucose monitor and reference analyzer blood glucose measurement (mg/dL, closed circles = chemistry analyzer, open circles = point-of-care monitor A). Linear correlation (left) and Bland-Altman plot (right). Bland-Altman is plotted as difference over average (reference analyzer value - continuous glucose monitor over the average of both values).

create a greater clinical risk potential on SEG analysis, as overcorrection of hyperglycemia with insulin administration could result in acute hypoglycemia.

Accurate assessment of blood glucose is an essential component of preclinical diabetic studies to ensure colony health and to provide reliable, repeatable study results. CGMs are an attractive means to monitor the animals in preclinical studies because they eliminate the stress associated with serial collection of blood samples. CGMs have been validated in humans, cats, dogs, and horses,[6,41,42] but to our knowledge, CGMs have not been previously validated in diabetic swine. We chose to test the CGM system for several reasons. The skin patch sensor has a low profile and a small size (5 mm high and 35 mm in diameter with the subcutaneous sensor filament approximately 0.4 mm thick). The CGM we used has been consistently accurate in human studies when compared with capillary blood glucose values, regardless of subject characteristics, for 14 d.[6] Porcine skin mirrors human skin in both extracellular matrix[10] and vascularization,[37,39] making human skin patch CGM appropriate for swine studies. In addition, in dogs, this glucose monitoring system has been validated in short term studies for accurately detecting blood glucose, showing potential veterinary applications for this technology.[12]

POCM A was our human point-of-care glucometer of choice, as it met both the requirements of the US Food and Drug Administration and the International Organization for Standardization. In a recent study investigating the accuracy of at-home blood glucose monitors, POCM A was one of 6 marketed monitors to meet accuracy standards in 3 separate studies.[23] The current American Animal Hospital Association Diabetic Management Guidelines does not recommend the use of human glucometers for the veterinary patient due to inaccuracies seen on human glucometers reading canine and feline blood. Rather, the use of POCM B is recommended as it has been calibrated in canines and felines.[7] Thus, our institution has historically used this point-of-care monitor for colony management, and it was incorporated in this study. The challenge arises in choosing

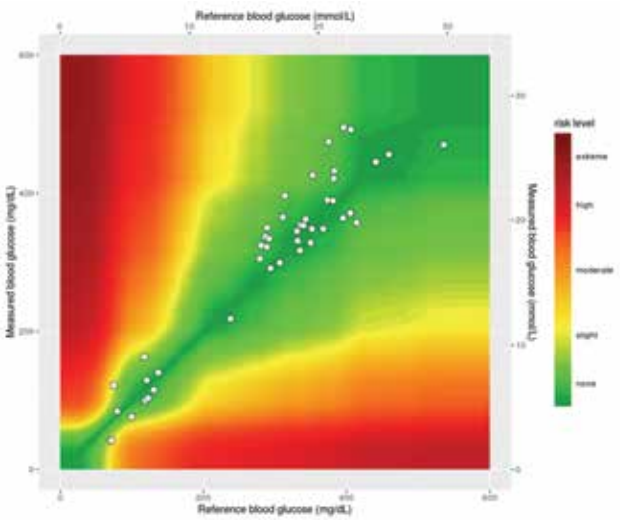

Figure 6. Color-coded surveillance error grid analysis of continuous glucose monitor blood measurements (y-axis), with chemistry and point-of-care monitor A values as the reference (x-axis).

a glucometer for preclinical studies that use nontraditional veterinary species, where a wealth of diabetic management experience may not be available. Often in studies using animals as diabetic models, point-of-care glucometers are used to monitor and manage blood glucose levels without established validations or backing from the literature. To our knowledge, no specific veterinary products are marketed with calibrations for swine capillary blood samples. The CGM used in this study was precalibrated by the manufacturer to enhance at-home use by the patient, but this calibration cannot be modified or repeated during use.

Continuous glucose monitors, point-of-care glucometers, and standard laboratory methods use different techniques for glucose measurement. Both point of care monitors operate on single-use strips and are designed to measure whole

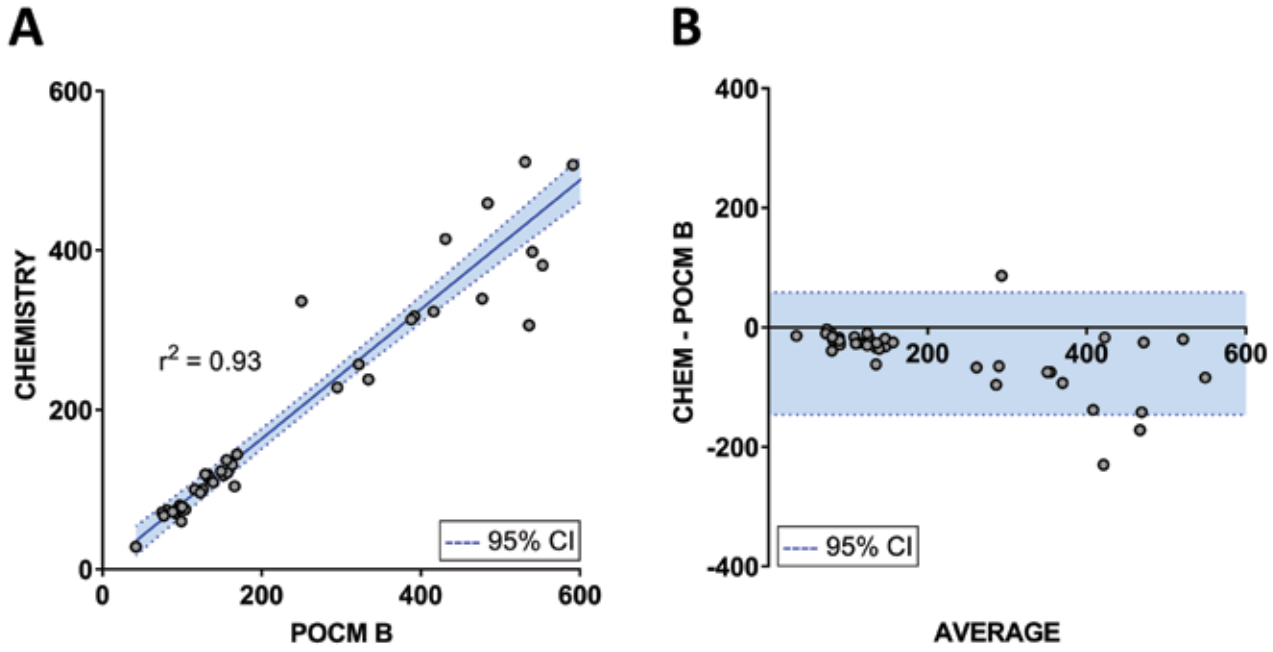

Figure 7. Comparison of point-of-care monitor B and chemistry analyzer blood glucose measurement (mg/dL). Linear correlation (left) and Bland-Altman plot (right). Bland-Altman is plotted as difference over average (chemistry analyzer value - point-of-care monitor A over the average of both values).

capillary or venous blood by glucose dehydrogenase assay.[38,45] The continuous glucose monitor measures interstitial glucose levels in the microenviroment around the subcutaneous filament by glucose oxidase technique.[12] The Heska Element DC measures serum glucose level in venous blood by dry chemistry and spectrophotometry.[18] While all of these assay techniques are well-established, a degree of error may be encountered due to the variability between blood glucose and interstitial glucose.[35] In the human literature, CGMs have demonstrated the potential to anticipate hypoglycemic events, as tissues first uptake available glucose within the interstitium, while glucose stores in the blood may take longer to deplete.[5] While this characteristic may be leveraged for clinical management in some cases, it can confound comparative blood glucose assessments. Glucose levels in the blood equilibrate with the interstitial space through diffusion, with time estimates ranging from almost zero "lag" time to over 20 min in studies of humans, rats, and dogs.[11,33,34,44] This lag period is affected by disease state and the magnitude of change.[35] As a result, CGMs may demonstrate variable sensitivity for characterizing acute glycemic manipulations.

In our dataset, this fluctuation could account for a portion of the error between the CGM and the reference analyzer. However, our pigs were evaluated in the fasting state, in which the interstitial and venous compartments are likely to be well-equilibrated.[24] While this fluctuation would be mild with regard to clinical risk for insulin administration, it would introduce an unacceptable degree of error in the study-driven assessment of novel glycemic interventions. One limitation of our study is that data-driven glycemic manipulations were not available to assess the responsiveness of the device. A recent study evaluated the responsiveness of the CGM used in this study in a swine model where the CGM was placed intraperitoneally, and found no differences between placements of the CGM in the 4 abdominal quadrants or between intraperitoneal or subcutaneous measurements.[2] Lastly, sedation could induce rapid changes in blood glucose, which may not be reflected in the CGM reading at time-matched sampling. Early in the course

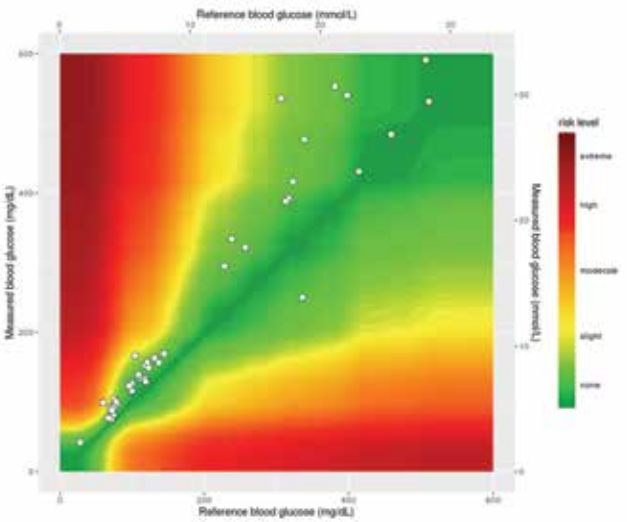

Figure 8. Color-coded surveillance error grid analysis of point-of-care monitor A blood glucose measurements (y-axis), with chemistry values as the reference (x-axis).

of sedation, we would expect a potential hyperglycemic shift secondary to stress. We considered this effect, and did not find a positive bias on venous samples in sedated pig; rather, the CGM tended to read slightly higher. Therefore, we determined that stress hyperglycemia did not contribute to experimental error. However, if sedation or restraint is required for study purposes, glycemic patterns should be monitored during this period.

Further study is necessary to determine whether other sensors or alterations in sensor placement could achieve improved accuracy for clinical diabetic management. Several other skin patch monitors on the market have the potential to be validated in swine for diabetic studies, each with slightly different features and drawbacks in size, durability, and filament design.

The device used in this study was a "flash" monitor, meaning that it only communicates real-time and stored data when scanned. Other CGMs communicate continuously and can be programmed to alert on low/high glucose ranges. In addition, a different skin location on the pig could be superior to the base of the ear. In the preliminary phase of this study, we placed the sensor at dorsal midline, just caudal to the scapulae. This placement proved unreliable, with sensors repeatedly failing to read within 24 h after placement. The sensor is designed to generate an error when unable to acquire sufficient contact for measurement, and suspected erroneous readings should be verified by a traditional blood glucose measurement technique. Pig skin thickness can range from 30 to 140 µm, with the thickest skin occurring over the dorsum and shoulders,[16,31] and the filament must achieve penetration into the interstitial bed. Pigs do have areas of thin skin, such as the base of the ear, but those areas are more mobile with a greater risk of sensor dislodgement and loss. Sensors may be more successful when placed on thinner areas of pig skin, such as the inguinal areas or the flanks.[13] CGM use in species with thinner skin, such as rabbits or primates, may be more successful and further testing of CGM in diabetes models using these species should be explored.

Because the GCM device is adhered to the skin and exposed to the external environment, it could potentially be dislodged under some conditions. The adhesive is constructed to withstand normal human activities, including showering and bathing, and did not appear affected by standard daily cage cleaning procedures in our facility. However, vigorous contact against cage surfaces could shorten the device's adhesive lifespan. In addition, inquisitive cage-mates could pose a significant threat to the device, so device use would likely preclude group housing of pigs. Once dislodged, it must be replaced with a new device, as the filament cannot be reinserted into the subcutis without the one-time use applicator. While the device filament itself is thin and superficial and unlikely to result in significant local inflammation, care should be taken to place the adhesive patch on a region of healthy skin. Due to the duration of adhesive contact and a predisposition to poor healing among diabetic individuals, dermatitis and local reactions have been reported in humans.[30] However, this was not observed in our porcine subjects.

Our results indicate that the continuous glucose monitor used in this study could be a useful adjunct to POCM for clinical blood glucose management in the stable diabetic pig and can provide a noninvasive and inexpensive alternative to serial blood collection for chronic colony maintenance. Of the methods assessed in this study, the veterinary point-of-care glucometer provided the least accurate blood glucose measurement when compared with reference serum chemistry analysis. While differences in agreement between the CGM and validated reference blood glucose assessments would likely permit clinically acceptable glycemic control, the CGM method is not a superior substitute for existing methods of blood glucose measurement in preclinical study designs. Further study is warranted to characterize the CGM in porcine physiology and in the presence of acute glycemic interventions.

Acknowledgments

The authors would like to thank Katherine Losurdo LVT, RLATg, Amanda Blanchard LVT, RLATg, Kaylene Milano LVT, RALAT, and Ashley Welch LVT, RALAT for their help in carrying out animal care, socialization, and treatments of the pigs used in this study. We would also like to acknowledge the both authors made equal contributions to the experimental design, data collection, analysis, and composition of the manuscript.

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