Evaluation of a continuous glucose monitoring system in healthy dairy calves and adult goats

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OBJECTIVE
To determine the accuracy of a continuous glucose monitoring system (CGMS) device by comparing glucose concentrations measured over time as determined by the CGMS to those of the chemistry analyzer (reference method).

ANIMALS
7 healthy goats and 7 dairy calves.

METHODS
A randomized, crossover design with 3 treatments: control, hypoglycemia, and hyperglycemia. The CGMS device was applied to the neck. Hypoglycemia and hyperglycemia were induced by insulin and xylazine, respectively. Glucose concentrations were measured by the chemistry analyzer CGMS, point-of-care glucometer, and intensive care unit machine at 0 (before treatment), 2, 4, 6, 8, 10, and 12 hours. Agreement between the CGMS and the chemistry analyzer was determined by Bland-Altman plots. The analytical and clinical accuracy of the CGMS was determined using the International Organization for Standardization (ISO) 15197:2013 criteria and the Parkes error grid analysis.

RESULTS
In goats, the CGMS overestimated glucose concentrations during the hypoglycemic, normoglycemia, and hyperglycemia treatments. In calves, the CGMS underestimated glucose concentrations during the hypoglycemic treatment but overestimated glucose concentrations in normoglycemia and hyperglycemic treatments. The CGMS met the ISO clinical accuracy criteria for goats and calves, with > 99% of the glucose measurements in zones A and B of the Parkes grid. However, the CGMS did not meet the ISO 15197:2013 criteria for analytical accuracy.

CLINICAL RELEVANCE
The CGMS evaluated in our study only met the ISO 15197:2013 clinical accuracy criteria, not the analytical accuracy. Therefore, the device might be considered for clinical use.

Keywords: calf, goat, accuracy, continuous, glucose

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glucometers, or ICU analyzers requires the physical restraint of animals to collect blood by venipuncture. The CGMS device is a 3.5-cm-diameter sensor placed on the skin, and test results are acquired with minimal physical restraint and no blood collection. The CGMS device sensor adheres to the skin and has a small filament that inserts directly under the skin and measures interstitial glucose concentrations, which have a set equilibrium with blood glucose concentrations.\(^2\) The glucose test result data are transmitted from the sensor wirelessly and displayed on the reader or through a mobile phone application. The CGMS device sensor can remain attached to the skin for up to 14 days and measures minute-to-minute glucose concentration changes. The CGMS device allows for monitoring glucose concentration changes multiple times per day. When a mobile phone application (the cost for the device is $90 and $0 for the mobile phone application) is used for reporting the results, the average daily cost for multiple readings using the CGMS over 14 days is $6 to $7. Thus, the CGMS might be a desirable alternative for hospitalized goats and calves requiring continuous glucose monitoring. Examples of diseases that require continuous glucose monitoring include diarrhea and septicemia in calves\(^7\) and pregnancy toxemia in goats.\(^10\)

Despite being evaluated in other species, information on the use of CGMS devices in calves and goats is limited. The objective of this study was to determine the accuracy of a CGMS device by comparing glucose concentrations measured over time as determined by the CGMS, the ICU analyzer, and the POC glucometer as compared to the chemistry analyzer (reference method) in normoglycemic, hypo-, and hyperglycemic healthy calves. The glucose test result data are transmitted from the sensor wirelessly and displayed on the reader or through a mobile phone application. The CGMS device sensor can remain attached to the skin for up to 14 days and measures minute-to-minute glucose concentration changes. The CGMS device allows for monitoring glucose concentration changes multiple times per day. When a mobile phone application (the cost for the device is $90 and $0 for the mobile phone application) is used for reporting the results, the average daily cost for multiple readings using the CGMS over 14 days is $6 to $7. Thus, the CGMS might be a desirable alternative for hospitalized goats and calves requiring continuous glucose monitoring. Examples of diseases that require continuous glucose monitoring include diarrhea and septicemia in calves\(^7\) and pregnancy toxemia in goats.\(^10\)

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**Methods**

**Study design and animals**

Sample size calculation was determined using JMP (JMP Pro, version 17.0; SAS Institute Inc). Sample size calculation was based on studies in foals\(^3\) that reported a mean bias of 6.2 mg/dL of glucose when comparing the CGMS test result to the chemistry analyzer, \(\alpha = 0.05\), power = 80%, assumption of a clinically relevant detectable difference of 10 mg/dL (standard deviation) of glucose by the CGMS, assuming a two-tailed test and a preferred Cohen’s \(d \geq 0.5\) for effect size. The minimal sample size required was 6 animals. To account for a 10% dropout, 7 goats and 7 calves were enrolled. A three-way, crossover-design clinical trial was performed with 3 treatments (normoglycemia, hypoglycemia, and hyperglycemia), with a two-day washout period. The sequence of treatments was randomized by picking, without replacement, 3 labeled cards representing the treatments from a hat. The study was approved by the University of California Davis Animal Care and Use Committee (Protocol #22821).

The research was performed from June 2022 through October 2022.

**Goat and calf enrollment**

Seven nonpregnant goats (3 Saanen, 2 Alpine, and 2 Lamancha and Alpine crossbreeds) aged 14 to 15 months were enrolled. Goats were housed in group pens and fed alfalfa, grass hay, and water ad libitum. The goats remained in their pens during the entire study period. Three goats were enrolled first, followed by the enrollment of 4 goats.

Seven Jersey bull calves with an age range of 4 to 9 days were enrolled. Calves were enrolled after observed birth and fed 2 L of colostrum thrice within the first 24 hours by orosophageal intubation. All calves were confirmed to have ingested adequate colostrum based on serum total solids concentrations > 6.0 g/dL.\(^11\) Calves were housed in individual calf hutch and fed 3 L of milk replacer (Suckle Pro; Manna Pro) and 0.5 kg of commercial calf concentrate twice daily. Four calves were enrolled first, followed by the enrollment of 3 calves. The calves were housed in individual calf hutches during the study.

**Continuous glucose monitoring system device placement**

The investigators performed the clinical examination. CBC and serum biochemical analysis were performed once at the University of California, Davis Veterinary Medical Teaching Hospital Clinical Pathology Laboratory to confirm that the animals were healthy. Intravenous catheters were placed in the jugular vein for subsequent blood collection in calves. Intravenous catheters were not placed in goats to prevent pulling or chewing the intravenous catheters in group housing. A 10 X 10-cm area in the neck was clipped, scrubbed with betadine solution, wiped with alcohol-soaked gauze, and let to dry. The CGMS device (FreeStyle Libre 2; Abbott Laboratories) was placed according to the manufacturer’s recommendations. Four drops of adhesive (Super glue; Elmer) were applied at 12, 3, 6, and 9 o’clock positions to ensure the device remained in place. The animals were not sedated for the placement of the CGMS device. In goats, the device was covered with an adhesive bandage (Elastikon; Johnson and Johnson) to prevent pen mates from removing the device (Figure 1). The device was left uncovered in the calves (Figure 2). The device was ready to acquire glucose concentrations after 1 hour. The devices were maintained on goats and calves for 10 days. The CGMS device had an operating ambient temperature range of 10 to 45°C. The devices were also checked during the wash-out period to confirm they were working.

**Treatments**

Hypoglycemia was induced by administering a short-acting (6 to 9 hours of duration of action) insulin (Novolin R; Novo Nordisk) at 0.1 units/kg intramuscularly once based on studies\(^2\) in horses. Hyperglycemia was induced by the administration of xylazine hydrochloride (AnaSed; Akorn Animal

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Health) at 0.15 mg/kg intravenously once. After administration of insulin, calves and goats were monitored for signs of severe hypoglycemia, including weakness, recumbency, or seizures. After administering xylazine hydrochloride, calves and goats were monitored for lateral recumbency and regurgitation. No medications were administered to the calves and goats during the normoglycemia treatment. For all treatments, animals had access to food and water. The experimental protocol is summarized in Figure 3.

**Sample collection and analysis**

Blood samples were collected directly from the jugular vein in goats, whereas samples were collected from intravenous catheters in calves. In calves, a 12-mL syringe was rinsed with heparin (heparin sodium; Fresenius Kabi) by drawing 1 mL and then flushing the syringe empty. Before sample collection, 6 mL of blood was scavenged into the 12-mL syringe to minimize the sample dilution due to the flushing of the catheters with heparinized saline (1:10, dilution of heparin:0.9% saline) between sample collections. Two investigators performed sample analysis. The first investigator collected a volume of 6 mL of blood into a syringe. A blood drop from the syringe was used to measure glucose concentrations immediately using a POC glucometer (AlphaTrak; Zoetis). The remaining blood sample was transferred to a 4-mL tube with heparin as an anticoagulant (BD Vacutainer; Beckton and Dickinson). The scavenged
blood was then returned to the animal through the catheter, and the catheter was flushed with heparinized saline. The second investigator measured glucose concentrations by holding the CGMS reader near the device immediately after the first investigator collected the blood sample. Only 1 device could be paired with 1 reader at a time.

### Statistical analysis

Data were analyzed using GraphPad (GraphPad Prism, version 10.0; GraphPad Software) and JMP (JMP Pro, version 17.0; SAS Institute Inc.). The Shapiro-Wilk test was used to test the data for normality. Mean ± SD was reported when data were normally distributed, whereas median (range) was reported when data were not normally distributed. Descriptive data, including body weight and procedure complications, were recorded. The effect of treatment and measurement method on glucose concentrations was determined by a two-way ANOVA and Friedman test when data were normally distributed and not normally distributed, respectively.

The chemistry analyzer was the reference method for measuring glucose concentrations. Agreement between the CGMS, ICU machine, or POC glucometer and the chemistry analyzer was determined by calculating bias and 95% limits of agreement using Bland-Altman plots for repeated measures. A positive bias indicated that the CGMS, ICU machine, or POC glucometer underestimated glucose concentrations compared to the chemistry analyzer. A negative bias indicated that the CGMS, ICU machine, or POC glucometer overestimated glucose concentrations compared to the chemistry analyzer.

The analytical and clinical accuracy of CGMS was determined by comparing glucose concentrations measured by the chemistry analyzer and the CGMS using the International Organization for Standardization (ISO) 15197:2013 criteria. The ISO 15197:2013 minimum criteria for acceptable system accuracy are: 1) for analytical accuracy assessment, at least 95% of CGMS results have to be within ±15 mg/dL at glucose concentrations <100 mg/dL and within ±15% at ≥100 mg/dL when compared to the chemistry analyzer and 2) for the assessment of clinical accuracy, at least 99% of results have to be within zones A and B in a consensus error grid. The clinical accuracy of the CGMS was determined using the Parkes consensus error grid analysis for diabetes mellitus type 1. The Parkes error grid was constructed using Microsoft Excel (Microsoft Corp) as previously described. The Parkes error grid analysis was performed by plotting the reference glucose measurements obtained by the chemistry analyzer (x-axis) against the interstitial glucose measurements obtained by the CGMS (y-axis). P < .05 was considered significant.

### Results

The data were normally distributed. The mean ± SD weight was 67.8 ± 3.6 and 29.5 ± 4.3 kg for goats and calves, respectively. The goats and calves were sedated from the administration of xylazine hydrochloride and were in sternal recumbency for 15 to 20 minutes. Regurgitation was not observed in any of the animals. Four and 2 CGMS devices were replaced once in different goats and calves because of detachment from rubbing against the feed bunk or calf hutch, respectively. All of the CGMS devices were replaced during the wash-out period; the time from placement to detachment ranged from 2 to 4 days. The goats and calves did not experience any other complications, including skin lesions from the placement of the CGMS devices.

### Glucose measurements and accuracy of CGMS in goats

Mean ± SD glucose concentrations before procedures (0 hours) based on the chemistry analyzer were 69.9 ± 2.4, 69.6 ± 2.1, and 72.6 ± 5.7 mg/dL for hypoglycemic, normoglycemic, and hyperglycemic treatments, respectively. Mean ± SD glucose concentrations at the first sample collection after procedures (2 hours) based on the chemistry analyzer were 63.0 ± 2.5, 67.6 ± 2.5, and 161.4 ± 51.3 mg/dL for hypoglycemic, normoglycemic, and hyperglycemic treatments, respectively. The glucose concentrations were not significantly different (69.9 vs 63.0 mg/dL; P = .297) at 2 hours after insulin administration compared to 0 hours. The glucose concentrations were higher at 2 hours (161.4 vs 72.6 mg/dL; P = .016) after xylazine hydrochloride administration compared to 0 hours. The treatment (P = .013) and method of glucose measurement (P = .004) had a significant effect on the glucose concentrations, whereas animal (P = .849) or interaction between treatment and method of glucose measurement (P = .997) did not have a significant effect on the glucose concentrations. Biases (95% limits of agreement) for the CGMS were 8.8 (~13.6, 31.2), 1.2 (~6.9, 29.2), and 1.7 (~43.5, 46.8) mg/dL during hypoglycemia, normoglycemia, and hyperglycemia treatments, respectively. Agreements between the chemistry analyzer and CGMS, ICU machine, and POC glucometer in goats are summarized in Table 1.

There were 147 paired samples possible from all goats. A total of 131 paired samples with glucose concentrations <100 mg/dL based on the chemistry analyzer were available. Based on the ISO 15197:2013 criteria, 86 of 131 (65.7%) CGMS glucose concentration results were within ±15 mg/dL at glucose concentrations <100 mg/dL. Eight samples had glucose concentrations >100 mg/dL, with 6 of 8 (75%) samples within ±15% of the chemistry analyzer measurements. Eight paired measurements were incomplete and were excluded from the analysis. Parkes consensus error grid analysis indicated that 139 of 139 (100%) of all glucose concentration measurements by the CGMS fell in the A and B zones (Figure 4).

### Glucose measurement and accuracy of CGMS in calves

Mean ± SD glucose concentrations before procedures (0 hours) based on the chemistry analyzer...
were 123.7 ± 19.6, 90.1 ± 8.3, and 132.0 ± 30.3 mg/dL for hypoglycemic, normoglycemic, and hyperglycemic treatments, respectively. The glucose concentrations were lower at 2 hours (101.7 vs 123.7 mg/dL; \(P = .016\)) after insulin administration compared to 0 hours. The glucose concentrations were not significantly different (132.0 vs 162.6 mg/dL; \(P = .156\)) at 2 hours after xylazine hydrochloride administration compared to 0 hours. The treatment

<table>
<thead>
<tr>
<th></th>
<th>Hypoglycemia</th>
<th>Normoglycemia</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats CGMS</td>
<td>8.8 (-13.6, 31.2)</td>
<td>10.2 (-8.9, 29.2)</td>
<td>1.7 (-43.5, 46.8)</td>
</tr>
<tr>
<td>ICU machine</td>
<td>-1.3 (-4.7, 2.2)</td>
<td>0.5 (-3.6, 3.6)</td>
<td>-0.5 (-4.7, 3.5)</td>
</tr>
<tr>
<td>POC glucometer</td>
<td>-12.4 (-17.8 to 7.0)</td>
<td>-13.2 (-19.2 to 7.3)</td>
<td>-16.8 (-24, 9.3)</td>
</tr>
<tr>
<td>Calves CGMS</td>
<td>-2.2 (-56.7, 52.5)</td>
<td>8.1 (-29.4, 45.5)</td>
<td>6.7 (-54.0, 67.3)</td>
</tr>
<tr>
<td>ICU machine</td>
<td>-0.2 (-15.2, 14.7)</td>
<td>0.8 (-24.3, 26.0)</td>
<td>-4.5 (-14.9, 6.0)</td>
</tr>
<tr>
<td>POC glucometer</td>
<td>-4.3 (-19.4, 10.7)</td>
<td>1.7 (-25.5, 28.9)</td>
<td>-6.0 (-22.5, 10.5)</td>
</tr>
</tbody>
</table>

Figure 4—Parkes error grid depicting the risk zones in 7 goats. Zone A represents clinically accurate measurement with no effect on clinical action. Zone B represents altered clinical action with little or no effect on clinical outcome. Zone C represents altered clinical action, likely affecting the clinical outcome. Zone D represents altered clinical action, could have significant clinical risk. Zone E represents altered clinical action, which could have dangerous consequences. CGMS = Continuous glucose monitoring system.
(P = .069), method of glucose measurement (P = .472), animal (P = .493), or interaction between treatment and method of glucose measurement (P = .937) did not have a significant effect on the glucose concentrations. Biases (95% limits of agreement) for the CGMS were −2.2 (−56.7, 52.5), 8.1 (−29.4, 45.5), and 6.7 (−54.0, 67.3) mg/dL during hypoglycemia, normoglycemia, and hyperglycemia treatments, respectively. Agreements between the chemistry analyzer and CGMS, ICU machine, and POC glucometer in calves are summarized in Table 1.

There were 147 samples possible from all calves. A total of 64 paired samples with glucose concentrations < 100 mg/dL based on the chemistry were available. Based on the ISO 15197:2013 criteria, 39 of 64 (60.9%) of CGMS glucose concentration results were within ± 15 mg/dL at glucose concentrations < 100 mg/dL. Seventy-three samples had glucose concentrations > 100 mg/dL, with 33 of 73 (45.2%) samples within ± 15% of the chemistry analyzer measurements. Ten paired measurements were incomplete and were excluded from the analysis.

Parkes consensus error grid analysis indicated that 136 of 137 (99.3%) of all glucose concentration measurements by the CGMS fell in the A and B zones, whereas 1 of 137 (0.7%) fell in zone C (Figure 5).

Discussion

Our study demonstrated that the CGMS had acceptable clinical accuracy for monitoring glucose concentrations in healthy goats and calves. However, the CGMS did not meet the ISO 15197:2013 criteria for the analytical accuracy of monitoring glucose concentrations in goats and calves. Currently, no peer-reviewed studies in goats or calves reporting the analytical accuracy of the CGMS are available. However, studies19 in cats applying a similar CGMS device reported that 67.7% of the values were within ± 15 mg/dL of those measured by the chemistry analyzer at glucose concentrations < 100 mg/dL. The report in cats is consistent with our findings.
which determined that 65.7% and 60% in goats and calves, respectively, of the glucose concentrations measured by the CGMS were within ±15 mg/dL of those measured by the chemistry analyzer at glucose concentrations <100 mg/dL.

Insulin administration in goats did not induce hypoglycemia, and no clinical signs of hypoglycemia were observed, likely due to physiologic responses maintaining glucose levels because the goats were healthy. Our first sample collection for glucose measurement was 2 hours post insulin administration. Therefore, we might have missed transient hypoglycemia before 2 hours. In contrast, xylazine hydrochloride administration in goats induced hyperglycemia. Although the glucose concentrations in calves were lower after insulin administration (101.7 vs 123.7 mg/dL), these concentrations were not in the hypoglycemic reference range. This is because the mean glucose concentrations before insulin administration were high. Furthermore, our clinical pathology laboratory’s recommended reference range for glucose might be appropriate for adult cattle and not dairy calves. The reported mean glucose concentrations for dairy calves at 2 weeks of age, consistent with the calf age in our study, were 110.6 ± 22.1 mg/dL, ranging from 65.4 to 222.5 mg/dL. Xylazine hydrochloride administration did not significantly affect the glucose concentrations in calves, likely because the glucose concentrations were high (123.0 vs 162.6 mg/dL) before the administration of xylazine. There was more variability in the glucose concentrations in calves compared to goats. This is likely because the calves in our study were preruminant, and therefore the glucose concentration changes are associated with feeding, similar to monogastrics.

In goats, the CGMS overestimated glucose concentrations during the hypoglycemia, normoglycemia, and hyperglycemia treatments by an average of 8.8, 10.2, and 1.7 mg/dL, respectively, compared to the chemistry analyzer. In calves, the CGMS underestimated glucose concentrations by an average of 2.2 mg/dL during the hypoglycemia treatment but overestimated glucose concentrations by an average of 8.1 and 6.7 mg/dL in normoglycemia and hyperglycemia treatments, respectively, compared to the chemistry analyzer. Considering hypoglycemia is acutely life threatening compared to hyperglycemia, the magnitude of bias between the CGMS and chemistry analyzer was smaller in calves compared to goats during the hypoglycemia treatment. Among the 3 methods (CGMS, ICU panel, POC glucometer), the ICU machine had the lowest magnitude of bias compared to the chemistry analyzer. It should be noted that the bias calculated from Bland-Altman plots only defines the limits of agreements (a measure of analytical accuracy) between the CGMS and the chemistry analyzer but does not state whether the limits are acceptable. In contrast, clinical accuracy assessed by the error grid analysis is helpful in making clinical decisions on patients.

Although the device can remain attached to the skin for 14 days, we maintained the device for the 10-day duration of the study. Placing a bandage around the device in goats reduced the detachment of devices from pen mates or rubbing against the feed bunk. Rubbing against the hutch in calves resulted in detachment. Therefore, the use of CGMS devices might be limited to individually hospitalized patients, and the placement of a bandage around the device should be considered. We anticipate that the impact of replacing the devices on the study results is minimal given that the devices are replaced after 14 days with a new device paired with the same reader in clinical practice.

The ISO 15197:2013 criteria compare glucose measurements from a single compartment, such as blood. Therefore, comparing 2 different compartments, such as blood and interstitial fluid, using ISO15197:2013 criteria is challenging because of the differences in tissue properties of the compartments. Currently, there are no established criteria for assessing the accuracy of glucose concentrations in interstitial fluid. Therefore, the ISO15197:2013 criteria are widely used in human and veterinary medicine. The CGMS evaluated in our study only met the ISO 15197:2013 clinical accuracy criteria, not the analytical accuracy. Therefore, the device might be considered for clinical use. However, further studies evaluating the accuracy of the CGMS in sick, hypoglycemic, or hyperglycemic patients are warranted.

Our study has limitations. The goats and calves enrolled in this study were healthy. Therefore, normoglycemia was reestablished effectively by physiologic mechanisms within 2 hours. It is possible that the accuracy of the CGMS in detecting changes in glucose concentrations in sick animals with hypoglycemia or hyperglycemia might be affected by altered physiologic status. Induction of hypoglycemia was ineffective in goats and calves, whereas induction of hyperglycemia was ineffective in calves. Therefore, the CGMS should also be evaluated in sick hypoglycemic and hyperglycemic goats and calves. In contrast to sick animals that might be anorexic or fed a different diet, all animals in our study were fed recommended diets during the study, allowing the animals to reestablish normoglycemia. Although the focus of our study was to assess the accuracy of the CGMS in detecting the changes in glucose concentrations due to treatment over time, the impact of feeding animals on the CGMS readings in our study remains unknown.

The CGMS met the ISO 15197:2013 clinical accuracy criteria but not the analytical criteria in healthy goats and calves. Thus, it might be considered for clinically monitoring glucose concentrations in goats and calves.

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Disclosures

The authors have nothing to disclose. No AI-generated technologies were used in the generation of this manuscript.
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