Use of Glucose Monitoring Systems in Horses

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ACADEMIC ABSTRACT

Traditional methods of blood glucose monitoring involve obtaining samples for measurement via laboratory methodology or point of care devices and require invasive collection techniques such as capillary stick, venipuncture, or the placement of intravenous catheters. Limitations of traditional methods include the limited information provided by intermittent testing and the stress associated with restraint and discomfort experienced by patients. The snapshot nature of the provided information restricts a clinician’s ability to truly monitor trends in glucose concentrations over an extended period of time, influencing clinical decision making. The stress of invasive sampling can cause stress hyperglycemia in many veterinary species, complicating interpretation.

Continuous interstitial glucose monitoring technology is widely used in the human medical field due to the expansive information provided in a minimally invasive manner. In recent years, the device technology has advanced and cost has improved, prompting application of these devices into the veterinary sector. Studies have shown good agreement between newer glucose monitoring systems and traditional methods in small animal patients with diabetes mellitus, allowing veterinarians to obtain comprehensive glucose data with minimal stress and discomfort to the patient. However, information regarding the use of this new technology in equine medicine is limited. The following study describes the evaluation of two widely available glucose monitoring systems, the Dexcom G6 and the FreeStyle Libre, in healthy adult horses.
Monitoring of glucose concentrations is essential for the diagnosis and monitoring of a variety of disorders within equine medicine. Traditional methods of obtaining samples for testing include capillary stick, venipuncture, or the placement of intravenous catheters, which can cause stress and discomfort to equine patients. The information obtained by this testing methodology only allows for intermittent assessment of glucose concentrations, limiting the amount of information available for clinicians to make clinical decisions. The use of continuous glucose monitoring systems in the human medical field have allowed clinicians to obtain continuous or near-continuous glucose concentrations, improving interpretation. These devices have nearly eliminated the need for blood sampling for glucose concentrations, instead relying on interstitial glucose concentrations which have been shown to compare favorably to blood concentrations. Studies in small animal veterinary species, such as dogs and cats, have shown good agreement between newer glucose monitoring systems and traditional methods in small animal patients with diabetes mellitus, allowing veterinarians to obtain comprehensive glucose data with minimal stress and discomfort to the patient. However, information regarding the use of this new technology in horses is limited. The study described in the manuscript following evaluates the use of two widely available glucose monitoring systems, the Dexcom G6 and the FreeStyle Libre, in healthy adult horse.
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attributions

the research and writing associated with this manuscript would not have been possible without aid from colleagues. contributions are defined below.

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Chapter 1: Introduction

The monitoring of blood glucose concentrations is essential for the diagnosis and treatment of a wide variety of conditions in equine medicine. The measurement of blood glucose using traditional methods of capillary stick, venipuncture, or catheterization for sampling are time consuming for staff, stressful for patients, and provide limited information as to the glucose profile of the animal over time. Continuous glucose monitoring system technology has allowed for the minimally invasive measurement of interstitial glucose concentrations while providing near continuous glucose data for interpretation in humans, addressing many of the limitations of traditional glucose monitoring. In recent years, improvement in technology and increased affordability have made these systems feasible for use in the veterinary sector with increasing use in small animal medicine cases, however information as to their accuracy and reliability in equine medicine is limited.

This thesis is compiled and formatted to discuss the current understanding of knowledge of continuous glucose monitoring devices and their application to equine medicine. The following literature review will provide information on glucose metabolism in equids and disorders resulting in glucose dysregulation requiring monitoring. Traditional glucose monitoring in veterinary medicine will then be discussed. A review of glucose monitoring system technology, application, and efficacy of use in human medicine followed by guidelines for evaluation of accuracy will be provided. The evaluation and use of glucose monitoring systems in other veterinary species will also be discussed prior to presentation of the manuscript describing the comparison of two glucose monitoring systems for use in horses. The manuscript reports the assessment of the accuracy and validity of the use of glucose monitoring systems in
healthy adult horses. Based on the conclusions of the manuscript, potential future research and clinical applications of the devices in equine medicine will be discussed.
Chapter 2: Literature Review

Glucose metabolism in equids

Blood glucose concentrations in equids are influenced by numerous factors, including diet, excitement, stress, illness, and obesity. In the horse, average fasting glucose concentrations range between 60 and 90 mg/dL. These concentrations are maintained by a balance between the rate of glucose entering circulation and rate of glucose removal from circulation.

The rate of glucose entering circulation is a result of an interplay between intestinal absorption, glycogenolysis, and gluconeogenesis. Though both processes result in glucose release from the liver during times of fasting, glycogenolysis is the breakdown of glycogen stores while gluconeogenesis is the formation of glucose primarily from lactate and amino acids.

Glucagon, a hormone produced by the liver exerts partial control over both processes, facilitating glycogenolysis during the first period of fasting, followed by gluconeogenesis as time progresses. Endogenous glucose production may be suppressed by the action of insulin either via direct action on the liver or via communication with the pancreatic alpha and beta cells, resulting in glucagon suppression.

Cortisol acts on several tissues within the body which effect glucose concentrations in circulation. The actions of cortisol in the liver result in an increase in gluconeogenesis and decreased glycogen synthesis. In adipose tissue, cortisol stimulates lipolysis, resulting in the release of fatty acids that may be used as substrates for increased gluconeogenesis. The action of cortisol on the pancreas includes potent inhibition of insulin release and signaling as well as
an increase in glucagon release.[3] The net effects of these processes result in increased glucose availability in systemic circulation.

Insulin is secreted from pancreatic beta cells in response to increases in blood glucose concentrations.[1] When glucose concentrations increase in circulation, insulin signals the cells of insulin sensitive peripheral tissues, especially skeletal muscles, to increase uptake of glucose. [2] Insulin also acts on the liver to promote the transformation of glucose into glycogen via glycogenesis.[1 4] Simultaneously, insulin inhibits glucagon secretion from the pancreatic alpha cells, ceasing production of glucose via glycogenolysis and gluconeogenesis as described above. [2] Due to normally tight regulation of glucose and insulin concentrations, abnormally elevated or decreased blood glucose levels in horses fasted for more than 12 hours may be indicative of disease or pathology.[1]

**Clinical approach to glucose disorders in equids**

A multitude of disorders in horses, discussed further below, may result from dysregulation of glucose metabolism, requiring close monitoring of glucose concentrations for diagnosis and treatment. In each of the following disease states, frequent glucose monitoring is necessary, providing a potential application for continuous glucose monitoring technology in equine medicine.

**Insulin resistance with systemic inflammation**

Insulin resistance is described as the failure of insulin sensitive tissues, including skeletal muscle, adipose tissue, and the liver, to respond to insulin, either endogenous or exogenous, resulting in hyperglycemia. Peripheral tissue insulin resistance has been associated with
significant systemic inflammation in humans, including cases of sepsis or severe trauma, resulting in hyperglycemia. Insulin resistance and resultant hyperglycemia have been associated with poor outcome and increased mortality in critically ill human patients.[5-7] Decreasing the incidence of hyperglycemia with glycemic control on a case by case basis is warranted in these patients, conceivably improving mortality rate and outcomes.[7-9]

Peripheral insulin resistance and hyperglycemia have also been reported systemically ill horses, resulting in increased mortality in this species as well. Several studies have evaluated the role of hyperglycemia in cases of abdominal disease in horses. An observational retrospective study evaluating a population of 269 horses admitted for acute abdominal disease found that 50.2% had glucose concentrations within reference intervals while 49.4% were hyperglycemic at admission. Non-survivors in this study had higher mean blood glucose concentrations at admission and subsequent sampling up to 48 hours after admission, as well as higher maximum and minimum blood glucose concentrations in the first 24 hours following admission.[10] In a separate population of 228 horses presenting for treatment of colic over a 3 year period, hyperglycemia was associated with failure to survive with 45% of the study population above reference range for glucose concentration and 16.7% of horses having severe hyperglycemia (>195mg/dL).[11] Systemic inflammatory response syndrome, or SIRS, is described as a non-specific, clinical, proinflammatory response. There is no consensus definition of SIRS in horses, however a patient may be diagnosed based on the presence of one or more of the following: body temperature >101.5 °F, heart rate > 60 beats/min, respiratory rate > 30 breaths per minute, and white blood cell count >12,500 cells/μL, less than 4,000 cells/μL, or presence of >10% band neutrophils.[12] In horses presenting with a diagnosis of SIRS, survivors were found to have significantly higher insulin and a significantly lower serum glucose concentration at admission.
Throughout hospitalization, hyperinsulinemic horses were four times more likely to survive and those that were hyperglycemic at any point were five times less likely to survive. Glucose to insulin ratio, reflecting insulin sensitivity, was significantly lower in survivors, however a glucose to insulin ratio of <10, indicative of insulin resistance, found in 38% of horses was not associated with a final outcome of survival.[13] In contrast to the previously described studies, a recent retrospective showed no significant difference in glucose concentration observed within 72 hours between survivors and non-survivors in cases of acute colitis with systemic inflammatory response syndrome.[14] However, in a group of critically ill foals, the presence of systemic inflammatory response syndrome was associated with derangements of blood glucose.[15] In this population, hypoglycemia, rather than hyperglycemia, was associated with non-survival to hospital discharge. Hypoglycemia may be more common in this neonatal population compared to adult horses due to low tissue energy stores in the form of fat and glycogen.[16]

**Diabetes mellitus**

The development of overt diabetes mellitus, defined by marked hyperglycemia resulting from lack of insulin production has been rarely reported in horses.[17-25] Due to the infrequent nature of diagnosis in horses, the classifications of type 1 (insulin dependent) and type 2 (noninsulin-dependent) diabetes mellitus commonly used in human medicine have not been widely used in the equine veterinary field.[26] Reported causes of cases of overt diabetes mellitus in horses have included chronic pancreatitis, granulosa cell tumor, and immune-mediated polyendocrinopathy.[17 20 21] Additionally, transient diabetes mellitus has been identified in a 3-day-old Thoroughbred foal, based on the presence of hyperglycemia in the absence of
hyperinsulinemia and positive response to administered insulin, similar to neonatal diabetes mellitus observed in human neonates.[27] Cases of diabetes mellitus have been observed in conjunction with a diagnosis of pituitary pars intermedia dysfunction and will be described in more detail below.[19 28]

Equine metabolic disease

In contrast to overt diabetes mellitus, insulin dysregulation secondary to metabolic disease is widely recognized in equine veterinary medicine.[26] The terms insulin resistance and insulin dysregulation are often used interchangeably, but insulin dysregulation is considered a broader term, encompassing tissue insulin resistance as well as hyperinsulinemia.[29] Insulin resistance is defined as the inability of tissues to respond appropriately to insulin for glucose uptake, resulting in hyperglycemia, in conjunction reduced suppression of gluconeogenesis in the liver. [30] Hyperglycemia stimulates beta cell secretion of insulin, resulting in hyperinsulinemia, which is reported as a common finding most frequently reported in horses with pituitary pars intermedia dysfunction or obesity resulting in metabolic disease.[1] Reduced hepatic insulin clearance and compensatory pancreatic secretion in cases of insulin resistance also contribute to insulin dysregulation. In horses, most cases of insulin dysregulation develop as a consequence of pituitary pars intermedia dysfunction (PPID) or equine metabolic syndrome (EMS).[31]

Pituitary Pars Intermedia Dysfunction (PPID)

PPID is one of the most common endocrinopathic conditions in older horses and is associated degeneration of dopaminergic nerves responsible for hormonal control. Insulin resistance is a commonly identified clinical abnormality in older horses with PPID.[32] Cases of overt diabetes
mellitus, defined by persistent hyperglycemia secondary to reduced insulin secretion due to pancreatic beta cell failure, have been observed in association with PPID.[31] PPID may also result in insulin resistance with no impairment of pancreatic insulin production. Proposed mechanisms for this connection have been proposed including antagonism of insulin by cortisol and other hormones, such as corticotropin-like intermediate hormone and alpha melanocyte-stimulating hormone concentrations, from the affected pars intermedia.[29 31]

Equine Metabolic Syndrome (EMS)

The American College of Veterinary Medicine consensus statement describes insulin dysregulation as a consistent key feature of the condition.[33] The presence of EMS is generally, but not always associated with obesity, as is also observed in humans. Both humans and horses with metabolic syndrome are at greater risk of chronic disease conditions, however, laminitis represents the most severe clinically important chronic disease for which EMS-affected horses are at increased risk. Although most horses with EMS remain normoglycemic, some horses with advanced disease may develop reduced glycemic control and hyperglycemia.[19]

Diagnostic Testing for Insulin Resistance and Dysregulation

Several different diagnostic tests are available for the identification of equine metabolic disease and different testing may provide insight into tissue insulin resistance or hyperinsulinemia, depending on the methodology. Diagnosis of insulin resistance may be assessed using fasting blood glucose levels or dynamic testing such as the euglycemic hyperinsulinemic clamp (EHC), insulin sensitivity test (IST), frequently sampled intravenous glucose tolerance test (FSIGTT), and combined glucose insulin tolerance test (CGIT). Because hyperglycemia is an indirect
measure of insulin resistance, fasting blood glucose has low sensitivity and specificity for the diagnosis of EMS.[34] The EHC is considered the gold standard to diagnose insulin resistance in the human and equine medical fields.[35-37] For this method, a priming dose of insulin is administered, followed by a continuous rate infusion of insulin and concurrent dextrose infusion.[36 37] Insulin sensitivity is determined by the rate of dextrose administration needed to maintain blood glucose concentrations within normal limits. A low glucose infusion rate indicates insulin resistance while a high glucose infusion rate is indicative of insulin sensitivity.[37]

The EHC directly assesses the response of insulin sensitive tissues to exogenous insulin, however its use is often limited to research settings due to the time commitment and dedicated personnel needed to perform testing.[36] The IST also directly assesses insulin sensitivity.[30] Different versions of this test have been described with one test completed over 5 hours and the other over 30 minutes, however a 50% decrease in blood glucose during the testing period indicates insulin sensitivity for both tests.[38 39] The IST has superior repeatability compared to other testing methodologies, but does carry a risk of inducing hypoglycemia in patients.

With the FSIGTT, up to 36 blood samples are obtained for determinations of glucose and insulin concentrations over a 6 hour period following intravenous dextrose and insulin injections.[37] The insulin sensitivity index is calculated as a ratio of glucose uptake relative to insulin concentration. A diagnosis of insulin resistance is made when the calculated insulin sensitivity index is low, whereas a high insulin sensitivity index indicates appropriate insulin sensitivity.[40] Some consider the frequently sampled intravenous glucose tolerance test to be
the new gold standard for assessment of tissue insulin resistance in horses, prompting widespread use in research settings.[35 40-42]

FSIGGT is also used to diagnose diabetes mellitus in cases of PPID as pancreatic beta cell responsiveness, or acute insulin response to glucose (AIRg), and insulin sensitivity (SI) may be calculated from glucose and insulin values obtained during testing. AIRg values may be variable with a diagnosis of diabetes mellitus, although a low value may be associated with severe pancreatic beta cell destruction. However the observations of persistent hyperglycemia and low SI are consistent with a diagnosis of diabetes mellitus in the horse [31]

The CGIT is performed with intravenous administration of both dextrose and insulin followed by blood sample collection to determine insulin and glucose levels, similar to the FSIGTT described above, however interpretation of the insulin and glucose concentrations obtained differs between the tests. With CGIT, the administration of both insulin and dextrose results in a biphasic curve consisting of a positive phase (relative hyperglycemia) and negative phase (relative hypoglycemia). The presence of a prolonged (>45 minute) positive phase, insulin concentration>100 µIU/ml, or both is indicative of tissue insulin resistance.[30] The CGIT is commonly used in clinical settings for dynamic testing of insulin resistance.[34 35]

Hyperinsulinemia, or the circulation of an inappropriate concentration of insulin in response to a stimulus, may be diagnosed using a basal serum insulin concentration or oral sugar/glucose tests.[30] Although one time testing of serum basal insulin is poorly sensitive, values >20µIU/ml in fasted horses or >60µIU/ml in fed horses combined with a clinical suspicion of metabolic disease is supportive of insulin dysregulation, indicating the need for future assessment using a dynamic test.[35 43 44] The oral sugar test (OST) measures the response to oral administration
of non-structural carbohydrates, such as corn syrup or oral glucose. With the OST, a serum insulin concentration >60µIU/ml 60- or 90-minutes post administration of corn syrup or >80µIU/ml 120-minutes after the administration of oral glucose in fasted horses provides a diagnosis of hyperinsulinemia.[45 46]

**Parenteral nutrition**

Parenteral nutrition, either partial or total, is used in many equine critical care cases, including neonates and adult horses with gastrointestinal disease, in order to avoid malnutrition when enteral feeding is contraindicated. It is recommended to monitor blood glucose concentrations at least every 4-6 hours after starting parenteral nutrition with the goal of maintaining blood glucose near established normal ranges. [47] The most common complication observed with parenteral nutrition is hyperglycemia.[48] In these cases, glucose concentrations are monitored closely to determine the need for altered rate of administration, or in the cases where hyperglycemia persists, insulin therapy may be indicated to stabilize glucose concentrations.[47]

In a study of 79 horses on parenteral nutrition with diagnoses of gastrointestinal disease, at least one blood glucose value was higher than 95.94 mg/dL (the upper limit for a normal horse) in 52 cases, or 65.82%, with values higher than 180mg/dL were observed in 15 cases and higher than 199.98 mg/dL in an additional 9 cases. [45] Due the degree of hyperglycemia, the rate of infusion was reduced temporarily in 5 cases and a continuous intravenous infusion of insulin was administered in 3 additional horses.[48] In two retrospective studies evaluating the use of parenteral nutrition in foals, 85% and 62% of foals developed hyperglycemia. [49 50] Myer et al. did not find an association between hyperglycemia in foals and mortality in this retrospective. [50]
Traditional blood glucose monitoring in veterinary medicine

Laboratory analyzers remain the gold standard for measurement of glucose concentrations in whole blood, plasma, or serum, although these methods are impractical for frequent serial monitoring of blood glucose. Three methods of determining glucose concentration have been developed, including reducing methods, condensation methods, or enzymatic methods. Today, almost all glucose measurements use indirect enzymatic methodology with most laboratories using the hexokinase method.[51] The hexokinase enzyme is responsible for catalyzing the reaction between glucose and adenosine triphosphate, which phosphorylates glucose into glucose-6-phosphate. Glucose-6-phosphate dehydrogenase then oxidizes the glucose-6-phosphate to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide, which can be measured and is proportional to the glucose concentration of the sample.[51 52] Artifactual hypoglycemia may be observed in samples due to delayed separation of serum or plasma from the red blood cells, resulting in continued uptake and metabolism of glucose by the cells.[53] Delays in separation and storage times have different effects among veterinary species, complicating interpretation of laboratory values. [53]

The development of portable, point-of-care glucometers has offered a practical solution for serial monitoring of glucose levels in ambulatory settings. These handheld devices typically use oxidoreductases, such as glucose oxidase or glucose dehydrogenase, in addition to coenzymes, mediator systems, and indicators to measure the concentration of glucose within a specimen.[51] Glucose meters designed for human use rely on a constant relationship between glucose concentrations within plasma and whole blood with erythrocytes and plasma each containing 50% of glucose, however this relationship is not uniform among all species. The glucose
distribution between plasma and erythrocytes in dogs is 87.5% and 12.5%, respectively. Only 7% of glucose is contained within erythrocytes in cats compared to 93% within plasma.[54] For this reason, several veterinary glucose meters have been developed for use in veterinary species. In particular, the AlphaTRAK meter is widely used due to the small volume of required blood and published studies showing high correlation with laboratory methods in dogs, cats, and horses.[55-57] Hackett and McCue evaluated the AlphaTRAK glucometer for use in both foals and adult horses in 2010. [57] The median absolute bias of the glucometer when compared to the reference chemistry analyzer was 6.1% in adult horses and 5.0% in foals. When the measurements from adult horses and foals were combined 96.94% of the values were within zone A and 100% of values were within zones A and B of the Clarke error grid, associated with values representing clinically correct decisions. It was concluded that the accuracy and precision of the veterinary glucometer evaluated were within acceptable limits for clinical use in horses.[57]

Traditional blood glucose monitoring requires either repeated capillary sticks, venipuncture or the placement of an indwelling intravenous catheter for sampling. However, due to the nature of veterinary species, stress induced hyperglycemia due to handling and restraint of the animal as well as removal from their normal environment and associated stress of hospitalization may confound results.[58-60]

**Glucose monitoring in human medicine**

Diabetes mellitus affects nearly 120 million human adults, in addition to an increasing number of children under the age of 18, and is currently the 7th leading cause of death in the United States.[61] Diabetes is best managed by tight glycemic control with hypoglycemia and glycemic
variability both associated with adverse outcomes in these patients.\textsuperscript{[61 62]} Self-monitoring of blood glucose is the cornerstone of management in patients with diabetes, however this can be inconvenient and difficult to maintain long term. It has been estimated that only 44\% of people with type 1 diabetes mellitus and 24\% of people with type 2 diabetes mellitus perform self-monitoring of blood glucose as recommended.\textsuperscript{[63 64]} Traditional methods of blood glucose monitoring only allow for the determination of glucose concentration at set intervals, limiting the amount of data available for interpretation and possible intervention. New technology in the form of continuous glucose monitoring systems can provide a comprehensive assessment of blood glucose, allowing a patient to identify glycemic variability and reducing the risk of hypoglycemia.\textsuperscript{[62]}

**What is continuous glucose monitoring (CGM)?**

Although the first self-monitoring glucose monitors were developed in the early 1970s, continuous glucose monitoring (CGM) systems were not released until the early 2000s.\textsuperscript{[65]} CGM systems employ minimally invasive, small, flexible sensors implanted under the skin which automatically monitor glucose levels in the interstitial fluid throughout the day and night. Glucose concentrations are reported on a monitor on either a handheld device or within a smartphone application available to the user.\textsuperscript{[61]} Historically, use of these devices has been limited due to perceived poor accuracy, increased cost, and short duration of sensor functionality.\textsuperscript{[66 67]} While self-monitoring of blood glucose using a glucometer provides patients and clinicians with glucose data at a single timepoint, CGM data provides comprehensive and dynamic information regarding glucose levels over time.\textsuperscript{[68]} Advancements in CGM technology have addressed many of these concerns with these systems, especially
improved accuracy, safely allowing sensor glucose values to be used in making therapeutic decisions.[68-70] As of 2019, 8 personal and professional CGM systems were FDA approved and their use has transformed the approach to the monitoring of diabetes in humans.[68] CGM usage is expected to increase globally with recent guidelines recommending that the technology be considered in all children and adolescents with type 1 diabetes mellitus.[71]

Research has shown clinical benefits of personal CGM use in human patients with type 1 diabetes mellitus, including reduction in HbA1c and glycemic variability.[68] HbA1c has long been used as the gold standard for assessing long term glycemic management, but it does not provide information about glucose trends and excursions.[64] The American Association of Clinical Endocrinologists stated in 2016 that CGM is likely to have benefits on any patient on intensive insulin therapy, regardless of the type of diabetes, however increasing evidence exists for the use of CGM in patients with type 2 diabetes mellitus not on insulin therapy due to the positive effects on glycemic control.[72-76] The use of these systems has resulted in improvement diabetes distress and hypoglycemic confidence as well as decrease in total health care costs and hospital admissions.[77-79]

Comparison of interstitial and blood glucose concentrations

The dynamic nature of blood glucose concentrations at different sites should be considered when evaluating monitoring devices. In humans, a 3-5mg/mL difference exists between arterial blood and venous blood with concentrations higher in the arterial blood due to diffusion of glucose from the plasma into the interstitial fluid as venous blood circulates through the capillary system.[80] Additionally, the concentration of glucose within the plasma may be up to 11% higher than in whole blood, which is the sample typically used for handheld glucose meters. [80]
Continuous glucose monitoring systems measure interstitial glucose concentrations. Blood and interstitial glucose concentrations are generally comparable, but can differ by 10-20%.[81] The observed discrepancy is attributed to sensor reaction time, signal processing delays, and interstitial fluid to plasma glucose equilibration time. Blood flow to the interstitial fluid dictates the amount of glucose delivered, as glucose is transferred from the capillary endothelium across a concentration gradient.[80] The time required for glucose to diffuse from the capillary to the tissue plays an important role in CGM systems and this lag time between changes in plasma and interstitial glucose may affect sensor readings.[80] A physiologic lag time in glucose concentration between the blood and interstitial fluid of about 3-12 minutes in humans has been reported, whereas the intrinsic electrochemical sensor lag is 1-2 minutes, contributing to the difference in values at one point in time.[82 83] A similar lag time of 5-12 minutes has been observed in dogs. [84 85] In cats, a lag time of about 11 minutes was observed after an intravenous bolus of glucose.[86] Limited information is available regarding a lag time between compartments for horses, but a recent study comparing the FreeStyle Libre to a handheld veterinary glucometer reported a lag time of 60 minutes at rest, 10 minutes during insulin induced hypoglycemia and 20 minutes for dextrose induced hyperglycemia.[60]

When interstitial fluid and plasma glucose variations were evaluated under hypoglycemic conditions, it was found that interstitial glucose concentrations may fall in advance of plasma glucose and remain below plasma glucose for an extended period of time.[87-89] In contrast, during times of glucose increases, the change in interstitial glucose was observed to be less than the change in blood glucose.[82] It has been theorized that this is due to a push-pull phenomenon, during which the glucose is pushed from the blood in to the interstitial space.
during times of hyperglycemia then it is pulled from the interstitial fluid by surrounding cells in response to decreasing glucose levels.[90]

To maintain accuracy of reading, older CGM devices required multiple calibrations per day. With technological advances, the use of a digital filter may compensate for the lag time and additionally allow for sensor signals indicating rate of glucose concentrating change, optimizing use of interstitial fluid for CGM systems and limiting the need for calibration.[64 83]

**CGM technology**

CGM systems may be divided into those for personal or professional use. For the purposes of this review, focus will be placed on those devices available for personal use. Personal use systems may be further divided into continuous or real-time systems and intermittently scanned or flash systems based on how the glucose values are obtained and reported by the device technology. Continuous systems passively transmit glucose information continuously to the reader or smart phone application whereas flash system only provide this information when the user scans the sensor.[64] To maintain consistent data collection, flash systems must be scanned at least every eight hours. Depending on the manufacturer of the product, the sensor may use a glucose oxidase enzymatic reaction or fluorescence sensing technology for detection of interstitial glucose levels.[68] Glucose values are recorded every 5-15 minutes, depending on the type of system. The need for calibration and duration of wear time varies between manufacturers. With the approval of factory calibrated systems, the need for confirmatory blood glucose levels via capillary stick has been virtually eliminated. Studies have shown that insulin dosing without confirmatory self-monitoring of blood glucose is safe, but only select devices have been approved for insulin dosing and/or are compatible with insulin pumps.[69] Many of the devices
also provide alarms during time of actual or impending hyper- or hypo-glycemia, which has been useful in those individuals with hypoglycemia unawareness.

Some devices allow remote monitoring where select individuals, such as parents or clinicians, may view glucose readings and alerts in real time.[91] Multiple CGM platforms have adopted the use of an ambulatory glucose profile (AGP) which combines input from multiple days of glucose data into one report, allowing review of glucose data over an extended period of time, allowing retrospective analysis of CGM data. It has been shown that review of at least 14 days of data on an individual patient will provide a good estimate of glycemic values over a 3-month period.[92] The review of this data also provides information on trends such as time in range and coefficient variation as a measure of glycemic variability over the time period.[93] The analysis of daily reports allows for review of glucose fluctuations and the evaluation of glucose concentrations in target range. Excursions into hypoglycemic or hyperglycemic states may then be analyzed and addressed as necessary.

**CGM Application**

CGM provides direct information of a patient’s glycemic status, but also provides information to guide interventions and facilitate personalized regimen changes. Episodes of hypoglycemia and hyperglycemia, resulting in increased glycemic variability, have been associated with increased complications and mortality in people with diabetes mellitus.[61 62 94] Because trends in glucose concentrations can be more readily identified with CGM, interventions may be more rapidly and accurately employed compared with traditional methods of intermittent measurement of blood glucose concentrations. Many studies have demonstrated an HbA1c reduction, increased time in range, and reduced hypoglycemia with CGM use.[75 95-101] Majority of the
evidence supports these benefits with CGM use in people with type 1 diabetes, however the evidence supporting use with type 2 diabetes is less robust. [64] No effect on hypoglycemic events have been observed in studies with type 2 diabetics, likely due to the low levels of hypoglycemia at baseline in people with this type of diabetes mellitus.[75] However, one study showed that CGM was more effective than capillary point-of-care testing for the detection of hypoglycemic episodes and asymptomatic hypoglycemia in hospitalized patients with type 2 diabetes mellitus with clinically valid glucose measurements.[76] CGM can also improve diabetes related satisfaction in people with diabetes and parents of children with diabetes in addition to the experience of the treating clinician as well as providing a cost-effective adjunct to management associated with reduced complications and hospitalization.[64]

**Evaluation of CGM technology**

Self-monitoring blood glucose systems using capillary blood are mainly assessed according to the International Standards Organization (ISO 15197-2013) accuracy requirements, however, CGM accuracy is not routinely assessed by these metrics and no consensus by which to assess these devices has been agreed upon.[102] Recently the Food and Drug Administration (FDA) released requirements for only for those CGM systems incorporated with insulin pumps, therefore no requirements currently exist for standalone CGM systems on the market.[102] Additionally, no guidelines for unified study design protocols for the assessment of CGM systems exist, further complicating interpretation of data.[103 104] Within each clinical trial, CGM readings from the interstitial fluid are compared to reference blood glucose measurements obtained for the same patients at the same time.[103] The concordance between these readings is dependent on the accuracy and precision of the CGM device being tested and the reference blood
glucose device being used, whether handheld or laboratory, with the understanding that the results may be affected by the fact that blood and interstitial fluid represent different physiological compartments with different dynamics.[104] Currently, there is no established reference method for determination of interstitial glucose concentrations.[102]

Accuracy is a measurement of how closely a series of values reflects the reference value whereas precision represents the reproducibility of a series of values independent of the reference value.[105] Because of the differences between blood and interstitial glucose concentrations, accuracy of the devices decreases at the extremes of glycemia and during times of rapid glucose change.[64 81] Certain commonly used medications, such as paracetamol and ibuprofen, as well as endogenous substances, such as bilirubin, cholesterol, and creatinine, may also affect accuracy of readings, however with improvements in technology, these interferences have lessened dramatically.[81] It is generally recommended to self-monitor blood glucose using capillary blood if CGM readings do not match clinical symptoms.[106]

In the absence of standardized criteria for the evaluation of the accuracy of CGM in interstitial fluid, systems are mainly assessed by mean absolute relative deviation (MARD) with additional analyses, such as point accuracy and error grid analysis, used supplementally to assess performance.[102]

**Mean absolute relative deviation/difference/error (MARD or MARE)**

Mean absolute relative deviation, or MARD, is also referred to in the literature as mean absolute relative difference or mean absolute relative error. For the purposes of this thesis, MARD will be used. MARD is considered the single best measure of accuracy and is the most frequently used
metric to characterize the analytical performance of CGM systems.[103] It is generally obtained from computing the individual absolute errors relative to the reference value, or the difference between CGM measurements and simultaneous measurements by a reference system.[61 105 107] Assessment based on MARD is advantageous in its simplicity, but it may be misleading due to the effects of variables, such as device performance and calibration, number of measurements, rate of change of glucose concentration or reference measurement error, affecting the true accuracy depicted.[103 104] Therefore, comparison of MARD values between different studies can be misleading.[108] This is evident in different MARD values observed in studies testing the same CGM system.[103] A lower percentage MARD represents better sensor performance. The current view is that a CGM system with a MARD of less than 10% is considered accurate enough to make treatment decisions without adjunctive self-monitoring of blood glucose via capillary stick.[104 108] One should also note that MARD values can vary with sensor life, with higher MARD values observed the first day of sensor use.[109 110]

**Point accuracy**

Point accuracy is calculated using the data points, or CGM values, within a certain percentage of the reference value. This method is generally used to determine the CGM values within 15% to 20% of the reference value. Points may also be plotted against the reference value in Bland-Altman plots to evaluate correlation between methods.

**Clark’s Consensus Error Grid**

Clark’s Error Grid was developed to compare readings of a particular system with those of a reference system. The grid is formatted by plotting the measured CGM value on the y-axis
against the reference value on the x-axis, and the grid is divided into zones A through E, creating a visual representation of clinical impact of system error. The highest degree of precision and accuracy, is observed within zone A where measured points are within 20% error of the reference method. Data points falling in zone A represent high accuracy while those points in zone B signify acceptable accuracy. The remaining zones have questionable clinical accuracy.[104] The Surveillance Error Grid is a more visual version which converts the error into color-coded risk levels.[61 111]

**Comparison of Dexcom G6 and FreeStyle Libre**

Two of the more widely used CGM systems are the Dexcom G6 (Dexcom Inc., USA), a continuous system, and the Abbott FreeStyle Libre (Abbott, USA), a flash system. Because of their current availability, these devices were chosen to be evaluated in horses, as described in the manuscript to follow. Many similarities and differences exist between the two systems. The FreeStyle Libre requires a 1-hour warm-up period while the Dexcom G6 needs 2 hours. Following warm-up, these systems use glucose-oxidase based technology to monitor interstitial glucose concentrations. Both the Dexcom G6 and the FreeStyle Libre have the ability to measure glucose readings within the range of 40 mg/dL to 400 mg/dL. Both systems generate glucose readings every 5 minutes. In the case of the Dexcom G6, these values are automatically uploaded to the chosen monitor whereas the FreeStyle Libre displays values once the sensor is scanned. The FreeStyle Libre sensor must be scanned at least every 8 hours to avoid gaps in reporting. The Dexcom G6 has a wear lifetime of up to 10 days while the FreeStyle Libre may be worn up to 14 days.
The costs associated with the use of each device varies. The FreeStyle Libre only requires the purchase of a one-time use sensor if the use of a compatible smartphone is available to obtain readings. The user has the option to purchase a handheld reader for the FreeStyle Libre if needed. In comparison, the Dexcom G6 requires the purchase of a one-time use sensor in addition to a transmitter, which may be used multiple times within 90 days. A compatible smartphone or reader purchased separately is also required with the Dexcom G6. Both these systems are factory calibrated, therefore they do not require calibration with capillary glucose, although the Dexcom G6 does have the option for calibration if warranted. The remainder of the devices available in the United States require some degree of calibration with capillary glucose.[112]

**Efficacy of the FreeStyle Libre and Dexcom G6**

As mentioned previously, numerous CGM systems are commonly used in the management of diabetes mellitus in humans, and both the FreeStyle Libre and Dexcom G6 have been evaluated extensively in these populations. The Abbott study in 2015 evaluated the use of the FreeStyle Libre system in patients with type 1 or type 2 diabetes across 4 clinical sites. An overall MARD of 11.4% with capillary blood glucose reference was observed and 86.2% and 85.5% of values were within 15% and 20% of the reference, respectively. Results from this study showed agreement between the system’s sensor readings and reference values, providing comprehensive glucose data in an easy-to-use experience for users.[113] A 2018 study evaluating the performance of the Dexcom G6 in adults and children with insulin-treated diabetes showed an overall MARD of 9.0% with 93.9% of the analyzed values within 20% and 83.3% within 15% when compared to the reference value. This study concluded that the Dexcom G6 system
displayed consistent accuracy in adults, adolescents, and children across a range of glucose values at different wear sites, across 10 days of system use.[114]

Ambulatory use of these devices is widely accepted, but investigations into monitoring of hospitalized in-patients has more recently progressed. Critical shortages in personal protective equipment and the need to maintain glycemic control in monitoring while minimizing infectious disease exposure during the coronavirus pandemic has led health professional to explore the feasibility of using CGM, particularly the Dexcom G6 and the FreeStyle Libre, in hospital.[115] Studies have shown that use of CGM in hospital resulted in increased detection of hypoglycemic events.[76 116 117] A recent study examining the use of Dexcom G6 monitoring in non-critically ill hospitalized patients with diabetes showed an overall MARD of 12.8% with 68.7% and 81.7% of values within 15% and 20% of the reference value, respectively.[115] Similar values were found with use of the FreeStyle Libre in hospitalized type 2 diabetic patients with an overall MARD of 14.8%, 62% of values within 15% of the reference value, and 76% within 20% of the reference value. Although fewer studies are available for this application, good correlation between CGM values and capillary and/or laboratory glucose values have been shown.[76 117-119]

**Use of CGM systems in veterinary species**

As the use of CGM systems in the human medical field has increased, the technology has been transferred over to veterinary medicine. Serial monitoring of blood glucose concentrations is a common diagnostic procedure in animals, including blood glucose curves in diabetic cats and dogs as well as glucose tolerance testing for malabsorptive or metabolic diseases in a variety of species.[120] The application of continuous glucose monitoring (CGM) technology is attractive
in these cases as it avoids limitations of traditional monitoring such as intermittent assessment of glucose concentrations, required hospitalizations for repeated venipuncture or catheterizations, and patient restraint.

One of the first studies to examine the use of CGM in veterinary species was performed by Wiedmeyer et al. in 2003. The aim of this study was to evaluate the use of the MiniMed (Medtronic, USA) continuous glucose monitoring system in dogs, cats, and horses. Results showed a positive correlation between interstitial and whole blood glucose concentrations in healthy subjects of each species, as well as dogs and cats with diabetes mellitus. The CGM system was also sensitive to abrupt changes in glucose concentrations while providing detailed information as to the animal’s glycemic status over an extended time period.[120]

Traditional 12-to-24-hour glucose curves used to determine peak and nadir blood glucose concentrations in diabetic small animals are generally performed in a hospital setting with blood sampling every 2-3 hours. This method is less than ideal because the practices are time consuming and labor intensive, while the stresses of hospitalization, restraint, and multiple venipuncture or catheter placement for sampling may affect the results. Additionally, peak and nadir concentrations may be missed due to the intermittent nature of resting.[121] For these reasons, the use of CGM technology in the evaluation of dogs and cats undergoing insulin therapy has become increasingly common.[121-124] Using CGM systems allow clinicians and owners to circumvent these pitfalls as the sensor may be placed in hospital then the animal sent home for the remainder of the testing. Additionally, with multiday studies, rather than the typical 12-to 24-hour testing period, more comprehensive data as to glucose variability may be obtained. The Freestyle Libre has been evaluated in dogs with diabetes mellitus, finding that measurement
of interstitial glucose was accurate when compared to the reference method, the technology was easy to use, and up to 14 days of glucose data was reported.[125] Similar findings were also observed when the same system was applied to diabetic cats, offering a practical, less stressful option for management in this species as well.[126] A recent study evaluated complications of use of the flash glucose monitoring system in cats, reporting the most common complication as early sensor detachment (5/33, 15%) while mild erythema/crusting was noted in 12% of cases. A small number, 2/33 cats, did develop more serious complications such as skin erosions or abscess formation.[127]

In addition to monitoring of insulin-dependent diabetes mellitus patients in small animal practice, conditions such as diabetic ketoacidosis, pancreatitis, insulinoma, head trauma, severe babesiosis, xylitol toxicosis, neoplasia, sepsis, and liver dysfunction, in addition to those patients receiving parenteral nutrition, require aggressive glucose monitoring during hospitalization. These patients would benefit from the minimally invasive, near continuous monitoring CGM technology provides.[128] While hyperglycemia in human critical care units and its association with increased morbidity and mortality is well described, the association in small animal medicine is not well established. A retrospective study of dogs and cats hospitalized with head trauma failed to show a correlation between hyperglycemia and survival.[129] In contrast, a more recent study showed a significant association was shown between the severity of hyperglycemia and length of hospital stay and survival in a population of critically ill dogs.[130] Although no association was found between hyperglycemia and survival in a population of hospitalized cats, hyperglycemic cats had a longer length of hospitalization compared to normoglycemic counterparts.[131] Recently, the application of FreeStyle Libre was evaluated for the monitoring of critically ill dogs with diabetic ketoacidosis and found to have acceptable
clinical accuracy, allowing more frequent, minimally invasive, intensive monitoring of the patients.[132]

The use of CGM technology in veterinary species besides dogs and cats has been limited. In 2014, a group applied the Dexcom Seven Plus to a population of healthy male alpacas for monitoring of glucose response to 3 different types of insulin.[133] When compared to the point of care glucometer, CGM showed a wide range in standard deviation, ranging from -27.9 mg/dL to 63.6 mg/dL. The modified error grid showed that 90% of the glucose readings were within zone A, indicating that CGM was unacceptable for determining clinical decisions regarding treatment. This group concluded that although the CGM system worked well for monitoring trends in blood glucose, it did not function well as a stand-alone system.[133]

Recent evaluation of use of the FreeStyle Libre and the Dexcom G6 in dairy cows revealed that due to low accuracy, neither devices are currently capable of replacing blood based glucose measurements.[134] For clinical use, accuracy should approach or exceed 90%, however the accuracy of the FreeStyle Libre throughout the study was 47%. The accuracy of the Dexcom G6 could not be determined because the vast majority of sensors would not deploy from the applicators, therefor minimal data was collected. The authors concluded that although aspects of the CGM systems would make them a useful tool in glucose monitoring of cows, obstacles included issues with application, calculation of algorithms, and inappropriate minimum detection limits would need to be further explored and resolved. [134]

CGM systems in equine medicine
The use of CGM technology in equine glucose monitoring has been limited. The first mention of their application was in 2003, where a Minimed (Medtronic, USA) system was applied to one horse with diabetes, two horses undergoing glucose tolerance testing, and 4 others. In this study, glucose values provided by the CGM correlated well with whole blood glucose concentrations, was sensitive to abrupt changes in glucose levels, and provided glucose data over an extended period of time.[120] Several years later in 2011, the same Medtronic system was evaluated in horses during a combined intravenous glucose and insulin test protocol, where again, the concentrations provided by the device corresponded to measured blood glucose concentrations.[135] In 2013, another group compared the use of the Guardian REAL-Time Continuous Glucose Monitoring System (Medtronic, USA) to a point-of-care glucometer in critically ill foals, however the results were not as favorable as previous, as the relatively poor agreement between values limited the device to adjunctive use only.[136]

As technology within these systems has advanced, CGM have become more affordable and more widely researched for application within the equine sector. Throughout the development of this research project and paper, several studies evaluating the FreeStyle Libre and Dexcom G6 systems have been published. Cunneen et al. in 2020 compared the FreeStyle Libre with the AlphaTRAK point-of-care glucometer in a population of healthy adult horses.[60] The devices were tested over a 3-phase study, including the assessment of resting glucose concentrations, insulin induced hypoglycemia, and dextrose induced hyperglycemia, and each sensor was left in place for a maximum of 31 hours. Overall, the FreeStyle Libre had good agreement with the AlphaTRAK, however, the authors reported that clinical relevance was limited by delays in detecting change in glucose concentrations and the need to replace the device as a total of 15 sensors were used for 10 horses throughout the study. The wide 95% limits of agreement
observed during insulin induced hypoglycemia and dextrose induced hyperglycemia could result in imprecise glucose recordings that under- or overestimate glucose concentrations for treatment decisions. This study did report a lag time between interstitial and blood glucose concentrations of 60 minutes at rest, 10 minutes for insulin induced hyperglycemia, and 20 minutes for dextrose induced hyperglycemia were consistently observed, which is useful information for clinical application of these monitors in horses. Prior to the conception of the project described in the manuscript below, a separate project was performed at Virginia-Maryland College of Veterinary Medicine and the Marion duPont Scott Equine Medical Center evaluating the use of the Dexcom G6 system in neonatal foals.[137] In this study, 200 matched glucose measurements collected from 8 neonatal foals, 4 healthy and 4 ill, showed acceptable correlation, providing accurate measurements when compared with laboratory analysis. Though the 95% limits of agreement were also large in this study, 67.5% and 85% of the Dexcom G6 values were within 10% and 15% of the laboratory chemistry value, supporting use for clinical decision making.

With recent advances in CGM technology, along with the findings of previously described studies investigating their use in horses, evaluation of the FreeStyle Libre and Dexcom G6 in a healthy adult population was of interest. The following manuscript describes this evaluation in depth.
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Chapter 3: Comparison of two glucose monitoring systems for use in horses

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Abstract

Serial blood glucose measurements are used to diagnose or monitor a variety of conditions in equine medicine; advances in near-continuous interstitial glucose monitoring allow for minimally invasive glucose assessment thereby reducing stress and discomfort to patients. Data from this study supports the use of the Dexcom G6 and Freestyle Libre 14-day interstitial glucose monitoring systems to estimate blood glucose concentrations in horses. The objective of the following study was to determine the accuracy of two interstitial glucose monitoring systems (GMS) for use in horses compared to a point-of-care glucometer (POC) and standard laboratory enzymatic chemistry method (CHEM). One of each GMS device (Dexcom G6 and Freestyle Libre 14-day) was placed on eight clinically healthy horses and blood glucose was measured via POC and CHEM at 33 time points and compared to simultaneous GMS readings. An oral glucose absorption test (OGAT) was performed on day 2 and glucose concentrations were measured and compared. Glucose concentrations were significantly ($P<0.05$) correlated with one another between all devices on days 1-5. Acceptable agreement was observed between Dexcom G6 and Freestyle Libre 14-day when compared with CHEM on days 1, 3, 4, and 5 with a
combined mean bias of 10.45 mg/dL and 1.53 mg/dL, respectively. During dextrose induced hyperglycemia on day 2, mean bias values for Dexcom G6 (10.49 mg/dL) and Freestyle Libre 14-day (0.34 mg/dL) showed good agreement with CHEM.

**Introduction**

Evaluation of blood glucose is a vital component of the diagnostic evaluation of various disease processes such as inflammatory and infiltrative bowel diseases [1], and insulin dysregulation associated with equine metabolic syndrome and pituitary pars intermedia dysfunction [2] in horses. Serial monitoring of blood glucose concentration in horses receiving carbohydrate containing intravenous fluids (e.g. anorectic or hyperlipemic horses) to avoid hyperglycemia [3] is commonly performed in hospitalized equine patients. Various studies in critically ill people suggests that prolonged hyperglycemia is associated with higher mortality. [4, 5] Thus, glucose monitoring is a common and important measured variable for both clinical and research purposes in equine medicine.

Glucose monitoring systems (GMS) are widely used in people, primarily to monitor type 1 diabetes mellitus but also in the critical care setting because of their agreement with traditional methods of measuring blood glucose concentration, ease of use, and ability to provide continuous or near continuous glucose data. [6-10] Two types of systems are available: continuous glucose monitoring systems (CGMS) and flash glucose monitoring systems (FGMS). Both systems report interstitial glucose concentrations every 5 minutes, but the continuous system automatically uploads glucose concentration measurements to a reader whereas flash glucose systems require direct scanning of the sensor with a reader at periodic intervals. Data may then
be uploaded to online software to view daily and multi-day graphic representation of glucose measurements.

Instead of utilizing finger pricks or venipuncture for obtaining blood samples, these systems measure glucose concentrations within the interstitial space of the subcutaneous tissue. The interstitial glucose reacts with glucose oxidase in a semipermeable membrane in the sensor, which converts glucose into gluconic acid and hydrogen peroxide. This reaction generates an electric signal proportional to glucose concentration and is translated into a milligram per deciliter value. Multiple studies have indicated that measurements of glucose concentrations within the interstitial space are comparable to measurements of whole blood glucose concentrations in both humans and dogs. Studies in horses have been limited, but an early study determined that interstitial glucose concentrations measured by CGMS correlated well with whole blood glucose concentrations, were sensitive to abrupt changes in glucose concentration, and provided a detailed and accurate representation of an animal’s glycemic status over an extended time period in dogs, cats, and horses. A more recent study evaluated the FGMS (FreeStyle Libre 14-day) in diabetic dogs and noted that it was a valid alternative for glucose monitoring when compared with traditional methods. However, other studies have raised concerns regarding the accuracy of these devices, necessitating the need for further investigation for clinical use. Additionally, although these devices are marketed for use for up to 10-14 days, no studies have evaluated their accuracy in adult horses past 31h (1.29 days) of use. Advancements in CGMS technology have produced more readily available and affordable devices, making their use a potentially viable tool for clinical application.

The objectives of this study were: 1) to determine the accuracy of two glucose monitoring systems (GMS) in horses compared to a point-of-care glucometer (POC) and a standard
laboratory enzymatic chemistry method (CHEM), and 2) to determine the accuracy of the devices during dextrose-induced hyperglycemia using an oral glucose absorption test (OGAT). We hypothesized that GMS would provide acceptable agreement with POC and CHEM and provide diagnostically useful OGAT glucose curves.

Materials and Methods

Eight privately owned animals were enrolled, following informed owner consent, with a mean age of 11 years (range 2 to 21 years) and a mean weight of 474 kilograms (range 373kg to 521kg). Four geldings, three mares, and one stallion were used and breeds included American Quarter horse (n=3), Paint (n=2), Appaloosa (n=1), Trakehner (n=1), and Arabian (n=1). All horses were determined to be healthy based on physical examinations, complete blood counts, and serum biochemistry profiles within acceptable reference intervals prior to initiation of the study. This study was approved by the university institutional animal care and use committee.

Horses were housed at least 12 hours prior to commencement of the study to allow for acclimatation to a new environment and were provided free choice hay and water throughout the study period, except for a 12-hour fast prior to performance of the OGAT. Physical examinations were performed every 12 hours over the 5-day study period. A jugular catheter was aseptically placed in each horse and catheter patency was maintained by flushing with 6 ml of heparinized saline every 6 hours.

Two 3”x 3” areas were clipped using a #40 clipper blade over the lateral neck and hindquarters, lateral to the tail head. The clipped areas were cleaned with isopropyl alcohol and allowed to dry prior to device application. Each device was applied according to manufacturer’s instructions: the FGMS (FreeStyle Libre 14-day, Abbott, USA) was placed on the neck and the
CGMS (Dexcom G6, Dexcom Inc., USA) was placed on the hindquarter. Adherence of the sensor pad to the skin was reinforced with cyanoacrylate adhesive placed on the periphery of the sensor pad. The devices were calibrated according to manufacturer’s recommendations which included a 2-hour calibration period for the Dexcom and 1-hour for the Libre, starting on day 1. The Dexcom system was recalibrated as prompted by the device using the value from the handheld glucometer whereas the Libre operated on a factory calibration system.

Following calibration on day 1, a 5 ml blood sample was collected in sodium fluoride tubes every hour for a total of 5 samples and then every 3 hours over the remainder of the 24-hour period. Readings from the Dexcom and Libre were recorded, just prior to blood collection. Blood glucose was immediately measured from each sample using a point-of-care (POC) handheld glucometer previously validated for horses (AlphaTRAK 2 glucometer, Zoetis, USA). The remainder of the sample was submitted for standard laboratory assay (gold standard) using the hexokinase method (CHEM; AU480 Chemistry Analyzer, Beckman-Coulter, USA). Blood samples acquired between 8 pm and 7 am were collected in similar fashion but stored at 4°C for blood glucose measurement via standard laboratory assay the following day. To evaluate both GMS in the hyperglycemic range, horses were fasted overnight, starting at 7pm on day one, to allow for an OGAT the following day.

On day 2, baseline glucose measurements were recorded from glucose monitoring devices and blood samples were drawn and measured via POC and CHEM. Horses were sedated with 0.4 mg/kg xylazine intravenously via IV catheter to facilitate nasogastric intubation associated with the OGAT. Subsequently, the OGAT was performed using a previously described protocol with a 20% dextrose solution, administered at a dose of 1g/kg by nasogastric intubation.\[16\] Blood samples (5 ml) were obtained every 30 minutes following dextrose administration for a total of 4
hours. Blood glucose was measured immediately after collection via POC glucometer with the reminder of the sample submitted for CHEM. Dexcom and Libre measurements were recorded immediately prior to the collection of each blood sample.

For the remainder of the study period (Days 3-5), blood samples were obtained every 4 hours for a total of 6 samples per day for blood glucose measurement. Corresponding Dexcom and Libre measurements were recorded immediately prior to sampling. Blood glucose was measured immediately after collection via POC glucometer with the remainder of the sample submitted for CHEM. Samples collected between 8 pm and 7 am were stored at 4°C overnight and submitted for CHEM the following morning. Following the final sample collection, the glucose monitoring devices and intravenous catheters were removed.

Statistical analysis

Data were tested for normality by a Shapiro-Wilk test and were noted to be normally distributed. Data were presented as mean ± SD. To compare measured glucose concentrations between the CHEM (considered the reference standard), POC glucometer, and GMS (Dexcom and Libre), the following paired comparisons were made: Dexcom-CHEM, Dexcom-POC, Libre-CHEM, Libre-POC, Dexcom-Libre, and POC-CHEM in both phases of the study.

Glucose concentrations on days 1-5 were compared among analyzers using Pearson’s linear correlation. Agreement between glucose concentrations for each method of glucose measurement was determined using the Bland and Altman, and Lin’s concordance analyses. The bias was calculated as the mean difference between the Dexcom-CHEM, Dexcom-POC, Libre-CHEM, Libre-POC, Dexcom-Libre, and POC-CHEM. A positive bias reflected overestimation of glucose as compared to CHEM. Likewise, when comparing the Dexcom and Libre to POC, a
positive bias reflected overestimation of the POC measurements as compared to CGMS. The limits of agreement were reported as bias ± (1.96 x standard deviation [SD] of the bias).

The mixed model for two factors repeated measures ANOVA was performed between glucose concentrations obtained by Dexcom, Libre, POC glucometer and CHEM to assess the effect of time or assay on glucose concentrations. When multiple comparisons between time points were performed, a Bonferroni correction was used to determine differences in glucose concentrations. A commercial statistics software program (IBM SPSS Statistics version 24, IBM corp., NY and Graph Pad Prism version 8, GraphPad Software, CA) and statstodo.com/Agreement_Pgm.php were used. Significance was set at $P \leq 0.05$.

An additional method used to compare the CGMS and POC devices with CHEM was the number of measurements that were observed within 15% of CHEM values.

**Results**

**Glucose concentration on days 1, 3, 4 and 5**

For glucose concentrations, blood samples were collected at time 0 and 1, 2, 3, 4, 7, 10, 13, 16, 19, and 24h on day 1, at time 0 and 30, 60, 90, 120, 150 and 180 min after glucose administration on day 2, at time 0, 4, 8, 12, 16, and 20h on days 3 and 4, and time 0, 4, 8h on day 5. There was an overall significant effect of time and method on glucose concentration on days 1, 3, 4 and 5 ($P<0.05$) (**Figure 1**). Glucose concentration was significantly higher when measured by Dexcom compared to CHEM at time points 0 on day 1 and 12h on day 3. No other differences in glucose concentrations between time points and devices reached the Bonferroni-adjusted $P$-value. The measurements from Dexcom, Libre, and POC were compared with CHEM and the
percent of total values within 15% of CHEM were calculated, resulting in 65.5%, 68.6%, and 35%, respectively.

Glucose concentrations were significantly correlated with one another (Table 1) between all devices on days 1-5 (Dexcom-CHEM, Dexcom-POC, Libre-CHEM, Libre-POC, Dexcom-Libre, and POC-CHEM). Acceptable agreement was observed between Dexcom G6 and Freestyle Libre 14-day when compared with CHEM with a combined mean bias of 10.45 mg/dL and 1.53 mg/dL, respectively. The mean bias (95% limits of agreement) amongst the other devices on days 1-5 was: Dexcom-POC 7.66 mg/dL (-27.7-43.05), Libre-POC 16.48 mg/dL (-26.2-60), Dexcom-Libre 8.9 mg/dL (-34.41-52.3), and POC-CHEM 18.13 mg/dL (-10.5-46.8) (Table 1 and Figure 2).

Lin’s concordance correlation coefficient tests how well bivariate pairs of observations conform relative to a gold standard. This test measures both precision and accuracy. The Lin’s coefficient between Dexcom-CHEM, Dexcom-POC, Libre-CHEM, Libre-POC, Dexcom-Libre and POC-CHEM on days 1-5 demonstrated strong agreement according to Altman et al. (Table 1).[17]

Dextrose-induced hyperglycemia on day 2

During the OGAT blood samples were collected on time 0, 30, 60, 90, 120, 150 and 180 min after dextrose administration. There was a significant (P<0.01) effect of dextrose administration on glucose concentration from 30-150 min for all 4 methods of glucose measurement (Figure 3). Glucose concentration was higher when measured by POC compared to CHEM at time 0 and 30 min (P<0.05). There were no other differences noted in glucose concentrations between time points and devices (P>0.05).
Glucose concentrations were significantly correlated with one another (Table 2) between all devices on day 2. During dextrose induced hyperglycemia on day 2, mean bias values for Dexcom G6 (10.49 mg/dL) and Freestyle Libre 14-day (0.34 mg/dL) showed good agreement with CHEM. The mean bias (95% limits of agreement) amongst the other devices on day 2 was: Dexcom-POC 20.16 mg/dL (-12.8-53.16), Libre-POC 30.33 mg/dL (-25.7-86.4), Dexcom-Libre 10.16 mg/dL (-41.3-61.6) and POC-CHEM 30.6 mg/dL (-10.6-71.9) (Table 3 and Figure 4). The Lin’s coefficient between Dexcom-CHEM, Dexcom-POC, Libre-CHEM, Libre-POC, Dexcom-Libre, and POC-CHEM in on day 2 demonstrated moderate to strong agreement (Table 2).

Figure 1: Mean ± SD glucose concentrations on days 1, 3, 4 and 5 measured via glucometer, CGMS (Dexcom, Libre), and chemistry analyzer (CHEM) in 8 horses. * P<0.05 Dexcom vs CHEM.
Figure 2 A-E: Bland-Altman-plots describing the degree of agreement between 2 glucose measuring techniques on days 1-5. The solid line shows the mean difference, whereas the upper dashed line represents the upper limit of agreement (Diff + 1.96 x SD), and the lower dashed line represents the lower limit of agreement (Diff + 1.96 x SD).

Table 1: Bland-Altman and Lin’s coefficient analysis of glucose concentration (mg/dL)
Figure 3: Mean ± SD glucose concentrations in dextrose induced hyperglycemia on day 2 measured via glucometer (POC), CGMS (Dexcom, Libre), and chemistry analyzer (CHEM) in 8 horses. ** P<0.01 compared to time 0; # P<0.05 POC vs CHEM.

Table 2: Bland-Altman and Lin’s coefficient analysis of glucose concentration (mg/dL) comparisons between POC, Dexcom, Libre, and CHEM during dextrose-induced hyperglycemia on day 2.
Discussion

In this study, both the Dexcom and Libre showed good agreement and were significantly correlated with the CHEM with the mean bias of 10.45 mg/dL and 1.53 mg/dL, respectively, indicating that the Dexcom and Libre overestimated blood glucose by 10.45 mg/dL and 1.53 mg/dL, respectively. Despite the close correlation with CHEM, the 95% confidence interval for the Dexcom and Libre was -22.68-43.58 mg/dl and -34.91-38 mg/dl, indicating a notable range.
in which the results of the device measurements could fall compared to CHEM. In comparison, the POC glucometer, which is the most frequently used POC glucose measuring device used in clinical practice, had the largest mean bias (18.13 mg/dL) and wide 95% confidence interval (-10.5 to 46.8 mg/dL). Because 65.5% of Dexcom values and 66.8% of Libre values were within 15% of the CHEM values, compared to only 35.9% of POC values within 15% of CHEM, the authors suggest that either GMS is an acceptable clinical tool to help guide therapy.

The OGAT is a long-standing and simple method to evaluate small intestinal absorption and was used to induce hyperglycemia and determine how the GMS performed at high glucose concentrations.[16] In order to safely administer the solution via nasogastric tube, all study horses were sedated with 0.4 mg/kg of xylazine intravenously. While the use of sedation may have induced hyperglycemia, it should not have affected the comparison of the values attained using different measurement methods on the same blood sample. Dextrose-induced hyperglycemia did not impact the accuracy of the devices used with the mean bias of Dexcom and Libre of 10.45 mg/dL and 0.34 mg/dL, respectively. The mean bias for the POC device was the highest amongst the devices tested at 30.6 mg/dL. In addition, both GMS provided more detailed OGAT glucose curves created by near continuous (every 5 minute) measurements of interstitial glucose during the OGAT period (Figure 5).
Figure 5: Daily view provided by the manufacture’s software systems for the FreeStyle Libre 14-day on LibreView (A) and the Dexcom G6 on Dexcom Clarity (B) from one horse on day 2 during OGAT.

Conventional blood glucose monitoring only allows for spot glucose determinations at set intervals, producing a snapshot of glucose concentrations at one point in time. This limits the amount of information available to base treatment decisions and increases workload on hospital staff. Frequency of venipuncture is also associated with patient discomfort and stress, which can cause transient hyperglycemia, complicating the interpretation of a single glucose value.

Utilizing interstitial GMS eliminates the need for venipuncture, lessens workload on staff, and provides dynamic information about glucose concentrations over time for clinician interpretation. Human subjects report little to no discomfort associated with GMS application, and the same was subjectively observed in the equine population in this study. Once applied, the Libre obtains glucose readings each minute while the Dexcom obtains readings every five minutes, resulting in 1,440 and 288 readings/day, respectively. Both devices have the ability to measure glucose readings within the range of 40 mg/dl to 400 mg/dl. Slightly different technologies between real-time continuous devices (e.g. Dexcom G6) and flash devices
(FreeStyle Libre 14-day) affect the way each device reports measurements. The Dexcom G6 automatically uploads readings to the reader via Bluetooth technology every 5 minutes. The Freestyle Libre also generates readings every 5 minutes, but the patient’s sensor must be scanned with a receiver or other compatible device to obtain readings at least every 8 hours due to limits of sensory memory. Both of these technologies allow for more comprehensive glucose data to be obtained with less patient disturbance and technical support.

Depending on clinical application, serial blood glucose monitoring by traditional methods can result in significant cost, but recent developments in GMS technology have increased their cost effectiveness, making them a potential option for clinical use. Both devices have the option to purchase receivers which cost approximately $75 (FreeStyle Libre 14-day) to $365 (Dexcom G6). The use of a compatible smart phone may negate the need to purchase a receiver. The FreeStyle Libre 14-day requires the purchase of an approximately $57 sensor. A sensor and transmitter are required for Dexcom G6 use, costing approximately $117 and $250 respectively. While the sensors for either device are limited to one time use, the Dexcom G6 transmitter is reusable up to 90 days.

A recent study evaluated the use of the Libre in horses,[15] comparing the Libre with the AlphaTRAK POC glucometer. Sensors were left in place for a maximum of 31 hours, whereas the study reported here evaluated sensors for approximately 108 hours. In the previously reported study, samples were collected every five minutes to evaluate a lag effect between change in blood glucose concentration and interstitial fluid glucose concentration under normal conditions, during insulin-induced hypoglycemia, and during dextrose-induced hyperglycemia. A lag effect was observed with significant differences detected in different phases of the study. A 60-minute lag time between glucose values generated by POC and Libre was observed with resting glucose
values, while lag times of 10 and 20 minutes were observed with insulin-induced hypoglycemia and dextrose-induced hyperglycemia, respectively. In the previous study, the mean bias (95% limits of agreement) for dextrose-induced hyperglycemia was -0.03 (-2.46-2.52) mmol/L as compared to 1.69 (-1.43-4.80) mmol/L in the current study. The previous study suggested that because of conflicting bias between phases of hypoglycemia and hyperglycemia, use in practice may be limited due to potential delay of clinical decisions and detriment to the patient. Although the POC represents the standard use of glucose measurement in clinical settings, it is not the gold standard of measurement and likely has inaccuracies, producing some degree of bias, and therefore effecting assessment of accuracy. The current study also compared both the Dexcom and Libre to the gold-standard laboratory assay, the results of which showed smaller mean bias and limits of agreement.

There are several limitations to the study reported here. Assessment of glucose concentrations during a hypoglycemic state was not evaluated in this study. In a previously reported study,[15] when hypoglycemia was induced by administration of 0.10 iu/kg of regular insulin; the GMS (FreeStyle Libre) measured higher glucose concentrations than POC during periods of hyperglycemia. Another limitation of this study was that the sensors were not investigated for the marketed longevity of 10- or 14-days for the Dexcom and Libre, respectively. Sensors were left in place for a maximum of approximately 106 hours. A total of 10 Dexcom sensors and 13 Libre sensors were used for the 8 horses over the 5-day study period due to dislodgement or “sensor error” readings. Additionally, several variables have been shown to interfere with glucose measurement using point-of-care glucometers, including oxygen pressure, hematocrit, pH, temperature, and medications such as mannitol, dopamine, and ascorbic acid. [18] These variables were not measured in the current study because the horses used were apparently
healthy. Further study is needed to investigate the effect of these variables on GMS performance in horses.

Veterinary medicine lacks a consensus regarding criteria for evaluation of glucometers for clinical use. Additionally, influences of sample type, glucose concentration, and delays in analysis on results in horses are largely unknown. [19] In the current study, all blood samples submitted to the laboratory for CHEM analysis were collected in sodium fluoride collection tubes. During business hours, these samples were immediately submitted to the laboratory for analysis. Any samples collected after hours were stored at 4°C until submission. Fluoride inhibits enolase, an enzyme in the glycolytic pathway, and sodium fluoride tubes have historically been used to prevent glycolysis by erythrocytes. Although the use of sodium fluoride within collection tubes prevents glycolysis, studies have shown their use can artifactually decrease plasma glucose concentration through loss of intracellular water from red blood cells, therefore diluting the plasma in the sample.[20] Ferrante et al. showed a 6-10% decrease in blood glucose concentration in samples obtained from horses collected in sodium fluoride tubes.[21] Recently, Rendle et al., 2019 evaluated the effect of sample type and storage on glucometer precision and accuracy, finding that blood stored in tubes containing EDTA and fluoride oxalate resulted in improved glucometer repeatability and agreement with laboratory standard, although the glucose concentration was underestimated.[19] The effect of delayed serum separation and storage temperature was evaluated by Collicutt et al. who found that the storage of whole blood at 4°C limits serum glucose concentration decline in equine blood samples for up to 8 hours of storage.[20] A combination of the use of fluoride collection tubes and the length of time of
sample storage due unavailability of after-hours laboratory testing represents a limitation in this study.

Glucose monitoring systems have multiple potential clinical applications in equine medicine. Serial monitoring of glucose concentrations is necessary for the diagnosis of several equine diseases, such as small intestinal malabsorptive disorders or insulin dysregulation. Glucose concentrations are also measured in horses with altered glucose homeostasis during critical illness or during parenteral administration of intravenous nutrition. Some of these applications require sampling as often as every 30 minutes, which can cause discomfort to the patient and require added technical support. GMS offer the ability to monitor glucose in these patients non-invasively while obtaining near continuous glucose data. Reducing fluctuation and variability in glucose concentrations has been shown to decrease morbidity and mortality in human patients in intensive care units. The results of this study support the use of these devices for application in clinical equine medicine.
References


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Chapter 4: Final Comments

This thesis evaluated the use of two glucose monitoring systems, the Dexcom G6 and FreeStyle Libre, in adult horses, revealing good agreement and significant correlation when compared to the laboratory reference method. Furthermore, over 65% of values obtained by either CGM device were within 15% of the laboratory value, compared to only 35.9% of the values obtained by the point of care device commonly used in clinical settings. These findings combined with potential benefits of providing more comprehensive glucose data and decreasing patient stress associated with sampling suggest further research on potential application is warranted. Of particular interest would be monitoring of patients receiving parenteral nutrition, who often have glucose monitored frequently, and in some cases, for an extended period of time. Additional applications in diagnostics such as oral glucose absorption testing, as was described in the study previous could provide additional information on glucose variability not readily available currently due to the intermittent nature of sampling. With the advancements in CGM technology, ease of use, affordability, and comprehensive nature of data obtained, the use of these devices in equine medicine may continue to prove to be useful clinical tools.