USE OF A REAL-TIME CONTINUOUS GLUCOSE MONITOR IN HEALTHY DOGS DURING ANESTHESIA

by

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Abstract

The use of continuous blood glucose monitors (CBGMs) has recently come into favor in human medicine for the control and monitoring of the diabetic patient. It allows for a higher degree of accuracy of the true glucose curve throughout a 72-hour period. With this information, physicians are better equipped to treat and manage diabetic patients. Recently, this modality has been verified for use in veterinary patients including cats and dogs. This is an excellent source of information, especially in the management of difficult to regulate veterinary patients. This device has potential for use in various applications, particularly for the monitoring of patients with various diseases under general anesthesia. In order to ensure accurate results do occur when an animal is under general anesthesia, the continuous blood glucose monitor was evaluated on apparently healthy patients under anesthesia for routine procedures such as ovariohysterectomies and orchiectomies. In this manner, the monitor was tested on anesthetized patients that had the potential to experience hypothermia, hypotension, and other anesthesia-associated complications that can be typical of patients that could benefit from the CGMS.

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Dedication

It is without question that I dedicate this thesis to my husband, John. There are no words to describe the immensity of support and encouragement he has given me. The sacrifices that he has made can only be demonstrated by the 8 years or our 17 year marriage that we have been apart for me to pursue my dream to become a surgeon. John has waited patiently and supported me financially and emotionally, supporting two households, tolerating long expanses of time without contact, and acting as my cheerleader when I was discouraged or defeated. He deserves an award for the unending assurance and comfort that has made it possible for me to be here and to achieve this degree.

CHAPTER 1 - Literature Review

Introduction – Background and Importance

Historically, plasma glucose determination has been accomplished through the use of peripheral blood sampling. Venous or capillary sampling has most often been utilized with infrequent use of arterial sampling. These methods may be simple and convenient, especially when sampling is performed at home by the patients themselves. The disadvantage of blood sampling is the potential pain associated with skin puncture, the trauma from required repeated sampling, and, in intensive monitoring cases, the reduction of the circulating red cell mass. The most important disadvantage of periodic sampling methods however, is that significant changes in plasma glucose levels can occur rapidly and, if undetected, can lead to increased patient morbidity. [1]

Intensive monitoring of anesthetized patients allows for early detection of life threatening changes in patient homeostasis. Currently, both human and veterinary patient parameters such as blood pressure, cardiac rate and rhythm, end-tidal carbon dioxide concentration, oxygen saturation, and temperature are routinely monitored continuously with real-time measurement. A simple method for continuous measurement of glucose has not been available until recently. Clinical trials in adult humans undergoing surgical procedures have shown that strict glycemic control results in a 35% to 53% reduction in mortality and even greater reduction in morbidity.[2, 3] This lends evidence to the importance of accurate and diligent monitoring of blood glucose concentration in anesthetized patients.

Development of several continuous glucose monitoring systems (CGMS) for use in human diabetic patients provide continuous, real-time monitoring of the interstitial glucose

concentration. Interstitial glucose measurements from CGMS have been shown to correlate well with plasma glucose concentrations.[4,5] The CGMS has been validated in veterinary patients and is currently used to assist in the regulation of diabetes mellitus.[6,7] The CGMS has not, however, been evaluated as a method for routine glucose monitoring of veterinary patients that are anesthetized. Real-time monitoring by the CGMS during the anesthetic period has great potential to improve patient monitoring if it can increase the detection of significant changes in glucose concentration which might be missed using intermittent glucose measurement. Additional advantages include elimination of the need for a sampling catheter, reduced need for blood sampling and preservation of red blood cell mass, and reduced manipulation of the patient during surgery.

Glucose Regulation

Glucose is present in the body in two stereoisomers, L-glucose and D-glucose. Only D-glucose is biologically active and available for energy production. D-glucose is utilized in both aerobic and anaerobic pathways to produce ATP and, during aerobic metabolism, the by-products carbon dioxide and water.[8] Glucose is the sole metabolic fuel for the brain except during periods of starvation in which ketones become a secondary fuel source. Organs and muscle tissue can utilize both glucose and fatty acids as a fuel source.[9,10] Sources of glucose include intestinal absorption, glycogenolysis or breakdown of glycogen stored within cells, and gluconeogenesis mainly in the liver and to a lesser degree in the kidneys.[9] Plasma glucose levels are the result of glucose entering the bloodstream from both liver and dietary sources as well as glucose leaving the bloodstream to enter the interstitium prior to tissue uptake.[8]

Regulatory and counter-regulatory mechanisms are in place to maintain plasma glucose levels within a narrow range. Regulatory mechanisms prevent hyperglycemia via the secretion

of insulin. Counter regulatory mechanisms prevent hypoglycemia and include hormones such as glucagon and epinephrine.[9]

Insulin is produced by the beta cells of pancreatic islets and is released in the presence of increasing plasma glucose levels. Briefly, the production of ATP has a direct influence on the release of insulin.[11,12] When glucose is in a steady state (ie: late post-prandial and early fasting), the ATP to ADP ratio is low. Under these circumstances, ATP-gated potassium channels, (K_{ATP}), are open and potassium is allowed to leave the cell maintaining a resting membrane potential of -60 mV. Increasing glucose concentrations in the blood lead to higher concentrations of glucose within the beta cells. This initiates increased production of ATP by cellular mitochondria. When the ATP to ADP ratio is high, ATP binds to K_{ATP} channels close, potassium efflux is decreased, and the cell is depolarized. At a membrane potential of – 40 mV, voltage gated calcium channels open and intracellular free calcium concentration rises rapidly. Influx of calcium stimulates the release of insulin from granules stored in the beta cell. Insulin regulates plasma glucose levels by suppressing both glucose production by the liver (gluconeogenesis) and release of stored glucose (glucogenolysis) as well as by stimulating glucose utilization. Insulin also has secondary effects on the circulating levels of fatty acids and glucagon through stimulation of vagal input and effects on the hypothalamus respectively. Decreasing levels of plasma glucose has an immediate inhibitory effect on insulin secretion.[9]

The counter-regulatory hormone glucagon is released from the alpha cells of the pancreatic islets and increases plasma glucose levels directly via activation of glycogenolysis and gluconeogenesis within the liver. Epinephrine is produced in the chromaffin cells of the adrenal medulla and increases circulating plasma glucose levels through $ß_2$ adrenergic activity. This

causes gluconeogenesis by the liver as well as mobilization of precursors for gluconeogenesis. Due to this second effect, epinephrine, unlike glucagon, can lead to persistent hyperglycemia.[9]

During fasting, as in patients prepared for anesthesia, glucose is preserved for utilization by the brain and the primary source of glucose is from production by the liver, which includes glucose stored in glial cells.[13] This source of glucose is depleted within 3-8 hours after a fast has begun. With continued fasting, fat and muscle tissue utilize fatty acids exclusively again conserving all remaining glucose for brain metabolism. Brain glucose utilization decreases as sources are depleted and there is an increase in lipolysis with ensuing ketone production and utilization. By using ketones and decreasing the amount of glucose utilized by the brain, muscle protein is preserved preventing muscle wasting in prolonged periods of fasting.[9]

Glucose Distribution and Kinetics

Glucose distribution and kinetics have been demonstrated by several different models in the literature. Mathematical models used to represent the complex physiology and distribution of glucose include lumped parameter models[10,14] and compartment models.[15-18] These will not be discussed here but can be summarized as follows. Lumped parameter models are named due to the number of parameters that cannot be measured and are therefore assumed. This can lead to inaccuracies within a particular study so the unknown parameters must be demonstrated to have a negligible effect in each case in which lumped parameter models are utilized.[10]

The measurement of interstitial glucose has led to the development of a two compartment minimal model.[15-18] A minimal model, as defined by Toffolo, is "the least complex mathematical representation capable of accounting for the observed dynamic relationship between insulin and glucose".[19] To understand this model, we must first discuss the current theories of glucose kinetics. The exact mechanism for glucose transport into the interstitial space

is not completely understood. One theory is that glucose moves from plasma into the interstitial space by simple diffusion down the concentration gradient across the capillary wall.[10,14,20] The rate of diffusion depends on the barrier characteristics of the capillary wall.[17] Tissues such as heart and liver are perfused with fenestrated capillaries[21] allowing for uninhibited flow of glucose into the interstitial space. Other tissues such as adipose and muscle tissue have unfenestrated capillaries. This leads to a delay in the equilibration of glucose from the capillary to the interstitial space. The significance of this delayed equilibration will be discussed later. The two compartment model best describes glucose transport into the interstitial space by simple diffusion with the interstitial space representing one compartment and the plasma being the other. Areas with no barrier to the flow of glucose across the capillary wall such as heart and liver are considered part of the plasma compartment and are represented by a one-compartment model. The 2-compartment model was first described in 1993 by Caumo and later validated by Vicini.[15,16] Vicini tested the model by performing intravenous glucose tolerance tests with radio-labeled glucose ([2-^{2}H] glucose) and by monitoring plasma glucose and insulin levels.[16] The 2-compartment model defines the compartments as an accessible pool (plasma) and the slowly equilibrating pool (interstitial space) and relates the two compartments through diffusion coefficients that account for the gradient of plasma glucose to interstitial glucose.

A study by Rebrin et al., also validated both the two-compartment model and the correlation between plasma glucose and interstitial glucose.[22] In Rebrin's study, three separate experiments were performed in dogs. First, hyperglycemia was induced by preventing endogenous insulin release with somatostatin during glucose infusion followed by return to euglycemia. Second, a glucose infusion was given while endogenous insulin was allowed to be released normally. And in the final experiment, a glucose infusion was given, endogenous

insulin was allowed to be released, and exogenous insulin was given as well. Plasma and interstitial glucose levels were monitored during all three experiments. Interstitial glucose levels were measured using an amperometric sensor placed in the subcutaneous tissue. The study was designed to determine if changes in insulin levels have a significant effect on the gradient between the plasma glucose and the interstitial glucose. In all three experiments, although there was a delay, the difference was not significantly different. The study showed that the two-compartment model was a good fit for description of glucose kinetics of the interstitial space since this model was used to determine the gradients utilized.

Steil also used the two compartment model to show the correlation of interstitial and plasma glucose.[18] Human subjects were given infusions of both insulin and glucose while the interstitial space and peripheral blood were sampled. An amperometric sensor was used in this study as well. During the infusions, glucose was clamped at three defined plasma glucose concentrations as hypoglycemia was induced and then the plasma glucose was allowed to recover to a euglycemic range. The important findings in this study were that there was not a significant change in the plasma to interstitial glucose gradient during insulin induced hypoglycemia, and when the two compartment model was used, the interstitial space accurately represented plasma glucose.

Boyne in 2003 performed a study that is perhaps the most relevant with regard to glucose dynamics in real-life situations.[23] The study was performed in humans with stable Type I diabetes. The interstial space was sampled every 5 minutes for eight hours using an amperometric sensor. Plasma glucose was also measured simultaneously. During the study period, subjects were administered insulin at their typical dose and time and a meal was given shortly thereafter. In contrast to the previous studies, large swings in glycemia were not

expected, but instead the normal daily glucose excursions expected in a typical controlled diabetic patient. In this study the change in plasma glucose consistently preceded the change in interstitial glucose as reported by the previous studies. Again, this delay was well defined at 6.7 min +/- 5.1 min and was predictable. This predictability has implications for continuous monitoring of glucose in clinical and research settings.

Not all studies agree with the data presented with regards to time course of changes in glucose of the interstitial space relative to plasma. Separate experiments by Aussedat, Kulcu, and Schmidtke were conducted in a similar manner to the studies which induced hyper- and hypoglycemia while both the interstitial and plasma glucose were sampled.[24-26] Again, a sensor-type continuous glucose monitor was used to sample the interstitial space. All of these studies demonstrated that under conditions leading to hypoglycemia, the interstitial glucose level actually decreased before plasma glucose. This is explained by Aussedat et al. as a "push-pull" phenomenon of glucose kinetics.[24] Since the interstitial space is an insulin sensitive tissue, when insulin is released (or administered), glucose from the interstitial space is transported into cells. This leads to a lower glucose level in the interstitium than the plasma until glucose from the plasma slowly diffuses down this gradient to once again establish equilibrium. This effect may be exaggerated by the fact that insulin inhibits release of endogenous glucose into the plasma pool. These same studies describe mathematical algorithms to account for this discrepancy when using interstitial glucose measurements to represent plasma glucose levels.[24-26]

A different theory of glucose dynamics has been described in a study by Quinn and others.[27] Plasma and interstitial glucose levels were compared during infusion of increasing doses of glucose. As the dose of glucose increased, the delay between the compartments was increased. For this reason it was speculated that a facilitated transport that could be saturated

must be present to account for the increasing delay. A similar study by Nielsen used increasing glucose boluses while measuring interstitial fluid in several different tissues including adipose tissue, as well as the interstitium of skeletal muscle and cerebral cortex.[28] The glucose curve was similar for all compartments compared to plasma glucose, however, glucose levels in the cerebral cortex were blunted compared to adipose tissue and skeletal muscle. During periods of hyper- and hypoglycemia, glucose levels in the brain paralleled the glucose curve of other compartments, but glucose was never quite as high or quite as low as those seen in the interstitium of other tissues. Nielsen speculated this may be due to a protective mechanism within the brain to prevent exposure to extremes of glucose. All tissues had a similar delay compared to plasma glucose and, contrary, to findings by Quinn et al., this was independent of the dose of glucose.

Because the capillary wall is a significant barrier to glucose diffusion, it is expected that a delay exists in the equilibrium between the plasma and interstitial glucose pools. This has been shown to be true in several experiments.[17,18,22,24,25,27] Quinn's study as described above describes a delay of between 7.5 and 10 minutes depending on the glucose load administered.[27] The study by Aussedat and others measured both plasma and interstitial glucose in rats in multiple scenarios with varying injections of glucose with and without insulin administration.[24] There was a consistent delay of between 5-12 minutes, most commonly < 10 minutes, during subtle changes in plasma vs interstitial glucose.[27] In Rebrin's study, the plasma glucose data was graphed and then the interstitial data was graphed with delay correction.[22] This meant graphing the interstital data by subtracting the calculated delay from the time of measurement. The delay correction did reveal a significant increase in accuracy of the data but only in one of three experiments tested in Rebrin's study. This experiment involved induced hypoglycemia with a

drop in plasma glucose of 70 mg/dl in just 10 minutes. In the development of a method to measure interstitial glucose, concerns have been voiced about how a delay could affect clinical decisions. For this reason, accounting for this delay has become an integral part of the processing of interstitial data by continuous glucose monitors today.

Continuous Glucose Monitoring Systems

A method for monitoring glucose levels continuously has been sought for almost half a century. The earliest mention in the literature was in 1962 when Clark described using an electrode with a permeable membrane that was bathed in diluted blood and sent data to a dialyzer.[29] In 1984 Abel described using a "Clark-type" glucose electrode in the earliest development of an "artificial beta cell".[30] This early attempt gave a foreshadowing of the eventual path of continuous glucose monitoring, but this was perhaps too soon. Years of development and modifications to ensure accuracy would be needed before a continuous glucose monitor could be relied upon for such a task. Abel's study did, however, lead to development decisions including the type of electrode membrane to use as well as serve as one of the earliest indicators of the reliability of the interstitial space. Pfeiffer, in 1987, presented a review that referred to the "dark past, the grey present, and the rosy future" of automated glucose regulation.[31] Until that time, continuous glucose monitoring involved a large amount of equipment that initially was not portable. Even when a "portable" version was developed, it was the size of a shopping cart that had to be pushed by the patient. These monitors pulled samples of venous blood into a reservoir, diluted the sample, and determined the glucose level. Although the equipment was bulky, accuracy was good. Eventually, however, electrode sensors fell out of favor because they tended to stop working within 24 hours of implantation. Scanning electron microscopy of the used sensors revealed a coating of cellular debris and fibrin indicating a local

inflammatory reaction to the sensor. It was not until the late 1980's that electrode sensors were once again investigated for interstitial sampling. At that time, changes to the design of the sensor and *in vitro* experiments led to a resurging interest in this technology.[31]

Electrode sensor development continued through the early 1990's with *in vitro* studies and a few *in vivo* animal studies including rats, sheep, and rabbits. The basic structure of the electrode is an amperometric sensor that relies on the production of a current between two dissimilar metals. Glucose is detected by the glucose oxidase reaction and occurs entirely at the electrode within the sensor component.[6,22,29,32-34] The sensor is coated with glucose oxidase and acts as a reaction surface for the enzymatic conversion of glucose to hydrogen peroxide. Free electrons formed as the glucose oxidase reaction proceeds are detected as the electrical current. The current is proportional to the glucose concentration.

As investigators began to conduct *in vivo* experiments, it was evident that the sensitivity of these sensors differed from *in vitro* trials.[35] This becomes important later with reference to calibration of electrode sensors. Using the compartment models previously discussed, algorithms were developed as *in vivo* experiments progressed to convert the data collected from the interstitial space by the electrode to a glucose concentration that adequately represented plasma glucose. By 1999, one company had developed a continuous glucose monitor that was reliable and accurate that was approved for use in humans by the Food and Drug Administration, (FDA).[36] Because of the fervent interest in this technology, multiple clinical trials were conducted throughout the next 10 years.

The first FDA approved CGMS consisted of an electrode sensor and control unit that stored the data. The sensor had a flexible probe that was placed through the skin into the subcutaneous space using a needle stylet. The needle stylet was removed leaving only the sensor

electrode remaining under the skin. Glucose data was acquired by the control unit every 10 seconds for a 5 minute period. The five minute averaged data was stored, providing 12 measurements per hour and 288 measurements per day. During use of the CGMS, several capillary blood glucose (CBG) measurements were required to calibrate the sensor. These were determined with a handheld glucometer and entered manually into the control unit at various times throughout the day. The dynamic range of the CGMS was 40 - 400 mg/dl glucose. The system was approved for use for 72 hours during which time the control unit would collect and analyze the data. Once removed, the data was uploaded to a computer to display 72 hours worth of continuous glucose data.

A pilot study by Kaufman in 2001 used the CGMS in 47 children with type I diabetes.[4] In this, and many subsequent studies, glycosylated hemoglobin (HbA_{1c}) was used as an indicator of glucose control. Glucose irreversibly binds hemoglobin during episodes of hyperglycemia and can be detected in a laboratory assay.[37] Physicians have used this assay to determine the effectiveness of diabetes therapy regimens. Target HbA_{1c} levels in humans are 4.3-6.3%. Levels higher than this indicate sustained episodes of hyperglycemia in the previous 1-3 months. This short timeframe allows for surveillance of the effectiveness of intensive glucose monitoring with a CGMS in a clinical trial setting. Kaufman's study determined the HbA_{1c} levels in children 3 months prior to the study period, at the time of placement of a CGMS and 3 and 6 months after CGMS use.[4] The CGMS was worn for 3 days and recommendations about therapeutic changes were made once the data was uploaded and analyzed by a physician. This study showed a significant improvement in HbA_{1c} levels throughout the study period in all but 4 out of 47 patients. Perhaps more important, however, was the detection of 191 episodes of hypoglycemia from the 47 children for the three days that the CGMS was worn. The CBG measurements taken

during the same three days detected only 42 episodes of hypoglycemia. The results of the CGMS led to significant changes in the therapy regimen for all children in the study.

Ludvigsson conducted a controlled crossover study with twenty-seven diabetic children.[38] Study patients wore the CGMS for 3 days every 2 weeks. For the first 3 months of the study, all patients were blinded to the CGMS data. During the second 3 month period one group continued to be blinded to the CGMS data while another group had access to the data at the end of each three-day wear period. Finally, during the third 3 month period, the groups switched allowing the previously blinded group access to the CGMS data and blinding of the previously aware group. This resulted in 642 days of combined glucose profiles. Glycolsylated hemoglobin assays improved for both groups, however, the most significant improvement occurred in the group that was blinded to CGMS data for the first 6 months but given access to the data during the last 3 months of the study. The initial improvement was attributed to the increased awareness and management due to inclusion in the study. Unfortunately, the group that was aware of the CGMS data during the middle 3 months and blinded during the last 3 months initially improved but returned to pre-study HbA_{1c} levels during the blinded month.

It was quickly evident that, although important information was being obtained with the CGMS, making the transition to real-time accession of data would be important not only for patient management, but also to again forge a path toward the artificial beta cell. After 6 years of CGMS use, modifications to the system allowed for immediate display of glucose data. The new system utilizes the same electrode sensor, but is now attached to a small transmitter that is the size of a quarter. This transmitter sends data wirelessly to the newly designed control unit/monitor that is about the size of a pager, half the size of the original unit. The excitement about the improvements to the system is evident by the rapid development of studies to test the

system. Two studies were published in 2006 using the new system.[39,40] Each study used a system by a different manufacturer also demonstrating that a race had begun. The goal of the race would be to incorporate a real-time continuous glucose monitor with an insulin pump, creating a closed-loop glycemic control unit.

First, a study by Garg placed a CGMS on two groups of diabetic patients.[39] Both groups were blinded for the first three days and the second group was unblinded for the second and third three-day period. As in all clinical studies, CBG measurements were continued as is normally required for diabetes management. With 91 subjects and 9 days of data, over 6700 paired (CGMS vs CBG) glucose measurements were obtained. Accuracy was considered good for these measurements. This study utilized a new way of analyzing the data because, with the availability of real-time data, deviation of any single value from the reference data is less important than the effect of that value on the clinical decision made by the patient or the physician.[41] The Clarke Error Grid[42] and later the Consensus Error Grid[41] attempt to analyze paired measurements (CGMS vs CBG) and place them on a grid with zones to describe the clinical relevance of the comparisons. The reference glucose (CBG) is plotted on the x axis while the CGMS glucose is plotted on the y axis. Zones are lettered A-E and are designated on the graph. The zones are designated as follows[41]:

Zone A: Clinically accurate (treatment decision correct)

Zone B: Benign errors (CGMS value outside the accepted accuracy range but does not lead to deliterious decision)

Zone C: Overcorrection errors (CGMS data outside the target range while CBG data is within the reference range and treatment decision may result in glucose outside the reference range)

Zone D: Failure to detect (failure to treat either hypo- or hyperglycemia)

Zone E: Erroneous errors (CGMS values opposite the reference values leading to treatment that is opposite of what is needed)

Values are considered valid when they fall in Zones A and B. In Garg's study, more than 95% of the 6700 data points fell in Zones A and B.[39] A study by Mastrototaro resulted in over 60,000 paired data points and found similar results with 95.9% of all data points falling in zones A and B.[40] The study by Mastrototaro was the first study to combine the CGMS with an insulin pump. The insulin pump was not integrated to make decisions based on the CGMS data. Instead, the study was designed to monitor patients on a continuous infusion of insulin who were already accustomed to using an insulin pump. Today a system is available that incorporates both the CGMS and an insulin pump. Again, the system is not integrated yet, but it allows pump users to periodically obtain 72 hours continuous glucose curves without having to purchase or wear a separate device. This data is used by the physician to make alterations to the continuous insulin regimen as well as diet and lifestyle changes.

Finally, a study published in 2009 by Mazze compared two popular systems with results similar to Mastrototaro.[43] The systems displayed either 93% or 98% of all paired data points in Zones A and B of Clark Error Grid analysis.[43] Despite these results, the author voiced that there is still enough incongruence of the data to delay incorporation of the CGMS with an insulin pump.

Accuracy in both absolute glucose measurement as well as temporal reporting of glucose data has not come easily. As stated above, a consistent delay of between 5 and 12 minutes of interstitial glucose achieving the levels found in the plasma is present in most studies.[17,18,23-25,27,28] Also, the actual value of glucose in the interstitial space is potentially 70%

of that in the plasma at any given moment.[24] To allow the interstitial glucose measurement (which may ultimately be the more relevant value equating the true tissue glucose levels) to represent the plasma glucose, mathematical algorithms have been developed using the compartment models discussed previously. These algorithms relied on calibration by manual input of CBG data several times throughout the day and analyzed the data retrospectively in the original CGMS system. The new system requires an entirely new set of algorithms to allow real-time reporting of data. The algorithms are both retro- and prospectively analyzed throughout the CGMS wear period. The retrospective aspect increases accuracy as the CGMS is worn allowing for incorporation of not just absolute values but also the rate of change detected by the CGMS.[44] As demonstrated by studies referenced in this review, accuracy leading to appropriate clinical decisions can be expected with the current CGMS system.

Further mathematical processing of CGMS data is required to account for the delay of interstitial glucose to plasma glucose as discussed previously. This is accomplished through a mathematical filter that takes into account several factors.[44] These factors include CBG data used for calibration of the CGMS, expected and actual delay, interstitial glucose measured by the CGMS, and the rate of change of interstitial glucose. As stated previously, the delay can be different depending on the rate of change of glucose at the time of glucose determination.[45] There was concern that CBG measurements used as calibrations for the CGMS would instill inaccuracy if they were taken during rapid increases or decreases in glucose levels. An advantage of the real-time system used today is that the wearer of the CGMS can access a graph of the previous glucose data on the monitor and determine if a rapid change is occurring. If so, a CBG measurement for calibration of the CGMS would not be taken at that time. This also alludes to the importance of real-time data vs single point CBG measurements that until now

have been used for diabetes regulation. Single point CBG measurements have been equated to a photograph whereas real-time CGMS data is more similar to a movie.[45] This places less importance on the accuracy of any one single measurement and more importance on the trends demonstrated by the monitor. This is the true advantage of continuous glucose monitoring and of real-time data technology.

CGMS Use in Veterinary Medicine

The first mention of CGMS use in veterinary patients occurred in two articles published simultaneously in 2003.[7,46] Wiedmeyer used the monitor in dogs, cats, and horses during both IVGTT and steady state conditions and found good correlation of CGMS data to plasma glucose levels.[46] Davison used the same system in 10 diabetic dogs with similar results.[7] In 2004, Ristic used the CGMS in diabetic cats and showed that not only did the device represent plasma glucose levels well, but also that it was tolerated by patients some who continued kneading behavior.[47] None of these studies used the real-time CGMS and these were purely studies to determine accuracy not efficacy in case management. A review article by Wiedmeyer in 2008 sited several advantages that could be exploited with CGMS use.[6] In cats, one problem in acquiring glucose curves is the stress induced with venipuncture. This can lead to erroneous hyperglycemia that does not accurately reflect the patient's true glucose excursions. Fewer blood samples are required (current recommendations require 2 calibration samples every 24 hours) and patients could potentially go home with the device on, decreasing the stress level even further. The CGMS also allows for input of data such as feedings and insulin administration that are then displayed on the graphs that are generated upon computer upload. This could lead to changes in case management decisions. And finally, with the new development of real-time measurement, Wiedmeyer proposes use of the CGMS in patients at risk for altered glycemia that

require anesthesia.[6] This includes patients with diabetes, sepsis, insulinomas, and portosystemic shunts. In fact, an abstract from a group in Japan has proposed that post-ligation seizure disorder in portosystemic shunt patients may have a link to global brain damage secondary to hypoglycemia during shunt attenuation surgery.[48] This could be an ideal population for this technology as point measurements during surgery could lead to missed periods of hypoglycemia. Unfortunately, this technology has not been tested in this manner and it will need to be validated in healthy patients before use in abnormal patients can be instituted.

CHAPTER 2 - Thesis Objectives

1. To determine the accuracy of a interstitial continuous glucose monitor when used in apparently healthy dogs placed under anesthesia.

CHAPTER 3 - Materials and Methods

Ten clinically healthy dogs, presented for elective ovariohysterectomy or orchiectomy, were included in the study. Criteria for inclusion in the study included a minimum body weight of 15 kg, a body condition score of 2 or 3 (on a 5-level scale), and documented normoglycemia at presentation. All dogs were expected to undergo a surgical procedure lasting at least 60 minutes. This study protocol was approved by the Kansas State University Institutional Animal Care and Use Committee and informed consent was obtained from owners of all dogs in the study.

Patient health status was verified by a board certified surgeon or surgical resident. A spun hematocrit and total serum protein (by refractometer) were determined for each dog prior to anesthesia. Normoglycemia was verified in all dogs at the time of CGMS calibration by measurement of whole blood glucose concentration by a portable chemistry analyzer[a] (PCA).

The Continuous Glucose Monitoring System

The CGMS[b] consists of an electrode sensor, a wireless transmitter, and a portable, pager-sized monitor. The sensor has a flexible probe that is placed through the skin into the subcutaneous space using a needle stylet. The needle stylet is removed leaving only the sensor electrode remaining under the skin. Glucose is detected by the glucose oxidase reaction and occurs entirely at the electrode within the sensor component. The sensor is coated with glucose

[a] I-Stat portable chemistry analyzer, Heska Corp, Fort Collins, Colo.

[b]Guardian-Realtime CGMS, Medtronic Minimed, Los Angeles, Calif.

oxidase and acts as a reaction surface for the enzymatic conversion of glucose to hydrogen peroxide. Free electrons formed as the glucose oxidase reaction proceeds are detected as an electrical current that is proportional to the glucose concentration. The sensor communicates to the monitor via a small (3 x 3.5 cm) wireless transmitter. Glucose data is acquired by the transmitter every 10 seconds for a 5 minute period. The five minute averaged data is relayed to and displayed by the monitor, providing 12 measurements per hour and 288 measurements per day. The CGMS reports glucose levels between 40 and 400 mg/dl. The small, discrete transmitter and portable monitor devices are well-tolerated by human and veterinary patients.[7,46,47,49]

The system requires calibration at its initiation and once every 12 hours thereafter. To calibrate the device, a whole blood sample is obtained either by peripheral venipuncture or capillary puncture. This sample is typically analyzed by a portable glucometer and the result is entered into the monitor. An internal prospective interpretive algorithm developed by the manufacturer uses calibration measurements to interpret glucose values acquired by the sensor. The sensor device can provide continuous data for up to 72 hours. The small, discrete transmitter and portable monitor devices are well-tolerated by human and veterinary patients.[7,46,47,49]

Placement of the Sensor

The CGMS[b] was placed and activated at least 18 hours prior to surgery. Sensor placement and CGMS set-up was performed as previously described[46] however, an upgraded system was used in the present study. Briefly, hair was shaved from a 10 cm^2 patch of skin on the right lateral thorax and the area was cleaned with alcohol. The sensor was introduced into the subcutaneous space using the needle stylet. The stylet was subsequently removed and the

sensor was adhered to the skin with adhesive tape. After a 5 minute 'wetting' period to establish sensor contact with the interstitial fluid, the wireless transmitter was connected to the sensor and the monitor was activated. Elastic adhesive tape was used to secure the sensor and transmitter to the skin once successful communication between the transmitter and the monitor was verified. Use of an Elizabethan collar prevented inadvertent removal or damage to the sensor by the patient. The monitor was placed on the door of the patient's cage and remained within a 5 ft radius the patient at all times, including during anesthesia and surgery.

CGMS Initialization and Calibration

After a 2-hour system initialization period, 0.2 mL of blood was drawn into a heparinized syringe via peripheral venipuncture for blood glucose concentration determination using a PCA that has been validated for accurate blood glucose measurement in dogs.[50] (Cohn) This measurement served to verify normoglycemia and was entered into the monitor as the first calibration of the system. Additional calibration measurements were performed 8 hours after initialization, and then every 12 hours thereafter as directed by the manufacturer's instructions. Technical failures, defined as a period during which the sensor failed to report data or ceased to function with or without sounding an alarm, were recorded throughout the study period.

Measurements to Ensure CGMS Function

Samples (referred to as 'verification samples') were obtained to determine the function and accuracy of the CGMS before anesthesia and then again 2-hr and 8-hr after anesthesia by comparing glucose readings registered by the CGMS with those from the PCA. Verification samples were obtained by collecting 0.2 mL of peripheral blood in a heparinized syringe for immediate analysis by the PCA. The verification sample results were used to confirm the CGMS was providing accurate readings in the pre- and post-anesthetic periods and were not used

as calibration samples. Values were considered accurate when CGMS values were within 20% of PCA values. The working range of the CGMS is between 40 – 400 mg/dl of glucose. As such all values below or above this range are reported by the CGMS as 40 mg/dl and 400 mg/dl respectively.

Anesthesia

The anesthetic period was defined as the period from induction (designated T=0) to extubation. Dogs were premedicated with acepromazine (0.04 mg/kg SQ) and morphine (0.5 mg/kg SQ). Anesthesia was induced with thiopental (10 mg/kg IV). Dogs were intubated and anesthesia was maintained with isoflurane in oxygen. The isoflurane vaporizer was adjusted as necessary to maintain an appropriate anesthetic plane. All dogs received IV infusion of lactated ringers solution (10 mL/kg/hr) throughout the anesthetic period. Immediately after induction, 0.2 mL of peripheral blood was collected in a heparinized syringe and analyzed immediately using the PCA. The PCA measurement and the concurrent CGMS measurement were recorded as time 0 (T=0). The patient's pulse, respiratory rate, and systolic blood pressure were recorded every 5 minutes and body temperature was recorded every 15 minutes. The glucose value displayed by the CGMS was recorded every 15 minutes beginning at T=0 and a peripheral venous blood sample was obtained for glucose determination by the PCA. The final PCA and CGMS measurements of the anesthetic period were acquired immediately after extubation. All surgical procedures were performed by senior veterinary students under the supervision of a veterinarian.

Statistical Analysis

Mean glucose concentration determined by the CGMS and the PCA were compared at each time period by paired T-test. Changes in glucose measured over time by each measurement

device were analyzed using repeated measures analysis of variance with a Newman-Keuls post-hoc comparison. A correlation coefficient matrix was used to assess effects of age, weight, surgery time, anesthetic time, temperature, and blood pressure on glucose determined by the CGMS and the PCA. A value of $P \leq 0.05$ was considered as significant for all statistical comparisons.

CHAPTER 4 - Results

The ten dogs (9 females, 1 male) deemed healthy on the basis of the pre-admission physical examination met criteria for inclusion in the study. Specific breeds represented included boxer, German shorthaired pointer, and German shepherd (1 dog from each breed); the remaining dogs were mixed breed (7 dogs). Mean age was 20 months (range 6-49 months). Mean body weight was 22 kg (range 15-38 kg). All 10 dogs underwent abdominal surgery. The 9 female dogs had an ovariohysterectomy and the male dog had a bilateral orchiectomy but required abdominal exploratory to locate a cryptorchid testicle. The mean surgical time was 110 min (range 86-145 min) and the mean anesthetic period was 171 min (range 128-215 min). The median number of paired venous glucose and CGMS measurements obtained per patient during the anesthetic period was 12 (range 8-15), yielding a total of 126 paired data points for analysis.

Blood glucose concentration measured by the PCA was used for CGMS calibration at the time of initial CGMS set-up. The mean blood glucose concentration (n=10 dogs) determined by the PCA at the time of initial calibration was 89 mg/dl (range 74-109 mg/dl; reference range 60-115 mg/dl). Preanesthetic verification samples were taken a mean of 136 minutes prior to induction (range 75-290 minutes). The mean preanesthetic glucose values from the CGMS and the PCA were 85.1 mg/dl (range 68-106 mg/dl) and 86.4 mg/dl (range 75-105 mg/dl) respectively. Verification samples were taken 2 and 8 hours following extubation. The mean values from the CGMS and the PCA 2 hours after extubation were 90.8 mg/dl (range 70-118 mg/dl) and 93.3 mg/dl (range 63-118 mg/dl) respectively. The mean values from the CGMS and the PCA 8 hours after extubation were 92.4 mg/dl (range 66-120 mg/dl) and 92.9 mg/dl (range 61-114 mg/dl) respectively. Analysis of all verification samples revealed no significant

differences between glucose concentrations determined by the CGMS and the PCA either before the anesthetic period or at 2-hr and 8-hr post-anesthesia.

Analysis of the 126 paired data points obtained during the anesthetic period showed agreement (< 20% difference) in 71 samples (56.8% of all samples; mean difference 5.54 mg/dl +/- 6.9 mg/dl). Disagreement (> 20 % difference) between CGMS and PCA measurements occurred in 54 samples (43.2% of all samples; mean difference 41.29 mg/dl +/- 17.81 mg/dl). Values from the CGMS were lower than the PCA in all discordant samples. Hypoglycemia (< 60 mg/dl) was reported by the CGMS in 25/126 data points, 4 of which were reported as 40 mg/dl (the lower limit of detection by CGMS). By contrast, hypoglycemia was documented by the PCA at only one of these 25 data points.

The majority of dogs (n=8) exhibited a distinct pattern when CGMS and PCA data from individual dogs was examined over the anesthetic period. In these dogs, glucose values determined by CGMS began to diverge from those determined by the PCA soon after anesthetic induction but became concordant near the end of the anesthetic period. Mean data from all dogs (n=10) is represented in Figure 4.1. To graphically represent the percent difference of paired data points during the anesthetic period (Figure 4.2), the mean percent difference was calculated using the following equation:

$$\mu\left(\frac{(PCR_g - CGMS_g)}{PCR_g} \times 100\right)$$

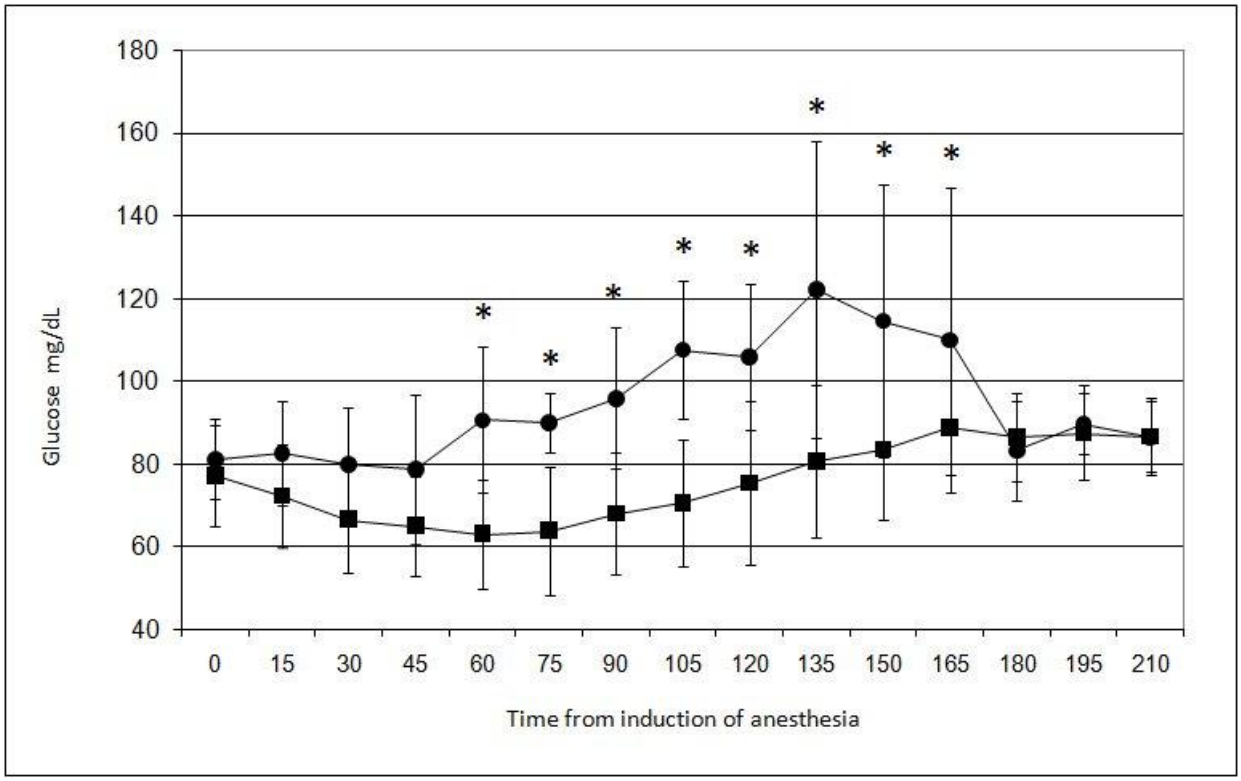
The anesthetic periods experienced by study dogs were not of equal length and it appeared from analysis of individual data that agreement between CGMS and PCA results improved near the end of the anesthetic period. To evaluate this further, the last five paired data points obtained from each dog were used to examine the relationship between CGMS and PCA

data in the 60 minutes immediately prior to extubation (Figure 4.3). The glucose concentration determined by the CGMS was significantly lower than the glucose determined by the PCA at 60 and 45 min prior to extubation but no significant differences were detected beginning 30 min prior to and including the time of extubation.

There were no significant correlations between age, weight, surgery time, anesthesia time, temperature, or systolic blood pressure on glucose measurements obtained by either the CGMS or the PCA.

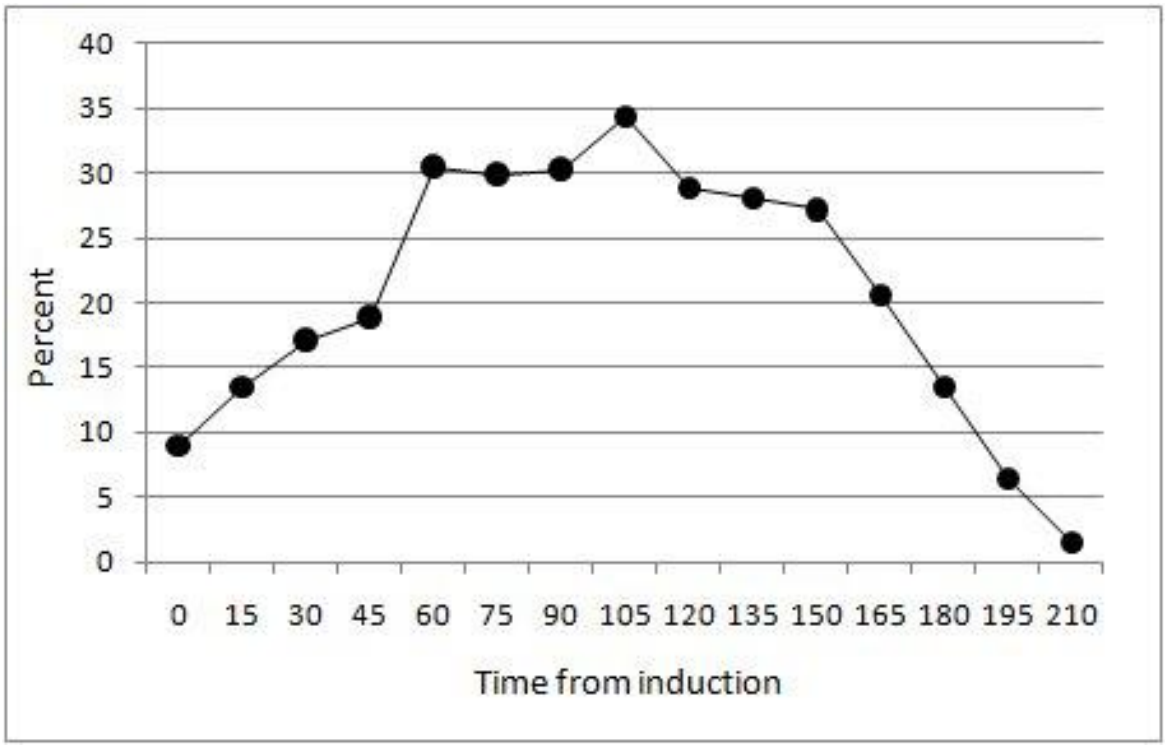
Figures

Figure 4.1 Mean of paired CGMS and PCR measurements during the anesthetic period



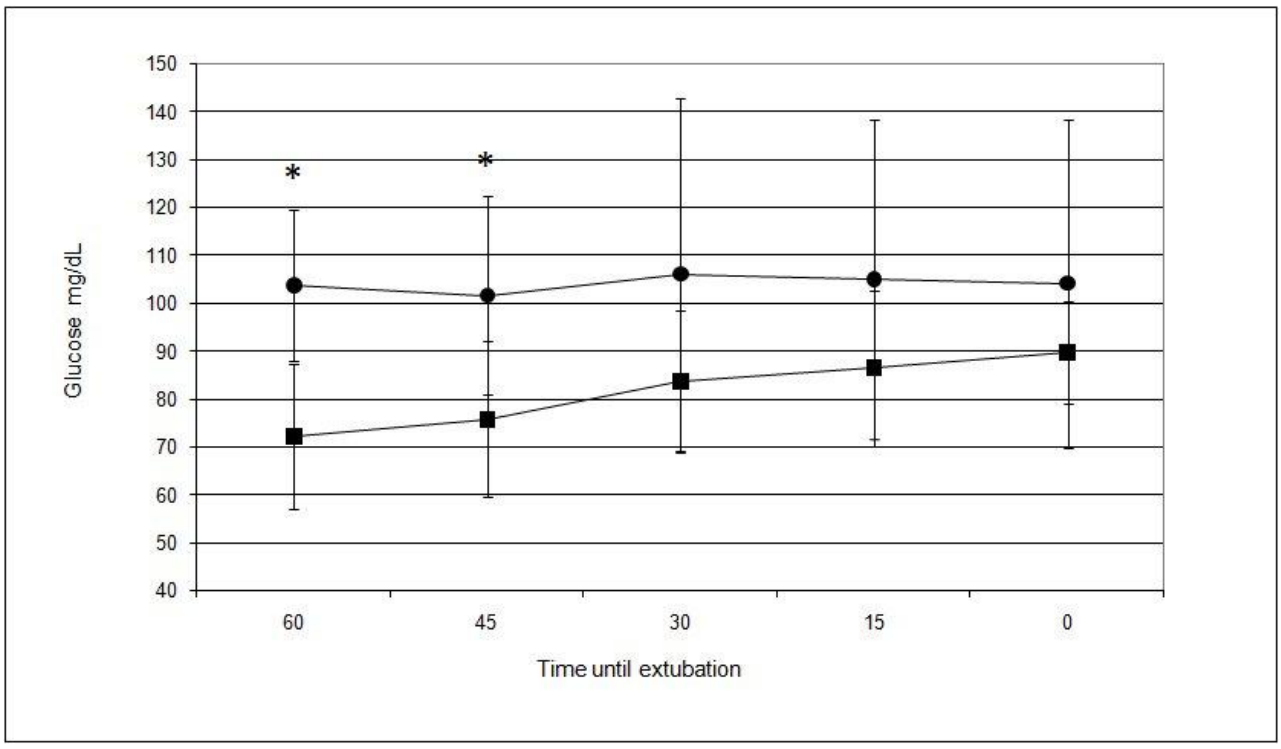
Mean and SD of CGMS (■) and PCA (●) measurements during the anesthetic period. Induction = 0. An asterisk (*) indicates CGMS and PCA measurements that are significantly different at individual time points.

Figure 4.2 Mean percent difference of paired data points during the anesthetic period



Mean percent difference of paired data points (●) during the anesthetic period. Induction = 0.

Figure 4.3 Mean of the last five paired CGMS and PCR measurements



Mean and SD of the last five CGMS (■) and PCA (●) measurements during the anesthetic period. Extubation = 0. An asterisk (*) indicates CGMS and PCA measurements that are significantly different at individual time points.

CHAPTER 5 - Discussion

The high percentage of discordant results provided by the CGMS suggests that it does not allow us to infer information about blood glucose in anesthetized dogs. Blood glucose concentration determined by the PCA was considered the reference data for comparison with data obtained by the CGMS in this study. The portable analyzer used to measure blood glucose in the current study has been shown to be an accurate method for blood glucose determination.[50] The PCA measurements in the study by Cohn indicated that 99% of all PCA readings were within 15% of the measurements by the reference laboratory. [50] This is a high degree of accuracy but even a 15% discrepancy might have interfered with the data in our study. The CGMS has a reported accuracy between 80-99% of reference values.[40,43] A small acceptable discrepancy in each device has the potential to cause a larger significant discrepancy in the study data due to a potential additive effect. This factor could have been addressed by either using in-house laboratory values as reference data or by randomly selecting samples throughout the study period to be analyzed by the in-house laboratory to verify the accuracy of our PCA.

The cause of the divergent results obtained during the anesthetic period is not clear. Erroneous data resulting from CGMS malfunction (defective sensor or electrode, transmitter error, unit calibration error, and incorrect sensor placement) is not a likely explanation as excellent agreement between the methods was observed between samples obtained before and after the anesthetic period and it is logical to expect equipment error to be problematic whether or not the dogs were anesthetized. Discordance between CGMS data and direct blood glucose measurement was previously observed in a study of nocturnal hypoglycemia in humans with tightly controlled type 1 diabetes. Hypoglycemic episodes recorded by the CGMS were found to

underestimate blood glucose by an average of 38% in the majority of patients when concurrent results from a glucose analyzer were available.[51] Although a true difference in the interstitial/plasma glucose gradient during sleep could not be ruled out, a calibration problem was thought the most likely cause of the low glucose readings recorded overnight.[51] In the current study, calibration error is an unlikely source of the discrepancy observed between glucose values determined by the CGMS and the PCA. First, the CGMS used in our study employed the latest software version provided by the manufacturer, including updated calibration algorithms, and great care was taken when obtaining and processing blood samples for calibration and entering the results in the CGMS monitor. Second, a calibration error is not a sufficient explanation for why inaccurate readings were only obtained when dogs were under general anesthesia, or why agreement between CGMS and PCA results improved near the end of the anesthetic period.

The validation and use of continuous glucose monitors has been addressed extensively in human medicine.[4,5,22] Only a few reports, however, studied the use of a CGMS in anesthetized humans.[33,34,52] There are no reports of use of the system for routine glycemic management of anesthetized veterinary patients. In one study of adult human patients undergoing a variety of abdominal procedures, the CGMS yielded a high rate of technical failures during the anesthetic period (66% of all time points during the anesthetic period were deemed invalid compared to only 18% of data obtained in the postoperative period).[33] Interestingly, the agreement was just 74% in anesthetized patients even when valid CGMS data was obtained. The cause for the technical failures was not definitively determined but the investigators speculated that the use of electrocautery during the anesthetic period interfered with the CGMS.[33] Interference by electrocautery was suspected to be the cause of technical malfunctions in 50% of the sensors in

another study which used a CGMS to monitor pediatric patients undergoing various cardiac surgeries.[34] Electrocautery was not used during any procedure in our study, and no incidents of technical failure occurred. All of the sensors used in this study continued to report data throughout the anesthetic period. Monitoring equipment used during this study included an ECG monitor and battery operated devices including a pulse oximeter, Doppler systolic blood pressure monitor, and an esophageal temperature probe. It is possible that one or more of these devices interfered with the function of the CGMS, but there are no published reports implicating any of these monitoring technologies in CGMS failure. Furthermore, CGMS and PCA measurements began to converge before the monitoring equipment was disconnected and removed from the vicinity of the patient (Figure 5.1).

Since glucose concentrations measured by the CGMS and the PCA represent interstitial fluid and blood concentrations, respectively, a temporary change in the gradient between the two compartments could cause the observed disparity. For example, changes in temperature, peripheral blood flow, or the size of the interstitial fluid compartment could introduce a discrepancy between interstitial glucose concentration and blood glucose concentration. The effect of temperature on CGMS function has not been studied extensively; however, hypothermia was reported to have a minimal influence on CGMS results in pediatric surgical patients.[34] Body temperature did not correlate to results from either the CGMS or the PCA in this current study. Body temperature of the study dogs remained relatively constant near the end of the anesthetic period while agreement between CGMS and PCA measurements improved, suggesting the improvement was independent of temperature. However, core body temperature in the study dogs was measured with an esophageal thermometer and temperature fluctuations may have been more pronounced in the skin and subcutaneous interstitial space.

Alteration in blood-interstitial fluid glucose dynamics due to anesthesia is another possible cause of discordance between CGMS and PCA results. A slight increase in blood glucose over baseline was present over most of the anesthetic period in the current study. The elevation in blood glucose may have resulted from catecholamine release stimulated by surgery or may have been an effect of anesthesia. Isoflurane, which was used to maintain anesthesia in the dogs in the current study, has been shown to increase blood glucose concentration in rats through a mechanism that involves K_{ATP} channel activation. This maintains the resting membrane potential of beta cells and ultimately inhibits insulin release.[53] A similar mechanism could explain the observed discrepancy between the CGMS and the PCA, but is unlikely because blood glucose and interstitial glucose have been shown to remain in equilibrium despite changes in insulin release or administration.[22]

Another factor to explain the disparity between CGMS and PCA glucose measurements is the equilibration delay between the blood and interstitial compartments. The time needed for glucose concentrations to equilibrate ranges between 5 and 12 minutes with changes in CGMS readings lagging behind changes in plasma glucose.[22] Despite the lag, accuracy of the CGMS is not significantly influenced due to use of internal calibration and a digital filter to correct for equilibration delay.[22,46] Rapid fluctuations in blood glucose were not observed in the current study, suggesting that large concentration differences did not develop between the blood and interstitial compartments at any time during the anesthetic period.

Prolonged immobility and intravenous fluid administration during the anesthetic period could alter fluid dynamics in the interstitial compartment. Dogs in this study received intravenous fluid at a pre-determined, standard rate. It is conceivable that the fluid volume administered during anesthesia might have expanded the interstitial compartment in some dogs.

Cardiopulmonary bypass can cause pronounced edema in children caused by both inflammatory and capillary leak mechanisms,[54] but the results from the CGMS were not affected by the development of subcutaneous edema in a study of anesthetized children managed on bypass.[34] No clinically detectable edema developed in any dog in the current study. Consequently, expansion of the interstitial compartment and subcutaneous edema are unlikely explanations for the discordant results provided by the CGMS and the PCA.

A limitation to this study is the working range of the CGMS. The manufacturer indicates accuracy of the system between glucose readings of 40 – 400 mg/dl. Glucose readings below or above this range are reported as simply 40 mg/dl and 400 mg/dl respectively. Although there were no CGMS readings of 400 mg/dl, the CGMS reported 4 readings of 40 mg/dl in this study. It is possible that the CGMS was reading a glucose measurement below 40 mg/dl during one or more of these readings. The corresponding PCA glucose levels corresponding to each of the 4 CGMS readings were between 100 – 108 mg/dl. This is an obvious divergence of the CGMS from the PCA. Thus, had the actual glucose measurements by the CGMS been available, it is not likely to have changed the major conclusion of this study.

The CGMS used in this study reports data in real-time. Use of this updated system has not been reported in veterinary medicine. The previous CGMS collected data similarly to the new system, but only reported data once uploaded to a computer. Since that data was reported retrospectively, the algorithm that was used to calibrate the system utilized a retrospective rolling average of calibration measurements to report the actual glucose readings. The new system has an updated algorithm to allow real-time data reporting. Despite the difference in calibration algorithm, if the disagreement during anesthesia was caused by a problem with calibration, one would expect all readings (during anesthesia, as well as pre- and post anesthetic measurements)

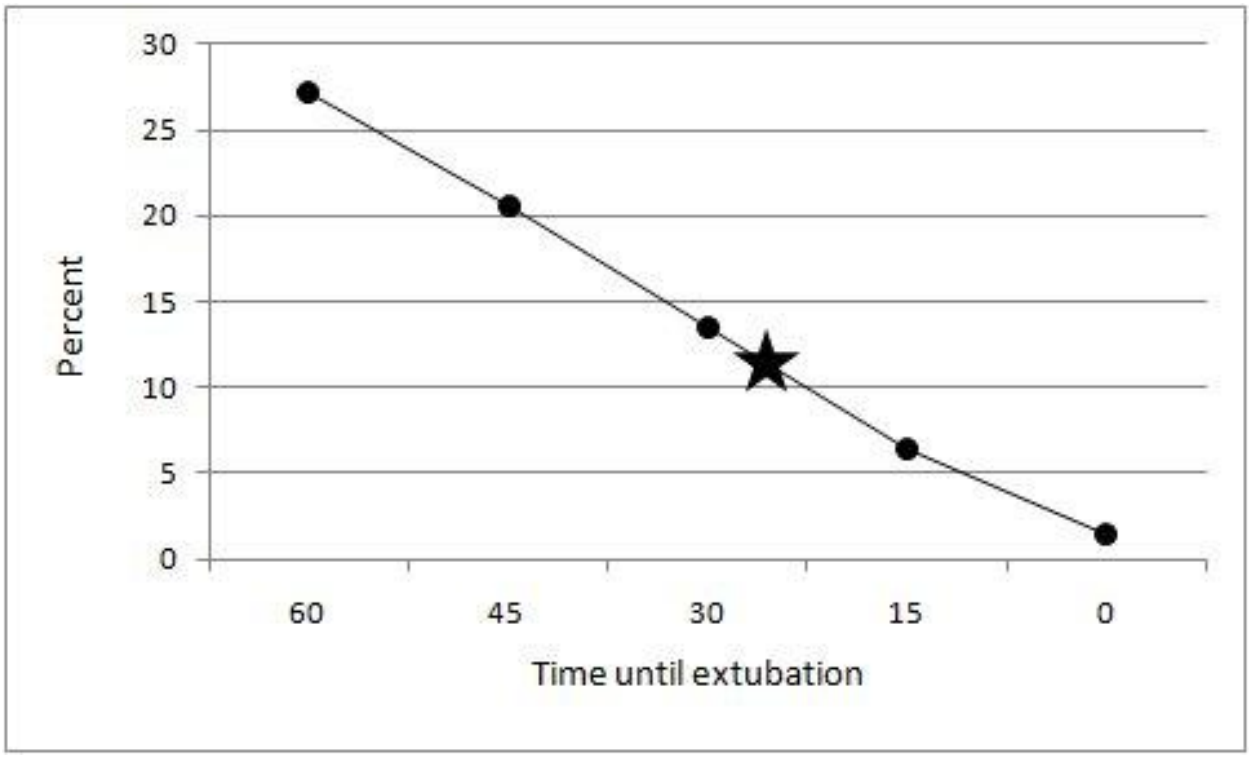
to be affected equally. Again, pre and post anesthetic readings by the CGMS were not significantly different than PCA readings as they were during the anesthetic period.

All dogs in this study were maintained with isoflurane in oxygen during the anesthetic period. High inspired oxygen concentrations are expected to increase the partial pressure of oxygen in blood and tissues, although this was not confirmed with blood gas analysis in the current study. The specific effects of increases in oxygen tension on the accuracy of the CGMS have not been investigated, therefore, it is not known if this is a potential source of disagreement between interstitial and blood glucose.

In conclusion, the CGMS apparatus was well tolerated and functioned well for over 36 hours in awake dogs. Although it has been shown previously that there is good agreement between interstitial fluid glucose measurements detected by a CGMS and blood glucose concentrations,[7,46,47] in the current study, the two values diverged significantly when the dogs were anesthetized. Only about 50% of CGMS results obtained during anesthesia agreed with the corresponding PCA results. The CGMS values were consistently lower than those recorded by the PCA. Agreement between the methods improved near the end of the anesthetic period and values were not significantly different once the dogs were awake. Although a specific cause for the discrepancy between CGMS and PCA glucose determinations during anesthesia was not identified, use of the CGMS for monitoring glucose in anesthetized dogs cannot be recommended at this time because the information obtained does not provide a useful estimate of blood glucose.

Figures

Figure 5.1 Cessation of monitor use during the anesthetic period



Mean percent difference of the *last five paired data points* (●) from each dog during the anesthetic period. Extubation = 0. The star (★) indicates the average time that monitoring equipment was turned off during the anesthetic period.

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