The influence of a dexmedetomidine - buprenorphine based anesthesia on the blood glucose of fasted dogs measured with blood glucose meters GlucoMen® GM meter (Arkray) and Xpress® meter (Nova biomedical).

Drs. Ing. S.S.G. Rossen, Drs I. Akkerdaas
University of Veterinary Medicine Utrecht, the Netherlands

Abstract

Background
Several factors influence the blood glucose level perioperative in an animal: anesthesia, additional medication, pre-operative stress (due to the rise of cortisol), fluid therapy, hypoxemia, temperature and underlying diseases such as Diabetes Mellitus (DM). In mice, inescapable electro-foot-shock stress induced a 30% rise in blood glucose levels *(S. Amir, 1982).* Medetomidine causes mild but significant hyperglycemia when measured with EDTA blood of dogs *(Ambrisko, 2005).* Buprenorphine administrated intravenously may variably affect insulin and glucagon secretion and therefore blood glucose levels *(Werther, 1984).* Another important factor is the method used for blood glucose validation.

Working hypotheses
Due to a premedication of dexmedetomidine and buprenorphine a significant increase of blood glucose is to be expected in fasted dogs measured with blood glucose meters (GlucoMen® GM meter and Xpress meter®). Because of pre-operative stress, an increase in blood glucose level before premedication is also to be expected.

Method
Venous blood was collected from 55 dogs, before and after premedication with dexmedetomidine (10 µg/kg IV) and buprenorphine (10 µg/kg IV or IM). 52 dogs received induction and 49 dogs received maintenance with isoflurane as well. From each dog, four blood samples were analyzed with a twenty minutes interval and measured with a GlucoMen GM® meter (Arkray) and an Xpress® meter (Nova biomedical).

Results
The mean blood glucose before the premedication (T=0) was calculated for both meters. GlucoMen GM® meter: 3.5+/- 0.58 mmol/L and Xpress® meter: 4,2 +/- 0,51 mmol/L. There was for both meters a significant increase in blood glucose level after 60 minutes. Q-Q plots showed a log normality of the glucose concentration in time.

Conclusions
A significant increase in blood glucose 60 minutes after premedication with dexmedetomidine and buprenorphine is shown. The increase measured with the GlucoMen® meter remained within the reference values. There was a high individual variance in the population. Despite, a normal log distribution is seen.

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Introduction

The brain uses glucose as its main fuel, a continuous flow of glucose to the brain is therefore very important. Hypoglycemia causes symptoms as drowsiness, unsteadiness, confusion and coma in humans *(Roach, 2003).* These effects can also been seen in dogs.
Hyperglycemia in critically ill dogs increases the risk of morbidity and mortality *(Torre DM, 2007).* In humans during hyperglycemia, the circulating levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are elevated *(K. Esposito, 2003).* This suggests a causal role in the immune activation of animals with hyperglycemia. Therefore, a regular blood glucose monitoring during anesthesia is needed, especially for DM patients who cannot regulate the glucose homeostasis properly.

Several factors influence the blood glucose level perioperative in the dog: anesthesia, additional medication, pre-operative stress (rise of cortisol), fluid therapy, hypoxemia, temperature of the animal and underlying diseases such as Diabetes Mellitus (DM) and pre-operative pain and/or stress. The method used for blood glucose measuring is also of influence. There are different ways to measure the blood glucose, each with different accuracy.

Pre-operative stress and pain hyperglycemia
Numerous studies demonstrate marked hyperglycemia following exposure to stress in both animals and humans. In mice, inescapable electro-foot-shock stress induced a 30% rise in blood glucose levels *(S. Amir, 1982).*
In a study, the analgetic effect of ketofen (NSAID) was studied in 8 dogs after given a maxillectomy or a mandibulectomy. There was a significant increase in blood glucose after one hour when only ketofen or only tramadol was used. A combination of both did not give a blood glucose increase *(T. Martins, 2010).*

Anesthesia
Anesthetic agents may have an important impact on endocrine and metabolic processes during anesthesia. α-adrenergic receptor agonists increase the blood glucose concentration in dogs. This hyperglycemic effect of α-adrenergic receptor agonists is reported and it is primarily attributed to the inhibition of insulin secretion *(Raekallio, 2005).* For example, a study measuring blood glucose during anesthesia with medetomidine was performed on 10 healthy Beagle dogs, which did not receive surgery. Medetomidine caused mild but significant hyperglycemia when EDTA blood samples were tested. When combined with butorphanol, fentanyl or ketamine, the hyperglycemic effect of medetomidine was reduced *(Ambrisko, 2005).* In horses, the plasma glucose concentration increased significantly after a detomidine-buprenorphine injection (within the first 3 hours). Although changes were statically significant, values remained within normal limits *(P. van Dijk, 2003).*
Buprenorphine administrated intravenously may variably affect insulin and glucagon secretion and therefore blood glucose levels *(Werther, 1984).* Parenteral administration of morphine given in a large doses produces hyperglycaemia in many animal species. A rise in glucose level as produced

by opioids represents an increased central sympathetic outflow to the adrenal medulla and peripheral sympathetic nerve endings, leading to peripheral catecholamine release *(Gigliano, 1988).* To our knowledge, no research has been done to study the effect of dexmedetomidine in combination with buprenorphine on the blood glucose levels in fasted dogs.

Medication

Antibiotics are used for antibacterial therapy but also as prophylaxis during surgery. These have little indirect influence on the blood glucose level. For example cefazolin (20 mg/kg IV) has inhibitory effects on glucose-6-phosphate dehydrogenase in erythrocytes. A deficiency of this enzyme in red blood cells causes haemolytic anemia *(M. Ciftici, 2000).* Anemia can be of influence on the blood glucose level.

Blood glucose levels stay relatively stable under propofol and propofol/buprenorphine anesthesia in fed rats *(Kitamura, 2009).*

In fasted rats, isoflurane exposure has no influence on the blood glucose level *(H. Zardooz, 2010).*

An increase of the blood glucose level by NSAIDs (carprofen 4 mg/kg IV) has not been described in the literature in dogs, except, in combination with insufficient post-operative pain management. In a dubbelblind study with healthy pigeons, after one day a slight but significant increase in blood glucose is described after given daily carprofen intramusculair (T. Zollinger, 2005) .

Method of glucose measurement

In veterinary practices, handheld glucose meters are often used but the efficacy is discussed.

A number of variables influence the reliability of the test results of a blood glucose meter, including hematocrit, hypoxemia, hypotension, altitude, temperature and humidity. Hematocrit causes the most significant error, especially in the intensive care unit *(E. Yoo, 2010).* Hematocrit values higher than normal (>45%), result in underestimation of laboratory values of blood glucose. Several hypotheses have been suggested to explain the impact of abnormal hematocrit values on blood glucose testing, such as altered viscosity of blood, prevention of plasma reaching the reaction surface of the test strip, change in diffusion kinetics, and/or increased packed red cell volume and displacement of plasma volume leading to insufficient plasma volume for accurate testing *(P.B. Musholt, 2011).* In this trial, two handheld glucose meters are evaluated, the Xpress® meter (corrects for hematocrit, vitamin c, lactose and urine acid) and the GlucoMen® GM meter.

Xpress® meter (NOVA biomedical)

The Xpress I Glucose Hospital Meter is made by Nova Biomedical in Walthram, USA and distributed by Nova Biomedical UK Cheshire, UK. The Xpress® meter is a professional meter with a mean coefficient variation of <4,5% for glucose concentrations between 4.14 and 20.70 mmol/L *(Lyon, 2008)* The coefficient variation for low glucose (<2.5 mmol/l) was 4.3%, at high glucose values (>24.4 mmol/l) this was 1.3%. No significant differences due to varying hematocrit were observed with the StatStrips from the

Xpress® meter *(Holzinger, 2008).* Linear regression analysis demonstrated a correlation coefficient of 0.99137 for the Xpress® meter in humans *(B. Bewley, 2007).* The correlation coefficient and reference values for dogs are still unknown. Mean bias from the reference method (meter value minus reference value) was 0,033 mmol/l *(Holzinger, 2008).* The Xpress® meter also eliminates interference due to maltose, galactose, xylose, oxygen, acetaminophen, uric acid, and ascorbic acid (vitamin c) *(A. Menarini Diagnostics).*

GlucoMen® GM meter (Arkray)
The GlucoMen® GM meter is made by Arkray Factory Ltd. In Sulfolk, UK and distributed by A. Menarini Diagnostics Firenze, Italy.
The GlucoMen GM® meter has a range of 0.6 – 33.3 mmol/L and the hematocrit range is 30 – 55% *(GlucoMen® GM manual).* The correlation coefficient tested on dogs and cats is 0.9749 in venous blood compared to the Spotchem system *(W. Kuik, 2001).* The GlucoMen GM® meter has a mean deviation of 31% between glucose concentrations measured at the lowest (25%) and the highest (60%) hematocrit value*. (P.B. Musholt, 2011)*.
From a study of 56 dogs, the references for blood glucose for dogs for the GlucoMen® GM meter were as follows:

- Venous blood 3.1 – 4.5 mmol/l
- Capillary blood 2.9 – 4.2 mmol/l *(Kuik, 2001)*

Working hypotheses

The following working hypotheses are evaluated during this trial:

- *The mean blood glucose before premedication (T=0) tested with the* GlucoMen GM® meter *will be increased (upper border of the reference values) due to pre-operative stress hyperglycemia.*
- *Due to a premedication of dexmedetomidine and buprenorphine a significant increase of blood glucose is to be expected in fasted dogs measured with handheld blood glucose meters (*GlucoMen GM® meter *and Xpress® meter).*

Materials and methods

Measurement devices and laboratory protocol
The following blood glucose meters were included: GlucoMen® GM meter (*Arkray)* and the Xpress® meter (Nova biomedical). The Glucomen® GM sensor strips (Arkray) contains glucose-oxidases which transforms the glucose in the blood to gluconolactone. The meter measures the electrons in this reaction and calculates the blood glucose *(Menarini manual).*
The Xpress® meter uses StatStrips (*NOVA Biomedical),* which contain a modified glucose oxidase-based amperometric test system with hematocrit and other interference correction *(R. Scot, 2007).*

Sample processing and anesthesia protocol
The study took place at the Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals, Utrecht University, the Netherlands.

Owner consent was necessary before the dogs could be included in the trial. A pre-anesthetic evaluation was performed to determine the dog's health.
The following exclusion criteria were followed during in this trial:

- Underlying diseases as Diabetes Mellitus or Cushing's disease;
- Dyspnea
- Additional medication (corticosteroids)
- Body temperature outside the normal range of 38 °C– 39.5°C
- Hypovolemia

To exclude other factors which may have influence on blood glucose, the fluidtherapy was kept at a constant rate of 10 ml/kg/hour. In some cases, breathing was supported by mechanical ventilation to prevent the to the influence of hypoxia. The bodytemperature of the dog was checked regularly and,if necessary, corrected.
To prevent hyperglycemia caused by pain, a suitable analgesia protocol was designed for each patient.

All animals had access to water but food was withheld for 12 hours before sedation. Blood was collected from the *V. Cephalica* or the *V Saphena* of 55 dogs. Premedication included dexmedetomidine (10 µg /kg IV) and buprenorphine (10 µg/kg IV or IM). Induction followed in 52 of the dogs ((94,5%) and anesthesia was maintained with isoflurane in 49 of the dogs (90%). 25 dogs (45.4%) received carprofen and 13 dogs (23.6%) received cefazolin intravenous at induction. Some dogs received surgery others were sedated only for diagnostics purposes. The blood samples were tested with the GlucoMen® GM meter *(Arkray)* and the Xpress® meter *(Nova Biomedical)* within a couple of seconds after blood collection. T = 0 was collected seconds before premedication with dexmedetomidine and buprenorphine. The other three samples were taken with a 20-minute interval (T= 20, T=40, T=60 respectively).

Analysis
All data of each meter were tabulated in excel and SPSS. A p-value < 0.05 was considered statistically significant. A linear mixed model with random dog effects was made with the following parameters: time, meter and time – meter interaction.
Also, to check normality of the values, Q/Q plots were made. To extrapolate the results to a generalized dog population, a t-distribution was made with a 95% confidence interval for the mean.

The following data were analyzed for both meters:

- Mean +/-SD in blood glucose (mmol/L) before premedication (T = 0)
- Mean starting +/- SD in blood glucose (mmol/L) before premedication for a generalized dog poopulation (T = 0)
- Mean difference +/- SD in blood glucose between T = 0 en T – 60 minutes. Q/Q plots were made to check normality of the values.

Results

Mean T=0 blood glucose trial

The mean blood glucose level before premedication with dexmedetomidine and buprenorphine of fasted dogs for the GlucoMen GM® meter was 3.54 +/- 0.58 mmol/L. The mean blood glucose at time = 0 for the Xpress® meter was 4.2 +/- 0.51 mmol/L.

Mean T=0 blood glucose for a generalized dog population

Because the value of the population standard deviation (SD) is unknown, we used the t- distribution: a 95% confidence interval was used where the multiple, $t_{0,05,}$ is the percentage point of the t-distribution with n-1 degrees of freedom.

Calculation

$$\text{Mean} \quad +/- \quad t_{0,05} \quad \frac{SD}{\sqrt{n}}$$

Explanation		
mean	=	mean of results
SD	=	Standard Deviation
n	=	number of animals
t 0,05	=	n – 1 degrees of freedom

GlucoMen: 3,5 Xpress: 4,2
GlucoMen: 0,58 Xpress: 0,51
n = 55
DF 54= 2,009

results

The mean glucose level before premedication for the population of fasted dogs lies within the following values:

GlucoMen GM® meter:	3,5 + / - 0,16	(3,3 – 3,7)
Xpress® meter:	4.2 + / - 0,14	(4.1 - 4.3)

Mean increase of blood glucose in one hour

In table 1 the mean glucose (mmol/L) in time is described for each time interval.

Mean Glucose (n=55)

Time (minutes)	GlucoMen GM® meter (SD)	Xpress® meter (SD)
0	3,5 (0,6)	4,2 (0,5)
20	3,5 (0,8)	4,5 (0,9)
40	3,8 (1,0)	4,4 (0,9)
60	3,8 (0,9)	4,5 (1,0)

Table 1 Mean glucose (mmol/L) in time (minutes). SD = standard deviation (mmol/L)

In Chart 1 an increase of glucose level is seen for the GlucoMen GM® meter and the Xpress® meter. In appendix 1, a linear mixed model table with random dog effects was made. From the table, it can be seen that the increase

of the glucose level within an hour is significant (2.7%). The increase of the GlucoMen GM® meter still remains within the reference values. Within the hour, each interval of 20 minutes differences is not significant.

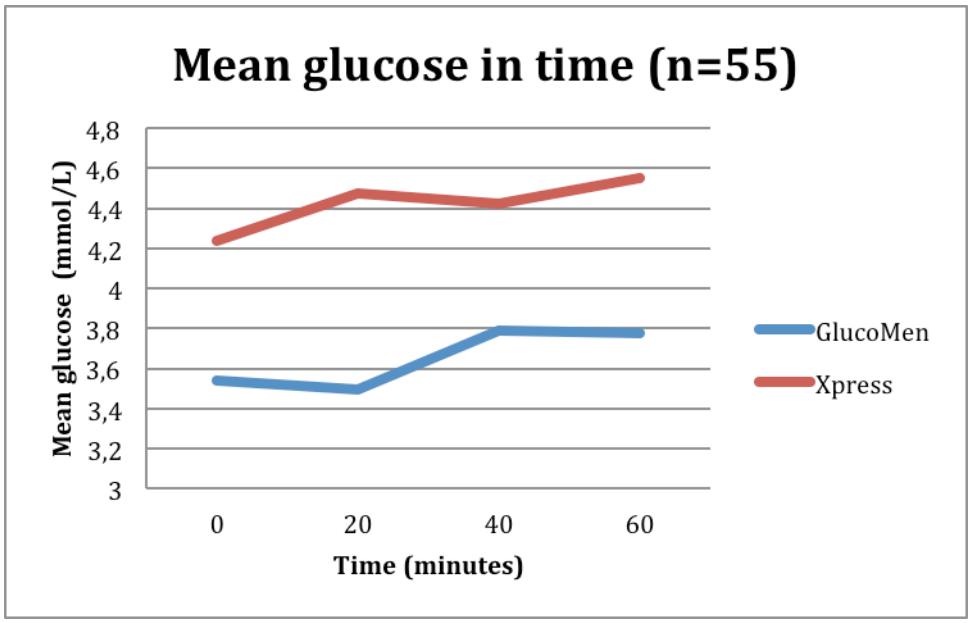

Chart 1 Mean glucose in time for the GlucoMen and the Xpress meter (mmol/L)

Establishing normality

To check normality of the values, Q-Q plots were made in appendix 2. The log transformed glucose concentration is the most normally distributed.

Variance

The individual variance between the dogs included in this trial is high. Appendix 3 shows that the variance is higher than the residue. From the high standard deviation seen in table 1 (mean glucose in time) this conclusion is confirmed.

Conclusion

The following working hypotheses were evaluated during this trial:

- *The mean blood glucose before premedication for the GlucoMen (T=0) will be increased (upper border of the reference values) due to pre-operative stress hyperglycemia.*
- *Due to a premedication of dexmedetomidine and buprenorphine a significant increase of blood glucose is to be expected in fasted dogs measured with blood glucose meters (GlucoMen and Xpress meter).*

Mean blood glucose at T = 0

The mean blood glucose measured with the GlucoMen GM® meter was 3.54 +/- 0.58 mmol/L. This is within the reference range (3.1 – 4.5 mmol/L). The mean blood glucose for the Xpress® meter was 4.3 +/- 0.51 mmol/L. The reference values for the Xpress® meter are not yet available.

Increase of the blood glucose

From this study, the hypothesis is confirmed that with both meters an increase of blood glucose with a dexmedetomidine – buprenorphine premedication in fasted dogs can be measured after one hour. This increase remains within the references given for the GlucoMen GM® meter.
The increase of the glucose within one hour is distributed as a log function (appendix 2).

Discussion

Because of the pre-operative stress caused by transport and the pre-anesthetic examination in this research, an increased glucose at the start of the anesthesia was therefore to be expected. Results show that the mean blood glucose levels before premedication stayed within the lower range of the reference values. A possible explanation could be that the increase of blood glucose in dogs, caused by stress hyperglycemia, is neglectible.
The standard variation for the mean blood glucose level is high (table 1: between 0.5 – 1,0 mmol/L) which showed that there is a high individual difference in blood glucose at T = 0.
9 dogs of the 55 were given buprenorphine intramuscularly instead of intravenously. Significance of the two groups were calculated but there was no difference in increase in blood glucose between the intramuscular group and the intravenously group.
Literature showed that medication such as NSAID's, corticosteroids, antibiotics, propofol, and isoflurane have no or small influence on blood glucose levels. Influences on blood glucose levels caused by the additional medication (besides the dexmedetomidine and buprenorphine) were not expected and are not calculated in the results.
By using interval measurement there is a risk of missing important changes in the blood glucose. In this study, there is chosen to measure with an interval of 20 minutes to decrease this risk. There were seen no abnormal increases or decreases in these intervals, so a 20 minute interval seems appropriate in this study.
The values of blood glucose, measured with the GlucoMen GM® meter and the Xpress® meter, cannot be compared because of the difference in of measuring.
The GlucoMen GM® meter has an accuracy of 31% (dependent of the hematocrit), for the Xpress® meter this is 4,3%. These values have to be taken in consideration when evaluating the blood glucose meters. Also, the GlucoMen GM® meter does not correct for hematocrit, which the Xpress® meter meter does.

In veterinary practices the blood glucose meters (Xpress® meter and GlucoMen GM® meter) are useful during the anesthesia of dogs. An increase of blood glucose is to be expected but this remains within the reference values of the GlucoMen GM® meter. For high risks patients it is important to establish a starting blood glucose level before premedication because of individual blood glucose levels in dogs.

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Appendix 1

Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	57,417	1051,558	,000
Meter	1	339,513	147,914	,000
Time	3	345,904	3,108	**,027**
Meter * Time	3	339,505	,793	,498

a. Dependent Variable: Glucose (mmol/L.)

Table 2 significance of blood glucose increase

Appendix 2

In a Q-Q plot (Q stands for quantile), the distribution of two variables is compared. As can be seen in the graphics, the plots which is log transformed is the most suitable for this trail.

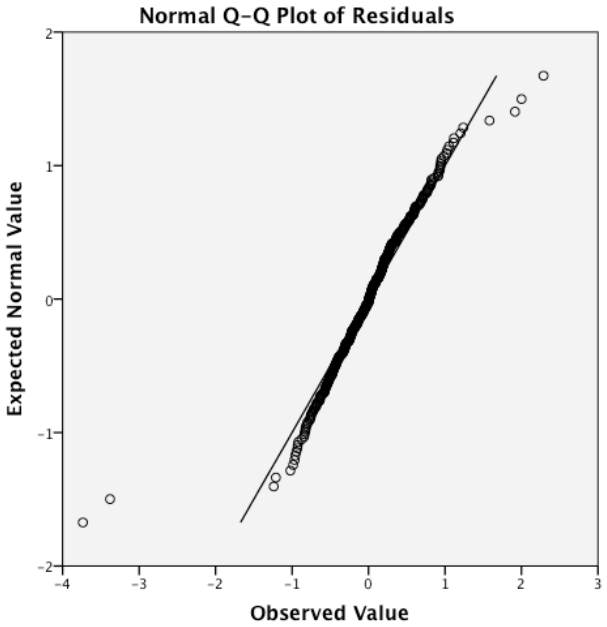

Chart 2 normal Q-Q plot

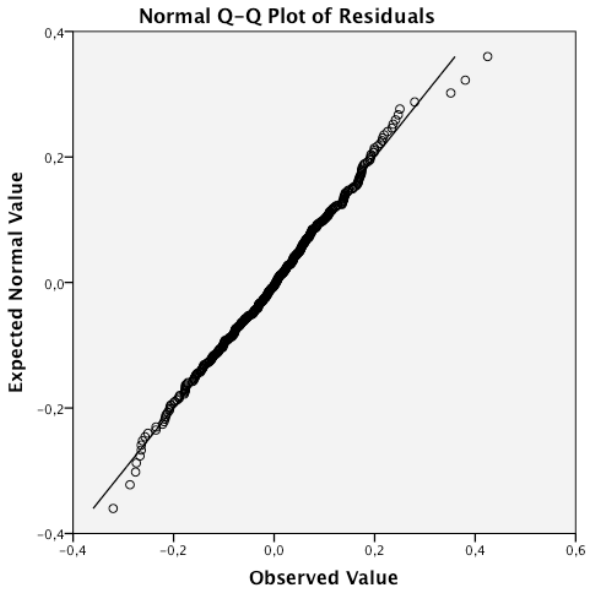

Chart 3 log Q-Q plot

Appendix 3

Estimates of Covariance Parameters

Parameter		Estimate	Std. Error
Residual		,365937	,028091
Intercept [subject = Dog]	Variance	,412118	,089123

Table 3 variance of individual blood glucose (mmol/L)

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