Serial plasma glucose changes in dogs suffering from dog bite wounds.

By

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CONTENTS

SUMMARY

Objective: To describe the changes in plasma glucose concentration in severely injured, canine to canine bite wound cases admitted for veterinary treatment. The changes were measured over a period of 72 hours from the initiation of the trauma. Historical, signalment, clinical and haematological factors were investigated to determine their possible relationship to blood glucose concentration. Hypo- and hyperglycaemia have been associated with death from sepsis and acute injury.

Method: Twenty dogs admitted to the Intensive Care Unit at Onderstepoort Veterinary Academic Hospital (OVAH) with severe bite wounds were evaluated. The time of injury was established by questioning the dogs' owners. Blood was taken on admission for haematology and plasma glucose concentration. Haematology was repeated every 24 hours and glucose every 8 hours, measured from the time the dogs were first bitten.

Results: On admission, 5% (1/20) of the dogs were hypoglycaemic, 40% (8/20) were normoglycaemic and 55% (11/20) were hyperglycaemic. No other dogs showed hypoglycaemia during the study period. The median glucose at each of the ten collection points, prior to the 56-hour collection point and at the 72 hour collection point, was in the hyperglycaemic range (5.8mmol/l to 6.2mmol/l). Puppies and thin dogs had considerably higher median plasma glucose concentrations than adult and fat dogs at 0 and 16 hours respectively ($P < 0.05$ for both). A high incidence of SIRS was encountered (65% to 80%). Fifteen dogs

were alive at 72-hours. Thirteen dogs (81.3%) eventually made a full recovery. Three out of four dogs (75%) that were recumbent on admission, died, whereas all dogs (12/12) admitted with either an alert or depressed mental status survived (P = 0. 004).

Clinical significance: The high incidence of hyperglycaemia may be explained by the 'diabetes of injury' phenomenon. The role of insulin therapy in the treatment of severe injuries should be explored in future studies, as its use in the treatment of human ICU cases, has resulted in a substantial reduction of fatalities resulting from acute injury. The high incidence of death and initial hyperglycemia in the collapsed group and the higher plasma glucose concentrations found in puppies and thin dogs warrants further investigation with a larger group of animals.

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CNS	Central nervous system
CoA	Co-enzyme A
CRT	Capillary refill time
GIT	Gastro intestinal tract
G6PDH	Glucose-6-phosphate dehydrogenase
g	Gravity
hr/s	Hour/s
Ht	Haematocrit
IQR	Inter quartile range
ml	Millilitre
Mm	Mucous membrane
mmol/l	Millimol/litre
mm^3	Cubic millimetre
n	Number
NADPH	Nicotinamide adenine dinucleotide phosphate
NaF/Ox	Sodium fluoride oxalate
nm	Nanometer

OVAH	Onderstepoort Veterinary Academic Hospital
P	Probability level
Pu	Pulse
R	Respiratory rate
r_s	Rho Spearman
SAS	Small animal surgery
SIRS	Systemic inflammatory response syndrome
STI	Since trauma interval
°C	Degree centigrade
%	Percentage

CHAPTER 1: Literature review

1.1 Epidemiology and pathophysiology of dog bite wounds.

Epidemiology:

Reports in the literature on the incidence of bite wounds seen in dogs and cats vary from 10.2% to 14.7% per 1000 (Kolata and others 1974).

The incidence of bite wounds recorded at the Onderstepoort Veterinary Academic Hospital (OVAH) can be seen in the table below:

Table 1 Statistical information of non-referred canine bite wound cases admitted to the OVAH for the period 1 March 1998 to 31 May 2005.

Year	Total Non-Referred Number of Canine Cases Admitted to OVAH Excluding Sterilizations and Castrations	Total Number of Non-Referred Canine Cases with Bite Wounds (OVAH)	Bite Wounds as a percent of total canine cases treated in OVAH
1998*	10469	194	1.85%
1999	15971	287	1.80%
2000	18123	371	2.05%
2001	18266	327	1.79%
2002	17054	400	2.35%
2003	16561	355	2.14%
2004	15705	337	2.15%
2005#	6035	130	2.15%
	118184	2401	2.03%

Year	Total Non-Referred Number of Canine Cases Admitted to Small Animal Surgery (SAS) Excluding Sterilizations and Castrations	Total Number of Non-Referred Canine Cases with Bite Wounds (SAS)	% Bite Wounds treated in SAS	Total Number of Non-Referred Canine Cases with Bite Wounds (OVAH)	Total Number of Non-Referred Canine Cases with Bite Wounds (SAS)	% Bite Wounds treated in SAS
1998*	1222	61	4.99%	194	61	31.44%
1999	1647	66	4.01%	287	66	23.00%
2000	1600	95	5.94%	371	95	25.61%
2001	1810	92	5.08%	327	92	28.13%
2002	1807	87	4.81%	400	87	21.75%
2003	1929	105	5.44%	355	105	29.58%
2004	1787	76	4.25%	337	76	22.55%
2005#	680	25	3.68%	130	25	19.23%
	12482	607	4.86%	2401	607	25.28%

* 1998 - Information for the period 1998/03/01 to 1998/12/31
\# 2005 - Information for the period 2005/01/01 to 2005/05/31

According to hospital records, the amount of money spent on bite wounds in the small animal surgery (SAS) clinic varies from year to year:

- 1998-03-01 – 1998-12-31 : R 22990.69
- 1999-01-01 – 1999-12-31 : R 22927.81
- 2000-01-01 – 2000-12-31 : R 41207.46
- 2001-01-01 – 201-12-31 : R 43641.12
- 2002-01-01 – 2002-12-31 : R 58217.99
- 2003-01-01 – 2003-12-31 : R 88361.58
- 2004-01-01 – 2004-12-31 : R 64629.70
- 2005-01-01 – 2005-05-31 : R 19127.38

These figures do not reflect any cases treated at the outpatients facility and therefore it can be assumed that the economic importance of dog bite wounds is much higher.

Pathophysiology:

The average dog's canine tooth can generate a crushing pressure of 150 - 450 pounds per square inch (Cowell and Penwick 1989, Pavletic 1995). It is important to realize that amount of energy cannot simply be defused; it can only change form as it is transferred from the teeth into tissue (Kolata 1993). Tissue blood flow decreases and infection rates increase in proportion to the amount of energy transferred during a bite (Holt and Griffin 2000). The shearing and tensile forces involved in the shaking of, especially smaller, dogs can cause a massive amount of damage to the underlying fascia, muscle, vasculature (collapse of the sub dermal plexus), nervous tissue, bone and parenchymatous organs (Pavletic 1995, Davidson 1998, Holt and Griffin 2000). Crushing leads to swelling, ischaemia and necrosis. This phenomenon is known as the 'iceberg effect' (Davidson 1998); where the original wound is the tip of the proverbial iceberg that obscures the extent of the trauma. Major tissue disruption can occur despite the absence of any physical penetrating wounds. This tissue disruption has the potential to cause large amounts of tissue breakdown products to be released into the circulatory system and predisposes these wounds to infection (Crane 1989).

1.2 Classification of wounds.

Wounds, whether surgical or traumatic, are classified as open or closed. Open wounds are further classified as clean, clean contaminated, contaminated, or dirty and infected (Waldron and Trevor 1993, Pavletic 1995, Davidson 1998).

Clean wounds are defined as non-traumatic with no inflammation observed, or as having no break in aseptic technique. The respiratory, gastro-intestinal tract (GIT) and urogenital tract have not been entered.

Clean contaminated wounds are defined as having a minor break in surgical technique. The GIT or respiratory tract have been entered into without significant spillage; the oropharynx or vagina entered; or the biliary or urogenital tract entered into in the absence of infected bile or urine.

Contaminated wounds are defined as being fresh, traumatic wounds with major breaks in aseptic technique and gross spillage from the GIT or biliary and urogenital tract.

Dirty and infected wounds are defined as being traumatic wounds with retained devitalized tissue; foreign bodies; faecal contaminants; delayed treatment; acute bacterial inflammation; and clean tissue that must be transected to access pus.

All bite wounds should be considered to be contaminated or dirty and infected (Waldron and Trevor 1993, Pavletic 1995, Davidson 1998, Davidson 1998, Holt and Griffin 2000). A wound is considered infected when more than 10^5 bacteria per gram of tissue are present (Davidson 1998). In untreated wounds, bacteria proliferate rapidly and invade healthy tissue, resulting in an infected wound. Griffin and Holt (2001), in an attempt to connect wound severity with infection, introduced a bite wound classification system. Wounds were classified as follows:

- Class 1 is defined as a partial thickness laceration without penetration of the dermis.
- Class 2 is defined as a full thickness laceration with penetration of the dermis.
- Class 3 is defined as a full thickness puncture wounds.
- Class 4 is defined as a full thickness laceration or puncture with avulsion of underlying tissue and dead space.

A laceration is defined as a wound with ragged edges and more than 10mm in length and a puncture wound as a wound less than 10 mm in length. It appears from the study that class 4 wounds may be more contaminated and are more likely to become infected.

1.3 Microbiology of dog bite wounds

Bacteria are introduced into wounds from environmental contaminants, direct inoculums from the canine mouth and, to a lesser extent, from organisms found on the victim's skin (Cowell and Penwick 1989, Pavletic 1995, Holt and Griffin 2000). A mixed aerobic and anaerobic population was present in 56% to 66% of the human wounds studied, with *Pasteurella spp* being the pathogen found most frequently (Pavletic 1995, Holt and Griffin 2000). In 36% to 74% of wounds only aerobes were cultured, whereas in 1% to 41% of wounds anaerobes were the only bacteria cultured (Pavletic 1995, Holt and Griffin 2000). When anaerobes are present, the severity of the infection is often increased (Pavletic 1995). Other bacteria frequently cultured include: *Staphylococcus intermedius* (23%), *Escherichia coli* (18%) and *Enterococcus* spp (Holt and Griffin 2000). The local environment of the wound is ideal for bacterial replication and infection (Pavletic 1995, Davidson 1998, Holt and Griffin 2000) and subsequent bacteraemia and/or endotoxaemia can be expected. No single antimicrobial agent has been demonstrated to be effective against all of the pathogens found in dog bite wounds (Cowell and Penwick 1989).

1.4 The role of glucose in wound healing

All wounds, whether surgical or traumatic, go through the same processes of healing. Healing begins with the inflammatory stage, and moves through the debridement stage, the granulation

stage, the repair stage and finally the maturation stage (Fowler 1989). Local and systemic aspects can influence the healing process. Local factors needed for proper healing include oxygen and nutrients. The metabolic demands of healing tissue are very high. Some of the systemic factors that influence wound healing include pathophysiological processes, like those present in diabetes mellitus (Fowler 1989). Excessively high glucose concentrations can inhibit neutrophil function by impairing phagocytosis and diminishing the production of oxygen radicals. Glucose is the primary energy source of leucocytes and fibroblasts and the predominating carbohydrate substrate for fibroblasts in their synthesis of proteoglycan polymers (Crane 1989). High glucose concentrations are needed in wound fluids. Efficient energy production from glucose is dependent on oxygen, as is found in granulation tissue (Fowler 1989, Crane 1989). During the early stages of healing, proteolysis of endogenous proteins mobilizes amino acids, which are used primarily for glucose production.

1.5 Carbohydrate metabolism in health and disease

Living animals require a constant input of glucose, fatty acids and protein. Maintenance of a euglycaemic state is dependant on carbohydrates entering and leaving the blood stream and is a function of the supply, storage, release and consumption of glucose. Glucose is supplied to the body via the GIT through the digestion and absorption of carbohydrates and proteins. Nutrients enter the liver where carbohydrates are broken down to glucose, which is used to regenerate adenosine triphosphate (ATP); secondly to replenish glycogen stores (glycogenesis) and lastly to replenish fatty acids and triglycerides from ‘leftover’ glucose. Glucose is used to produce fatty acids through the carboxylation of acetyl CoA into malonyl CoA. Acetyl CoA also has the option of entering the citric acid cycle for conventional energy production.

Ingestion of nutrients stimulates insulin secretion from the endocrine pancreas, which promotes the uptake of glucose, with potassium as a co-transport mechanism, into cells. Insulin inhibits degradation of stored glycogen, protein and triglycerides. Cell metabolism and function depends on the delivery of energy sources, such as glucose, via the circulation. Ingested carbohydrates, fat and protein provide fuel for approximately 4 to 8 hours of cell metabolism. After this period, fuel must be obtained from endogenous sources (Feldman and Nelson 1996).

During anorexia, a catabolic state results from reversal of the metabolic pathways. The endocrine pancreas secretes glucagon. The liver then releases glycogen, as primary energy source from its stores, through glycogenolysis. Fat is broken down to fatty acids and acetyl-CoA. Protein is metabolized to amino acids. Glucose is re-synthesized from glycogen and gluconeogenic amino acids. Acetyl CoA, derived from fatty acids, is then introduced into the Krebs cycle to produce energy. There is a change in the body's use of fuel sources, with a shift to fatty acid and ketone metabolism. The central nervous system (CNS) and erythrocytes remain dependent on glucose as an energy source (Bone and others 1992, King and Hammond 1999). Hepatic glycogen reserves are depleted over a two to three day period under normal circumstances in adult animals (Abood and Mauterer 1993).

These normal metabolic alterations do not occur during the stressed and diseased state; instead, the initial response is centered on the release of glycogen and the mobilisation of lipid stores. The activity of the animal declines during the 'ebb phase' of trauma. The metabolic rate declines during this initial phase, but increases later. The 'ebb phase' is recognized by massive sympatho-adrenal discharge and typically lasts approximately 24 hours. This phase can be associated with hyperglycemia (Mizock 1995).

If the animal survives this ‘ebb phase’ it progresses to the ‘flow phase’, which is recognized by an increased metabolic rate and enhanced breakdown of lean body mass. The afferent signals from the wound via pain and other peripheral receptors, as well as cytokines, interleukin 1 and tumor necrosis factor, initiate the hypermetabolic process (Douglas and Shaw 1989, Abood and Mauterer 1993, Feldman and Nelson 1996, King and Hammond 1999). These signals are integrated in the hypothalamus and stimulate the secretion of glucagon, cortisol, catecholamines and growth hormone (Rosin 1981, Douglas and Shaw 1989).

1.6 Hypoglycaemia in dogs

Syndromes associated with hypoglycaemia include (Breitschwerdt and others 1981, King and Hammond 1999):

- Neoplasia and Paraneoplastic syndrome
- Hyper-insulinism (iatrogenic or neoplastic)
- Hypoadrenocorticism
- Hepatic insufficiency
- Sepsis / SIRS
- Iatrogenic
- Anorexia
- Laboratory error

Possible consequences of hypoglycaemia include (Leifer and Peterson 1984, Nelson 1985, Crane 1989, Fowler 1989, Walters and Dobratz 1992):

- Reduced resistance to infection

- Impaired immune function
- Slow wound healing
- Slow recuperation time of diseased animals
- Decreased wound strength
- Weight loss
- Weakness
- Permanent blindness
- Seizures
- Coma
- Death

1.7 Hyperglycaemia in dogs

Causes of hyperglycaemia include (Nelson 2005, Van den Berhge 2004):

- Diabetes mellitus
- Postprandial hyperglycaemia, especially with diets containing sugars
- Hyperadrenocorticism
- Dioestrus (bitch)
- Phaeochromocytoma
- Pancreatitis
- Exocrine pancreatic neoplasia
- Renal insufficiency
- Drug therapy

  - Glucocorticoid
  - Progestagen
  - Megestrol acetate
  - Thiazide diuretics
- Dextrose-containing fluids
- Parenteral nutrition
- Head trauma
- Acute injury

Possible consequences of hyperglycaemia in humans include (Mizcock 2001, Van den Berghe 2004):

- Osmotic diuresis with hypovolaemia
- Electrolyte abnormalities
- Hyperosmolar non-ketotic coma.
- Inhibited neutrophil function by impairing phagocytosis and diminishing the production of oxygen radicals.
- Death

In a recent study on *Babesia canis* infection, hyperglycaemia, although present, was not shown to be a reliable indicator of disease severity (Keller and others 2004). In dogs there have been reports of hyperglycemia associated with head trauma (Syring and others) and heart failure (Brady and others).

In a recent human ICU study on acute injury, a positive linear correlation has been established between the degree of hyperglycaemia and the risk of death (Van den Berghe 2004).

1.8 Potential risk factors for blood glucose perturbations in dog bite wounds

Dogs with dog bite wounds can be in a shocked state when admitted and, as a result, are thus under sympathetic control with high concentrations of adrenaline and cortisol in their systems (Rosin 1981, Douglas and Shaw 1989, Devey and Crowe 1997). After injury there is a rapid increase in plasma cortisol levels which peak within 4 to 6 hours and then decrease to resting levels within 24 hours during the 'ebb phase' (Rosin 1981). The effects of cortisol include an influence on inflammation (immuno suppression in high doses, stabilizing the membranes of cellular lysozymes, decreasing capillary permeability, diminishing the vasodilation effects of histamine, enhancing the vasoconstriction effects of norepinephrine and epinephrine) and an increased metabolism of carbohydrate, protein and fat as energy sources (Rosin 1981).

Changes in blood volume and the afferent sensory nerve stimulation of the hypothalamus resulting from injury, release catecholamines into the blood stream. These hormones increase cardiac output and raise blood pressure while epinephrine increases basal metabolic rate by as much as 100%. Epinephrine also stimulates metabolic activities such as glycogenolysis in the liver, glucose release into the blood, hydrolysis of fat and release of free fatty acids into the blood (Rosin 1981)

Patients with disease therefore undergo an accelerated form of starvation and tissue depletion (Rosin 1981, Cerra 1987, Douglas and Shaw 1989, Remilard and Martin 1990, Chandler and others 1992, Devey and Crowe 1997). Hypermetabolism follows the 'ebb phase' after injury and results in a marked increase in visceral and muscle blood flow and oxygen and carbohydrate consumption. Fat and protein is broken down and used as an energy source (Rosin 1981, Cerra 1987, Douglas and Shaw 1989, Remillard and Martin 1990, Chandler and

others 1992, Mizock 1995, Patiño and others 1999). The estimated increase in metabolic rate for injuries range from is 15% for relative minor injuries, to 100% for large thermal burns, and approximately 80% in sepsis (Long and Nelson 1993). The increase in respiratory quotient (the ratio of consumption of oxygen to the production of carbon dioxide, reflecting the different types of fuel used for body energy) from 0.7 in uncomplicated starvation to 0.8 in hypermetabolism indicates a mixed fuel oxidation of which glucose supplies approximately 40% to 60% (Long and Nelson 1993).

During the hypermetabolic state, a number of alterations in carbohydrate metabolism are induced, (Bretschwerdt and others 1981, Měszăros 1987, Mizock 1995, Patiño and others 1999, Andersen and others 2004, Van den Berghe 2004) these include:

- Enhanced peripheral uptake and utilization of glucose by the wound and other organs involved in the immune response, such as the liver and spleen.
- Hyperlactataemia - due to possible hypoperfusion.
- Increased glucose production - adrenaline, cortisol, glucagon, growth hormone.
- Decreased glucose production - in severe sepsis due to gram-negative bacterial endotoxin, interleuken-6, insulin-like factors, decreased hepatic export, or increased utilization.
- Depressed glycogenesis – suppressed by persistent high rate of glycogen breakdown under the influence of adrenaline and glucagon, decreased glycogen synthatase activity or TNF presence.
- Glucose intolerance and insulin resistance – rise in extra cellular glucose concentrations, due to defective suppression of gluconeogenesis and resistance to the peripheral action of insulin, also termed ‘diabetes of injury’ (Van den Berghe 2004).

Tissue trauma with, or without, infection can initiate the systemic inflammatory response syndrome (SIRS), in which multiple inflammatory, immunologic, coagulation and fibrinolytic cascades are activated and interact (King and Hammond 1999, Holt and Griffin 2000). SIRS is characterised by hypermetabolism, a hyperdynamic cardiovascular state, and clinical manifestations of fever or hypothermia, tachycardia, tachypnoea and leucocytosis or leucopaenia (Bone and others 1992, Welzl and others 2001, King and Hammond 1999).

Sepsis has been defined as SIRS with a documented infection and severe sepsis as SIRS with a documented infection and haemodynamic compromise (King and Hammond 1999, Holt and Griffin 2000). Septic shock is defined as severe sepsis that requires both volume replenishment and inotropes to restore tissue perfusion (King and Hammond 1999). All these syndromes are commonly seen in dogs with bite wounds (Holt and Griffin 2000).

An alteration in blood glucose represents one of the most consistent findings in models of experimentally induced sepsis (Breitschwerdt and others 1981). Sepsis and SIRS can lead to hypoglycaemia due to impaired gluconeogenesis, especially from amino acids, (Woolf and others 1979) and increased peripheral uptake and utilization of glucose (Mizock 1995, King and Hammond 1999).

Stress is typically associated with increased gluconeogenesis and therefore, hyperglycaemia. Septic stress however is distinguished by a biphasic response. Lethal models of sepsis in animals seem to demonstrate an initial hyperglycaemia, followed by a phase of hypoglycaemia during which glucose production is suppressed (Breitschwerdt and others 1981, Mizock 1995).

Hypothermia (Oncken and others 2001), anorexia (Abood and Mauterer 1993), age (McKelve and Powers 1966, Strombeck and Rogers 1974, Atkins 1984, McMicheal and Dhupa 2000),

pregnancy (Jackson and Bruss 1980, Leifer and Peterson 1984) and breed (Atkins 1984, Leifer and Peterson 1984) have been shown to be able to influence plasma glucose concentrations.

1.9 Normal values for plasma glucose concentrations for dogs

Normal plasma glucose values vary between 3.3mmol/l and 5.5mmol/l which is the reference range used by the Clinical Pathology Laboratory of the Onderstepoort Veterinary Faculty. Hypoglycaemia is taken as plasma glucose concentrations below 3.3mmol/l (Walters and Dobratz 1992, Feldman and Nelson 1996), and hyperglycaemia as plasma glucose concentrations above 5.5mmol/l (Walters and Dobratz 1992).

1.10 Determination of plasma glucose concentrations

Precautions should be taken to prevent significant changes in glucose concentrations in stored blood. Glucose declines in routinely stored blood due to the metabolism of the red blood cells through glycolysis (Sonnewirth and Jarret 1980). To minimize this artifactual decrease in glucose the plasma or serum should be promptly separated from the red blood cells or sodium fluoride oxalate added to the sample. Sodium fluoride oxalate (NaF/Ox) prevents coagulation and inhibits red cell glycolysis (Sonnewirth and Jarret 1980, Kaplan 1984). Glucose levels were stable at room temperature for up to ten days in sterile blood samples taken in NaF/Ox, and for 30 days if the blood was centrifuged and stored at -20 °C (Sonnewirth and Jarret 1980).

Plasma glucose can be measured by several different methods. In this study plasma glucose concentrations were measured using the hexokinase method. The enzyme hexokinase catalyses the reaction of glucose with adenosine triphosphate (ATP) to glucose-6-phosphate and adenosine diphosphate (ADP). A second enzyme, glucose-6-phosphate dehydrogenase

(G6PDH), catalyses the reaction of the already produced glucose-6-phosphate, with nicotinamide adenine dinucleotide phosphate (NADP) to form 6-phosphogluconate and reduced NADPH. Since NADPH absorbs strongly at 340 nm, the increased absorption at this wavelength is used as a measure of the initial glucose concentration (Sonnewirth and Jarret 1980). This method minimizes interference by proteins and other serum constituents (Kaplan 1984).

CHAPTER 2: Objectives

2.1 Problem statement

No studies, evaluating plasma glucose alterations over time in dogs with dog bite wounds, could be found in the literature. Dog bite wounds are acute injuries inflicted on an otherwise healthy animal and can, therefore, not be compared with a 'chronic' illness like canine babesiosis (in which marked serum glucose perturbations have been shown), where animals are presented, sometimes, days after the start of the disease process (Keller and others 2004). A previous veterinary study has shown that blood glucose concentrations are significantly affected by the severity of head trauma in dogs and cats. The study showed that blood glucose concentration is considerably higher in dogs and cats with head trauma than in control animals. Blood glucose concentration, however, was not associated with outcome (Syring and others 2001).

Hypo- and hyperglycaemia have been associated with death from sepsis and acute injury. Possible risk factors for glucose perturbations in dog bite wounds are currently unknown.

2.2 Objectives of this study

The primary aim of this study was to prospectively describe the prevalence and incidence, over a 72-hour period, of hypo-, normo-, or hyperglycaemia in dogs admitted with canine bite wounds. Historical, signalment, clinical and haematological factors were also investigated to determine their relationship to blood glucose concentrations.

2.3 Research questions

1) Does hypo- or hyperglycaemia occur in admitted bite wound cases on admission, or during the 72-hour period following the infliction of trauma?
2) If hypo- or hyperglycaemia does occur during the 72-hour period, how long after the infliction of trauma does it happen?
3) What is the correlation between historical, signalment, clinical, and haematological findings and plasma glucose concentrations on admission, and during the study period?

2.4 Benefits

1) This project will provide important information on the blood glucose status of dogs admitted with bite wounds.
2) This project may strengthen the case for the routine serial assessment of blood glucose concentrations in canine bite wound cases so as to better predict, treat or prevent complications.
3) This project is in partial fulfillment of the requirements for the degree MMed Vet (Chir) (small animals).

CHAPTER 3: Materials and methods

3.1 Model system

Twenty dogs, bitten by another dog within 24 hours, with at least one open wound, and per attending clinician's judgement required intravenous fluid therapy, were enrolled in this study. These animals enrolled serially, taking into consideration the availability of the researcher. As no severity scores are available for dog bite wounds, the criteria set out above were used to collect the most severely affected animals. All owners signed a consent form, allowing the animal to be studied (Addendum A). The dogs were admitted to the small animal surgery clinic and hospitalised in the Intensive Care Unit of the OVAH. Possible *Babesia canis* was excluded by means of a peripheral blood smear and, in order to minimize confusing influences in plasma glucose concentrations, no alpha$_2$ agonists were used in these cases on admission, or over the study period.

3.2 Observations

Signalment, history and clinical data

The dogs' owners were asked to complete a questionnaire (Addendum B) regarding the approximate time their dog was bitten, the time since the dog's last full meal, pregnancy, the dog's known medical conditions, and any medication given to the dog.

On admission, the primary investigator performed a full clinical examination on each dog where the following data was recorded (Addendum C): Habitus (alert, depressed or collapsed), time since last meal, time since bite wound was inflicted, age, sex, body weight (size), body condition using the Purina Nine Point Body Condition Score (Addendum D), temperature,

pulse and respiration. Temperature, pulse and respiration were recorded at each collection point thereafter.

The study period extended the 72 hours from the time since trauma infliction (STI), or up to euthanasia or death, whichever came first. The study did not interfere with the normal clinical management of these cases.

OVAH patient records

Patient records were photocopied and filed according to their research number.

Plasma glucose determination

Blood was collected from all dogs by the cephalic or jugular vein into a paediatric EDTA (0,5ml) and an evacuated tube containing Sodium Fluoride Oxalate (NaF/Ox), (1ml). Additional NaF/Ox samples for plasma glucose determination were taken at 8-hour intervals and haematology at 24-hour intervals from the time the bite was inflicted, as indicated by the owners. These collection points were selected with the purpose of comparing the dogs at similar times in the trauma pathway. A full haematology was done by the Clinical Pathology laboratory of the OVAH on admission, and at each 24-hour collection point thereafter. The NaF/Ox samples were centrifuged within minutes of collection at 1730 x g for eight minutes. The plasma was separated into a cryopreservation tube, labelled and immediately frozen at -20 °C. Analysis of glucose was performed in a single batch using the hexokinase method.

No glucose containing fluids were administered to any of the dogs during the study period. The dogs were fasted for two hours before blood was drawn (McKelvie and Powers 1966).

The primary investigator was blinded to the plasma glucose concentrations for the duration of the study.

Sample size

Twenty dogs, admitted to the SAS clinic and ICU facility of the OVAH, were used. These cases presented over an 8-month period, and were selected according to the criteria set out in paragraph 3.1.

Data analysis

Data was entered on an Excel® spreadsheet (Microsoft Corporation). A veterinary epidemiologist performed the statistical analysis.

Hypoglycaemia was defined as plasma glucose concentration below 3.3 mmol/l (Walters and Drobatz 1992, Feldman and Nelson 1996), and hyperglycaemia as plasma glucose concentration above 5.5mmol/l (Walters and Dobratz 1992).

Habitus on admission; time since last meal; time since bite occurred; age (<6 months = puppy; > 6 months = adult); sex, body weight (size); body condition and outcome (died versus survived) were included as predictor variables for plasma glucose concentrations at each time point. Patients were classified into discrete, clinically meaningful categories of each predictor variable. The median plasma glucose concentration over time for each category of a variable was compared using Kruskal-Wallis one-way ANOVA on ranks.

The presence of SIRS was determined using the criteria described by Hauptman and others (1997) and Welzl and others (2001) from the admission data and at 24-hour intervals after the initial trauma. To be considered SIRS positive, patients had to have (Hauptman and others 1997, Welzl and others 2001):

- A white cell count < 6000/mm^3 or > 16000/mm^3, and / or > 3% band cells plus, at least one of the following:
    - A rectal temperature of < 38.1 or > 39.2 °C
    - A heart rate of > 120 beats per minute
    - A respiratory rate of > 20 breaths per minute

The plasma glucose concentration of SIRS+ and SIRS- groups were compared at each time point using the Wilcoxon rank-sum test.

The correlation of plasma glucose concentration with temperature, pulse, respiratory rate, red cell count, haematocrit, total white cell -, mature neutrophil -, immature neutrophil -, eosinophil -, lymphocyte -, monocyte - and thrombocyte count was assessed at each time point using the Spearman rank correlation coefficient.

The association between habitus on admission and at outcome was assessed using Fisher's exact test. Statistical analysis was done using NCSS 2004 (NCSS, Kaysville, UT, U.S.A.).

CHAPTER 4: Results

A complete data set is provided in addendum F

Outcome

Two dogs were euthanased within 8 hours of being bitten and only admission samples were obtained from them. One dog was euthanased after 48 hours. All euthanasia's were attributable to financial reasons and not prognosis. One dog was collected by the owner after 16 hours and taken to an alternative facility because of financial constraints; the outcome is unknown. This left 16 dogs in the study from which mortality could be objectively assessed. One dog died during the study period, and two died after cessation of the study period (one on day 5, the other on day 7). Fifteen out of 16 dogs (93.8%) survived the 72-hour study period, and 13 out of 16 dogs (81.3%) or survived to discharge. All dogs (12/12) admitted as either alert or depressed and not euthanased or collected by the owner, survived, whereas 3/4 dogs (75%) admitted in a state of collapse, died ($P = 0.004$). A statistically significant difference in plasma glucose concentrations was only found at the 32-hour since trauma interval (STI), with collapsed dogs having a higher median plasma glucose concentration than depressed dogs, but not compared to alert dogs.

Prevalence of glucose abnormalities

The medians and interquartile ranges of plasma glucose concentrations, and the proportion of dogs that were hypo-, normo- and hyperglycaemic at each given time point, are shown in Table 2. The median plasma glucose concentration at each of the ten collection points was consistently in the hyperglycaemic range (5.7mmol/l – 6.2mmol/l), excluding only the 56-hour (5.4mmol/l) and the 64-hour (5.5mmol/l) STI, which were normoglycaemic. No dogs were

found to be severely hypo- (< 2.2 mmol/l) or hyperglycaemic (> 10 mmol/l) during the study. On admission, 5% (1/20) of the dogs were hypoglycaemic, 40% (8/20) were normoglycaemic and 55% (11/20) were hyperglycaemic. No dogs showed hypoglycaemia during the remainder of the study period.

Table 2 Descriptive statistics of plasma glucose concentrations in mmol/l on admission, and at 8-hour time intervals from the time the dog was bitten

	admission	8 hr	16 hr	24hr	32hr	40hr	48hr	56hr	64hr	72hr
Count	20	16*	18*	17	17	17	17	16	15	15
Median	6	6.2	6.2	5.8	5.8	6.2	5.9	5.4	5.5	5.7
IQR	5 - 7	5.4-7.3	5.6-6.8	5.2-6.8	5.6-6.3	5.5-6.8	5.1-6.5	4.8-5.9	5.1-6.2	5.1-6.8
Hypoglycaemic (< 3.3 mmol/l)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normoglycaemic (3.3 – 5.5 mmol/l)	8 (40%)	5 (31%)	4 (22%)	5 (29%)	4 (24%)	4 (24%)	6 (35%)	10 (63%)	9 (60%)	5 (33%)
Hyperglycaemic (> 5.5 mmol/l)	11 (55%)	11 (69%)	14 (78%)	12 (71%)	13 (76%)	13 (74%)	11 (65%)	6 (37%)	6 (40%)	10 (67%)

* - Two cases presented between 8 and 16 hours from the time the bite occurred and therefore, do not have 8-hour samples.

Systemic Inflammatory Response Syndrome (SIRS)

The prevalence of SIRS at each 24-hour STI is shown in Table 3. Median plasma glucose concentrations for SIRS+ and SIRS- dogs are also shown, however, no significant differences existed at any time.

Table 3 Prevalence and median values of plasma glucose concentrations in mmol/l for SIRS+ and SIRS- cases on admission and at 24-hour intervals from the time the dogs were bitten.

		Admission		**24 hours***		**48 hours**		**72 hours**	
Variable	**Category**	**n**	**Median**	**n**	**Median**	**n**	**Median**	**n**	**Median**
SIRS	SIRS +	13	6.6	13	5.8	13	5.9	12	5.7
	SIRS -	7	5.4	3	6.8	4	5.9	3	5.9
	% SIRS+	65%		81%		76%		80%	

- Incomplete haematology data for one case

Putative risk factors/predictor variables

The median plasma glucose concentrations for subgroups of dogs defined by different levels of the predictor variables are shown in Table 4.

There was a significant statistical difference ($P < 0.05$) in the median plasma glucose concentrations at the following time intervals, for the following variables:

- Habitus: at the 32-hour STI (depressed = 5.6 mmol/l; collapsed = 6.3 mmol/l).
- Time since bite occurred: at the 32-hour STI (<8hrs = 6 mmol/l; >8hrs 5.5 mmol/l)
- Age: on admission (puppy = 7 mmol/l; adult = 5.4 mmol/l)

  At the 8-hour STI (puppy = 7.6 mmol/l; adult = 5.7 mmol/l)

  At the 16-hour STI (puppy = 7 mmol/l; adult 6 mmol/l)
- Body condition: on admission (thin = 6.8 mmol/l; fat = 4.7 mmol/l)

  At the 16-hour STI (thin = 6.9 mmol/l; fat = 4.5 mmol/l)

There was no significant statistical difference in median plasma glucose concentrations for the total study period for the following variables:

- Time since last meal
- Sex
- Size

Table 4a Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds from admission to 24 hours.

			Admission		8 hour		16 hour		24 hour	
Variable	**Category**	**n**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	5.4	4.5;6.8	6.1	5.3;7.1	5.9	4.8;6.6	5.9	5.4;6.5
	Depressed	8	6	5;7.1	7.3	6.2;8	6.4	5.9;7	5.4	4.7;6.1
	Collapsed	5	6.6	5;7.7	5.5	3.9;7.15	6.4	5.7;7.6	6.8	5.6;7.2
Time since last meal	≤8 hrs	9	6.8	5.7;7.1	6.8	5;7.7	6.8	6.2;7	6.1	5.2;6.8
	>8 hrs	11	5.4	4.7;6.6	5.8	5.4;7	5.9	5.2;6.3	5.8	5.1;.6.5
Time since bitten	≤8hrs	16	6.4	5;7	6.5	5.6;7.6	6.5	5.9;6.9	5.9	5.7;6.9
	>8hrs	4	5.7	3.8;7.2	5.4	3.3;5.9	5.7	5.1;6.3	5.2	4.9;5.8
Age	Puppy (≤ 6 mths)	4	7*	6.8;8.1	7.6*	7.1;8.5	7*	6.8;8.1	6.7	6.3;7.4
	Adult (> 6 mths)	16	5.4*	4.8;6.6	5.7*	5;6.5	6*	5.3;6.4	5.8	5;6.2
Sex	Male	9	6.6	5.4;7.2	5.6	4.7;7.3	6	5.7;6.7	6	5.2;6.9
	Female	5	6.1	5;7.2	6.9	6.5;8	6.5	5.4;7.2	5.7	4.8;5.9
	Female sterilised	6	5	4.2;6.9	6.3	5;7.15	6.3	4.7;6.8	6.1	5.1;6.8
Size	≤5 kg	3	7.2	6.8;8.4	7.3	7;8.7	7	6.8;8.4	6.9	6.3;7.4
	>5 kg ≤10 kg	13	5.4	4.8;6.6	5.9	5.2;6.9	6	5.7;6.4	5.7	4.8;5.9
	>10 kg	4	6.1	3.8;7	5.5	3.8;7.3	6.1	5.1;6.9	6.4	5.5;7
Body condition	Thin (1-4)	6	6.8*	6.4;7.5	7.2	5.8;8	6.9*	5.9;7.4	6.3	5.3;7.1
	Ideal (5-6)	11	5.4	5.1;7	5.7	5;6.9	6.2	5.6;6.6	5.9	5.5;6.8
	Fat (7-9)	3	4.7*	4.5;4.9	4.8	4.8;4.8	4.5*	4.3;4.7	4.7	4.5;4.8
Outcome	Recovered	13	5.4	4.9;6.8	6.5	5.4;7.2	6	5.2;6.7	5.7	4.9;6.2
	Died	3	6.6	6.6;7	5.5	4.5;5.6	6.4	5.9;6.8	6.8	5.8;7

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference.

Table 4b Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds, from 32 to 56 hours.

			32 hour		40 hour		48 hour		56 hour	
Variable	**Category**	**n**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	6	5.6;6.5	6.6	5.1;7.3	5.6	4.9;6.2	5.7	4.1;7.2
	Depressed	8	5.6*	5.3;5.8	6.1	5.2;6.2	5.7	5.4;6.6	5.5	5.3;6
	Collapsed	5	6.3*	5.9;7.9	6.6	6;7.7	6.1	5.3;7.6	4.9	4.5;5.7
Time since last meal	≤8 hrs	9	5.9	5.6;6.9	6.2	5.3;6.6	5.9	5.5;6.5	5.5	5.2;5.8
	>8 hrs	11	5.8	5.4;6.3	6.2	5.5;7.7	5.9	5;6.5	5.3	4.5;6.9
Time since bitten	≤8hrs	16	6*	5.7;6.7	6.2	5.7;6.8	5.9	5.3;6.5	5.4	4.8;5.8
	>8hrs	4	5.5*	5.2;5.8	5.9	5.2;6.7	5.4	4.8;6.3	6.1	4.8;6.9
Age	Puppy (≤ 6 mths)	4	7.1	5.7;7.7	6.9	6;8.7	6.1	5.6;8	5.6	5.3;5.9
	Adult (> 6 mths)	16	5.8	5.5;6.2	6.2	5.3;6.6	5.8	5;6.5	5.4	4.6;6
Sex	Male	9	5.8	5.5;7	6.4	5.8;7.7	5.9	5.4;6.9	5.3	4.5;6.4
	Female	5	5.7	5.2;6.2	6.2	5.5;6.2	5.4	5;6.4	5.4	5.3;5.6
	Female sterilised	6	6.1	5.8;6.9	5.9	5;6.8	6	4.9;6.1	5.9	4.2;7.9
Size	≤5 kg	3	7.4	7.1;7.7	7.8	6.9;8.7	7.1	6.1;8	5.9	5.9;5.9
	>5 kg ≤10 kg	13	5.8	5.5;6.3	6.2	5.1;6.4	5.9	5;6.6	5.4	4.6;5.7
	>10 kg	4	5.7	5.2;6.1	6.3	5.7;6.8	5.4	4.8;6	5.6	4.7;6.5
Body condition	Thin (1-4)	6	6.3	5.8;7.4	6.6	6.1;7.8	6.1	5.8;7.3	5.6	4.8;6.7
	Ideal (5-6)	11	5.8	5.5;6.2	6.2	5.5;6.7	5.8	5.2;6.5	5.5	5;6
	Fat (7-9)	3	5.6	5.1;6	5.2	5.1;5.3	4.8	4.6;5	4.4	3.6;5.2
Outcome	Recovered	13	5.7*	5.5;6	6.2	5.2;6.6	5.6	5;6.3	5.4	4.9;6.3
	Died	3	6.3*	6.2;8.1	6.6	6.4;6.6	6.1	5.9;7.2	5.2	4.6;5.8

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference.

Table 4c Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds, from 64 to 72 hours.

			64 hour		72 hour	
Variable	**Category**	**N**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	6.1	5.3;7.2	6.1	5.4;6.7
	Depressed	8	5.5	4.9;6.3	5.7	5.1;7.2
	Collapsed	5	4.9	4.6;5.5	5.1	4.5;6.8
Time since last meal	≤8 hrs	9	5.5	5.1;5.8	5.7	5.1;6.7
	>8 hrs	11	5.9	4.9;7.3	6	5;7.4
Time since bitten	≤8hrs	16	5.5	5.1;5.9	6	5.1;6.8
	>8hrs	4	6.2	5.1;7.2	5.7	4.8;7.1
Age	Puppy (≤ 6 mths)	4	5.9	5.5;6.2	5.8	5.1;6.4
	Adult (> 6 mths)	16	5.5	5;6.4	5.7	5.1;7
Sex	Male	9	5.5	4.9;6.8	5.7	5.1;6.8
	Female	5	5.2	4.6;5.8	5.9	5.2;6.9
	Female sterilised	6	5.9	5.2;7.9	5.8	4.6;7.2
Size	≤5 kg	3	6.2	6.2;6.2	6.4	6.4;6.4
	>5 kg ≤10 kg	13	5.4	5;6.3	6.1	5.5;7.2
	>10 kg	4	5.5	5.1;6.5	5.1	4.7;5.6
Body condition	Thin (1-4)	6	6.2	5.5;7.3	6.4	5.1;7.5
	Ideal (5-6)	11	5.5	5.1;6.1	5.9	5.6;6.9
	Fat (7-9)	3	4.8	4.4;5.1	4.7	4.4;5
Outcome	Recovered	13	5.5	5.1;6.5	5.7	5.1;6.8
	Died	3	5.1	4.6;5.5	6	5.1;6.8

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference

Clinical and haematological variables

Few significant correlations were found between clinical or haematological variables and plasma glucose concentration:

- No statistically significant correlation to temperature or pulse rate on each STI.
- A positive correlation (r_s = +0.63; P = 0. 0051) with respiratory rate only on admission.
- A negative correlation (r_s = -0.5467; P = 0.0126) with the total red cell count on admission; a negative correlation (r_s = -0.5184; P = 0.0477) with the total red cell count at 72-hour STI.
- A negative correlation (r_s = -0.5663; P = 0.0092) with haematocrit at admission; a negative correlation (r_s = - 0.5135; P = 0.0503) with haematocrit at the 72-hour STI.
- No statistically significant correlation with total white cell -, mature neutrophil -, immature neutrophil -, lymphocyte -, monocyte - and total platelet count at each time since trauma interval.
- A positive correlation (r_s = +0.5406; P = 0.0306) with eosinophil count only at 24-hour STI.

CHAPTER 5: Discussion

Outcome

This study has found a significantly lower survival rate in bite wound cases that were presented as collapsed. In a recent study on canine babesiosis cases, collapsed states were strongly associated with hypoglycaemia (Keller and others 2004). In this study a significant statistical difference in plasma glucose concentrations was only found at the 32-hour STI, with collapsed dogs, ironically, having a higher median plasma glucose concentration than depressed dogs, but not compared to alert dogs. Similar to the findings of Syring (2001) in head trauma cases, this study found no association between degree of hyperglycaemia and eventual outcome. The low survival rate of collapsed animals should be investigated further as this may prove to be a relevant measure of the severity and a predictor of outcome in dog bite wounds.

Prevalence of glucose abnormalities

This study found a consistently high median plasma glucose concentration at all but the 56-hour and 64-hour STI (Table 2). A low prevalence of hypoglycaemia was encountered only on admission (one case), with no other cases becoming hypoglycaemic during the study period.

During the post-traumatic ‘ebb phase’, the body’s initial response is centered on the release of glycogen, the mobilization of lipid stores, and reducing the activity of the animal. The metabolic rate declines during this phase, but increases later. This phase is recognized by massive sympatho-adrenal discharge and characteristically lasts approximately 24 hours. This phase can be associated with hyperglycaemia (Douglas and Shaw 1989, Mizock 1995).

If the animal survives this 'ebb phase', it progresses to the 'flow phase', which is characterized by an increased metabolic rate and enhanced breakdown of lean body mass. The afferent signals from the wound via pain and other peripheral receptors, as well as cytokines, interleukin 1 and tumor necrosis factor, initiate the process (Abood and Mauterer 1993, Feldman and Nelson 1996, King and Hammond 1999). These signals are integrated in the hypothalamus and stimulate the secretion of glucagon, cortisol, catecholamines and growth hormone (Rosin 1981, Douglas and Shaw 1989). The net effect of these hormones, coupled with a defective suppression of gluconeogenesis and resistance to the peripheral action of insulin, is hyperglycaemia. This is all part of the hypermetabolic response to trauma (Měszăros and others 1987, Douglas and Shaw 1989, Patiňo and others 1999) which may last for seven to ten days post trauma (Douglas and Shaw 1989). This phenomenon has been described in the human literature on acute injury. It has been termed 'diabetes of injury' (Van den Berghe 2004). A positive linear correlation has been found between the degree of hyperglycaemia and the risk of death (Van den Berghe 2004). In human critical care medicine, insulin resistance has been implicated as a major contributor to this phenomenon (Andersen and others 2004, Van den Berghe 2004, Langouche and others 2005). Aggressive control of even mildly elevated glucose concentrations to within normal levels, via insulin therapy, has shown a decline in death rates in human ICU's from 20.2% to 10.6% (Van den Berghe 2004). Insulin therapy also prevented complications such as severe nosocomial infections, acute renal failure, liver dysfunction, muscle weakness and anaemia (Van den Berghe 2004, Langouche and others 2005). Insulin has been shown to have the inherent capability of counteracting the metabolic changes observed in sepsis. It is hypothesized that insulin protects the endothelium (thereby contributing to the prevention of organ failure), modulates inflammatory pathways, protects

cells from direct glucose toxicity, preserves hepatic mitochondrial function, improves dyslipidemia and shows an anabolic effect by suppressing proteolysis and activating protein synthesis (Andersen and others 2004, Van den Berghe 2004, Langouche and others 2005).

The low incidence of hypoglycaemia was surprising, however, as hypoglycaemia has been associated with profound metabolic illness and severe sepsis in dogs (Sonnewirth and Jarret 1980, Breitschwerdt and others 1981, Mizock 1995).

All animals were maintained on Ringers Lactate as intravenous infusion. Lactate can act as a precursor for glucose, and, although unlikely, may have influenced the results (Sonnewirth and Jarret 1980). Food was allowed ad lib, but removed two hours before sampling. These dogs could not be starved for long periods as they were in the hypermetabolic phase of trauma, with markedly elevated basal metabolic rates (Long and Nelson 1993). McKelvie and Powers (1966), in a study on normal beagles, have shown that one to two hours of food deprivation is sufficient not to statistically influence blood glucose concentrations. The feeding should therefore not have added to artificially high glucose concentrations.

Systemic Inflammatory Response Syndrome (SIRS)

A high prevalence of SIRS was encountered (Table 3), on admission and during the study period. Sepsis has been defined as SIRS with a documented infection. All bite wounds should be considered contaminated, or dirty and infected (Pavletic 1995, Davidson 1998, Holt and Griffin 2000) and the local environment of the wound ideal for bacterial replication and infection (Pavletic 1995, Davidson 1998, Holt and Griffin 2000) with subsequent bacteraemia and or endotoxaemia. Consequently, the dogs in this study can be classified as septic with a high degree of certainty. Previous reports state that these syndromes are commonly seen in

dogs with bite wounds (Holt and Griffin 2000) and this study found similar results. However, no statistically significant differences in the mean plasma glucose concentrations could be established between dogs with and without SIRS.

Putative risk factors/predictor variables

Few significant associations were found between these variables and plasma glucose concentration (Table 4). Nevertheless, it is interesting to note that puppies and thin dogs tend to have higher plasma glucose concentrations than adults or obese dogs, particularly during the first 16 hours of trauma. This may warrant further investigation, as it seems to be contrary to accepted norms.

Clinical and haematological variables

The negative correlation of total red cell count and haematocrit (Ht) with plasma glucose concentrations was not expected, as low values of these variables have been correlated with lower plasma glucose levels in a previous babesiosis study (Keller and others 2004), however, if the dog had significant bleeding to lower the haematocrit, this would be consistent with more severe injury and greater stress response, which may lead to higher plasma glucose concentrations.

Increased utilisation of glucose by bacteria and polymorphonuclear leucocytes has been implicated in hypoglycaemia associated with sepsis (Breitschwerdt 1981), and it is therefore surprising that this study did not find any correlation between glucose concentration and leucocyte counts.

CHAPTER 6: Conclusions

The aim of this study is to describe the serial changes in plasma glucose concentrations occurring in severely bitten dogs, at 8-hour intervals. The results show an almost uniformly high median plasma glucose concentration over the 72-hour sampling period. This can probably be ascribed to the 'diabetes of injury' response. The role of insulin therapy should be explored in future studies, as its use in humans has shown a significant reduction of fatalities resulting from acute injury. The low incidence of hypoglycaemia was unexpected, considering the high incidence of SIRS and possible subsequent sepsis encountered. There was no significant statistical difference between the plasma glucose concentrations of the SIRS positive and negative cases. Few significant associations were made between historical, clinical and haematological variables and plasma glucose concentration. However, the high incidence of death in the collapsed group, and the higher plasma glucose concentrations found in puppies and thin dogs warrants further investigation with a larger group of animals.

Possible limitations (apart fro those noted in the text) to the study are:

- The low number of patients used
- The differing levels of severity (although an attempt was made to select the most severely bitten animals) and the current unavailability of a meaningful severity score
- The "white jacket" effect/response that may have added to the stress response of the dogs
- The low specificity of the SIRS criteria used.

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Addendum A

Consent form bite wound study

Your dog has been diagnosed with bite wounds. At present, we are conducting a study to evaluate blood glucose (sugar) levels in dogs with severe bite wounds. This requires the collection of blood samples (+- 1ml of blood – approximately 1/4 teaspoon) at admission, and 1ml every eight hours (+-3 ml per day), over a 3-day period of your dog's hospital stay. At no time will this study interfere with the treatment of your pet. By conducting these tests, we hope to improve our knowledge and treatment of bite wounds in future.

The costs of these tests will not be added to your account; we will pay the extra cost. You will still be responsible for other routine tests and treatment given to your dog as you normally would.

The ethics committee of the University of Pretoria has passed this study.

Thank you for your willingness in allowing your animal to be a part of this study and your patience in filling out the questionnaire.

Should you require more information please contact:

Dr CJ Du Plessis
Companion Animal Surgery
Onderstepoort Veterinary Academic Hospital
Tel: (012) 529 8000 or 529 8087.
I, …… hereby give permission

that my dog (name) …….., a (sex) ……...,

(breed)……., (colour)……..,
may participate in the clinical study, as set out above, of bite wounds at the Onderstepoort Veterinary Academic Hospital. I understand that this study will in no way harm my dog or result in the withholding of treatment that would otherwise have been given. I understand that no additional expenses will be incurred by me in respect of the trial for blood sampling and testing.
Signed at Onderstepoort on the …… day of …… 2005.

Signature owner/authorized person:……..

Tel (h)……. Tel (c)……
Tel (w).....

Addendum B

Bite wound history questionnaire

STUDY NO:

Date:…….

Owner:
Surname:……..Owner number:……..

Patient:
Name:……Number:……

Age:……Weight:……Breed:……Sex……

1. At what approximate time and date did the wound happen?

…….

2. When was the last time your dog had a full meal?

< 8hrs	8 – 16 hrs	16 – 24 hrs	24 –48 hrs	>48hrs

3. If your dog is female, is she pregnant?

Yes	No	Do not know

4. Does your dog suffer from any medical conditions?

Yes	No

4.1 If yes, please specify: ……

……..

5. Is your dog on any medication or was any medication given?

Yes	No

5.1 If yes, please specify: ……..

Addendum C

Clinical and Laboratory Data Recording Sheet

Habitus at admission.

Alert	Depressed	Collapsed

Plasma glucose concentrations:

1)Admission:……mmol/lT……., Pu……., R……, MM……..,CRT……..

2)8hr:..…….... mmol/l T……., Pu……., R……, MM……..,CRT……..

3)16hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

4)24hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

5)32hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

6)40hr:……... mmol/l T……., Pu……., R……., MM……., CRT……..

7)48hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

8)56hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

9)64hr:……mmol/l T……., Pu……., R……., MM……., CRT……..

10)72hr:……mmol/l T……., Pu……, R……., MM……., CRT……..

Haematology results to be attached dated and timed.

Discharged: Date:……

Died: Date:…… Reason:……

……

Euthanased: Date:…… Reason:……

……

Addendum D

Purina Nine Point Body Condition Score

Too Thin

1 Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.

2 Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.

3 Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist.

Ideal

4 Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.

5 Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed.

Too Heavy

6 Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.

7 Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and

base of tail. Waist absent or barely visible. Abdominal tuck may be present.

8 Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distension may be present.

9 Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distension.

Addendum E

Journal article

Serial plasma glucose changes in dogs suffering from severe dog bite wounds.

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Key words: Glucose, bite wounds, trauma, SIRS.

Summary

Objective: To describe the changes in plasma glucose concentration in severely injured, canine bite wound cases admitted for veterinary treatment. The changes were measured over a period of 72 hours from the initiation of trauma. Historical, signalment, clinical and haematological factors were investigated as to their possible effect on blood glucose concentration.

Method: Twenty dogs with severe bite wounds admitted to the Intensive Care Unit at OVAH were used. The time of injury was established by questioning the dogs' owners. Blood was taken on admission for haematology and plasma glucose concentration. Haematology was repeated every 24 hours and glucose every 8 hours, measured from the time the dogs were first bitten.

Results: On admission, 5% (1/20) of dogs were hypoglycaemic, 40% (8/20) were normoglycaemic and 55% (11/20) were hyperglycaemic. No dogs showed hypoglycaemia at

any other stage during the study period. The medians at each of the ten collection points, excluding the 56-hour and 64-hour collection points, which showed normoglycaemia, were in the hyperglycaemic range (5.8mmol/l - 6.2mmol/l). Puppies and thin dogs had significantly higher median plasma glucose concentrations than adult and fat dogs at 0 and 16 hours, respectively ($P < 0.05$ for both). A high prevalence of SIRS was encountered (65% - 80%). Fifteen dogs survived the 72-hour study period. Thirteen dogs (81. 3%) eventually made a full recovery. Three out of four dogs (75%), admitted as collapsed, died, whereas all dogs (13/13) admitted as either alert or depressed, survived ($P = 0. 004$).

Clinical significance: The high incidence of hyperglycaemia can possibly be explained by the 'diabetes of injury' phenomenon. The role of insulin therapy should be explored in future studies, as its use in humans has resulted in a significant reduction of fatalities as a result of acute injury. The high incidence of death in the collapsed group, and higher plasma glucose concentrations found in puppies and thin dogs warrants further investigation with a larger group of animals.

Introduction

Reports in the literature on the incidence of bite wounds seen in dogs and cats vary from 10.2% to 14.7% of trauma cases (Kolata and others 1974). The average dog's canine tooth can generate a crushing pressure of 150 to 450 pounds per square inch (Cowell and Penwick 1989, Pavletic 1995). The shearing and tensile forces involved in shaking of, especially smaller, dogs can cause a large amount of damage to the underlying fascia, muscle, vasculature (collapse of the sub dermal plexus), nervous tissue, bone and parenchymatous organs (Pavletic 1995, Davidson 1998, Holt and Griffin 2000). Crushing leads to swelling, ischaemia and necrosis.

This phenomenon is known as the 'iceberg effect' because the external wounds conceal the severity of the underlying damage (Davidson 1998).

Local and systemic factors can influence the wound healing process. Some of the local factors needed for proper healing include oxygen and nutrients. During the early stages of healing, proteolysis of endogenous proteins mobilizes amino acids, which are used primarily for glucose production (Davidson 1998). Glucose is the primary source of energy for leucocytes and fibroblasts and the predominating carbohydrate substrate for fibroblasts in their synthesis of proteoglycan polymers during the wound healing process (Crane 1989). Some of the systemic factors that influence wound healing include pathophysiological processes like those present in diabetes mellitus (Fowler 1989). Excessively high glucose concentrations can potentially inhibit neutrophil function by impairing phagocytosis and diminishing the production of oxygen radicals (Fowler 1989, Andersen and others 2004).

Dogs with dog bite wounds are generally in a shocked state when admitted and are therefore under excessive sympatho adrenal control (Rosin 1981, Douglas and Shaw 1989, Devey and Crowe 1997). After injury there is a rapid increase in plasma cortisol levels which peak within 4 to 6 hours and decrease to resting levels within 24 hours during the 'ebb phase' (Rosin 1981). The effects of cortisol include an influence on inflammation and an increased metabolism of carbohydrates, protein and fat as energy sources (Rosin 1981). Changes in blood volume and the afferent sensory nerve stimulation of the hypothalamus from an injury result catecholamines being released into the bloodstream. These hormones increase cardiac output, raise blood pressure and epinephrine increases the basal metabolic rate by as much as 100%. Epinephrine also stimulates metabolic activities such as glycogenolysis in the liver, glucose release into the blood, hydrolysis of fat and release of free fatty acids into the blood (Rosin

1981). Patients with disease therefore undergo an accelerated form of starvation and tissue depletion (Remilard and Martin 1990, Chandler and others 1992, Devey and Crowe 1997). This hypermetabolism results in a marked increase in visceral and muscle blood flow and oxygen and carbohydrate consumption. Fat and protein is broken down and used as an energy source (Chandler and others 1992, Mizock 1995, Patiňo and others 1999). The estimated increase in metabolic rate is 25% to 50% for multiple injuries and approximately 80% in sepsis (Long and Nelson 1993). The increase in respiratory quotient (the ratio of consumption of oxygen to the production of carbon dioxide, reflecting the different types of fuel used for body energy) from 0.7 in uncomplicated starvation to 0.8 in hypermetabolism indicates a mixed fuel oxidation of which glucose supplies approximately 40% to 60% (Long and Nelson 1993). During the hypermetabolic state, a number of alterations in carbohydrate metabolism are induced, (Patiňo and others 1999, Andersen and others 2004, Van den Berghe 2004) these include:

- Enhanced peripheral uptake and utilization of glucose by the wound and other organs involved in the immune response like the liver and spleen.
- Hyperlactataemia - due to possible hypoperfusion.
- Increased glucose production - adrenaline, cortisol, glucagon, growth hormone.
- Decreased glucose production - in severe sepsis due to gram-negative bacterial endotoxin, interleuken-6, insulin-like factors, decreased hepatic export or increased utilization.
- Depressed glycogenesis – suppressed by persistent high rate of glycogen breakdown under the influence of adrenaline and glucagon, decreased glycogen synthatase activity or TNF factor presence.

- Glucose intolerance and insulin resistance – a rise in extra cellular glucose concentrations, due to defective suppression of gluconeogenesis and resistance to the peripheral action of insulin, also termed 'diabetes of injury' (Van den Berghe 2004).

Tissue trauma with, or without, infection can initiate the systemic inflammatory response syndrome (SIRS), in which multiple inflammatory, immunologic, coagulation and fibrinolytic cascades are activated and interact (King and Hammond 1999, Holt and Griffin 2000). SIRS is characterised by hypermetabolism, a hyperdynamic cardiovascular state and clinical manifestations of fever or hypothermia, tachycardia, tachypnoea and leucocytosis or leucopaenia (Bone and others 1992, Welzl and others 2001, King and Hammond 1999).

Sepsis has been defined as SIRS with a documented infection and severe sepsis as SIRS with a documented infection and haemodynamic compromise (King and Hammond 1999, Holt and Griffin 2000). Septic shock is defined as severe sepsis that requires both volume replenishment and inotropes to restore tissue perfusion (King and Hammond 1999). All these syndromes are commonly seen in dogs with bite wounds (Holt and Griffin 2000). Alterations in blood glucose represent one of the most consistent findings in models of experimentally induced sepsis (Breitschwerdt and others 1981). Sepsis and SIRS can lead to hypoglycaemia due to impaired gluconeogenesis, especially from amino acids (Woolf and others 1979), and increased peripheral uptake and utilization of glucose (Mizock 1995, King and Hammond 1999). Stress is typically associated with increased gluconeogenesis and therefore hyperglycaemia. Septic stress however is distinguished by a biphasic response. Lethal models of sepsis in animals seem to demonstrate an initial hyperglycaemia followed by a phase of hypoglycaemia during which glucose production is suppressed (Breitschwerdt and others 1981, Mizock 1995).

Hypothermia (Oncken and others 2001), anorexia (Abood and Mauterer 1993), age (Atkins 1984, McMicheal and Dhupa 2000), pregnancy (Jackson and Bruss 1980, Leifer and Peterson 1984) and breed (Atkins 1984, Leifer and Peterson 1984) have been shown to influence plasma glucose concentrations.

No studies, evaluating the plasma glucose alterations over time in dogs with dog bite wounds, could be found in the literature. Dog bite wounds are acute injuries inflicted on an otherwise healthy animal and cannot, therefore, be compared with a 'chronic' illness like canine babesiosis (in which marked serum glucose perturbations have been shown), where animals are presented sometimes days after the start of the disease process (Keller and others 2004). In dogs there have been reports of hyperglycemia associated with heart failure (Brady and others). Another veterinary study has shown that blood glucose concentrations are significantly affected by the severity of head trauma in dogs and cats, and was significantly higher in dogs and cats with head trauma than in the control animals. However, blood glucose concentration was not associated with the outcome (Syring and others 2001).

The aim of this study is to describe the prevalence and prospective incidence, over a 72-hour period, of hypo-, normo- or hyperglycaemia, in admitted canine bite wound cases. Historical, signalment, clinical and haematological factors were investigated to determine their possible effect on blood glucose concentrations.

Method

Twenty dogs, bitten within 24 hours, with at least one open wound, and whose clinical condition, in the opinion of the attending outpatient's clinician, warranted the use of intravenous fluid therapy, were used. As no severity scores are available for dog bite wounds,

the criteria set out above were used to collect the most severely affected animals. They were admitted to the small animal surgery clinic and hospitalised in the Intensive Care Unit of the Onderstepoort Veterinary Academic Hospital. Possible *Babesia canis* was excluded by means of a peripheral blood smear and, in order to minimize any confusing influences on plasma glucose concentrations, no alpha$_2$ agonists were used in these cases at any time during the study period. These cases presented over an 8-month period.

The dogs' owners signed a consent form, and were asked to complete a questionnaire regarding the approximate time their dog was bitten, the time since the dog's last full meal, pregnancy, the dog's known medical conditions, and any medication given to the dog.

On admission the primary investigator performed a full clinical examination on each dog, and the following data was recorded: habitus (alert, depressed or collapsed), time since last meal, time since bite wound was inflicted, age, sex, body weight (size), body condition (Purina Nine Point Body Condition Score), temperature, pulse and respiration. Blood was collected from all dogs via the cephalic or jugular vein into a paediatric EDTA tube and an evacuated tube containing Sodium Fluoride Oxalate (NaF/Ox). The dogs were starved for two hours before blood was drawn (McKelvie and Powers 1966). Additional NaF/Ox samples for plasma glucose determination were taken at 8-hour intervals and haematology at 24-hour intervals from the time the bite was inflicted, as indicated by the owners. These collection points were selected with the purpose of comparing the dogs at similar times in the trauma pathway. A full haematological examination was done on admission and at each 24-hour collection time point thereafter. The NaF/Ox samples were centrifuged within minutes of collection at 1730 x g for eight minutes. The plasma was separated in a cryopreservation tube, labelled and immediately frozen at -20°C. Glucose analysis was performed in a single batch using the hexokinase

method. Hypoglycaemia was defined as plasma glucose concentration below 3.3 mmol/l (Walters and Drobatz 1992, Feldman and Nelson 1996), and hyperglycaemia as plasma glucose concentration above 5.5mmol/l (Walters and Drobatz 1992). No fluids containing glucose were administered to any of the dogs during the study period. The primary investigator was blinded to the plasma glucose concentrations for the duration of the study.

Temperature, pulse and respiration were recorded at each collection point. The study period extended from infliction of trauma to 72 hours, or until euthanasia or death, whichever came first. The study did not interfere with the normal clinical management of these cases.

Habitus at admission, time since last meal, time trauma was inflicted, age (<6 months = puppy; > 6 months = adult), sex, body weight (size), body condition, and outcome (died versus survived) were included as predictor variables for plasma glucose concentrations at each time point. Patients were classified into discrete, clinically meaningful categories of each predictor variable. The median plasma glucose concentration for each category of a variable was compared using Kruskal-Wallis one-way ANOVA on ranks.

The presence of SIRS was determined, using the criteria described by Hauptman and others (1997) and Welzl and others (2001), from the admission data and data collected at 24-hour intervals after the initial trauma. To be considered SIRS positive patients had to have (Hauptman and others 1997, Welzl and others 2001):

- A white cell count < 6000/mm^3 or > 16000/mm^3, and / or > 3% band cells, plus at least one of the following:
  - A rectal temperature of < 38.1 or > 39.2 deg C
  - A heart rate of > 120 beats per minute
  - A respiratory rate of > 20 breaths per minute

The plasma glucose concentration of SIRS+ and SIRS- groups were compared at each time point using the Wilcoxon rank-sum test.

The correlation of plasma glucose concentration with temperature, pulse, respiratory rate, red cell count, haematocrit, total white cell -, mature neutrophil -, immature neutrophil -, eosinophil -, lymphocyte -, monocyte - and thrombocyte count at each time point was done using the Spearman rank correlation coefficient.

The association between habitus on admission and at outcome was assessed using Fisher's exact test. Statistical analysis was done using NCSS 2004 (NCSS, Kaysville, UT, U.S.A.).

Results

Outcome: Two dogs were euthanased within 8 hours of being bitten and only admission samples were obtained from them. One dog was euthanased after 48 hours. All euthanasia's were attributable to financial reasons and not prognosis. One dog was collected by the owner after 16 hours, and taken to an alternative facility because of financial constraints; the outcome is unknown. This left 16 dogs in the study in which mortality could be objectively assessed. One dog died during the study period, and two died after cessation of the study period. Fifteen out of 16 dogs (93.8%) survived the 72-hour study period, and 13 out of 16 dogs (81.3%) recovered. All dogs (13/13) admitted as either alert or depressed and not euthanased or collected, survived, whereas 3/4 dogs (75%) admitted as collapsed, died (P=0.004). A significant statistical difference in plasma glucose concentrations was only found at the 32-hour interval (STI), with collapsed dogs having a higher median plasma glucose concentration than depressed dogs, but not compared to alert dogs.

Prevalence of glucose abnormalities: The medians and interquartile ranges of plasma glucose concentrations, and the proportion of dogs that were hypo-, normo- and hyperglycaemic at each given time point, are shown in Table 1.

The median plasma glucose concentration at each of the ten collection points was consistently in the hyperglycaemic range (5.7 mmol/l to 6.2 mmol/l), excluding the 56-hour (5.4 mmol/l) and the 64-hour (5.5 mmol/l) STI's, which were normoglycaemic. No dogs were found to be severely hypo- (< 2.2 mmol/l) or hyperglycaemic (> 10 mmol/l) during the study. On admission, 5% of the dogs (1/20) were hypoglycaemic, 40% (8/20) were normoglycaemic and 55% (11/20) were hyperglycaemic. None of the dogs showed hypoglycaemia during the remainder of the study period.

SIRS: The prevalence of SIRS at each 24-hour STI is shown in Table 2. Median plasma glucose concentrations for SIRS+ and SIRS- dogs are also shown. No significant differences existed at any time.

Putative risk factors/predictor variables: The median plasma glucose concentrations for subgroups of dogs defined by different levels of the predictor variables are shown in Table 3.

Few significant differences ($P < 0.05$) in the plasma glucose concentration were found, when assessing the groups within the different risk factors/predictor variables, with the exception of:

- Habitus: at the 32-hour STI (depressed = 5.6 mmol/l; collapsed = 6.3 mmol/l).
- Time since bite occurred: at the 32-hour STI (<8hrs = 6 mmol/l; >8hrs 5.5 mmol/l)
- Age: On admission (puppy = 7 mmol/l; adult = 5.4 mmol/l)

  At the 8-hour STI (puppy = 7.6 mmol/l; adult = 5.7 mmol/l)

  At the 16-hour STI (puppy = 7 mmol/l; adult 6 mmol/l)
- Body condition: On admission (thin = 6.8 mmol/l; fat = 4.7 mmol/l)

At the 16-hour STI (thin = 6.9 mmol/l; fat = 4.5 mmol/l)

Few significant correlations were found between clinical or haematological variables and plasma glucose concentration, with the exception of:

- A positive correlation with respiratory rate (r_s = 0.63; P = 0.0051) only on admission.
- A negative correlation (r_s = -0.5467; P = 0.0126) with the total red cell count on admission; a negative correlation (r_s = -0.5184; P = 0.0477) with the total red cell count at 72-hour STI.
- A negative correlation (r_s = -0.5663; P = 0.0092) with haematocrit on admission; a negative correlation (r_s = - 0.5135; P = 0.0503) with haematocrit at the 72-hour STI.
- A positive correlation (r_s = +0.5406; P = 0.0306) with eosinophil count only at the 24-hour STI.

Discussion

Outcome: This study has found a significantly lower survival rate in bite wound cases that presented as collapsed. In a recent study on canine babesiosis cases, collapsed states were strongly associated with hypoglycaemia (Keller and others 2004). In this study a significant statistical difference in plasma glucose concentrations was only found at the 32-hour STI, with collapsed dogs, ironically, having a higher median plasma glucose concentration compared to depressed dogs, but not compared to alert dogs. Similar to the findings of Syring (2001) in head trauma cases, this study found no association between the degree of hyperglycaemia and the eventual outcome. The low survival rate of collapsed animals should be investigated further as this may prove to be a relevant measure of severity, and predictor of outcome, in dog bite wounds.

Prevalence of glucose abnormalities: This study found a consistently high median plasma glucose concentration at all except the 56- and 64-hour STI (Table 1). Hypoglycaemia was encountered only on admission and only in one case.

During the post-traumatic 'ebb phase', the body's initial response is centered on the release of glycogen, the mobilization of lipid stores, and reducing the activity of the animal. The metabolic rate declines during this phase, but increases later. This phase is recognized by massive sympatho-adrenal discharge and lasts approximately 24 hours. This phase can be associated with hyperglycaemia (Douglas and Shaw 1989, Mizock 1995).

If the animal survives this 'ebb phase' it progresses to the 'flow phase', which is characterized by an increased metabolic rate and enhanced breakdown of lean body mass. The afferent signals from the wound via pain and other peripheral receptors, as well as cytokines, interleukin 1 and tumor necrosis factor, initiate the process (Abood and Mauterer 1993, Feldman and Nelson 1996, King and Hammond 1999). These signals are integrated in the hypothalamus and stimulate the secretion of glucagon, cortisol, catecholamines and growth hormone (Rosin 1981, Douglas and Shaw 1989). The net effect of these hormones, coupled with a defective suppression of gluconeogenesis and resistance to the peripheral action of insulin, is hyperglycaemia. This is all part of the hypermetabolic response to trauma (Měszăros and others 1987, Douglas and Shaw 1989, Patiňo and others 1999) which may last for seven to ten days post trauma (Douglas and Shaw 1989). This phenomenon has been described in the human literature regarding acute injury. It has been termed 'diabetes of injury' (Van den Berghe 2004). A positive linear correlation has been found between the degree of hyperglycaemia and the risk of death in human cardiac patients in a surgical ICU (Van den Berghe 2004). In human critical care medicine, insulin resistance has been implicated as a

major contributor to this phenomenon (Andersen and others 2004, Van den Berghe 2004, Langouche and others 2005). Aggressive control of even mildly elevated glucose concentrations, using insulin therapy, has shown a decline in death rates in human ICU's from 20.2% to 10.6% (Van den Berghe 2004). Insulin therapy also is associated with fewer complications such as severe nosocomial infections, acute renal failure, liver dysfunction, muscle weakness and anaemia (Van den Berghe 2004, Langouche and others 2005). Insulin has been shown to have the inherent capability of counteracting the metabolic changes observed in sepsis. It is hypothesized that insulin protects the endothelium (thereby contributing to the prevention of organ failure), modulates inflammatory pathways, protects cells from direct glucose toxicity, preserves hepatic mitochondrial function, improves dyslipidemia and shows an anabolic effect by suppressing proteolysis and activating protein synthesis (Andersen and others 2004, Van den Berghe 2004, Langouche and others 2005). The low incidence of hypoglycaemia in these dogs with bite wounds was surprising considering hypoglycaemia has been associated with profound metabolic illness and severe sepsis in dogs (Sonnewirth and Jarret 1980, Breitschwerdt and others 1981, Mizock 1995).

All animals were maintained on Ringers Lactate as intravenous infusion. Lactate can act as a precursor for glucose, and, although unlikely, may have influenced the results (Sonnewirth and Jarret 1980). Food was allowed ad lib, but removed two hours before sampling. These dogs cannot be fasted for long periods as they are in the hypermetabolic phase of trauma, with markedly elevated basal metabolic rates (Long and Nelson 1993). McKelvie and Powers (1966), in a study on normal beagles, showed one to two hours of food deprivation as sufficient not to statistically influence blood glucose concentrations. The feeding should therefore, not have added to artificially high glucose concentrations.

SIRS: A high prevalence of SIRS was encountered (Table 2) on admission and during the study period. Sepsis has been defined as SIRS with a documented infection. All bite wounds should be considered contaminated, or dirty and infected (Pavletic 1995, Davidson 1998, Holt and Griffin 2000), and the local environment of the wound ideal for bacterial replication and infection (Pavletic 1995, Davidson 1998, Holt and Griffin 2000) with subsequent bacteraemia and or endotoxaemia. Consequently, the dogs in this study can be classified as septic with a high degree of certainty. Previous reports state that these syndromes are frequently seen in dogs with bite wounds (Holt and Griffin 2000), and this study found similar results. However, no significant statistical differences in the mean plasma glucose concentrations could be established between dogs with and without SIRS.

Putative risk factors/predictor variables: Few significant associations were found between these variables and plasma glucose concentration. It is interesting to note that puppies and thin dogs tended to have higher plasma glucose concentrations than adult or obese dogs, particularly during the first 16 hours of trauma. This may warrant further investigation, as it seems to contradict accepted norms. The negative correlation between total red cell count and haematocrit (Ht) with plasma glucose concentrations was not expected, as low values of these variables have correlated with lower plasma glucose levels in a previous babesiosis study (Keller and others 2004), however, if the dog had significant bleeding to lower the haematocrit, this would be consistent with more severe injury and greater stress response, which may lead to higher plasma glucose concentrations.

Increased utilisation of glucose by bacteria and polymorphonuclear leucocytes has been implicated in hypoglycaemia associated with sepsis (Breitschwerdt 1981). Consequently, it is

surprising that this study did not find any correlation between glucose concentration and leucocyte counts.

Conclusion and limitations

The aim of this study is to describe the serial changes in plasma glucose concentrations at 8-hour intervals, in severely bitten dogs. The results show an almost uniformly high median plasma glucose concentration over the72-hour sampling period. This can probably be ascribed to the 'diabetes of injury' response. The role of insulin therapy should be explored in future studies, as its use in humans has shown a significant reduction of fatalities resulting from acute injury. The low incidence of hypoglycaemia is surprising considering the high incidence of SIRS and the subsequent sepsis encountered. There was no significant difference between the plasma glucose concentrations of the SIRS positive and negative cases. Few important associations were made between historical, clinical and haematological variables and plasma glucose concentration. However, the high incidence of death in the collapsed group, and the higher plasma glucose concentrations found in puppies and thin dogs warrants future investigation with a larger group of animals.

Possible limitations (apart from those noted in the text) to the study are:

- The low number of patients used.
- The differing levels of severity (although an attempt was made to select the most severely bitten animals) and the current unavailability of a meaningful severity score.
- The "white jacket" effect/response that may have added to the stress response of the dogs.
- The low specificity of the SIRS Criteria used.

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Table 1 Descriptive statistics of plasma glucose concentrations in mmol/l at admission, and at 8 hr time intervals since time bitten as indicated by owners.

	admission	8 hr	16 hr	24hr	32hr	40hr	48hr	56hr	64hr	72hr
Count	20	16*	18*	17	17	17	17	16	15	15
Median	6	6.2	6.2	5.8	5.8	6.2	5.9	5.4	5.5	5.7
IQR	5 – 7	5.4-7.3	5.6-6.8	5.2-6.8	5.6-6.3	5.5-6.8	5.1-6.5	4.8-5.9	5.1-6.2	5.1-6.8
Hypoglycaemic (< 3.3 mmol/l)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normoglycaemic (3.3 – 5.5 mmol/l)	8 (40%)	5 (31%)	4 (22%)	5 (29%)	4 (24%)	4 (24%)	6 (35%)	10 (63%)	9 (60%)	5 (33%)
Hyperglycaemic (> 5.5 mmol/l)	11 (55%)	11 (69%)	14 (78%)	12 (71%)	13 (76%)	13 (74%)	11 (65%)	6 (37%)	6 (40%)	10 (67%)

* - Two cases presented between 8 and 16 hrs since bitten, and therefore do not have 8 hr samples.

Table 2 Prevalence and median values of plasma glucose concentrations in mmol/l for SIRS+ and SIRS - cases at admission and at each 24 hr interval since time bitten as indicated by owners.

		Admission		**24 hours***		**48 hours**		**72 hours**	
Variable	**Category**	**n**	**Median**	**n**	**Median**	**n**	**Median**	**n**	**Median**
SIRS	SIRS +	13	6.6	13	5.8	13	5.9	12	5.7
	SIRS -	7	5.4	3	6.8	4	5.9	3	5.9
	% SIRS +	65%		81%		76%		80%	

* - Incomplete haematology data for one case

Table 3a Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds from admission to 24 hours.

			Admission		8 hour		16 hour		24 hour	
Variable	**Category**	**n**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	5.4	4.5;6.8	6.1	5.3;7.1	5.9	4.8;6.6	5.9	5.4;6.5
	Depressed	8	6	5;7.1	7.3	6.2;8	6.4	5.9;7	5.4	4.7;6.1
	Collapsed	5	6.6	5;7.7	5.5	3.9;7.15	6.4	5.7;7.6	6.8	5.6;7.2
Time since last meal	≤8 hrs	9	6.8	5.7;7.1	6.8	5;7.7	6.8	6.2;7	6.1	5.2;6.8
	>8 hrs	11	5.4	4.7;6.6	5.8	5.4;7	5.9	5.2;6.3	5.8	5.1;.6.5
Time since bitten	≤8hrs	16	6.4	5;7	6.5	5.6;7.6	6.5	5.9;6.9	5.9	5.7;6.9
	>8hrs	4	5.7	3.8;7.2	5.4	3.3;5.9	5.7	5.1;6.3	5.2	4.9;5.8
Age	Puppy (≤ 6 mths)	4	7*	6.8;8.1	7.6*	7.1;8.5	7*	6.8;8.1	6.7	6.3;7.4
	Adult (> 6 mths)	16	5.4*	4.8;6.6	5.7*	5;6.5	6*	5.3;6.4	5.8	5;6.2
Sex	Male	9	6.6	5.4;7.2	5.6	4.7;7.3	6	5.7;6.7	6	5.2;6.9
	Female	5	6.1	5;7.2	6.9	6.5;8	6.5	5.4;7.2	5.7	4.8;5.9
	Female sterilised	6	5	4.2;6.9	6.3	5;7.15	6.3	4.7;6.8	6.1	5.1;6.8
Size	≤5 kg	3	7.2	6.8;8.4	7.3	7;8.7	7	6.8;8.4	6.9	6.3;7.4
	>5 kg ≤10 kg	13	5.4	4.8;6.6	5.9	5.2;6.9	6	5.7;6.4	5.7	4.8;5.9
	>10 kg	4	6.1	3.8;7	5.5	3.8;7.3	6.1	5.1;6.9	6.4	5.5;7
Body condition	Thin (1-4)	6	6.8*	6.4;7.5	7.2	5.8;8	6.9*	5.9;7.4	6.3	5.3;7.1
	Ideal (5-6)	11	5.4	5.1;7	5.7	5;6.9	6.2	5.6;6.6	5.9	5.5;6.8
	Fat (7-9)	3	4.7*	4.5;4.9	4.8	4.8;4.8	4.5*	4.3;4.7	4.7	4.5;4.8
Outcome	Recovered	13	5.4	4.9;6.8	6.5	5.4;7.2	6	5.2;6.7	5.7	4.9;6.2
	Died	3	6.6	6.6;7	5.5	4.5;5.6	6.4	5.9;6.8	6.8	5.8;7

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference.

Table 3b Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds from 32 to 56 hours.

			32 hour		40 hour		48 hour		56 hour	
Variable	**Category**	**n**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	6	5.6;6.5	6.6	5.1;7.3	5.6	4.9;6.2	5.7	4.1;7.2
	Depressed	8	5.6*	5.3;5.8	6.1	5.2;6.2	5.7	5.4;6.6	5.5	5.3;6
	Collapsed	5	6.3*	5.9;7.9	6.6	6;7.7	6.1	5.3;7.6	4.9	4.5;5.7
Time since last meal	≤8 hrs	9	5.9	5.6;6.9	6.2	5.3;6.6	5.9	5.5;6.5	5.5	5.2;5.8
	>8 hrs	11	5.8	5.4;6.3	6.2	5.5;7.7	5.9	5;6.5	5.3	4.5;6.9
Time since bitten	≤8hrs	16	6*	5.7;6.7	6.2	5.7;6.8	5.9	5.3;6.5	5.4	4.8;5.8
	>8hrs	4	5.5*	5.2;5.8	5.9	5.2;6.7	5.4	4.8;6.3	6.1	4.8;6.9
Age	Puppy (≤ 6 mths)	4	7.1	5.7;7.7	6.9	6;8.7	6.1	5.6;8	5.6	5.3;5.9
	Adult (> 6 mths)	16	5.8	5.5;6.2	6.2	5.3;6.6	5.8	5;6.5	5.4	4.6;6
Sex	Male	9	5.8	5.5;7	6.4	5.8;7.7	5.9	5.4;6.9	5.3	4.5;6.4
	Female	5	5.7	5.2;6.2	6.2	5.5;6.2	5.4	5;6.4	5.4	5.3;5.6
	Female sterilised	6	6.1	5.8;6.9	5.9	5;6.8	6	4.9;6.1	5.9	4.2;7.9
Size	≤5 kg	3	7.4	7.1;7.7	7.8	6.9;8.7	7.1	6.1;8	5.9	5.9;5.9
	>5 kg ≤10 kg	13	5.8	5.5;6.3	6.2	5.1;6.4	5.9	5;6.6	5.4	4.6;5.7
	>10 kg	4	5.7	5.2;6.1	6.3	5.7;6.8	5.4	4.8;6	5.6	4.7;6.5
Body condition	Thin (1-4)	6	6.3	5.8;7.4	6.6	6.1;7.8	6.1	5.8;7.3	5.6	4.8;6.7
	Ideal (5-6)	11	5.8	5.5;6.2	6.2	5.5;6.7	5.8	5.2;6.5	5.5	5;6
	Fat (7-9)	3	5.6	5.1;6	5.2	5.1;5.3	4.8	4.6;5	4.4	3.6;5.2
Outcome	Recovered	13	5.7*	5.5;6	6.2	5.2;6.6	5.6	5;6.3	5.4	4.9;6.3
	Died	3	6.3*	6.2;8.1	6.6	6.4;6.6	6.1	5.9;7.2	5.2	4.6;5.8

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference.

Table 3c Putative risk factors and their medians and interquartile ranges (IQR) of plasma glucose concentrations in mmol/l in severe dog bite wounds from 64 to 72 hours.

			64 hour		72 hour	
Variable	**Category**	**n**	**Median**	**IQR**	**Median**	**IQR**
Habitus	Alert	7	6.1	5.3;7.2	6.1	5.4;6.7
	Depressed	8	5.5	4.9;6.3	5.7	5.1;7.2
	Collapsed	5	4.9	4.6;5.5	5.1	4.5;6.8
Time since last meal	≤8 hrs	9	5.5	5.1;5.8	5.7	5.1;6.7
	>8 hrs	11	5.9	4.9;7.3	6	5;7.4
Time since bitten	≤8hrs	16	5.5	5.1;5.9	6	5.1;6.8
	>8hrs	4	6.2	5.1;7.2	5.7	4.8;7.1
Age	Puppy (≤ 6 mths)	4	5.9	5.5;6.2	5.8	5.1;6.4
	Adult (> 6 mths)	16	5.5	5;6.4	5.7	5.1;7
Sex	Male	9	5.5	4.9;6.8	5.7	5.1;6.8
	Female	5	5.2	4.6;5.8	5.9	5.2;6.9
	Female sterilised	6	5.9	5.2;7.9	5.8	4.6;7.2
Size	≤5 kg	3	6.2	6.2;6.2	6.4	6.4;6.4
	>5 kg ≤10 kg	13	5.4	5;6.3	6.1	5.5;7.2
	>10 kg	4	5.5	5.1;6.5	5.1	4.7;5.6
Body condition	Thin (1-4)	6	6.2	5.5;7.3	6.4	5.1;7.5
	Ideal (5-6)	11	5.5	5.1;6.1	5.9	5.6;6.9
	Fat (7-9)	3	4.8	4.4;5.1	4.7	4.4;5
Outcome	Recovered	13	5.5	5.1;6.5	5.7	5.1;6.8
	Died	3	5.1	4.6;5.5	6	5.1;6.8

* - Medians for different categories of the same variable differ significantly ($P < 0.05$, Kruskal-Wallis one-way ANOVA on ranks). No asterisk = no significant difference

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