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Determination of the pathophysiological consequences of capture and capture-induced hyperthermia in blesbok

(*Damaliscus pygargus phillipsi*)

by

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Abstract

An unacceptable number of wild animals die or experience morbidity as a result of capture-related complications. Capture-induced hyperthermia is believed to play a role in the morbidity and mortality of captured animals. The aims of this research were to gain a better understanding of the pathophysiological consequences of capture and capture-induced hyperthermia and to better understand the association between capture-induced hyperthermia and capture myopathy. We aimed to determine whether cooling could reduce the pathophysiological consequences of capture and protect against capture related complications like capture myopathy.

Forty wild blesbok were captured from the wild and then housed in bomas at Groenkloof Nature Reserve for the duration of the study. These animals were divided into three groups which included a group of animals that were chased and cooled during immobilization (C+C), a group that was chased but not cooled during immobilization (CNC) and a control group that was not chased before or cooled during immobilization (Ct). The control group received powder diazepam in their feed two hours before they were darted to minimize their stress response to capture. The treatment groups were chased for 15 minutes before they were darted. All the animals were darted and immobilized with etorphine and azaperone. The chased and cooled group were doused with 10L of 4°C water over a 10 minute period during the immobilization. The immobilization period lasted for forty minutes in all the animals.

A number of clinical and physiological parameters were measured in all the animals directly after induction into immobilization, 40 minutes later, and then on day 2, 16 and 30 post the initial capture. On these subsequent days the animals were not chased but they were simply all immobilized in their holding bomas, for data and sample collection, after they received in feed

diazepam. The parameters measured and analysed included muscle and rectal temperature, PaO_2, $PaCo_2$, pH, lactate, GGT, GLDH, creatinine, BUN, CK, AST, cardiac troponin I and cortisol.

The animals in the control group had normal values for all the variables measured except they were hypoxic during the immobilization. The animals that were chased developed hyperthermia, hypoxia and mild acidaemia, and they had mildly elevated concentrations of GLDH, AST, CK and creatinine indicating mild hepatic, renal and skeletal muscle damage. These animals also had severe increases in lactate and cardiac Troponin I concentrations indicating cardiac damage possibly as a result of the hypoxia that occurred during the chase and the immobilization. Despite cooling correcting the hyperthermia, it did not prevent or protect against any of these pathophysiological effects. Therefore, this capture-induced hyperthermia appeared to play a limited role in causing these effects.

Therefore, capture that involves chasing animals before immobilization appears to primarily cause cardiac muscle damage. This capture-induce cardiomyopathy may have profound secondary consequences, which could result in mortality or other capture related complications.

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List of Abbreviations

Ach: acetylcholine

ACTH: adrenocorticotropic hormone

AST: aspartate transaminase

ATP: adenosine triphosphate

AVP: arginine-vasopressin

BE: base excess

BILI: bilirubin

BUN: blood urea nitrogen

Ct: control

CNC: chase non-cooling

C+C: chase plus cooling

$cHCO_3^-$: calculated bicarbonate

cSO_2: calculated oxygen haemoglobin

Ca^+: calcium

CK: creatine-kinase

CREA: creatinine

cTnI: cardiac troponin I

CRP: C-reactive protein

CRH: corticotropin-releasing hormone

CTM: critical thermal maximum

DIC: disseminated intravascular coagulation

EDTA: ethylylenediaminetetra-acetic

EH: exertional hyperthermia

EHS: exertional heat stroke

ER: exertional rhabdomyolysis

EHI: exertional heat illness

G: gauge

GGT: gama-glutamyl transferase

GLDH: glutamate dehydrogenase

HPA: hypothalamic-pituitary-adrenal

Htc: haematocrit

IU^{-1}: international units

K^+: potassium

MT: Muscle temperature

MH: Malignant hyperthermia

M: meters

μmol: micromoles

Mm: millimetres

mmHg: millimetres of mercury

Mmol.l^{-1}: milimole litre

Na$^+$: sodium

Ng.ml^{-1}: nanogram per milliliter

pH: potential of hydrogen

PaO$_2$: partial pressure of oxygen

PaCO$_2$: partial pressure of carbon dioxide

PSS: porcine stress syndrome

PVC: polyvinyl chloride

RT: rectal temperature

SAA: serum amyloid-A

SAM: sympathetic-adrenal-medullar

Chapter 1

Introduction

Human-wildlife conflict is one of the most critical challenges that wildlife species are facing today (Dickman, 2010), therefore wildlife conservation and preservation is gaining importance not only in South Africa but also internationally. During the last few decades the value of wildlife has increased not only from a conservation perspective but also in terms of animal production and sales through wildlife ranching, eco-tourism and the need to harvest wildlife as a food source (Chardonnet *et al*., 2002; Dry, 2010). In the last ten years, wildlife ranching in South Africa has been designated as the new sustainable green economy (Dry, 2010) being financially viable, particularly on marginal land.

In order for game ranching and conservation activities to occur, wild animals need to be translocated and therefore successful capture, immobilization and transportation of wild animals is essential. In spite of the current use of immobilizing and tranquilizing drugs, which decreased morbidity and mortality (Harthoorn, 1972), an unacceptable number of animals still succumb during and after capture and translocation procedures. Therefore, veterinarians and game capturers need to develop safer capture and immobilizing techniques to ensure the welfare and survival of wild animals that they work with.

Every capture poses a threat to the animal that is being caught and easily results in alarm and acute psychological stress. This alarm and stress response was first explored by Cannon (1929) and was named the "Flight-or-Fright response" in which the sympathetic nervous system is rapidly triggered resulting in a stimulation of the adrenal gland and the release of adrenaline, noradrenaline and other catecholamines.

Although the alarm and stress response prepare an animal for "flight or fright", they may also cause several negative consequences especially during capture when these response are extreme or prolonged. Some of the negative consequences include an increase in lactic acidaemia, hyperthermia and hypoxia. How these consequences affect an animal's organs and bodily functions in the short (< 5 days) and medium-term (< 30 days) post capture is not fully known. Capture-induced hyperthermia (acute temperature rise to as high as 43-44°C) is a common consequence of capture and is believed to play a major role in causing morbidity and mortality. This acute rise in body temperature is mainly caused by stress and secondly affected by environmental temperature and exercise (Meyer *et al.*, 2008 a; Meyer, 2010). As a treatment for this hyperthermia, animals are often cooled down by dousing them with water (Sawicka, 2011). How capture-induced hyperthermia affects an animal and whether this cooling reduces the negative consequences of capture needs to be determined.

The aim of this study is therefore to determine what the short and medium-term pathophysiological consequences of capture and capture-induced hyperthermia on the health of captured animals and whether cooling affects or alters these consequences.

Chapter 2

Literature Review

2.1 Capture, a stressful event

The capture of wild animals, either by physical restraint or chemical immobilization, causes a certain amount of stress in these animals (Harthoorn & Young, 1974; Kock *et al.,* 1987 a & b; Kock *et al.*, 1990; Morton *et al.*, 1995). Different stimuli perceived by an animal, such as the perception of danger, new environments and crowded conditions are important psychological stressors that cause distress (physical or mental anguish or suffering) and induce fear and anxiety (Spraker, 1993). These stressful situations commonly induce alterations in an animal's metabolism (Elsasser *et al.*, 2000).

The impact of stress on an animal's metabolism is in positively correlation with metabolic alterations that occur (Elasser *et al*., 2000). The ability of an animal to cope with stressful situations will determine the magnitude of the impact of these metabolic alterations and derangements. This ability is inherent and different in each individual, and whenever the break point, which is the limit for stress adaptation, is passed, disease occurs.

There are different types of stress that animals can suffer from like physical, physiological and psychological stresses (Bouwknecht *et al*., 2007). These different types of stress can appear either separately or in combination. When a wild animal is chased, a psychological, physical and a physiological stress occurs. The stress response firstly results from an activation of the central nervous system after perceiving a threat or physiological alteration in the body. The perception of the threat or physiological alteration is critical as it activates key stress responses that may

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determine an individual's survival. Depending on the severity and duration of a threat or physiological alteration, four different biological responses may occur, these include; behavioural, autonomic nervous system, neuroendocrine and immune responses (Moberg, 2000).

The acute activation of the stress system by a perceived threat prepares an animal, through stress responses, for a flight or fight response (Fowler, 1986; Guyton, 1991; Stratakis & Chrousos, 1995). In these circumstances both the sympathetic-adrenal-medullar (SAM) and hypothalamic-pituitary-adrenal (HPA) systems are activated (Fowler, 1986; Stratakis & Chrousos, 1995). Through the stimulation of the SAM system the sympathetic nerves release acetylcholine (Ach) in the adrenal gland which causes the release of adrenaline and small amounts of noradrenaline (Fowler, 1986). In the HAP system activation, corticotrophin-releasing hormone (CRH) together with arginine-vasopressin (AVP) hormone are released; together they stimulate the pituitary gland to release corticotrophin (ACTH) (Fowler, 1986; Stratakis & Chrousos, 1995). Circulating ACTH stimulates the adrenal cortex to produce cortisol or corticosterone depending on the species of animal (Fowler, 1986, Stratakis & Chrousos, 1995). Other factors like angiotensin II, cytokines and lipid mediators of inflammation are also released into the blood stream during a stress response and are believed to potentiate the activity of the HPA axis (Stratakis & Chrousos, 1995).

Some of the primary actions of the sympathetic activation is vasoconstriction of vessels in the digestive system, connective tissue and skin. Concurrently there is vasodilation of vessels in the brain, skeletal muscle, heart and lungs, thus increasing the blood flow to these organs (Spraker, 1993). Another important sympathetic effect results in the increase in the availability of energy in the form of ATP for fuelling the muscles and other organs during the animal's attempt to escape. These actions are achieved by increasing the rate of cellular metabolism, gluconeogenesis and glycogenolysis. There is also a rise in blood pressure and an increase in mental activity and blood coagulation (Spraker, 1993).

Capture stress possess a challenge to an animal's metabolic capability. The effects of a stress response may cause pathophysiology that could manifest as per-acute, acute or chronic effects (Spraker, 1993), which vary according to the intensity and duration of the chase as well as an individual's, or species makeup. The result of that pathophysiology can be moderate to severe (Spraker, 1993). Moderate pathophysiological effects include tachycardia, hypertension, a moderate increase in haematocrit and haemoglobin concentrations and muscle fatigue (Fowler, 1986). These effects generally have a protective function in the animal, unless their activation is extreme or prolonged, which will lead to more harmful consequences. Whereas, severe pathophysiological effects, which may have harmful and even lethal consequences, include ventricular fibrillation, muscle exhaustion, severe hypoglycaemia, hyperthermia, hypoxia and shock (Fowler, 1986).

Relative to acute stress, chronic stress persists for a much longer period of time. Prolonged exposure to a stressor can lead to a continuous negative feedback on the adenohypophysis and adrenal gland exhaustion, and both will alter the production of adrenocortical steroids (Fowler, 1986). Chronic stress can lead to long-term effects due to chronic exposure to cortisol. Some of these effects involve alterations in normal behaviour, decreased food intake, reproductive failure, gastric and intestinal ulceration, metabolic and electrolyte imbalances, immunological deficiencies and changes in general behaviour (Fowler, 1986; Spraker, 1993; Moberg, 2000).

Stress-induced hyperthermia has been reported in a large variety of mammalian species including humans (Bouwknecht et al., 2007). In wild animals that are subject to capture procedures, psychological (exposure to foreign sights, sounds and smells from helicopters, trucks and humans), physical (restraint) and physiological (muscle exertion from a chase) stress occurs which may trigger an acute hyperthermia. This hyperthermia, called capture-induced hyperthermia, is essentially a stress-induced hyperthermia and is caused predominantly by acute psychological

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stressors which an animal is exposed to during capture and results in an increase in metabolism (Bouwknecht *et al.*, 2007; Meyer *et al.*, 2008 a & b).

2.2 Hyperthermia

Capture procedures cause several side effects that can alter an animal's homeostasis (Kock *et al.*, 1987 a & b; Montane *et al.*, 2007; Schumacher, 2008; Meyer, 2010). Respiratory depression, poor muscle relaxation, regurgitation, acidosis and hyperthermia are some of the most relevant side-effects caused by capture (Kock *et al.*, 1987 a & b; Harthoorn, 1972; Schumacher, 2008; Meyer, 2010). Hyperthermia is believed to play a major role in the development of the pathophysiological effects seen during capture and has been implicated in the development of capture myopathy (Harthoorn, 1965; Hofmeyer *et al.*, 1973; Harthoorn, 1975; Meyer *et al.*, 2008 a; Meyer, 2010)

Hyperthermia is the rise of an individuals' body temperature above its hypothalamic set point generally due to the dysfunction of thermoregulatory mechanisms (Bouchama & Knochel, 2002). Imbalances between heat gain (from high environmental temperatures) and generation (from metabolism) and heat dissipation will result in hyperthermia (Bouchama & Knochel, 2002). Hyperthermia can trigger a number of different pathophysiological effects in an individual that can range from mild heat illness to a systemic inflammatory response with activation of coagulation, resulting in disseminated intravascular coagulation (DIC), shock and even death (Bouchama & Knochel, 2002). The extent of the individuals' reaction to hyperthermia will be determined by different factors such as the individual's cellular ability to adjust and respond to different temperatures (Miller & Ziskin, 1989) and the extent of cell injury will depend on the critical thermal maximum (CTM) of that individual (Bouchama & Knochel, 2002). The critical thermal maximum is the body temperature above which the cells of an individual will start to suffer lethal injury or begin to denature (Bouchama & Knochel, 2002). The extent of cellular injury is not only dependant on the magnitude of the hyperthermia but also the duration at which temperature is above the critical thermal maximum (Miller & Ziskin, 1989). Cellular injury from hyperthermia will produce metabolic

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disturbances and multi-organ dysfunction (Bouchama *et al.*, 2005). The magnitude and time course of these perturbations are dependent on the magnitude and duration of this hyperthermia (Bouchama *et al.*, 2005).

Hyperthermia affects many different organ systems. The organ that is affected first is the gastrointestinal system (Lambert *et al.*, 2002; Lambert, 2009). Hyper-permeability and disruption of the intestinal barrier of the gastro intestinal tract caused by severe hyperthermia results in the release of endotoxins which in turn activate an inflammatory response (Miller & Ziskin, 1989; Bouchama *et al.*, 2005; Lambert, 2009). Simultaneously, coagulation is also activated and together with the inflammatory response, plays a major role in the pathological alterations that occur during severe hyperthermia (Bouchama *et al.*, 2005). Capture-induced hyperthermia is believed to produce vascular collapse (shock), liver necrosis, hypoxemia, metabolic and electrolyte imbalances, metabolic acidosis and disseminated intravascular coagulation (Fowler, 1978; Miller, 2000). Capture-induced hyperthermia is also believed to cause tissue changes with irreversible damage in the kidneys (kidney failure), heart (myocardial failure) and brain (cerebral oedema), (Fowler, 1978; Gfeller, 2005).

2.2.1 Capture-induced hyperthermia and exertional hyperthermia

Wild animals typically develop acute hyperthermia irrespective of whether they are chemically or physically captured (Hofmeyer *et al.,* 1973; Kock *et al.*, 1987 a & b; Gericke *et al.*, 1978; Cheney & Hattingh, 1988; Martucci *et al.*, 1992; Montane *et al.*, 2003; Montané *et al.*, 2007; Meyer *et al.*; 2008 a & b; Meyer, 2010). It has been proposed that this hyperthermia may play a major role in capture myopathy and acute deaths during capture (Gericke, 1978; Cheney & Hattingh, 1988; Antognini *et al.*, 1996; Meltzer and Kock, 2006; Meyer *et al.*, 2008 b). Measures are usually taken to prevent or diminish the incidence and severity of capture-induced hyperthermia in order to reduce the incidence of morbidity and mortality (Harthoorn, 1965; Hofmeyer *et al.,* 1973, Gericke &

Hofmeyer, 1975; Harthoorn, 1981; Kock *et al*., 1987 c; Burroughs & McKenzie, 1993; Montane *et al*., 2007; Meyer, 2010; Kock & Burroughs, 2012)

Due to a fright and flight response, an animal's muscles can over-exert during a fast and stressful attempt to escape from a dangerous situation (Harthoorn, 1965; Hofmeyer *et al*., 1973, Gericke *et al*., 1978). During this escape, an animal's body temperature increases as a result of an animal's stress response to danger and activity when it attempts to escape from that life-threatening situation (Montané *et al*., 2007). Although Meyer *et al*., (2008 a & b) demonstrated that hyperthermia is produced predominantly by stress, current texts (Kock & Burroughs, 2012) still recommend not to chase animals for distances greater than 2 kilometres or for a duration of 60-90 seconds, since hyperthermia and muscle exertion are believed to be the primary cause of capture myopathy (Harthoorn & Young, 1974; Kock & Burroughs, 2012). Moreover, recommendations are made that capture procedures should not take place during hot seasons and should be restricted only to times of the day that are cool, so that captured animals are able to easily dissipate the excess heat produced by their bodies (Burroughs & McKenzie, 1993; Kock *et al*., 2006; Kock & Burroughs, 2012).

Animals may tolerate a certain degree of hyperthermia, but when they reach and exceed their critical thermal maximum, their ability to thermoregulate is lost (Bouchama & Knochel, 2002) and irreversible pathological changes start to occur in tissues with potential fatal consequences (Miller & Ziskin, 1989). Even before this temperature is reached, they may begin to show signs of heat-induced illness (Williams & Thorne, 1996).

The exact mechanisms causing stress or capture-induced hyperthermia have not yet been fully elucidated (Meyer, 2010). In a similar way, the role that capture-induced hyperthermia plays in causing morbidity and the pathogenesis of capture myopathy in wild animals is not completely

known. Because the syndrome of capture myopathy and multiple organ failure often occur after capture (Hofmeyer *et al*., 1973; Harthoorn, 1975; Spraker, 1993; Williams & Thorne, 1996; Meyer *et al*., 2008 a; Meyer, 2010; Kock & Burroughs, 2012), many attempts have been made to establish the best ways to minimise and even prevent these from occurring. For this reason, and because of the alleged link between capture-induced hyperthermia, capture myopathy and multiple organ failure, there is a common practice in the field of dousing hyperthermic animals with water in order to treat or prevent hyperthermia (Sawicka, 2011; Broekman, 2012). Whether these methods have a preventive or protective effect against capture myopathy and other organ damage has yet to be determined.

Further research is needed in order to establish the real consequences, both in the short and the long-term, of capturing wild animals. Whenever an animal is captured and survives, it is presumed that no, or limited side effects and suffering have been induced. Unless there is severe morbidity or mortality in the days following capture, usually no close monitoring is done on an animal, and therefore the long-term consequences of capture are seldom identified and recorded (Meyer, 2010). On some occasions, animals are put in bomas after being captured from the wild. Commonly, these animals' behaviour changes while they are confined and in many instances they lose their appetite (Burroughs, 1993; La Grange *et al*., 2010; Harthoorn, 1965; Kock & Burroughs, 2012). Whether this is a maladaptation to a stress response resulting from prolonged penning or a consequence of the capture procedure has not been determined. What is clear is that capture stress alters an animal's physiology and in certain circumstances results in the development of capture myopathy (Harthoorn, 1965; Gericke *et al*., 1976; Meyer *et al*., 2008 a & b; Meyer, 2010). One of the predisposing factors for capture myopathy is the intensity of the chase prior to the capture (Harthoorn & Young, 1974; Hofmeyer *et al*., 1973). After capture, animals that had the greatest acidosis were found to be predisposed to develop capture myopathy. These animals were those that were chased intensively by vehicles over a short distance (approximately 2 km) (Harthoorn & Young, 1974). This finding is noteworthy and can easily be applied to today's common method of helicopter-assisted capture, where animals are chased intensely for short

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distances. Therefore, recommendations are made that special care must be taken while chasing animals using a helicopter for long periods of time (Harthoorn, 1965; Harthoorn, 1972; Hofmeyer *et al.*, 1973; Gericke *et al.*, 1978; Kock & Burroughs, 2012). Even over long but low intensity chases, animals tend to overexert with negative consequences (Hofmeyr *et al.*, 1973; Harthoorn & Young, 1974). Some animals that have been chased develop depression, ataxia and unsteady movements, incoordination, weakness, muscle stiffness, tremors, torticollis, recumbency and shock, with acute death sometimes occurring (Williams & Thorne, 1996).

In humans, exertional hyperthermia (EH) or exertional heat stroke (EHS) appears to mainly occur in untrained individuals when they exercise intensively or participate in endurance events without proper training. Exertional heat illness is a disturbance of the thermoregulatory process where excess heat production from exercise surpasses the body's heat dissipation mechanisms and can quickly progress into exertional heat stroke (Cappachione & Muldoon, 2009). The progression into exertional heat stroke is due to direct heat cytotoxicity in the endothelium, brain, gut, kidney, spleen, liver and skeletal muscle cells (Leon & Helwig, 2010). This cytotoxicity triggers the release of cytokines producing a systemic inflammatory response and possibly the release of coagulation factors which activate disseminated intravascular coagulation (Leon, 2006; Leon & Helwig, 2010). If these responses are severe multi-organ system failure and finally death may occur (Leon, 2006; Leon & Helwig, 2010).

Athletes, military personnel and certain occupational workers, such as fire fighters are predisposed to developing exertional heat stroke (Leon & Helwig, 2010). Primary predisposing factors for exertional heat stroke are excessive physical work or exercise in hot and humid conditions. Other factors include the use of recreational drugs, alcohol and pre-existing illness (Leon & Helwig, 2010)

Exertional rhabdomyolisis (ER) is a complication of exertional heat illness (EHI) and exertional heat stroke and can occur irrespective of whether humans exercise at high environmental temperatures or have high core body temperature (Capacchione & Muldoon, 2009). Its aetiology is diverse but normally occurs as a response to strenuous exercise which results in damage to skeletal muscles which leads to hyperkalaemia, myoblobinuria and renal failure (Capacchione & Muldoon, 2009).

According to Valberg *et al.,* (1999), the aetiology of exertional rhabdomyolisis in breed horses is similar to that of exertional rhabdomyolisis in humans. In Quarter horses, rhabdomyolisis is produced mainly by a glycogen storage disorder and it mostly affects untrained horses (Valberg *et al.*, 1999). In the case of Thoroughbreds, this disease is mostly due to an alteration of the muscle contractility and it is only expressed, albeit inconsistently, when they suffer stress from exertion (Valberg *et al.*, 1999).

2.2.2 Heat Stroke

Hyperthermia can also affect humans and potentially result in myopathy, especially if they develop heat stroke (Bouchama & Knochel, 2002). Heat stroke is induced by thermoregulatory failure resulting from prolonged exposure to high environmental temperatures (classic or non-exertional heat stroke) or from strenuous exercise (exertional heat stroke) (Bouchama & Knochel, 2002). The pathogenesis of heat stroke arises from a breakdown of complex metabolic responses that try to protect the body against the effects of heat cytotoxicity (Bouchama *et al*., 2005). Some of these responses include thermoregulation, acclimatization, acute phase response and the production of heat proteins (Bouchama & Knochel, 2002).

Thermoregulation is a process where the body automatically tries to balance metabolic heat production, heat dissipation and environmental heat gain to maintain a constant or defined range in body temperature (Bouchama & Knochel, 2002; Leon, 2006; Fowler, 2008). In cases where

environmental heat is excessive (summer seasons) animals have difficulty in dissipating excess heat produced by their bodies (Bouchama & Knochel, 2002). On the other hand, acclimatization stimulates the body to adjust metabolically by improving cardiovascular performance, activating the renin-angiotensin-aldosterone axis, increasing sweat production and other evaporative cooling routes, expanding plasma volume and improving the ability to cope with heat (Bouchama & Knochel, 2002). According to Harthoorn (1979) wild animals can also become acclimatized and habituated to different environments and procedures in order to reduce the side-effects of capture.

Subsequent to acclimatization, exposure to heat induces an acute phase response, a coordinated reaction involving endothelial cells, leukocytes and epithelial cells that protect against tissue injury and promote repair, and the production of heat shock proteins (Bouchama & Knochel, 2002).

Chronic or extreme exposure to heat results in heat stroke (Bouchama & Knochel, 2002, Bouchama et al., 2005) which is caused by an exaggerated response in the acute phase that leads to an intensified production of heat shock proteins and inflammatory mediators (Bouchama & Knochel, 2002, Bouchama et al., 2005). In rats the intestinal mucosa becomes permeable to endotoxins when core temperatures are above 45°C. In primates that have been heat stressed, a core temperature of only 40°C is necessary to increase the permeability of the gut to endotoxins (Bouchama & Knochel, 2002). Meddings & Swain (2000) demonstrated that not only heat but also physical and psychological stress (like manual restraint or forced swimming) in rats can result in an alteration in the gastrointestinal barrier leading to the endotoxaemia.

Patients with heat stroke also have a strong probability of developing infections (Bouchama & Knochel, 2002; Bouchama et al., 2005; Lambert, 2009; Tong et al., 2013). Furthermore, endothelial cell injury and diffuse micro-vascular thrombosis together with activation of coagulation, often

resulting in disseminated intravascular coagulopathy (DIC), are all changes commonly associated with heat stroke (Bouchama & Knochel 2002).

Whether hyperthermia is the main cause or not, it is the common factor that seems to play a role in several similar diseases that have different aetiologies, such as heat stroke, capture myopathy and exertional myopathy. Other diseases or syndromes where hyperthermia plays a role include malignant hyperthermia (both in humans and animals), exertional heat illness and in porcine stress syndrome. Irrespective of whether it is triggered by stress, anaesthetic drugs, environmental heat load or exercise, excessive and acute hyperthermia, which occurs during capture, most likely provokes pathological metabolic alterations that can have a negative consequence on an animal.

2.2.*3* Malignant Hyperthermia, Porcine Stress Syndrome and Azoturia in Horses

A number of other diseases or syndromes that are well described in the literature may have bearing on capture-induced hyperthermia. Malignant hyperthermia (MH) is a life-threatening skeletal muscle disease which is induced by the exposure to caffeine, volatile anaesthetics and other central nervous system drugs in mammals (Rosenberg *et al*., 2007). In humans, pigs, dogs and horses, malignant hyperthermia has been shown to have a genetic basis, which results from the dysfunction in the coding of the RyR1 gene (Aleman *et al.*, 2004). This gene is involved in the formation of the calcium release channels in the sarcoplasmic reticulum of skeletal muscle fibres (Bhadane *et al*., 2007; Barbut *et al*., 2008; Manea *et al*., 2008). The disruption of this channel leads to an excessive release of calcium into the myoplasm of the muscle cells, resulting in a series of hyper-metabolic events which result in severe hyperthermia and death (Bhadane *et al*., 2007). Similar to humans, horses also suffer from malignant hyperthermia (Equine Malignant Hyperthermia) when they are exposed to volatile anaesthetics such as halothane (Aleman *et al*., 2004). A genetic component in the aetiology has also been described with a special predisposition shown in quarter horses, thoroughbreds, appaloosas, arabs and ponies (Aleman *et al*., 2004; Valberg *et al*., 1999)

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Pigs succumb to a similar disease to malignant hyperthermia known as porcine stress syndrome (PSS). Porcine stress syndrome (PSS) is triggered by a stressful stimuli and it presents with muscle rigidity, severe lactacidosis and hyperthermia and occurs in the absence of administration of any drugs (Bhadane *et al*., 2007; Barbut *et al*., 2007). Since this syndrome shares the same genetic defects as malignant hyperthermia, pigs have become good research model for malignant hyperthermia in humans (Mitchell & Heffron, 1980). It has been shown that porcine stress syndrome and malignant hyperthermia can be induced by similar stimuli resulting in rapid glycolysis, the formation of lactate and severe hyperthermia (Mitchell & Heffron, 1980). Transport, high environmental temperature, exercise, fighting, service and parturition are some of the stimuli that can trigger porcine stress syndrome (Mitchell & Heffron, 1980). Both malignant hyperthermia and porcine stress syndrome cause muscle necrosis and discoloration with a pale and sometimes soft and exudative oedematous appearance (Allen *et al*., 1970; Lawrie, 1960). These macroscopic changes to the muscle are similar to those seen in other animal species like zebra, antelope and birds that develop capture myopathy and therefore Mitchell & Heffron (1980) speculated that there may be a potential stress and genetic predisposition in animals that develop capture myopathy.

2.3 Consequences of Hyperthermia

Hyperthermia has different consequences according to the species, the age, the general state of an animal and environment they are in. In pregnant females, hyperthermia has shown to have teratogenic effect in the foetus (Miller & Ziskin, 1989). According to Edwards and co-workers (Edwards, 1968; Edwards *et al*., 1974) who have studied the effects of hyperthermia in guinea pigs, there is an important reduction in cellular multiplication in the embryo and a rise in only 3°C above the foetus' normal temperature can cause embryonic death. Studies on sheep have shown that hyperthermia during the last two thirds of gestation results in microencephaly and alterations of the white matter of the lamb's brains (Hartley *et al*., 1974). Similar studies have investigated the effects of hyperthermia in pregnant primates and humans and have found that a number of foetal

disorders develop, thus indicating that there is a close relation between foetal abnormalities and hyperthermia (Miller & Ziskin, 1989). With regards to wild antelope, this is particularly important to consider if capture occurs while females are pregnant.

As discussed previously, the heat from hyperthermia has a direct cytotoxic effect on cells and can cause dysfunction or necrosis which ranges from local tissues to extensive organ failure and damage (Haskins, 1995). Severe hyperthermia leads to multiorgan dysfunction and failure: renal failure, liver failure, gastrointestinal failure, disseminated intravascular coagulation, hypoxemia, metabolic acidosis, myocardial failure, skeletal muscle necrosis, tachycardia, hypotension and cerebral oedema (Haskins, 1995).

2.4 Capture myopathy and the consequences of capture.

Several short-term consequences of capture have been described (Harthoorn, 1975; Gericke *et al*., 1978, Kock *et al*., 1987 a & b; Cattet *et al*., 2008, Meyer, 2010). According to Harthoorn (1975), animals that have been exposed to a stressful event will become hyper-susceptible to the effects of additional stress for a period of about two weeks. The short-term (<5 days post-capture) consequences of capture include dehydration, hyperthermia, shock (Shepherd *et al*., 1988), aggression and physical injuries (Shepherd *et al*., 1988; Cattet *et al*., 2008). Depression and loss of appetite also occur and further exacerbate these consequences and thus predispose animals to suffer from capture myopathy.

Very little is known about the long-term (> 30 days post-capture) pathophysiological consequences of a stressful capture, and how this capture affects the future wellbeing of an animal. Cattet *et al.,* (2008) found that after capture, grizzly and black bears had a slower rate of movement than that before capture; normal activity patterns only returned to normal three to six weeks post capture. In this same study, researchers were able to determine the body condition after each capture. They

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found that loss in body condition was a direct effect of the number of captures performed in each animal. This effect they attributed to either a decrease in food intake or an increase in energy used for healing injured tissues, or a combination of both (Cattet *et al*., 2008). These researchers were also able to provide strong evidence that capture and handling of bears caused significant muscle damage which was evident for more than 30 days after capture. They found that 6% of grizzly bears and 18 % of black bears captured by leghold snares developed capture myopathy (Cattet *et al*., 2008). Other negative long-term effects that they found were alterations in reproduction and a decrease in lean body growth, especially in bears captured several times (Cattet *et al*., 2008).

Capture myopathy is a multi-causal and multi-factorial syndrome that results in multiple organ dysfunction in wild animals. It is induced mainly by external stimuli that cause distress and over exertion. According to Harthoorn (1976), Spraker (1993) and Mann (1996) there are four different general categories of shock that can influence capture myopathy. These are cardiac shock (decompensated cardiac failure), hypovolemic or haemorrhagic shock, anaphylactic or septic shock and neurogenic or psychogenic shock (Mann & Helmick, 1996). All four categories can occur singly or in combination in the same animal (Mann & Helmick, 1996). There are different situations in the capture procedure that can be associated with each form of shock. The hyper-acute phase of capture myopathy described by Harthoorn (1976) (described as capture shock syndrome by Spraker, 1993) has similar pathogenic features to those of neurogenic or psychogenic shock. It is initiated by many intense stress-producing factors which results in continuous and extreme adrenergic stimulation which ends up exhausting the animal. This exhaustion together with the influence of hyperthermia results in the loss of an individual's ability to maintain its internal balance (Spraker, 1993). At this point, blood pressure falls, tissue hypoxia occurs and consequently carbonic acid and lactic acid levels increase and accumulate in the circulatory system (Mann & Helmick, 1996). This acidity, combined with hyperthermia and oxidative stress products can induce disseminated intravascular coagulation (Lamb & Stephenson, 2006; Bangsbo & Juel, 2006; Haskins, 1995). With the onset of hypoxia, cellular deterioration and hyperpermeability of cells occurs and electrolyte shifts take place (Lambert, 2009). Death can

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result suddenly due to circulatory collapse and cardiac arrest. This hyper-acute phase or capture shock syndrome can occur within minutes to six hours post-capture.

According to Harthoorn (1976), a second acute phase of myopathy can be induced between six to 48 hours after capture and it corresponds to the "ataxic myoglobinuric syndrome" of capture myopathy described by Spraker (1993). This phase is characterized by renal failure, azotaemia and acidosis (Spraker, 1993). At this point animals show clear clinical signs of renal failure and muscle necrosis. The kidneys suffer from hypoxia due to vasoconstriction caused by the release of catecholamines and the effects of excessive myoglobin released from necrotic muscles. The hypoxia causes tubular necrosis and renal damage (Harthoorn, 1976; Spraker, 1993). Muscle damage occurs and is believed to be a result of hypoxia and hyperthermia, and is often associated with severe muscle acidosis and acidaemia (Harthoorn, 1976; Spraker, 1993). Acidaemia, cellular deterioration due to hypoxia and blood stagnation together with electrolyte imbalance often result in renal damage which can lead to death (Harthoorn, 1976; Spraker, 1993).

If animals survive the acute phase of myopathy the lesions in the muscle often progress to a point that extensive areas of necrosis occur (Spraker, 1993). The weight of the animal causes the muscle fibres to rupture. Often the gastrocnemius, semimembranosus, semitendinosus and middle and deep gluteal muscles are ruptured, showing a marked drop of the hindquarters and hyperflexion of the hock, resulting in severe lameness (Spraker, 1993). This stage of capture myopathy is described by Spraker (1993) as the "ruptured muscle syndrome" and corresponds to certain aspects of the sub-acute phase syndrome of capture myopathy described by Harthoorn (1976).

Animals that are not affected by any of the stages or phases described above may still die a number of days or weeks after capture. This stage is described by Spraker (1993) as the "delayed

– peracute syndrome" and by Harthoorn (1996) as a "chronic state" of capture myopathy and occurs when an animal is moderately stressed and exerted at first capture, but death, most likely due to heart failure, only occurs when the animal is exposed briefly to another stressful event or capture (Spraker, 1993; Harthoorn, 1995; Williams & Thorne, 1996).

Chapter 3

Materials and methods

3.1 Model system and justification of the model

3.1. a Study site and animals

A group of 42 blesbok *(Damaliscus pygargus phillipsi)* consisting of males and females, both adult and sub-adult animals, were used in this study (Table 1). These animals were captured, using a mass capture technique, at Voortrekker Monument, Pretoria, Gauteng (25.7764° S, 28.1758° E) by a certified game capture team. The whole group of animals was slowly herded by a helicopter, at a walking pace, into a funnel shaped temporary boma (Fig. 3.1), build out of sail sheeting (Fig. 3.2) that guided them to a loading ramp which led into a customised game capture transport truck (Fig 3.3).

Figure: 3.1 Aerial view of the temporary mass capture funnel.   Figure: 3.2 Close view of the

plastic funnel set up. Boma enclosure with closing curtains.

Figure: 3.3 Truck and loading ramp used for the mass capture at Voortrekker Monument.

Once the blesbok got to the loading ramp they were confined in the funnel shaped boma and then

were hand restrained, injected with the tranquilizing drug haloperidol, 5 mg per animal (IM

Haloperidol, Kyron, RSA) and their horns were covered with PVC piping before the animals were

loaded into the truck. The animals were transported in two different groups (twenty one each time)

in order to avoid overcrowding and the consequent stress response. Once in the truck, the animals

travelled for 30 minutes until they were off-loaded into bomas at Groenkloof Nature Reserve, Pretoria, Gauteng (25.7934° S, 28.2039° E).

The bomas that housed the animals at Groenkloof Nature Reserve were custom designed for this specific study and located at a 1300 meters above sea level. They consisted of ten small enclosures of 5 m x 5 m and were built out of wooden pole walls held up by wire meshing and support poling (Figure 3.4). These enclosures were suitable to house the blesbok in groups of four to five animals each. Both males, females and subadults were allocated to form these groups and adult males were not mixed together. Allocating the animals into groups of four or five per boma allowed better control over the management of the animals in the boma and aided in the darting, restraint and data collection procedures (Table 1). These smaller groups were arranged to form three larger study groups in a way that age and sex of the animals were approximately balanced across these three study groups.

All animals were given 6 weeks of boma adaptation before data collection for the study started. During the adaptation period and during the study the animals were fed hay and lucerne and were provided with water *ad libitum*. Each animal also received 150g of antelope cubes (Voermol Game Pellets, Maidstone, RSA) per day. Bomas and water troughs were cleaned once a week to avoid any possible hygiene or management problems. At the end of the 6 week boma adaptation period, only 38 animals were in a body condition suitable to include in the study; four animals that were in poor body condition were removed from the study. All animal's body conditions were evaluated according to body condition scoring system for goats (Villaquiran *et al.,* 2005).
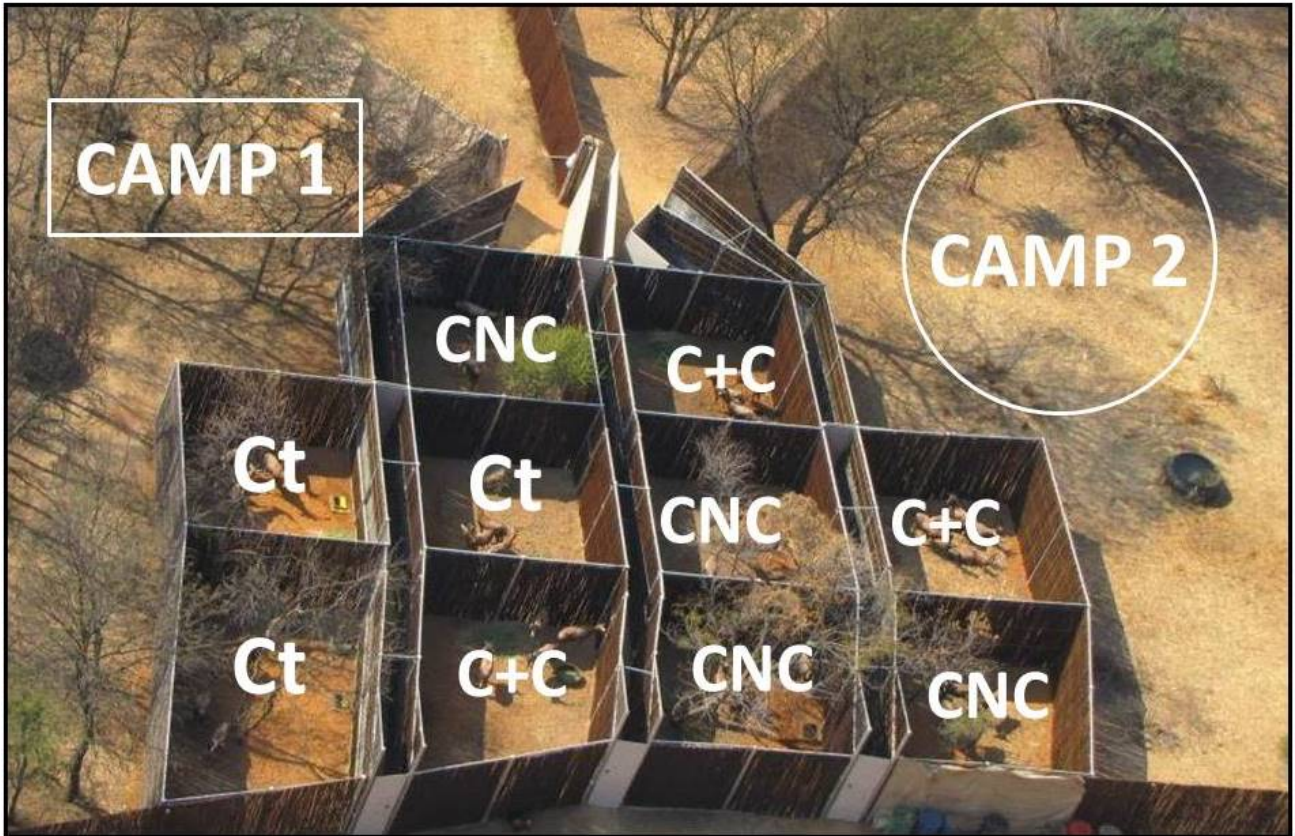
Figure 3.4 Boma layout at Groenkloof Nature Reserve, Pretoria, Gauteng (25.7934° S, 28.2039° E) showing how the animals were housed and arranged into the different study groups including a Control group (Ct), a Chase plus Cooling group (C+C) and a Chase No Cooling group (CNC). Camp # 2, which was part of the larger boma complex, was connected to the holding enclosures through the corridors and gates and was where the animals were chased for 15 minutes.

Table 1. Sex and age distribution of the 42 captured blesbok in the different study groups and boma compartments.

GROUP	BOMA	ANIMAL ID	SEX	AGE	WEIGHT
CONTROL (Ct)	# 1	B1R1	M	SUB-ADULT -2 TOOTH	62KG
CONTROL (Ct)	# 1	B1B1	M	ADULT - FULL MOUTH	69 KG
CONTROL (Ct)	# 1	B1W1	F	ADULT - 5/6 YEARS	70 KG
CONTROL (Ct)	# 1	B1O1	M	ADULT - 4 TOOTH	68 KG
CONTROL (Ct)	# 2	B2B2	F	-------	65 KG
CONTROL (Ct)	# 2	B2O2	M	ADULT - 4 TOOTH	65 KG
CONTROL (Ct)	# 2	B2R2	M	ADULT – 4 TOOTH	76 KG
CONTROL (Ct)	# 2	B2W2	F	ADULT - 3-4 YEARS	69 KG
CONTROL (Ct)*	# 2	B2Y2		SUB-ADULT- Removed	-----
CONTROL (Ct)	# 4	B4B4	F	ADULT - FULL MOUTH	66 KG
CONTROL (Ct)	# 4	B4O4	F	ADULT - FULL MOUTH	64 KG
CONTROL (Ct)	# 4	B4R4	M	SUB-ADULT - 2 TOOTH	61 KG
CONTROL (Ct)	# 4	B4W4	F	SUB-ADULT - 1 YEAR	43 KG
CHASE NON COOLIG (CNC)	# 6	B6B6	M	ADULT – 8 TOOTH	67 KG
CHASE NON COOLIG (CNC)	# 6	B6O6	F	ADULT - FULL MOUTH	61 KG
CHASE NON COOLIG (CNC)	# 6	B6R6	M	SUB- ADULT	40 KG
CHASE NON COOLIG (CNC)	# 6	B6W6	F	SUB-ADULT - 1 YEAR	39 KG
CHASE NON COOLIG (CNC)	# 3	B3B3	F	ADULT – 8 TOOTH	70 KG
CHASE NON COOLIG (CNC)	# 3	B3O3	M	SUB-ADULT – 2 TOOTH	46 KG
CHASE NON COOLIG (CNC)	# 3	B3W3	F	ADULT – 8 TOOTH	77 KG
CHASE NON COOLIG (CNC)*	# 3	B3R3		ADULT – Removed	-----
CHASE NON COOLIG (CNC)	# 5	B5B5	M	ADULT – 8 TOOTH	82 KG
CHASE NON COOLIG (CNC)	# 5	B5O5	M	SUB-ADULT – 2 TOOTH	42 KG
CHASE NON COOLIG (CNC)	# 5	B5R5	M	ADULT – 4 TOOTH	60 KG
CHASE NON COOLIG (CNC)*	# 5	B5W5		ADULT –Removed	-----
CHASE NON COOLIG (CNC)	# 7	B7B7	F	ADULT – 8 TOOTH	70 KG
CHASE NON COOLIG (CNC)	# 7	B7O7	M	ADULT – 8 TOOTH	73 KG
CHASE NON COOLIG (CNC)	# 7	B7R7	M	ADULT – 8 TOOTH	85 KG
CHASE NON COOLIG (CNC)*	# 7	B7W7		SUB-ADULT – Removed	----
CHASE PLUS COOLING (C+C)	# 8	B8B8	M	SUB-ADULT - 2 TOOTH	61 KG
CHASE PLUS COOLING (C+C)	# 8	B8O8	F	ADULT – 8 TOOTH	70 KG
CHASE PLUS COOLING (C+C)	# 8	B8R8	M	ADULT – 8 TOOTH	73 KG
CHASE PLUS COOLING (C+C)	# 8	B8W8	F	ADULT – 8 TOOTH	74 KG
CHASE PLUS COOLING (C+C)	# 9	B9O9	F	ADULT – 8 TOOTH	63 KG
CHASE PLUS COOLING (C+C)	# 9	B9B9	M	ADULT – 8 TOOTH	74 KG
CHASE PLUS COOLING (C+C)	# 9	B9R9	M	ADULT – 8 TOOTH	71 KG
CHASE PLUS COOLING (C+C)	# 9	B9W9	M	SUB-ADULT - 4 TOOTH	41 KG
CHASE PLUS COOLING (C+C) ●	# 10	B10B10	F	ADULT – Data removed	62 KG
CHASE PLUS COOLING (C+C) ●	# 10	B10O10	M	SUB-ADULT- Data removed	58KG
CHASE PLUS COOLING (C+C)	# 10	B10R10	M	ADULT – 8 TOOTH	81 KG
CHASE PLUS COOLING (C+C)	# 10	B10W10	F	SUB-ADULT - 4 TOOTH	40 KG
CHASE PLUS COOLING (C+C) ●	# 10	B10Y10	M	SUB-ADULT-Data removed	65 KG

* Animals not included in the study (red) because of poor body condition before the trials commenced. ● Three animal's data from group C+C were removed because of confounding effects that occurred before capture.

3.1. *b* Experimental design

This study was an unpaired comparative interventional study.

Forty two blesbok were captured from the wild and housed in bomas for a 6 week period of adaptation. Only 38 animals, that adapted best to the bomas, were used in the study.

These 38 animals were randomly selected and allocated to three different groups (Ct, CNC & C+C). One of these groups acted as a Control group (Ct) while the other two groups (CNC & C+C) were subject to different experimental interventions.

Each animal in the study groups was captured four times: capture at day 0 (experimental interventions and data collection) and on day 2, 16 and 30 for data collection only. Captures on day 2, day 16 and day 30 were performed in a way that minimal stress was induced in the animals, minimizing the effects of capture in the animals so that the data collected at these times would mainly reflect the effects of the capture that occurred on day 0 rather than the effects of the capture at these times.

At day 0, Group C+C (Chase plus cooling) was chased through the corridor and around the camp for 15 minutes continuously by being exposed to different sonorous stimuli. Immediately after the chase the blesbok were pushed back into their housing bomas where they were simultaneously darted (with etorphine and azaperone) and kept immobilized for 40 minutes. Samples were taken twice on the interventional day, directly after they became recumbent (Day 0-1/2) and just prior to reversal of the immobilization (Day 0-2/2). One set of samples were taken on day 2, day 16 and day 30.

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These animals were cooled by dousing them with 10 litres of cold water at 4°C for 10 minutes between taking the two sets of samples.

Group CNC (Chase non-cooling) was chased and immobilized in the same way as group C+C. Data collection took place in the same way as for group C+C but this group was not doused with cold water and therefore they remained hyperthermic during the 40 minutes of immobilization.

To try and keep the Control group calm (Group Ct) before darting they received diazepam (approximately 0.2 mg/kg) , mixed in maize meal in the antelope cubes, two hours before they were darted. They were not chased or disturbed before they were captured by this darting. The capture was done in a manner to create the smallest stress response possible. The animals were darted simultaneously with the same drug combination as for group C+C and CNC and the immobilization procedure lasted for 40 minutes. Data collection took place in the same way as in group C+C & CNC but this group was not doused with cold water.

In all three of the Groups captures were repeated on day 2, day 16 and day 30 using the same capture method used initially in group Ct (pre-tranquilization with darting only). Blood, urine and muscle biopsy samples were collected during these immobilization procedures.

During the capture on day 16th, after samples were taken, half of the animals (five from each group) were selected for euthanasia. These animals were euthanized with sodium pentobarbital to perform a full post mortem examination and to collect organ specimens for histopathological and biochemical analysis.

On day 30, the remaining antelope were captured for sample collection as described above. These animals were then euthanized following the immobilization procedure for post mortem examination and specimen collection.

3.2 Experimental procedures

3.2.1 Animal capture

3.2.1. a Experimental intervention and data collection capture (Day 0)

- <u>Chase animals that were cooled (C+C Group n = 10)</u>

Blesbok were herded through the enclosure gates into a boma corridor and then into camp # 2 (Figure 3.4), a bigger boma enclosure (30 x 30 m) within the 120 x 110 m main boma complex. Once in this camp, they were chased continuously by being exposed to sonorous stimuli (clapping hands, shouting, hissing and whistling) for 15 minutes. After that period, they were herded back into their housing boma and immobilized with the immobilizing drugs etorphine hydrochloride (3 mg for sub-adults, 3.5 mg for adults, Captivon, Wildlife Pharmaceuticals, South Africa) and azaperone (40 mg for sub-adults, 60 mg for adults, Kyron, Johannesburg, South Africa) delivered via a projectile dart (3 ml syringe, needle 1.5 x 25 mm with collar, Dan-Inject International RSA). Once all the animals in the boma were recumbent, they were placed in a sternal position with the vertebrae and neck aligned, the head held up and the muzzle facing down to allow ruminal gas to be released and to prevent bloat. A blindfold was placed over the animal's face and cotton-wool was used as ear plugs to limit external stimuli during the immobilization. The animal's vital signs including rectal temperature, pulse and respiratory rate were monitored throughout the immobilization.

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Directly after the animals became immobilized, the first 0.5 ml of arterial blood from the medial auricular artery was drawn in a 1 ml heparinised syringe for blood gas determination. Simultaneously, venous blood from the cephalic vein was drawn for haematology (one tube of 10 ml EDTA tube) and for biochemistry and endocrine concentration determinations (four tubes of 10 ml serum blood tubes). All serum blood samples were centrifuged and aliquoted into cryovials and placed in a freezer on site. These venous blood samples were transported in a freezer to the Faculty of Veterinary Science, University of Pretoria's Clinical Pathology Department where they were analysed.

Urine samples were collected by catheterization in female animals only. Cystocentesis and catheterization in males was not feasible due to the deep position of the bladder and the sigmoid shape of the penis. Urine samples were frozen at - 80°C and stored for later determination of markers for nephron damage, myoglobinuria and haemoglobinuria.

Muscle biopsy samples were taken using a biopsy needle (Bergstrom biopsy needle, size 6 mm, Dixons Surgical Instruments Ltd, UK) from the *Vastus lateralis* muscle. Before sampling, the skin above the biopsy site was shaved and disinfected with chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa) and 1 ml of local anaesthetic (Lignocaine injection 2%, Centaur Labs, Johannesburg, South Africa) was injected subcutaneously. A small skin incision was made before inserting the biopsy needle. Two muscle samples were taken from this site. One set of samples was placed in cryovials for storage in liquid nitrogen tubes at –192 °C for muscle biochemistry analysis later (data not presented in this dissertation). The second sample was fixed in a standard 10% buffered formalin solution (Bancroft & Cook, 2000) for future histopathological examination. The skin incision was treated with disinfectant before it was glued (Loctite, China) and sprayed with a topical disinfectant (Supona Aerosol 400 #, Chlorfenvinphos 0.5% m/m, dichlorphos 0.83% m/m and gentian violet 0.15 m/m, Zoetis, South Africa) to help the healing process and prevent miasis.

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Although urine and muscle samples were collected, the analysis and results from these are not reported in this dissertation for the sake of brevity.

The antelope from this study group were then cooled by dousing them with 10 litres of cold water at 4°C for a period of 10 minutes. After 10 minutes of water dousing and rubbing the water into the animal's hair, a second set of arterial and venous blood sample were taken in the same way as the first samples. These samples were taken at 40 minutes after the animals became recumbent. Once this procedure was finished the immobilization was reversed by using naltrexone (1.2 ml total IM, Trexinol, Wildlife Pharmaceuticals, South Africa) which antagonised etorphine's immobilizing effects. The animals were released in their housing bomas and were closely monitored until they had fully recovered from the immobilization.

-Chase non-cooled animals (CNC group n = 13)

This group was also chased, immobilized, sampled and reversed in the same way as the C+C group. All data and sample collection, clinical monitoring, blood, urine and muscles samples were also taken in the same way and at the same time points during the immobilization as in the C+C group. However, these animals were not cooled by dousing them with cold water.

-Control group animals (Ct group n = 12)

This group was tranquilized using in-feed diazepam dosed approximately at 0.2 mg/kg (Kock & Burroughs, 2012) per os (diazepam powder 10 mg in 1 kg of maize meal, V-Tech, Johannesburg, South Africa) fed to them with antelope cubes 2 h before they were darted. The in-feed tranquilization was to ensure that the animal's stress response to darting was kept to a minimum. This group was not chased and was not cooled with water during the immobilization. Chemical

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immobilization was achieved by darting the animals in their housing enclosure using the same drug combination and dose as used in the other groups. Reversal of the immobilization was also done in a similar way at 40 minutes after the start of immobilization.

All data and sample collection, clinical monitoring, blood, urine and muscles samples were also taken in the same way as for the C+C and CNC groups.

In this first immobilization numbered and coloured ear tags, for identification of each animal, were placed in both ears.

3.2.1. b Capture for data collection: Day 2, 16 and 30

The subsequent immobilizations and procedures were done in exactly the same way as for the Control group on day 0. To minimise the stress response at each of these captures, the animals were tranquilized with in-feed diazepam using the same procedure as done for the Control group. Once tranquilized, they were chemically immobilized via darting with etorphine and azaperone in their holding enclosures.

On day 2, 16 and 30, only one sample of arterial (1 mL heparinised syringe) and venous blood (one tube of 10 ml EDTA tube and four tubes of 10 mL red serum blood tubes) were taken as soon as the antelope were recumbent. Urine samples and muscle biopsies (alternating on the opposite side of the animal to the previous capture) were taken in the same way and with the same standard procedures as on the interventional day (day 0). Two minutes after the samples were collected, the effects of the immobilization were reversed and the animals were monitored until fully recovered in their housing enclosures.

On day 16, after data collection and sampling, approximately half of the animals (19 animals in total) from each study group were randomly selected and euthanased with sodium pentobarbital. A full post-mortem examination and organ specimens were collected for histopathological and biochemical analysis by veterinary specialist pathologist (data not shown in this dissertation).

On day 30, the remaining animals were captured, data collected, sampled, euthanased and subject to full post-mortem examination and specimen collection in the same way as done on the other animals on day 16.

3.2.2 Data and sample collection

General clinical monitoring

General monitoring of animal's vital signs and depth of immobilization took place whenever the animals were immobilized. Respiratory rate, counted as breaths per minute, was measured by placing a hand at the nostril of the antelope to feel the air movement together with chest movement observations. Pulse was evaluated by palpating the auricular artery.

General body condition was determined using an ovine body condition scoring system and each animal was weighed at each capture using a hanging manual scale that was connected to a stretcher that the animals were placed on.

Body temperature measurements

Rectal and muscle temperature readings were recorded every 5 minutes during the immobilization. Rectal temperature was measured by using a thermocouple probe (ESO-1 Oesophageal,

Physitemp Instruments inc, NJ, USA) connected to a digital thermometer (BAT-12, Physitemp Instruments, Clifton, NJ, USA) that was inserted 7 cm in the animal's rectum. Muscle temperature was measured with a fine thermocouple probe (IT-18, Physitemp Instruments, Clifton, NJ, USA) which was also connected to the digital thermometer (BAT-12, Physitemp instruments, Clifton, NJ, USA). This probe was inserted sterilely 20 mm into the gluteus muscle of the animals through an 18 G needle.

Environmental conditions

During each trial, environmental conditions were measured using a portable weather station (Kestrel, Nielsen – Kellerman Co., PA, USA). Recorded data included ambient temperature, relative humidity, black globe temperature, Wet Bulb Globe Temperature, Wet Bulb temperature, Wet Bulb natural temperature, barometric pressure, altitude and wind speed.

Arterial blood samples

Arterial blood (0.5 ml) was drawn into a 1ml heparinised syringe from the median auricular artery for determining blood gases, acid-base and electrolytes. The samples were immediately placed on ice and analysed within 10 min of sampling using EPOC™ BGEM blood test cards and an EPOC Reader (Epocal, Inc. Ottawa, Ontario, Canada).

The following variables were determined or calculated by the reader: blood pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), calculated bicarbonate ($cHCO_3$-, not reported on), base excess (BE, not reported on), calculated oxygen haemoglobin saturation (cSO_2, not reported on), haematocrit (Htc, not reported on) and calculated haemoglobin (cHgb, not reported on). The reader also determined the following electrolyte concentrations; sodium (Na^+, not

reported on), potassium (K$^+$, not reported on), and calcium (Ca^{++}, not reported on), and other metabolic variables such as lactate and glucose concentrations.

Venous blood samples:

Four 10 mL red serum tubes for serum collection were filled with blood from the cephalic vein for biochemistry and cortisol determination. These samples were left to clot for 30 minutes and then centrifuged at 2000g for 8 minutes to separate the red cells from the serum. Volumes of 0.5 - 2.0 mL of serum were aliquoted into 2 mL cryo vials with screw tops and then snap-frozen on site in a liquid nitrogen container in the field. Within the same day, these samples were transferred into a -80°C freezer in the Clinical Pathology Laboratory at the Onderstepoort Veterinary Academic Hospital. For the biochemistry determination a Cobas Integra 400 plus device (Roche, Johannesburg, South Africa) was used and for hormone determination an Immulite 100 device (Siemens, Johannesburg, South Africa) was used. The concentrations of the following variables were determined:

- Biochemistry enzyme determination: gama-glutamil transferase (GGT), glutamate dehydrogenase (GLDH), aspartate transaminase (AST), creatine kinase (CK), urea (BUN), creatinine (CREA).
- Endocrine determination: cortisol.
- Cardiac enzyme determination: cardiac troponin-I.

Although blood for haematology, urine samples and muscle biopsies were collected, analysis of these samples, and other variables highlighted above, were not performed for this dissertation.

3.3 Statistical analysis

Data was analysed and compared using Graph Pad Prism program version 6.01. Due to the small sample size it was not possible to test the data for normality so the data was treated as parametric or non-parametric based on an estimated data distribution of a variable in a normal antelope population. For non-parametric data (cardiac troponin I, lactate, CK and GLDH), the data was log10 transformed. All measured variables, and log transformed data were compared over time in each group and between the groups using a two–way analysis of variance (two-way ANOVA). A post-hoc Tukey's multiple comparison test identified where the differences were between the three groups, and a post-hoc Dunnett's multiple comparison test for comparison within each group was used to identify where differences occurred compared to the first sample. In all tests a p-value below 0.05 was considered significant.

Transformed data was used to calculate mean and standard deviations and this data was transformed back so that mean and standard deviations of the actual data could be depicted in the figures.

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Chapter 4

Results

4.1 Environmental conditions at chase and immobilization

The study was done in August and September of 2014, winter time in the Southern hemisphere. During the different immobilization procedures, environmental conditions varied according to the month and the time of the day. During the first chase and immobilization procedure (28-August) ambient temperature ranged from 16.9°C in the early morning to 27.1°C at noon. Relative humidity ranged from 10% to 25% while wet bulb globe temperature (WBGT) ranged from 12.6°C in the early morning to 19.8°C at midday.

On day 30 (23 Sept, 2014) minimum air temperature was 17.2°C with a maximum of 28.7°C, relative humidity ranged from 30% to almost 70% and WBGT ranged between 16°C and 22°C.

On day 16 (9 Sept, 2014) was the hottest day; air temperatures ranged between 20°C and 31.8°C, relative humidity between 20% and 36% and WBGT between 14.5C° and 24.9°C. The coldest temperature recorded during the study was on Day 2 where air temperature ranged between 3.8°C in the early morning up to a maximum of only 17°C in the afternoon and relative humidity ranged from 30% to almost 70% and WBGT ranged between 16°C and 22°C. The altitude of Groenkloof Nature Reserve is 1300 meters above sea level.

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4.2. Capture

All chased groups were darted immediately after the 15 minute chase once they were back in their holding bomas. After darting it took on average 3 to 6 minutes until all the animals in a boma compartment became recumbent. The immobilization lasted for approximately 40 minutes after the animals became recumbent. The drug combination used was adequate to maintain the immobilization without complications over this time; no animals required cardiopulmonary support or resuscitation.

We observed that three animals (B10O10, B10B10 and B10Y10) from boma compartment # 10 (from the C+C group, Fig 3.4) were disturbed while other animals were being chased in camp # 2 which was right next to this boma compartment. These animals showed signs of excessive pacing, running and were seen jumping against the boma compartment wall. All other animals in the boma complex were calm. Even though the jumping and excitement was obvious, there were no signs of physical injury to the animals at the time, but after blood analysis, CK concentrations from the first sample (Day 0-1/2) taken directly after darting were abnormally elevated in these animals (B10O10 = 8788 $IU.l^{-1}$, B10B10 = 4090 $IU.l^{-1}$ and B10Y10 = 3634 $IU.l^{-1}$) compared to mean concentrations in animals in the other groups (Ct group = 135.42 ± 46.53 $IU.^{-1}$, CNC group = 481.86 ± 296.87 $IU.^{-1}$). CK concentrations in these three animals were 64, 30 and 26 times more than mean concentrations of the animals from the Ct group and 18, 8.5 and 7.5 times increased compared to those of the CNC group. Because abnormally high CK concentrations are indicative of direct muscle damage, we believed that these three animals had muscle damage from jumping against the boma walls. We therefore removed their data from the study to minimise any bias created by this damage.

4.3 Effects of capture and cooling on rectal and muscle temperatures

The mean rectal temperature for blesbok in the Ct group on Day 0-1/2 was 38.1 ± 0.7°C and was 3.8 to °C ($F_{2, 157}$ = 40.79, p < 0.0001, Fig. 4.1 A) lower than animals that were chased and then immobilized in the CNC (41.9 ± 0.7°C) and the C+C groups (41.9 ± 0.9°C). The rectal temperature of the animals in these treatment groups (CNC and C+C) did not differ at this sampling time (p > 0.999, Fig. 4.1 A).The rectal temperature of the Ct group was the same throughout, with the exception of Day 16 where it was higher (p = 0.042).

After 40 minutes into the immobilization procedure (Day 0-2/2), the rectal temperature in the Ct group was the same compared to that at the beginning of the immobilization (p > 0.886, Fig. 4.1 A). In the CNC group the rectal temperature decreased (p < 0.0001) to 40.4 ± 1.3°C which was still higher compared to that of the Ct Group (p < 0.0001) at this time. Dousing the animals with cold water reduced the rectal temperature of blesbok (sample Day 0-2/2) in the C+C group to 37.9°C; ± 1°C (p < 0.0001), a level similar to the animals in the Ct group (p =0.520), and significantly lower than the CNC group (p < 0.0001, Fig 4.1 A). The rectal temperature of both treated groups (CNC and C+C) differed from each other at this same sampling point (p < 0.0001) (Fig. 4.1 A).

Rectal temperatures measured during immobilization on the data collections days (Day 2, 16 and 30, Fig 4.1A) were no different between the treatment groups (CNC and C+C) and the Ct group (p > 0.05) and were on average 37.8 ± 0.2°C at Day 2, 38.7 ± 0.3°C at Day 16 and 38.4 ± 0.2°C at Day 30.

Muscle temperature changes followed a similar pattern to rectal temperatures, but were on average one degree higher than rectal temperature. On Day 0 (sample Day 0-1/2) the Ct group had mean muscle temperatures of 38.9°C ± 0.7°C (Fig 4.1 B) on average (±) 4°C lower than the CNC (42.7 ± 0.8°C; p < 0.0001) and C+C (43.2 ± 0.7 °C; p < 0.0001) groups.

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After 40 minutes of immobilization, the muscle temperature in the Ct group (sample Day 0-2/2) was similar to the initial temperature recorded at the first sample (Day 0-1/2, $p = 0.019$; Fig 4.1 B). In the CNC group the muscle temperature decreased ($p < 0.0001$) to $40.8 \pm 0.7°C$ which was still higher compared to that of the Ct group ($F_{(2, 142)} = 36.42$; $p < 0.0001$) at this time. Dousing with cold water reduced the muscle temperatures (sample Day 0-2/2) of the animals in the C+C group by 5°C to $37.9 \pm 1.7°C$ ($p < 0.0001$), a similar temperature to that of the animals in the Ct group ($p < 0.0001$), but significantly lower than the CNC group ($p < 0.0001$, Fig 4.1 B).

The muscle temperatures measured during immobilization on the following data collections days (Day 2, 16 and 30, Fig 4.1 B) were no different between the groups that were chased (CNC and C+C) and the Ct group ($p > 0.05$) and were on average $38.7 \pm 0.2°C$ at Day 2, $39.4 \pm 0.2°C$ at Day 16 and $39.3 \pm 0.1°C$ at Day 30.
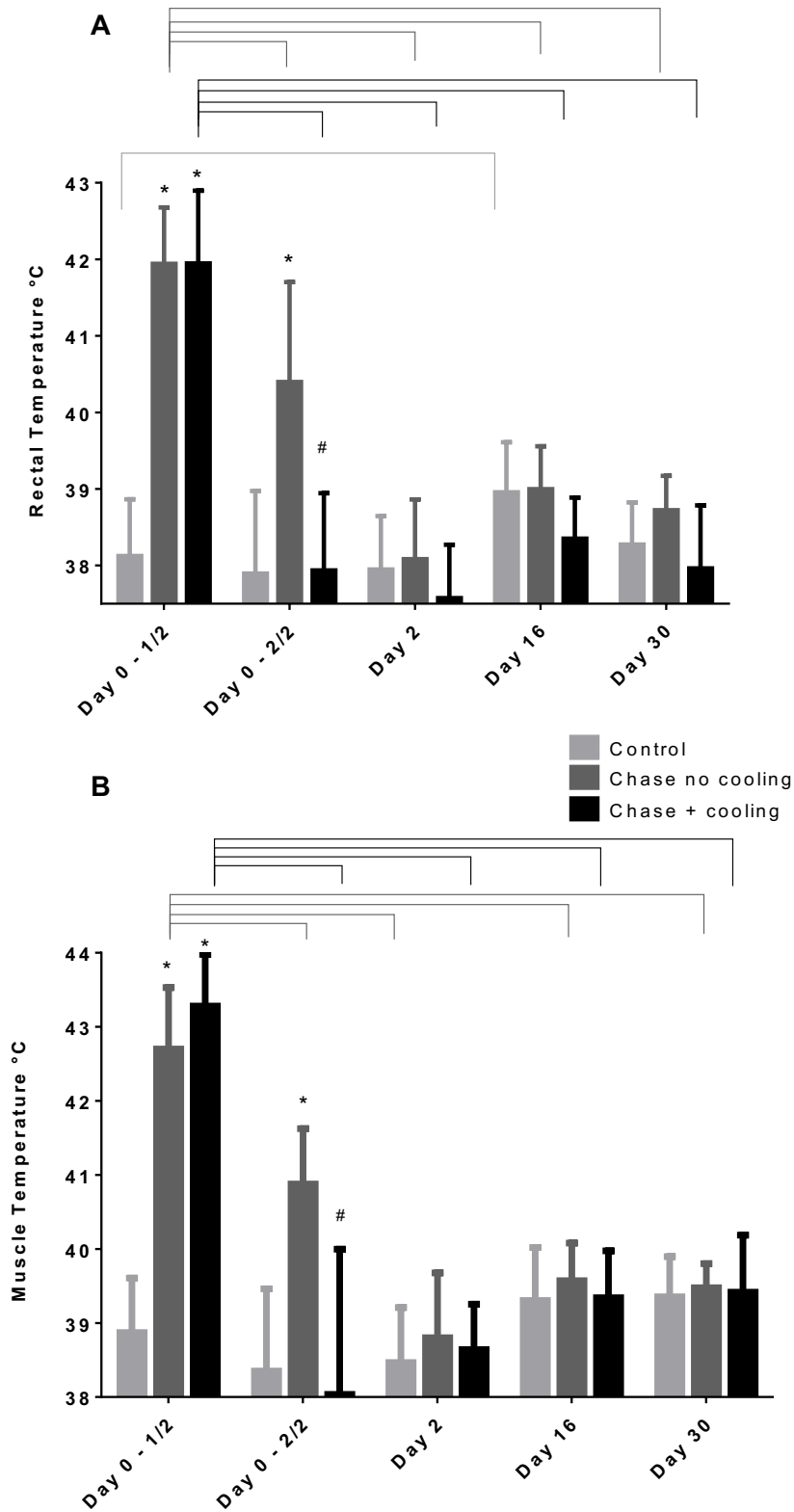
Figure 4.1 Rectal (A) and muscle (B) temperatures (mean ± SD) of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the rectal temperatures in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct),

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and the # indicates where the rectal temperatures in the Chase non-Cooling and Chase plus Cooling groups were significantly different to each other. The brackets above the bars represent significant differences between the temperatures measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days

4.4 Effects of capture and cooling on arterial blood gases

Using the alveolar gas equation (Fahlman *et al.*, 2012) the expected PaO_2 in the blesbok at the study site (an altitude of 1300 m) would be on average 76.6 mmHg (Fig. 4.2 A).

 At all the sampling time points, the PaO_2 of the animals in the three treatment groups were below this expected PaO_2 and there were no differences between the groups at any of these times ($F_{(2, 157)}$ = 2.363; p = 0.097, Fig. 4.2 A).

There were no differences in the PaO_2 during immobilization of the animals in the Ct group when comparing the first sample (PaO_2 = 48.73 ± 8.26 mmHg, Day 0-1/2) to samples taken at Day 0-2/2 (PaO_2 = 52.13 ± 8.12 mmHg, $F_{(4, 49)}$ = 8.064; p = 0.827), Day 2 (PaO_2 = 42.79 ± 6.81, p =0.218), Day 16 (PaO_2 = 42.81 ± 6.18, p = 0.257) and Day 30 (PaO_2 = 45.81 ± 4.6 mmHg, p = 0.890) (Fig 4.2 A). When the animals in the CNC group were immobilized, they had higher PaO_2 values on Day 0-1/2 (PaO_2 = 52.95 ± 8.83 mmHg) compared to Day 2 (PaO_2 = 40.72 ± 5.65 mmHg, p =0.0005), Day 16 (PaO_2 = 39.81 ± 4.93 mmHg, p < 0.0001) and day 30 (PaO_2 = 39.0 ± 7.08 mmHg, p = 0.0004) (Fig 4.2 A). When animals in the C+C group were immobilized, their PaO_2 at the first sampling time (PaO_2 = 51.53 ± 11.76 mmHg, Day 0-1/2) was only higher (p < 0.0001) compared to Day 2 (PaO_2 = 40.23 ± 6.7 mmHg, p = 0,035, Fig. 4.2A).

The $PaCO_2$ values from the immobilized animals were only different between the groups at the first and second sample (Day 0-1/2 and Day 0-2/2); animals in the CNC (Day 0-1/2 = 28.38 ± 3.99 mmHg, Day 0-2/2 = 35.25 ± 5.22 mmHg) and C+C (Day 0-1/2 = 27.07 ± 5.99 mmHg, Day 0-2/2 = 39.61 ± 4.70 mmHg) groups had significantly lower $PaCO_2$ values compared to animals in the Ct group (Day 0-1/2 = 46.24 ± 4.95 mmHg, Day 0-2/2 = 49.86 ± 5.76 mmHg) ($F_{(2, 144)}$ = 33.03; p < 0.0001).

On the first day (Day 0, samples 1/2 and 2/2), animals in both chased groups, CNC (Day 0-1/2 = 28.38 ± 3.99, Day 0-2/2 = 35.25 ± 5.22 mmHg) and C+C (Day 0-1/2 = 27.07 ± 5.99 mmHg, Day 0-2/2 = 39.61 ± 4.70 mmHg) had similar $PaCO_2$ values (Day 0-1/2, p=0.265 and Day 0-2/2, p=0.267, Fig 4.2 B). In the Ct group, there was no difference in $PaCO_2$ over time (sample Day 0 vs. Day 2, 16 and 30, ($F_{(4, 49)}$ = 1.9, p = 0.125). $PaCO_2$ values in the CNC (28.38± ± 3.99 mmHg) and the C+C (27.07 ± 5.99 mmHg) group were lower at the first sample collection (Day 0-1/2) compared to samples taken on Day 2 (CNC = 49.34 ± 3.48 mmHg, C+C = 50.03 ± 5.10 mmHg), Day 16 (CNC = 46.42 ± 3.14 mmHg, C+C = 44.81 ± 5.24 mmHg), and Day 30 (CNC = 47.51 ± 3.57 mmHg, C+C = 45.07 ± 4.74 mmHg) ($F_{(4, 144)}$ = 59.16, p < 0.0001).

Cooling did not have any effect on PaO_2 and $PaCO_2$ in the animals in the C+C group; these variables did not change after they were cooled, and they were no different to those of the animals in the CNC group on Day 0 sample 2/2 or the other sampling days.

A

B

Figure 4.2 Arterial partial pressure of oxygen (A, PaO_2; mean ± SD) and carbon dioxide (B, $PaCO_2$; mean ± SD) in immobilized blesbok from the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the PaO_2 or the $PaCO_2$ in the Chase non-Cooling (CNC) and the Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct) (Two way ANOVA, Tukey post hoc test, p

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< 0.05). The brackets above the bars represent significant differences between the PaO_2 or the $PaCO_2$ measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days.

4.5 Effects of capture and cooling on pH and lactate

4.5.1 pH

When blesbok from the Ct group were immobilized, their blood pH was similar between (F $_{(4, 49)}$ = 0.798, p = 0.532) the respective sampling days and was on average 7.4 ± 0.033 throughout the different immobilizations (F $_{(4, 49)}$ = 0.798, p = 0.532) (Fig 4.3).

After the chase, animals in the CNC group had pH values lower than the animals in the Ct and C+C groups at the first sampling point (Day 0-1/2, F $_{(2, 144)}$ = 4.131, p = 0.003) and lower than the animals in the Ct group at the second sampling point (Day 0-2/2, p = 0.014).There were no differences in the pH between the C+C group and the Ct group (p = 0.125) at any of the sampling times.

On days 2, 16 and 30 there was no difference in the blood pH between the three groups (p > 0.999) and pH did not change over time compared to the first sample in the groups.

Cooling did not have any effect on any animal's pH in the C+C group; pH did not change after they were cooled, and was no different to those of the animals in the Ct and CNC group on Day 0 sample 2/2 or the other sampling days.
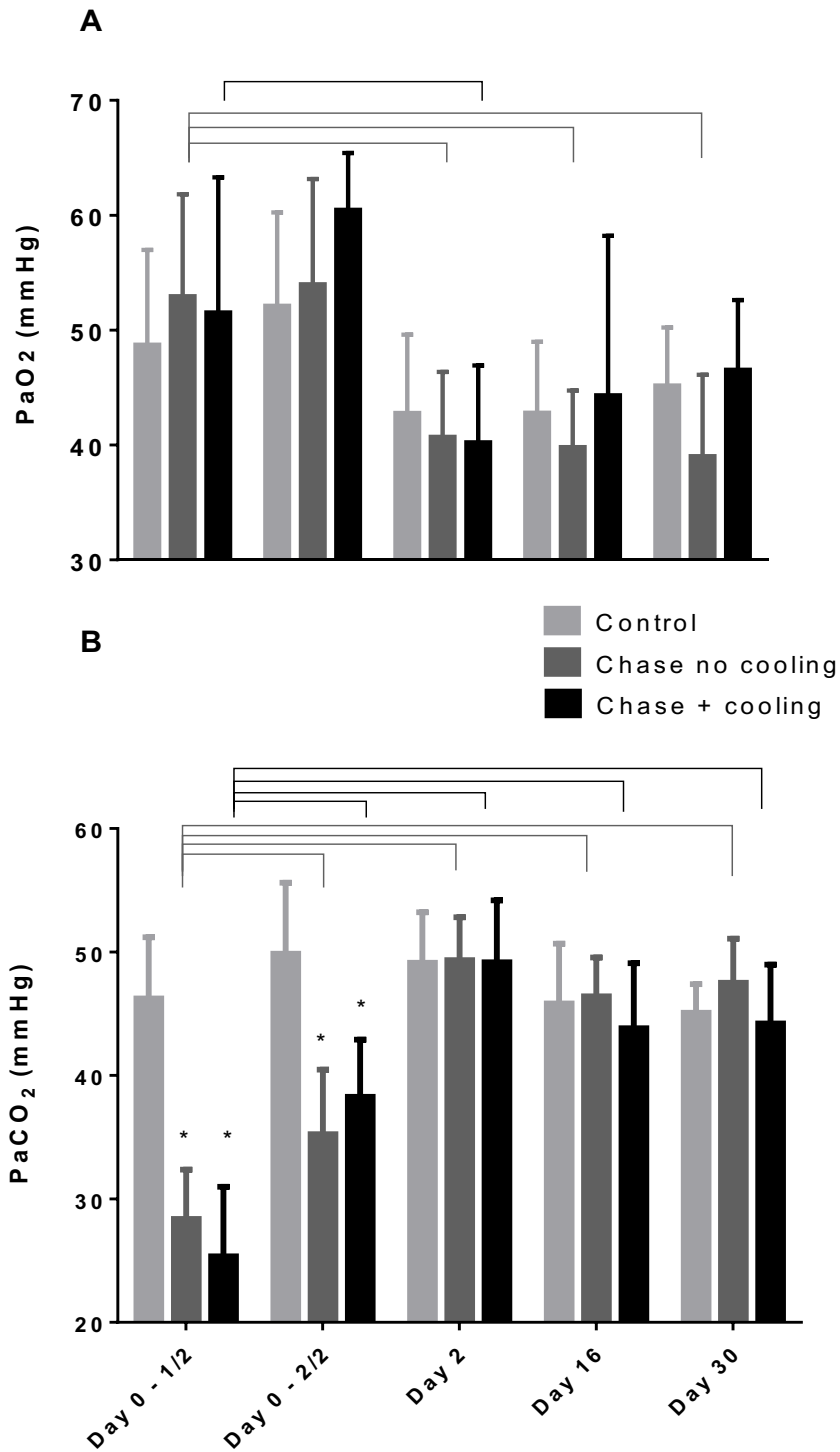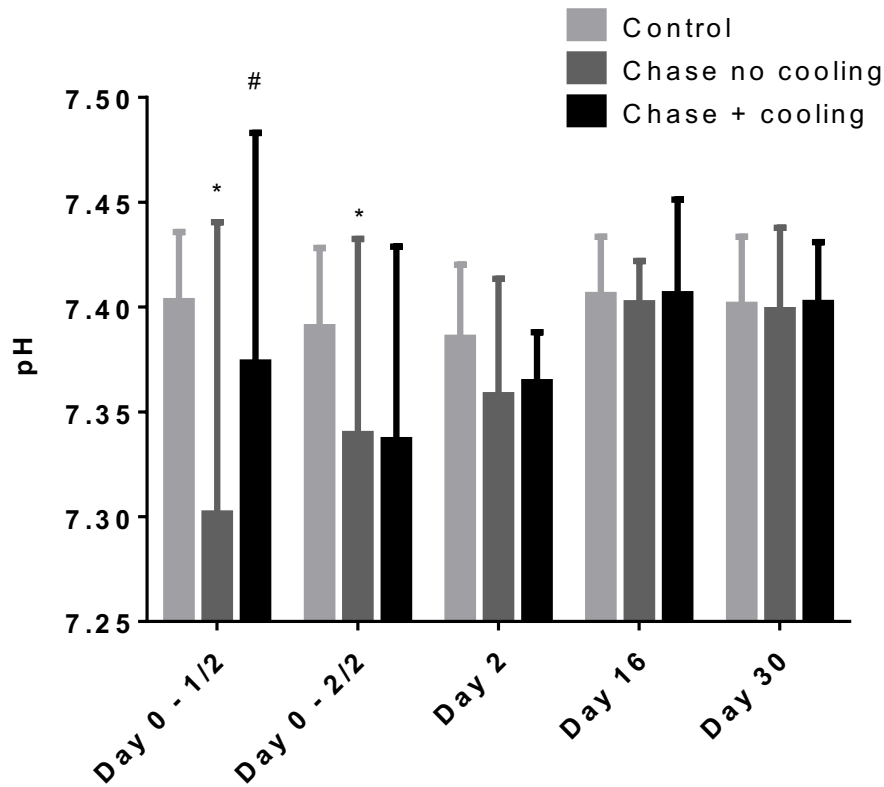
Figure 4.3 pH values (mean ± SD) of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the pH values in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct), and the # indicates where the pH values in the Chase non-Cooling and Chase Plus Cooling groups were significantly different to each other (Two way ANOVA, Tukey post hoc test, $p < 0.05$).

4.5.2 Lactate

At the first immobilization (Day 0-1/2), the animals in the Ct group had plasma lactate concentrations of 1.68 ± 0.81 mmol.l^{-1} (Fig. 4.4). These lactate concentrations did not differ to those on Day 2, 16 and 30 in this group ($F_{(4, 49)} = 2.266$; $p = 0.075$). The animals that were chased and then immobilized in the CNC and C+C groups had lactate values that were 8 – 9 times higher at the first sample and between 8 – 9 times higher at the second sample ($F_{(2, 145)} = 83.94$, $p < 0.0001$) compared to values measured in the Ct group on day 0. There were no differences in

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plasma lactate concentration between the two chased groups at the first (day 0-1/2, p = 0.976) or second (Day 0-2/2, p = 0.719) sample.

Compared to lactate values on Day 0, values measured on the sampling days (Day 2, Day 16 and Day 30) had decreased in the CNC (p < 0.0001) and the C+C (p < 0.0001) groups to levels that were similar to lactate concentrations measured in the Ct group (p = 0.950 – p > 0.999).

Cooling did not have any effect on the animal's plasma lactate concentration in the C+C group; plasma lactate did not change after they were cooled, and was no different to those of the animals in the CNC group on Day 0 sample 2/2 or the other sampling days.
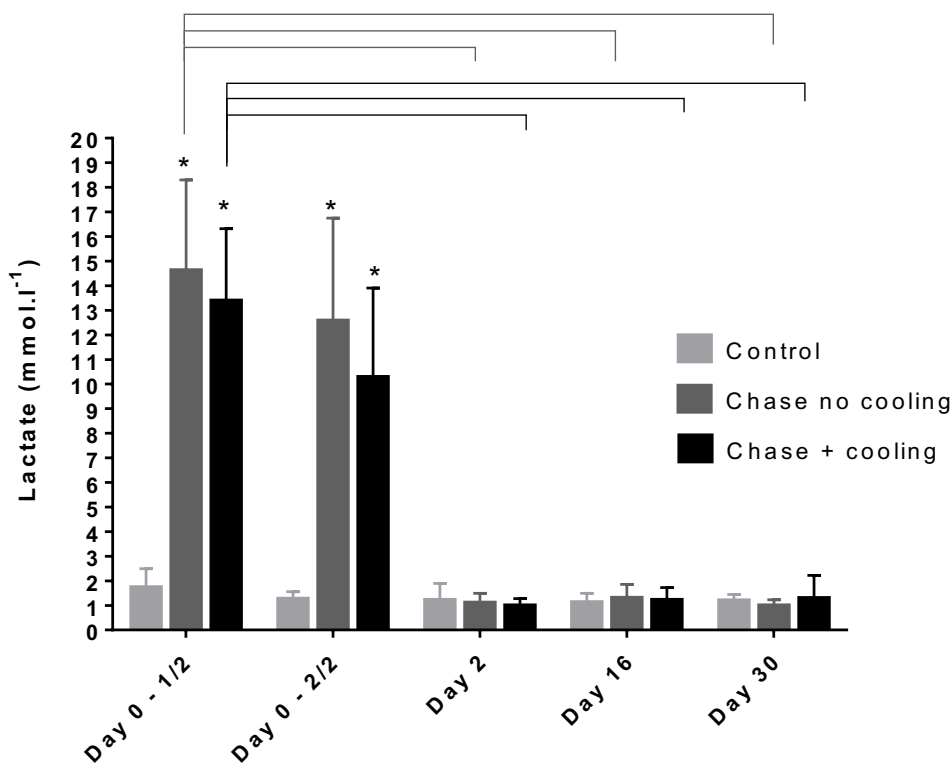

Figure 4.4 Lactate values (mean ± SD) of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the lactate values in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups

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were significantly different compared to the Control group (Ct) (Two way ANOVA, Tukey post hoc test, $p < 0.05$). The brackets above the bars represent significant differences between the lactate values measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.6 Effect of capture and cooling on hepatic enzymes

4.6.1 Gamma-Glutamyl Transferase (GGT)

The mean GGT concentration was 64.08 ± 11.23 IU.l^{-1} in the Ct group in the first sample on Day 0 and did not differ at any of the other sampling times ($F_{(4,49)} = 0.357$, $p = 0.200$) in these animals (Fig. 4.5). These GGT concentrations also did not differ between the animals in the different groups ($F_{(2, 145)} = 1.216$; $P = 0.299$) at any of the sampling times. Cooling had no effect on the animal's GGT concentrations in the C+C group.
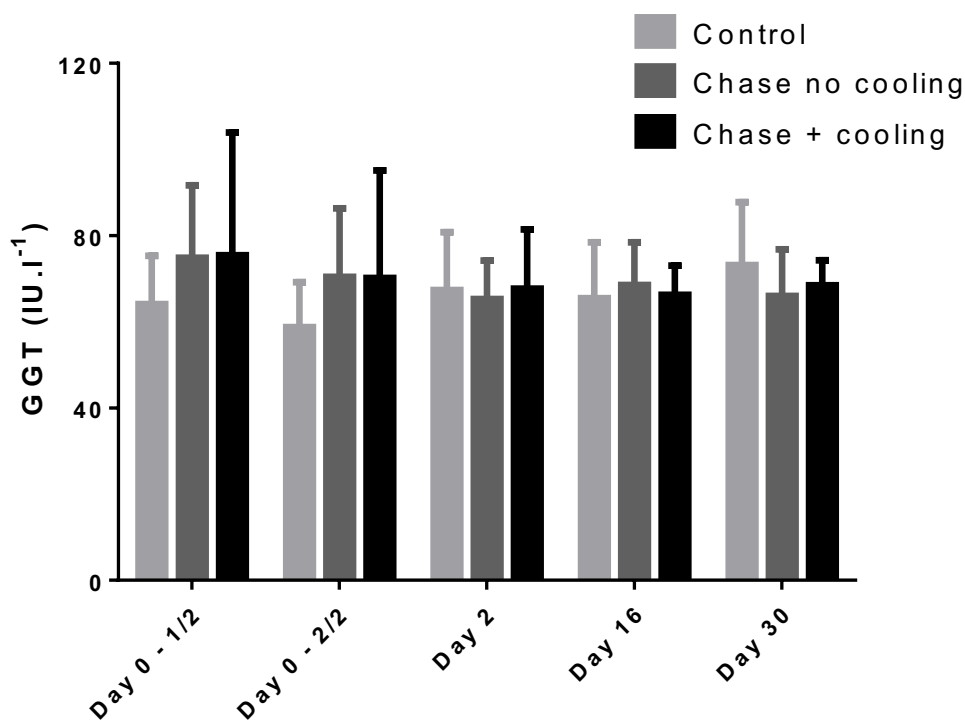
Figure 4.5 Gamma-glutamyl transferase (GGT; mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). No differences occurred between the groups at any of the sampling points (Two way ANOVA, Tukey post hoc test, $p < 0.05$) or between the GGT concentrations measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.6.2 Glutamate Dehydrogenase (GLDH)

Mean GLDH plasma concentration in the Ct groups was 8.7 ± 6.5 IU.l^{-1} at the first sample on Day 0 and did not differ at any of the other sampling times ($F_{(4, 49)} = 0.377$; $p = 0.635$) in these animals (Fig. 4.6). Immediately after immobilization, (Day 0-1/2) the blesbok in the CNC ($p = 0.008$) and the C+C ($p = 0.013$) had higher GLDH plasma concentrations compared to the animals in the Ct group. However, there was no significant difference in the plasma GLDH concentration between the animals in the two chased groups ($p = 0.984$). Similarly, at the second sample on the first day (day 0-2/2) the animals in the CNC ($p = 0.012$) and the C+C ($p = 0.016$) groups had GLDH plasma concentrations that were higher than those of the animals in the Ct group but no different between the chased groups ($p = 0.984$).

On the following sampling days (Day 2, Day 16 and Day 30) the GLDH plasma concentration was no different between the three groups ($p = 0.169 – p = 0.996$).

Cooling did not have any effect on the animal's plasma GLDH concentration in the C+C group; plasma GLDH did not change after they were cooled, and was no different to those of the animals in the CNC group on Day 0 sample 2/2 or the other sampling days.

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Figure 4.6 Glutamate dehydrogenase (GLDH; mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the GLDH levels in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct) (Two way ANOVA, Tukey post hoc test, $p < 0.05$). The brackets above the bars represent significant differences between the GLDH levels measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.7 Effects of capture and cooling on renal function

4.7.1 Creatinine

The mean creatinine concentration was 98.42 ± 11.72 mmol.$^{-1}$ in the Ct group in the first sample on day 0 and did not differ from any of the other sampling times ($F_{(4, 49)} = 1.236$; $p = 0.225$) in these animals (Fig. 4.7).

However, immediately after immobilization (Day 0-1/2) the blesbok in the CNC and the C+C had higher creatinine plasma concentrations compared to the animals in the Ct group ($p < 0.0001$). There was no difference between the two chased groups at that time ($p = 0.782$). Similarly, at the second sample on Day 0 (Day 0-2/2) the animals in the CNC ($p < 0.0001$) and the C+C ($p < 0.0001$) groups had creatinine plasma concentrations that were higher than those of the animals in the Ct group but no different to each other ($p = 0.673$).

On sampling Day 2, the creatinine plasma concentration was no different between the three groups ($p = 0.496 – p = 0.991$).

However, on sample Day 16, the animals in the CNC group had lower creatinine plasma concentrations compared to those of the Ct ($p = 0.042$) but no different to those of the C+C group ($p = 0.938$).

On the last sample day (Day 30), the animals in Ct group had higher creatinine plasma concentrations compared to animals in both the CNC ($p = 0.005$) and C+C ($p = 0.006$) groups. These concentrations did not differ between the animals in the two chased groups ($p = 0.986$).

Cooling did not have any effect on creatinine plasma concentrations in the animals in the C+C group; these variables did not change after they were cooled, and they were no different to those of the animals in the CNC group on Day 0 sample 2/2 or the other sampling days.
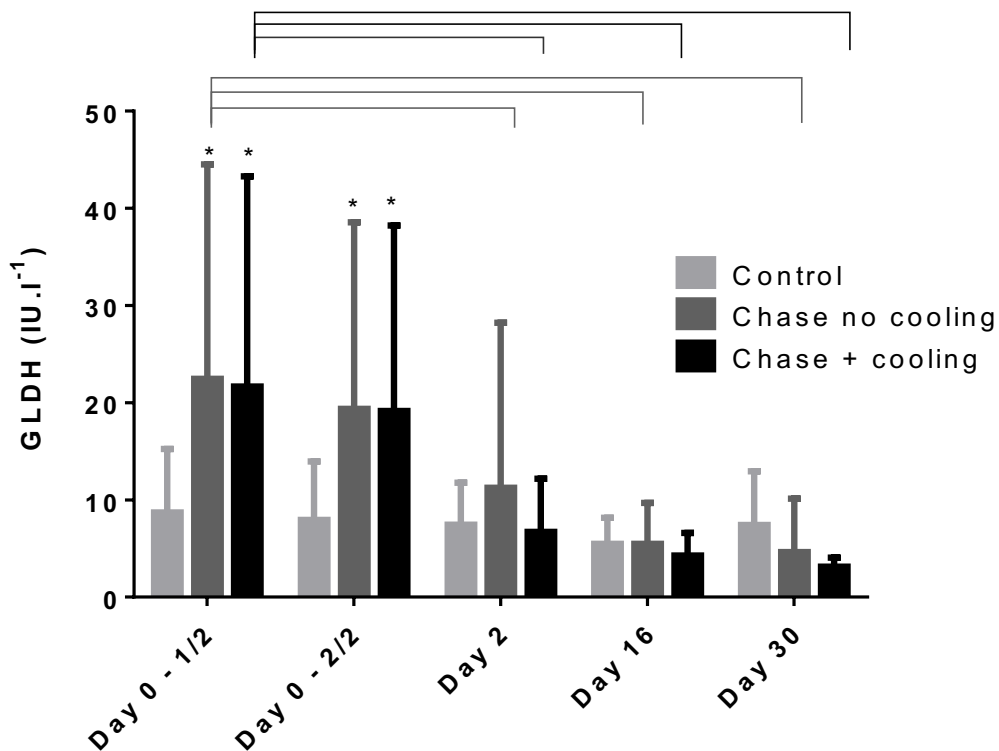
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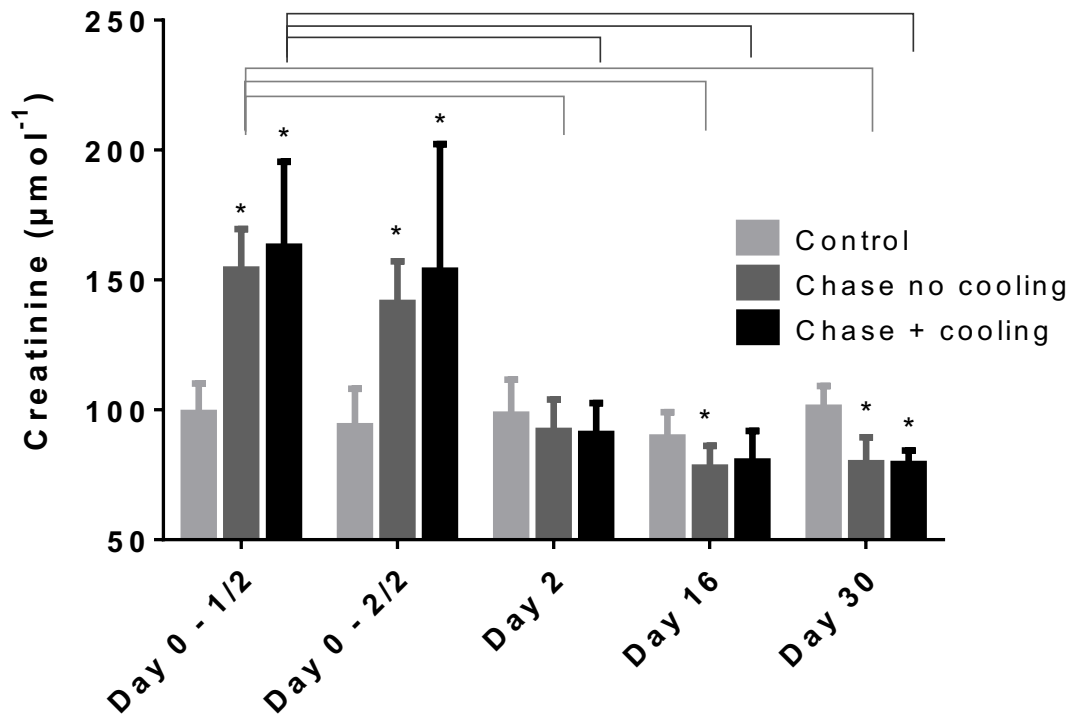
Figure 4.7 Creatinine (mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the creatinine levels in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct) (Two way ANOVA, Tukey post hoc test, $p < 0.05$). The brackets above the bars represent significant differences between the creatinine levels measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.7.2 Blood Urea Nitrogen (BUN)

The mean BUN concentration in the Ct group was 8.8 ± 1.2 mmol.L^{-1} in the first sample on Day 0 (sample Day 0-1/2) and did not differ compared to the values measured at the other sampling time points (Day 0-2/2, 2, 16 and 30) (Fig. 4.8). The BUN concentration also did not differ between the animals in the three groups ($F_{(2, 144)} = 2.911$; $p = 0.057$) at sampling points Day 0-2/2, Day 2 and Day 30.

On sample Day 16, the animals in the CNC group had elevated BUN concentrations compared to animals from the Ct (p = 0.005) and C+C (p = 0.005) groups. However, at this time, the BUN concentration in the Ct and the C+C group animals did not differ (p = 0.974).

Cooling did not alter the BUN concentration in the animal's in the C+C group.

Figure 4.8 Blood Urea Nitrogen (BUN; mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the BUN concentration in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct), and the # indicates where the BUN concentration in the Chase non-Cooling and Chase plus Cooling groups were significantly different to each other (Two way ANOVA, Tukey post hoc test, p < 0.05). The brackets above the bars represent significant differences between the BUN concentrations measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes

later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.8 Effects of capture and cooling on markers of muscle damage

4.8.1 Creatine Kinase (CK)

On sample Day 0-1/2 the mean CK concentration in the animals in the Ct group was 135.42 ± 46.53 $UI.I^{-1}$ and it did not change at any other sampling time except for day 30 where it was lower compared to the first sample (Day 0) (Fig. 4.9). The CK concentration from the animals in the two chased groups, the CNC ($p < 0.05$) and C+C ($p < 0.05$) group was higher than that of the animals from the Ct group at sampling point Day 0-1/2, Day 0-2/2 and Day 2. However, there was no difference between the two chased groups at these sampling points ($p = 0.718$, $p = 0.658$, $p = 0.610$, respectively). On the following sampling days (Day 16 and Day 30) the CK plasma concentration was no different between the three groups ($p = 0.795 – p = 0.999$).

Cooling did not have any effect on the animal's plasma CK concentration in the C+C group; plasma CK did not change after they were cooled, and was no different to those of the animals in the CNC group on Day 0 sample 2/2 or the other sampling days.
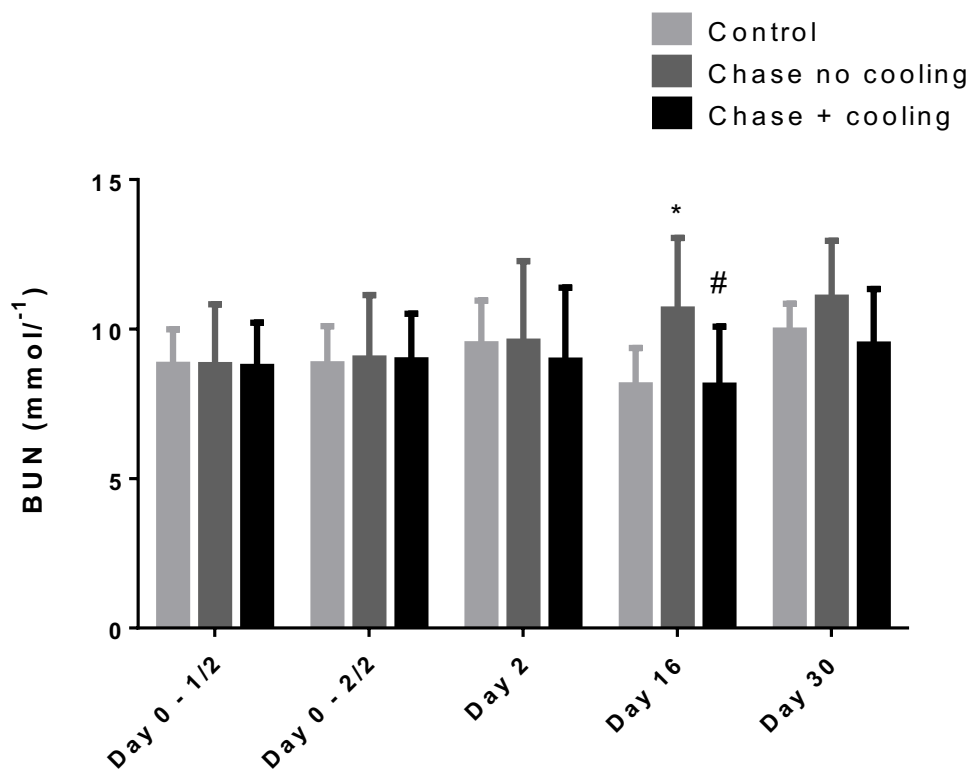
Figure 4.9 Creatine Kinase (CK; mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the CK concentrations in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct), and the # indicates where the CK concentrations in the Chase non-Cooling and Chase plus Cooling groups were significantly different to each other (Two way ANOVA, Tukey post hoc test, $p < 0.05$). The brackets above the bars represent significant differences between the CK concentrations measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.8.2 Aspartate Transaminase (AST)

On sample Day 0-1/2 the mean AST concentration was 109.08 ± 13.16 UI.l^{-1} in the Ct group (Fig. 4.10). At the other sampling time points it was similar except at Day 2 it was higher and day 30 it was lower in these Ct animals.

AST concentration differed between the different groups ($F_{(2,144)}$ = 3.87; p = 0.023) at the first sample Day 0-1/2 (p = 0.039), the second sample Day 0-2/2 (p = 0.019) and on Day 2 (p = 0.010). On Day 2, in animals that were chased in the CNC (p = 0.0/19) and the C+C (p = 0.003) group, the AST concentration was significantly higher compared to the Ct group. However, on this same day, there was no difference between the two chased groups (CNC vs. C+C, p = 0.610).

Cooling did not have any effect on the animal's plasma AST concentration in the C+C group; plasma AST concentration did not change after they were cooled, and was no different to those of the animals in the CNC group on Day 0 sample 2/2 or on the other sampling days.
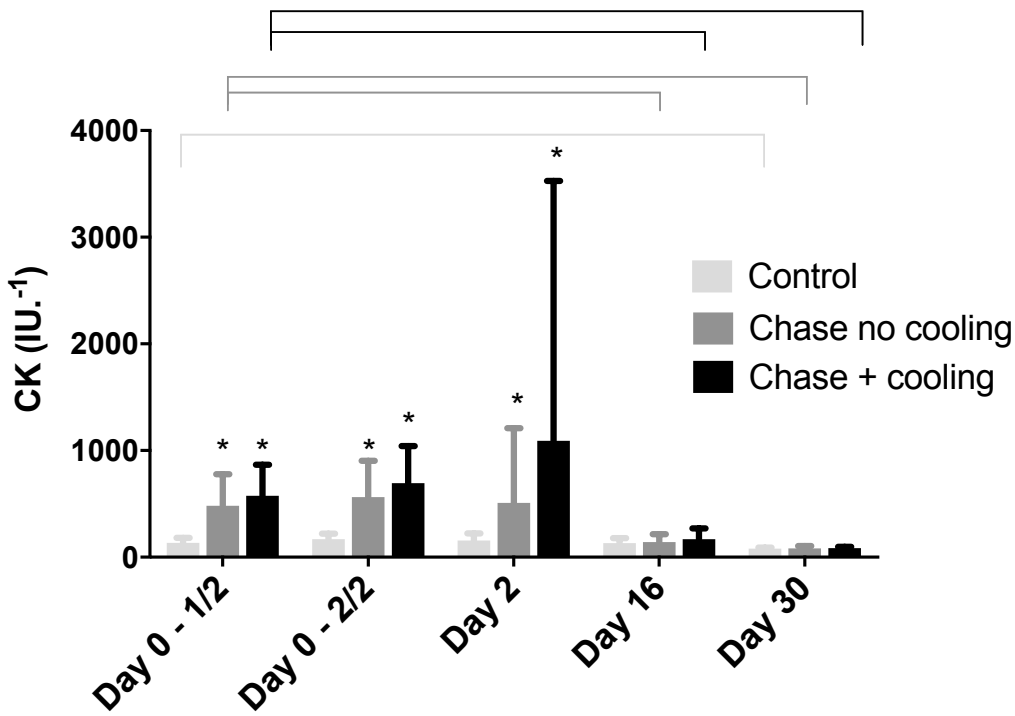

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Figure 4.10 Aspartate transaminase (AST; mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the AST concentration in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct) (Two way ANOVA, Tukey post hoc test, $p < 0.05$). The brackets above the bars represent significant differences between the AST concentration measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

4.8.3 Cardiac Troponin I

At the first capture and sample Day 0-1/2, the animals in the Ct group had cardiac troponin concentrations of $0.022 ± 0.014$ ng.ml^{-1} (Fig 4.11). These concentrations did not differ in the Ct animals at the other time points except at sample Day 2 ($p = 0.0052$) where it was lower.

The animals that were chased and then immobilized in the CNC and C+C groups had cardiac troponin I concentrations that were 11 to 18 times higher at the first sample and 40 to 42 times higher, respectively, at the second sample ($F_{(2, 144)} = 126.6$; $P < 0.0001$) compared to values measured in the Ct group. There were no differences in plasma cardiac troponin I concentrations between the animals in the two chased groups at the first (Day 0-1/2, $p = 0,904$) or second (Day 0-2/2, $p = 0,959$) sample.

On sample day 2, animals in the chased groups had 87 (CNC) to 68 (C+C) times higher cardiac troponin I concentrations compared to those of the Ct group ($p = < 0.0001$) at that time. There were no differences in cardiac troponin I concentrations between the animals in the chased groups ($p = 0.999$).

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On sample day 16, the animals in the CNC group had higher cardiac troponin concentrations compared to the animals in the Ct group (p = 0.026), but there were no differences in concentrations between the two capture groups (p = 0.973) or the Ct and the C+C (p = 0.089) groups at this time.

Cooling did not reduce the animal's plasma cardiac troponin I concentrations in the C+C group; in fact it increased after cooling. However, this increase was no different to that of the animals in the CNC group on Day 0 sample 2/2 and also did not differ at the other time points.
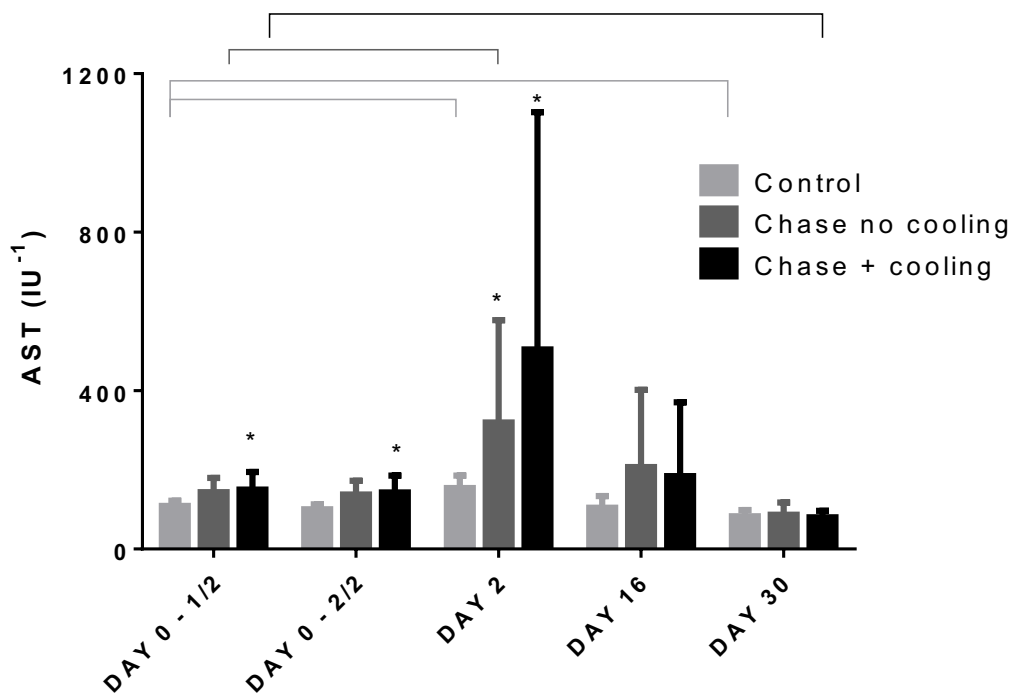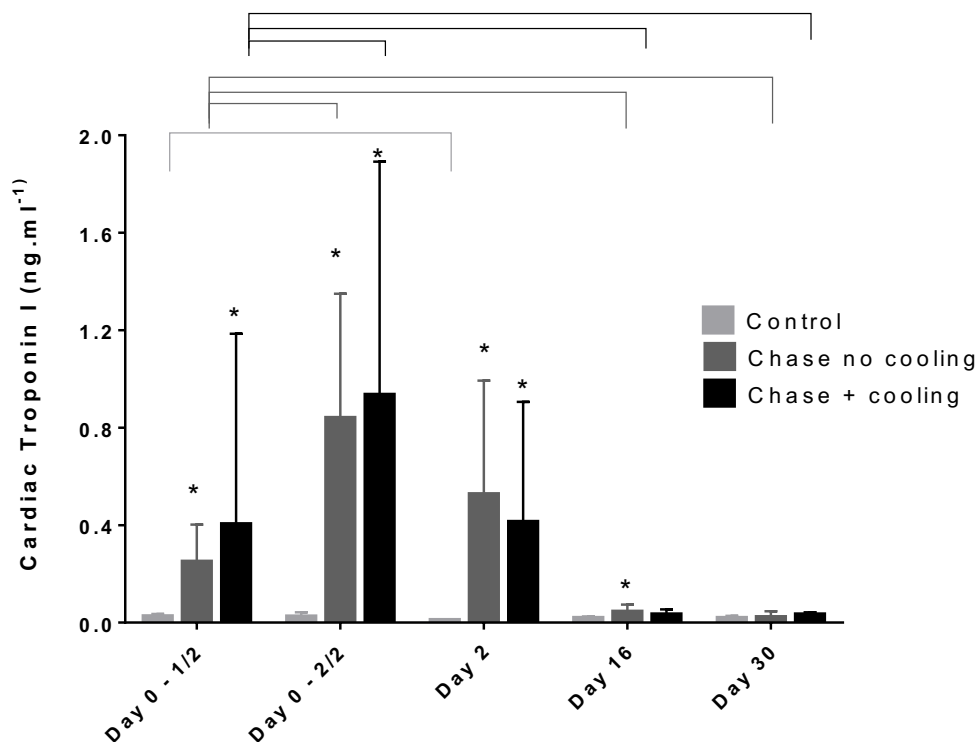

Figure 4.11 Cardiac troponin I (mean ± SD) values of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The * indicates where the cardiac troponin I concentration in the Chase non-Cooling (CNC) and Chase plus Cooling (C+C) groups were significantly different compared to the Control group (Ct)

(Two way ANOVA, Tukey post hoc test, p < 0.05). The brackets above the bars represent significant differences between the cardiac troponin I concentration measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test p < 0.05).

4.9 Effects of capture and cooling on cortisol

Cortisol values were no different at any sampling time points between the groups (Fig. 4.12). Cortisol values were only different over time in the Control group (Ct) animals; compared to the first sample (D0-1/2) the cortisol was higher at 40 minutes into the immobilization on the first day (Day 0-2/2, $F_{(4, 49)} = 4.358$; P = 0.004) and lower in the animals on Day 30 (P = 0.023).
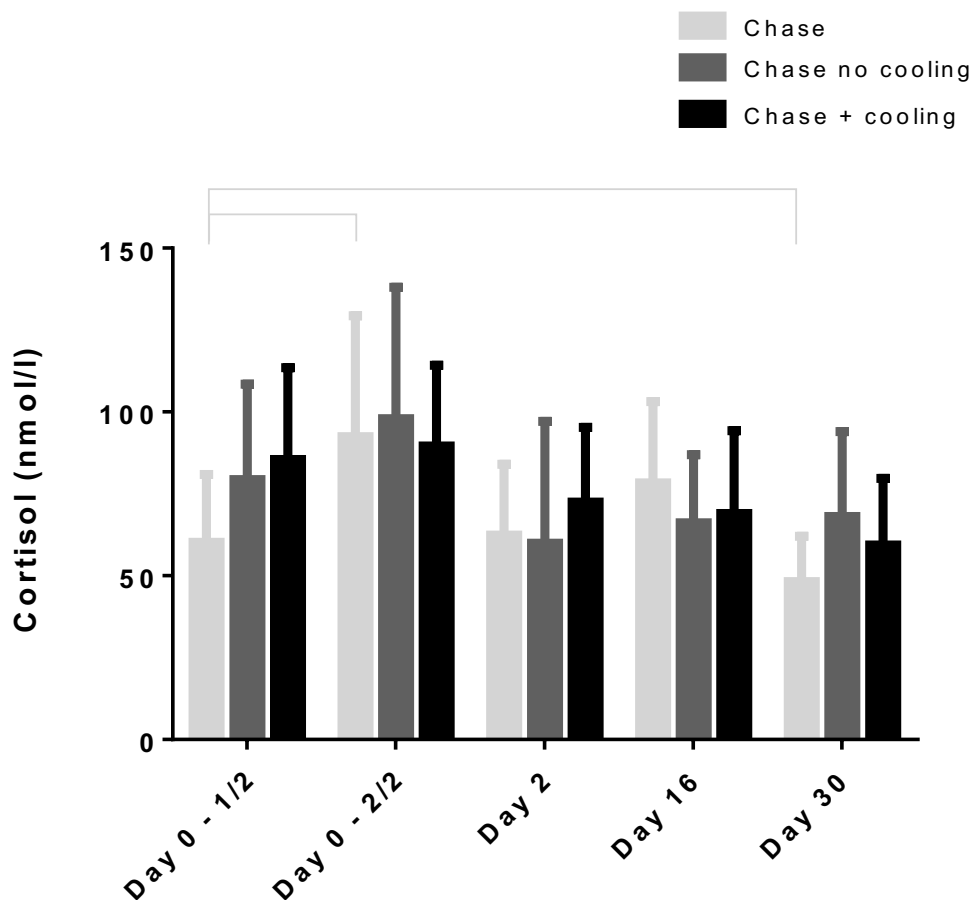
Figure 4.12 Cortisol (mean ± SD) of immobilized blesbok in the three study groups on the day of the intervention (Day 0) and the three data collection days (Day 2, 16 & 30). The brackets above the bars represent significant differences between the cortisol concentration measured directly after immobilization on the intervention day (Day 0-1/2) compared to 40 minutes later (Day 0-2/2) and directly after immobilization on the data collection days (Two way ANOVA, Dunnett's post hoc test $p < 0.05$).

Chapter 5

Discussion

In the absence of published normal clinical and biochemical values for blesbok, I compared values for parameters measured to those reported for blesbok in the ISIS data base (ISIS, 1999). This data base reports reference ranges for values obtained from both healthy and sick zoo animals and therefore may not strictly reflect normal values for wildlife. However, currently this data base gives the only reported reference range values for blesbok. Comparing to this data base the animals in the Control group, that were darted and immobilized in the bomas without first being exposed to the stress of been chased, had "normal", or below normal values for clinical and physiological parameters during the first capture (on the interventional day – Day 0).

For parameters not reported in the ISIS data base we made comparisons with healthy domestic ruminants or wild ruminants, like white tail deer, that were captured in a way that we felt would not negatively influence these parameters. As an example, we found that the cardiac troponin I enzyme (cTnI) in the animals in the Control group at the first capture (Day 0- 1/2 - 0.021 ± 0.005 ng/ml) was slightly greater than that measured in ground-darted white-tailed deer (0.00 ± 0.01 ng/ml) (Boesch *et al*., 2011) but less than that measured in normal domestic ruminant's (bovine: 0.18 ng/ml, sheep: 0.15 ng/ml, goats: 0.20 ng/ml) (Basbugan *et al.,* 2010; Leonardi *et al.,* 2008). These "normal" values were not only measured at the first capture, but also on the other sampling days in the Control group. Therefore, it is likely that these normal values from the Control group serve as an adequate control at all the time points for the animals in the treatment groups.

The mean rectal temperature from the Control animals was 38.0 ± 0.4°C for all the sampling times combined. These body temperatures were within a normal range, as they were similar to those measured by implanted temperature sensitive data loggers (38.7 ± 0.3°C) in healthy free-living blesbok (Broekman, 2013; Sawicka *et al.,* 2015), and lower than those reported by the ISIS data base (39.1 ± 1°C) for zoo blesbok. Mean muscle temperature in blesbok from the Control group were also similar to those measured using mini-temperature sensitive data loggers implanted in the caudal thigh muscles of free-living blesbok (Broekman, 2013). These normal temperatures indicate that in the Control group animals this stress response (indicated as a stress-induced hyperthermia) to darting and immobilization was minimal and no major muscle activity occurred during these captures.

Blesbok in both treated groups (C+C and CNC groups) that were exposed to the stress of being chased before they were darted and immobilized, had profound changes to some of their normal physiology. The primary clinical sign that was observed in these animals, directly after they became immobilized, was an acute hyperthermia. These animals had rectal and muscle temperatures that were approximately 4°C higher than the animals in the Control group at the first sampling time. In addition, at this time, animals in these chased groups had elevated blood lactate levels (14.58 ± 3,73 mmol.l^{-1} CNC group and 13.34 ± 2.98 mmol.l^{-1} C+C group vs. 1.68 ± 0.81mmol.l^{-1} in the Control group), indicating anaerobic metabolism, and a mild acidaemia (pH: 7.39 ± 0.1 for CNC group and 7.30 ± 0.14 for C+C group vs 7.40 ± 0.03 for Ct group). Despite differences in lactate the animals in all three groups had a similar degree of hypoxaemia immediately after capture; hypoxaemia was similar in the Chased groups (PaO$_2$ = 52.95 ± 8.9 mmHg CNC and 51.5 ± 11.7 mmHg C+C groups) compared to the Control group (PaO2 = 48.7 ± 8.2 mmHg). Therefore, the high level of anaerobic metabolism detected by lactate directly after capture in the chased groups was unlikely to be mainly due to the hypoxaemia measured at that time, but more likely as a result of the hypoxia that occurred during the chase.

The $PaCO_2$ measured directly after the chase, was 28.4 ± 4.0 mmHg in the CNC group and 27.1 ± 6.0 in the C+C group, while in the Control group it was 46.24 ± 4.95 mmHg ($P < 0.0001$). At an altitude of 1300 meters we would expect a normal $PaCO_2$ to be between 35 - 40 mmHg. Therefore, animals in the chased groups were hypocapnic, while animals in the Control group were hypercapnic indicating hypoventilation, which is normally expected from etorphine-induced respiratory depression. That the animals in the chased group were hypocapnic indicates that they did not suffer from etorphine-induced respiratory depression but rather were hyperventilating, possibly as a compensation for the hyperthermia they developed. That this hyperventilation did not improve hypoxaemia in these animals is an interesting finding that requires further research which should possibly focus on changes in pulmonary dynamics and metabolism.

Serum liver and kidney enzyme concentrations were used to assess liver and kidney function in the blesbok. GGT is a membrane bound enzyme located mainly in the hepatic biliary tree, but also in renal and mammary epithelial cells. If it is elevated it mostly indicates hepatic necrosis (Latimer, 2001). Compared to ISIS (68 ± 47 U/L) the animals in the Control group (64.08 U/L ± 11.23, Day 0-1/2) had relatively lower GGT values. In contrast, animals in the chased groups had moderately higher GGT values compared to ISIS. However, these values were not significantly different to the animals in the Control group (74.85 ± 16.82 U/L for CNC group and 75.42 ± 28.51 U/L for C+C group at sample Day 0) neither were they clinically elevated when comparing to the ISIS values. Therefore, there was no indication of any damage to the membranes of hepatic epithelial cells. In contrast, serum GLDH concentrations in the chased animals (22.5 ± 22.1 IU/L for CNC group and 21.7 ± 21.6 IU/L for C+C group on Day 0-1/2) were 2.5 times greater compared to those in the Control group animals (8.7 ± 6.5 IU/L). GLDH is an important measure in the determination of hepatic necrosis in ruminants (Kaneko *et al.,* 2008) and is a cytosolic enzyme that, if elevated in serum, indicates intracellular hepatic damage (Latimer, 2011). This damage usually occurs due to hypoxia which leads to hepatic necrosis (Kaneko *et al*., 2008). Hypoxia causes a decreased supply of cellular energy in the form of ATP (Jungermann and Kietzamann, 2000; Zhu *et al*., 2012;

Vollmar *et al*., 1994) in the hepatocytes. This insufficient availability of energy leads to alterations in parenchymal and non-parenchymal hepatocytes, which results in cell damage in the form of mitochondrial swelling, vacuolization, cytoplasmic degeneration and disruption of the rough endoplasmic reticulum (Jungermann and Kietzamann, 2000; Vollmar *et al*., 1994; Zhu *et al*., 2012). In our study, chased blesbok had a mild increase of GLDH enzymes right after capture (Day 0-1/2) as well as 40 minute into immobilization (Day 0-2/2), but not beyond this time. Therefore we believe that only mild temporary liver damage occurred in the chased animals, probably mainly as a result of the hypoxia during the chase. Generalised systemic hypoxia was most likely caused by excessive oxygen demand from muscle exertion during the chase and was worsened by respiratory compromise that occurred during the immobilization.

BUN values were similar between the three groups (8.8 ± 1.2 mmol/L for Ct group, 8.8 ± 2 mmol/L for CNC and 8.36 ± 1.56 mmol/L for C+C group on Day 0) and to those values in the ISIS data base (11.07 ± 3.2 mmol/L). Serum creatinine concentrations were similar in the two chased groups (153.64 ± 15.95 µmol/L for CNC group and 160.67 ± 28.5 µmol/L for C+C group on Day 0) and to ISIS data base values (133 ± 35 µmol/L), but they were significantly higher compared to values from animals in the Control group (98.42 ± 11.72 µmol/L). Both, BUN and serum creatinine are indicators of renal damage (Clarkson *et al*., 2006) and even though normal values can be slightly modified due to pre-existing conditions or diet, they should not go over 240 µmol/L for creatinine and 8.58 mmol/L for BUN (values based on Eland, Silberova *et al*., 2010). Because BUN was not elevated above this critical value, it suggests that the blesbok may not have had renal damage. More specifically, serum creatinine was not elevated above its critical clinical value, thus confirming that renal damage was not present in these animals. However, creatinine was moderately elevated in the chased compared to the Control group animals. Serum creatinine is a metabolic product that derives from the breakdown of muscle and therefore its moderate elevation in the chased animals may have been as a result of muscle damage (Silberova *et al*., 2010; Shemesh *et al*., 1985).

Serum enzymes (CK and AST) specific to skeletal muscle were measured to determine the degree of skeletal muscle damage in the blesbok. CK concentrations from the animals in the Control group (135.42 ± 46.53 U/L on Day 0) were much lower than those reported from the ISIS data base for blesbok (351 ± 377 U/L). The chased animals had significantly elevated CK concentrations on Day 0 and Day 2 (481.86 ± 296.87 U/L CNC group and 1807 ± 296.87 U/L C+C group Day 0-1/2) compared to the Control animals. Similarly, AST values in the Control group animals (109.08 ± 13.16 U/L) were lower than the ISIS data base values (143 ± 74 U/L) and these were either similar or slightly higher in the animals that were chased, but only on Day 2 (143.79 ± 35.51U/L CNC group and 185.08 ± 79.02 U/L C+C group). Although both CK and AST values were elevated in the chased groups these elevations were not that high. If serum CK is elevated by 3 to 5 times it usually indicates necrosis in 20 g of muscle tissue (Kaneko *et al*., 2008). The CK in the animals from the chased groups was 5 -13 times elevated, which would not have equated to a large amount of damage and therefore these animals probably only had mild skeletal muscle damage.

To determine if any cardiac damage occurred, we measured serum cardiac troponin I (cTnI) concentrations. Although normal reference ranges for cTnI have not been determined in blesbok, nor has it been validated as an indicator of cardiac damage in this species, I believe it indicated potential cardiac damage because it is a protein enzyme that appears to be highly homologous between mammals and a good indicator of cardiac damage across species (Basbugan *et al*., 2010; Varga *et al*., 2013; Boesch *et al*., 2011; Leonardi *et al*., 2008). As mentioned above the animals in the Control group had similar concentrations of cTnI compared to normal values from other species in which this enzyme has been validated as an indicator of cardiac muscle damage. In the chased animals the concentration of cTnI was 15 (CNC mean 0.033 ± 0.3 ng/ml) and 17 (C+C mean 0.36 ± 0.3 ng/ml) times elevated directly after the animals became immobilized on Day 0 compared to the Control animals. Peak concentrations in the chased animals occurred at 40 minutes into the immobilization and were approximately 45 times greater than those in the Control group. Even after 2 days (sample Day 2) of the interventional capture (Day 0) the concentrations in the chased

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groups were 20 times higher than the Control group. I believe that these findings indicate that acute cardiac damage occurred in these animals as a result of the chase.

Even though the body temperature of the animals in the CNC group naturally decreased over the 40 minutes of immobilization, they were still hyperthermic at the end of the immobilization (40.40 ± 0.7°C). Cooling the animals by dousing them with 4°C water quickly resolved this hyperthermia in the Chased + Cooling group. Although the cooling was effective at normalising core (rectal) body and muscle temperature, unexpectedly, when comparing to the Chased Non-Cooled animals, cooling had no major effect on lowering or modifying any of the parameters (GLDH, creatinine, CK, AST or cTnI) that indicated either mild to severe pathophysiological effects.

Ideally, it would have been better to do this project in the wild, using free-living animals, and a capture procedure that normally occurs in the field. However, because of the lack of data for healthy free-living blesbok a controlled study was required. To implement proper controls in a study in the field is impossible, therefore the study was designed around using wild animals in a captive environment (bomas) using a capture procedure that mimicked a capture situation in the wild. This design also allowed for better control of the animals in the treatment groups which reduced variability and bias associated with working in the field. In order to obtain and keep wild blesbok in a controlled environment (bomas) they had to go through an initial mass capture which could have caused pathophysiological changes and influenced their health. However, these animals were captured by an experienced game capture team in a manner that minimised a stress response and did not cause over-exertion of the animals. To further reduce the possible complications of this initial capture the animals were tranquilized with long-acting tranquilizers and transported for only 30 minutes before they were released into their holding bomas. To avoid injuries caused by fighting only one adult male was placed in each small holding boma. After this initial capture, the blesbok

were allowed 6 weeks to adapt to confinement, feeding and other management procedures in the boma. During this time the animal's health and behaviour was closely monitored.

Blesbok were selected as the subjects for this study because of their relative abundance and the ease to which they can be handled in captive conditions in this type of study. Additionally, they have been used successfully in other studies investigating the hyperthermic effects of capture (Broekman, 2013) and cooling methods used to treat capture-induced hyperthermia (Sawicka *et al.,* 2011). A possible criticism of using blesbok in this study is that they are considered to be "hardy" animals that only occasionally develop capture myopathy. However, they do develop severe acute capture-induced hyperthermia (Broekman, 2013; Sawicka *et al.,* 2011) and other capture related complications (McKenzie, 1993; Kock & Burroughs, 2012). Additionally, it was not our aim or intention to induce overt capture myopathy or other capture related complications but merely to investigate the short and medium-term pathophysiological effects that are caused by a mock capture procedure. Of primary importance was to determine the consequences of capture-induced hyperthermia and whether cooling altered these consequences. Therefore, we felt that blesbok served as a good species to use in this type of study and possibly they may be a good model for informing us of the effects of capture and cooling in other antelope species.

Unfortunately the boma layout did not allow for animals from smaller sub-groups to be chased without causing a disturbance to other animals while they were still in their holding bomas. Fortunately, it appeared that only one sub-group belonging to study group C+C (Boma #10) was visibly disturbed. Some animals in this group ran around this holding boma and jumped into the boma walls as other animals were being chased in the adjacent area (Camp # 2; Figure 3.4).Three out of five animals from this sub-group had increased CK concentrations directly after they were immobilized. These results indicated muscle damage in these animals before the capture

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procedure, and therefore the data from these animals was removed before data analysis took place.

To further reduce variability it would have been optimal for our subjects to be of the same sex and age group. Unfortunately, due to logistics and costs we had to work with a mixture of males, females and sub-adult animals. Although this situation was not optimal it did represent what normally occurs in a field capture and animals were assigned randomly by age and gender so that the groups were approximately balanced. Ideally more sampling points could have been included between day 2 and 16 to better understand the pathophysiological changes during that time, but unfortunately this was not feasible due to costs. However, future studies should consider focusing sampling at regular intervals before day 16 post-capture. We collected tissue samples for histopathological analysis at day 16 and 30, but the analysis and interpretation of these was beyond the scope of this study. We expect that these samples will not reveal active damage but only chronic or healing changes, if any. In the future, if associations between clinical pathology and histopathology changes are required, then both blood and tissue samples need to be collected earlier than day 16 post-capture.

The sampling times we used after capture were chosen *a priori* so that we could gain insights into the short and medium-term pathophysiological effects of capture and the consequences of capture-induced hyperthermia. Sampling over a month gave a general indication that active pathophysiological changes occurred only up to, or earlier than day 16 post-capture; only a few active changes occurred at day 16 and almost none at day 30. Coincidentally, Harthoorn (1976) identified this 16 day window as a "stress period" where animal's enzymes, which indicate pathophysiology, were still elevated, and he deemed this period to be a time where animals were very susceptible to additional stress. Harthoorn (1976) recommended that animals should not be

re-captured within this period, but rather allowed to recover beyond this time until their body condition improved.

It is well known that capture of wild animals induces a stress response, especially when animals are chased using helicopters or land-based vehicles like quad-bikes or trucks. The use of vehicles enhances the success and efficiency of capture procedures but can cause detrimental consequences to an animal's health. Chasing wild animals, whether it is for a short or long duration (more than 5 minutes), triggers complex alterations in an animal's physiology, which if severe, can cause disability or even death (Harthoorn, 1975; McKenzie, 1993; Kock & Burroughs, 2012). Death and disability is normally attributed to the capture myopathy syndrome. Wild animals that suffer from the capture myopathy syndrome can develop various forms of shock which result from common pathological lesions (Harthoorn, 1974, 1975, 1976). Some of the typical post-mortem lesions that can be seen in animals with capture myopathy are related to irreversible damage to skeletal and heart muscles, blood vessels, kidney and parenchymatous organs like the liver (Harthoorn, 1976).

When an animal encounters a stressor, two pathways of the normal stress response are activated: the fast "fight-or-flight" response (Meyer *et al*., 2008 a; Romero, 2004) mainly triggers the sympathetic pathway which causes acute catecholamine release, and simultaneously the HPA-axis is also activated and results in a slower release of glucocorticoid hormones (Romero *et al*., 2004). The combination of effects of both of these responses constitutes an acute stress response (Romero *et al*., 2004). This response diverts physiological and behavioural (Proulx, 1999) processes from normal homeostatic functions to those that focus on immediate survival needs (Spraker, 1993; Romero *et al.,* 2004). If the stressor continues then the HPA response persists leading to a chronic stress response which results in immunosuppression, weight loss and gastric ulcers amongst other pathophysiological effects.

Capturing wild animals using techniques that cause a severe stress response and over-exertion predisposes them to suffer from capture-induced hyperthermia (Boroughs & McKenzie, 1993; Meyer *et al*., 2008; Fahlman *et al*., 2011). Hyperthermia is one of the main consequences of capture (Burroughs and Mc Kenzie, 1993) and the amount of exertion from a chase is not necessarily the main cause of this hyperthermia but rather the stress induced by the chase (Meyer *et al*., 2008 a). Previously it was believed that this hyperthermia was caused by similar mechanisms that caused malignant hyperthermia in humans and other animals exposed to stressors or anaesthetic drugs like halothane (Mitchell & Heffron, 1982). However, this hyperthermia occurs even when anaesthetics drugs are not administered, and unlike malignant hyperthermia it resolves as soon as a stressor is removed (Meyer *et al*., 2008 a). Antognini *et al*, (1996) investigated a possible link between malignant hyperthermia and capture myopathy in black-tailed deer when exposed to halothane, caffeine and succinylcholine. After several experiments, which included muscle biopsies to determine the muscle susceptibility to these drugs, they concluded that the deer did not develop malignant hyperthermia and suggest no direct link between malignant hyperthermia and capture myopathy (Antognini *et al.,* 1996). Harthoorn (1975, 1976), Fowler (1978) and Mackenzie (1993) all suggested that capture-induced hyperthermia occurs when animals are captured in the heat and therefore equated this hyperthermia to heat illness or heat stroke. However, Meyer *et al.,* (2008 a & b) found that capture-induce hyperthermia in impalas was not influenced by ambient temperature but was mainly associated with the stress response that was induced during capture. Similarly, they found that hyperthermia was not associated with activity levels during capture (Meyer *et al*., 2008 b).

It is well known that hyperthermia causes cytotoxic effects and results in damage to many organs including muscles (Miller & Ziskin, 1989; Bouchama & Knochel, 2002; Lambert *et al*., 2002; Leon, 2006). It is also known that cooling is effective in reducing the pathophysiological effects of hyperthermia in animals and humans that suffer from heat stroke (Bouchama & Knochel, 2002; Smith, 2005; Epstein & Roberts., 2011). However, I found that although dousing animals with cold

water effectively cooled the hyperthermic blesbok it did not treat, or prevent, the pathophysiological effects that occurred post capture. This finding indicates that the pathophysiology that developed in the chased blesbok was unlikely to be due to the capture-induced hyperthermia but possibly had another primary cause. A likely primary cause is the hypoxia that would have occurred during the chase, when metabolic demands of specific organs like the heart muscle and liver, and to a lesser degree the skeletal muscle, exceeded that of their aerobic and anaerobic capacity. Evidence of this hypoxia is not only reflected in abnormal concentrations of enzymes from these organs but the marked hyperlactaemia and elevated GLDH that was evident in both groups of chased animals. Because hypermetabolism from stress and exertion were possibly the primary causes of hypoxia and also hyperthermia, it is plausible that the magnitude of the hyperthermia could indicate the extent of damaged induced by the hypoxia. Whether the magnitude of capture-induced hyperthermia, or other parameters that can be measured at capture, like lactate, can be used as good indicators for the extent of organ damage is worth further investigation.

Although, capture-induced hyperthermia may not be the primary cause of the pathophysiology that develops during capture its effects should not be ignored. Prolonged hyperthermia, if not treated, may worsen the pathophysiology that occurs, especially because it increases metabolic demands and may worsen hypoxia via the Q10 effect (Bennett, 1985; Nybo *et al*., 2002). Prolonged hyperthermia is also known to produce vascular collapse (shock), liver necrosis, kidney and myocardial failure, metabolic and electrolyte imbalances, metabolic acidosis, disseminated intravascular coagulation (DIC) and cerebral oedema (Fowler, 1978; Miller, 2000; Gfeller, 2005). Since hyperthermia triggers inflammation and cytotoxicity (Bouchama *et al*., 2005; Lambert, 2009) the time period an animal suffers from hyperthermia is as important as the magnitude of this hyperthermia (Bouchama *et al.,* 2002). The Cytotoxic effects of hyperthermia start occurring at 41°C in humans and most likely in animals too (Miller & Ziskin, 1989). When body temperature reaches 42°C to 43°C it approaches an animal's critical thermal maximum (CTM) (Leon, 2007). Such CTM is the body temperature which is incompatible with life as it produces cell necrosis and

denaturation of proteins (Leon, 2006). This CTM differs between species: 41.7°C for dogs, 43.5 for cats and higher than 44.5 for monkeys, just to mention some examples (Leon, 2006). There is no published information about CTM in blesbok or other antelope.

Harthoorn & Young (1974) found that when animals were over-exerted (pushed too hard) over a short distance they had higher body temperatures and lactic acid concentrations compared to animals that were chased for longer times, but not over-exerted. Similarly, when the study blesbok were chased in both treatment groups they developed high rectal and muscle temperatures (4x that of the control animals) as well as lactate concentrations. However, unlike the animals in Harthoorn & Young's (1974) study that develop a marked acidaemia, the blesbok in the treated groups only had a mild acidaemia. This finding was unexpected in light of their very high lactate levels, which normally would equate to a severe lactic-acidaemia and a low pH. The mild acidaemia can possibly be explained by the fact that these animals were hyperventilating, as indicated by hypocapnia. Therefore, the acidaemia was most likely compensated for by a respiratory alkalosis induced by hyperventilation.

Normally during moderate exercise the aerobic pathways in the mitochondria (Krebs cycle and B oxidation of free fatty acids) produce energy mainly from glucose, fatty acids (from circulating glucose or stores in muscle as glycogen) and oxygen in the form of ATP (Valberg, 2008). ATP is the main source of fuel for active muscles. During intense exercise, or over-exertion, like that seen during the capture of wild animals, oxygen requirements are outstripped by demand and therefore ATP can no longer be produced by oxidative means, but rather by less efficient anaerobic metabolism (Hinchcliff et al., 2004; Valberg, 2008). Anaerobic metabolism, occurring in the cell cytoplasm, triggers the production of pyruvic acid to lactate (Hinchcliff et al., 2004; Philp et al., 2005) by the enzyme lactate dehydrogenase. This lactate is either utilised as an energy source in the cells or if in excess is released into the blood where it is either excreted in the kidneys or

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converted back to glucose mainly in the liver and kidneys (Hinchcliff *et al.*, 2004; Valberg, 2008). Previously it was believed that if lactate accumulated in cells it formed lactic acid which was responsible for "muscle fatigue" (Lamb & Stephenson, 2006). However, it is now believed that muscle fatigue mostly occurs because of a disturbance in excitation contraction (EC) coupling (Lamb & Stephenson, 2006) which occurs with an accumulation of hydrogen ions (Valberg, 2008; Gladden, 2008 a) and electrolytes (like phosphorus and magnesium) together with a decrease in energy substrates in muscle fibres (ATP, creatine phosphate, glycogen) (Lamb & Stephenson, 2006; Bangsbo & Juel, 2006).

The chased blesbok had peak lactate concentrations that were between 8 (CNC) and 9 (C+C) times higher than animals in the Control group (1.6 mmol/L) just after the chase. The lactate concentrations remained elevated even 40 minutes into the immobilization. Lactate is normally metabolised very quickly (Hodgson & Rose, 1994; Gladden, 2008 a & b) therefore its high level at 40 minutes indicates that a large degree of anaerobic metabolism continued throughout the immobilization despite the animals only suffering from mild hypoxaemia. High lactate concentrations directly after the chase indicates that this chase caused the animals to exercise beyond their normal aerobic capacity and therefore anaerobic metabolism occurred to meet the energy requirements needed for continued muscle activity. Studies done in horses that were intensively exercised at maximal effort showed that they reached the threshold for anaerobic metabolism (where blood lactate concentrations increased) at 20-120 seconds after the start of this exercise (Piccione *et al.,* 2010). Other studies done in horses showed that, even though exercise stops, anaerobic metabolism continues and therefore blood lactate concentrations keep on rising (Hinchcliff *et al.*, 2008). In our study chased animals had an 8 (C+C group) – 9 (CNC group) fold increase in lactate concentrations immediately after the chase. However, after 40 minutes into the immobilization lactate levels of both chased groups were even higher (8.5x for C+C and 10x for CNC groups) indicating a greater activation of anaerobic metabolism or possibly saturation of its metabolism or excretion.

The running of wild animals while being chased can easily be compared to the performance of sprint athletes with the exception that wild animals are not usually trained or fit enough for this type of exertion. Athletic performance, from a metabolic point of view, involves fitness through creating adequate capacity of both aerobic and anaerobic metabolic pathways (Reeta Poso *et al*., 2008). The aerobic capacity of an individual is determined by the availability of oxygen and the ability of muscle tissue to optimally use oxygen for energy (Reeta Poso *et al*., 2008) and training can improve these. However, both the brain and heart muscles are specifically sensitive to oxygen deprivation and both organs have a very limited anaerobic capability (Hill *et al*., 2008). Therefore, when hypoxaemia occur, these organs are the first to be damaged. Excessive muscle activity can predispose animals to muscle breakdown (Clarkson *et al*., 2006) and exertional rhabdomyolisis leading to renal failure from the release of myoglobin (Vanholder *et al*., 2000; Lin *et al*., 2006; Paterson, 2007; Capacchione & Muldoon, 2009; Alpers & jones, 2010; Silberova *et al*., 2010).

In animals that are excessively exerted during capture, pathological lesions are normally found mainly in skeletal muscle, heart muscle, kidneys and in extreme cases even pulmonary oedema can occur (Harthoorn, 1975, 1976). In our chased blesbok skeletal muscle was only mildly damaged and mild hypoxic hepatic damage occurred (indicated by a moderate increase in GLDH). However, I believe that the high cTnI concentrations indicated major damage to the heart muscle of these chased animals. That there was greater damage to the heart muscle compared to skeletal muscle, and other organs, is probably a reflection of how sensitive the hearts of the blesbok were to the hypoxia induced by the intense chase, and to its limited anaerobic capacity.

Harthoorn (1976) also showed that excessive stress and over-exertion caused a greater hyperthermia and risk of capture myopathy in blesbok when he compared an intense chase over a short distance (< 2 km) to a slower but longer (> 2 km) chase. Generally, many wild antelope species are not accustomed to running fast and hard or over long distances and therefore are not

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prepared for intense or long chases associated with many capture procedures. Therefore, when they are pushed beyond their normal exercise limits this can lead to them developing both mental and physical exhaustion (Harthoorn, 1974). In the studies done by Harthoorn (1974 &1976) animals were specifically chased in a manner that caused over-exertion and excessive stress in order to induce capture myopathy. In this study even though the blesbok were chased continuously for 15 minutes, I don't believe that they over-exerted. Because the animals were accustomed (from boma husbandry procedures) to the humans that chased them they did not appear to run too hard when they were chased and the boma design did not allow them to run in a straight line and therefore they did not appear to reach maximum speed and thus a maximal "work out".

Kock *et al.*, (1987 a & b) also studied the pathophysiological effects of capture, which included a chase, in bighorn sheep. They suggested that body temperature, respiratory rate, glucose, pH, cortisol, creatine phosphokinase (CPK), serum glutamic oxaloacetic transaminase (SGOT), lactate deshidrogenase (LDH), potassium and white blood count (WBC) were parameters that could be used together to indicate the stress status and risk of morbidity and mortality. Bighorn sheep that had lower core body temperatures had a better outcome after capture than animals that had temperatures greater than 42.2°C at capture. A similar situation occurred in our Control group animals where their temperatures (mean 38.1°C) were approximately 4°C lower than the chased groups (CNC: 41.9°C and C+C: 42°C) and they had no indication of pathophysiological changes in their organs.

Several authors consider the measurement of plasma cortisol concentrations as a useful tool, and a single indicator for the assessment of a "stress response" in wild animals (Bubenik, 1982; Foreyt & Jessup, 1982; Spraker, 1982). However, Kock *et al.,* (1987 b) found that cortisol concentrations were not a good indicator of stress in captured bighorn sheep because it did not differ significantly between the animals based on their classification of stress status (normal, stressed, capture

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myopathy mortality and accidental mortality). However, they did find that cortisol concentrations were different depending on the method used for capture. Drive net and chemical immobilization caused greater increases in cortisol concentrations than drop net capture (Kock *et al*., 1987 a), an interesting finding considering that chemical immobilization is considered to be less stressful than methods which involve physical capture (Kock & Burroughs, 2012).

Unexpectedly, in our study cortisol concentrations did not differ significantly between the animals in the control and chased groups. Although there appeared to be differences between the groups, especially at the first sampling point (Figure 4.12, Day 0-1/2) there was a large degree of variability (SD) in the cortisol concentrations, and hence no difference could be detected. A possible explanation for this finding could be that the effects of the potent opioid etorphine, and the hypoxia caused by the immobilization, may have had a greater effect on cortisol concentration, thus masking any differences in the cortisol or stress response induced by the chase. Opioids are known to directly influence the release of cortisol (Drolet *et al*., 2001), and etorphine and the effects of hypoxic, are both known to be strong drivers for the activation of the sympathetic nervous system and potentially the release of cortisol too (Raff *et al*., 1981; Petraglia *et al*., 1990).

The validity of using only serum cortisol as a measure of a stress response during the capture of wildlife has been questioned by a number of researchers (Kock *et al*., 1987 a & b; Hattingh, 1988; Knox *et al*., 1991; Hattingh & Petty, 1992; Knox *et al*., 1993). These scientists propose that other parameters which reflect a stress response and pathophysiological changes should also be taken into account. Hattingh (1988) proposed a more objective assessment of the physiological response to the stress induced by capture. This assessment involves the evaluation of species specific relative increases, to maximum measurements, of eight different blood variables including haematocrit, osmolality, lactate, glucose, total protein, total lipid, cortisol and total catecholamines, and is named the species-specific response to stress (SSERTS) (Hattingh, 1988).

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The objective of our study wasn't to focus on evaluating the stress response to capture, therefore we did not measure all the parameters required to evaluate the SSERTS. However, because we believe that stress played a major role in the pathophysiology that developed in the blesbok we propose that these parameters should be measured and evaluated in similar future studies.

Although a better objective evaluation of a stress response is important during capture the effects of this stress on the outcomes of capture is equally, if not more important. Therefore, like Kock *et al.,* (1987 a & b) I believe that a stress status, that better predicts the risk of morbidity and mortality, should rather be determined. This status should be determined not only by measuring the parameters used to calculate the SSERTS, but also those that indicate pathophysiological effects including cardiac troponin I, muscle and liver enzymes, rectal and muscle temperatures, lactate and potentially other parameters like glucose, pH, K and white blood cell counts.

When Boesch *et al.,* (2011) compared two different capture methods (clover traps and ground-darting) in white-tail deer they found that body temperature, lactate, glucose, cortisol, CK, AST, LDH and cTnI increased when animals were captured and physically restrained in clover traps. Worth noting is that these animals were not chased but only physically restrained and probably developed greater pathophysiological changes as a result of a greater stress response to this method of capture. Results from our study revealed a similar outcome in the chased blesbok that likely had a greater stress response compared to animals in the Control group. Rectal temperatures were 38.2°C in the darted white tailed deer, which is similar to that of the blesbok in our Control group (38.1°C), while in trapped deer their temperatures were 39.5°C, which most likely reflects a greater stress response in these animals. Changes in muscle enzymes in these white tailed deer also revealed interesting insights into the effects of capture. Creatine kinase values in deer caught in clover-trapped were much greater (2919, 940 - 20.060 IU/l median, minimum - maximum) than those of ground darted deer (294, 1811 - 911 IU/l median, minimum -

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maximum), and even higher than those we observed in our chased blesbok (481 ± 297 IU/l in the CNC and 1807 ± 297 IU/l in the C+C groups). Clover-trapped deer also had higher levels of cTnI enzyme values (0.08, 0.01 – 1.11ng/ml median, minimum - maximum) than ground darted deer (< 0.01 ng/ml) and our Control blesbok group (0.02 ± 0.1 ng/ml), but these values were a lot lower than those measured in our chased blesbok (0.25 ± 0.16 ng/ml CNC group and 0.4 ± 0.48 ng/ml C+C group). The comparison of these parameters measured in Boesch's study (2011) and this study highlights that the stress of capture may play a major role in causing pathophysiology. However, it appears that differences occur in this pathophysiology, especially in certain organs, depending how animals are captured. Clover trapping, where animals are physically restrained, appeared to cause greater damage in skeletal muscles whereas capture involving a chase, as in our blesbok, appeared to cause greater cardiac muscle damage.

Cardiac troponin I enzyme is currently used in mammals to measure damage in cardiac myocytes. In humans, cardiac troponins are used in preference to creatine kinase – myocardial band (CK-MB), AST and LDH, which were all used previously as biomarkers to diagnose ischemic heart disease like myocardial infarctions (Sleeper *et al*., 2001; Babuin *et al*., 2005; Wells & Sleeper, 2008; Boesch *et al*., 2011). Cardiac troponins are members of a protein complex that include cTnI and cTnT and are part of the cardiac and skeletal contractile muscle apparatus (Wells & Sleeper, 2008; Scolletta *et al*., 2012). Cardiac troponin I is highly specific to myocardial tissue (Scolletta *et al*., 2012) and is determined as the biomarker of choice to assess cardiac damage in humans. Because it is highly homologous in mammals it has been used in different species like dogs (Sleeper *et al*., 2001; Oyama & Solter, 2004), cats (Sleeper *et al*., 2001; Connolly *et al*., 2003), horses (Rishniw & Simpson, 2005; Durando *et al*., 2006; Nostell & Häggström., 2008), ruminants (Leonardi *et al*., 2008; Varga *et al*., 2013) and even in wild animals like the white tailed deer (Boesch *et al*., 2015), to detect cardiac damage.

In humans cTnI is normally released, and only starts to increase, between 4 to 6 hours post cardiac ischaemic damage, with maximum values at 18 to 24 hours (Babuin *et al*., 2005). That we detected elevated values within a few minutes of the chase and capture, and very high values at 40 minutes into immobilization, may reflect a species difference in release or an acute more generalised damage in the blesbok. Cardiac troponin I concentrations remain elevated in humans with cardiac ischaemia for about 7 to 10 days (Babuin *et al*., 2005). In both chased groups cTnI was significantly elevated on day 2 and was even mildly elevated on day 16 in the CNC compared to the Control group.

Currently, human cardiac troponin I is used in combination with CK-MB as the most common biomarkers in the diagnoses of cardiac muscle ailments (Scolletta *et al*., 2012). However, new biomarkers are showing promising results in the diagnosis, monitoring and determining prognosis of different heart conditions. One of these biomarkers is brain natriuretic peptide (BNP) (Scolletta *et al*., 2012). BNP is produced by the cells of the cardiac ventricle and is released in response to pressure or volume overload (Scolletta *et al*., 2012). Currently, species specific BNP is used in domestic animals (cats & dogs) to assess and screen for different cardiomyopathies (Wess *et al.,* 2011). Other useful biomarkers for the detection of cardiac arrest and other cardiac diseases are acute phase proteins (C-reactive protein-CRP), cytokines and necrosis factors (TNF-α) (Scolletta *et al*., 2012) which can detect inflammation. The value of these biomarkers in determining the pathophysiological effects of capture and the prognosis of capture-related complications should be explored in the future.

I believe that the acutely and decidedly elevated cTnI concentration clearly indicates that a stressful chase negatively affects an animal's heart during capture. Despite the high cTnI concentrations none of the blesbok in the study succumbed post capture. It would be interesting to determine if chronic cardiac lesions developed in these animals (histopathology samples from day

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16 and 30 still need to be analysed) and if these lesions could result in subsequent mortalities if the animals were captured again. Harthoorn (1976) describes a chronic form of capture myopathy where animals only succumb, mainly from acute heart failure, to the effects of capture when they are captured for a second time, weeks to months later. Also worth clarifying in blesbok, is whether different cTnI concentrations reflect the magnitude of cardiac damage and if there is a threshold concentration that indicates irreparable damage or damage that causes acute mortality.

As mentioned previously, most of the pathophysiologic effects that we observed occurred in the short-term, within 16 days post-capture and were probably primarily as a consequence of the "fight or flight response" and being chased. However, work done by Cattet *et al.,* (2008) and Macbeth *et al.,* (2010) on the long-term effects (> 30 days) of capture in ursids (bears) showed clear evidence of detrimental effects of capture over a longer time. Some of these detrimental effects were assumed to be as a result of increased muscle injury, where movement rates of most bears were negatively affected for a number of weeks post-capture as well as a decrease in body condition (Cattet *et al.,* 2008). Other negative effects seen were immunosuppression, decreased reproduction and low growth rates (Macbeth *et al*., 2010). These detrimental effects were proportionally associated to the amount of times the animals were captured and were worse in older animals. The long-term consequences of capture, in African antelope, have not been assessed properly. Therefore, future studies need to be done to assess these consequences and how they are associated with the short-term pathophysiological effects of capture.

Why some animals succumb to the consequences of capture, while others survive, needs further investigation. What role cardiac muscle damage plays in the long-term survival of animals post capture, and whether cardiac lesions predispose animals to damage in other tissues, like skeletal muscle (i.e. classic capture myopathy), needs to be determined. Future efforts should also be made to find a practical way to assess animals that have been captured in the field to determine

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whether they will suffer from capture-related complications after capture procedures. A simple but effective assessment method is urgently needed to help wildlife veterinarians identify and treat animals that may suffer from capture-related complications. What the ideal treatment is also needs to be determined and whether exercise training before capture, as suggested by Harthoorn (1975, 1976), prevents capture-related complications should be investigated. The answers to all these questions are urgently needed in order for the wildlife community to improve wild animal welfare during and after capture procedures.

Chapter 6

Conclusion

Blesbok that were captured by darting in their holding bomas, in a way that caused minimal disturbance, did not develop hyperthermia or any other pathophysiological effects. However, when a mock field capture procedure was mimicked by chasing blesbok for 15 minutes continuously, before they were immobilized by darting, they developed pathophysiological effects. These effects included hyperthermia, hyperlactaemia, hypoxaemia, hypocapnia, mild acidaemia and mild skeletal muscle and liver damage. These chased animals also had very high concentrations of serum cardiac troponin I, most likely indicating extensive cardiac muscle damage. All these pathophysiological effects, as indicated by serum biochemistry, appeared only to be active in the short term (< 16 days post capture).

Dousing chased animals with cold water corrected their hyperthermia but it did not treat or prevent the pathophysiology effects that the chase caused; hepatic and muscle enzymes remained elevated for up to two days irrespective of whether chased animals were cooled or not. Therefore, I believe that this capture-induced hyperthermia played a limited role in causing the pathophysiological effects seen, but rather it is itself a pathophysiological outcome. Its magnitude, therefore, may be a good indicator of the extent of the other pathophysiology that occurs during capture.

The elevated lactate and GLDH in the chased animals, measured soon after the chase, indicated that hypoxia occurred during the chase and negatively affected some tissues. This hypoxia, and its affects, continued after the chase and persisted throughout the immobilization. I believe that it is this hypoxia, rather than the hyperthermia, that was the primary cause of organ damage and the

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metabolic derangements seen in the chased blesbok. This hypoxia, which was most likely caused by hypermetabolism induced by exertion and stress, appeared to mainly affect the heart, an organ that has limited anaerobic metabolic capacity.

Therefore, based on the data collected, capture that involves chasing animals before immobilization appears to mainly cause cardiac muscle damage. This capture-induce cardiomyopathy may have profound secondary consequences, which could result in mortality, or play a role in the pathophysiology that results in skeletal muscle, kidney and other tissue damage that normally is seen in classical capture myopathy.

Appendices A

Animal Ethics Committee

PROJECT TITLE	Determination of the pathophysiological consequences of capture and capture-induced hyperthermia in wildlife
PROJECT NUMBER	V047-14
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. A Fitte

STUDENT NUMBER (where applicable)	1411 0289
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	Wild antelope (Blesbok)	
NUMBER OF ANIMALS	40	
Approval period to use animals for research/testing purposes		August – November 2014
SUPERVISOR	Dr. L Meyer	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	30 June 2014
CHAIRMAN: UP Animal Ethics Committee	Signature	

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Bibliography

Adin, D. B., Oyama, M. A., Sleeper, M. M., & Milner, R. J. (2006). Comparison of canine cardiac troponin I concentrations as determined by three analyzers. Journal of Veterinary Internal Medicine, 20(5), pp. 1136-1142.

Aleman, M., Aldridge, B. M., Riehl, J., LeCouteur, R. A., Stott, J. L. & Pesaah, I. N. (2004). Equine malignant hyperthermia. In Proceedings of the 50th Annual Convention of the American Association of Equine Practitioners, Denver, Colorado, USA. American Association of Equine Practitioners (AAEP), pp. 51-54.

Allen, W. M., Patterson, D. S., Harding, J. D., & Berrett, S. (1970). Experimentally induced acute stress syndrome in Pietrain pigs. Veterinary Record, 87(3), pp. 64-69.

Alpers, J. P., & Jones, L. K. (2010). Natural history of exertional rhabdomyolysis: A population-based analysis. Muscle & Nerve, 42(4), pp. 487-491.

Antognini, J. F., Eisele, P. H. & Gronert, G. A. (1996). Evaluation for malignant hyperthermia susceptibility in black-tailed deer. Journal of Wildlife Diseases, 32(4), pp. 678-681.

Babuin, L., & Jaffe, A. S. (2005). Troponin: the biomarker of choice for the detection of cardiac injury. Canadian Medical Association Journal, 173(10), pp. 1191-1202.

Banci, V. & Proulx, G. (1999). Resiliency of furbearers to trapping in Canada. Mammal Trapping. Alpha Wildlife Publications, Sherwood Park, Alberta, Canada, pp.175-203.

Bancroft, J. D., Cook, H. C., & Turner, D. R. (2000). Manual of histological techniques and their diagnostic application–Churchill Livingstone. Edinburgh, London, Madrid, Melbourne, New York, Tokyo.

Bangsbo, J., & Juel, C. (2006). Reply: The Last Word: Point: Counterpoint authors respond to commentaries on "Lactic acid accumulation is an advantage/disadvantage during muscle activity". Journal of Applied Physiology, 101(1), pp. 369-370.

Barbut, S., Sosnicki, A. A., Lonergan, S. M., Knapp, T., Ciobanu, D. C., Gatcliffe, L. J., Huff-Lonerganc, E. & Wilson, E. W. (2008). Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Science, 79(1), pp. 46-63.

Başbuğan, Y., Ağaoğlu, Z., & Yüksek, N. (2010). An investigation on serum troponin concentration in healthy ruminants. Kafkas Univ Vet Fak Derg, Turkey, 16(4), pp. 641-645.

Bennett, A. F. (1985). Temperature and muscle. Journal of Experimental Biology, 115(1), pp. 333-344.

Bhadane, K. P., Upadhyay, S. R., Roy, S. & Soodan, J. S. (2007). Metabolic disorders in pigs -an overview. Intas Polivet, 8(2), pp. 509-511.

Bouchama, A., Roberts, G., Al Mohanna, F., El-Sayed, R., Lach, B., Chollet-Martin, S., Olivier, V., Al Baradei, R., Nakeeb, S., Eldali, A. & de Prost, D. (2005). Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. Journal of Applied Physiology, 98(2), pp. 697-705.

Bouchama, A. & Knochel, J.P. (2002). Heat stroke. New England Journal of Medicine 346(25), pp. 1978-1988.

Bouwknecht, J. A., Olivier, B., & Paylor, R. E. (2007). The stress-induced hyperthermia paradigm as a physiological animal model for anxiety: a review of pharmacological and genetic studies in the mouse. Neuroscience & Biobehavioral Reviews, 31(1), pp. 41-59.

Broekman, M. S. (2013). Detection of hyperthermia during capture of wild antelope (MSc dissertation, Faculty of Science, University of Witswatersrand).

Boesch, J. M., Gleed, R. D., Erb, H. N., & Solter, P. F. (2015). Laboratory validation of a point-of-care cardiac troponin I assay for use in white-tailed deer (Odocoileus virginianus). Journal of Zoo and Wildlife Medicine, 46(3), pp. 468-475.

Boesch, J. M., Boulanger, J. R., Curtis, P. D., Erb, H. N., Ludders, J. W., Kraus, M. S., & Gleed, R. D. (2011). Biochemical variables in free-ranging white-tailed deer (Odocoileus virginianus) after chemical immobilization in clover traps or via ground-darting. Journal of Zoo and Wildlife Medicine, 42(1), pp. 18-28.

Bubenik, G.A. (1982). Chemical immobilization of captive white-tailed deer and the use of automatic blood samplers. In Chemical immobilization of North American Wildlife, L. Nielsen, J. C. Haigh, and M.E. Fowler (eds). Wisconsin Humane Society, Milwaukee, Wisconsin, pp. 335-354.

Burroughs R. (1993). Chemical capture of antelope in The Capture and Care Manual. McKenzie, A. Wildlife Decision Support Services CC and the South African Veterinary Association, Pretoria, South Africa, pp. 348-380.

Burroughs R. (1993). Chemical capture of antelope in The Capture and Care Manual. McKenzie, A. Wildlife Decision Support Services CC and the South African Veterinary Association, Pretoria, South Africa, pp. 440-464.

Capacchione, J. F. & Muldoon, S. M. (2009). The relationship between exertional heat illness, exertional rhabdomyolisis, and malignant hyperthermia. Anesthesia and Analgesia, 109(4), pp. 1065-1069.

Cattet, M., Boulanger, J., Stenhouse, G., Powell, R. A. & Reynolds-Hogland, M. J. (2008). An evaluation of long-term capture effects in ursids: implications for wildlife welfare and research. Journal of Mammalogy, 89(4), pp. 973-990.

Chardonnet, P., Clers, B. D., Fischer, J., Gerhold, R., Jori, F. & Lamarque, F. (2002). The value of wildlife. Revue Scientifique et Technique-Office International des Epizooties, 21(1), pp. 15-52.

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Cheney, C. S. & Hattingh, J. (1988). Effects of chemical immobilisation on the blood composition of impala (Aepyceros melampus) (Lichtenstein). Journal of the South African Veterinary Association, 59(1), pp. 13-18.

Clarkson, P. M., Kearns, A. K., Rouzier, P., Rubin, R., & Thompson, P. D. (2006). Serum creatine kinase levels and renal function measures in exertional muscle damage. Medicine and Science in Sports and Exercise, 38(4), pp. 623

Cooper, J.E. (1996). Physical Injury, in Fairbrother, A., Locke, N.L. & Hoff, G.L. (2nd eds). Non-infectious Diseases of Wildlife. Iowa State University Press. Iowa, USA, pp. 157-172

Connolly, D. J., Cannata, J., Boswood, A., Archer, J., Groves, E. A., & Neiger, R. (2003). Cardiac troponin I in cats with hypertrophic cardiomyopathy. Journal of Feline Medicine and Surgery, 5(4), pp. 209-216.

Dickman, A. J. (2010). Complexities of conflict: the importance of considering social factors for effectively resolving human–wildlife conflict. Animal Conservation, 13(5), pp. 458-466.

Dimsdale, J. E. (2008). Psychological stress and cardiovascular disease. Journal of the American College of Cardiology, 51(13), pp. 1237-1246.

Durando, M. M., Reef, V. B., Kline, K., & Birks, E. K. (2006). Acute effects of short duration, maximal exercise on cardiac troponin I in healthy horses. Equine and Comparative Exercise Physiology, 3(04), pp. 217-223.

Drolet, G., Dumont, É. C., Gosselin, I., Kinkead, R., Laforest, S., & Trottier, J. F. (2001). Role of endogenous opioid system in the regulation of the stress response. Progress in Neuro-Psychopharmacology and Biological Psychiatry, *25*(4), pp. 729-741.

Dry, G. C. (2010). Commercial wildlife ranching's contribution to the "green economy". Proceedings of the South African Wildlife Management Association, pp. 2-4.

Edwards, M. J. (1968). Congenital malformations in the rat following induced hyperthermia during gestation. Teratology, 1(2), pp. 173-177.

Edwards, M. J., Mulley, R., Ring, S. & Wanner, R. A. (1974). Mitotic cell death and delay of mitotic activity in guinea-pig embryos following brief maternal hyperthermia. Journal of Embryology and Experimental Morphology, 32(3), pp. 593-602.

Elsasser, T. H., Klasing, K. C., Filipov, N., & Thompson, F. (2000). The metabolic consequences of stress: targets for stress and priorities of nutrient use. The Biology of Animal Stress. Eds. by G. P. Moberg & J. A. Mench, CABI, UK, pp. 77-110.

Epstein, Y., & Roberts, W. O. (2011). The pathophysiology of heat stroke: an integrative view of the final common pathway. Scandinavian Journal of Medicine & Science in Sports, 21(6), pp. 742-748.

Esch, T., Stefano, G. B., Fricchione, G. L., & Benson, H. (2002). Stress in cardiovascular diseases. Medical Science Monitor, 8(5), RA93-RA101.

Fahlman, Å., Arnemo, J. M., Swenson, J. E., Pringle, J., Brunberg, S., & Nyman, G. (2011). Physiologic evaluation of capture and anesthesia with medetomidine-zolazepam-tiletamine in brown bears (*Ursus arctos*). Journal of Zoo and Wildlife Medicine, 42(1), pp 1-11.

Foreyt, W. J., & Jessup, D. A. (1982). Fatal pneumonia of bighorn sheep following association with domestic sheep. Journal of Wildlife Diseases, 18(2), pp 163-168.

Fowler, M. E., & Miller, R. E. (2008). Zoo and wild animal medicine: current therapy (Vol. 6). Elsevier Health Sciences.

Fowler, M. E. (1986). "Zoo and wild animal medicine." In Zoo and Wild Animal Medicine, 2nd Ed. WB Saunders Co., Philadelphia, Pennsylvania, USA.

Fowler, M. E. (1978). Restraint and handling of wild and domestic animals. Iowa State University Press, Ames. Iowa, USA.

Gericke, M. D., Hofmeyr, J. M. & Louw, G. N. (1978). The effect of capture stress and haloperidol therapy on the physiology and blood chemistry of springbok (Antidorcas marsupialis). Madoqua, 11(1), pp. 5-18.

Gericke, M.D & Hofmeyer, J.M. (1976). Aetiology and treatment of capture stress and myopathy in springbok (Antidorcas marsupialis). South African Journal of Science. Vol 72(1), pp. 28-28.

Gfeller, R., (2005). Heatstroke in Ettinger, S.J. & Feldman, E.C. Textbook of Veterinary Internal Medicine (6th eds). Elsevier. (I) pp. 437-439

Gladden, L. B. (2008a). Current trends in lactate metabolism: introduction. Medicine and Science in ports and Exercise, 40(3), pp. 475-476.

Gladden, L. B. (2008b). A lactatic perspective on metabolism. Medicine and Science in Sports and Exercise, 40(3), pp. 477-485.

Guyton textbook of medical physiology. (1991) (8th ed.). Saunders, Philadelphia, pp. 667.

Haas, E. (1981). 50 Diagnostic special stains for surgical pathology. J B Lippincott Company, Philadelphia.

Hattingh, J., & Petty, D. (1992). Comparative physiological responses to stressors in animals. Comparative Biochemistry and Physiology Part A: Physiology, *101*(1), pp 113-116.

Hattingh, J. (1988). Comparative quantitation of the physiological response to acute stress in impala and roan antelope. Comparative Biochemistry and Physiology Part A: Physiology, 89(4), pp. 547-551.

Harthoorn, A.M., (1981). Comparison of 2 methods of capture of wild animals. Veterinary Record 108: 37

Harthoorn, A. M. (1979). The use of corrals to capture and train wild ungulates prior to relocation. Veterinary Record, 104(15), pp. 349-349.

Harthoorn, A.M. (1976). Quinquennial Annual Report. Physiology of capture myopathy. Transvaal Nature Conservation Division. Pretoria, South Africa.

Harthoorn, A.M. (1975). Fourth Annual Report. Physiology of capture myopathy: part three. Transvaal Nature Conservation Division. Pretoria, South Africa.

Harthoorn, A. & Young, E. (1974). A relationship between acid-base balance and capture myopathy in zebra (Equus burchelli) and an apparent therapy. Veterinary Record 95(15), pp. 337-342.

Harthoorn, A. M. (1972). Restraint and neuroleptanalgesia in ungulates. Veterinary Record 91(3), pp. 63-67.

Harthoorn, A. M. (1965). Application of pharmacological and physiological principles in restraint of wild animals. Wildlife Monographs, (14), pp. 3-78.

Harthoorn, A.M. (1965). Continuous radiotelemetric records of the deep body temperature of some unrestrained African mammals under near-natural conditions. Journal of Physiology. 176, pp. 145-162

Harthoorn, A. M. (1962). Translocation as a means of preserving wild animals. Oryx, 6(04), pp. 215-227.

Hartley, W.J., Alexander, J. & Edwards, M.J. (1974). Brain cavitation and microencephaly in lambs exposed to prenatal hyperthermia. Teratology 9:299-300.

Haskins, S. C. (1995). Thermoregulation, hypothermia, hyperthermia. Veterinary Internal Medicine, 1, pp 26-30.

Hattingh, J. (1988). Comparative quantitation of the physiological response to acute stress in impala and roan antelope. Comparative Biochemistry and Physiology Part A: Physiology, 89(4), pp. 547-551.

Hattingh, J., & Petty, D. (1992). Comparative physiological responses to stressors in animals. Comparative Biochemistry and Physiology Part A: Physiology, 101(1), pp. 113-116.

Hill, R. W., Wyse, G. A., & Anderson, M. (2008). Animal physiology. Sunderland, MA, Sinauer Associates.

Hinchcliff, Kenneth William, Andris J. Kaneps, and Raymond J. Geor. (2008). Equine exercise physiology: The science of exercise in the athletic horse. Elsevier Health Sciences. pp. 350-382

Hinchcliff, Kenneth W., Andris J. Kaneps, and Raymond J. Geor. (2004) (First ed.). Equine Sports Medicine and Surgery. Elsevier Health Sciences,

http://www2.isis.org/Pages/Home.aspx, Accessed on 11 July 2016.

https://ahdc.vet.cornell.edu/sects/clinpath/reference/chem.cfm

Hodgson, D. R., & Rose, R. J. (1994). Evaluation of performance potential. Hodgson, DH, Rose, RJ Principles and practice of equine sports medicine: The Athletic Horse. WB Saunders Company, pp. 361-365

Hofmeyr, J. M. (1974). Developments in the capture and airlift of roan antelope *Hippotragus equinus equinus* under narcosis to the Etosha National Park. Madoqua, 8, pp. 37-48.

Hofmeyr, J.M., Louw, G.N. & du Preez, J.S. (1973). Incipient capture myopathy as revealed by blood chemistry of chased zebras. Madoqua, I (7) pp. 45-50.

Hopkins, P. M. (2007). Is there a link between malignant hyperthermia and exertional heat illness?. British Journal of Sports Medicine, 41(5), pp. 283-284.

ISIS, (1999). International Species Information System, Damaliscus pygargus. http://www.species360.org

Jungermann, K., & Kietzmann, T. (2000). Oxygen: modulator of metabolic zonation and disease of the liver. Hepatology, 31(2), pp. 255-260.

Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (Eds.). (2008). Clinical biochemistry of domestic animals. Academic press.

Knox, C. M., Zeller, D. A., & Hattingh, J. (1993). Comparison of two methods for the capture of boma-confined impala. South African Journal of Wildlife Research-24-month delayed open access, 23(1), pp. 1-5.

Knox, C. M., Hattingh, J., & Raath, J. P. (1992). Physiological responses of boma-confined impala to repeated capture. *South African Journal of Wildlife Research*, *22*(1), pp. 1-6.

Knox, C. M., Hattingh, J., & Raath, J. P. (1991). Physiological changes related to nutritional status in boma-confined impala. South African Journal of Wildlife Research, 21(4), pp. 109-113.

Kock M.C & Burroughs R, (eds.) (2012), Chemical and Physical Restraint of Wild Animals, A Training and Field Manual for African Species. (2nd eds). IWVS, South Africa.

Kock, M. D., Meltzer, D. & Burroughs, R. (2006). Chemical and physical restraint of wild animals. A training and field manual for African species. Zimbabwe Veterinary Association Wildlife Group and International Wildlife Veterinary Services (Africa), South Africa.

Kock, M. D., du Toit, R., Kock, N., Morton, D., Foggin, C., & Paul, B. (1990). Effects of capture and translocation on biological parameters in free-ranging black rhinoceroses (Diceros bicornis) in Zimbabwe. Journal of Zoo and Wildlife Medicine, pp. 414-424.

Kock, M. D., Clark, R. K., Franti, C. E., Jessup, D. A. & Wehausen, J. D. (1987 a). Effects of capture on biological parameters in free-ranging bighorn sheep (Ovis canadensis): evaluation of normal, stressed and mortality outcomes and documentation of post capture survival. Journal of Wildlife Diseases, 23(4), pp. 652-662.

Kock, M.D., Clark, R.K., Franti, C.E. & Jessup, D.A., (1987 b). Effects of capture on biological parameters in free-ranging bighorn sheep (Ovis canadensis): evaluation of drop-net, drive-net, chemical immobilization and the net-gun', Journal of Wildlife Diseases 23(4), pp. 641-651.

Kock, M. D., Jessup, D. A., Clark, R. K., Franti, C. E., & Weaver, R. A. (1987 c). Capture methods in five subspecies of free-ranging bighorn sheep (Ovis Canadensis): an evaluation of drop-net, drive-net, chemical immobilization and the net-gun. Journal of Wildlife Diseases, 23(4), pp. 634-640.

Kregel, K. C. (2002). Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology, 92(5), pp. 2177-2186.

La Grange M., Van Rooyen, J. & Ebedes, H. (2010). Capturing wild animals in du P Bothma, J. & du Toit, J.G. (5th eds) Game Ranch Management. Van Schaik Publishers. Pretoria.

Lambert, G. P. (2009). Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. Journal of Animal Science, 87 (14 suppl), E101-E108.

Lambert, G. P., C. V. Gisolfi, D. J. Berg, P. L. Moseley, L. W. Oberley, and K. C. Kregel. (2002). Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. Journal of Applied Physiology. 92, pp.1750–1761.

Lamb, G. D., & Stephenson, D. G. (2006). Point: Counterpoint: Lactic acid accumulation is an advantage/disadvantage during muscle activity. Journal of Applied Physiology, 100(4), pp. 1410-1412.

Latimer, K. S. (Ed.). (2011). Duncan and Prasse's veterinary laboratory medicine: clinical pathology. John Wiley & Sons.

Lawrie, R. A. (1960). Post mortem glycolysis in normal and exudative longissimus dorsi muscles of the pig in relation to so-called white muscle disease. Journal of Comparative Pathology and Therapeutics, 70, 273-IN14.

Leon, L. R., & Helwig, B. G. (2010). Heat stroke: role of the systemic inflammatory response. Journal of Applied Physiology, 109(6), pp. 1980-1988.

Leon, L. R. (2007). Heat stroke and cytokines. Progress in Brain Research, 162, pp. 481-524.

Leon, L. R. (2006). The thermoregulatory consequences of heat stroke: Are cytokines involved? Journal of Thermal Biology, 31(1), pp. 67-81.

Leonardi, F., Passeri, B., Fusari, A., De Razza, P., Beghi, C., Lorusso, R., Corradi, A., & Botti, P. (2008). Cardiac troponin I (cTnI) concentration in an ovine model of myocardial ischemia. Research in Veterinary Science, 85(1), pp. 141-144.

Lin, H., Chie, W., & Lien, H. (2006). Epidemiological analysis of factors influencing an episode of exertional rhabdomyolysis in high school students. The American Journal of Sports Medicine, 34(3), pp. 481-486.

Macbeth, B. J., Cattet, M. R. L., Stenhouse, G. B., Gibeau, M. L., & Janz, D. M. (2010). Hair cortisol concentration as a non-invasive measure of long-term stress in free-ranging grizzly bears (Ursus arctos): considerations with implications for other wildlife. Canadian Journal of Zoology, 88(10), pp. 935-949.

Manea, M. A., Kevorkian, S., Georgescu, S. E., Dinischiotu, A. & Costache, M. (2008). Diagnostication of malignant hyperthermia in swine. Scientific Papers Animal Science and Biotechnologies, 41(1), pp. 117-121.

Mann, F.A & Helmick, K.E. (1996). Shock in Fairbrother, A., Locke, N.L. & Hoff, G.L. (2nd eds). Non-infectious Diseases of Wildlife. Iowa State University Press. pp. 173- 180.

Martucci, R. W., Jessup, D. A., Gronert, G. A., Reitan, J. A., & Clark, W. E. (1992). Blood gas and catecholamine levels in capture stressed desert bighorn sheep. Journal of Wildlife Diseases, 28(2), pp. 250-254.

McKenzie, A. A. (1993). Capture and care manual. Wildlife Decision Support Services; South African Veterinary Foundation.

Meddings, J. B. & Swain, M. G. (2000). Environmental stress-induced gastrointestinal permeability is mediated by endogenous glucocorticoids in the rat. Gastroenterology, 119(4), pp. 1019-1028.

Meltzer, D., & Kock, N. (2006). Stress and capture related death. Chemical and physical restraint of wild animals. International Wildlife Veterinary Services (Africa), Greyton, pp. 68-76.

Meyer, L. C. R., Hetem, R. S., Mitchell, D., & Fuller, A. (2015). Hypoxia following etorphine administration in goats (Capra hircus) results more from pulmonary hypertension than from hypoventilation. BMC Veterinary Research, 11(1), pp. 1.

Meyer, L. C. R. (2010). Reduction of capture-induced hyperthermia and respiratory depression in ungulates (Doctoral dissertation, Faculty of Science, University of the Witwatersrand).

Meyer, L. C., Fick, L., Matthee, A., Mitchell, D. & Fuller, A. (2008 a). Hyperthermia in captured impala (Aepyceros melampus): a fright not flight response. Journal of Wildlife Diseases, 44(2), pp. 404-416.

Meyer, L. C. R., Hetem, R. S., Fick, L. G., Matthee, A., Mitchell, D. & Fuller, A. (2008 b). Thermal, cardiorespiratory and cortisol responses of impala (Aepyceros melampus) to chemical immobilisation with 4 different drug combinations. Journal of the South African Veterinary Association, 79(3), pp. 121-129.

Miller, J.B. (2000). Hyperthermia and Hypothermia in Ettinger, S.J. & Feldman, E.C. Textbook of Veterinary Internal Medicine (5th eds). Saunders. (2) pp. 6-10.

Miller, M. W., & Ziskin, M. C. (1989). Biological consequences of hyperthermia. Ultrasound in medicine & biology, 15(8), pp. 707-722.

Mitchell, G., & Heffron, J. J. A. (1982). Porcine stress syndromes. Advances in Food Research, 28, pp. 167-230.

Mitchell, G. & Heffron, J. J. A. (1980). Porcine stress syndromes: a mitochondrial defect? South African Journal of Science, 76(12), pp. 546-551.

Moberg, G. P., & Mench, J. A. (Eds.). (2000). The biology of animal stress: basic principles and implications for animal welfare. CABI, pp. 1-21

Montané, J., Marco, I., López-Olvera, J. R., Rossi, L., Manteca, X. & Lavín, S. (2007). Effect of acepromazine on the signs of capture stress in captive and free-ranging roe deer (Capreolus capreolus). Veterinary Record 160(21), pp. 730-738.

Montané, J., Marco, I., López-Olvera, J., Perpinan, D., Manteca, X. & Lavin, S. (2003). Effects of acepromazine on capture stress in roe deer (Capreolus capreolus). Journal of Wildlife Diseases, 39(2), pp. 375-386.

Morton, D. J., Anderson, E., Foggin, C. M., Kock, M. D., & Tiran, E. P. (1995). Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. The Veterinary Record, 136(3), pp. 60-63.

Nybo, L., Møller, K., Volianitis, S., Nielsen, B., & Secher, N. H. (2002). Effects of hyperthermia on cerebral blood flow and metabolism during prolonged exercise in humans. Journal of Applied Physiology, 93(1), pp. 58-64.

Nostell, K., & Häggström, J. (2008). Resting concentrations of cardiac troponin I in fit horses and effect of racing. Journal of Veterinary Cardiology, 10(2), pp. 105-109.

Oyama, M. A., & Solter, P. F. (2004). Validation of an immunoassay for measurement of canine cardiac troponin-I. Journal of Veterinary Cardiology, 6(2), pp. 17-24.

Paterson, J. (2007). Capture myopathy. Zoo Animal and Wildlife Immobilization and Anesthesia. G. West, D. Heard, and N. Caulkett, ed. Blackwell Publishing Ltd., Oxford, UK, pp. 115-122.

Petraglia, F., Modena, A. B., Comitini, G., Scazzina, D., Facchinetti, F., Fiaschetti, D., Genazzani, A.D., Barletta, C., Scavo, D. & Genazzani, A.R, (1990). Plasma beta-endorphin and beta-lipotropin

levels increase in well trained athletes after competition and non-competitive exercise. Journal of Endocrinological Investigation, 13(1), pp. 19-23.

Philp, A., Macdonald, A. L., & Watt, P. W. (2005). Lactate–a signal coordinating cell and systemic function. Journal of Experimental Biology, 208(24), pp. 4561-4575.

Piccione, G., Messina, V., Casella, S., Giannetto, C., & Caola, G. (2010). Blood lactate levels during exercise in athletic horses. Comparative Clinical Pathology, 19(6), pp. 535-539.

Raff, H., Tzankoff, S. P., & Fitzgerald, R. S. (1981). ACTH and cortisol responses to hypoxia in dogs. Journal of Applied Physiology, 51(5), pp. 1257-1260.

Reeta Poso, A., Hyyppa, S., Geor, R.J, (2008). Metabolic responses to exercise and training, in Animal Physiology. pp. 771-792.

Rishniw, M., & Simpson, K. W. (2005). Cloning and sequencing of equine cardiac troponin I and confirmation of its usefulness as a target analyte for commercial troponin I analyzers. Journal of Veterinary Diagnostic Investigation, 17(6), pp. 582-584.

Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. Trends in Ecology & Evolution, 19(5), pp. 249-255.

Ronderos, R. E., Avegliano, G., & Dallasta, G. (2009). Stress-induced cardiomyopathy. Current Cardiovascular Imaging Reports, 2(5), pp. 332-342.

Rosengberg, H., Davis, M., James, D., Pollock, N. & Sotwell, K. (2007). Malignant Hyperthermia: Review. Orphanet Journal of Rare Diseases. (2) pp. 21.

Sawicka, J. (2011). Cooling methods to treat capture-induced hyperthermia in blesbok (Damaliscus dorcas phillipsi) (MSc dissertation, Faculty of Science, University of Witswatersrand).

Sawicka, J., Fuller, A., Fick, L. G., Hetem, R. S., & Meyer, L. C. (2015). Efficacy of different cooling methods for capture-induced hyperthermia in antelope. African Journal of Wildlife Research, 45(1), pp. 100-110.

Schumacher, J. (2008). Side effects of Etorphine and Carfentanil in non-domestic hoofstock in Miller, R.E & Fowler, M.E. Zoo and Wildlife Medicine: Current Therapy 6. Saunders, Elsevier. (56) pp. 455-461.

Scolletta, S., Donadello, K., Santonocito, C., Franchi, F., & Taccone, F. S. (2012). Biomarkers as predictors of outcome after cardiac arrest. Expert Review of Clinical Pharmacology, 5(6), pp. 687-699.

Shemesh, O., Golbetz, H., Kriss, J. P., & Myers, B. D. (1985). Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney International, 28(5), pp. 830-838.

Shepherd, N. C., Hopwood, P. R. & Dostine, P. L. (1988). Capture Myopathy-Two Techniques for Estimating Its Prevalence and Severity in Red Kangaroos (Macropus-Rufus). Wildlife Research, 15(1), pp. 83-90.

Silberová, P., Lukešová, D., & Pinc, L. (2010). The determination of blood profile in captive wild ungulates with focus on eland (*Taurotragus oryx*). Agricultura Tropica et Subtropica, 43, pp. 4.

Sleeper, M. M., Clifford, C. A., & Laster, L. L. (2001). Cardiac troponin I in the normal dog and cat. Journal of Veterinary Internal Medicine, 15(5), pp. 501-503.

Smith, J. E. (2005). Cooling methods used in the treatment of exertional heat illness. British Journal of Sports Medicine, 39(8), pp. 503-507.

Spraker, T.R., (1993), 'Stress and Capture Myopathy in Artiodactylids', M.E. Fowler (Ed), Zoo and Wild Animal Medicine – Current Therapy 3, W.B. Saunders, Philadelphia, pp. 481-488

Stratakis, C. A., & Chrousos, G. P. (1995). Neuroendocrinology and pathophysiology of the stress system. Annals of the New York Academy of Sciences, 771(1), pp. 1-18.

Thompson, James M., and Howard H. Meyer. Body condition scoring of sheep. [Corvallis, Or.]: Oregon State University, Extension Service, 1994.

Tong, H., Wan, P., Zhang, X., Duan, P., Tang, Y., Chen, Y., Tang, L. & Su, L. (2013). Vascular Endothelial Cell Injury Partly Induced by Mesenteric Lymph in Heat Stroke. Inflammation, 37(1), pp. 27-34.

Valberg, S. J., Mickelson, J. R., Gallant, E. M., Mac Leay, J. M., Lentz, L. & Corte, F. (1999). Exertional rhabdomyolysis in quarter horses and thoroughbreds: one syndrome, multiple aetiologies. Equine Veterinary Journal, 31(S30), pp. 533-538.

Valberg, S. J. (2008). Skeletal muscle function. Clinical Biochemistry of Domestic Animals, 6, pp. 459-484.

Varga, A., Angelos, J. A., Graham, T. W., & Chigerwe, M. (2013). Preliminary investigation of cardiac troponin I concentration in cows with common production diseases. Journal of Veterinary Internal Medicine, 27(6), pp. 1613-1621.

Vanholder, R., Sever, M. S., Erek, E., & Lameire, N. (2000). Rhabdomyolysis. Journal of the American Society of Nephrology, 11(8), pp. 1553-1561.

Villaquiran, M., Gipson, T. A., Merkel, R. C., Goetsch, A. L., & Sahlu, T. (2005). Body condition scores in goats. Langston University, Langston, OK, USA.

Vollmar, B., Glasz, J., Leiderer, R., Post, S., & Menger, M. D. (1994). Hepatic microcirculatory perfusion failure is a determinant of liver dysfunction in warm ischemia-reperfusion. The American Journal of Pathology, 145(6), pp. 1421.

Wells, S. M., & Sleeper, M. (2008). Cardiac troponins. Journal of Veterinary Emergency and Critical Care, 18(3), pp. 235-245.

Wess, G., Daisenberger, P., Mahling, M., Hirschberger, J., & Hartmann, K. (2011). Utility of measuring plasma N-terminal pro-brain natriuretic peptide in detecting hypertrophic cardiomyopathy and differentiating grades of severity in cats. Veterinary Clinical Pathology, 40(2), pp. 237-244.

Williams, E. & Thorne, E. (1996). Exertional myopathy (capture myopathy) in Fairbrother, A., Locke, N.L. & Hoff, G.L. (2nd eds). Non-infectious Diseases of Wildlife. Iowa State University Press., Iowa, USA, pp. 181-193.

Zhu, R., Wang, Y., Zhang, L., & Guo, Q. (2012). Oxidative stress and liver disease. Hepatology Research, 42(8), pp. 741-749.