PHYSIOLOGICAL RESPONSES OF WILD ANTELOPE TO EXERCISE TRAINING AS A PROSPECTIVE TREATMENT TO PREVENT CAPTURE MYOPATHY

by

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ABSTRACT

Capture myopathy is a lethal condition associated with physiological stress in wildlife and is responsible for most deaths during game capture. Exercise training to improve fitness has been proposed as a preventative management strategy for capture myopathy but lacks scientific evidence. The aim of this study was to determine whether regular exercise training of wild antelope is indeed feasible, and whether physiological adaptations occur that could mitigate the response to capture stress.

Forty wild blesbok (*Damaliscus pygargus phillipsi*) were habituated for two weeks to a boma. Twenty were randomly selected to be exercise trained for four weeks, consisting of thirty-minute low, medium and high intensity running sessions four or five times per week. Ten of the exercise trained and ten untrained blesbok were subjected to a twenty-minute capture stress event that included chasing by humans, a motorised quadbike, and a helicopter. Immediately following this event, the animals were immobilised, physiological variables recorded, and blood samples obtained at 0 and 40 minutes. The same sampling occurred on days 2 and 5 after the original stress event but only at 0 minutes. Untrained and unstressed animals served as controls for the effect of training and the acute stress response, respectively.

The exercise trained blesbok responded physiologically better to the capture stress event , indicated by lower blood lactate concentrations [exercised: Median (Mdn) = 9.4 mmol/l , Interquartile range (IQR) = 7.9 – 12.4 mmol/l vs. non-exercised: Mdn = 11.0 mmol/l, IQR = 10.5 – 14.1 mmol/l] and reduced post-capture stress rectal temperatures [exercised: Mdn = 41.4 °C, IQR = 41.1 – 41.6 °C vs. non-exercised: Mdn: 41.8 °C, IQR = 41.6 - 41.8 °C]. Although the pH did not differ between the groups - the exercise trained blesbok group had less animals presenting acidaemic, there was an increased $cHCO_3^-$ [mean (M) ± standard deviation (SD) of exercised: 20 ± 3 vs. non-exercised: 16 ± 5 mmol/l) and BE_{ecf} (M ± SD of exercised: - 5 ± 3 vs. non-exercised: - 10 ± 6 mEq/L) that supports an increased buffering ability for the exercised group. These findings conform to previous exercise training studies in humans, horses, and rodents that indicate similar adaptations in trained groups exposed to exertional stress compared to untrained groups. The data also confirmed the severe hypoxaemia that is caused by the opioid immobilisation drugs. This is the first study showing that wild antelope can be successfully exercise trained, which led to physiological adaptations resulting in improved fitness. Whether this training programme will prevent the onset of capture myopathy and reduce fatalities still needs to be further investigated.

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DEDICATION

To my late parents, Pieter and Francis Breed, for the love they instilled for the natural world, and the example and upbringing they provided that assisted me on this journey.

Capture Myopathy Project – May 2017 @ Ngongoni farm, White River, Mpumalanga, South Africa
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LIST OF KEY ABBREVIATIONS

A-a gradient	alveolar-arterial oxygen partial pressure gradient
ADP	adenosine diphosphate
AGap	anion gap
AGapK	anion gap including potassium
AST	aspartate transferase
ATP	adenosine triphosphate
ATV	all-terrain vehicle
BE_b	base excess of blood
BE_{ecf}	base excess of extracellular fluid
bili	bilirubin
ble	blesbok
BUN	blood urea nitrogen
ca	caprine
Ca^{2+}	ionised calcium
$cHCO_3^-$	calculated bicarbonate
Cl^-	chloride
CK	creatine kinase
Crea	creatinine
cSO_2	haemoglobin oxygen saturation
cTnI	cardiac muscle troponin I
D0	day 0
D2	day 2
D5	day 5
DOMS	delayed onset muscle weakness

EDTA	ethylenediamine tetra-acetic acid
ENS	exercise trained non-capture stressed group
ES	exercise trained capture stressed group
eq	equine
F	F-statistic
Fig.	figure
FiO_2	fractional inspired oxygen
GGT	gamma glutamyl transferase
GM	geometric mean
GLDH	glutamate dehydrogenase
Glu	glucose
GSD	geometric standard deviation factor
Km/h	kilometre per hour
H^+	hydrogen ion
H_2O_2	hydrogen peroxide
$cHCO_3^-$	actual bicarbonate
HCO_3^-	bicarbonate
H_2CO_3	hydrogen bicarbonate
Hct	haematocrit
Hgb	haemoglobin
HIE	high intensity exercise
HIIT	high intensity interval training
hu	human
IL-6	interleukin 6
IQR	interquartile range
IU/ml	international units per millilitre

K^+	potassium ion
km	kilometre
L	litre
lac	lactate
LDH	lactate dehydrogenase
LIE	low intensity exercise
m	metre
M	mean
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCT4	monocarboxylate transporter 4
MCV	mean corpuscular volume
Mdn	median
Mg^{2+}	magnesium ion
MIE	medium intensity exercise
min	minute
mm	millimetre
mmHg	millimetres mercury
MPV	mean platelet volume
n	usable sample size
Na^+	sodium
NENS	non-exercised trained non-capture stressed group
NES	non-exercised trained capture stressed group
OH^-	hydroxyradical
O_2^-	superoxide
ov	ovine

P	probability value
$PaCO_2$	partial pressure of carbon dioxide in arterial blood
PaO_2	partial pressure of oxygen in arterial blood
Pb	measured barometric pressure in millimetres mercury (mmHg)
pH	potential of hydrogen
pCO_2	partial pressure of carbon dioxide
PCV	packed cell volume
pH	potential of hydrogen
PLT	platelet count
pO_2	partial pressure of oxygen
PO_4^{3-}	phosphate orthophosphate
PTH	parathyroid hormone
r	Pearson's correlation coefficient
R^2	R-squared
RBC	red blood cell count
RDW	red cell distribution width
REML	restricted maximum likelihood
ROS	reactive oxygen species
SD	standard deviation
SOD	superoxide dismutase
TnI	troponin I
TP	total protein
u/l	units per litre
µmol	micromoles
V_E	minute ventilation
V_E/VO_2	ventilatory equation for oxygen

VCO_2	validation of carbon dioxide production
VO_2	validation of oxygen production
VO_{2max}	maximum oxygen consumption
W	walking
WBC	white blood cell count
WBGT	wet bulb globe temperature

PRELUDE

The lethal stress condition of capture myopathy in wildlife is often mentioned in hushed tones in wildlife translocation circles, causing disrepute for capture operations, large financial deficits, and devastating animal losses. Multiple theories by experts and laypeople alike exist on the causes, pathophysiology, treatments, and prevention of capture myopathy. The renowned wildlife veterinarian, Dr Antonie Harthoorn, hypothesised that exercise training might improve stress resilience and prevent capture myopathy. On a physiological level, this theory has merit, as exercise training improves human exertional-stress resilience. No study has established a measurable difference in the physiological resilience in so-called fit wild animals compared to those considered unfit to substantiate such efforts.

The present study forms the foundation to further our understanding of the condition known as capture myopathy. The study is part of a more extensive research programme investigating the different aspects that may contribute to the underlying mechanisms of capture myopathy. Stress is considered the primary contributing factor that causes capture myopathy, but what predisposes a specific animal to develop capture myopathy when all the animals were exposed to the same stressors is still unknown. A study by Meyer et al. (2009) using wild antelope found that neither environmental temperature nor drugs dictated the capture-related hyperthermia. Only stress was a predictor of the level of hyperthermia experienced (Fig. 1). The human condition, known as *exertional heat stroke,* is considered to have very similar pathophysiology to capture myopathy. A report by Rae *et al*. (2008) confirmed that the level of exertion did not determine whether exertional heatstroke will develop in humans, suggesting that there might be other underlying pathomechanisms for excessive hyperthermia.

The present study aimed to investigate the impact of exercise training on the physiological response to a simulated capture event and whether physical inactivity is a plausible co-factor contributing to the development of capture myopathy.

The specific aims of this study were:

1) To determine if daily exercise training is an achievable endeavour in a specific wild antelope species.
2) To evaluate if any physiological adaptations occurred from such exercise training.
3) To assess whether exercise training and associated physiological adaptations provide a protective function to wild antelope during a capture event.

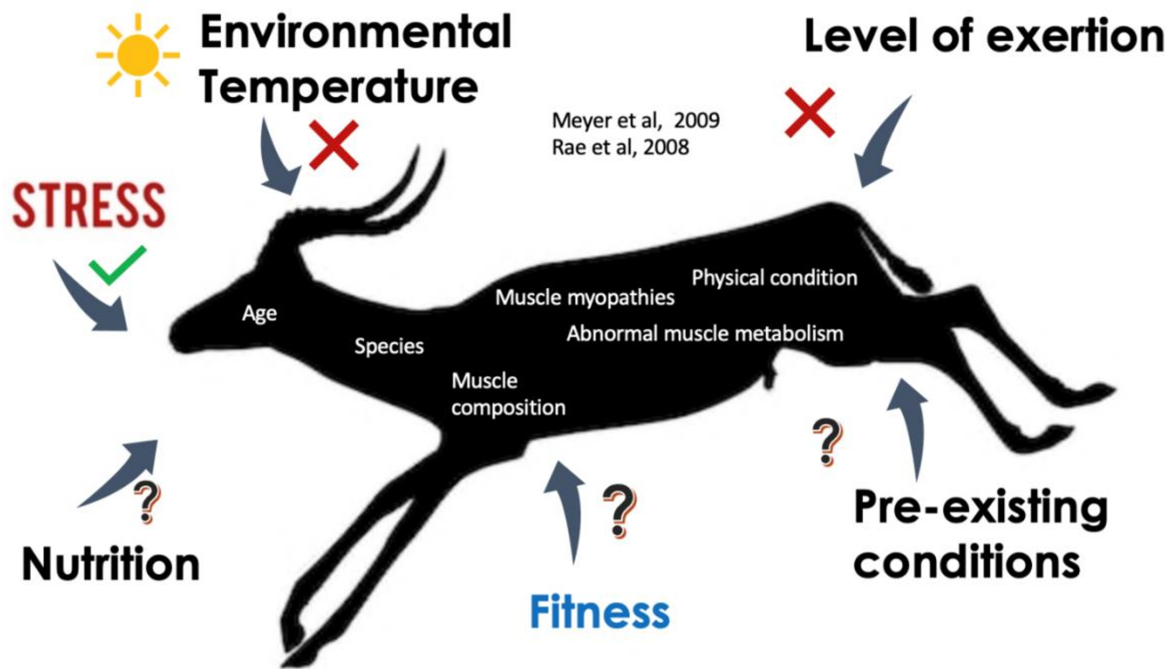

Figure 1 Factors that may contribute to the development of capture myopathy

The objectives of this thesis are:

- to provide a comprehensive overview of what is currently known about capture myopathy in the literature (Chapter 1 - review published in Conservation Physiology (2019) Conserving wildlife in a changing world: Understanding capture myopathy a malignant outcome of stress during capture and translocation),
- to provide an overview of the adaptations relevant to exercise training pertinent to this study observed in other species for the specific physiological variables measured and the potential responses that could be anticipated from the animals in this study (Chapter 2),
- to design, execute and assess the feasibility of exercise training 20 wild antelope for four weeks (Chapter 3),
- to record, measure and determine differences in the physiological response of trained and untrained antelope subjected to a capture stress event (Chapter 4-6), and
- to summarise the main findings of the study and provide directions for future research (Chapter 7).

CHAPTER 1

LITERATURE REVIEW: CAPTURE MYOPATHY

Conserving wildlife in a changing world: Understanding capture myopathy - a malignant outcome of stress during capture and translocation. Conservation Physiology, Volume 7, Issue 1, 2019, coz027,

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Title

Conserving wildlife in a changing world: Understanding capture myopathy - a malignant outcome of stress during capture and translocation.

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Lay Summary

The rapid threat of extinction confronts mammals globally and potentially affects almost a quarter of mammals on the African continent. The threats facing wildlife are numerous and include climate change, habitat loss and poaching. The need to capture and translocate wildlife is essential to attempt to conserve species and maintain ecological balance. Stress during these operations is inevitable, and a life-threatening condition called capture myopathy is still the cause for most deaths during and after such efforts. There is an urgent need to understand and address the cause(s) for this condition to improve conservation efforts and enhance animal welfare during such operations.

Total Word Count

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Keywords

Capture stress, myopathy, wildlife translocation, rhabdomyolysis, myoglobinuria, hyperthermia, exertional heatstroke, malignant hyperthermia, oxidative stress

Abstract

The number of species that merit conservation interventions is increasing daily with ongoing habitat destruction, increased fragmentation and loss of population connectivity. Desertification and climate change reduce suitable conservation areas. Physiological stress is an inevitable part of the capture and translocation process of wild animals. Globally, capture myopathy - a malignant outcome of stress during capture operations - accounts for the highest number of deaths associated with wildlife translocation. These deaths may have considerable impacts on conservation efforts, but also have direct and indirect financial implications. Such deaths usually are indicative of how well animal welfare was considered and addressed during a translocation exercise. Importantly, devastating consequences on the continued existence of threatened and endangered species succumbing to this known risk during capture and movement may result. Since first recorded in 1964 in Kenya, many cases of capture myopathy have been described, but the exact causes, pathophysiological mechanisms and treatment for this condition remain to be adequately studied and fully elucidated.

Capture myopathy is a condition with marked morbidity and mortality that occurs predominantly in wild animals around the globe. It arises from inflicted stress and physical exertion that would typically occur with prolonged or short intense pursuit, capture, restraint or transportation of wild animals. The condition carries a grave prognosis and despite intensive, extended and, largely non–specific supportive treatment, the success rate is poor. Although not as common as in wildlife, domestic animals and humans are also affected by conditions with similar pathophysiology.

This review aims to highlight the current state of knowledge related to the clinical and pathophysiological presentation, potential treatments, preventative measures and, importantly, the hypothetical causes and proposed pathomechanisms by comparing conditions found in domestic animals and humans. Future comparative strategies and research directions are proposed to help better understand the pathophysiology of capture myopathy.

Glossary

Name	Description
Acidosis	Blood pH lower than 7.35
Akathisia	Motor restlessness
Alkalosis	Blood pH higher than 7.45
Anorexia	Below normal body weight caused by inability to consume adequate nutrients
Anxiolytic	Drug that relieves anxiety
Apoptosis	Programmed cell death
Ataxic	Losing control of bodily movements
Azotaemia	Abnormally high levels of nitrogen-containing compounds in the blood
Azoturia	Abnormally high levels of nitrogen-containing compounds in the urine. Associated with horses; associated with muscle rhabdomyolysis
Bradykinesia	Slow movements
Cast formation	Solidification of protein in the lumen of the kidneys
Diabetic ketoacidosis	Breakdown of fats into ketones while blood glucose is above normal
Dystonia	Continuous muscle spasms and contractions
Exertional rhabdomyolysis	Muscle breakdown during exercise

Extrapyramidal signs	Symptoms caused by psychotic drugs which can include dystonia, akathisia, parkinsonism, bradykinesia, tremor, and tardive dyskinesia
Exudative	Oozing of fluid from pores or wounds
Fibrosis	Formation of excessive connective tissue
Heatstroke – classic	Hyperthermia caused by environmental conditions and with lack of sweating
Hyperadrenalism	Excessive amounts of hormones secreted by the adrenal glands
Hyperkalaemic	Above normal blood potassium concentrations
Hyperthermia	Above normal body temperature
Hypokalaemia	Below normal blood potassium concentrations
Hypophosphataemia	Blood phosphate concentrations below normal
Hypoxaemia	Below normal arterial oxygen pressure
Hypoxic	Inadequate oxygenation of tissues
Idiopathic	Medical condition with no known cause
Ischaemic hypoxia	Poor oxygenation due to poor blood flow to the tissue
Metabolic acidosis	Excessive production of H^+ by the body or failure of the buffers and kidneys to neutralise or remove H^+
Myalgia	Muscle pain
Myoglobin casts	Solidification of myoglobin in the nephron
Myoglobinuria	Myoglobin in the urine
Myopathy	Muscle disease
Necrosis	Death of cells
Nephrotoxic	Substances that can damage the kidneys
Neurological vasogenic shock	Response of, or injury to, the central nervous system resulting in sudden loss of sympathetic tone, leading to autonomic instability that is manifested in hypotension, bradyarrhythmia, and temperature dysregulation
Nuclear pyknosis	Irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis
Parasitosis (trichinellosis)	A type of parasitic cyst formation within the tissue
Parkinsonism	Rigidity of the muscles, tremors and postural instability

Parturition	Giving birth
Prognosis	The likely course of a medical condition
Proteinuria	Abnormal high concentrations of protein in the urine
Pupillary mydriasis	Dilation of the pupil
Rhabdomyolysis	Muscle breakdown
Tachypnoea	Rapid breathing
Tardive dyskinesia	Irregular twitchy movements
Tetraplegia	All four limbs are paralysed
Torticollis	A condition where the neck is asymmetrically bent to one side

1.1 Introduction

Globally over the last 40 years 25% of carnivores and ungulates have moved towards extinction (Cardillo et al., 2005; Di Marco et al., 2014). In this time, within African protected areas, large mammal populations have reduced by 59% in the same time period and currently 23% of (African) mammals are threatened according to the IUCN Red List (Di Marco et al., 2014; Schipper et al., 2008).

Introducing or reintroducing species through translocation, to areas in their former range is widely and increasingly practiced in response to aggravating loss of habitat, devastating poaching rates, and the hotter and drier climates experienced (Brown, 2015; Tarszisz et al., 2014). Climate change in continents that are already largely dry such as Africa has already resulted in decreased habitable areas for wildlife (Brown, 2015). There are more translocation efforts with the aim of restoring ecological balance or preserving individual threatened species as more species require conservation efforts (Tarszisz et al., 2014). The introduction of extralimital large mammal species is also widely practiced for economic and public game viewing purposes in protected areas and often requires intensive active management (including movement of animals) thereafter (Bro-Jorgensen & Mallon, 2016; Miller, 2013).

Translocation of wildlife is a widely used conservation tool, but unfortunately can have a low success rate (Dickens et al., 2010; Tarszisz et al., 2014). The reasons for this have not been clearly identified – it may be related to the lack of proper investigation of deaths and long-term follow-up of translocated individuals and groups (Dickens et al., 2010).

The physiological mechanisms that ensure the survival of a lifeform when its homeostasis is altered (such as fleeing from a predator, reproduction or exercise) are inherent to that organism. Capture myopathy is a pathophysiological manifestation where the inherent biological stress defences of an animal have failed or are in the process of failing (Blumstein et al., 2015; Dickens et al., 2010). It is a condition that causes marked morbidity and mortality predominantly in wild animals from around the world (La Grange et al., 2010; Paterson, 2014; Spraker, 1993) and is often documented during capture and translocation procedures in Southern Africa and Africa (Atkinson et al., 2012; Garai et al., 2005; La Grange, 2006; Oberem & Oberem, 2011). It is not only limited to land mammals, but the literature has reported sea mammals, reptiles and birds that acquired and succumbed to this fatal condition (Phillips et al., 2015; Rogers et al., 2004; Sierra et al., 2017; Ward et al., 2011).

The animal welfare issues related to the management and translocation of wildlife can be highly emotive. There is an increased awareness of the plight of wildlife around the globe and the need for their ethical treatment. The tragic high mortality rate of black rhinoceroses losses during the much publicised translocation projects in Kenya and South Africa in 2018 received severe criticism, due to what was perceived as preventable deaths (Africa Geographic, 2018). Increasingly, where the intention has been a major conservation objective, losses cannot be afforded.

Although the well-being of animals are considered in translocations by conservators, in-depth knowledge of the physiology of individuals and species is still largely lacking (Tarszisz et al., 2014). The rate of capture myopathy during and after translocation, as a malignant outcome of stress, can be indicative of how well animal welfare in a species was addressed in a capture operation (Dechen Quinn et al., 2014; A. A. McKenzie, 1993). Knowledge of a species, handling and expertise all assist in reducing stress during translocation (Dechen Quinn et al., 2014).

Conventionally, it is believed to arise from the inflicted stress and physical exertion that typically occur with prolonged or short intense pursuit, capture, restraint or transportation of wild animals (Hartup et al., 1999; Wallace et al., 1987). The condition is not only limited to animals of the wild subjected to capture (and translocation), but may also occur in zoo animals, as they may be chronically stressed due to confinement (Dickens et al., 2010; Mason, 2010). Clinically, the animal usually presents with a combination of any of the following signs: lethargy, muscular stiffness, weakness, incoordination, recumbence, partial paralyses (paresis), metabolic acidosis, myoglobinuria and death (Chalmers & Barrett, 1977; Spraker, 1993).

Macropathology typically reveals muscle necrosis, dark red stained renal medullae and dark coloured urine (Harthoorn & Van der Walt, 1974). Variations on these classic macroscopic findings exist and seem dependent on various factors.

There is no specific duration before death sets in, but can occur within a few minutes, hours, days or even weeks after the precipitating event (Harthoorn, 1976; Spraker, 1993). The condition carries a grave prognosis, but there are occasional reports of successful treatment (Rogers et al., 2004; Spraker, 1993). Intensive efforts are needed for treatment and these are normally prolonged, mostly non–specific and supportive in nature (Businga et al., 2007; Rogers et al., 2004; K. M. Smith et al., 2005). Therefore, in the wild, treatment is often not feasible (Paterson, 2014).

This review provides a broad overview of what is known about capture myopathy in wildlife. The focus will be on the clinical and pathophysiological presentation of the condition, potential treatments, preventative measures and, importantly, the proposed biological causes and hypothesised mechanisms. In order to improve our understanding and to devise new strategies to study this condition, capture myopathy will be compared to other myopathy–causing conditions that present with similar pathophysiology in humans and domestic animal species.

1.2 Background of capture myopathy

The first recorded pathological description of capture myopathy was from 1964 in a Hunter's hartebeest (*Beatragus hunteri*), currently one of the most critically endangered antelope species (Jarrett et al., 1964). Since this first recording, more veterinarians and conservationists started recognising the condition, and reported it in the literature. However, very little research has been performed to better understand the pathogenesis or biology thereof. The condition also goes by many names, including capture or white muscle disease, exertional rhabdomyolysis, transit myopathy, diffuse muscular degeneration, stress myopathy, muscular dystrophy, idiopathic muscle necrosis, but capture myopathy is the preferred term (Jarrett et al., 1964; Spraker, 1993). As a result of its similar presentation, capture myopathy has been compared to a human condition known as either exertional rhabdomyolysis or exertional heatstroke.

Capture and translocation of wildlife are essential tools in wildlife conservation and management, and both have played a major role in securing the survival of many species. In southern Africa, capture and relocation of wildlife have become commonplace due to the multibillion–dollar private game farming industry that has developed over the last four decades

(Trust & Endangered Wildlife Trust, 2016). Largely as a result of this industry, the conservation and economic value of certain species have increased dramatically, with many individual wild animals reaching auction prices exceeding a million US dollars (Bosveld Vleissentraal, 2017). Thus, the loss of an individual animal may present a conservation or substantial economic loss. In addition, more wildlife have succumbed to capture myopathy than any other disease in the last few decades (La Grange et al., 2010). The high financial risk to the loss of wild animals from capture myopathy thus emphasise the importance to better understand the causes, treatment and prevention of this fatal condition.

Human presence, restraint and the added fear of motorised vehicle noise during capture have been hypothesized to be the primary stressors that activate the fight or flight response in these animals. Spraker (1993) proposed that good management practices reduce the stress that the animals experience during capture and relocation, lowering the incidence of capture myopathy to below 2%. Despite these recommendations, capture myopathy still occurs, primarily from not knowing the underlying pathophysiological mechanisms causing this condition, and how stress and exertion can give rise to muscle damage (La Grange et al., 2010; Mason, 2010).

Finally, there are no published data accurately reflecting how many animals die from capture myopathy. The reasons may vary but could merely be kept secret for financial or animal welfare reasons or were just not recorded at all and perceived as unimportant.

1.3 Capture myopathy presentation in animals

(i) Pathophysiology

The condition of capture myopathy is an often fatal, exertion– or stress–induced muscle degenerative condition affecting captured wild animals. The myopathy referred to relates to the muscle damage and weakness observed after a strenuous event. Muscle damage (rhabdomyolysis) is central to the pathogenesis of capture myopathy (Harthoorn, 1976; Spraker, 1993).

When the basal membrane and sarcolemma of the injured muscle fibres are compromised (such as in rhabdomyolysis), the consequential result is that cytoplasmic components, such as myoglobin and creatine kinase (CK) are released from the injured muscle fibres into the blood stream (Spraker, 1993; Vanholder et al., 2000). In addition, blood lactate concentration is elevated leading to a decrease in pH and acidosis. Although not confirmed, this change in

metabolism may contribute to the high body temperatures observed in the early stages of capture myopathy (La Grange et al., 2010; Meyer, Fick, et al., 2008).

Acute kidney injury often occurs and is believed to be one of the devastating effects associated with myoglobinaemia. Myoglobinuric acute kidney injury is induced by three mechanisms: (i) vasoconstriction, (ii) intraluminal cast formation and (iii) haem–protein induced cytotoxicity (Vanholder et al., 2000). Prolonged splanchnic vasoconstriction from the fight or flight phase of the stress response, may also result in renal ischaemia. The vasoconstriction, causing decreased renal perfusion, results in hypoxic damage to the glomeruli and tubules, and proteinuria that can cause the obstruction of the renal tubules, reducing the glomerular filtration rate (Spraker, 1993; Vanholder et al., 2000). Myoglobin is usually easily filtered by the glomerular basement membrane, but the underlying metabolic acidosis is a driver for myoglobin precipitation, which results in cast formation. A breakdown product from myoglobin, called ferrihemate (a form of free iron) has direct nephrotoxic effects, by catalysing free radical production (e.g. hydroxy radicals) resulting in oxidative cell injury (Baxter & Moore, 2003; Vanholder et al., 2000). The haem centre of myoglobin also results in direct kidney injury by initiating lipid peroxidation (Vanholder et al., 2000). It is also now evident that, using a rhabdomyolysis mice model, myoglobin released from damaged muscle may increase vasoconstriction within the renal afferent arterioles (Wan et al., 2017). Eventually, multiple organ failure and death follow the myoglobin-induced acute kidney failure (La Grange et al., 2010). Thus, rhabdomyolysis can be considered the primary malignancy in capture myopathy.

(ii) Clinical signs and pathology

Classically, the initial clinical signs observed in animals suffering from capture myopathy are anxiety, shivering, rapid breathing, bent neck (torticollis), dark red urine and hyperthermia. In more protracted cases, animals may also present with lame or stiff limbs, appetite loss, constipation and can appear weak or lethargic. Once the animal presents with these signs, the probability of recovery is very poor (La Grange et al., 2010; Spraker, 1993; Wallace et al., 1987).

Although animals suffering from capture myopathy often present with these symptoms, there is a wide variation in their presentation, which has led to various authors classifying capture myopathy into different syndromes (Harthoorn, 1976; La Grange et al., 2010; Paterson, 2014;

Spraker, 1993). Harthoorn (1976) and Spraker (1993) each described four syndromes that closely resemble one another. The main difference, however, is that Harthoorn (1976) refers more to a time frame and associated clinical signs, whereas Spraker (1993) focusses more on clinical presentations (Harthoorn, 1976; Montané et al., 2002; Paterson, 2014; Spraker, 1993).

The four syndromes described are:

(a) Hyper acute or capture shock syndrome

Sudden death of a wild animal may occur during capture or up to a few hours thereafter (1 to 6 hours after capture). The animal may present with tachypnoea, tachycardia, a weak pulse, hyperthermia, lethargy and may even die. Macroscopic lesions on post-mortem include intestinal, hepatic and pulmonary congestion. Blood may be found in the lumen of the small intestine. On histopathology, multifocal areas of necrosis are evident in the brain, liver, adrenal glands, lymph nodes, spleen, pancreas, kidneys, heart and skeletal muscles (Harthoorn, 1976; Spraker, 1993). Laboratory serum biochemistry may illustrate elevated enzymatic activity for lactate dehydrogenase (LDH), CK, and aspartate aminotransferase (AST) (Paterson, 2014; Spraker, 1993). Metabolic acidosis may be present, as indicated by a low blood pH (Harthoorn, 1976). The acidosis, if severe enough, results in electrolyte imbalances causing cardiac fibrillation and death. However, haemolysis and muscle damage will result in hyperkalaemia, and will affect normal neuronal conduction in the heart, leading to cardiac fibrillation and ultimately death. This fibrillation will be exacerbated if high circulating adrenalin from the adrenals are present (Guis et al., 2005; Harthoorn, 1976).

This capture shock syndrome has a similar underlying pathophysiology to neurological vasogenic shock. The main distinguishing factor between the two is the presence of rhabdomyolysis in capture shock syndrome and the resultant myoglobin protein detected in the renal tubules, which is the main cause of the kidney injury (Guis et al., 2005; Oberem & Oberem, 2011; Vanholder et al., 2000). Distinguishing between these two conditions does not make them mutually exclusive. Additionally, it is considered that capture myopathy is a continuation of the initial shock condition. During the shock phase, kidney and muscle injury is believed to be initiated by ischaemic hypoxia. Surviving this phase, kidney injury in animals is compounded by the protein by–products of severe rhabdomyolysis (Spraker, 1993).

(b) Acute or ataxic myoglobinuric syndrome

This syndrome is the most frequently observed and can occur hours to a few days after the capture event. The animals may show ataxia, torticollis and myoglobinuria in varying degrees. The same elevations in serum enzymes are seen as before (AST, CK and LDH), but blood urea nitrogen (BUN) is also elevated (Harthoorn, 1976; Spraker, 1993). Fortunately, an animal with mild symptoms may be better off surviving. On gross pathology, the kidneys are dark red and swollen, and the bladder may be empty or contain a small volume of red–brown fluid. In the flexor and extensor muscles of the limbs and the cervical and lumbar muscles, soft, pale and dry areas are observed with central white foci (severe muscle necrosis – a classical sign of capture myopathy) (Blumstein et al., 2015; Roe & Spraker, 2012; Spraker, 1993).

The primary muscles affected are the quadriceps and the gastrocnemius muscles. The longer the animal survives, the more prominent the muscle pathology (Harthoorn, 1976; Meyer, Fick, et al., 2008; Spraker, 1993). On histopathology, the main lesions are found in the kidneys and muscle. The kidney tubules appear dilated and necrotic, and myoglobin casts are present. The muscle fibres are swollen with striation loss, fragmentation of myofibrils and sub-sarcolemmal nuclear pyknosis, the latter representing an irreversible condensation of chromatin in the nucleus of a cell that is undergoing programmed cell death (Harthoorn, 1976; La Grange et al., 2010; Montané et al., 2002; Spraker, et al., 1987; Wallace et al., 1987). The other distinguishing feature of this syndrome is that cardiac tissue is often severely affected, supporting a possible renaming of this syndrome to capture–induced cardiomyopathy.

(c) Sub–acute or ruptured muscle syndrome

Animals with this syndrome appear normal, but within one to two days after capturing clinical signs appear. They mostly die within a few days or may survive for a few weeks (Harthoorn, 1976; Spraker, 1993). The animals present typically with ruptured gastrocnemius muscles that result in dropped hindquarters and hyper flexion of the hocks (Harthoorn, 1976; Paterson, 2014; Spraker, 1993). Often, these animals are unable to stand, giving rise to tetraplegia. Torticollis is evident as a result of cervical muscle injury (Harthoorn, 1976).

The muscular lesions are similar to the ataxic myoglobinuric syndrome but are more severe and extended. Lesions also occur in the forelimb, diaphragm, cervical and lumbar muscles (Harthoorn, 1976; Lewis et al., 1977; Spraker, 1993). Monophasic myonecrosis is the main histopathological finding in these muscles. Sarcolemma proliferation with muscular

regeneration and fibrosis is more evident in this condition and used to distinguish between the sub–acute and chronic form of capture myopathy (Paterson, 2014; Spraker, 1993). LDH, CK and AST are markedly elevated, whereas BUN is either normal or slightly elevated (Harthoorn, 1976; Paterson, 2014; Spraker, 1993).

(d) Chronic debility or delayed per–acute syndrome

This syndrome occurs rarely. Harthoorn (1976) also referred to this phase as the indefinite phase. Typically, these animals have been captured at least once in the past. When they are exposed to a second, usually mild stressful event (often another capture), death occurs within a few minutes (Montané et al., 2002). The animals will try to escape, but will suddenly come to a standstill, show pupillary mydriasis and die (Spraker, 1993). Lesions on gross pathology are mild with either a few or no pale necrotic areas visible in the muscles. However, histopathology does reveal rhabdomyolysis in skeletal muscle – typically in the hind limbs (Harthoorn, 1976; Lewis et al., 1977). The cardiac tissue also shows variable interstitial fibroses (Harthoorn, 1976). The underlying pathogenesis appears to be related to sudden hyperadrenalism that promotes a hyperkalaemic incident, disrupting cardiomyocytic cell membrane depolarisation, resulting in fatal ventricular fibrillation (Montané et al., 2002).

What is evident in the classification of the various phases or syndromes, is that muscle rhabdomyolysis plays a central role in the pathological presentation and outcome of capture myopathy.

1.4 Treatment of capture myopathy

Currently, there is no treatment that ensures recovery from capture myopathy. The most successful approach is adopting good preventative practises (Businga et al., 2007; Roe & Spraker, 2012). The use of tranquillizers during and after capture has aided in reducing the occurrence of capture myopathy but remains anecdotally at best. It has, however, not eliminated capture myopathy related deaths due, in part, to human factors and capture methods employed (La Grange et al., 2010). For example, it is well-known that if field staff are not adequately trained in the correct handling or restraint of a specific species this may pose a risk to these animals. Also, cost constraints may eliminate the option of using a helicopter, or a veterinarian with access to capture drugs, which may result in a capture operator opting to use

less than optimal methods for capture and translocation purposes. Furthermore, if appropriate capture methods are not performed correctly by well-trained staff who have an in-depth knowledge of the species in question, it may result in increased mortalities. Additionally, failure to recognise and record capture myopathy deaths may have a negative impact on accurate reporting of its prevalence.

Capture myopathy therapy consists mainly of supportive treatment with anecdotal successes reported following some intensive efforts (Businga et al., 2007; K. M. Smith et al., 2005; Spraker, 1993). Most success in treatment seems to be achieved in animals that develop the acute or ataxic myoglobinuric syndrome. The treatment of the sub–acute or ruptured muscle syndrome is symptomatic and protracted. By its nature, the prognosis for animals with this syndrome is very poor due to the severe, often permanent, vital tissue injury that occurs. In the hyper–acute or capture shock syndrome there is often not enough time to initiate treatment, and due to the severity of the pathophysiology, it is difficult to implement effective treatment. The only advice that can be provided in the delayed or chronic syndrome is that animals are handled gently (stress free) after capture events (Harthoorn, 1976).

Current therapies that are used for the treatment of capture myopathy and other conditions causing rhabdomyolysis are summarised in Table 1.1. The efficacy of many of these therapies has not been validated, and most are difficult to apply practically under field conditions. Unfortunately, once an animal shows clinical signs of capture myopathy, especially the appearance of dark coloured urine, the prognosis is poor. This highlights the need for proper clinical trials in capture myopathy.

Table 1.1 A summary of reported and, or, proposed treatment regimens for capture myopathy and other forms of rhabdomyolysis

Treatment management regime	Practicality	Evidence of success	References
Analgesics			
Opioids To alleviate any pain the animal may experience due to capture myopathy.	Easy to administer. Expensive	No clinical trials or studies exist indicating its efficacy or success in treating capture myopathy.	(Paterson, 2014; Spraker, 1993)
Inhibitors of inflammation			

Table 1.1 A summary of reported and, or, proposed treatment regimens for capture myopathy and other forms of rhabdomyolysis

Corticosteroids and non–steroidal anti-inflammatory medication To inhibit the inflammatory response and act as an analgesic.	Relatively cheap.	Multiple drug combinations including other treatment showed some improvements in birds. A dog with rhabdomyolysis recovered using a combination of treatments. No clinical trials or studies exist indicating its efficacy or success in treating capture myopathy.	(Paterson, 2014; Spraker, 1993; Ward et al., 2011; Wells et al., 2009)
Chemical inhibition of muscle contraction			
Dantrolene Is a drug registered for treatment of malignant hyperthermia. Acts directly on the ryanodine receptor to prevent calcium release from the sarcoplasmic reticulum.	Very expensive, sensitive to light, poor solubility in water and large quantities required in large animals. May cause adverse effects including muscle weakness, hepatoxicity and neurological impairment.	Used successfully in the treatment of neuroleptic malignant syndrome and spasticity in humans. Some success reported to prevent recurrent exertional rhabdomyolysis in horses. A dog with rhabdomyolysis recovered completely using a combination of treatments. No direct benefit in treatment of exertional heatstroke in humans. No clinical trials or studies exist indicating its efficacy or success in treating capture myopathy.	(Choi et al., 2017; Edwards et al., 2003; E. C. McKenzie et al., 2004; Paterson, 2014; Watson et al., 1993; Wells et al., 2009)
Correction of acidosis			
Sodium bicarbonate Solution of $NaHCO_3$ in saline.	Relatively inexpensive. Titration against blood pH values is very difficult, time consuming and expensive.	Treatment of wild zebra (*Equus zebra*) after capture:	(Bagley et al., 2007; Forsythe & Schmidt, 2000; Harthoorn &

Table 1.1 A summary of reported and, or, proposed treatment regimens for capture myopathy and other forms of rhabdomyolysis

Infusion titrated against blood pH. To maintain circulatory volume and alleviate metabolic acidosis, hyperkalaemia and myoglobinuria.	Very difficult to perform in field conditions.	Nine from 12 treated animals survived. Untreated animals all succumbed to capture myopathy. Post-mortem signs were typical of capture myopathy. Immediate alleviation of cardiac dysfunction and dyspnoea. Bicarbonate treatment for acidosis lost favour over the years. Other cases reported no effect on capture myopathy outcome.	Young, 1974; Paterson, 2014; Wells et al., 2009)
Fluid therapy Saline or ringers lactate infusion	Relatively inexpensive.	Although frequently used to prevent kidney damage in human and equine rhabdomyolysis, no clinical studies exist that determined its efficacy in wildlife with capture myopathy.	(Bagley et al., 2007; Valberg, 2009)
Nutritional support and antioxidant supplementation			
Added nutritious feed during the protracted treatment and rehabilitation process of animals recovering from capture myopathy. Intravenous or oral administration of antioxidant compounds, such as: Vitamin E	Relatively inexpensive May have adverse effects in high doses?	Although some success was reported in human metabolic myopathies, no direct evidence or controlled studies exist to suggest efficacy of any antioxidant supplementation or	(Beech, 1997; Chalmers et al., 1979; Graffam et al., 1991, p. 199; Landau et al., 2012; Montgomery et al., 2011; Parikh et al., 2009; Rogers et al.,

Table 1.1 A summary of reported and, or, proposed treatment regimens for capture myopathy and other forms of rhabdomyolysis

Selenium Co–enzyme Q10 L–carnitine		nutritious feed to treat capture myopathy.	2004; K. M. Smith et al., 2005; Valberg, 2016; van Adel & Tarnopolsky, 2009; Ward et al., 2011; Wells et al., 2009)
Anxiety alleviation			
Anxiolytic (e.g. benzodiazepines and some tranquilisers). To reduce muscular rigidity and any further stress.	Relatively inexpensive. Possible side effects.	No clinical studies or evidence exist to suggest that it successfully treats capture myopathy.	(Ward et al., 2011; Wolfe & Miller, 2016)
Hyperbaric oxygen supplementation			
Drug–induced rhabdomyolysis in a human. Successfully treated with the conjunctive use of hyperbaric oxygen.	Impractical in animals in field conditions. Concern of oxygen toxicity.	No clinical trials exist to prove its efficacy in wildlife suffering from capture myopathy.	(Abdullah et al., 2006; Parikh et al., 2009)
Cooling			
Ice water immersion and water dousing with or without fanning, infusion of cold saline solution. Alleviating hyperthermia in humans and animals with exertional heatstroke or hyperthermia.	Inexpensive. Can be time consuming and may take a long time for temperature to reach normality. If hyperthermia is not diagnosed and treated adequately morbidity and mortality can occur. Some methods are impractical in the field.	Efficacy of cold water immersion has been proven in case studies of humans with exertional heatstroke. No clinical trials exist to show that cooling can successfully treat or prevent capture myopathy.	(Fahlman et al., 2008; Rae et al., 2008; Sawicka et al., 2015)

1.5 Prevention of capture myopathy

The best measure to prevent capture myopathy is to ensure that animals are limited to external causes of stress. Although not yet clinically tested in controlled settings, the preventative measures include capture method, adequate planning, the use of tranquilisers and immobilisation drugs, habituation, cooling of animals and the environment (e.g., time of day, temperature).

(i) Capture method

Deciding on whether to use chemical or physical restraint and which type of these restraints to use, is a decision that will be dictated by economics, level of operator experience, ethics, environment and the species to be captured.

For chemical restraint, the type of immobilisation drugs used is determined by the species to be captured, availability of the immobilisation drugs, route of administration and the delivery system (oral, pole syringe, or dart gun). Failure of chemical restraint and subsequent tranquilisation will lead to an increased stress response, which can result in trauma and possibly even capture myopathy. This failure results from equipment and darting failure, lack of preparation and planning, poor staff communication and execution, and poor selection and under–dosing of immobilisation drugs (Atkinson et al., 2012; La Grange et al., 2010; Spraker, 1993a).

It is possible to restrain animals physically with or without tranquilisation. Due to the hands–on nature of this type of technique, adequate experience of an operator is essential to ensure that animals are handled in a way that minimise the stress response. Different methods tailored to the operation at hand are used in the field, and include:

Direct physical restraint – mostly appropriate for small birds and reptiles and not suited for use in larger free–roaming wildlife.

Ropes – often utilised to restrain and manipulate animals but can cause stress and trauma to both animal and handler if not used appropriately.

Drive nets or drop nets – preferred for mass capture of smaller antelope. Excitable species might quickly succumb, therefore rapid tranquillisation is necessary to calm animals.

Net gun – used for individual animals and requires a skilled and trained operator to ensure safety of the animal.

Physical barriers – erecting a fence or shaded netting to manipulate the directional movement of animals into large bomas is often used in mass capture operations (Atkinson et al., 2012; La Grange, 2006; La Grange et al., 2010).

(ii) Adequate planning of the capture process

The primary goal of the capture process should be to reduce stress during the operation through adequate risk assessment and contingency planning (La Grange, 2006). In particular, the duration that animals are exerted, handled and transported should be minimised. Achieving these goals require significant resources and planning that include reduction of the time spent gathering, loading, restraining or immobilising and transporting of the animals (Atkinson et al., 2012; La Grange, 2006; La Grange et al., 2010).

A qualified experienced capture team with adequate knowledge of the specific species being captured is vital to reduce stress and trauma to the animals (Atkinson et al., 2012). These practices reduce the incidence, but do not eliminate the occurrence of capture myopathy. It raises an important question as to why certain species or specific individual animals within the same species are more susceptible to developing capture myopathy than others (Atkinson et al., 2012; La Grange et al., 2010; Mason, 2010; Meyer, Fick, et al., 2008).

(iii) The use of tranquilisers and immobilisation

The administration of tranquilisers, sedatives and sometimes immobilising drugs during capture procedures are recommended practices that appear to decrease the incidence of capture myopathy and improve survival rates. However, the use of drugs should not be a substitute for poor planning, lack of habituation or poor handling techniques of animals (La Grange et al., 2010). In fact, the use of these drugs have no effect on reducing the inevitable increase in body temperature of captured animals, and if prolonged, this hyperthermia may contribute to the development of capture myopathy (Meyer, Fick, et al., 2008).

Immobilising drugs can also disrupt thermoregulation. Opioids (immobiliser) and α_2–agonists (sedative) are known to alter thermoregulation and may contribute to the capture–induced hyperthermia (Fahlman et al., 2008; Meyer, 2009). Although not yet understood, opioids are thought to cause hyperthermia through sympathetic stimulation, which increases metabolism and resultant heat production (Atkinson et al., 2012; Meyer, 2009). Furthermore, many of the

drugs that are used in wildlife like ketamine, haloperidol, diazepam, naltrexone and succinyl choline are known to induce rhabdomyolysis directly in humans (Guis et al., 2005).

The use of long-acting tranquilisers post-capture has shown great benefit by lowering the incidence of capture myopathy and mortalities from more than 20% to less than 2% (La Grange et al., 2010). However, these drugs need to be used with caution as cases of overdosing can result in unwanted side effects, such as anorexia and extrapyramidal signs (Atkinson et al., 2012). Extrapyramidal effects cause an increase in uncontrolled muscular activity and thus may play a role in the development of capture myopathy.

(iv) Habituation

Before a capture is executed, it has been recommended that, wherever possible, wild animals should be exposed to the presence of humans and motorised vehicles (habituated) to attempt to reduce the stress response to capture. Animals should also be allowed adequate time to habituate to a new environment before repeated handling events (La Grange et al., 2010). For some species, habituation before minor procedures such as vaccinations, venepuncture and physical examinations, can be adequate to allow for no or minimal restraint (Meyer, Hetem, et al., 2008; Paterson, 2014). For this degree of habituation and reduction in stress response to occur, continuous gradual desensitisation is required, but is very difficult to attain in free–living wild animals.

(v) Cooling

Hyperthermia is believed to play a role in the development of capture myopathy. Therefore, it has become common practice to attempt to actively cool animals that develop capture–induced hyperthermia (Meyer, 2009). This practice originates from knowledge gained from classic and exertional heatstroke (Rae et al., 2008). If core temperatures surpass 41 °C, the individual is submerged in an ice bath, where after active fanning may be required until the body temperature returns to normal. The duration of hyperthermia, and not its magnitude, is thought to be the main differentiating factor in the outcome of heatstroke in humans. Hence, effective and rapid cooling is required (Casa et al., 2012). The adverse impact of the duration and magnitude of hyperthermia is still to be determined in captured wildlife.

Despite not knowing the actual consequences of capture–induced hyperthermia, many methods are employed to cool hyperthermic captured wild animals, varying from water dousing, ice packing, cold intravenous fluid administration, mist sprayers and the use of cold water enemas (Sawicka et al., 2015). Water dousing seems the most practical and effective method of cooling hyperthermic animals in the field (Sawicka et al., 2015). Although effective cooling did not protect against or prevent any of the associated pathophysiological changes that were induced in captured blesbok (Damaliscus pygargus phillipsi), the findings suggest that the contribution of capture–induced hyperthermia may be less than previously thought.

(vi) Environmental conditions

A customary recommendation to prevent capture myopathy or limit hyperthermia–associated complications is to avoid capture operations on hot days, or during the time of day when temperatures are high. The prescribed recommendation is not to capture wild animals when the ambient temperature exceeds 25˚C (Atkinson et al., 2012). These recommendations may stem from the belief that capture myopathy is similar to the human condition of classic heatstroke, where hot ambient conditions are the primary catalyst (Meyer, 2009; Rae et al., 2008).

Additionally, the clinical presentation of capture myopathy is like that of exertional rhabdomyolysis and exertional heatstroke found in horses and humans, respectively, where hyperthermia and muscle rhabdomyolysis are primary symptoms. However, an in–depth analyses of humans suffering from exertional heatstroke concluded that ambient conditions were not associated with the elevated body temperatures and that excessive production of endogenous heat within individuals was more likely the cause (Rae et al., 2008). A similar conclusion was drawn where the magnitude of capture–induced hyperthermia in impala (*Aepyceros melampus*) was not associated with environmental conditions or the intensity and amount of exertion during the capture process, but rather the stress response itself (Meyer, Fick, et al., 2008). Furthermore, cooling did not prevent or reduce the capture-induced pathophysiological effects, suggesting that the role of capture–induced hyperthermia in the pathogenesis of capture myopathy may be overplayed (Fitte, 2017).

Although all these preventative measures are implemented, capture myopathy still arises, is unpredictable and highlights a lack of understanding of the biological mechanisms that cause this condition. Fortunately, several conditions with similar symptoms and pathophysiology exist that may assist in studying capture myopathy.

1.6 Similar myopathy conditions in mammals

Many conditions presenting with hyperthermia and rhabdomyolysis exists in mammals, but each with a different causation. These include amongst others, exertional heatstroke, crush injury, malignant hyperthermia and exertional rhabdomyolysis.

(i) Exertional heatstroke in humans

Exertional heatstroke may occur after excessive exercise in unfit humans, and rarely in highly trained athletes. It has also been termed "march myoglobinuria" as it is a prominent condition presenting after severe exertion in the first six months of basic military training in new recruits (Capacchione & Muldoon, 2009; Landau et al., 2012; Rae et al., 2008; Spraker, 1993). The condition is rare, but once present, often fatal. Although hyperthermia occurs in this condition, it should be distinguished from classical or environmental associated heatstroke. The latter results from inefficient thermoregulatory control when individuals are subjected to high environmental temperatures and occurs mostly in the very young and old. Secondly, patients with classic heatstroke do not sweat, whereas in exertional heatstroke, patients sweat profusely (Rae et al., 2008).

Poor heat dissipation from endogenous heat production is believed to cause exertional heatstroke (J. E. Smith, 2005). The prominent symptoms are elevated core body temperatures (> 41 °C), myalgia, muscle weakness (caused by muscle rhabdomyolysis) and dark red coloured urine (myoglobinuria). The exact mechanism that triggers the pathophysiology is still unclear (Baxter & Moore, 2003; J. E. Smith, 2005). Some risk factors have been associated with this condition and include dehydration, concurrent illness, sleep deprivation, obesity, alcohol consumption, poor fitness and excessive clothing (Guis et al., 2005; J. E. Smith, 2005). These are all difficult to reproduce or study in humans.

A puzzling occurrence is that exertional heatstroke can occur in healthy fit individuals that are prepared and conditioned for the event that they are participating in. This perplexity poses the question whether these individuals may be predisposed to developing exertional heatstroke by a mechanism that is not associated with fitness or health (Capacchione & Muldoon, 2009; Rae et al., 2008). Importantly, it is quite normal for elite endurance runners to develop core body temperatures above 40 °C without developing any abnormalities. They can therefore tolerate excessive heat better than untrained individuals, and that this exercise–induced hyperthermia is most likely not the inciting cause of exertional heatstroke (Marino et al., 2004; Wyndham,

1977). Some of the underlying factors that may precipitate rhabdomyolysis in this condition may include abnormalities in metabolism e.g. glycogen storage diseases, diabetic ketoacidosis, mitochondrial myopathies, hypokalaemia and hypophosphataemia. Other proposed causes include muscular parasitosis (trichinellosis) and toxins. Chronic medication, the ingestion of over-the-counter drugs (e.g. non–steroidal anti–inflammatories) to improve performance, or illicit drug use have also been associated with the development of rhabdomyolysis (Baxter & Moore, 2003; Watson et al., 1993).

Like capture myopathy, treatment for exertional heatstroke is limited and usually involves rapid cooling by submerging the patient in ice water. However, if the diagnosis and treatment is delayed, this treatment can be unsuccessful (Baxter & Moore, 2003). The hyperthermia that occurs during exertional heatstroke episodes usually continues after exercise has ceased, indicating that the origin of the heat is not from contracting muscles, but from yet unknown "heat generators" (Rae et al., 2008).

Many similarities between the ataxic myoglobinuric syndrome of capture myopathy in animals and exertional heatstroke in humans exist. These similarities include hyperthermia, rhabdomyolysis and myoglobinuria. It may be that the suspected triggers and predisposing factors for these conditions are similar, but poorly understood or researched (Bartsch et al., 1977).

(ii) Compartment syndrome and crush injury rhabdomyolysis

Compartment syndrome may occur after severe trauma like crush injury or fracture(s). It results in an increased interstitial pressure within a closed osteofascial compartment that limits local circulation. This latter effect results in ischaemic damage, which, if prolonged, can cause irreversible damage. Compartment syndrome is believed to contribute towards rhabdomyolysis and vice versa. It has also been implicated in the pathophysiology of capture myopathy. Whether acute kidney injury follows, is most likely dependent on the extent of the rhabdomyolysis (Baxter & Moore, 2003; Malinoski et al., 2004).

Rhabdomyolysis caused by crush injury is well documented in humans, especially in victims of car accidents and earthquakes. The presenting pathophysiology is similar to exertional heatstroke, except that the inciting cause is external trauma to the muscles (Malinoski et al., 2004).

(iii) Malignant hyperthermia

A well–known genetic defect primarily described in humans and pigs cause a condition known as malignant hyperthermia (Wappler, 2001). It stems from a mutation in the ryanodine 1 receptor (RYR1), with its function to regulate Ca^{2+} release from the sarcoplasmic reticulum (Wappler, 2001). To date, approximately 30 mutations in the over 300 variations of the RYR1 gene have been associated with causing malignant hyperthermia in humans (Schneiderbanger et al., 2014). The mutation makes the RYR1 very sensitive to certain triggers, like caffeine and general anaesthetic agents (e.g. halothane). The triggers cause uncontrolled release of Ca^{2+} from the sarcoplasmic reticulum into the muscle fibres resulting in prolonged muscle contraction. This contraction results in a hypermetabolic state that presents with increased aerobic and anaerobic metabolism, leading to hypoxia, metabolic acidosis, increased CO_2 production and hyperthermia. In addition, sarcoplasmic Ca^{2+} resorption utilises vast amounts of adenosine triphosphate (ATP), depleting intracellular ATP, phosphocreatine and glycogen stores. These effects result in protracted rigidity and rhabdomyolysis (Schneiderbanger et al., 2014; Wappler, 2001).

Volatile halogenated inhalation anaesthetics and the muscle relaxant succinylcholine are the most common triggers of malignant hyperthermia. Exposure does not always trigger the condition and it is postulated that the condition might be dose dependent (Capacchione & Muldoon, 2009; Schneiderbanger et al., 2014). However, malignant hyperthermia does not necessarily need to be drug induced. Severe emotional or physical stress can also be a trigger and has been well described in pigs (Schneiderbanger et al., 2014; Wappler, 2001).

Malignant hyperthermia in pigs is also known as porcine stress syndrome, which causes an economically important condition called pale, soft, exudative meat. A single mutation in the RYR1 gene is the cause for this syndrome in all pig breeds, and animals present with muscle rigidity, acidosis and hyperthermia (Fujii et al., 1991; Moochhala et al., 1994). Rapid glycolysis, increased lactate formation and muscle necrosis result in pale areas of skeletal and heart muscle – thus giving rise to the name "Pale Soft Exudative" meat syndrome (Mitchell & Heffron, 1980). Identified triggers include high environmental temperatures, exertion, fighting, mating and parturition (Mitchell & Heffron, 1980). Its aetiology and presentation are very similar to that of capture myopathy. Therefore, as in pigs, Mitchell and Heffron (1980) suggested that a genetic anomaly may be present that results in an abnormal response to stress that causes this condition to occur in wildlife.

A small cohort of clinical studies have proposed an association between exertional heatstroke and malignant hyperthermia in humans (Schneiderbanger et al., 2014). In a controlled study by Wappler *et al*. (2001), twelve patients who survived exertional heatstroke were tested with the standard European test for malignant hyperthermia known as the In Vitro Contracture test. This test involves exposing an excised piece of muscle tissue to various concentrations of halothane and caffeine. Ten of the twelve patients tested positive, one showed an equivocal result, and another was negative for the condition (Wappler, 2001). Therefore, a mutation in the RYR1 receptor is very likely to make individuals acquire exertional heatstroke. The mutation causing malignant hyperthermia has also been found in horses and dogs, but too few studies have been performed in wildlife to conclude its presence (Aleman, 2008). One study did attempt to determine if capture–induced hyperthermia and capture myopathy could be associated with malignant hyperthermia by subjecting muscle specimens from 4 black–tailed deer to the In Vitro Contracture test. All specimens were negative, indicating no association (Antognini et al., 1996). However, the small sample size and the fact that healthy animals and not animals that developed capture myopathy, were tested, make the findings from this study inconclusive. Therefore, it is imperative that this area in capture myopathy be reopened for investigation.

(iv) Exertional rhabdomyolysis in horses

Exertional rhabdomyolysis is a popular condition in horses, frequently diagnosed, and known by many names, such as tying–up, set fast, Monday–morning disease, azoturia, chronic intermittent rhabdomyolysis and equine rhabdomyolysis syndrome. Horses that partake in Polo Cross have the highest incidence of acquiring exertional rhabdomyolysis (~13%), with Thoroughbreds competing in horse racing having a much lower incidence (~6%) (Aleman, 2008; Beech, 1997; Valberg, 2009). Anecdotal risk factors to this condition include being two years of age, female, a highly strung nature, continuous exercise with minimal rest days, high concentrate carbohydrate diets and lameness before the event (McGowan et al., 2002).

Clinical presentation of this condition is muscle stiffness, tachypnoea, sweating, painful hindquarter muscles and the reluctance to move. The diagnosis is made on history of exercise, elevated serum CK and AST concentrations. Moderate to severe rhabdomyolysis may result in myoglobinuria, metabolic alkalosis and azotaemia due to the effects of the myoglobin on the kidneys (Beech, 1997; Valberg, 2009).

The condition can be divided into two syndromes: i) horses that develop a sporadic episode of exertional rhabdomyolysis, and ii) chronic exertional rhabdomyolysis, where it occurs frequently, and the horses appear to have an underlying susceptibility. Triggers of sporadic exertional rhabdomyolysis are believed to include excessive exertion, heat exhaustion and electrolyte imbalances. Glycogen storage myopathies and nutritional imbalances have also been associated with the manifestation of chronic exertional rhabdomyolysis (Lentz et al., 1999; Valberg, 2009).

(a) Polysaccharide storage myopathy

Stephanie Valberg and colleagues first described polysaccharide storage myopathy (PSSM) in 1992, when a cohort of horses with recurrent exertional rhabdomyolysis tested positive for abnormal glycogen depositions in their muscle biopsies (Valberg et al., 1992). Over the years, PSSM was found to be mainly prevalent in Quarter horses (between 6 to 12%) and draught horses (36% of Belgian draught horses) (Aleman, 2008; McCue et al., 2008). The condition manifests in horses when exercised and results from the inability of their muscle fibres to utilise glycogen for ATP synthesis. The cause of PSSM has been attributed to a mutation in the gene that encodes for the muscle glycogen synthase enzyme, Gys1 (McCue et al., 2008). The gold standard for diagnoses of PSSM is the amylase periodic acid Schiff (PAS) stain on histologically prepared sections. A muscle fibre that harbours the mutation would reveal granular precipitate in type II muscle fibres, indicating the presence of abnormal glycogen (Aleman, 2008; Sierra et al., 2012). Additional mutations in conjunction with the Gys1 mutation may aggravate the symptoms of PSSM (McCue et al., 2009)

A condition with the same histological presentation as PSSM has been found in the muscle of 11 species of aquatic mammals (cetaceans) (Sierra et al., 2012). Out of 148 beached cetaceans, PAS positive, diastase resistant inclusions were found in 26 of these animals, being consistent with abnormal glycogen deposits or complex polysaccharide. In addition, these inclusions also stained positive for ubiquitin, with type II fibres specifically affected. Whether this condition is also caused by a glycogen synthase mutation still needs to be established (Sierra et al., 2012). Thus, there could be a link between PSSM and capture myopathy, warranting further investigation (Roe & Spraker, 2012).

(b) Other unknown causes of recurrent exertional rhabdomyolysis

Recurrent exertional rhabdomyolysis in horses is diagnosed when a horse that develops the condition tests negative for the Gys1 mutation associated with PSSM, as well as the RYR1 mutation associated with malignant hyperthermia. Histologically, the muscle fibres of these horses show no sign of excessive or abnormal glycogen storage but have vast numbers of fibres with central nuclei (Aleman, 2008; Lentz et al., 1999; Valberg, 2009). Recurrent exertional rhabdomyolysis is frequently found in thoroughbred horses with an average prevalence of 5–10%. During the racing season, up to a fifth of horses may develop this type of rhabdomyolysis and the cause is believed to be a genetic predisposition associated with abnormal intramuscular Ca^{2+} regulation (Spraker, 1993). The involvement of important defects in RYR1, such as the dihydropyridine receptor–voltage sensor and sarcoplasmic reticulum Ca^{2+}-ATPase, has been excluded as the triggers of this condition. However, these horses do test positive using the In Vitro Contracture Test (Valberg, 2009). Thus, another mechanism for abnormal muscle Ca^{2+} regulation may exist in these animals and determining this mechanism may reveal insights into why some wildlife develop capture myopathy.

To summarise, there are stark similarities between known rhabdomyolysis and hyperthermic conditions in humans, domestic animals and other species. Studying their similarities can guide research aimed at unravelling the pathomechanisms and causes of capture myopathy.

1.7 Hypothetical causes of capture myopathy

Capture myopathy has been proposed to be an "inherent mechanism" that causes wild animals to die quicker when caught by a predator, and indirectly assists the predator in conserving energy (Spraker, 1993). However, this theory seems highly unlikely. For example, a predator would never chase wild prey for prolonged periods of time in their natural habitat, whereas in a capture situation, the chase might be considerably longer and involve more stressors (La Grange et al., 2010). Additionally, many animals have escaped the jaws of their predators and subsequently survived. Thus, the concept of accelerated death when caught by a predator contradicts the widely accepted theory of "survival of the fittest", as prey will never evolve mechanisms to assist its predator in conserving energy. Furthermore, the theory of natural selection postulates that an evolutionary adaptation of a trait requires the continued reproduction of such a trait. ‘Dying quicker’ does not assist in transferring any traits to any future generations and would result in their extinction. Some predators, like wild dogs, may

use exertion myopathy to their advantage since these animals are renowned for chasing prey over long distances, inducing exhaustion of their prey to complete the kill (Bartsch et al., 1977). Similarly, indigenous humans of southern Africa, known as the Koi San, are known for tracking and chasing a single animal to the point of exhaustion to get closer to the animal for bow and arrow shots (Liebenberg, 2006). It is more conceivable that prey species have evolved physiological mechanisms in their muscles that aid the animal in escaping predation during the fight and flight response. Thus, the outcome of these mechanisms is a successful escape if the chase is of short duration, but the disadvantage is that the probability is high for these mechanisms to fail when over-exerted, and manifests as capture myopathy (Bartsch et al., 1977).

The stress experienced by wild animals appears to be one of the key precipitating factors of capture myopathy (La Grange et al., 2010). However, as is witnessed with malignant hyperthermia and recurrent exertional rhabdomyolysis, factors other than stress can trigger the development of rhabdomyolysis (Capacchione & Muldoon, 2009; Lentz et al., 1999). Even by minimising the stress response, some wild animals still develop capture myopathy (La Grange et al., 2010). The stress–induced pathophysiological events that lead to capture myopathy are just not that well understood.

Old hypotheses have since been refuted for exertional heatstroke and capture myopathy. For example, the intensity and duration of exercise performed or high ambient temperatures during endurance events, or both, was considered primary risk factors to develop hyperthermia and exertional heatstroke in humans (Rae et al., 2008). The same factors were extrapolated to causing hyperthermia and capture myopathy in wildlife. However, evidence from studies has since questioned these claims (Meyer, Fick, et al., 2008; Rae et al., 2008). In fact, Meyer, Fick, *et al.*, (2008) showed that neither environmental temperature, the level of exertion nor the use of different drugs was associated with the extent of hyperthermia that developed during the capture of impala (Meyer, 2009; Meyer, Fick, et al., 2008). Rae *et al.* (2008) supported these findings in humans. Exertional heatstroke cases were reported at ambient temperatures as low as 4˚C at low exercise intensities of short duration and distance (e.g. one athlete acquired exertional heatstroke after only 2 km of running at 7.4 km/h for only 16 minutes (min) at an ambient temperature of 16.7˚C) (Rae et al., 2008).

The above clearly indicates some underlying condition that induces excessive endothermy, of which the mechanisms are not yet understood (Bartsch et al., 1977; Capacchione & Muldoon, 2009; Rae et al., 2008; J. E. Smith, 2005). Therefore, many external factors, like high ambient

temperatures may only be playing a secondary or aggravating role. Additionally, why certain species and individual animals are more prone to the development of capture myopathy is still not known, but suggest an inherent genetic predisposition (Antognini et al., 1996; La Grange et al., 2010; Mason, 2010). The proposed causes discussed below should also be contextualised with the response to fear and capture, as reviewed in section 1.3 of this review.

(i) Inherent predisposition to capture myopathy

(a) Species and size

Although numerous vertebrate species can be affected by capture myopathy, mammal and bird species seem most frequently affected, fish and amphibians less so and only a few cases have been reported in reptiles (Phillips et al., 2015; Spraker, 1993). Roan (*Hippotragus equinus*), nyala (*Tragelaphus angasii*), tsessebe (*Damaliscus lunatus*), red hartebeest (*Alcelaphus buselaphus caama*), springbok (*Antidorcas marsupialis*), kudu (*Tragelaphus strepsiceros*) and giraffe (*Giraffa camelopardalis*) are considered some of the most susceptible African ungulate species to capture myopathy (Oberem & Oberem, 2011). In North America, the condition has been observed in a few species including white–tailed deer (*Odocoileus virginianus*) (Beringer et al., 1996; Dechen Quinn et al., 2014), black–tailed deer (*Odocoileus hemionus columbianus*) (Antognini et al., 1996), pronghorn (*Antilocapra americana*) (Chalmers & Barrett, 1977) and elk (*Cervus elaphus*) (Lewis et al., 1977). Southern chamois (*Rupicapra pyrenaica*) (López-Olvera et al., 2007) and roe deer (*Capreolus capreolus*) (Montané et al., 2002) are the most frequent European species affected by capture myopathy. Wild turkeys (*Meleagris gallopavo*) (Spraker, T. R et al., 1987), sandhill cranes (*Grus canadensis*) (Businga et al., 2007), rheas (*Rhea americana*) (K. M. Smith et al., 2005), bar–tailed godwits (New Zealand) (*Limosa lapponica*), and long–legged shore birds are some of the recorded cases of capture myopathy in bird species (Blumstein et al., 2015; Rogers et al., 2004).

Marine animals are not excluded from acquiring capture myopathy, especially various whale species like finned pilot whale (*Globicephala melas*), Risso's dolphin (*Grampus griseus*), pygmy sperm whale (*Kogia breviceps*) and Blainville's beaked whales (*Mesoplodon densirostris*). The dolphin species in which capture myopathy was found include the false killer whale (*Pseudorca crassidens*), striped dolphin (*Stenella coeruleoalba*), Atlantic spotted dolphin (*Stenella frontalis*), spinner dolphin (*Stenella longirostris*) and bottlenose dolphin (*genus Tursiops*). The often poor success rate of cetacean rehabilitation is frequently attributed

to stress–associated myopathies (Herráez et al., 2013). PSSM may play a significant role in cetaceans that develop rhabdomyolysis, suggesting a possible genetic cause of capture myopathy in these animals (Herráez et al., 2013; Roe & Spraker, 2012; Sierra et al., 2012). What was not clear from the literature was whether these aquatic mammals also presented with hyperthermia. Nevertheless, although certain species seem more susceptible, and, hence, may have a genetic predisposition, it does seem evident that any animal can acquire and succumb to capture myopathy (Mason, 2010).

With large stranded cetaceans prolonged muscle compression may contribute to the rhabdomyolysis and myoglobinuric nephrosis that follows (Herrá Ez et al., 2007). This effect is also a recognised complication in rhabdomyolysis of large muscle masses in humans, and may also increase the likelihood of renal failure in these individuals (Percy, 2014). It is well known that immobilised large animals, such as rhinoceros, run the risk of rhabdomyolysis due to reduced blood flow and hypoxaemia (i.e. ischaemia), especially in their limbs, during recumbency (Cole et al., 2017; Meyer et al., 2015). Sadly, the incidence of rhinoceros that have been chemically immobilised by poachers and not killed, has increased over the years, particularly due to this type of poaching method being more discreet compared to the noise of gunshots. These animals are often deserted without reversing the immobilising drugs (most often opioid anaesthetic drugs) and may remain immobilised for hours before they are found or when the anaesthesia wears off (Cole et al., 2017; Meyer et al., 2015). The consequence is usually extensive myopathy, characterised by rhabdomyolysis and myoglobin–induced kidney injury, which carries a poor prognosis for many of these animals (Meyer et al., 2015).

(b) Age and physical condition

Young and old animals are reported to be more prone to develop capture myopathy, but the reasons for this anomaly is still unclear. Susceptibility to the condition seems to be increased by poor physical condition or being overweight (Harthoorn, 1976; La Grange et al., 2010). The former state is commonly found in young and old animals, since these age groups are often of the lowest social rank in a herd. Interestingly, obesity seems to play an important role in predisposing humans to exertional heatstroke (Casa et al., 2012). These conditional factors have merely been associated with capture myopathy and their specific roles need further investigation.

(c) Skeletal muscle composition: fibre type, metabolism and oxidative stress defence

An alternative cause may be related to metabolism. Exercise increases the metabolic demand for ATP synthesis from aerobic and anaerobic metabolism of glucose and glycogen through glycolysis and the Krebs cycle, and from fats through β–oxidation (Hawley & Hopkins, 1995). The metabolism of skeletal muscles from wild animals differs substantially from that seen in humans. Antelope species, such as springbok, kudu, mountain reedbuck (*Redunca fulvorufula)* and black wildebeest (*Connochaetes gnou*) have muscle with very high mitochondrial numbers and high oxidative capacities, equating to capacities found in highly trained human endurance athletes (Curry et al., 2012; Kohn, 2014). Additionally, these animals (including some wild felid species) also have an enormous glycolytic capacity to metabolise glucose and glycogen through their glycolytic pathway to either feed into the Krebs cycle or to produce lactate (Curry et al., 2012; Kohn, Burroughs, et al., 2011). It was also shown that individual muscle fibres from wild felids produce three times more power than their human equivalent, indicating the large demand for ATP from these metabolic pathways once muscle contraction commences (Kohn & Noakes, 2013).

Although not yet measured, it is postulated that during a fight or flight episode, the muscles of these animals possess the capacity to generate enormous quantities of ATP (Kohn, 2014; Kohn, Curry, et al., 2011). In stressed animals, β_2–adrenergic receptor stimulation by adrenalin results in the production of cyclic AMP, which in turn increases glycogenolysis and glycolysis, with a resultant additional increase in ATP syntheses (Levy, 2006). With this increase in metabolism, there is a concomitant increase in reactive oxygen species (ROS) and reactive nitrogen species production in the tissue via a number of pathways (e.g. within the mitochondria, the xanthine oxidase pathway, NADP oxidase) (Powers et al., 2011). In humans and animals, ROS also act as signalling molecules to aid in adaptation (e.g. increasing mitochondrial biogenesis) of muscle systems to be able to cope with increased contraction demand. Some of these adaptations include improved blood flow through capillarisation, increased enzyme activities of the metabolic pathways and the upregulation of antioxidant pathways (French et al., 2008; Powers et al., 2016). Enzymes in the antioxidant pathways, like superoxide dismutase require zinc, copper and manganese for optimal function. Superoxide dismutase converts superoxide to hydrogen peroxide, where after it is further neutralised to water by peroxiredoxins and glutathione peroxidase (requiring selenium as a co–factor) or reduced to water and oxygen (Cleveland & Kastan, 2000).

Fear and resultant flight during escape, both cause an increase in muscle metabolism and therefore increased ROS production. Hence, if capture fear and escape–exertion are excessive, then overproduction of ROS may become a possibility (Barth et al., 2007; Reardon & Allen, 2009). Excessive ROS are known to cause mitochondrial oxidative phosphorylation to uncouple, leading to heat production, which in turn may trigger cell death (Busiello et al., 2015; Powers et al., 2016). Thus, in theory, if the overproduction of ROS overwhelms the antioxidant defence system of an animal, it may lead to the build–up of highly reactive superoxide ions, that could be the cause of rhabdomyolysis, and the elevated body temperature observed in capture myopathy.

In the presence of iron molecules, superoxide may be converted to hydroxyl radicals, which is the strongest oxidant produced in biological systems and a potent trigger of cell death (Augusto & Miyamoto, 2012; Schrader & Fahimi, 2006). Large variations in iron content exist in skeletal muscles between species and, hence, may affect the rate of hydroxyl radical formation. Although not yet linked to capture myopathy, the higher iron concentration may be a cause or be a predisposing factor in certain species that are more susceptible to capture myopathy (Mostert & Hoffman, 2007). In support of this argument, iron supplementation in mice increased iron carriers (i.e. ferritin) by approximately 200%, and it also increased the activities of glutathione reductase and glutathione peroxidase by 30% and 220%, respectively. Exercise performance in these mice decreased substantially and they were more prone to oxidative stress (Barth et al., 2007). Additionally, when dietary iron is excessive, it causes a copper deficiency, possibly by preventing copper absorption (Dashti et al., 2016). Copper deficiency is known to result in decreased antioxidant (superoxide dismutase) activity in muscle tissues (Dashti et al., 2016). With superoxide known to cause uncoupling in the mitochondria, leading to increased thermogenesis, continuous production of superoxide may be the cause of hyperthermia in capture myopathy and exertional heatstroke, even when muscle contraction has stopped for a prolonged duration (Echtay et al., 2002).

(d) Abnormal response of muscle metabolism to hormones may cause rhabdomyolysis

Past research has shown that hyperthermia and rhabdomyolysis may be caused by rapid surges in hormone levels. Specifically, higher than normal levels of thyroid hormone or noradrenalin have been shown to increase mitochondrial uncoupling (Mills et al., 2004; Rusyniak & Sprague, 2006; Sprague et al., 2007). Additionally, rhabdomyolysis has been induced when

α_1– and β_3–adrenoreceptors were activated using various drugs that stimulate the sympathetic nervous system (Mills et al., 2004, 2004). In theory, different responses to stress, the amount of hormone released, the sensitivity of skeletal muscles to these hormones and differences in mitochondrial uncoupling between species and individual animals may therefore explain why some animals are more susceptible to develop capture myopathy, but requires further investigation.

(e) Rhabdomyolysis can be caused by inherent muscle myopathies

Although metabolic myopathies are not believed to be the most common aetiology for exertional rhabdomyolysis in humans, they should be considered and eliminated as a possible cause (Rawson et al., 2017). Similarly, they should be considered and investigated in cases of capture myopathy (Bartsch et al., 1977; Capacchione & Muldoon, 2009). A number of metabolic myopathies identified in humans may cause muscle rhabdomyolysis (Guis et al., 2005; van Adel & Tarnopolsky, 2009). These include genetic mutations in mitochondria, fatty acid oxidation and glycogen metabolism, resulting in an imbalance between energy supply and demand. Of these, carnitine palmitoyl transferase II and myophosphorylase deficiencies are well-known to cause rhabdomyolysis (Guis et al., 2005; Rawson et al., 2017).

There is evidence that supports the involvement of metabolic myopathies in the various conditions that present with rhabdomyolysis. Wappler *et al.* (2001) found that 10 out of 12 patients that developed exertional heatstroke, tested positive for malignant hyperthermia. As mentioned before, this condition can be triggered in pigs by emotional stress, but whether this is the case in wild animals, still needs to be determined (Aleman, 2008; Antognini et al., 1996; Capacchione & Muldoon, 2009). PSSM in horses (and potentially in cetaceans) is a common cause of exertional rhabdomyolysis, and therefore a highly plausible cause for capture myopathy (Aleman, 2008; Roe & Spraker, 2012; Sierra et al., 2012; Valberg et al., 1992). Thus, the presence of metabolic myopathies in wildlife should be investigated in more detail in susceptible species.

(ii) External predisposition to capture myopathy

(a) Nutritional factors associated with oxidative stress and rhabdomyolysis

Antioxidants play an important role in reducing the ROS produced from the increased metabolism during capture (Bagley et al., 2007; Reardon & Allen, 2009). Many of the antioxidant pathway enzymes require cofactors in the form of minerals such as zinc, copper, selenium or manganese to function optimally (Powers et al., 2011). Previous research on a similar condition, namely porcine stress syndrome, indicated that zinc supplementation, either in fodder or injected prior to stress, decreased the formation of pale soft exudative lesions typically found in the heart (Häggendal et al., 1987). Similar results were obtained when animals were pre–treated with a combination of vitamin E and selenium (Liu et al., 2018). Therefore, a deficiency in these mineral cofactors for optimal antioxidant functioning may prevent the neutralisation of ROS and lead to excessive cell damage and excessive uncoupling of oxidative phosphorylation in mitochondria (Schrader & Fahimi, 2006).

Sadly, there is limited to no evidence that any supplementation has a protective effect against the development of rhabdomyolysis and capture myopathy, but must be studied (Banerjee et al., 2003; Powers et al., 2004; Valberg, 2009). Anecdotal reports that vitamin E and selenium supplementation may prevent chronic exertional rhabdomyolysis in horses do exist, but lacks scientific backing from clinical trials (Beech, 1997). A greater understanding of ROS metabolism and the antioxidant status in healthy wild animals is needed, and further investigations are required to determine the role that ROS plays in capture myopathy.

(b) Lack of adaptive physiological mechanisms to protect against rhabdomyolysis

A lack of exercise may play a role in the development of exertional heatstroke in humans and tying up in horses (Harthoorn, 1976; Rae et al., 2008). Poor fitness levels, which usually occur in wild animals kept in confined spaces (i.e. enclosures, paddocks, and public exhibits), may predispose them to capture myopathy (Harthoorn, 1976; Rae et al., 2008; J. E. Smith, 2005). However, although free roaming wild animals are likely to be fitter than the above, their fitness level is unlikely adequate to endure the overexertion caused by a capture event. In fact, impala in a wild setting probably do not have a high level of fitness as they only run for less than 5% of the distance, they normally travel in one day (Powers et al., 2004). Harthoorn (1979) developed methods that involved exercise training of wild animals before translocation, based on the assumption that training would aid in reducing capture–related deaths. This training may

increase fitness and have the benefit of increasing habituation to capture procedures, thus reducing stress responses. However, whether fitness or habituation to stressful procedures play a role in reducing capture myopathy have not yet been determined.

The proposed mechanism by which exercise training could protect against capture myopathy would be through the upregulation of antioxidant pathways. Production of ROS is a normal occurrence of physical exercise and the inherent antioxidant pathways provide sufficient means of neutralising these free radicals (Powers et al., 2004). However, when the intensity of exercise is severe and coupled with stress and anxiety, the antioxidant pathways may be overwhelmed with subsequent oxidative damage (Aleman, 2008; Banerjee et al., 2003). Regular exercise training may be protective against these effects as it readily upregulates the amount and activity of antioxidant enzymes, thus effectively reducing ROS and increasing cellular protection against oxidative damage (Banerjee et al., 2003; French et al., 2008; Yamashita et al., 1999).

Another cellular adaptation to exercise is increased levels of heat shock proteins. These proteins are crucial protectors of cellular components during periods of stress. Specifically, exertion (such as exercise) leads to hyperthermia, oxidative stress and altered fuel metabolism. It has been shown that both heat shock proteins and antioxidant enzyme expression levels can increase within 3 to 5 days after exposure to stressors induced by mild exercise intensities (Noble et al., 2008). In the event of a subsequent exposure to stressors, these adaptations protect the cellular components and result in a higher survival rate of cells (Tupling et al., 2008). Thus, the integrity and function of heat shock proteins in cellular protection during episodes of stress and capture in wild animals also needs to be investigated.

(c) Pre–existing conditions

Pre–existing diseases, infections and severe verminoses cannot be excluded in predisposing animals to capture myopathy. Additionally, underlying kidney damage from drinking water with high salinity in certain habitats can contribute to the development of capture myopathy. Conversely, although marine mammals live in a ‘high salinity habitat’, they do not consume salt water as a norm and get most of their water requirements from their food or as a metabolic by-product (Ortiz, 2001). Female animals in their final trimester of gestation may also be at greater risk of developing capture myopathy. All these different factors should be investigated further to evaluate the risk that each condition may contribute (Herráez et al., 2013; La Grange et al., 2010).

1.8 Hypothesis of rhabdomyolysis in capture myopathy

An integrated hypothesis for the mechanisms that can possibly contribute to causing rhabdomyolysis in capture myopathy (and exertional heat stroke) is proposed in Figure 1. Once an animal is in survival mode and fearing for its life (fight or flight), over-compensation of its physiological responses may be detrimental to its survival. Exertion increases several metabolic pathways, leading to an increase in metabolic (e.g. ROS) and physiological (e.g. hyperthermia) by–products that stress normal cellular functions. Usually, these stressors are neutralised to some extent by internal processes, such as the antioxidant system and increase blood flow to the periphery to dissipate heat or feedback to the brain to stop exercising. Chronic and repetitive exposure to low levels of these stressors should lead to positive adaptations that may delay the onset and protect against the development of rhabdomyolysis. However, aggravating elements, such as genetic (e.g. metabolic myopathies) and environmental (e.g. lack of minerals in diets) factors may predispose a stressed animal to fatal rhabdomyolysis. It therefore is essential that these hypothetical causes and proposed mechanisms be systematically investigated in wildlife models.

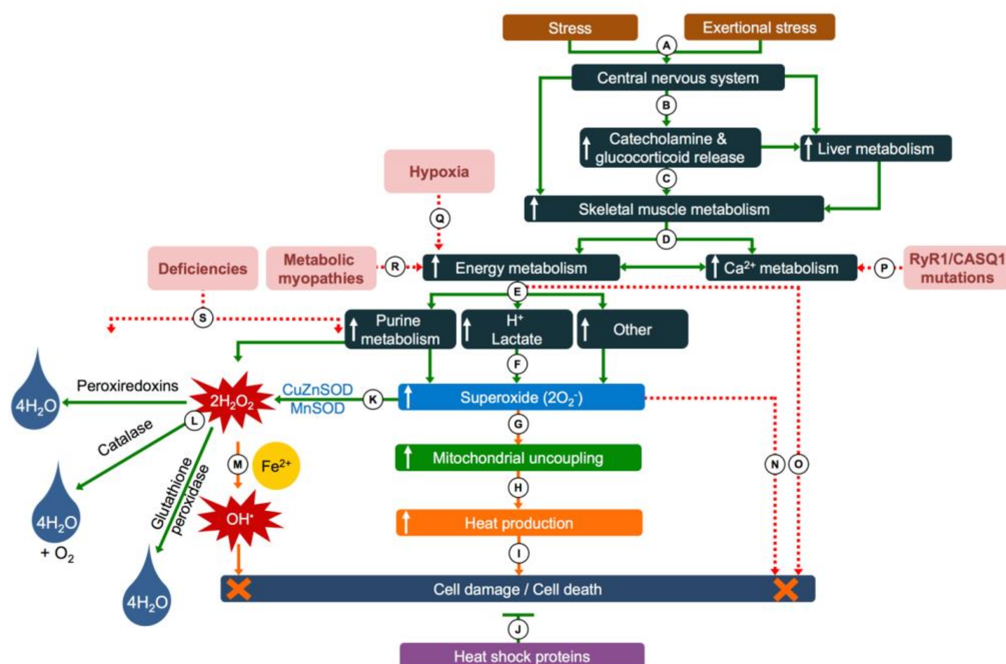

Figure 1 Simplified hypothesis for the possible pathomechanisms of capture myopathy and rhabdomyolysis of wild animals

(A) Stimuli in the form of fear and, or, exertional stress (typical fight or flight response), with the central nervous system reacting to the stimuli. (B) Increase in sympathetic nervous activation and

increased adrenalin, noradrenalin, dopamine and glucocorticoid secretion and release, as well as increased liver metabolism and skeletal muscle activity. (C) Increased catecholamine secretion upregulates skeletal muscle metabolism. (D) Increased ATP production from glycogen breakdown and phosphagen pathways in response to the demand from skeletal muscle contraction—myosin ATPase activity, active Ca^{2+} resorption into sarcoplasmic reticulum and the Na^{+}/K^{+}ATPase pumps. (E) The increased demand for ATP replenishment results in elevated purine metabolism, increased lactate and H+ production and other pathways resulting in (F) increased generation of reactive oxygen species (ROS), such as superoxide (O_2^{-}). (G) The increase in O_2^{-} results in greater uncoupling of oxidative phosphorylation and (H) increases heat production from the skeletal muscle. (I) An elevation in muscle temperature increases the risk of muscle fibre damage and necrosis (J) but is counteracted by the protective effect of heat shock proteins. (K) O_2^{-} is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), which requires zinc, copper and manganese to function optimally. (L) Three pathways neutralize the H_2O_2 to water (peroxiredoxins and glutathione peroxidase that requires selenium to function optimally) and oxygen (catalase). (M) If not neutralized, H_2O_2 may be converted to hydroxyradical molecules (OH^{-}) through the Fenton reaction (involving iron) that can cause severe cellular damage. (N) Excess ROS especially in the form of O_2^{-} may cause cellular damage. (O) A lack of ATP replenishment as a result of excessive metabolism [e.g. glycogen depletion or (Q) hypoxia prevents the myosin–actin cross-bridges to detach (form of rigor) and leads to damaged muscle fibres through mechanical stretch. Mutations in receptors involved in (P) Ca^{2+} regulation or (R) ATP production can result in muscle damage through the same mechanism proposed in (O). (S) Mineral deficiencies (co-factors) within the oxidative stress pathway enzymes can lead to diminished antioxidant capacities, leading to excess ROS that may injure cell membranes.

1.9 Conclusion

(1) Capture myopathy is a condition that can kill many wildlife species, it is characterised by severe muscle rhabdomyolysis, kidney failure and elevated body temperatures. It presents with very similar symptoms and pathophysiology compared to human and domestic animal conditions with a rhabdomyolysis component.

(2) To date, no cure exists for capture myopathy.

(3) Good planning and available resources before a capture event are currently the best way in improving survival rates of wild animals.

(4) Due to a lack of scientific studies, various intrinsic and extrinsic factors that are known causes of muscle rhabdomyolysis in humans and animals have not yet been implicated as a cause for capture myopathy.

(5) There is an urgency to adequately determine the pathophysiology, triggers and predisposing factors that induce capture myopathy in order to identify targets for treatment, to ensure animal welfare and the survival of already endangered species.

Conservation implications

Climate change, poaching and habitat loss increasingly threatens wildlife globally and is of increased concern for conservation in arid and semi-arid continents like Africa. The continent currently faces a tragic extinction epidemic of a multitude of species where translocation efforts may often be the only hope.

The need for increased success rates in the capture and translocation efforts of large mammals is thus of crucial importance and improvements can only be achieved by better understanding the causes and pathophysiology of capture myopathy.

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Dedication

We dedicate this review to the late Dr Antonie Marinus Harthoorn, whose pioneering work on capture myopathy has inspired our research endeavour.

CHAPTER 2

LITERATURE REVIEW: PHYSIOLOGICAL RESPONSES TO EXERCISE AND ADAPTATIONS TO EXERCISE TRAINING

2.1 Introduction

The terms 'physical exertion', 'physical activity', 'physical fitness' and 'exercise training' are different terms but are often wrongly used interchangeably. Physical exertion or activity is the use of skeletal muscles to create movement and result in energy expenditure (Campbell et al., 2013; Caspersen et al., 1985; Vanhees et al., 2005). Exercise or exercise training refers to a repeated and planned form of physical exertion or activity with the aim to improve or maintain physical fitness (Campbell et al., 2013; Caspersen et al., 1985; Vanhees et al., 2005).

Increased fitness through exercise has been proposed as an important management intervention to prevent mortalities resulting from capture myopathy in wild antelope (Breed et al., 2019; Harthoorn, 1979; La Grange et al., 2010). Although proposed as far back as 1979 by Harthoorn, the hypothesis that poor animal fitness is associated with increased mortality from capture myopathy has not been tested (Chapter 1). Similarly, there appear to be no studies that have attempted to exercise-train wild caught antelope or determine the adaptation effects this may have on physiological parameters of the animals (Breed et al., 2019).

Using untrained human participants, measurable adaptations to exercise have been shown to occur as early as two weeks after starting high intensity interval training sessions of 20 minutes, three times per week (Gibala et al., 2012). However, these adaptive changes decrease within a week or two after cessation of training (Thyfault *et al.*, 2015). Most adaptations are completely lost within a few months of sedentary behaviour in humans (Thyfault *et al.*, 2015), suggesting that captive or semi-captive animals, with limited space to move, may lose any naturally acquired fitness adaptations. In addition, animals can walk great distances when searching for food, running away from predators or when chasing prey, or in the rutting season (fighting). Captive animals are often overfed due to obligations to comply to well-meaning legislation, wrongful perceptions of animal well-being or to enhance production (Altman et al., 2005; Goodchild & Schwitzer, 2008; Schiffmann et al., 2018). Fitness level is generally considered to be cardioprotective and is associated with decreased levels of anxiety and stress-related disorders in humans (Alleman et al., 2015; Stubbs et al., 2017). Numerous beneficial

adaptations occur in response to exercise, including improved cardiovascular response to exercise, improved temperature regulation, hormonal, metabolic and musculoskeletal adaptations that improve physical performance (Geor & McCutcheon, 1998; MacInnis & Gibala, 2017). During the capture of wild animals, psychological and physiological stress is experienced. Thus, fitness may play a significant role in increasing physical resilience to capture stress. In wild animals, sedentary captive situations may increase the vulnerability of these animals to a stressful incident during follow-up capture operations.

The typical fitness indicators used in humans are body fat percentage, resting heart rate, maximum oxygen consumption (VO_{2max}) and lactate threshold. VO_{2max} is defined as the maximal amount ability of the pulmonary, cardiovascular, and musculoskeletal systems to uptake, transport and utilise oxygen per minute (Katch et al., 2010; Kemnitz et al., 2002; Smirmaul et al., 2013).

VO_{2max} is usually measured by an incremental exercise test on a treadmill or cycle ergometer (Smirmaul et al., 2013). This methodology is clearly problematic in wild caught animals and cannot be used. Additionally, although exercise can be facilitated in a less intrusive and natural manner in wild caught animals, many of the physiological measurements and biological samples would still require some form of physical restraint at some point, resulting in a stress response (fight or flight). Thus, for most wild animal studies, chemical immobilisation is frequently required at a predetermined time point to perform physiological measurements and biological sampling, but VO_{2max} cannot be measured in this manner. This method of obtaining physiological measurements and samples have a significant effect on the results obtained and implications for the interpretation of the results obtained (Pfitzer et al., 2021).

The normal physiological responses of wild animals during any hands-on sampling are significantly influenced by the immobilisation required using potent opioid agonists. Opioids in particular cause significant respiratory depression that is often aggravated by the tranquilisers administered in combination, which leads to hypoventilation, hypoxia, hypercapnia, hypertension and acidaemia. Opioids may also cause central nervous system depression or stimulation, impaired thermoregulation, and bradycardia (Meyer, 2009; Meyer et al., 2015; Pfitzer et al., 2020). Medetomidine, a sedative that is often used in combination with opioids, may have dose dependent cardiopulmonary effects that include bradycardia and hypoxaemia (Williams et al., 2018). Azaperone, another tranquiliser that is often used in combination with opioids, antagonises α_1-receptors in peripheral arterioles, limiting vasoconstriction and resulting in hypotensive effects (Buss et al., 2016; Lees & Serrano, 1976).

This vasodilatory effect of azaperone may counter some of the hypertensive effects of opioids when used in combination (Buss et al., 2016; Lees & Serrano, 1976).

Mortalities may occur during the capture of wild animals by humans and the factors that lead to such an occurrence are compounding. Wild caught animals are unhabituated to the noise of vehicles, humans and helicopters and psychological stress may result due to these perceived threatful situations. They may be forced to run at high intensities for prolonged periods during a capture operation, that is not a natural occurrence as most predators do not chase their prey for extended periods. In natural environments, animals are forced to be physically active to obtain food (or grazing), water, reproduce, escape danger and migrate depending on environmental factors (Halsey, 2016). In the game ranching industry, it is likely that predators have been removed or are separated off in camps, and the wild animals are contained in small camps or kept in bomas for prolonged periods. These animals may have severely reduced or limited daily movement and may therefore become detrained or unfit. The period of severe physical exertion and psychological stress during a capture would be followed by immobilisation with opioids and tranquilisers. The opioids and tranquilisers severely reduce the physiological compensating mechanisms required after exertional stress like hyperventilation, thermoregulation, increased cardiac output etc. With these limitations and compounding factors in mind for wildlife in a current day context, this study was designed to measure whether trained differ to untrained wild antelope in their physiological responses during immobilisation after a stressful capture event.

The following review looks at the responses and adaptations to exercise that have been well studied in humans, rodents, horses, and dogs, but very rarely in wild-caught animals. The focus of the review is on variables that were measured in this study, as a fully comprehensive review of fitness adaptations would be too exhaustive.

2.2 Body conditioning

Body conditioning refers to the process of exercise training to become physically fit (Harkins et al., 1993; Katch et al., 2010). Body mass changes in humans and animals is the sum of energy consumed versus energy expended in the form of physiological maintenance and physical activity (Meunier, 2006; Swift et al., 2014). In humans, increased energy expenditure occurs with exercise training, which is matched by increased energy intake as performance will decrease in the event of insufficient energy intake and thus, ATP production. In elite athletes,

consuming enough energy to maintain body and specifically, muscle mass, is often problematic (Manore, 2015). For individuals that aim to lose body fat, consuming less energy to lose fat and retain muscle mass often requires conscious effort and planning (Sikiru & Okoye, 2014). Exercise training without appropriate dietary adjustment to achieve designated weight loss goals may only result in small weight changes (Katch et al., 2010; Swift et al., 2014). This is proven by manual labour workers maintain their body weight despite high energy expenditures during their daily workday, supporting a natural increase in food consumed to match the energy required for the work performed (Swift et al., 2014). Wild animals with lower or lean body condition scores, that are exposed to increased exercise or exercise training and receive inadequate nutritional intake may lose muscle mass (Bright Ross et al., 2021).

Changes in body mass may represent a body composition shift that is accompanied by an increased lean muscle weight and decreased body fat percentage (Sikiru & Okoye, 2014). Increased muscle weight, or decreased fat, improves the power-to-weight ratio and increases resistance to fatigue (Sikiru & Okoye, 2014). In contrast, sedentary behaviour or poor nutrition may have the opposite effect resulting in decreased muscle mass as is seen in humans and other animals (Goodchild & Schwitzer, 2008; Mugahid et al., 2019; Schwitzer & Kaumanns, 2001). In wild animals, a layer of adipose tissue may protect against starvation when access to food is limited but can reduce speed when escaping from a predator (Halsey, 2016). The reduced threat of predators is proposed as one of the reasons for increased body fat percentages seen in laboratory and zoo animals (Schwitzer & Kaumanns, 2001). Some animals can maintain their muscle mass despite inadequate exercise or diet (Lohuis et al., 2015; Mugahid et al., 2019). Hibernating polar bears, squirrels and grizzly bears are highly resistant to muscle atrophy and able to maintain their muscle mass by mobilising their accumulated fat reserves and reabsorbing urea from urine which decreases the protein need from muscles (Lohuis et al., 2015; Mugahid et al., 2019).

2.3 Body temperature response to exercise

During exercise heat is produced from increased energy expenditure (70–75% of the energy is converted to heat) needed for muscle contraction and increased metabolism in various assisting organs (Burton et al., 2004; Cain et al., 2006). Thus, it can be deduced that only 25-30% of energy is used to perform actual work (Burton et al., 2004; Cain et al., 2006). Heat is dissipated through cutaneous vasodilation of blood vessels in cool environments, allowing increased

blood flow to the areas that facilitate heat exchange between the organism and the environment, so called non-evaporative heat loss. Increased evaporative cooling through sweating and panting are additional pathways to rid the body of excess heat (Burton et al., 2004; Cain et al., 2006).

Hyperthermia during heat exhaustion or heat stroke can result in the neurological and consequent haematological dysfunction firstly and may ultimately lead to multi organ failure (kidneys, liver, heart etc.) (Kenny et al., 2018; Smith, 2005). Due to the brain's sensitivity to elevated temperatures, selective brain cooling was initially thought to be an adaptive mechanism to keep the brain below a critical temperature when the body temperature rises in artiodactyls (Cain et al., 2006; Taylor & Lyman, 1972). However, hyperthermia is not a prerequisite for selective brain cooling and may occur at any time of the day, even when the carotid arterial blood temperature is relatively low (Strauss *et al.*, 2017). The carotid rete is an area of the carotid that divides into small blood vessels behind the brain and encounters small venules returning from the nasal passages (Cain *et al.*, 2006). The heat is dissipated through evaporative cooling in the nasal passages. The blood that enters the brain is almost 4 °C cooler than the core body temperature (Cain et al., 2006). None of the ungulates that employ selective brain cooling (springbok, eland, wildebeest) use this mechanism to protect the brain from overheating during exertional hyperthermia (Mitchell *et al.*, 2002). Selective brain cooling appears to favour non-evaporative compared to evaporative heat loss routes and is thought to be primarily a water-conservation mechanism (Strauss *et al.*, 2017; Mitchell *et al.*, 2002).

Exercise heat tolerance refers to the ability of an organism to cope with heat produced internally as well as the external environmental heat load (Kenny & McGinn, 2017). Factors that affect exercise tolerance in humans during high environmental temperatures include heat acclimatisation, exercise training in equivalent conditions, sex, gender, and body fat percentage (Kenny & McGinn, 2017). Exercising under the prevailing environmental conditions and allowing for heat acclimatisation are an integral part of preventing heat related injuries. Both exercise training and heat acclimatisation, facilitate a reduction in core temperature through increases in skin vasodilation, sweating, and cardiovascular and fluid balance adjustments (Fortney & Vroman, 1985). Adaptations to exercise training allow for trained individuals, including humans and a wide array of animals that include horses, dogs, lizards, ducks, salmons, crocodiles etc. to have a greater resistance to fatigue (Butler & Turner, 1988; Geor & McCutcheon, 1998; Husak et al., 2015).

Each species have different adaptations to cope with the metabolic and environmental heat load (Reece et al., 2015). Animals can evaporatively cool through cutaneous water loss (sweating), panting, spreading saliva or wetting of the body (Fuller et al., 2016). There is a correlation between body size and increased cutaneous evaporation (Robertshaw & Taylor, 1969). Larger animals tend to favour cutaneous evaporation, while smaller animals tend to favour getting rid of heat through the respiratory tract (Robertshaw & Taylor, 1969). Most African mammals, including cheetah and springbok, have sparse fur allowing them to quickly dissipate heat through cutaneous evaporation during high intensity exercise (Fuller et al., 2016). In antelope, sweating and respiratory evaporative cooling are used to reduce body temperature (Robertshaw & Taylor, 1969). Eland lost 78% of heat through sweating, 20% through respiratory evaporative cooling (panting) and stored 2% of heat for release at night when it was cooler (Finch De, 1972). The hartebeest lost 33% of heat through sweating, 61% through panting and 6% of heat was stored for later release when cooler (Finch De, 1972). The two smallest antelopes, the dik-dik and suni, rely mostly on panting (Musewe et al., 1976). Reliance on sweating appears to be the highest among equids and primates, and a reason that these species do not extend into arid zones due to their reliance on consuming water (Fuller et al., 2016).

It is believed that cellular damage occurs when body temperatures are 2 to 3 °C above normal. In humans the normal body temperature is 36.6 °C and damage may occur at greater than 40 °C (Protsiv et al., 2020; Walter & Carraretto, 2016). In blesbok, this threshold is estimated to be 41 to 42 °C, and temperatures exceeding 43 °C in wild antelope often result in fatalities (Meyer et al., 2008). Capture-induced hyperthermia is believed to be a prerequisite for the development of the fatal capture myopathy syndrome (Protsiv et al., 2020; Walter & Carraretto, 2016). However, little is known about the exact mechanism that induces this hyperthermia or the duration and extent at which it is likely to be deleterious (Sawicka et al., 2015).

Wildlife translocation guidelines prescribe that capture occurs at cooler environmental conditions (<25 °C), but hot conditions are often unavoidable (A. A. McKenzie, 1993). Furthermore, these guidelines have been questioned since a pivotal study that showed that capture-induced hyperthermia in impala occurs regardless of environmental conditions at capture and is independent of the level of exertion (Meyer et al., 2008). In this study it was shown that the hyperthermia is predominantly associated with the stress response induced by capture (Meyer et al., 2008). Whether exercise training and acclimation could improve thermoregulation in response to the exertional stress experienced during capture is currently not known or evident in the literature.

2.4 Cardiovascular response to exercise

The adaptations in the cardiovascular system to prolonged exercise are similar in humans, and many animals like horses, dogs, mice, pigs, rabbits that are often used as animal models in exercise studies (Betros et al., 2013; Husak & Lailvaux, 2019; Pellegrino et al., 2014; Poole et al., 2020). The adaptations do quantitatively vary and are correlated to body size and weight (Physick-Sheard, 1985; Reece et al., 2015). During exercise, splanchnic blood flow is decreased, whereas blood flow to the muscles, lungs, heart, and skin is increased. Blood flow is maintained to vital organs like the kidneys and the brain (Burton et al., 2004). The metabolites produced in muscle during exercise result in vasodilation and increased blood flow to the muscles. The decrease in pH and increase in temperature during muscle exertion results in a right shift in the oxygen disassociation curve and improved unloading of oxygen from haemoglobin in muscle tissue (Burton et al., 2004).

The capacity of the cardiovascular system is the primary factor limiting delivery of oxygen to the muscles during exercise (Vella, 2005). The increased oxygen demand requires increased blood flow and is directly related to increased cardiac output, where cardiac output is the product of the heart rate (beats per min) and stroke volume (volume of blood ejected per min) (Vella, 2005). This increased demand is met by an increased heart rate (chronotropy) and an increased strength of contraction (inotropy), described by Starling's law (Vella, 2005).

Trained human athletes achieve the same cardiac output at rest compared to untrained individuals but with a lower heart rate, primarily from an increased stroke volume (Bahrainy et al., 2016). Proposed mechanisms for this reduction in heart rate are an increased vagal tone that reduces the heart rate, or a decreased sensitivity of the heart to beta-adrenergic stimulation (Bahrainy et al., 2016). The current school of thought is that the heart rate is reduced by intrinsic adaptations in the heart, such as increased parasympathetic activity (Bahrainy et al., 2016). This lower heart rate allows for increased filling of the ventricles. The increased filling of the ventricle and strengthened hypertrophic heart muscle of trained individuals result in a larger volume of blood that can be ejected with each contraction (Burton et al., 2004; Katch et al., 2010). During maximal exercise, heart rate and stroke volume reach 90% of maximal capacity for fit and unfit individuals, but fit individuals can eject a greater volume of blood due to their improved stroke volume, thus being able to maintain greater oxygen delivery to the working muscles (Burton et al., 2004).

The phenomena of a decreased heart rate at rest with physical conditioning is not a standard finding in other species. In dogs, physical fitness has been shown to be associated with a lower resting heart rate, but in horses the finding is variable, and one study only found a lower resting heart rate in young Standardbred mares and not for older mares (Betros et al., 2013; Shave et al., 2017). In horses and greyhounds, heart rate is reduced during submaximal exercise as an adaptation in response to exercise training (Reece et al., 2015). However maximum heart rate does not change in response to exercise training in horses and greyhounds, which is like humans where only age affects the maximum heart rate (Reece et al., 2015).

2.5 Respiratory response to exercise

During exercise, proprioceptors in the muscle and joints of active limbs provide feedback to the motor cortex which stimulates the respiratory centres in the brain stem to increase ventilation, before any blood gas changes are evident (Burton et al., 2004; Katch et al., 2010; Reece et al., 2015). Ventilation is further increased as exercise continues or intensifies, in response to the decrease in O_2, rise in the production of CO_2, hydrogen ions (H^+) and body heat (Burton et al., 2004; Katch et al., 2010; Reece et al., 2015). Ventilation is directly proportionate to oxygen requirements and is increased by an increase in tidal volume and the respiratory rate (Burton et al., 2004).

No structural adaptations have been recorded in the lungs resulting from repetitive exercise training, with the main adaptations being functional (D. C. McKenzie, 2012). The respiratory muscle strength does however increase, which facilitates a higher respiratory rate and depth, and thus ventilation (D. C. McKenzie, 2012). Respiratory rate is a reliable indicator of the anaerobic threshold, but it is better to use minute ventilation (V_E), and the ventilatory equation for oxygen (V_E/VO_2), when evaluating small adaptive changes in highly fit athletes (Carey et al., 2005; Linderman et al., 2008). Trained human athletes maintain adequate ventilation during exercise through increased tidal volume and to a lesser extent from an increase in respiratory rate (D. C. McKenzie, 2012). There is also increased blood flow to the lungs that facilitates greater pulmonary diffusion, and thus training improves ventilation perfusion ratios (D. C. McKenzie, 2012; Tedjasaputra et al., 2016). Breathing rate does not reach maximum capacity during exercise and is not the main limiting factor when it comes to oxygen delivery to the muscles (Burton et al., 2004). Respiratory rate can, however, be used to measure effort during exercise (Nicolò et al., 2019).

The respiratory rate of healthy untrained human adults increases by 35 - 45 breaths/min in response to exercise, with a tidal volume more than 2.0 L and a minute ventilation of up to 100 L/min. In well-trained human athletes during maximal exercise, respiratory rate can increase to ~70 breaths/min and minute ventilation can increase as high as 200 L/min compared to untrained individuals (Katch et al., 2010). The horse has a minute ventilation of 100 L/min at rest and can increase to 1800 L/min during exercise. The latter is achieved mainly by increasing the respiratory rate from 10 – 15 breaths/min to 120 – 148 breaths/min. By comparison, tidal volume only increases three-fold from 5 to 15 L (Franklin et al., 2012).

2.6 Blood gas tensions and acid-base balance response exercise

In horses and humans, the differences in the arterial pressure of oxygen (PaO_2), carbon dioxide ($PaCO_2$) and arterial pH between trained and untrained individuals during moderate exercise is not significant (Azizi, 2011; Reece et al., 2015; Spoo et al., 2015). The blood gas response to exercise is facilitated through increased cardiac output, increased pulmonary blood flow and improved gas exchange (Azizi, 2011; Reece et al., 2015; Spoo et al., 2015). Following relatively exhaustive anaerobic exercise in trained and untrained individuals, mitochondria may not have sufficient oxygen for optimal function, and anaerobic glycolysis increases (Douglas et al., 2012; Levy, 2006; Reece et al., 2015). This produces lactate and H^+ which decreases muscle pH (Douglas et al., 2012; Levy, 2006; Reece et al., 2015). Lactate is exported from muscle fibres by monocarboxylate transporter 4 (MCT4), which is a H^+ symporter (Harley et al., 2009; Messonnier et al., 2013). H^+ must be buffered to maintain blood pH (Harley et al., 2009; Messonnier et al., 2013). When excessive lactate is produced and the free H^+ exceeds the buffering systems, the blood pH decreases and fatigue sets in (Atherton, 2015). Chemical buffers are the first line of defense and consist of bicarbonate (HCO_3^-), phosphates and blood proteins. Haemoglobin (Hgb) is considered the most important blood protein buffer (Atherton, 2015). The secondary mechanisms of pH buffering are only activated when there is a change in pH (Atherton, 2015). The pH is then also adjusted through changes in ventilation (described below in detail) and the renal buffering system (Atherton, 2015).

HCO_3^- buffers the excess free H^+ during lactate production in the following reaction (Azizi, 2011; Katch et al., 2010; Robertson, 1989) :

The buffering from HCO_3^- results in a decreased blood HCO_3^- concentration with increased $PaCO_2$, initially compensated for by increasing ventilation, and as a result the $PaCO_2$ decreases (Robertson, 1989). The increased ventilation also results in increased PaO_2. The main driver to maintain increased ventilation, since blood gases are generally normalised quickly with increased ventilation, is the increased H^+ ions in the blood that originate from the export of lactate via the MCT4 transporters. The increased cardiac output assists with improving oxygen delivery and consumption by muscle tissue (Maina, 2014).

High intensity anaerobic exercise training may improve the ability to buffer acidic metabolites and allow more H^+ to be produced during exercise (Edge et al., 2006; McGinley & Bishop, 2016). In some human studies it has been shown that increasing HCO_3^- reserves within circulation prior to exercise can improve buffering and performance during anaerobic exercise (Hadzic et al., 2019; Krustrup et al., 2015). Some studies on wildlife claimed that infusing HCO_3^- after strenuous exercise could be beneficial in treating severe acidosis (Harthoorn et al., 1974; Harthoorn & Young, 1974), but this practice has largely been refuted in human and animal clinical studies (Forsythe & Schmidt, 2000).

Base excess is a variable that is often measured and can provide additional information on the acid base balance during exercise. Base excess is the amount of base required to normalise the pH of a litre (L) of blood to its normal pH (7.4) at a pCO_2 of 40 mmHg. It is described as the metabolic component of the acid-base balance or the non-respiratory component (Peake and White, 2002; Berg and Meyer, 2008) and is calculated from blood pH and pCO_2 as follows:

$$\text{Base excess} = 0.028 \times pCO_2 \times 10(pH - 6.1) + 13.8 \times pH - 124.6 \qquad \text{(Berg \& Meyer, 2008)}$$

The base excess is often used increases in metabolic alkalosis and decreases in metabolic acidosis, but only provides information about one component in the acid-base balance overview. This calculation does not take into consideration the fact that the metabolic response might be due to respiratory acidosis or alkalosis, or other disorders causing an imbalance that require compensation (Peake & White, 2002).

The anion gap is a concept used to determine the cause of a pH shift in the body. The concentrations of the cations (K^+ and Na^+) must balance with the anions (Cl^- and HCO_3^-) to maintain electrical ion homeostasis. The anion gap uses this principle and measures the difference between Na^+ and Cl^- and HCO_3^- i.e.

$$\text{Anion gap} = Na^{+} - (Cl^{-} + HCO_3^{-}) = \text{Unmeasured anions – Unmeasured cations}$$

(Reece et al., 2015)

Unmeasured cations and anions include K^{+}, Ca^{2+}, Mg^{2+}, albumin ($^{-}$), PO_4^{3-}, various organic acids and sulphates. A low anion gap is usually associated with hypoproteinaemic alkalosis, whereas a high anion gap with metabolic acidosis results from increased H^{+} accumulation (Reece et al., 2015; Stringer et al., 1992; Zeiler & Meyer, 2017). During aerobic exercise, when the lactate threshold is reached, lactate increases in the blood. If a metabolic acidosis (reduced pH) is apparent, with a hyperlactaemia, the increased H^{+} production caused by anaerobic metabolism during exercise is the likely cause of the acidosis (Berg & Meyer, 2008). In unfit individuals, aerobic exhaustion will occur earlier due to lack of exercise training adaptations to prevent fatigue (Rivera-Brown & Frontera, 2012). The earlier onset of anaerobic metabolism will result in a lactate and H^{+} surge that will require buffering. As buffering capacity is an acquired exercise adaptation, the unfit individuals will have a reduced pH buffering capacity compared to fit individuals, resulting in a decreased pH (Mairbäurl, 2013; McGinley & Bishop, 2016; Weston et al., 1996). The point at which this threshold will be exceeded will vary, depending on the intensity of the exercise and the innate aerobic fitness of individuals exposed to aerobic exercise.

2.7 Electrolyte response to exercise

Electrolyte regulation plays an important role in the onset of muscle fatigue, and exercise trained individuals are believed to be better able to respond to electrolyte changes during exercise (McKenna et al., 2008). Muscle contraction consists of the flow of action potentials along the sarcolemma and transverse tubules, and the consequent release of Ca^{2+} from the sarcoplasmic reticulum. During depolarisation of the action potential there is a Na^{+} influx, and for repolarisation, a K^{+} efflux occurs. Cl^{-} also moves into the sarcoplasm during repolarisation (McKenna, 1995; McKenna et al., 2008). Physical fatigue post exercise is partly related to multiple electrolyte disturbances (McKenna et al., 2008).

Many factors challenge the electrolyte balance during exercise. For example, the sweat rate of a horse exercising in a hot environment may approach 10 to 12 L per hour and is the main route of fluid and electrolyte loss (Geor & McCutcheon, 1998). There are large differences between the composition of horse and human sweat. Human sweat is largely hypotonic, whereas equine

sweat contains concentrations of Na^+ that are similar to serum concentrations, but the K^+ concentrations found in the horse's sweat is 10 to 20-fold higher than serum concentrations (Geor & McCutcheon, 1998). Smaller animals tend to dissipate more of their heat through the respiratory tract (e.g. dogs) and larger animals through sweating (Robertshaw & Taylor, 1969). In trained dogs that compete at submaximal exercise in agility events, disturbances in electrolytes were not found (Rovira et al., 2007). This lack of overt changes in electrolytes may be due to the minimal electrolyte loss that occurs through panting (Alves & Santos, 2016; Otto et al., 2017). In sheep and red deer, sweating only plays a minor role in thermoregulation as they are reliant on heat loss through ventilation (Jenkinson, 1973). Most small to medium sized herbivores also have fewer sweat glands and reduced sweat ability, relying more on ventilatory mechanisms to thermoregulate (Musewe et al., 1976). When addressing electrolyte imbalances post exercise, the magnitude and mechanism of fluid loss is an important consideration which can assist in determining how to correct the fluid and electrolyte imbalances effectively (McKeever & Lehnhard, 2014; Périard et al., 2015; Reece et al., 2015; Rovira et al., 2007).

(i) Potassium

Electrolytes are strictly regulated to prevent cellular swelling or dehydration. Potassium (K^+) is the main intracellular cation, and Na^+ is the main extracellular fluid cation maintaining electrical homeostasis - both cations are major role players in acid-base balance physiology (Lindinger & Sjøgaard, 1991; Reece et al., 2015). During exercise, muscles release K^+ into the interstitial space, which promotes vasodilation and increased blood flow to the muscles (Lindinger & Sjøgaard, 1991; Medbø & Sejersted, 1994). The plasma K^+ concentration subsequently rises, and the Na^+/K^+-ATPase pump retrieves the K^+ from the extracellular fluid in exchange for intracellular Na^+, to prevent K^+ from increasing to toxic concentrations (Warburton et al., 2002). Hypokalaemia may follow the initial hyperkalaemia due to the intracellular movement of K^+ because of increased blood flow and likely, intracellular acidosis that may further support the movement of K^+ into cells (Warburton et al., 2002). The loss of K^+ and gain of Na^+ in muscle is a major contributor to fatigue and may contribute to the pain reported with prolonged exercise (Lindinger & Sjøgaard, 1991; McKenna et al., 2008).

Regular exercise may increase the number of Na^+/K^+-ATPase pumps by up to 15% to address the elevated K^+ caused by exercise (McKenna, 1995). Exercise training may therefore improve the ability to regulate K^+ post exercise. Studies in animals have shown that inactivity results in

reduced Na^+/K^+-ATPase pump density, thus inactivity causes impaired K^+ regulation and a larger increase in K^+ is seen during exercise (McKenna, 1995; Medbø & Sejersted, 1994).

Another factor that influences K^+ homeostasis is the pH. When an extracellular alkalaemic environment dominates, H^+ ions leave the cells in exchange for Na^+ via the Na^+/H^+-ATPase pump. This in turn activates the $Na/^+K^+$-ATPase pump that returns K^+ to the cell and pumps Na^+ out. This exchange results in hypokalaemia. In acidaemia, the opposite occurs and H^+ ions enter the cells via the Na^+/H^+-ATPase pump (Aronson & Giebisch, 2011; McKenna et al., 2008). K^+ ions subsequently leave the cells to maintain electrical neutrality (Aronson & Giebisch, 2011; McKenna et al., 2008), and the excess K^+ in the extracellular fluid results in hyperkalaemia. In time, excess K^+ can be excreted by the kidneys and facilitate retention of Na^+ (McKenna et al., 2008; Reece et al., 2015). In dogs, a lower resting concentration of K^+ was recorded in fit dogs, and vigorous exercise did not result in a significant increase of K^+ (Alves & Santos, 2016).

(ii) Sodium

Animals with normal serum Na^+ concentrations prior to exercise will be able to maintain Na^+ homeostasis during submaximal exercise, but during strenuous exercise these mechanisms may be disrupted (Otto et al., 2017). In a study investigating agility dogs during exercise, hyponatraemia was evident but was attributed to increased consumption of water resulting in a dilution effect (Gregerson et al., 1996). A similar exercise associated hyponatraemic state is seen in humans that consume excess fluid during exercise (Noakes, 2003). Herbivores may even present with hyponatraemia at rest, as plants contain low sodium (Na^+) concentrations, and NaCl needs to be regularly supplemented (salt lick) (Kaspari, 2020; Molina et al., 2014). Hyponatraemia may be more evident in wildlife as they often reject consuming a supplementary salt lick if not accustomed to the practice (La Grange et al., 2010). Animals that are sub-clinically hyponatraemic will often have suboptimal responses to exercise. This is due to early on muscle fatigue during exercise due to lack of Na^+ to facilitate muscle contraction and further loss during of Na^+ during exercise through sweating (Reece et al., 2015; Warburton et al., 2002).

(iii) Chloride

The major anions in the extracellular fluid are chloride (Cl^-), HCO_3^- and the phosphates (McKeever & Lehnhard, 2014). Cl^- is the main anion and has an important role in maintaining extracellular fluid volume. During exercise, the hydration reaction of CO_2 results in an accumulation of HCO_3^- in red blood cells and this in turn results in an accumulation in plasma due to the concentration gradient. To counter this electrical shift of HCO_3^- into the plasma, Cl^- moves into the red blood cells in what is known as the "chloride shift". When CO_2 leaves the red blood cells in the lungs and HCO_3^- returns to the red blood cells, Cl^- re-enters the plasma (Reece et al., 2015; Westen & Prange, 2003). The chloride shift is important as it enhances the ability of the blood to carry HCO_3^- and is thus an important part of blood acid base regulation (Reece et al., 2015; Westen & Prange, 2003). The movement of Cl^- is also known to determine Hgb-O_2 dissociation in the brown bear and a few species of bats and ruminants (Brix et al., 1990; Westen & Prange, 2003). Haematocrit (Hct), and in turn Hgb that carries O_2, is integral for this Cl^- shift and therefore the acid base balance regulation during exercise (Westen & Prange, 2003).

In dogs, plasma Cl^- was unaltered post exercise (Rovira et al., 2007). Increased plasma concentrations of K^+ and Cl^- have been related to capture stress in wildlife and are likely due to the metabolic acidosis that is present (Paterson, 2014). Another possible contributing factor may be catecholamine-induced renal vasoconstriction and failure to facilitate electrolyte excretion in the urine (Kock et al., 1987). In untrained humans that were exposed to an acute bout of 15 min cycling exercise, a decrease in blood pH and increased Cl^- was observed, where in the trained individuals no changes were observed (Foran et al., 2003).

(iv) Calcium

Calcium (Ca^{2+}) is the most abundant electrolyte in the body and is very important for nerve function, muscle contraction, blood clotting, enzyme function, vitamin D function and fluid transport across membranes. Half of the Ca^{2+} is bound to albumin, with the remainder occurring as the ionic form or bound to citrate, HCO_3^- or PO_4^{3-} (Constable et al., 2019; Katch et al., 2010; Reece et al., 2015). Ca^{2+} plays an important role in muscle fatigue (Wan et al., 2017). Decreased blood Ca^{2+}, exercise or metabolic acidosis may stimulate parathyroid hormone (PTH) release and quickly activate bone resorption resulting in Ca^{2+} release concentration (Kohrt et al., 2018; Lin & Hsieh, 2005, 2005). The disruption of plasma Ca^{2+} concentrations

seem to be related to exercise intensity and duration (Kohrt et al., 2018; Lin & Hsieh, 2005). In one study that investigated PTH and Ca^{2+} in response to exercise (60 min cycling bouts) in adult men, the Ca^{2+} concentration increased during the first 15 min, where after it steadily decreased during the later phase of the exercise period (Lin & Hsieh, 2005). The initial increase in Ca^{2+} is likely due to haemoconcentration and not an actual increase in the Ca^{2+} concentration (Kohrt et al., 2018; Lin & Hsieh, 2005). Research into the long-term effects of regular exercise training on Ca^{2+} homeostasis is still lacking (Kohrt et al., 2018; Lin & Hsieh, 2005).

2.8 Blood biochemistry in response to exercise

(i) Exercise metabolites

Vigorous exercise causes a dramatic increase in ATP production from aerobic and anaerobic pathways. The pyruvate produced from glycolysis is converted to acetyl-CoA by pyruvate dehydrogenase and enters the citric acid cycle, where, in conjunction with the electron transport system it produces ± 32 ATPs, 6 CO_2 and 6 H_2O (Bitschnau et al., 2010; Goodwin et al., 2007; Swart & Jennings, 2004). If anaerobic conditions prevail, then the pyruvate is converted to lactate, by lactate dehydrogenase (LDH), producing approximately 2 ATPs. Lactate is no longer considered a waste product as it is converted to glucose in the liver through gluconeogenesis, following export from working muscles (Foucher & Tubben, 2021). Lactate produced by the muscles is also an important source of energy during recovery when sufficient oxygen is available, where it is converted to pyruvate and can enter the citric acid cycle (Bitschnau et al., 2010; Goodwin et al., 2007; Swart & Jennings, 2004).

In the horse, lactate is on average only produced when the heart rate exceeds 150 beats/min but would be determined by the individual animal's inherent fitness level (Evans, 2000; Evans et al., 1993; Reece et al., 2015). This point is known as the onset of blood lactate accumulation (OBLA), and the blood lactate concentration that this occurs at is approximately 4 mmol/L. This usually occurs between 55 to 65% of VO_{2max} in untrained individuals, and in trained endurance athletes can exceed 80% of VO_{2max}. The OBLA is largely an imbalance between blood lactate production and clearance, and not necessarily the exact point of anaerobic muscle metabolism. The OBLA has been shown to be a better indicator of aerobic fitness than VO_{2max} (Goodwin et al., 2007; Katch et al., 2010). The measurement of lactate as an indicator of fitness has not been validated in dogs or any other species except humans and horses (Otto et al., 2017).

An increase in the plasma lactate concentration, where release surpasses uptake, is a normal reaction to vigorous exercise (Goodwin et al., 2007; Swart & Jennings, 2004). A high concentration of plasma lactate is not always a sign of muscular hypoxaemia or ischaemia (Goodwin et al., 2007; Swart & Jennings, 2004). Lactate plasma concentration increases incrementally until a 'threshold' is reached, whereafter it increases rapidly (Goodwin et al., 2007; Swart & Jennings, 2004). This 'lactate threshold' is identified by measuring the work rate at which the lactate increases rapidly, and the intersection can be visually inspected on a graph (Goodwin et al., 2007; Swart & Jennings, 2004). Aerobic exercise training has been shown to increase this lactate threshold by improving lactate clearance, thus delaying the rapid increase in lactate (Goodwin et al., 2007; Swart & Jennings, 2004). An exercise trained individual will achieve a higher lactate threshold than an untrained person during any intensity of exercise (Goodwin et al., 2007; Swart & Jennings, 2004). The lactate plasma concentration can be used to evaluate the intensity of exertion in an individual and is a better measure of severity of exertion than heart rate or blood gas values (Goodwin et al., 2007; Swart & Jennings, 2004).

In humans and dogs, regular moderate intensity exercise improves insulin sensitivity and results in better blood glucose regulation (Adams, 2013; Alves & Santos, 2016). The level of fitness (e.g. VO_{2max}) is positively correlated to improved insulin sensitivity (Adams, 2013; Alves & Santos, 2016). Glucose is the main energy substrate for metabolism during intense exercise but at lower intensities other substrates, e.g. free fatty acids, are also used (Burton et al., 2004). In humans, at lower intensity exercise, plasma glucose decreases after postprandial exercise, but remains constant in exercise that occurs after food absorption has occurred (Marliss & Vranic, 2002). The response to intense exercise is hypoglycaemia for up to 60 min post exercise (Marliss & Vranic, 2002). With improved fitness, improved blood glucose concentration is reported post exercise in fasting and non-fasting individuals (Adams, 2013; Marliss & Vranic, 2002).

An initial decrease in plasma glucose concentrations in horses for the first 6 minutes of exercise occurs, after which plasma glucose increases (Ferraz et al., 2008). This increase can be apparent for up to 30 min post exercise (Ferraz et al., 2008). The point where blood glucose concentration starts rising during exercise is referred to as the "glucose threshold" and results from an increase in liver glycogenolysis in reaction to adrenalin (Ferraz et al., 2008). There is a correlation between the lactate and glucose threshold, suggesting that the glucose threshold may also be an indicator of fitness (Ferraz et al., 2008).

(ii) Markers of muscle damage

Strenuous exercise may result in minute tears or damage that results in the release of aspartate AST, CK, myoglobin and troponin I from the damaged muscle fibres (Katch et al., 2010; Padilha et al., 2017). AST is an enzyme that is not specific to skeletal muscle and may originate from the liver, kidneys, brain, red blood cells, cardiac – and skeletal muscle (Pettersson et al., 2008; Reece et al., 2015). CK is an enzyme that catalyses the phosphorylation of creatine or the phosphorylation of ADP to ATP when rapid energy is required (Tietze, 2012; Washington & Van Hoosier, 2012). It has many different isoforms that are found in skeletal muscle, heart, brain, bladder, stomach, colon, and mitochondria (Tietze, 2012; Washington & Van Hoosier, 2012). The main origin of serum CK is from muscular damage and rises due to muscle necrosis or disease (Vanholder et al., 2000). Regular exercise is not associated with CK increases, but CK can increase after strenuous exercise (Vanholder et al., 2000). Serum CK concentration can be variable, and some individuals may not show a rise in CK activity post exercise (Vanholder et al., 2000). Large increases in CK (100 000 U/mL) are usually associated with an increase in AST (Kindermann, 2016). Exertional rhabdomyolysis in humans and horses, and capture myopathy in wildlife, are conditions that are characterised by significantly elevated blood CK concentration (Paterson, 2014). Strenuous unaccustomed exercise performed by humans usually results in a condition known as delayed onset muscle soreness (DOMS) (Grobler et al., 2004). DOMS is not fatal but is characterised by muscle pain 1-2 days post exercise and a peak in serum CK activity 48 hours post exercise (Grobler et al., 2004). The pain usually subsides after the third day, with CK activities returning to normal within five days (Grobler et al., 2004). In horses, the rise and peak in serum CK activity is within 24 hours and the post exercise elevated LDH and AST activities return to normal within 72 hours (Padilha et al., 2017).

Troponins are part of the contractile proteins of skeletal and cardiac muscle (Boesch et al., 2015). Ca^{2+} binds to troponin, changing its conformation, which in turn pulls tropomyosin away from the myosin binding sites located on the actin, allowing myosin-actin interaction (Katch et al., 2010; Reece et al., 2015; Weber & Murray, 1973). Currently, three types of troponins exist, namely troponin I (TnI), troponin T and troponin C (Boesch et al., 2015). Troponin I has three different isoforms, two of which originate from skeletal muscle and one that originates from cardiac muscle (cTnI) (Boesch et al., 2015). When cardiac myocytes are injured from hypoxic conditions or ischaemia, the cardiac isoform of troponin (cTnI) is released into the blood (Boesch et al., 2015). This blood biomarker is currently the gold standard diagnostic tool to detect acute myocardial infarction in humans and is a good indicator

of cardiac damage across species (Boesch et al., 2015). There is evidence that exercise training has an inverse relationship with myocardial damage. Individuals with adequate exercise training had a lower likelihood of developing elevated cTnI compared to those that did not (Florido et al., 2017). These cTnI elevations in inactive individuals can potentially be used as a prognostic indicator for underlying myocardial damage and increased heart failure risk (Florido et al., 2017). Cardiac troponin I (cTnI) is also known to increase in healthy untrained individuals after exercise (Gresslien & Agewall, 2016). Cardiac troponin I (cTnI), however, remains unchanged in trained individuals, unless exercise intensity and duration deviates from their accustomed training regime (Nie et al., 2018). A study in trained horses showed cTnI increases post exercise and peaks between 2 – 6 hours post exercise, returning to normal within 24 hours (Rossi et al., 2015). This is similar to humans where the cTnI increases up to 4 hours after exercise and then gradually decreases in the following 24 hours (Baker et al., 2019; Nie et al., 2018). Although the concentration of serum troponins varies significantly between individuals, evidence suggests that individuals with markedly elevated concentrations of cTnI compared to others post exercise may be more susceptible to cardiac pathology when stressed (Baker et al., 2019; Nie et al., 2018). This observation conforms to research showing that cTnI increased significantly post exercise in dogs with asymptomatic mitral valve regurgitation, compared to healthy dogs (Wall et al., 2018).

(iii) Renal function

Creatinine is a by-product of phosphocreatine metabolism, with increased serum creatinine concentrations frequently found after exercise training, rhabdomyolysis, and dehydration (Otto et al., 2017; Reece et al., 2015). Creatinine moves freely through the kidneys and is neither absorbed nor excreted and is routinely used as an indicator of kidney function (Reece et al., 2015; Wyngaert et al., 2018). A high serum creatinine concentration is one of the first indicators of kidney disease (Reece et al., 2015; Wyngaert et al., 2018). Any significant rise in creatinine following exercise suggests muscle injury and, or dehydration (Otto et al., 2017). Following exercise, elevated creatinine concentrations should return to normal within 24 hours if the rise was caused by muscle fibre micro tearing, and renal function is not compromised (Padilha et al., 2017). Creatinine increases have also been reported in wildlife subjected to capture stress and the resultant catecholamine renal vasoconstriction (López-Olvera et al., 2007).

During the deamination process of amino acids for energy metabolism in the liver, urea is formed (Foran et al., 2003; Katch et al., 2010). Urea is water soluble, osmotically active, and excreted by the kidney's urine (Foran et al., 2003; Katch et al., 2010). Thus, protein catabolism with subsequent urea excretion results in fluid loss. As exercise increases, so do the plasma urea concentration in humans and horses (Foran et al., 2003; Larsson et al., 2013; Reece et al., 2015) and the excess urea is excreted in sweat and urine (Foran et al., 2003; Katch et al., 2010). Increased plasma urea concentrations are often found when dietary protein increases, or during muscle catabolism when carbohydrate and lipid energy sources have been depleted (Reece et al., 2015). Plasma concentrations of urea and creatinine are consistently elevated in endurance horses even at rest (Reece et al., 2015). Elevated resting urea is a well-known finding in human athletes and is most likely due to the continuous increased protein metabolism that takes place during training, as mentioned before (Warburton et al., 2002).

(iv) Hepatic function

The liver has a primary role in providing glucose energy for active muscles during sustained exercise (Hoene & Weigert, 2010). The liver has also been shown to significantly adapt to regular exercise to improve lipid and glycogen metabolism (Hoene & Weigert, 2010). Short bursts of exercise do not appear to create a substantial increase in liver biomarkers, but prolonged or exhaustive exercise may result in increased aspartate transaminase (AST), bilirubin, and inflammatory markers (IL-6 and C-reactive protein) in humans and animals (Hoene & Weigert, 2010).

Gamma glutamyl transferase (GGT) is used as an indicator for liver function, but it is non-specific and may originate from the liver, biliary epithelial cells, renal tubules, pancreas, or intestinal cells (Gowda et al., 2009). In horses that were trained, no changes were seen in GGT concentrations (Padilha et al., 2017). Additionally, Fitte (2017) found that there were no changes in GGT concentrations in blesbok subjected to exertional (capture) stress. In the same study glutamate dehydrogenase (GLDH) increased in response to the exertional capture stress in the blesbok, but returned to normal by day 2 (Fitte, 2017).

Although AST may originate from many sources, it primarily originates from the liver and is an indicator of liver function. AST has been reported to increase in severe exertion and increases in animals exposed to severe capture-induced exertion have been demonstrated (Hartup et al., 1999; Pettersson et al., 2008; Schomaker et al., 2020).

Bilirubin concentrations increase in athletes due to increased haemolysis and catabolism of Hgb by the liver (Witek et al., 2017). The increased haemolysis is due to oxidative damage and mechanical factors, such as direct muscle damage to red blood cells and red blood cells squeezing through vasoconstricted capillaries (Witek et al., 2017). An example of this is marching haemolysis in military trainees (Witek et al., 2017). Decreased blood bilirubin concentration is associated with an increased risk of cardiovascular disease (Musa et al., 2016; Swift et al., 2012). In human athletes, elevations in serum bilirubin appear to be an adaptation to exercise and increased concentration are associated with a reduction in weight and lower body fat (Witek et al., 2017). Our understanding of the response of liver biomarkers to exercise, adaption to continuous exercise or damage from severe exertional stress is still limited (Shephard & Johnson, 2015).

Single laboratory tests are of limited value in assessing liver damage, as some liver diseases may result in normal values and abnormal levels may be found in some healthy individuals (Gowda et al., 2009). It is thus important to evaluate changes in enzyme abnormalities over time, as such changes may indicate liver damage (Gowda et al., 2009).

2.9 Cortisol

The acute response to intense exercise is an increased sympathoadrenal response with an increased concentration of pituitary hormones, resulting in a rise in most hormones except for insulin (Duclos & Tabarin, 2016; Hill et al., 2008). Cortisol is one of the hormones that increases during and after exercise in most species (Morton et al., 1995; Reece et al., 2015). In humans, the concentration of cortisol post exercise is determined by the duration and intensity of the exercise (60% of VO_{2max} or above is required to elicit an increase in cortisol), how long after exercise sampling occurred and time of day. Blood cortisol concentration naturally fluctuates during the day and is related to the circadian rhythm in humans and diurnal animals. The highest levels of cortisol are recorded in the morning after waking up, and lower levels in the evening at the start of sleeping (Budde et al., 2015; Hill et al., 2008; Tsai et al., 2014).

This increase may be a result of physical and psychological stress. The rise in cortisol concentration stimulates proteolysis, gluconeogenesis, hepatic glycogenolysis and lipolysis, which assist in providing adequate ATP synthesis for extended exercise. In fit individuals, a larger surge in cortisol is usually observed (Duclos & Tabarin, 2016; Reece et al., 2015).

2.10 Haematology in response to exercise

(i) Red blood cells

The total blood volume is critically important in the response to exercise (Mairbäurl, 2013). The components that make up the total blood volume (i.e. plasma volume and red blood cell volume) are vital to support the increased oxygen demands during aerobic exercise (McKeever & Lehnhard, 2014). The plasma volume is an important factor which ensures adequate cardiac filling during diastole and the red blood cell count (RBC) dictates the amount of oxygen that can be delivered to working tissues. Total plasma volume is difficult to measure as it requires a special dye technique, but Hct can be used to indicate the RBC (McKeever & Lehnhard, 2014). Hct can also be used to indicate changes in plasma volume (dehydration) but should be interpreted with caution (McKeever & Lehnhard, 2014). Acute splenic contraction may also increase RBC initially in relation to the same amount of plasma volume (Rovira et al., 2007; Wan et al., 2017). Horses, humans, and dogs show increased plasma volume in response to training to allow for the increased fluid loss that takes place during exercise (Rovira et al., 2007; Wan et al., 2017).

Packed cell volume (PCV) and total protein concentration (TP) is expected to increase during exercise in response to the fluid loss (Mairbäurl, 2013). The spleen also contracts during exercise in many animals, but most notably in the dog and horse. In the horse, the spleen holds a reserve of between 6 and 12 L (16 to 30% of blood volume) of red blood cells, which are released into circulation and improve gas transport (Mairbäurl, 2013). The PCV and splenic response is reduced in animals that are not physically trained or have underlying health conditions that compromise red blood cell availability, such as anaemia (Mairbäurl, 2013). In one study using dogs, PCV increased with exercise but the mechanism (i.e. splenic contraction or fluid shifts) could not be determined (Rovira et al., 2007). Another study in agility dogs, indicated a decrease in PCV and TP in response to exercise, suggesting that plasma volume expansion may be causing a dilution effect (Otto et al., 2017).

Hgb, as previously mentioned, is one of the most important blood protein buffers (Berg & Meyer, 2008). Hgb is known to increase during exercise training in animals and can be an indicator of cardiovascular system adaptations (Mairbäurl, 2013; Rovira et al., 2007). If an animal is anaemic and deficient in Hgb, its buffer reserve is also reduced and thus acidaemia may result during periods of exertion (Mairbäurl, 2013; Reece et al., 2015).

(ii) White blood cells

The acute response to exercise includes an increase in white blood cells such as T-lymphocytes and neutrophils, which are released from the lymphatic system (Walsh et al., 2011; Wan et al., 2017). The release of white blood cells is a non-specific inflammatory reaction in response to hypoxic tissue (Wan et al., 2017). An acute inflammatory response to high intensity exercise was not recorded in Standardbred trotter horses, although an inflammatory response has been recorded in horses during endurance racing, due to muscular damage (Kristensen et al., 2014). The cortisol released in stressful situations, such as exercise, results in a stress leukogram, inducing right shift neutrophilia, lymphopaenia and eosinopaenia (Satué et al., 2014). No differences or adaptations have been shown in the white blood cell response in relation to fitness in humans (Sand, 2013). Suppression of the antibody response has been shown in severe acute exertional exercise in humans, whereas moderate exercise training can improve antibody response and have a protective function against health stressors and aging (Walsh et al., 2011).

Exercise positively affects the number and function of circulating white blood cells that contribute to the innate immune system (Walsh et al., 2011). Additionally, evidence suggests that each bout of exercise may have an anti-inflammatory effect, which explains the protective effects of exercise against many degenerative diseases. The reduction of visceral fat that is often associated with regular training contributes to the reduced inflammatory response, as fat has been established to play an important inflammatory role in many degenerative diseases (Walsh et al., 2011). The link between exercise and its pro-immunity and anti-inflammatory effects are still poorly established (Walsh et al., 2011).

2.11 Conclusion

An exhaustive review of all exercise adaptations that occur, was beyond the scope of this review. The aim was to explore the expected physiological response to exercise and adaptions to exercise training of the variables that were measured in this study (temperature, heart rate, respiration, blood biochemistry, blood gasses, acid-base balance, cortisol and haematology) by looking at studies done in humans and other species. The beneficial physiological, physical, and even psychological adaptations to exercise are overwhelming, but as evident, vary between species. Theoretically if these adaptations occur during the exercise training of wild antelope, they may be of benefit to wildlife during an exertional capture stress event and may be protective against capture myopathy. In the following chapters, the methodology of exercise

training wild caught antelope, exposing them to an exertional capture stress event and obtaining the physiological variables are described in Chapter 3. The results, analysis and discussion of the variables recorded are captured in Chapter 4 – 6. In Chapter 7, the differences recorded and what differences and benefits were evident for exercise trained antelope exposed to the exertional stress event are summarised and what conclusions can be derived, if any.

CHAPTER 3

METHODOLOGY: BLESBOK EXERCISE TRAINING INTERVENTION AND CAPTURE STRESS STUDY

3.1 Introduction

The capture, restraint and translocation of wildlife has become a frequent occurrence for conservation and trade purposes. Individual animals and species can have significant conservation or economic value. Physiological stress is an inevitable part of the capture and translocation process of wild animals and capture myopathy is one of the most important conditions threatening wildlife interventions (Breed et al., 2019; Dickens et al., 2010; Spraker, 1993). Briefly, capture myopathy is a varied syndrome that primarily presents with acute severe hyperthermia and later as severe rhabdomyolysis (muscle breakdown). This rhabdomyolysis results in grave pathological ramifications, one of which is systemic myoglobin (muscular protein) release, which can result in acute kidney damage. Limiting and managing stress is thus a very important welfare consideration, but also vital to ensuring the survival of species and reducing animal losses (for an extensive review on this topic, refer to Chapter 1 or Breed *et al.*, 2019).

Multiple methods have been proposed to reduce, limit, and remove the stress experienced by wild animals during translocation. These methods have mainly focused on reducing the external causes of stress inflicted on the animal, such as improving capture methods, reducing the duration of stress exposure, and treating the psychological stress component by using tranquillizers. These approaches greatly improved survival rates, but deaths still occur during and after translocation (Breed et al., 2019; Dechen Quinn et al., 2014; Paterson, 2014; Spraker, 1993).

An aspect that has received some attention in the literature is capture-induced hyperthermia, which is thought to be one of the initial pathological manifestations of the capture myopathy syndrome (Breed et al., 2019; Meyer et al., 2008). Methods to prevent, manage or treat capture stress hyperthermia have been investigated. Environmental temperature, level of activity and the specific drugs that are used during capture were thought to contribute significantly to the typical hyperthermia displayed by wild animals during capture (capture-induced hyperthermia). However, these factors were shown to have a poor association with body

temperature changes in captured impala (Meyer et al., 2008). The subsequent hypothesis is that there might be other predisposing, intrinsic factors or physiological mechanism(s) that trigger capture-induced hyperthermia, and in turn, the capture myopathy syndrome, which warrants investigation (Breed et al., 2019; Meyer et al., 2008).

The intrinsic factors that may predispose wild animals to develop capture myopathy have not yet been studied. Some species also seem to be more susceptible to developing this condition, but the exact cause remains unclear (Graffam et al., 1991; Ward et al., 2011; Young, 1972). Some researchers suggested that inherent muscle myopathies, abnormal muscle metabolism or differing muscle fibre type compositions may play a role in those animals that are more susceptible, but their link to capture myopathy remains to be proven (Breed et al., 2019; Roe & Spraker, 2012; Wappler et al., 2001). Anecdotal evidence has also indicated that pre-existing factors like age, physical condition, verminoses and pregnancy, to name but a few, can predispose animals to succumb to the syndrome when subjected to stress and physical exertion (Breed et al., 2019; Paterson, 2014). However, there may be intrinsic physiological factors, such as the level of fitness, that could be improved to increase the resilience of animals to the stress and physical exertion of capture.

In humans, the leading cause of exertional rhabdomyolysis is when strenuous exercise is performed, especially in untrained overweight individuals, which results in severe muscle damage (Rawson et al., 2017). Dr Antonie Harthoorn, a pioneer in wildlife veterinary medicine and conservation, proposed a similar theory - that inadequate fitness in wild animals may be a predisposing factor in some animals to develop capture myopathy (Harthoorn, 1979; Harthoorn & Van der Walt, 1974). He proposed that wild animals might sometimes be unable to cope with the severe physical exertion they are exposed to during a capture operation. Wild animals are often unaccustomed to the type and level of physical exertion that occurs during these capture operations and, hence, develop severe muscle rhabdomyolysis, muscle tears and haemorrhaging. Harthoorn likened capture myopathy to the condition of azoturia or ‘tying-up’ in horses, which has been partially associated with inadequate fitness before being exerted. This could be similar to wildlife that are not adequately conditioned for the exertion experienced during capture (Harthoorn, 1979).

Most wildlife species do not perform much running in their day-to-day life, except when they may have to flee from a predator or catch prey (Harthoorn & Van der Walt, 1974; Spraker, 1993). During capture operations, animals may have to exert themselves at a very high intensity

for abnormally extended periods, which can result in severe rhabdomyolysis and the syndrome of capture myopathy (Breed et al., 2019).

Many studies report the beneficial physiological adaptations that occur in response to exercise training in humans and domestic animals (e.g. horses, racing reindeer, dogs and rodents) (Katch et al., 2010; Physick-Sheard, 1985; Reardon & Allen, 2009; Reece et al., 2015). However, none have reported the effects of training animals that are wild and unaccustomed to humans and handling. The difficulty in training wild animals is that they naturally flee from humans due to their fight or flight response, are naturally strong and can cause harm to handlers and themselves. Furthermore, animals may succumb to capture stress during this stressful type of direct unaccustomed handling.

3.2 Background to the methodological approach

i. Exercise training intervention

Almost 15 years later Harthoorn described the stress-induced syndrome of capture myopathy in 1964, he motivated that animal death during translocation could be avoided if animals were i) allowed to acclimatise to bomas, ii) physically conditioned through exercise before capture and iii) not to be subjected to severe continued exertion during the capture process (Harthoorn, 1979). Harthoorn suggested that animals be exercise-trained using camps connected by a central corral so that they could be driven from one side to the other (Fig. 3.1). Capture deaths usually occur when these three factors are ignored and unconditioned animals are chased, handled and transported.

Although the design of the camp allowed for the movement of animals, it did not allow for any standardisation in terms of distance and pace of exercise that the animals were subjected to. Thus, the challenges involved in exercise training a wild species for a research study would include:

1) how to efficiently execute exercise training in a group of animals and
2) how to quantify distance, speed, time, and level of exertion during the exercise.

The specific aims of this part of the study were therefore to determine whether captive wild animals can be successfully exercise-trained and the training variables accurately quantified. The objectives were to habituate 20 recently captured wild blesbok (*Damaliscus pygargus*

phillipsi) for 2 weeks in bomas and then exercise train the antelope for 4 weeks (MacInnis & Gibala, 2017; Nicolò & Girardi, 2016; Nie et al., 2018).

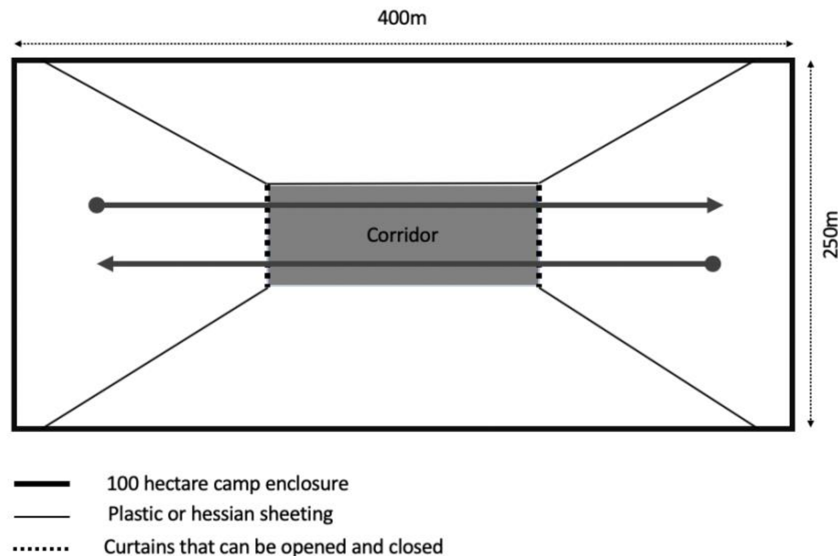


Figure 3.1 Harthoorn's exercise training wildlife camp design

This wildlife camp design is derived from a proposed model by Harthoorn (1979) describing a method where wild antelope could be exercise trained. A corridor-like-capture boma (a coral to keep wildlife) is constructed in the center of a 100-hectare camp. Both openings of the corridor have curtains that allow the movement of animals from one side to the other.

ii. The capture stress event

To test the theory that exercise-trained animals may be more physiologically resilient to capture stress compared to their untrained counterparts, this study had to create an exertional stress event that would simulate a typical wildlife capture operation (Breed et al., 2019; Meyer, 2009). Exercise trained and untrained animals had to be subjected to this exertional capture stress and their physiological measurables were evaluated to measure any differences in their physiological response.

3.3 Ethical approval

All procedures were approved by the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee (ref. no. 015/033) and the University of Pretoria's Animal Ethics Committee (project no. V021-16). A section 20 Research permit was obtained from the South

African Department of Agriculture Forestry and Fisheries (DAFF) in terms of the Animal Diseases Act, 1984 (Act no 35 of 1984).

3.4 Research venue

The study was executed at the Wildlife Pharmaceuticals Wildlife Research Facility, South Africa (25º31’25.2” S, 31º06’50.8” E) on Ngongoni farm (wildlife ranch) (Fig. 3.2, 3.3). The research facility was situated near Nelspruit, South Africa and is approximately 670 m above sea level. The study commenced the 3rd of April 2017 and concluded on the 20th of May 2017. The study took place during autumn, with day temperatures being cooler and dry.

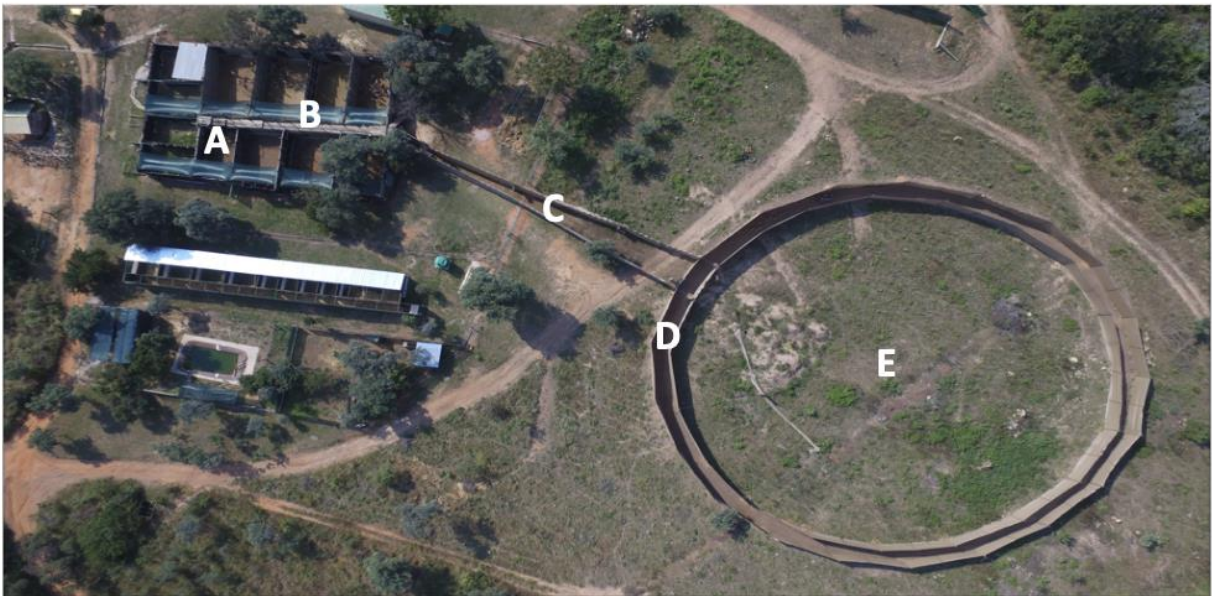


Figure 3.2 Aerial image of the housing bomas and training doughnut

Location: Wildlife Pharmaceuticals Wildlife Research Facility, Nelspruit, South Africa.

A. Ten adjacent permanent bomas, eight containing blesbok.
B. A central corridor between adjacent bomas.
C. Temporary funnel connecting the bomas and the temporary circular run or ‘training doughnut’.
D. Circular 200 m run or ‘training doughnut’ for exercising blesbok.
E. The central area of the doughnut or ‘stress rink’ was utilized for the capture stress event.

The movement and direction of the blesbok were controlled through doors and plastic curtains diverting animals to the direction desired.

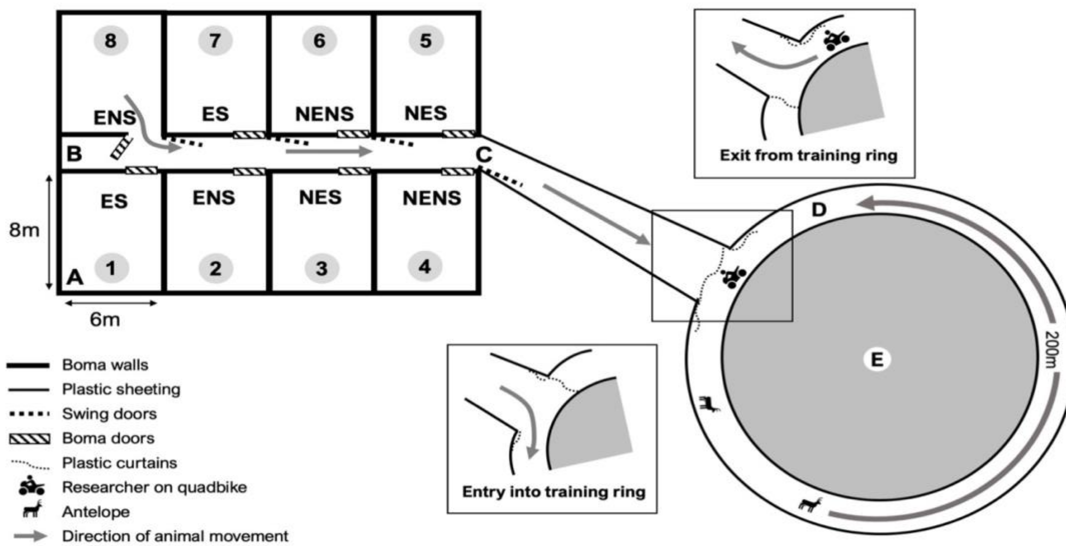


Figure 3.3 Schematic drawing of the housing bomas and "training doughnut"

A. Eight of the ten bomas that were adjacent to one another, were used in the study. Different groups were assigned to the bomas as indicated in the figure, and were exercised stressed (ES), exercised non-stressed (ENS), non-exercised stressed (NES) and non-exercised non-stressed (NENS) (further details on the groups in section 3.6).
B. The central corridor between adjacent bomas.
C. Temporary corridor connecting permanent bomas and temporary circular run.
D. Circular 200 m run or 'training doughnut' for exercising blesbok.
E. The central area of the doughnut or 'stress rink'.

The movement and direction of the blesbok were controlled through doors and plastic curtains diverting animals to the direction desired.

The facility included 10 permanent bomas (corrals constructed of wooden beams for the keeping of wildlife), 5 bomas on either side with a central connecting run. Eight of these bomas were used in the study, each with an area of 48 m^2, boma walls extending 3 m upwards an *ad libitum* supply of freshwater (Fig. 3.3). Game capture sails (Condere Marketing and Engineering, Kempton Park, South Africa) and metal poles extending 2.5 m upwards (used in the mass capture of game) were erected to construct a 50 m connecting funnel/corridor between the bomas and a 200 m circular run (termed the *training doughnut*) that was used to train the animals (Fig. 3.2, 3.3). When exercised, the blesbok could be directed into or out of the training

doughnut by employing strategically erected plastic curtains within the corridor and circular run (Fig. 3.3). Following the exercise-training period of 4 weeks, 10 trained and 10 untrained animals were exposed to the "capture stress event". For this event, the animals were directed through the funnel and into the unfamiliar central area of the doughnut (termed the *stress rink*) (Fig 3.2 E).

3.5 Animals

Forty-four wild blesbok (*Damaliscus pygargus*) were acquired before the study commenced, in the last week of March 2017. Thirty-six of the blesbok were obtained from game auction bomas in Mookgopong (Naboomspruit), which is in the neighbouring province of Limpopo. An effort was made to obtain the history of the blesbok before their stay in the Mookgopong bomas, but it could not be obtained and is unfortunately unknown. This is a typical situation when game is procured in such a manner. A professional game capture company was hired to load and transport the animals by truck to Ngongoni farm. To supplement the number, an additional eight animals were caught shortly after (± 3 days) on the farm where the Wildlife Pharmaceuticals Wildlife Research Facility is located, Ngongoni, and were evenly introduced into the existing groups. The blesbok were general young to adult blesbok ewes, with a smaller percentage of young blesbok rams. The blesbok had an average body conditions score (2.5 - 3 out of 5) and any poor condition outliers were removed before the study.

All animals were allowed to habituate for at least two weeks (the first introduced group had a few days extra of habituation) to boma conditions (bomas described in section 3.4) before the study commenced. This period of habituation allowed for animals to recover from the capture and transport, but also allowed for the return to a normal physiological and psychologically calm state. This was evaluated by the daily evaluation of body condition scores and habitus. Animals showed either maintenance or improved body condition scores, an adequate appetite in a boma during/after feeding, and lastly a decreased state of alertness which was evident by less easily being startled. This period provided the opportunity to observe pecking order issues within a group, and where a ranking order issue may result in a "poor doer" depicted by constant bullying, injury or inadequate access to food provided. All of which may inadvertently result in loss of condition and poor habitus. It also served as a quarantine period that allowed for the removal of blesbok before the study commenced that presented with a poor body condition score and/or poor habitus (lethargy, listless etc.) to decrease physiological variation.

After the two weeks of habituation, all the blesbok were immobilised (details on immobilisation and drugs used described in section 3.9 (iii)) by darting intramuscularly in the neck with non-barbed darts to minimise trauma, from an elevated ramp above the boma enclosure. During this first immobilisation event, a few (2-3) animals in each group were reallocated into the four groups of 11 to distribute the rams as equally as possible between the groups and attempt to mitigate any dominance issues that were observed. All the animals were weighed, examined for pregnancy, sexed, age estimated, and their general body condition and health evaluated. All the blesbok received an ectoparasiticide (Dectomax®, Zoetis (Pty) Ltd, South Africa) according to their weight (1 ml/50 kg or ±1.2 ml). Each animal was fitted with an ear identification tag and adhesive coloured tape, corresponding to the ear tag colour, tied around their horns to easily identify their respective grouping and animal number during visual inspections.

All animals were fed the same diet throughout the study. This diet consisted of lucerne (alfalfa - *Medicago sativa*) twice daily in an *ad libitum* fashion. The lucerne was well dispersed in the boma to prevent intra-group dominance-related aggression and allow for equal feeding opportunities. A supplemental concentrate feed cube (Wildskorrels 100®, Voermol Feeds (Pty) Ltd, Maidstone, South Africa) was provided twice daily to address any additional nutritional requirements and *ad libitum* fresh water was provided. The feeding areas and throughs could be accessed from outside and thus humans did not have to enter the bomas. The bomas were however cleaned once weekly. This was achieved by directing the blesbok into an adjacent boma with the sorting doors (Fig. 3.3). The staff would clean the boma, and thereafter the blesbok were returned to their boma in the same manner.

3.6 Animal groups

The study design aimed to physically exercise specific groups of blesbok for 4 weeks and then expose a trained and an untrained group to a stressful and physically exhaustive event that would simulate a typical game capture operation, referred to as the *capture stress event* (Fig. 3.4). The latter event was introduced to determine whether the exercise training intervention had an overall beneficial effect on the physiological exercise tolerance of the animals and stress resilience compared to the animals that received no training.

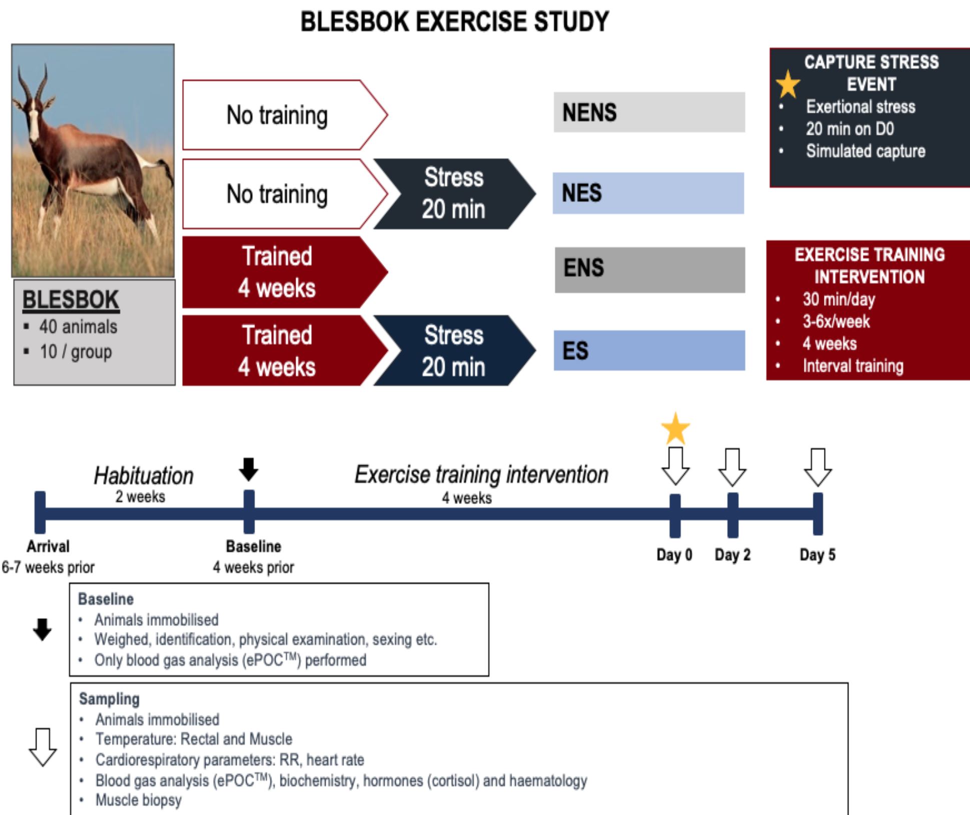


Figure 3.4 Blesbok exercise study timeline

Top: The blesbok group consisted of forty animals that were divided into four groups of 10. Two of the groups were exercise-trained for four weeks and two were not exercise trained. After the four weeks, one of the non-exercised trained and one of the exercise-trained groups were exposed to the capture stress event that consisted of 20 min of exertional stress and typical capture stressors. The groups were assigned the following acronyms: NENS = non-exercised non-stressed; NES = non-exercised stressed; ENS = exercised non-stressed; ES = exercised stressed. The training intervention consisted of four weeks of 30 min/day interval training (see Table 3.3 for detail) three to six times per week. During the capture stress event, the animals were exerted for 20 min and exposed to typical capture stress associated stressors (Fig. 3.6).

Middle/bottom: The animals were all habituated for 2 weeks in the bomas, only the ES and ENS groups were subjected to a four-week exercise training intervention. On D0, only the NES and ES were subjected to the capture stress event. Monitoring and sampling as described in the image occurred at the Baseline, day 0 (D0), day 2 (D2) and day 5 (D5) timepoints (RR = respiratory rate).

Upon the arrival of the initial 36 animals, they were divided into four groups in the bomas described before, and soon after the additional 8 animals were evenly added to each group to a total of eleven animals in each group i.e., a total of forty-four animals. After the two-week

habituation period, a few were moved during the immobilisation to distribute males evenly and sort out dominance issues as was described in section 3.5. The groups that were trained, were trained in groups of eleven. Each group was further reduced to contain only ten animals during the four weeks of the exercise training intervention by separating individuals in the central corridor through the swing gates and boma doors (Fig. 3.3). Reasons for removal of an animal from a group ranged from excessive loss of body condition, an undetected pregnancy that became evident, or removal of a surplus male, to balance the sex ratio within and between the groups. It was realised during the study that with the number of staff that would be available for the sample collection week, smaller groups and more time would be needed to facilitate sample collection and physiological monitoring for individual animals. Thus, at the end of week three, the animals were further divided into eight groups of five animals each, by passively sorting using the central corridor, swing doors, curtains and boma doors (Fig. 3.3). The exercise training intervention groups were trained in groups of 5 thereafter. The group allocations and descriptions are in the table below (Table 3.1).

Table 3.1 Blesbok group descriptions

Groups are described according to size, exposure to the exercise training intervention and, or capture stress event.

Group	Exercise training intervention	Simulated capture event	Description
Not-Exercised-Not-Stressed (NENS) n = 10; Male 3; Female 7	No	No	Received no exercise training and was not exposed to the capture stress event.
Not-Exercised-Stressed (NES) n = 10; Male 2; Female 8	No	Yes	Received no exercise training and was exposed to the capture stress event.
Exercised-Not-Stressed (ENS) n = 10; Male 2; Female 8	Yes	No	Received exercise training and was not exposed to the capture stress event.
Exercised-Stressed (ES) n = 10; Male 1; Female 9	Yes	Yes	Received exercise training and was exposed to the capture stress event.

3.7 Exercise training intervention

For the majority of the exercise intervention period, three technical assistants were required to exercise the blesbok - two assistants to direct the animals from the bomas into the run and back, and a driver for the all-terrain-vehicle (also known as an ATV, four-wheeler or quadbike, 2012 Polaris Sportsman® 90 model) that was able to easily adjust to the exercise training speed required in the training schedule for the animals. The exercise regimen for the blesbok was designed to last for 4 weeks, each training session to last ~ 30 min, three to six times per week (Gibala et al., 2012; MacInnis & Gibala, 2017; Munk et al., 2013). The intensity of the exercise training for the blesbok was based on previous gradings used for horse training i.e. walking (W - walking), trotting (LIE – low-intensity exercise), cantering (MIE - medium intensity exercise) and galloping (HIE - high-intensity exercise) to determine exertion level (Padalino et al., 2014; Robilliard et al., 2007). The intensity of the interval training was incrementally increased from walking to peak at galloping for intermittent periods towards the end of the training period as is depicted in Table 3.3.

The blesbok groups were individually guided from an opened boma enclosure, through the central corridor, to the connecting corridor and finally, with guidance by the plastic sail curtains, anti-clockwise into the training doughnut. The initial walking phase (days 1 to 3) was facilitated by three staff members that walked behind the blesbok in the training doughnut for the duration of the exercise period. Initially, the animals would gallop the moment they saw the staff, for the first few rounds on the first day, but they very quickly habituated and walked, walking on only when they saw the staff appearing in the training doughnut. On day 5, when LIE was introduced, a human-operated ATV was used to drive the blesbok forward, control their movement and maintain a constant exercise speed. The speed of the blesbok was maintained at a constant by using the speedometer of the ATV and maintaining a set speed. The use of clapping on the dashboard, whistling and verbal encouragement was used to ensure movement according to the ATV speed when the antelope would lag. Walking was maintained at 5-7 km/h, LIE 7-10 km/h, MIE 10-15 km/h and HIE between 15-20 km/h. The number of laps (distance), speed and environmental variables were recorded throughout each training event (see section 3.9 (i)).

The distance, duration of exercise time and calculated average speeds attained weekly for the exercise period is reported in Table 3.2. Light intensity exercise (LIE) was the major category of exercise (225 min), followed by walking (W - 120 min), moderate (MIE - 95 min) and lastly high-intensity exercise (HIE - 40 min). A total of 480 min or 8 hours of exercise was achieved

over 23 days including 6 intermittent rest days (Table 3.3). On a typical training day, the average distance covered was 2.7 ± 0.1 km and the average speed was 7.9 ± 0.4 km/h. Figure 3.5 provides a breakdown of the distance and average speed per day during the exercise training intervention period. The average speed per week increased from 5.8 ± 0.6 km/h to 9.0 ± 0.5 km/h from the first to fourth week. The average speed achieved by the fourth week equates to a 55% increase from the first to last week of the exercise training intervention period.

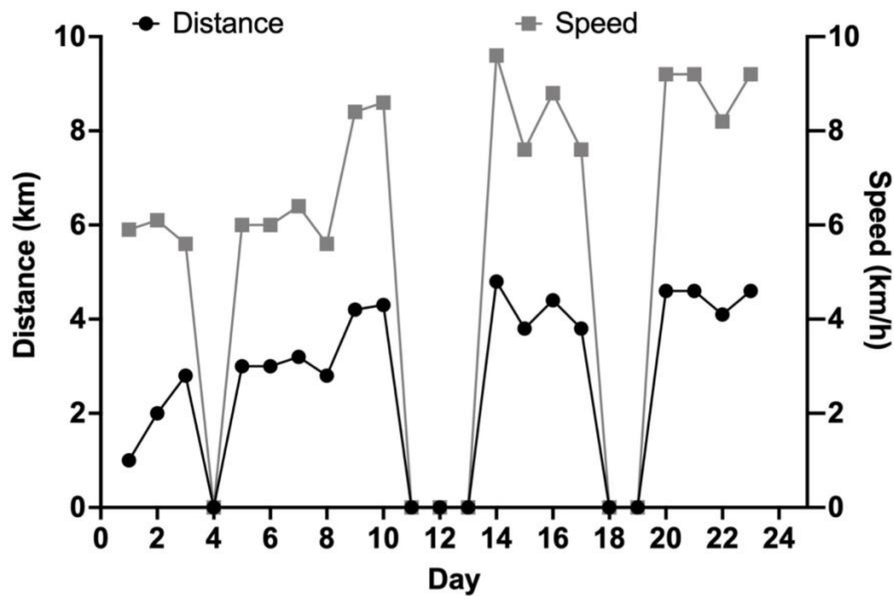


Figure 3.5 Exercise programme daily distance and average speed per day

Table 3.2 Physical exercise recorded for the exercise intervention period

Distance covered, time exercised, average speed and time spent on different exercise categories for each week are shown.

Week	Distance (km)	Duration (hours)	Speed (km/h)	W (min)	LIE (min)	MIE (min)	HIE (min)
1	5.8 ± 0.6	1.0	5.8 ± 0.6	60	0	0	0
2	22.5 ± 1.2	3.0	7.5 ± 0.4	55	115	10	0
3	16.8 ± 0.8	2.0	8.4 ± 0.4	5	50	55	10
4	17.9 ± 0.8	2.0	9.0 ± 0.4	0	60	30	30
Average			**7.9 ± 0.4**				
Total	**63.0 ± 3.4**	**8.0**		**120**	**225**	**95**	**40**

W = walking; LIE = light intensity exercise; MIE = medium intensity exercise; HIE = high intensity exercise

Table 3.3 Blesbok exercise programme

W = Walking, LIE = Low Intensity Exercise, MIE = Medium Intensity Exercise, HIE = High Intensity Exercise

Day	Exercise	Distance (m)
1	10 min W	1000 ± 200 m
2	20 min W	2000 ± 200 m
3	30 min W	2800 ± 200 m
4	Rest	0
5	10 min W → 10 min LIE → 10 min W	3000 ± 200 m
6	5 min W → 5 min LIE → 5 min W → 5 min LIE → 5 min W → 5 min LIE	3000 ± 200 m
7	5 min W → 10 min LIE → 5 min W → 10 min LIE	3200 ± 200 m
8	15 min LIE → 5 min W → 10 min LIE	2800 ± 200 m
9	30 min LIE	4200 ± 200 m
10	10 min LIE → 5 min MIE → 5 min W → 5 min MIE → 5 min LIE	4300 ± 200 m
11	Rest	0
12	Rest	0
13	Rest	0
14	5 min LIE → 10 min MIE → 5 min W → 5 min MIE → 5 min LIE	4800 ± 200 m
15	5 min LIE → 10 min MIE → 5 min LIE → 10 min MIE	3800 ± 200 m
16	10 min MIE → 10 min LIE → 10 min MIE	4400 ± 200 m
17	10 min LIE → 5 min HIE → 10 min LIE → 5 min HIE	3800 ± 200 m
18	Rest	0
19	Rest	0
20	10 min LIE → 5 min HIE → 10 min LIE → 5 min HIE	4600 ± 200 m
21	5 min MIE → 5 min HIE → 5 min LIE → 5 min MIE → 5 min HIE → 5 min LIE	4600 ± 200 m
22	10 min LIE → 10 min HIE → 10 min LIE	4100 ± 200 m
23	10 min HIE → 10 min LIE → 10 min HIE	4600 ± 200 m

3.8 Capture stress event

The central area of the training doughnut, the *stress rink* (Fig. 3.2 and 3.3), was levelled prior and prepared to ensure that the blesbok would not injure or be able to escape during the capture stress event. All holes needed to be covered in the containment walls of the stress rink, as any animal when chased and/or stressed would be looking for any possible escape routes. The stress rink also provided a foreign and unfamiliar environment for all the animals during the capture stress event, different from the 'training doughnut', that the exercised trained groups were at this point well habituated to.

The capture stress event occurred at the end of the four-week training period (referred to as day 0 or 'D0'). The sampling for 'D0' spanned over two consecutive days and only four sub-groups were immobilised and sampled on a day (section 3.5), thus all eight groups over two days. This was done to allow sufficient time for the execution of the capture stress event before immobilisation on the second day. The non-stressed groups were all immobilised on the 1st day of D0. These 10 non-exercised-not-stressed (NENS) and 10 exercised-not-stressed (ENS) groups, served as physiologically non-stressed control groups.

The capture stress event occurred on the 2nd day of D0, where 10 untrained (non-exercised-stressed (NES) and 10 exercised-stressed (ES) blesbok were exposed to the capture stress event and then only immobilised. Thus, the animal groups that were exposed to the capture stress event (NES and ES), were first guided into the stress rink, whereafter they were chased circularly by an overhead helicopter, an ATV and a minimum of 5 humans. The people in the stress rink were using various noises from shouting to whistling (Fig. 3.6). The animals were chased in a circular direction and were only exposed to 20 min of this planned capture stress event. The goal of this intervention was to physically exert while simultaneously exposing the animals to typical capture stressors e.g. multiple foreign vehicles, noises and humans. The animals were evaluated for fatigue by monitoring for open mouth breathing, reluctance to move despite exposure to the stressors and displaying a stiff gait. A stiff gait can be described as the apparent rigid 'stick' movement of the legs compared to the usual natural movement.

For all groups, non-stressed and the capture stress event groups, darting took place from elevated platforms on the perimeter of each boma. During the habituation and exercise training intervention period, all the blesbok became accustomed to a human being present for 5 – 10 min on these platforms daily to evaluate their condition and habitus. An experienced wildlife veterinarian darted (refer to section 3.9 for details on the drugs used) all five animals from the

same platform on their own in quick succession (± 2 min) with a gas-driven dart projector (Pneu-Dart X-caliber™, Pneu-Dart Inc., USA), that produced minimal noise to ensure that stress was limited before immobilisation. After darting, the animals were not disturbed to allow for smooth induction of immobilisation and not stress them during this time.

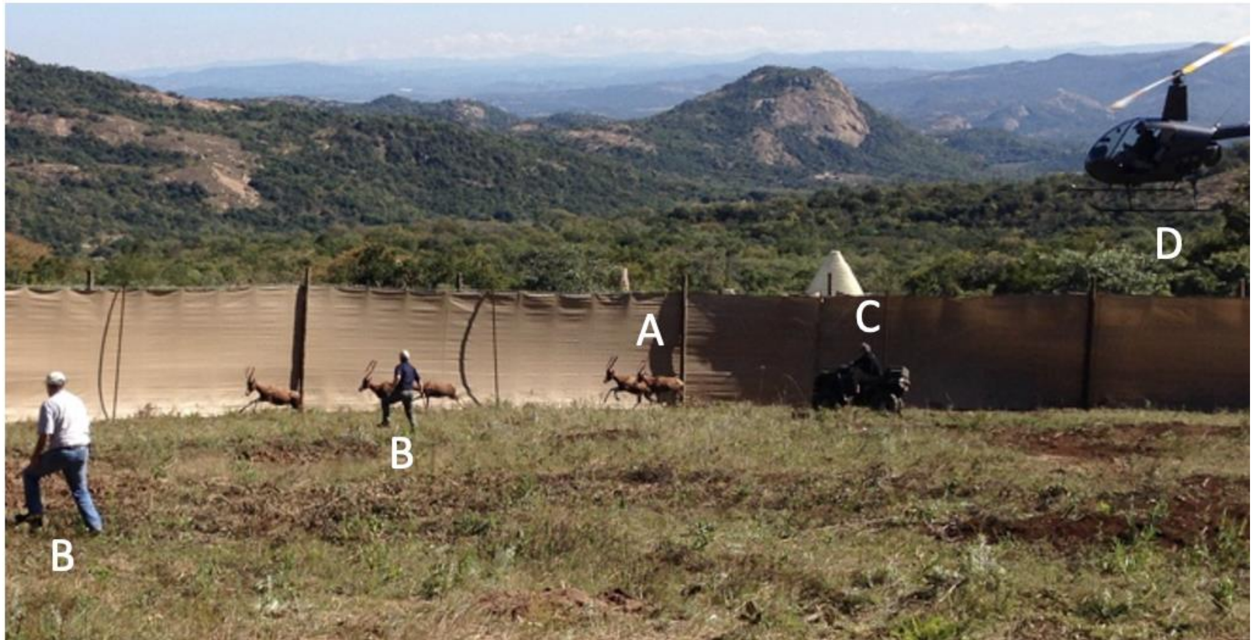


Figure 3.6 Capture stress rink

Groups of five blesbok (A) were exposed to the capture stress event that simulated a typical wildlife capture, by being chased by humans (B), an ATV (C) and a helicopter (D).

Four sub-groups of five animals each (10 NES and 10 ES), were exposed to the capture stress event. The distance covered by the four groups, calculated from the number of laps recorded for each group during the twenty-minute capture stress event varied from 7.4 to 8.6 km. The speed of the animals was calculated by dividing the distance (counting laps) over the time exposed to the capture stress event and found to be an average of between 22 to 26 km/h. This speed was more than double the speed at which the animals trained during the last week of the exercise training intervention, so would qualify as a speed that the exercise-trained and non-exercise trained groups were unaccustomed to.

3.9 Data collection

(i) Environmental

A portable weather station (Kestrel 5400 Heat Stress Tracker, Nielsen – Kellerman Co., PA, USA) was used to measure weather conditions every day throughout the study duration, and included ambient temperature, relative humidity, black globe temperature, wet bulb globe temperature (WBGT), wet bulb temperature, wet bulb natural temperature, barometric pressure, altitude, and wind speed. WBGT has become the global standard measurement to evaluate environmental stress during the physical activity of humans and animals (Cooper et al., 2017). The WBGT measures the ambient temperature but calculates the level of radiation, wind chill factor and humidity to determine the actual heat stress experienced by the animal or person (D'Ambrosio Alfano et al., 2014).

Sample collection for D0 occurred over two days to facilitate the groups of five animals at a time that had to be immobilised, sampled and required the capture stress event (Appendix: Table 9.1). On the first morning of collections for D0, it had rained the night before so the animals in the ENS and NENS groups that were immobilised and sampled on that day, were wet (Appendix: Table 9.1). By the time the ES and NES groups that were sampled on the second day of collections for D0, it had dried up after the rain and the animals from these groups were doused with tepid tap water. This was done to simulate the dousing effect of the rain the previous day, that the ENS and NENS groups experienced. Approximately 10 L of water was applied to the dorsum of the body of the blesbok and rubbed into the animal's hair to ensure penetration of the coat. This water application was done once off at the beginning of recumbent immobilisation. The evaporation of the water from the animals would result in a cooling effect on the animals (Sawicka et al., 2015). The dousing would thus reduce the core temperature similar to what the rain dousing would have achieved the previous day. A previous study comparing the different cooling methods that are used post wildlife capture, showed that applying tepid water also resulted in a cooling effect albeit at a reduced rate compared to water that was cooled (Sawicka et al., 2015).

(ii) Weight

After the two-week habituation period, the blesbok were immobilised for an initial health check and weighed. The method of weighing consisted of placing an immobilised blesbok in a

stretcher and using a hanging scale suspended on a pole held by two attendants. The blesbok were again weighed at the end of the study on Day 5 (Fig. 3.4).

(iii) Immobilisation

To obtain the samples and measurements required at Baseline, D0, D2 and D5 (Fig 3.4), the blesbok were immobilised by darting intramuscularly in the neck, from an elevated ramp above the boma enclosure. A 0.5 ml P-type Pneu-Darts (with gel collar) loaded with 3 mg etorphine (Captivon 9.8 mg/ml, Wildlife Pharmaceuticals, [Pty] Ltd, South Africa) and 40 mg azaperone (Zapnil 100mg/ml, Wildlife Pharmaceuticals [Pty] Ltd, South Africa) was fired from a gas-powered dart projector (X-Caliber; Pneu-Dart Inc., Pennsylvania, United States). The drugs were reversed with 30 mg of Naltrexone (Trexonil 50 mg/ml, Wildlife Pharmaceuticals [Pty] Ltd, South Africa). Whenever additional drugs were required to maintain immobilisation, 100 mg of ketamine (Anaket V 100 mg/ml Injection, Bayer, South Africa) was injected intravenously (Fitte, 2017). Additional drugs were rarely required and only administered when the animals were too alert and resisted recumbency or handling during immobilisation.

The control animals (ENS and NENS), that were not subjected to the 20 min capture stress event, were immobilised the day before the stress event to avoid any exposure to the capture stressors of the stress event (e.g. noise). The ES and NES groups were immediately immobilised after the capture stress event, and samples and physiological variables were obtained within 5 min of recumbency.

(iv) Body temperatures, heart – and respiratory rate

The heart rate, respiratory rate, rectal and additionally, muscle temperatures variables were recorded every 5min on D0, D2 and D5. On D0 the animals were monitored for 50 min post immobilisation until reversal, and on D2 and D5 for 15 to 20 min, until reversal (Fig. 3.7). The heart rate was measured by cardiac auscultation and the respiratory rate was recorded by visualising the chest and abdomen for breathing movements and by placing a hand in front of the animal's nostrils and feeling for airflow.

The probe of a thermometer (Hanna HI98509 Checktemp®1, Fig. 3.7) was inserted ±7 cm into the rectum to obtain rectal temperature. Muscle temperature was recorded by aseptically inserting an 18-gauge needle ± 20 mm deep into the gluteal muscle and feeding a fine

thermocouple probe (IT-18 Physitemp Instruments, Clifton, NJ, USA) through the hub and needle into the muscle. This probe was connected to the IT-18 monitor (Physitemp Instruments, Clifton, NJ, USA).

The response recorded and analysed for the heart rate, respiratory rate, rectal and additionally, muscle temperatures is specific to D0, which is where the effect of the capture stress response was evident. There was a slight time delay post immobilisation and recumbency to obtain all the measurements required from all five animals. The delay between the exercise stress event, immobilised recumbency (2.5 to 5 min) and obtaining muscle temperature from the muscle probe for all animals (another 5 to 10 min) may have resulted in a 7.5 to 15 min delay post-exercise. The time point where all animals had measurements for all variables was at 10 min post immobilisation.

(v) Sample collection

A previous similar study done in blesbok revealed that the most relevant and significant pathophysiological responses occurred within the first few days (2 to 5 days) after a capture stress event (Fitte, 2017). This also holds true in other species and humans post severe exertional events, where muscle damage markers (i.e. plasma CK) peak at day 2 post-exercise and decreases rapidly (Kristensen et al., 2014; Spada et al., 2018). Therefore, based on these results, the sampling for the present study was limited to multiple samplings within five days after the capture stress event.

Time delays in obtaining biological samples and physiological recordings are inherent to a study like this. For example, after the capture stress event, the blesbok were returned to their bomas and immobilised in short succession, but recumbency took between 3–5 min after darting and the animals not yet recumbent could not be disturbed. To mitigate the lag in sample collection, staff were assigned a specific blesbok to ensure minimal time delays after recumbency to obtain arterial samples and record the heart rate, respiratory rate, rectal and muscle temperatures, and, hence, reduce sampling variability.

Only one arterial sample was obtained during the Baseline sample collection day, which occurred before the exercise training intervention. Arterial and venous blood samples were collected at 0- and 40-min post immobilisation on D0, whereas arterial and venous samples, were only collected once directly after immobilisation on D2 and D5.

Arterial blood samples were obtained from the medial auricular artery. A 5000 IU/ml Na^+-heparin solution was flushed through a 23-gauge needle and 1 ml syringe. This small amount of heparin primed the syringe with enough heparin that prevented the samples collected from clotting. The sample was immediately analysed on-site (Fig. 3.8) with the ePOC™ Blood Gas, Electrolyte and Critical Care Analyser using EPOC BGEM blood test cards (Alere, USA) (F. Chen et al., 2017; Fitte, 2017). Results were obtained within 10 min of drawing the arterial sample.

The variables analysed by the ePOC™ Analyser include blood pH, Na^+, K^+, Cl^-, Ca^{2+}, glucose (Glu), lactate (Lac), Hct, creatinine (Crea), blood urea nitrogen (BUN), partial pressure of oxygen (PaO_2) and the partial pressure of carbon dioxide ($PaCO_2$). Calculated values obtained were actual HCO_3^-, total CO_2, base excess of extracellular fluid (BE_{ecf}), base excess of blood (BE_b), calculated oxygen, Hgb saturation (cSO_2), anion gap (AGap), anion gap K^+ (AGapK) and Hgb (J. Chen et al., 2016). The variables that were relevant to the current exercise study are discussed later.

Two venous blood samples were obtained from either the jugular or cephalic vein, into an EDTA primed tube for haematology and a serum tube for biochemistry analysis. The device used for haematological analysis was the Abaxis (Zoetis, Johannesburg, South Africa), for biochemistry a Cobas Integra (Roche, Midrand, South Africa) and an Immulite 1000 (Siemens, Midrand, South Africa) for cardiac troponin I and cortisol analysis.

Haematological variables included the white blood cell count (WBC), RBC, Hgb, Hct, mean corpuscular volume (MCV), corpuscular Hgb (MCH), mean corpuscular Hgb concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), differential and immature neutrophil count, differential lymphocyte, monocyte, eosinophil and basophil count. The biochemical variables that were measured included GGT, GLDH, AST, CK, TBil, urea, creatinine, cardiac troponin I and the hormone cortisol.

On D0, D2 and D5 a muscle biopsy was obtained from the *vastus lateralis* muscle. The site was shaved and disinfected with chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa). Local anaesthesia (1ml of Lignocaine injection 2%, Centaur Labs, Johannesburg, South Africa) was administered subcutaneously. The skin was incised, and a sample was obtained with a biopsy needle (Bergström biopsy needle, size 6 mm, Dixons Surgical Instruments Ltd, UK) using the suction-assisted method (Evans *et al.*, 1982).

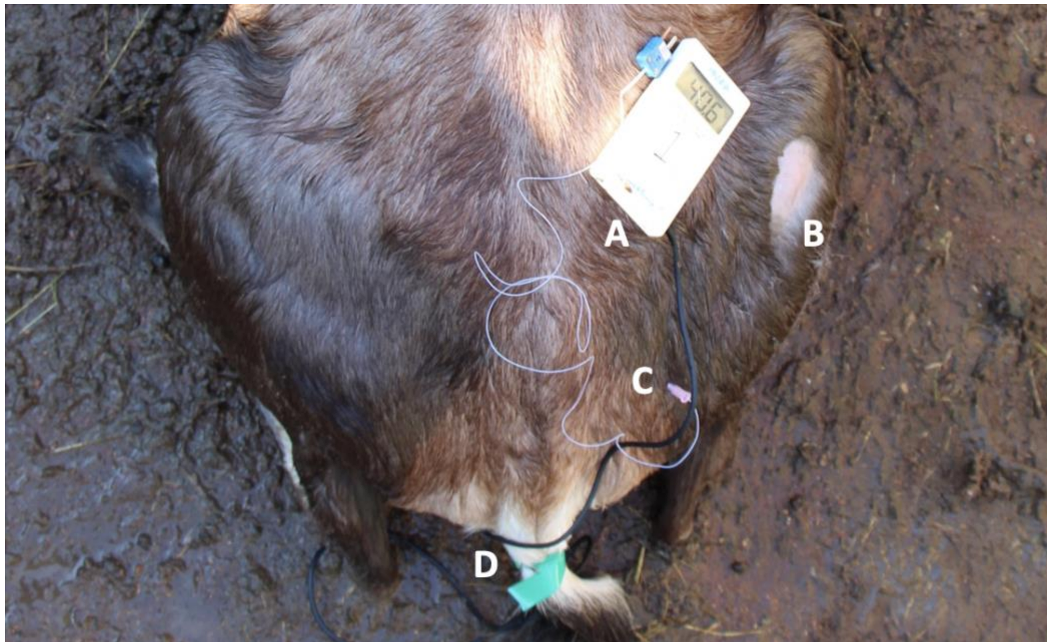


Figure 3.7 Monitoring of rectal and muscle temperatures in blesbok

(A) The Hanna HI98509 Checktemp®1 was used to measure rectal temperature and muscle temperatures.

(B) The muscle biopsy site where the local anaesthetic was injected, and a *vastus lateralis* muscle sample was obtained with a biopsy needle.

(C) The 18-gauge needle and inserted fine thermocouple muscle probe that was used to obtain muscle temperatures in the gluteal muscle area.

(D) The temperature probe inserted into the rectum was used to obtain rectal temperatures.

Figure 3.8 A. Monitoring of blesbok while immobilised.

B. ePOC™ Blood Gas, Electrolyte and Critical Care Analyser.

The muscle biopsies were frozen in liquid nitrogen at -196 °C and stored in cryovials in liquid nitrogen tanks for muscle biochemistry analysis later. The wound was closed using surgical glue (Loctite, Henkel AG & Co, China) and by applying pressure to the wound. Finally, a topical disinfectant (Supona Aerosol 400, Zoetis, South Africa) that assists in preventing myiasis was applied. The data from the muscle biopsies are not presented in this study as it falls beyond the scope of this dissertation.

D5 was the endpoint of the study and after sample collection, all animals were humanely euthanised using a captive bolt while immobilised. A full necropsy was performed by a veterinary pathologist and samples were collected. This data is not presented as it falls beyond the scope of this dissertation.

3.10 Statistical analysis

The physiological measured data from the capture stress event were analysed using GraphPad Prism 9 (GraphPad Software, USA) version 9.4.0 for macOS. The Shapiro-Wilk test was performed to test for normality (Ghasemi & Zahediasl, 2012) and when required, data were log10 transformed for further analysis and interpretation. For all statistical analysis, a P-value < 0.05 was considered significant. Where data had a normal distribution, and where not otherwise stated, results are reported as mean ± standard deviation (SD). Where the data did not have a normal distribution, the results are reported as mean (M) ± standard deviation (SD), median (Mdn) and interquartile range (IQR), and geometric mean (GM) and geometric standard deviation factor (GSD) (Ghasemi & Zahediasl, 2012). The data was transformed back when required so that the mean (GM) and standard deviations (GSD) of the transformed data could be presented graphically (GraphPad Software, 2022). The original data (M ± SD) for all variables are available in the Appendix (Table 9.4 – 9.7).

An average value was determined for the variable measured that were recorded at regular intervals of 5 min, that included rectal, muscle temperatures, heart and respiratory rate. Four time points were included in calculating the time point labelled "D0: 0 min". This was due to more difficulty in obtaining values for all animals initially at every time point, and therefore more missing values, that would result in the skewing of data. The initial values recorded at 0, 5, 10 and 15 min were thus used to calculate an average and labelled "D0: 0 min" to reflect the physiological status of the animal over this period. For the labelled time point "D0: 40 min", the variables collected at 35 and 40 min were averaged to give a more accurate indication of

the physiological status of the animals. Fewer time points were included as there were more values recorded at these time points that gave a more accurate indication of the physiological status of the animals. On D2 and D5, the immobilisation period was shorter as only one set of samples was collected at 0 min, and the regular monitoring was only completed for 15 min. An average value was also obtained for 0, 5, 10 and 15 min on these days. Data were analysed using a mixed model two-way ANOVA (GraphPad Prism 9). This mixed model uses a compound symmetry covariance matrix, and the data are fitted using a Restricted Maximum Likelihood (REML).

A Tukey's multiple comparison post hoc test was applied to determine the differences between groups. A post-hoc Dunnett's multiple comparison test, for comparison within a group, was performed to determine specific differences using the values obtained at Baseline values as a control, or the D0: 0 min value when the specific Baseline values were not collected for the variable. Additionally, at D0: 0 min and D0: 40 min, the NES group was compared to the ES with a Welch's *t*-test to detect any significant differences between these groups. An F-test was used to compare the variances between the NES and the ES group at D0: 0 min and D0: 40 min.

The relationships between multiple physiological variables at a single time point, D0: 0 min, were analysed within the stressed groups (n = 20) using a Pearson's correlation matrix. The Pearson's coefficient was used as the preferred analysis as it is the best measurement of the association of two variables and has the ability to establish the presence of a linear relationship (Hauke & Kossowski, 2011; Schober et al., 2018). Most of the physiological data did not display a normal distribution as was revealed by the Shapiro-Wilk test and the Pearson's correlation matrix requires normality of measurables. Original data cannot be correlated to log10 transformed data, therefore all values were log10 transformed prior to performing the correlation analysis. The time point 'D0: 0 min', was calculated as a single value (as above) using an average for variables recorded at 0, 5, 10 and 15 min to allow for an accurate value to depict the animal's physiological status at that point. The physiological variables included rectal - and muscle temperature, respiratory - and heart rate, pH, $PaCO_2$, PaO_2, $cHCO_3^-$, $BE_{(ecf)}$, Na^+, K^+, Ca^{2+}, Cl^-, AGap, glucose, lactate, creatinine, WBC, RBC, Ht, Hgb, platelet count, GGT, GLDH, AST, CK, Tbili, urea, troponin and cortisol. Where relevant to the discussion, r, R^2, P and sample size (n) are reported. All correlation results are reported in the Appendix (Appendix: Table 9.2 & 9.3). Where the sample size varies in reporting (e.g. n = 18 or n = 19),

it was due to a laboratory error and failure to obtain a specific physiological variable value for an animal.

3.11 Calculated values

The alveolar-arterial oxygen partial pressure gradient (A-a gradient) was calculated to assist with the interpretation of the blood gas values. The values were calculated for a constant pressure open system from the following formula:

$$\text{A-a gradient} = FiO_2 (Pb - PH_2O) - PaCO_2 - PaO_2 \quad \text{(Dill \& Penrod, 1948)}$$

Where FiO_2 is the fractional inspired oxygen (0.209), Pb is the measured barometric pressure (mmHg) and PH_2O is the water vapour pressure of saturated air in the alveoli. PH_2O was calculated as follows:

$$PH_2O \text{ (mmHg)} = 4.58\, e([17.27Tb]/[237.3+Tb]) \quad \text{(Barenbrug, 1974)}$$

Where Pb is body temperature, it was assumed that the partial pressure of CO_2 in the alveoli is equal to the arterial partial pressure of CO_2 ($PaCO_2$). The same statistical analysis as described in section 3.8 was applied to the A-a gradient values obtained using GraphPad Prism 9 (GraphPad Software, USA). Data were analysed using a mixed model two-way ANOVA (GraphPad Prism 9). This mixed model uses a compound symmetry covariance matrix, and the data are fitted using a Restricted Maximum Likelihood (REML).

A Tukey's multiple comparison post hoc test was applied to determine the differences between groups. A post-hoc Dunnett's multiple comparison test, for comparison within a group, was performed to determine specific differences using the values obtained at Baseline values as a control, or the D0: 0 min value when the specific Baseline values were not collected for the variable. Additionally, at D0: 0 min and D0: 40 min, the NES group was compared to the ES with a Welch's *t*-test to detect any significant differences between these groups. An F-test was used to compare the variances between the NES and the ES group at D0: 0 min and D0: 40 min. The results were reported as in section 3.9.

3.12 Discussion

This part of the study aimed to determine if *wild-caught* antelope could be successfully exercise-trained for four weeks. These exercise-trained antelope would then be subjected to a capture stress event to induce hyperthermia and exhaustion, and record and compare physiological responses to non-exercise-trained antelope. It is difficult to work with wild animals that can injure themselves and the people working with them. Many factors had to be considered to successfully execute the intervention of training wild antelope as well as inducing the exertional stress that would be comparable to conditions in a game capture operation.

(i) Research venue

The well-designed bomas of the Wildlife Pharmaceutical Wildlife Research Facility proved to be the ideal study venue as only minor temporary modifications had to be erected to facilitate and link the training doughnut to the existing bomas (Fig. 3.2). The existing boma complex was already designed in such a way as to facilitate the movement of animals within the complex.

Clearing and levelling of the area, erecting poles and fencing for the corridor and training doughnut connected to the existing bomas, were the most labour intensive, as this was considered a temporary structure (Fig. 3.2 and 3.3). The construction phase lasted approximately 2 days using a group of workers, which is not ideal when in the veld under a normal game capture operation, where resources and time may be limited. Daily inspection of the training doughnut was required to ensure that the sheets were adequately secured to prevent escape and that the ground was adequately level to prevent injuries. The on-site staff that was available and were experienced in captive wildlife management, husbandry and handling within the bomas, was of critical importance to the success of this study.

(ii) Animals

For this study, an antelope species was required that was relatively easy to manage concerning size and temperament (not dangerous to the handlers) and would readily take flight. Blesbok (*Damaliscus pygargus*) had been used in previous studies investigating forced exercise (Harthoorn & Van der Walt, 1974), cooling methods (Sawicka et al., 2015), and capture-induced hyperthermia (Dill & Penrod, 1948). This species is of lesser concern on the CITES

red list, readily traded and economical to procure (Bosveld Vleissentraal, 2017; Dalton et al., 2016). Other species that could be considered for similar future studies are species like springbok and impala. They are small enough to handle with ease, are known to be 'flighty' species, and are well-known to easily succumb to capture complications.

(iii) Animal grouping and exercise training intervention

Physiological adaptations to training are evident within 2 to 6 weeks of initiating high-intensity interval training in humans (Kohn et al., 2011; Nicolò & Girardi, 2016). The blesbok of the present study were not accustomed to any forced controlled physical activity. Thus, additional exercise protocols were implemented initially to habituate the animals to any running, which were recorded, to mitigate the risk of injury to the animals. There was therefore a gradual increase in intensity and intervals, as depicted in the Figure 3.5 and Table 3.3. Previous studies using untrained human participants that were subjected to high-intensity interval training found adaptations within two weeks with ~ 20 min training sessions three times per week (Gibala et al., 2012; MacInnis & Gibala, 2017; Nie et al., 2018). The present exercise intervention study used interval training, starting at a low intensity, gradually increasing the intensity over the 4-week period to minimise stress and habituate to the level of exercise (Table 3.3).

The blesbok could quickly be habituated. Initially, the animals were a bit fractious as could be seen by animals splintering off from the main group or trying to find escape routes during training. Within a few days, the blesbok had become accustomed to the exercise training, as was evident by calmly moving uniformly from the bomas and in the 'training doughnut' during training. Two people were required to direct the animals out of the boma, whereafter one would ride the ATV to exercise train the blesbok into the 'training doughnut'. The other staff member would assist at the end with directing the blesbok back into the bomas again. The exercise interventions could also be applied over an extended period and quantified in terms of distance by measuring laps or having a pedometer-like device attached to the animals, time (stopwatch) and speed (an average speed can be calculated by taking distance over time). One of the difficulties faced during exercising the blesbok was motivating the animals to move at a particular speed, as they would habituate to the ATV and stop moving. Alternative methods in addition to the ATV had to be employed to ensure the pace of exercise was kept constant throughout the training period. These methods included vocal encouragement, whistling, clapping of hands and 'revving' the ATV to increase the noise produced.

Dominant animals would also prevent subordinate animals from moving to the front and intergroup rivalry would delay exercising effectively at the intended speed. The most dominant behaviour during exercise was shown by adult blesbok ewes. This group behaviour could be explained by the fact that most of the males were young and had not reached full maturity. The division of the groups into smaller groups of five at the end of week 3 reduced dominance interactions and improved ease of exercise training. However, it substantially increased the time needed to exercise all four groups and subsequently 2 hours were required to train all groups rather than 1 hour. In practice, this would considerably increase the labour and time required to execute daily exercise for animals and reduce the feasibility of such an intervention.

(iv) Capture stress event

Surprisingly, the blesbok quickly habituated to being chased by the helicopter and refrained from running - like what occurred in the exercise training intervention using the ATV. Only the presence of humans and a human-operated ATV, with a variety of noises and different gestures, maintained continuous running, but only up to the point that physical exhaustion was observed, thereafter the animals became less responsive to all stimuli.

During the capture stress event, signs of exhaustion became evident, some blesbok presented with open mouth breathing within 6 to 9 min of starting to run, and after 10 min, some blesbok would run with their heads in a downward position. One to two of the blesbok in each group started running with visibly stiff limbs, which displayed as a change of gait and legs moving more rigidly. From approximately 15 min into the stress event, some blesbok also presented with visible lethargy and they would frequently try to stop. Finally, the response to the above-mentioned stressors decreased until a point was reached that they could not be encouraged to run at all because of exhaustion.

Anecdotal evidence suggests that blesbok can reach speeds of up to 70 km/h, but their true maximum running speed and endurance capability have not been measured (Kohn, 2014). The average speed attained in the stress event (22 - 26 km/h) was considerably higher than the average and maximal speeds attained during the exercise training intervention. The maximal speeds during exercise training of 15 - 20 km/h (as indicated by the speedometer on the ATV) was for short periods and the maximal average speed during the last week of training was up to 9.1 ± 0.5 km/h (Table 3.3).

In this study, all groups were immobilised after the 4-week exercise training period including the untrained groups. Of the four groups, one of the untrained and one of the trained groups were exposed to the simulated capture event to obtain comparative data between the different groups and to assess responses to their allocated training intervention and capture stress event. The results of these measurements will be discussed later in Chapters 4-7.

(v) Limitations

Inter-individual variation can cause vast variability in results obtained in physiological studies. In domesticated species, this is overcome by having specialised breeding facilities for selected species and an intense selection process to standardise the animals used as much as possible (Meunier, 2006; Poole et al., 2020). The ideal animal model would be animals that have limited physical, behavioural and clinical variation to reduce the variability that might skew results (Meunier, 2006). The ability to select wild animals to reduce inter-individual physiological variation is limited. Animals are obtained from willing conservation bodies and farmers, who often sell a particular species through game auction houses as intermediaries. The animals obtained often have limited information on their history, nutrition and disease status. For this study, all the animals could not be sourced from one supplier and had to be supplemented with eight blesbok that were available on the Ngongoni farm. It was attempted to procure only adult female blesbok, but a small percentage of young males were included in the stock procured. To try to reduce variability between the groups, an attempt to balance the sex ratios in the groups was made (Table 3.1) and the distribution of animals from the different locations equally between the groups.

The drugs administered for immobilisation were not given according to weight and were given as a standard dose. The animals were weighed initially but working out individual doses and ensuring that the correct blesbok received the correct dart would have been difficult to achieve as swiftly as the darting was executed (± 2 min). Trying to locate the correct animal in a group to dart with the correct dart, would have required additional staff while darting, likely led to mistakes being made and it would have increased the time to dart and therefore stress inflicted on the animals. Giving standardised doses is also the method employed in wildlife game capture operations, as the weights are not known of the animals, and the only difference in dosages may be divided into a ‘female’, ‘male’ and ‘juvenile’ dose.

Another limiting factor of this study was that it was difficult to assess the efficacy of the training intervention in the wild animals in real-time during the intervention or capture event. In human and equine exercise studies, that usually occur on treadmills, the physical measurement of heart rate, blood pressure, VO_2 max (maximal rate of oxygen consumption measured during incremental exercise and widely used as an indicator of cardiorespiratory fitness) during exercise and, or, after any exercise provides additional insight into the changing physiology of individuals during and after exercise (Bitschnau et al., 2010; Physick-Sheard, 1985). With wildlife, invasive remote sensing methods have been developed but currently are limited to heart rate and temperature (Laske et al., 2014). At the time when this study was planned (2015 - 2016), these methods were prohibitive due to the number of animals that would require implants, the associated cost and availability. To measure multiple physiological variables, animals had to be immobilised, which limited the number of measurement events. The darting and drugs used would affect the physiological measurements obtained and inflicted temporary stress but would enable as much data as possible to be collected.

3.13 Conclusion

Human alteration has often converted wildlife habitats into small confined fenced areas. Game farming results in selected wild species living in small camps with feeding stations or larger camps, without predators, and with artificial watering holes. These changes have restricted the natural movement, behaviour and likely, inherent physiology of the animals, which may decrease their resilience to stress and exertion (Beringer et al., 1996). The history of most of the blesbok procured for this study was unknown, except that they had been standing in bomas when procured and were then further confined to bomas during the habituation phase for 2 weeks. The 20 exercise-untrained blesbok, additionally had another 4 weeks of boma confinement. Thus, the exercise-untrained blesbok had a minimum of 6 weeks of very limited movement and would be sufficient to have resulted in the loss of any aerobic physiological adaptations they may have had.

The methodology and results from exercise-training the blesbok confirmed that:

(1) a temporary built facility to execute exercise interventions for blesbok was feasible,
(2) wild-caught blesbok quickly adapted to a regular regimen of exercise training and handling,

(3) blesbok could be exercise-trained on an almost daily basis with limited staff for an extended period,
(4) distance, time, speed and intensity could be controlled and recorded,
(5) the methodology described proved that exercise training is feasible in wild-caught blesbok, a finding that is fundamental for developing future research in studying the effects of exercise training on the physiological and biochemical adaptations of wild animals, and
(6) these findings may provide and justify methods to develop feasible ways of introducing prior exercise management interventions to reduce losses incurred during translocation due to capture myopathy.

The feasibility and practicality of introducing exercise training in wildlife may seem onerous, but if the derived benefits result in increased survival of an endangered species, or highly valued animal(s), it may be a well worth endeavour (Harthoorn and Van der Walt, 1974). Physical exercise is seen as vital for health in humans and some domestic animals (e.g. dogs and horses) but is not often considered in the health and welfare of other species.

The chapter also describes the successful execution of a capture stress event that simulated a real-life game capture event. After the 4-week exercise training intervention, the exercise-trained (ES) and non-exercise trained blesbok (NES) were exposed to the 20-minute capture stress event on D0, physiological variables and samples obtained from all blesbok including the non-stressed groups, and the analysis was completed on the physiological data collected. The data were analysed according to the methodology described and the results from the sampling and data analysis are discussed in Chapters 4 – 6, that include the environmental data, weight, heart rate, respiratory rate, rectal and muscle temperatures, blood gasses, electrolytes, biochemistry and also haematology. This data will provide valuable insight into the effect of immobilisation, capture stress and the effect, if any, that exercise training had on the capture stress response of the blesbok.

CHAPTER 4

RESULTS & DISCUSSION: ENVIRONMENTAL DATA, WEIGHT, RECTAL & MUSCLE TEMPERATURE, HEART RATE, AND RESPIRATORY RATE

4.1 Introduction

Four groups of blesbok were all immobilised on D0, D2 and D5 of the study period (Fig. 3.1) to obtain samples. Two of the groups were exercise trained (ENS and ES) and two exposed to the capture stress event (NES and ES). The control group (NENS) did not receive any exercise training intervention or exposure to the capture stress event. The ENS and ES groups that received the exercise training may be referred to as the *trained* groups, and the NES and ES groups exposed to the capture stress may be referred to as the *stressed* groups. In this chapter the effect of environmental data, weight, rectal and muscle temperature, and the heart and respiratory rate in each group are reported and discussed.

The blesbok were immobilised with a combination of an opioid, namely etorphine, and a tranquiliser called azaperone (see section 3.9 (iii) for details on immobilisation drugs). All results must be interpreted with the physiological effects of these drugs in mind and the known individual differences in sensitivity to the drugs. Opioids have well-known depressive effects on respiration rate and minute ventilation that may result in hypoventilation, hypoxaemia, hypercapnia and progressive acidaemia when administrated (La Grange, 2006; Morkel et al., 2010). Opioids have multiple other effects depending on the drug used and dose (Meyer, 2009), including decreased gastro-intestinal motility, a decrease in urine output, excitement and heart rate and an increase in blood pressure (La Grange, 2006). The depressant effect of opioids is mainly achieved through its activation of μ_2-opioid receptors (Pfitzer et al., 2020). The doses used in wildlife immobilisation usually result in systemic and pulmonary hypertension leading to decreased blood circulation to tissues and shunting of blood through the lungs (Meyer, 2009; Pfitzer et al., 2020). The ventilation perfusion mismatching that ensues decreases O_2 diffusion across the alveolar membrane leading to hypoxaemia (Meyer, 2009). Individual sensitivity to opioids also varies and can affect time to immobilisation. This delay in immobilisation can affect the duration that stress is experienced by the animal, which can alter other physiological

variables. The varying sensitivity can also result in differing depressive effects on ventilation (Meyer, 2009).

Azaperone is a short acting butyrophenone tranquiliser. Butyrophenones are antipsychotic drugs that block the action of D_2-dopamine receptors in the central nervous system (Wolfe & Miller, 2016). They also have some effect on other dopamine, serotonin, and α-adrenergic receptors (West et al., 2007; Wolfe & Miller, 2016). In wildlife azaperone causes decreased emotional arousal and responsiveness to environmental stressors (West et al., 2007; Wolfe & Miller, 2016). Opioids combined with azaperone in immobilisation darts allows for quicker recumbency and an overall smoother immobilisation by reducing some of the side effects, in particular the excitatory effects of the opioids (La Grange, 2006). Azaperone is thought to have minimal effects on respiration but has an antagonist effect on α_1-adrenergic receptors that results in vasodilation which can increase heat loss during immobilisation (Hodgkinson, 2007; Sawicka et al., 2015). The α-adrenergic blocking effect may also result in decreased stress-induced renal vasoconstriction during capture, which may have a renal protective function (López-Olvera et al., 2007).

In summary, the normal physiological changes are likely to be altered by the effects of these drugs, and in the present study, the most significant effects will likely result from the use of etorphine. We expect the effects of the drugs to be universal as the same drugs and doses were used in all the groups (section 3.9 (iii)), thus allowing the physiological response of the exercise training intervention and capture stress event in the study to become evident in the variables measured.

4.2 Environmental conditions

<u>Results</u>

The four-week exercise training was performed during moderate daily ambient temperatures, ranging between 16 °C and 30 °C, a humidity between 40 and 81%, and WBGT of 15 °C to 29 °C. The ambient temperatures on the sampling days ranged between 14 °C and 25 °C, with a humidity between 38 and 100%, and WBGT of 13 °C and 23 °C.

The WBGT data were found to have a normal distribution and did not require log transformation before further analysis. The ambient conditions during the capture stress event

and sampling periods (D0, D2 and D5) are reported in Table 4.1. There was no difference in the WBGT between the various training weeks and sampling days ($F_{(6,24)} = 1.31$; $P = 0.29$).

Table 4.1 Wet bulb globe temperatures (WBGT)

The WBGT recorded during the study period per week and on the D0, D2 and D5 of sample collection.

WBGT (°C)	Exercise training intervention period				Sampling period		
	Week 1	Week 2	Week 3	Week 4	D0	D2	D5
WBGT min	17	17	16	15	13	16	14
WBGT max	24	25	29	21	20	21	23
WBGT mean & SD	20 ± 2	22 ± 3	21 ± 4	19 ± 2	16 ± 1	18 ± 1	18 ± 3

Discussion

The weather is considered cooler during the autumn in the lowveld of South Africa than in summer. There were no extreme weather conditions that were endured during the exercise or sampling period. The sampling times for each group was not consistent and were randomised throughout the sample collection period to reduce the variability that may be produced by differing environmental conditions (Appendix: Table 9.1). The only effect that was not planned for was the rain that occurred the night before the first day of sampling that resulted in wet animals upon sample collection, but as mentioned in section 3.8 (i), the wetting effect of the rain was accounted for by dousing the second set of animals, on the second sampling day, with tepid tap water.

4.3 Animal weights

Results

The weight of the animals was only measured at two time points during the study period, i.e., at Baseline and again at D5 (endpoint). The weight data were found to have a normal distribution and did not require log transformation before further analysis. There were no differences in weight at Baseline between the groups, and at D5 only the ES group weighed comparatively less than the NES group ($P = 0.04$). There was no significant weight loss in the exercised groups (ES and ENS) over the study period using a post hoc Tukey's multiple comparisons test (Table 4.2). However, when the exercised (ENS and ES) and non-exercised

(NES and NENS) groups were grouped together to see the specific effect of exercise training on weight, to form two larger groups (n = 20) significant differences were found (Table 4.2). A post hoc Sidak's test revealed no difference in weight between exercised and non-exercised groups at Baseline (before training), but at D5 (endpoint), the exercised trained groups weighed less than the non-exercised trained groups ($P = 0.005$). With this larger sample size (n = 20) for exercise training vs. non exercise trained (n = 20), exercise training accounted for 12.5% of the variation observed in weight over the study period and the effect was significant ($F_{(1, 38)} = 7.04$, $P = 0.01$).

Table 4.2 Weights recorded during the study period

Means and SD in kilograms for the different groups at the Baseline day (beginning) and day 5 of sampling (D5 - endpoint) of the study. * Different from Baseline within group (P < 0.05).

	Exercised groups	ENS	ES	Non-exercised groups	NENS	NES
Baseline	60 ± 4	61 ± 5	59 ± 3	63 ± 6	63 ± 5	63 ± 7
D5 (End)	58 ± 6 *	59 ± 7	57 ± 4 *	63 ± 6	63 ± 4	64 ± 7

Discussion

Weight loss can occur during exercise if dietary adjustments to increase energy consumed are not achieved (Swift et al., 2014). Weight loss due to calorie restriction and physical training occurs in humans (Herrera Uribe et al., 2016) and domestic species like dogs (Herrera Uribe et al., 2016; Swift et al., 2014) and horses (Bamford et al., 2019). The exercised groups would have increased energy demands but receiving the same feed ration as all the other groups. The energy deficit in the exercised trained groups should result in weight loss, which the data is supportive of in this exercise study. The unfortunate thing is that weight loss is often disadvantageous in wild animals where a thinner condition may give the appearance that the animal is sick or render their monetary value less. The effect of exercise on weight loss was limited in this study (± 2 kg) due to the relatively short exercise period of four weeks and would have not likely resulted in a poor body condition. Weight loss during exercise can also be ascribed due to favourable body composition change (Katch et al., 2010; Swift et al., 2014). Body fat percentage measurements were not included in this study. More frequent weight measurements may have revealed increased information on weight fluctuations during the study period. It is important to understand the impact that exercise training wild antelope may

have on energy requirements, body mass and composition. To support favourable body composition changes during exercise training, feeding rations need to be adjusted to ensure that nutritional and energy requirements are adequate and can be easily consumed. Additionally, animals must be managed to allow for adequate feed intake to meet the increased energy demands.

4.4 Rectal and muscle temperatures

Results

The rectal and muscle temperature data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the rectal temperature were time ($F_{(2, 100)} = 74.89$, $P < 0.0001$), exercise and stress ($F_{(3, 36)} = 17.5$, $P < 0.0001$) and the interaction of these factors ($F_{(9, 107)} = 6.97$, $P < 0.0001$). The factors that affected the muscle temperature were time ($F_{(2.63, 92)} = 50.97$, $P < 0.0001$), exercise and stress ($F_{(3, 36)} = 30.06$ $P < 0.0001$) and the interaction of these factors ($F_{(9, 105)} = 13.51$, $P < 0.0001$).

The rectal and muscle temperatures of the capture stressed groups (ES and NES) at D0: 0 min and 40 min were compared to the non-stressed groups (NENS and ENS) (Fig. 4.1 and 4.2) and were found to be markedly elevated (ES: $F_{(3, 36)} = 17.52$, $P < 0.0001$ and NES: $F_{(2.67, 89.80)} = 50.07$, $P < 0.0001$). The rectal temperatures for the non-stressed groups of animals on D0 decreased at a gradual rate. For the animals in the ENS group the temperature went from 38.0 ± 0.6 °C (GM = 37.6 °C, GSD = 1.03; Mdn = 38.1 °C, IQR = 37.0 - 38.3 °C) to 36.1 ± 0.7 °C (GM = 36.1 °C, GSD = 1.02; Mdn = 36.2 °C, IQR = 35.5 – 36.7 °C, $P < 0.0001$) and for the NENS group animals from 37.8 ± 1.0 °C (GM = 38.0 °C, GSD = 1.03; Mdn = 41.8 °C, IQR = 41.6 – 42.8 °C) to 36.3 ± 1.2 °C (GM = 36.3 °C, GSD = 1.04; Mdn = 37.9 °C, IQR = 37.3 – 38.6 °C; $P < 0.0001$) (Fig. 4.1). In the groups exposed to the capture stress event, a sharp decline in animals' rectal temperature of approximately 4 °C was observed in both the ES and NES group. The ES group from 41.1 ± 0.5 °C (GM = 41.3 °C , GSD = 1.01; Mdn = 41.4 °C, IQR = 41.1 – 41.6 °C)] to 37.6 ± 0.5 °C (GM = 37.6 °C GSD = 1.01; Mdn = 37.6 °C , IQR = 37.3 – 37.9 °C; $P < 0.0001$] and the NES group from 41.8 ± 0.4 °C (GM = 42.2 °C, GSD = 1.02; Mdn = 41.8 °C , IQR = 41.6 – 42.8 °C) to 38.0 ± 0.8 °C (GM = 38.0 °C, GSD = 1.02; Mdn = 37.9 °C, IQR = 37.3 – 38.6 °C; $P < 0.0001$] over the 40-minute immobilization period. Comparing only the NES and ES groups with each other, the NES group had a 0.5 °C higher rectal temperature (41.3 °C) than the ES group at D0: 0 min (40.8 °C; $P = 0.024$), but no

difference at D0: 40 min (Fig. 4.1). There were no significant differences in the muscle temperatures between NES and ES groups on D0 (Fig. 4.1).

The muscle temperatures for blesbok from the capture stressed groups followed a similar trend to the rectal temperatures. The muscle temperatures dropped gradually from 40.4 ± 0.5 °C (GM = 41.4 °C, GSD = 1.02; Mdn = 41.3 °C, IQR = 40.9 – 41.8 °C) to 37.3 ± 0.8 °C (GM = 37.3 °C, GSD = 1.02; Mdn = 37.0 °C, IQR = 36.6 – 37.8 °C) for the ES group's animals ($P < 0.0001$) and 41.0 ± 0.7 °C (GM = 41.3 °C, GSD 1.01; Mdn = 41.2 °C, IQR = 41.2 – 41.6 °C) to 38.3 ± 1.0 °C (GM = 38.0 °C, GSD = 1.03; Mdn = 38.4 °C, IQR = 37.7 – 38.6 °C) for the NES group's animals, at 40 min ($P < 0.0001$). The muscle temperatures for the non-stressed groups decreased from 37.6 ± 0.4 °C (GM = 37.9 °C, GSD = 1.00; Mdn = 37.9 °C, IQR = 37.9 - 37.9 °C) to 35.7 ± 0.7 °C (GM = 35.7 °C, GSD = 1.02; Mdn = 36.1 °C , IQR = 35.4 – 36.2 °C) for the ENS group ($P < 0.0001$), and 37.0 ± 0.9 °C (GM = 38.0 °C, GSD = 1.004; Mdn = 38.0 °C, IQR = 37.8 – 38.1 °C) to 35.9 ± 0.8 °C (GM = 35.8 °C, GSD = 1.02; Mdn = 36.0 °C, IQR = 35.1 – 36.5 °C) for the NENS group ($P < 0.0001$) (Fig. 4.1). From D0: 0 min to D0: 40 min, the rectal and muscle temperatures decreased with approximately 2 °C in the non-stressed groups.

A post-hoc Tukey analysis was performed to reveal if there were any significant differences between the rectal and the muscle temperatures at each recorded 5-minute time point from 10 to 40 minutes post immobilisation on D0. There were no significant differences within the stressed (ES and NES) or the non-stressed groups between the muscle and rectal temperatures (NENS and ENS). The only differences noted, were, as expected, between the stressed and the non-stressed group's temperatures. The muscle and rectal temperatures were positively correlated to each other in the stressed groups at D0: 0 min ($r = 0.48$, $R^2 = 0.23$, n = 20, $P = 0.036$) as depicted in Fig. 4.2. The muscle temperature of the stressed groups at D0: 0 min were positively correlated to the respiratory rate ($r = 0.48$, $R^2 = 0.23$, $P = 0.04$, n = 19), Ht ($r = 0.50$, $R^2 = 0.25$, $P = 0.03$, n = 19) and Hgb ($r = 0.49$, $R^2 = 0.24$, $P = 0.03$, n = 19). No other correlations were noted (Appendix: Table 9.2 & 9.3 and Fig. 9.1).

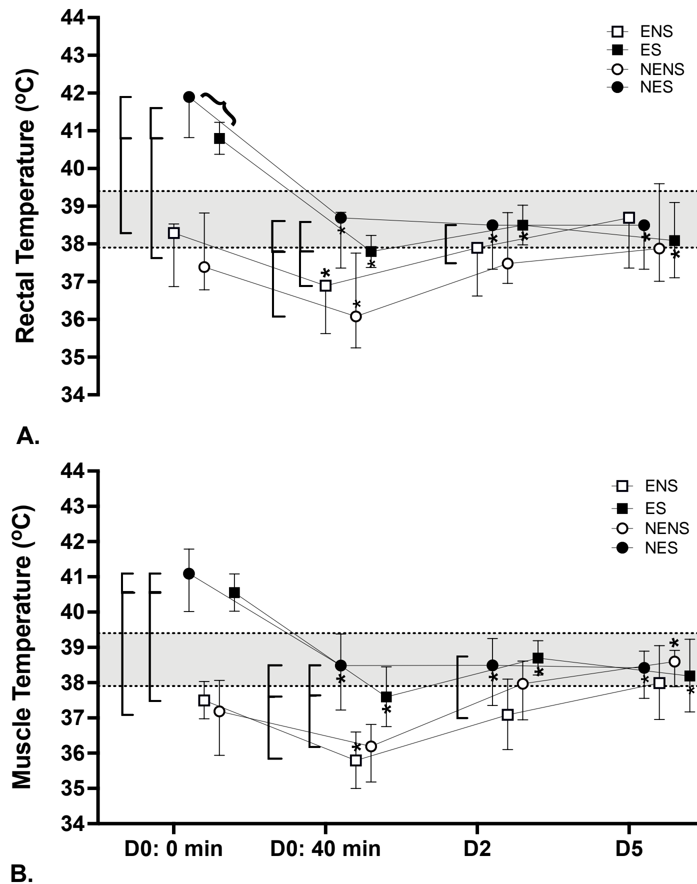


Figure 4.1 Rectal temperature & muscle temperatures

A. Back transformed mean and SD of the rectal temperatures (°C) for each group measured at D0:0 and 40 min, D2 and D5.

B. Back transformed mean and SD of the muscle temperatures (°C) for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal body temperature interval for blesbok (Sawicka *et al.*, 2015). Vertical bars: different between groups (P < 0.05). * Different from D0: 0 min within group (P < 0.05). Curly bracket: difference between the NES and ES group (t-test, P < 0.05).

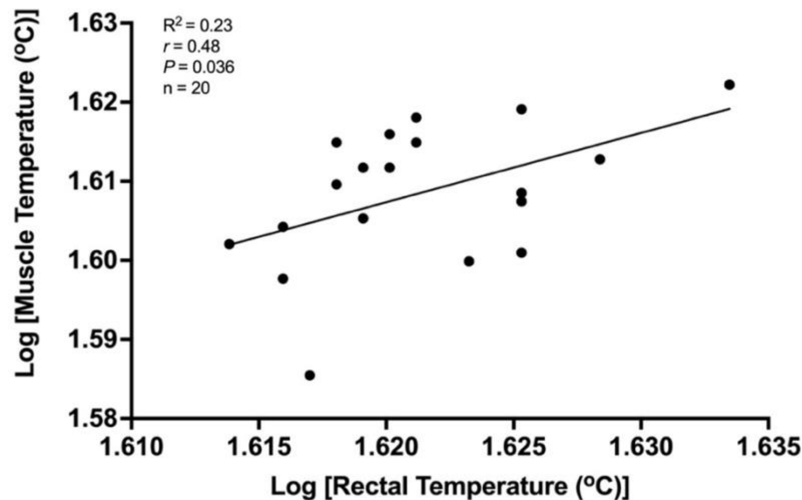


Figure 4.2 Correlation of the rectal and muscle temperatures

Pearson's correlation plot of the animals in the stressed groups (NES and ES, n = 20) on D0: 0 min using the log transformed values of the rectal and muscle temperatures.

Discussion

The capture stressed groups, ES and NES, experienced elevated rectal and muscle temperatures post the capture stress event in comparison with the normal temperatures of the non-stressed groups. This is otherwise known as a capture induced hyperthermia. There was a rectal temperature difference of 0.4 °C ($P < 0.05$) at D0: 0 min post-capture stress between the non-exercise trained (NES: Mdn = 41.8 °C , IQR = 41.6 – 42.8 °C) and exercise-trained (ES: Mdn = 41.4 °C, IQR = 41.1 – 41.6 °C) groups in this study. This result is clinically relevant as capture-induced hyperthermia is believed to play an important role in the development of capture myopathy in wildlife (Breed et al., 2019; Meyer, 2009; Meyer et al., 2008). In horses and human studies, aerobic fitness, and heat acclimation allow for improved thermal-tolerance and heat dissipation (Geor & McCutcheon, 1998; Kenny & McGinn, 2017; Périard et al., 2015). The core temperature recovers quicker in fit individuals when exposed to an exertional stress event. Muscle temperature is a good measure of core body temperature during exercise (Saltin et al., 1968).

For this study the rectal and muscle temperatures were specifically selected to measure due to the associated rhabdomyolysis that is seen in capture myopathy (Fortney & Vroman, 1985; Geor & McCutcheon, 1998; Périard et al., 2015). Core body temperatures for non-immobilised blesbok at rest have been measured with implanted data loggers to be 38.8 ± 0.4 °C reaching a minimum of 37.9 °C to a maximum of 39.4 °C during the day (Sawicka et al., 2015). Elevated

rectal and muscle temperatures are expected after exertional stress (Kenny et al., 2007). The heat generated after exercise is independent of aerobic capacity (i.e. fitness) and directly related to workload in humans (Saltin & Hermansen, 1966). However, factors such as age, sex, obesity, and poor fitness all compromise heat loss capacity and contribute to excessive heat retention post-exercise (Kenny et al., 2007). In antelope, the number of sweat glands per surface area may also play a role in heat loss (Fortney & Vroman, 1985; Kenny & McGinn, 2017; Périard et al., 2015; Robertshaw & Taylor, 1969).

The higher rectal temperature of the animals in the NES than the ES group after the capture stress event at D0: 0 min supports likely improved thermoregulatory adaptations, like improved heat loss, for the ES group. The training of the blesbok was not performed in extreme heat compared to human studies where heat acclimation during exercise was achieved (Geor & McCutcheon, 1998; Kenny et al., 2003; Périard et al., 2015). However, the blesbok would have experienced acclimation to the local environmental conditions during training (Geor & McCutcheon, 1998; Kenny et al., 2003; Périard et al., 2015). In this study, it does appear that fitness did result in a reduced rectal temperature response, which is supported by the similar studies in humans and horses where environmental acclimation and aerobic fitness resulted in reduced rectal temperatures (Geor & McCutcheon, 1998; Kenny et al., 2003; Périard et al., 2015). However, as Meyer *et al.* (2008) showed, post-capture stress hyperthermia is affected by the level of psychological stress the animal experiences before and during capture. Exercise training habituates the animals to human presence and capture associated stimuli, and this may reduce the stress experienced during a capture event, and consequently, the temperature exhibited post-capture. The reduced hyperthermic response in the capture stressed exercise-trained group could be attributed to fitness and habituation. However, the contribution of each factor remains undetermined.

It was not possible to record the temperatures of the blesbok until they were immobilised post the capture stress event i.e., no real time tracking could occur of the temperature changes during and immediately post the stress event. Recording the body temperatures prior, during and immediately after the stress event may have accentuated the overt difference in body temperatures between the exercise-trained and non-exercise trained groups even more. Future studies would be recommended where data loggers are implanted to monitor muscle and core body temperature in real-time to better evaluate the differences in temperatures at critical moments during and after exercise.

An important consideration in interpreting findings is that the capture-induced hyperthermia experienced was not strongly associated with the intensity of the exercise or the environmental temperatures during capture in a previous study (Meyer et al., 2008). The capture stressed animal's rectal temperature at D2 and D5 was significantly lower than their temperatures at D0: 0 min due to lack of activity on those days. Although no physical activity was performed on D2, there is a difference in muscle and rectal temperatures in the ES groups (rectal: M ± SD = 38.4 ± 0.2 °C; GM = 38.4 °C, GSD = 1.01; Mdn = 38.4 °C, IQR = 38.2 – 38.6 °C and muscle: M ± SD = 38.9 ± 0.5 °C; GM = 38.9 °C, GSD = 0.01; Mdn = 38.9 °C, IQR = 38.3 – 39.4 °C) and ENS groups (rectal: M ± SD = 37.1 ± 0.1°C; GM 37.3 °C, GSD = 1.003; Mdn = 37.3 °C, IQR = 37.2 – 37.5 °C and muscle: M ± SD = 37.0 ± 0.4 °C; GM = 37.0 °C, GSD = 1.01; Mdn = 37.0 °C, IQR = 36.7 – 37.5 °C). The times that the measurements for the groups were obtained throughout the day were randomised (Appendix: Table 9.1). However, the difference in body temperatures could partially be attributed to the natural diurnal body temperature variation, which can differ up to a degree or more (Kelly, 2006). In humans, younger and fitter individuals have shown to have a greater amplitude in average body temperatures measured throughout the day and this could also explain the variation recorded in the trained ES and ENS groups on D2 (Kelly, 2006).

A delay of a few minutes in obtaining rectal and muscle temperature recordings post immobilisations could have also resulted in decreased values in the ENS group compared to the ES group. A steady decline of body temperatures is expected post immobilisation. The higher WBGT that the ES groups experience during sampling of 19.9 – 20.8 °C compared to the cooler WBGT experienced by the ENS group of 16.4 – 17.4 °C could also contribute. However, a previous study has indicated that ambient temperature did not influence body temperatures recorded (Meyer et al., 2008).

There was no difference found between the rectal and muscle temperatures within capture stressed groups. A previous study, using a similar approach of chasing blesbok, muscle temperatures, on average, were 1 °C higher than rectal temperature after a capture stress event (Fitte, 2017). A study in immobilised rhinoceros also revealed higher muscle temperatures than rectal during immobilisation (Morkel et al., 2012). In horses, the gluteal muscle temperature rises much quicker and reaches a much higher temperature during and immediately post-exercise than rectal temperatures (Weishaupt & Staempfli, 1996). In this study, the rectal and muscle temperatures did not differ. Muscle temperature results from localised balances between heat produced from the muscle, blood flow removing heat from the muscle and

allowing for its release at the periphery (skin) and heat storage. In humans, 3 to 5 min after the commencement of exercise, the muscle temperature rises above rectal temperature and reaches an equilibrium after 10 to 20 min (Saltin et al., 1968). In horses, muscle temperature immediately decreases after completion of exercise, while the rectal temperature may continue increasing (Jones et al., 1989; Reece et al., 2015). Between 10 to 12 min post-exercise in studies in horses, the rectal and muscle temperature showed little difference (Jones et al., 1989). As described in Chapter 3 (section 3.8), there was a delay between the exercise stress event, immobilised recumbency (2.5 to 5 min) and obtaining muscle temperature from the muscle probe for all animals (another 5 to 10 min) which may have resulted in a 7.5 to 15 min delay post-exercise. This lapse in time may have missed recording a difference. Muscle temperature may also vary according to the specific muscle, the site and depth of measurements. In this study this was standardised to minimise this effect (Chapter 3). When the ambient temperatures are elevated, it may result in inadequate heat loss, with excess heat retention in the muscle and a more considerable difference between muscle and the rectal temperature is then expected (Saltin et al., 1968). In this study the ambient temperatures were not elevated so this would have not aggravated any differences, but in real capture situations this may contribute to muscle heat retention. The groups on the first day, D0, were wet from rain the night before, experienced milder temperatures and a higher relative humidity that day than the next day where the groups experienced no rain, the relative humidity was lower and the ambient temperatures higher. The latter group of animals that were doused with water, and experienced lower humidity and higher ambient temperatures, could have experienced significant effects on their body temperatures and cooling capacity by improved evaporative cooling due to an increased vapor pressure. The vapor pressure would need to be measured to confirm this effect.

The muscle temperature of the animals in the non-stressed groups, NENS and ENS, dropped significantly during immobilisation (± 2 °C) from low normal body temperatures to hypothermic levels (± 36 °C). A gradual reduction in temperature is expected during anaesthesia (Díaz & Becker, 2010). The gradually worsening hypothermia experienced in these groups was likely caused by the cooling effects of the rain and aggravated by the lower environmental temperatures on that day (minimum ambient temperature of 14 ºC).

4.5 Heart rate

Results

The heart rate data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the heart rate was time ($F_{(2.23, 79.61)} = 8.32$, $P < 0.001$), exercise and stress ($F_{(3, 36)} = 3.16$, $P = 0.04$) and the interaction of these factors ($F_{(9, 107)} = 6.97$, $P < 0.0001$). The differences were only found at D0: 0 min, where the capture stressed groups differed from the non-stressed groups ($P < 0.05$) (Fig. 4.3). There was no difference at any other time points or between exercised and non-exercised trained groups. When comparing D2 and D5 with D0 heart rates, only the ES and NES group had an increased heart rate at D0 ($P < 0.05$) (Fig. 4.3).

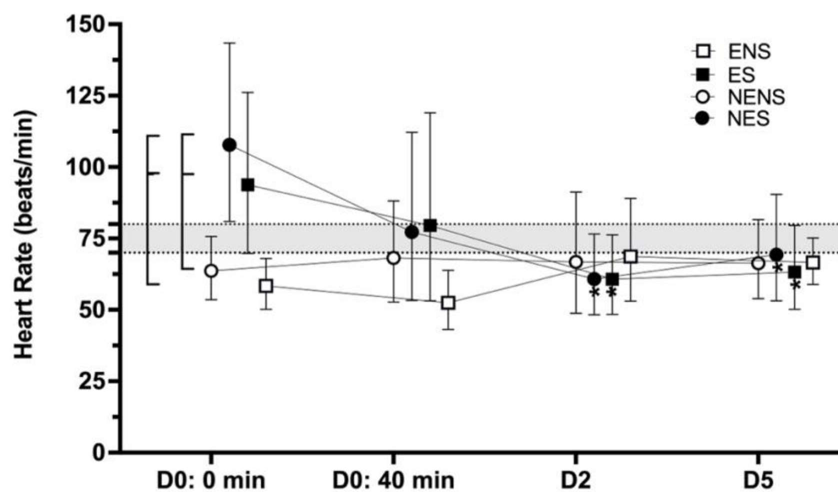


Figure 4.3 Heart rate

Back transformed mean and SD of the heart rate (beats/min) for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal heart rate interval for non-immobilised goats (70 to 80 beats/min). Vertical bars: different between groups (P < 0.05). * different from D0: 0 min within-group (P < 0.05).

The heart rate decreased (Fig. 4.3) from D0: 0 min to D0: 40 min for the capture stressed groups. When the ES and ES group were compared (*t*-test, $P < 0.05$) at D0: 0 min and D0: 40 min there were no differences between the trained and untrained animals. The immobilised non-stressed heart rates of the control groups, ENS and NENS, were also compared at D0: 0 min and D0: 40 min (*t*-test, $P < 0.05$) to see if there was a difference between the resting immobilised heart rate of the exercise-trained and non-exercise trained blesbok. No statistical

difference was noted. At D0: 0 min in the stressed groups (NES and ES) the heart rate was negatively correlated to $cHCO_3^-$ (r = - 0.54, R^2 = 0.29, n = 20, P = 0.02) (Fig 4.4) and positively to $BE_{(ecf)}$ (r = 0.55, R^2 = 0.30, n = 19, P = 0.02). There were no other significant correlations (Appendix: Table 9.2 & 9.3).

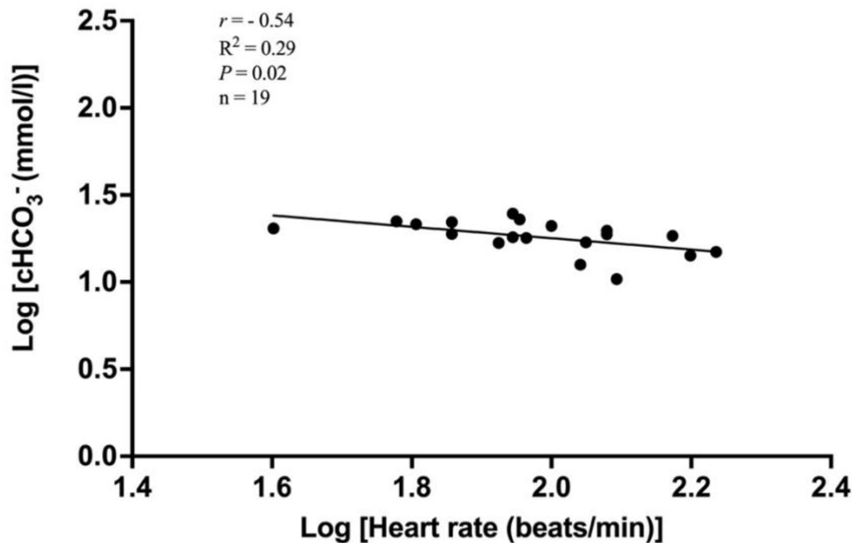


Figure 4.4 Correlation between the heart rate and $cHCO_3^-$

Pearson's correlation plot of the heart rate and $cHCO_3^-$ of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

Discussion

The capture stress event increased the heart rates of the ES (M ± SD = 92 ± 25 beats/min; GM = 89 beats/min, GSD = 1.36 , Mdn = 98, IQR = 63 – 66 beats/min) and NES group (M ± SD = 108 ± 8 beats/min; GM = 108 beats/min, GSD = 1.07; Mdn = 105 beats/min, IQR = 103 – 117 beats/min) on D0: 0 min compared to the non-stressed groups, ENS (M ± SD = 59 ± 1 beats/min; GM = 59 beats/min, GSD = 1.02; Mdn = 59 beats/min, IQR = 58 – 60 beats/min) and NENS (M ± SD = 64 ± 2 beats/min; GM = 64 beats/min, GSD = 1.07; Mdn = 64 beats/min, IQR = 63 – 66 beats/min). There were no differences noted for the exercise-trained versus non-exercise trained groups at any time point. The normal heart rate for blesbok at rest is not well established. The closest domesticated species where resting heart rate reference values are well known are goats and sheep. The resting heart rate of goats and sheep is between 70 - 80 beats/min, similar to humans (60 – 90 beats/min) (Aiello et al., 2016; Nanchen, 2018). Du Plessis (2018) recorded the heart rate for two non-immobilised blesbok via remote sensing methods (Equivital EQ02 biotelemetry system), but the sample size was small (2) and varied

considerably for the two animals. Blesbok 1 had a stationary mean heart rate of 85.6 ± 0.7 beats/min, and Blesbok 2 was 53.1 ± 0.2 beats/min. The reference range for goats was used for heart rates due to the large sample sizes and increased accuracy of the normal interval ranges. In goats, heart rates below 55 beats/min are considered bradycardic, and above 110 beats/min are deemed tachycardic in resting non-immobilised small ruminants (Grimm et al., 2015).

There are several factors to consider when interpreting all the heart rate responses for the immobilised animals in this study. First, and most likely, the opioids used may have varying cardio-effects that are dose-dependent and may directly influence heart rate (Meyer, 2009). As the weight of the animals varied slightly and the dose administered was standardised (i.e. all darts contained the same amount of opioid and tranquilizer) it would have resulted in the actual dose of opioid per kilogram varying. Individual animals would also have had varying responses to the cardio-effects of opioids. A study in blesbok (Pfitzer et al., 2020) found the mean heart rate for blesbok immobilised with etorphine alone to be 37 beats/min. In the present study, the mean heart rates recorded were higher for the non-stressed groups, ENS (59 ± 1 beats/min) and NENS (61 ± 2 beats/min) at D0: 0 min than the animals in the study by Pfitzer (2020). The etorphine dose was higher in the study by Pfitzer (2020), as no azaperone was added, and the etorphine was given at a per kilogram dosage. A standard dose of etorphine and azaperone were used (see Chapter 3, section 3.8 (iii)) on all immobilised blesbok in the present study. The addition of azaperone is likely to have caused the increased heart rate observed in this study. Azaperone has an α_1 antagonist effect that causes vasodilation and a decrease in blood pressure. The baroreceptor reflex response is activated by the decrease in blood pressure. The heart rate increases to attempt to increase the cardiac output to increase and stabilise the blood pressure (Lees and Serrano, 1976).

A reduction in resting heart rate in human athletes is one of the most well-known fitness measurements and is accounted to an increased achievable stroke volume by the heart of trained individuals (Edwards et al., 1969; Katch et al., 2010; Ramos-Jiménez et al., 2008). The effect of training on resting heart rate on other species, except the horse and dog, is not well explored, and even in dogs and horses, the data are inconclusive (Betros et al., 2013; Nanchen, 2018; Reece et al., 2015; Shave et al., 2017). The heart rate data in this study cannot be used to evaluate whether a lower heart rate for the exercise trained (ENS) versus the non-exercise trained blesbok (NENS) were observed, and could not be equated to an increased fitness level for the ENS vs the NENS group. The immobilizing drugs would have altered the heart rate too severely. To evaluate the effect of training on resting heart rate - remote data loggers would

have been required to obtain non-immobilised resting heart rate values. The period of training of four weeks may have also not been long enough to see significant adaptations in heart rate.

There was no difference in the heart rates obtained on D0 of the blesbok exposed to the capture stress (Fig. 4.3). A distinction was not expected in the blesbok maximal heart rate between the stressed groups, as findings in humans indicate that the heart rate during maximal exertional stress in humans does not differ between trained and untrained individuals, but only in the work rate where fatigue sets in (Edwards et al., 1969; Goodwin et al., 2007).

The weak negative correlation of heart rate to blood HCO_3^- and positively to BE_{ecf} (Fig. 4.4) can be explained in that as exertion and heart rate increase, so would plasma lactate (Chapter 6) and H^+ increase and buffering excess H^+ with HCO_3^- would reduce its blood HCO_3^- concentration. Another plausible explanation is that exertion results in an increased H^+ concentration that increases heart rate through sympathetic stimulation. Interestingly the heart rate was not correlated to any other blood gas variables like PaO_2, $PaCO_2$, blood pH and lactate that are linked to exertion. The heart rate is controlled through the autonomic nervous system by adjustments in the sympathetic and parasympathetic stimulation. Baroreflex mechanisms regulate the sympathetic and parasympathetic activity, and in turn the heart rate and blood pressure. The immobilisation drugs (etorphine and azaperone) that effect the sympathetic and parasympathetic activity and blood pressure regulating mechanisms would have had the main impact on heart rate for these animals and not the level of exertion (Buss et al., 2016; Lees & Serrano, 1976; Pfitzer et al., 2020).

4.6 Respiratory rate

Results

The respiratory rate data were found to not have a normal distribution and required to be log transformed before further analysis. The only factor that affected the respiratory rate was time ($F_{(1.93, 69.50)} = 3.28$, $P = 0.05$), neither the exercise training and stress ($F_{(3, 36)} = 1.16$, $P = 0.34$) or the interaction of time, exercise, and stress ($F_{(9, 108)} = 1.17$, $P = 0.32$) showed any significant effect on the respiratory rate. There were no differences between the four groups at any time point (Fig. 4.5). Within each group, only the D5 respiratory rate of the animals in the NES group were lower ($P = 0.019$) than D0: 0 min. When the two stress groups were compared (NES vs ES, *t*-test) respiratory rate was higher in the non-trained animals at D0: 0 min, but not at D0: 40 min.

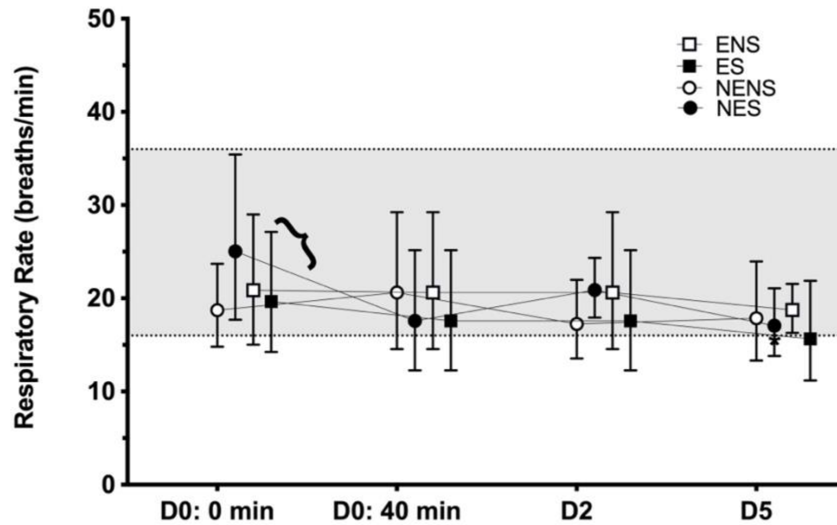


Figure 4.5 Respiratory rate

Back transformed mean and SD of the for each group measured at D0:0 and 40 min, D2 and D5. Grey shaded area and gridlines: normal respiratory rate for non-immobilised goats (16 to 36 breaths/min). * different from D0: 0 min within-group ($P < 0.05$). Curly bracket: different between NES and ES group (*t*-test, $P < 0.05$)

The respiratory rate correlated positively with PaO_2 ($r = 0.64$, $R^2 = 0.41$, n = 20, $P = 0.003$) and negatively with $PaCO_2$ ($r = -0.45$, $R^2 = 0.20$, n = 19, $P = 0.036$) (Fig. 4.6). The respiratory rate was also positively correlated to haemoglobin ($r = 0.46$, $R^2 = 0.21$, $p = 0.04$, n = 20) and negatively to creatinine ($r = 0.48$, $R^2 = 0.23$, $P = 0.03$, n = 20). No other correlations were noted (Appendix: Table 9.2 & 9.3 and Fig. 9.1).

Discussion

The respiratory rates recorded of the blesbok post immobilisation in response to the capture stress event was not the typical hyperventilation response expected (Fig. 4.5). Physical exercise causes increased O_2 consumption (VO_2) and CO_2 production (VCO_2), and depends on the type of exercise, load and individual. Exercise directly stimulates the cardiopulmonary centres to activate increases in the depth and rate of breathing to increase pulmonary ventilation to maintain adequate oxygen supply and carbon dioxide exhalation (Azizi, 2011).

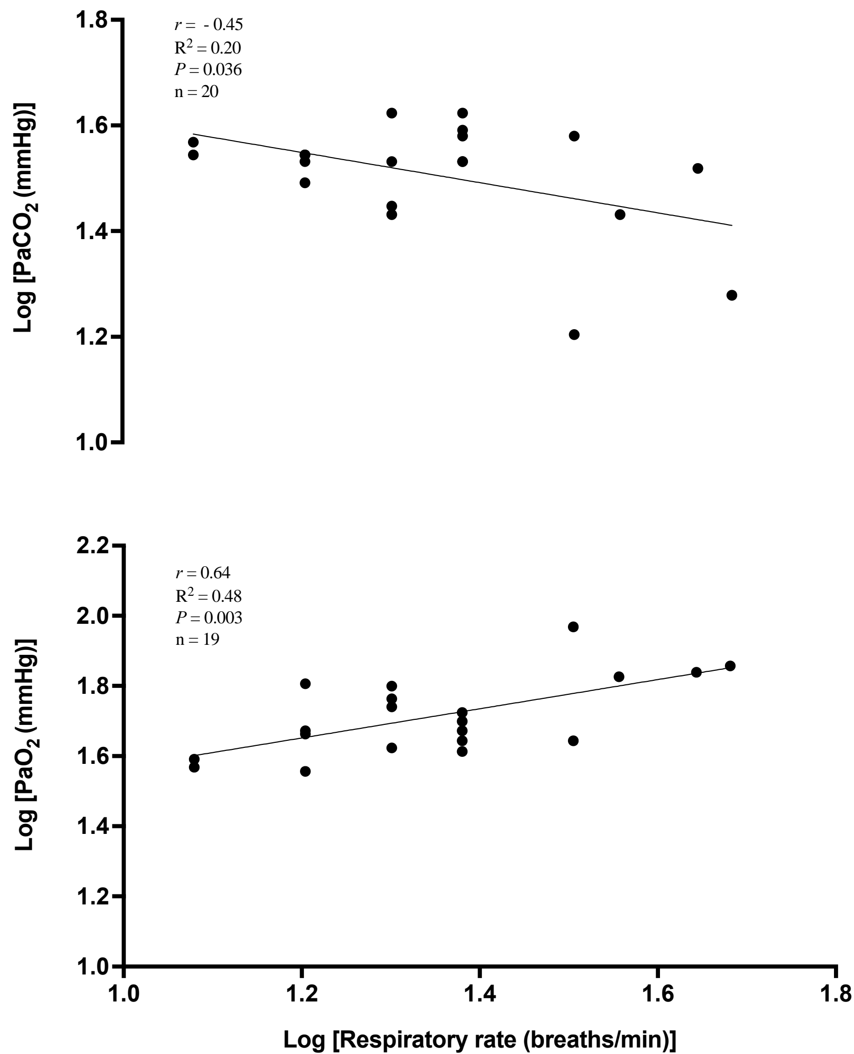


Figure 4.6 Correlation between respiratory rate, PaO_2 and $PaCO_2$

Pearson's correlation plot of the respiratory rate, PaO_2 and $PaCO_2$ of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

The respiratory rate has been found to be an accurate indication of the anaerobic threshold (Carey et al., 2005), but in this study respiratory rates should be interpreted with caution due to the severe respiratory depression that the opioids cause during immobilisation (McKenzie, 1993; Meyer, 2009; Paterson, 2014). The normal respiratory rate for goats and sheep, indicated as the grey shaded area in Figure 4.5, is between 16 to 36 breaths/min (Reece et al., 2015). The respiratory rates of the blesbok groups exposed to the capture stress event (NES and ES) should have increased. As can be seen from Fig. 4.5, the respiratory rate does not appear adequately elevated post the capture stress event as all the breathing rates were within the normal non-

stressed reference range. The respiratory rates in the capture stressed groups are not adequately elevated in response to exertion, due to opioid suppression.

The NES group's respiratory rate was significantly higher for the capture stress event compared to the ES group. This increased respiratory rate may be supportive of a delayed return to normal respiratory rate post exercise compared to the ES group, which is a well-known occurrence in untrained individuals (Depiazzi & Everard, 2016; Folinsbee et al., 1983). An increased respiratory rate does not necessarily indicate that increased ventilation is occurring. Tidal volume needs to be monitored in addition to respiratory rate to achieve a true reflection of ventilation (Holley et al., 2016). In this study, ventilation was indirectly assessed by measuring partial pressure of carbon dioxide. $PaCO_2$ is a very good indicator of ventilation, whereas PaO_2 is not. With regards to PaO_2 an individual may have good ventilation and still be hypoxaemic due to poor oxygen diffusion into the pulmonary vasculature (Morkel et al., 2010).

The respiratory rate's positive correlation with PaO_2 and negative correlation with $PaCO_2$ (Fig. 4.6 and Fig. 9.1) supports that an increased respiratory rate improved ventilation and blood oxygenation (increased PaO_2 and decreased $PaCO_2$). The relative higher respiratory rate in the NES group at D0: 0 min (Fig. 4.3) could indicate a greater drive to increase ventilation in this group. Increased plasma $PaCO_2$ and H^+ produced as by-products in energy metabolism stimulate the drive to increase ventilation (Delpierre et al., 1978).

An important finding is that the ES respiratory rate did not differ from the NES and NENS groups. The failure of the ES group animals to increase their respiratory rate post the capture stress event may be due to an improved recovery time after the capture stress event, but in addition, may also be because of opioid respiratory depression (Meyer, 2009; Meyer et al., 2006). The lack of a proper elevated respiratory rate response for the capture stressed groups re-emphasises the lack of normal compensatory mechanisms. This is due to animals that are immobilised with the opioid etorphine post-exercise (a common drug combination in field wildlife capture) that results in concomitant hypoxia, hypercapnia and acidaemia.

4.7 Conclusion

There were no extreme weather conditions that were endured during the exercise or sampling period. The exercise trained blesbok (n = 20) weighed less than the non-exercise trained groups (n = 20) at the end of the study, indicating weight loss due to exercise training. An expected finding if calorie consumption is not increased with increased activity. The exercise trained

blesbok had a decreased capture or stress-induced hyperthermia compared to the non-exercise trained (NES) which possibly indicates improved thermoregulation as a fitness adaptation, or possibly a decreased stress response due to habituation. The capture stress groups had an increased heart rate on D0. The non-exercise stressed group (NES) had a higher respiratory rate than the exercise trained stress group (ES), also an expected finding in unfit animals when exertional stressed. Any changes that exercise training might have had on heart rate or respiratory rates will be affected by the cardiorespiratory effects of opioids, making interpretation difficult.

The monitoring of heart and respiratory rate, muscle and rectal temperatures are an initial indication of the physiology and possible adaptations that might have occurred in the blesbok. In the following chapter the blood gas and electrolyte values are discussed and related to previous observations to provide a deeper understanding of the possibly physiological mechanisms that were at play.

CHAPTER 5

RESULTS & DISCUSSION: ARTERIAL BLOOD GASES & ELECTROLYTES

5.1 Introduction

In this chapter, the ePOC blood analysis results (Chapter 3, section 3.8) from the study's four groups are discussed. The physiological variables obtained consist of arterial blood gases, electrolytes and the calculated A-a gradient (Chapter 3, section 3.8). The initial discussion focuses on reference intervals used for this study, the difficulties associated with their use, and the data interpretation.

Published reference interval values for physiological variables for wild species are scarce and can be difficult to obtain. The validated reference interval values that are readily available are for humans and domesticated species like dogs, cats, sheep, cows, goats and horses. Reference interval values are available for a few selected wild animal species, but the small sample sizes are often problematic, and herd influence (known as 'herd effect') can significantly affect reference interval values obtained (Stevens et al., 1994). The 'herd effect' must be considered when using such reference intervals (Stevens et al., 1994). In this study, the immobilised animals in control groups (i.e. those *not* exposed to the capture stress event, NENS and ENS) served to gain reference interval values for the measured variables. The control groups were compared to immobilised blesbok exposed to the capture stress event in the non-exercise trained and exercise-trained groups (NES and ES). As an additional comparison, the values obtained from the current study were compared to other studies with similar protocols, including species used and drugs used for immobilisation.

The ePOC user guide only provides reference interval values for dog, cats and horses for the variables measured by their cassettes (Epocal Inc., 2012). Values obtained for these variables using other conventional methods exist for blesbok (Fitte, 2017). Most of these values are from *immobilised* animals and likely represent physiologically altered values different from true non-immobilised reference intervals. A closely related domestic species (similar in weight, stature, family), the domestic goat (*Capra aegagrus hircus*), may serve as a valid model for gaining *non-immobilised* reference interval values to compare the variables obtained from blesbok in this study (Stevens et al., 1994). Goats have a similar physiology to blesbok, and

published reference values could be obtained from a study with a large sample size and non-immobilised animals. Additionally, horse and human reference interval values were added as an extra comparison due to large sample sizes, non-immobilised participants, and the fact that numerous exercise studies have been published in these species.

Harthoorn (1976) obtained blood gas values from a handful of tame blesbok and tsessebe (non-immobilised blesbok and partially immobilised tsessebe) and found that the values obtained were the same as the reference intervals for humans. The reference interval values obtained from previous studies of non-immobilised animals are indicated in Table 9.5 (Appendix), comparing the values obtained for this study's immobilised animals. The table highlights how immobilisation affects the physiological variable values obtained. Du Plessis (2018) collected some non-immobilised blesbok values, but the sample size (n = 2) was too small to refer to as 'normal' blesbok reference intervals in this study. In the discussions of the results obtained, reference is made, where applicable, to similar studies to the current study.

Another factor that influences reference interval values, especially blood gases, is the altitude at which they were obtained. The altitude above sea level will impact the partial pressure of the gases breathed, which in turn will affect blood gas values and the reference values obtained (Meyer et al., 2006). For example, with increasing altitudes above sea level, the atmospheric pressure decreases and so too does the inspired partial oxygen pressure, which results in lower 'normal' PaO_2 values compared to at sea level. The blesbok exercise intervention study occurred at 670m above sea level (Chapter 3, section 3.4). The altitude at which the comparison reference intervals were obtained varied from near sea level (96m) (Stevens et al., 1994) to 670m (Pfitzer et al., 2020) and 1753m (Meyer et al., 2006) above sea level. The horse and human reference intervals (Appendix: Table 9.5), were obtained from a large sample base and although not directly stated in the documents assumed to be from varied altitudes (Castro et al., 2021; Epocal Inc., 2012).

5.2 Blood gases and acid-base balance

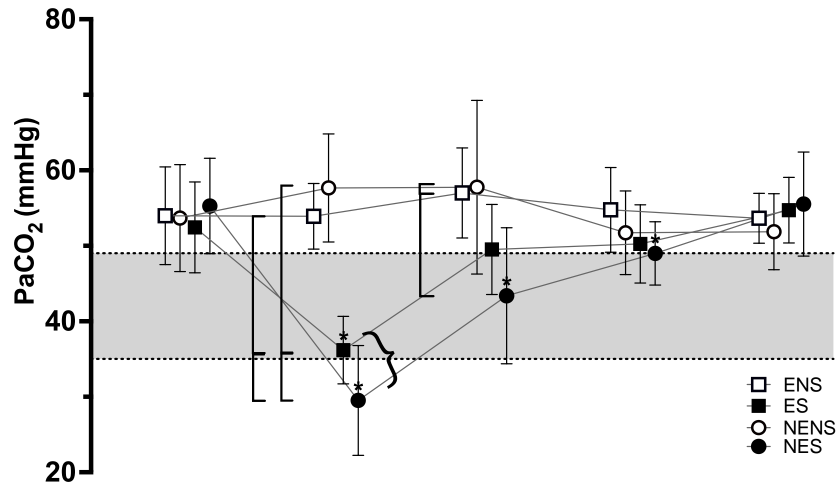
(i) $PaCO_2$, PaO_2, A-a gradient and the Anion Gap

Results

The $PaCO_2$ data were found to have a normal distribution and did not require log transformation before further analysis. The factors that significantly affected the $PaCO_2$ were time ($F_{(3.05, 108.3)}$

= 23.35; $P < 0.0001$), exercise training and capture stress ($F_{(3,36)} = 8.96$; $P < 0.0001$) and the interaction between these factors ($F_{(12, 142)} = 14.54$; $P < 0.0001$). The capture stressed groups had lower $PaCO_2$ values than the non-stressed groups at D0: 0 min ($P < 0.05$). At D0: 40 min, only the NES group had a lower $PaCO_2$ value than the non-stressed groups (ENS and NENS, Fig. 5.1, Appendix: Table 9.5). Only within the ES group was the $PaCO_2$ lower at D0: 0 min than the Baseline value. Whereas the $PaCO_2$ values for the NES groups were significantly lower at D0: 0 min, D0: 40 min and D2 from the Baseline values ($P < 0.05$). Excluding the D0: 0 min values for the capture stressed groups, the $PaCO_2$ values were elevated above the reference interval values at all other time points (Fig. 5.1, Appendix: Table 9.5). When the two capture stress groups were compared (NES vs ES, *t*-test), the $PaCO_2$ for the untrained (NES) group was significantly lower at D0: 0 min ($P = 0.027$), but there was no difference at D0: 40 min. The $PaCO_2$ for the stressed groups has a positive correlation to $cHCO_3^-$ ($r = 0.83$, $R^2 = 0.68$; n = 20; $P < 0.001$) and cortisol ($r = 0.52$, $R= {}^2 = 0.28$; n = 20; $P = 0.02$). The $PaCO_2$ for the stressed groups had a negative correlation to the respiratory rate ($r = - 0.45$, $R^2 = 0.22$; n = 20; $P = 0.036$) (Fig. 4.6), PaO_2 ($r = - 0.73$, $R^2 = 0.53$; n = 20; $P < 0.001$) (Fig. 5.2), BE_{ecf} ($r = - 0.69$, $R^2 = 0.48$; n = 20; $P = 0.0.001$), AGap ($r = - 0.63$, $R^2 = 0.39$; n = 20; $P < 0.001$), glucose ($r = - 0.47$, $R^2 = 0.22$; n = 20; $P = 0.04$), lactate ($r = - 0.63$, $R^2 = 0.40$; n = 20; $P = 0.003$), urea ($r = - 0.70$, $R^2 = 0.49$; n = 20; $P = 0.001$), and creatinine ($r = - 0.53$, $R^2 = 0.28$; n = 20; $P = 0.02$).

The PaO_2 data were found to have a normal distribution and did not require log transformation before further analysis. The factor that affected the PaO_2 was time ($F_{(2.78, 98.80)} = 77.85$, $P < 0.0001$), but neither exercise training or capture stress ($F_{(12, 142)} = 1.18$, $P = 0.23$) nor the interaction between exercise training, capture stress and time $F_{(3, 36)} = 1.16$, $P = 0.34$) had an influence on the PaO_2 values. The arterial PaO_2 within the NES group's animals were higher at D0: 0 min ($P = 0.03$), D0: 40 min ($P < 0.0001$) and D2 ($P = 0.025$) than at Baseline. When the two stress groups were compared (NES vs ES, *t*-test) PaO_2 was higher in the untrained (NES) group at D0: 0 min, but not at D0: 40 min.



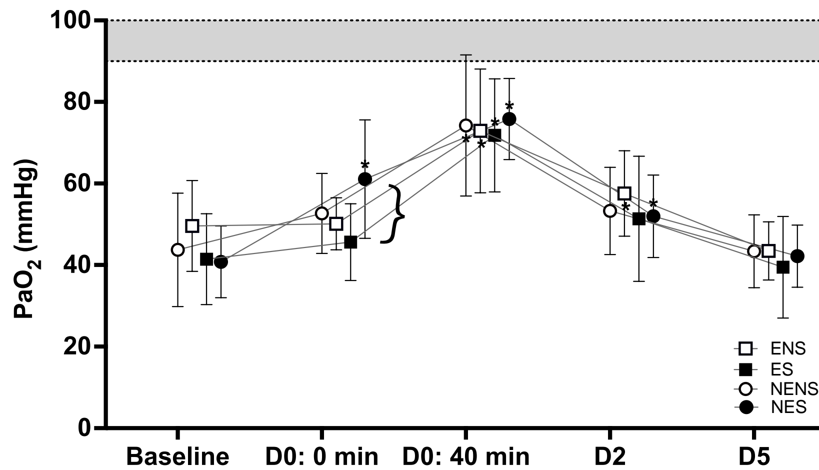


Figure 5.1 PaO_2 and $PaCO_2$

A. Mean ± SD of $PaCO_2$ for each group measured at D0:0 and 40 min, D2 and D5.

B. Mean ± SD of PaO_2 for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal $PaCO_2$ interval for blesbok (Stevens et al., 1994; Meyer et al., 2006; Pfitzer et al., 2020). Vertical brackets: different between groups ($P < 0.05$). * Different from D0: 0 min within group ($P < 0.05$). Curly bracket: difference between the NES and ES group (t-test, $P < 0.05$).

The PaO_2 in the capture stressed groups on D0: 0 min was positively correlated to the respiratory rate ($r = 0.64$; $R^2 = 0.40$; n = 20; $P = 0.003$) (Fig. 4.6), $BE_{(ecf)}$ ($r = 0.52$; $R^2 = 0.27$; n = 19; $P = 0.02$), AGap ($r = 0.45$; $R^2 = 0.21$; n = 20; $P < 0.001$), glucose ($r = 0.24$; $R^2 = 0.06$; n = 19; $P = 0.03$), lactate ($r = 0.51$; $R^2 = 0.26$; n = 20; $P < 0.001$), haematocrit ($r = 0.42$; $R^2 = 0.18$; n = 20; $P = 0.02$), haemoglobin ($r = 0.38$; $R^2 = 0.14$; n = 20; $P = 0.05$), creatinine ($r = 0.51$; $R^2 = 0.26$; n = 20; $P = 0.02$) and cortisol ($r = 0.52$; $R^2 = 0.28$; n = 20; $P = 0.02$). The PaO_2 was negatively correlated in the capture stressed groups on D0: 0 min to the $PaCO_2$ ($r = -0.73$; $R^2 = 0.51$; n = 20; $P < 0.001$), $cHCO_3^-$ ($r = -0.49$; $R^2 = 0.68$; n = 19; $P < 0.001$) and urea ($r = -0.70$; $R^2 = 0.49$; n = 20; $P = 0.001$).

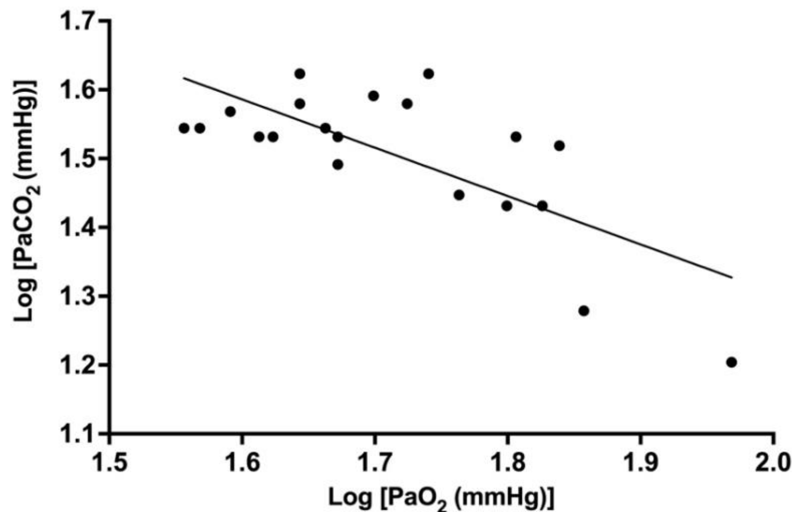
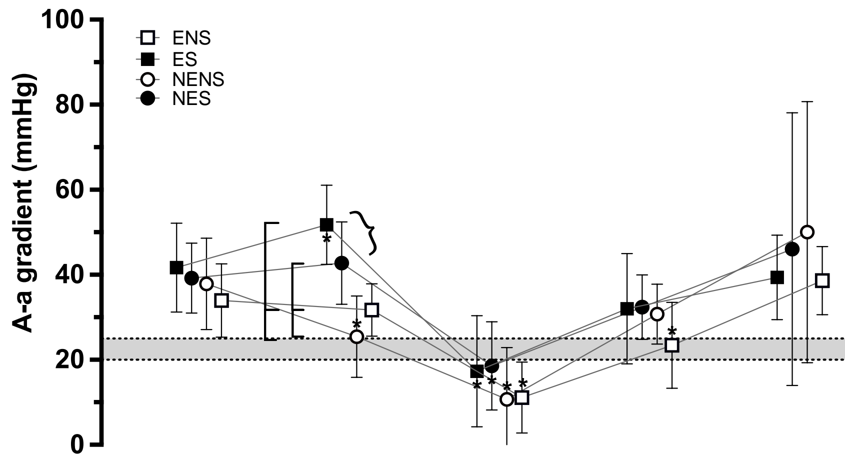


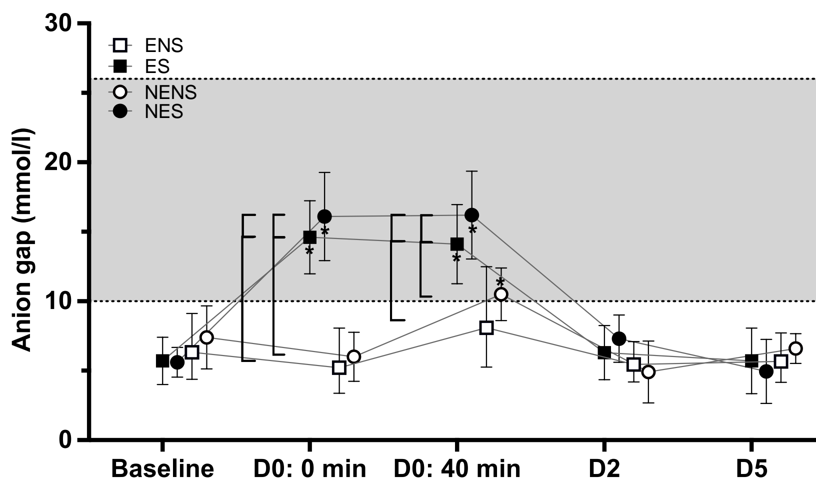
Figure 5.2 Correlation between PaO_2 and $PaCO_2$

Pearson's correlation plot of the PaO2 and $PaCO_2$ of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

The A-a gradient data were found to have a normal distribution and did not require log transformation before further analysis. The factors that affected the A-a gradient were time ($F_{(2.122, 68.44)} = 21.41$, $P < 0.0001$), exercise and capture stress ($F_{(3, 36)} = 3.14$, $P < 0.037$) and the interactions between exercise, time and capture stress ($F_{(12, 129)} = 1.82$, $P = 0.049$). The A-a gradient was higher ($P < 0.05$) at D0: 0 min for the capture stressed than the non-stressed groups. Within all groups, the D0: 40 min values were all significantly lower ($P < 0.05$) compared to the Baseline values (Fig. 5.1, Appendix: Table 9.5). When the two stress groups were compared (NES vs ES, *t*-test), the A-a gradient was higher in the trained (ES) animals.



A.



B.

Figure 5.3 A-a gradient and AGap calculations

A. Mean ± SD of A-a gradient (mmHg) for each group measured at D0:0 and 40 min, D2 and D5.

B. Back transformed mean ± SD of AGap (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal $PaCO_2$ interval for blesbok (Stevens et al., 1994; Meyer et al., 2006; Pfitzer et al., 2020). Vertical brackets: different between groups (P < 0.05). * Different from D0: 0 min within group (P < 0.05). Curly bracket: difference between the NES and ES group (t-test, P < 0.05).

The anion gap data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the anion gap were time ($F_{(3.37, 120.4)} = 39.76$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 9.38$; $P = 0.0001$) and the interaction of these factors ($F_{(12, 143)} = 8.69$; $P < 0.0001$) on the groups. The capture stressed groups had significantly higher anion gap values compared to the non-capture stressed groups ($P = 0.05$) (Fig. 5.3). When the two stress groups were compared (NES vs ES, *t*-test), there were no differences noted at D0: 0 min ($P = 0.26$) and D0: 40 min ($P = 0.054$). Within groups, the time points that differed from the Baseline were for the capture stressed groups (ES and NES) at D0: 0 and 40 min ($P < 0.05$). NENS also differed at D0: 40 min from Baseline values ($P = 0.006$). The anion gap in the capture stressed groups on D0: 0 min was positively correlated to $BE_{(ecf)}$ ($r = 0.78$; $R^2 = 0.61$; n = 19; $P < 0.001$), Na^+ ($r = 0.80$; $R^2 = 0.64$; n = 20; $P < 0.001$), Cl^- ($r = 0.71$; $R^2 = 0.51$; n = 20; $P < 0.001$), glucose ($r = 0.51$; $R^2 = 0.30$; n = 20; $P = 0.02$), lactate ($r = 0.88$; $R^2 = 0.77$; n = 20; $P < 0.001$) (Fig. 5.4) and haematocrit ($r = 0.47$; $R^2 = 0.21$; n = 20; $P = 0.04$). The anion gap in the capture stressed groups on D0: 0 min was negatively correlated to pH ($r = - 0.75$, $R^2 = 0.57$, n = 20; $P < 0.001$) (Fig. 5.4), $PaCO_2$ ($r = - 0.63$, $R^2 = 0.39$, n = 20; $P = 0.003$), $cHCO_3^-$ ($r = - 0.74$, $R^2 = 0.54$, n = 19; $P < 0.001$) (Fig. 5.4) and WBC ($r = - 0.46$, $R^2 = 0.21$, n = 20; $P = 0.04$).

Discussion

The general hypercapnia presented in all groups (excluding the capture stressed groups on D0: 0 min) is typical of opioid anaesthesia. Opioids result in hypoventilation by directly depressing respiratory neurons and decreasing the rate, depth, and breathing rhythm (Meyer et al., 2010). Opioids also cause a decreased sensitivity of peripheral and central receptors to CO_2, and concurrent hypermetabolism leading to increased CO_2 production (Meyer et al., 2006; West et al., 2007; Zeiler & Meyer, 2017). The capture stressed groups were panting during the capture stress event (recorded observation) supportive of a higher respiratory rate. The NES group had a comparatively higher respiratory rate than the ES group, which is expected for untrained animals (Vanhees et al., 2005). An increased respiratory rate is not necessarily indicative of increased ventilation, but a below reference limit value of $PaCO_2$ observed in the NES group at D0: 0 min supports that the group was hyperventilating (Fig. 5.1, Appendix: Table 9.5). The respiratory rate and $PaCO_2$ at D0: 0 min were negatively correlated (Fig. 4.6) and is additional support that hyperventilation took place. CO_2 is more soluble and is not as affected by pulmonary gas diffusion as oxygen, therefore, $PaCO_2$ is good indicator of the efficacy of ventilation, whereas PaO_2 is not (Azizi, 2011; Wagner, 2015).

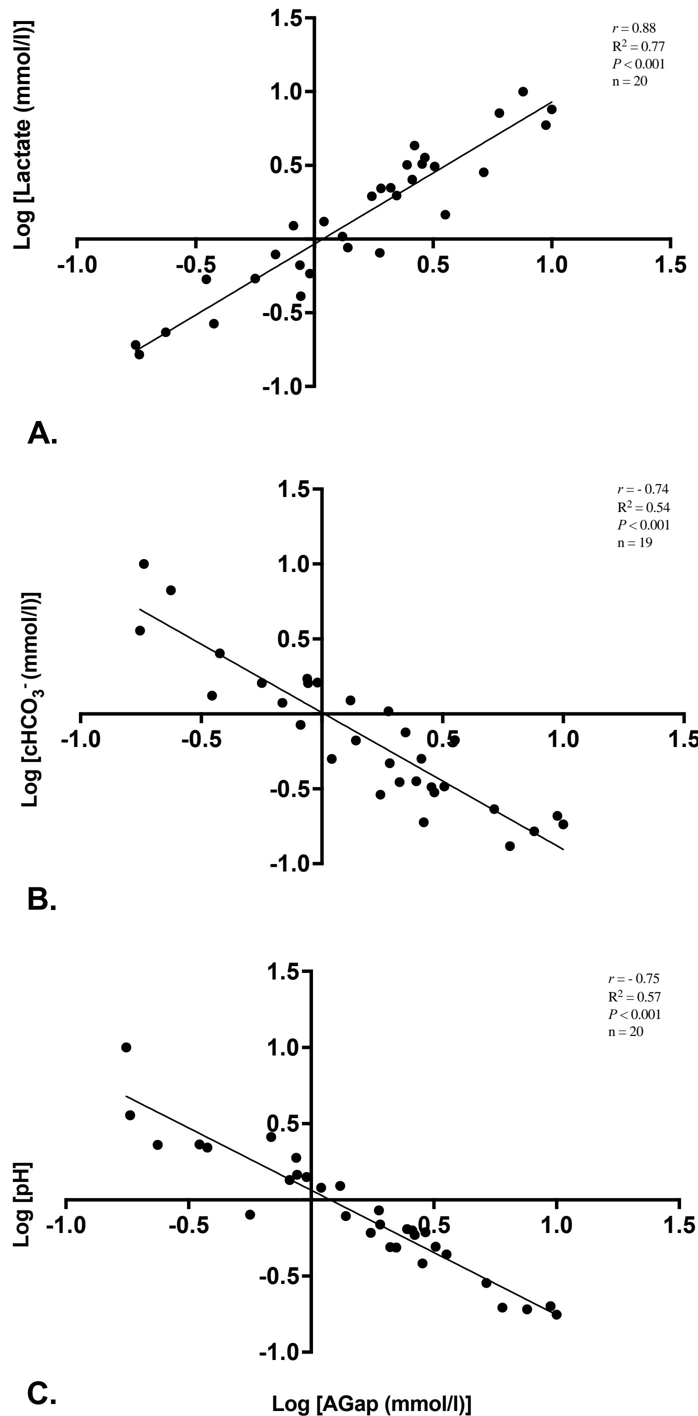


Figure 5.4 Correlation of pH, $cHCO_3^-$ and lactate to AGap

A. Pearson's correlation plot of the AGap and blood lactate concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the AGap and $cHCO_3^-$ concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Pearson's correlation plot of the AGap and the blood pH of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

PaO_2 is influenced by barometric pressure which influences inspired oxygen concentration, oxygen diffusion from alveoli to lung capillaries and ventilation: perfusion matching (Treacher & Leach, 1998). PaO_2 is more easily affected by factors that alter gas exchange, which are reflected as increases in the A-a gradient (Wagner, 2015). At sea level, normal PaO_2 is > 90 mmHg, and at the altitude that the study occurred (670m), the expected PaO_2 would be > 81 mmHg (Sharma et al., 2021). Most of the PaO_2 measurements from the immobilised blesbok at the various time points were low values that would be considered clinical hypoxaemia (< 60 mmHg) (Manninen & Unger, 2016; Pfitzer et al., 2020). Only the values at D0: 40 min (Fig. 5.3, Appendix: Table 9.5) were closer to the reference intervals indicated, but many blesbok still had a moderate hypoxaemia (60 – 80 mmHg) that can be explained by the subsiding effect of the etorphine induced vasoconstriction (Manninen & Unger, 2016; Prabhakar, 2016). The A-a gradient is the difference between the alveolar and arterial oxygen partial pressures, which indicates the effectiveness of pulmonary gas exchange (oxygen diffusion) and can also reflect on the integrity of the alveolar-capillary membrane. Hypoventilation, on its own, will not result in an elevated A-a gradient (Sarkar et al., 2017). The increased A-a gradient in the blesbok indicates reduced oxygen diffusion across the alveoli membrane, resulting from several causes, including ventilation-perfusion mismatching or right-left shunting of lung portions (Buss et al., 2016; Pfitzer et al., 2020; Sarkar et al., 2017). Additionally, etorphine-induced vasoconstriction, with resulting pulmonary hypertension, reduces oxygen diffusion due to congestion and oedema (Meyer et al., 2006). When cardiac output is high, pulmonary vasoconstriction may also result in an increased speed of blood flow through the pulmonary capillary beds with a reduced time for adequate oxygen diffusion (Buss et al., 2016; Sarkar et al., 2017).

The A-a gradients for most of the blesbok groups were increased substantially above the indicated reference interval at Baseline, D0: 0 min, D2 and D5. However, at D0: 40 min, the A-a gradients for all groups improved to below the reference interval for goats and sheep but within human reference interval limits (Fig. 5.3, Appendix: Table 9.5). In rhinoceros, systemic hypertension caused by etorphine subsided by 25 min, likely due to the redistribution and metabolism of the drug (Buss et al., 2016). Thus, the A-a gradient at D0: 40 min may have improved in all groups due to etorphine's redistribution and metabolism. The stress response and its associated pulmonary hypertensive effect would have also waned, further reducing the A-a gradient (Buss et al., 2016; Kim & Ha, 2016). There may have been a change in pulmonary dynamics that allow for improved ventilation-perfusion and less shunting to occur at this time

(Sarkar et al., 2017). More in-depth cardiopulmonary assessment and monitoring is required to verify the possible physiological mechanisms proposed.

The A-a gradients were higher ($P < 0.05$) for the stressed groups (Fig. 5.3, Appendix: Table 9.5) compared to the non-stressed groups at D0: 0 min, but there was no difference between these groups at D0: 40 min. Exercise requires increased oxygen delivery to working muscles. The increased demand for oxygen activates the sympathetic nervous system which causes an increase in cardiac output, but this also increases pulmonary blood pressure (Kim & Ha, 2016). There is a subsequent increase in pressure in the pulmonary capillary beds resulting in congestion, and thus exercise may cause a decrease in pulmonary oxygen diffusion and an increase in A-a gradients. In normal circumstances, a reduction of pulmonary oxygen diffusion (elevated A-a gradient) from exercise would be countered by an increase in respiratory rate and ventilation. However, in etorphine immobilised antelope, the compensatory increase in respiratory rate and ventilation is suppressed by this opioid. The higher A-a gradients of the stressed groups compared to the non-stressed groups could additionally be due to a stress-induced sympathetic response, with resultant pulmonary blood pressure increases (Buss et al., 2016; Kim & Ha, 2016). Thus, the stress from the simulated capture event, exercise and etorphine's effects may have contributed to an increase in pulmonary vascular pressure causing congestion, and possibly oedema, resulting in reduced oxygen diffusion and increased A-a gradients.

The NES group was hypocapnic at D0: 0 min (Fig. 5.1, Appendix: Table 9.5), indicating that the blesbok were hyperventilating. The hyperventilation in the NES group likely improved the pulmonary ventilation: perfusion ratio during exercise. The A-a gradient was lower, and the PaO_2 higher for the NES group compared to the ES group. The PaO_2 values were positively correlated to the respiratory rate of the capture stressed groups (Fig. 4.6), and support that increased ventilation resulted in increases seen in PaO_2. Etorphine-induced hypoxia and hypoxaemia are the drivers of the pulmonary hypertensive effect (Buss et al., 2016). The higher PaO_2 of the NES, compared to the ES group may have proportionally reduced the stimulus to increase cardiac output and subsequent pulmonary hypertension in this group. The reduced pulmonary hypertension possibly improved pulmonary oxygen diffusion, indicated by lower A-a gradients, increasing the PaO_2 further.

The anion gap is used to determine the cause of a blood pH shift. The anion gap uses the principles that the cations (K^+ and Na^+) must balance with the anions (Cl^- and HCO_3^-) to maintain electrical homeostasis. If there is a shift, it is due to an unknown rise or fall in an

anion concentration. A low anion gap is associated with decreased unmeasured anions, e.g. a hypoproteinaemic alkalosis. However, low anion gaps are rarely clinically significant. A high anion gap can indicate acidosis due to increased unmeasured anions such ketoacids, sulphates, salicylate, lactate and ethylene glycol metabolites (Element POC Blood Gas & Electrolyte Analyzer, 2013; Reece et al., 2015; Stringer et al., 1992; Zeiler & Meyer, 2017). For a detailed discussion on the anion gap, please refer to Chapter 2, section 2.6.

The capture stressed groups (ES and NES) had higher anion gap values than the non-capture stressed groups at D0: 0 min and D0: 40 min. The anion gap values for the capture stressed groups were within high normal or above reference limits (Fig. 5.3). When a metabolic acidosis presents with hyperlactaemia, the excess H^+ from lactate production will likely cause the acidosis (Berg & Meyer, 2008). The anion gap was positively correlated to the blood lactate concentration and negatively correlated to the pH (Fig. 5.4). The pH for the ES group was normal, and the NES group was slightly below normal at D0: 0 min (Fig. 5.5). Both groups (ES and NES) had elevated anion gaps and lactate values on D0 (Chapter 6, section 6.3). The cause of the slight acidaemia in the NES group is likely due to the exertion that resulted in lactate and H^+ build up. At D0: 0 min both groups had a severe metabolic acidosis that was compensated for by both respiratory (ventilation indicated by $PaCO_2$) and metabolic compensation (HCO_3^-). At D0: 40 min there would still be a metabolic acidosis, but also a respiratory acidosis (opioid induced), as is evident by the increased $PaCO_2$ for the ES group. The metabolic and respiratory acidosis at D0: 40 min would be compensated through metabolic means i.e. HCO_3^-.

Aerobic exhaustion should occur earlier in untrained individuals due to higher lactate production and an increase in H^+ ions. The buffering capacity would also be exhausted at an earlier stage than aerobically exercise-trained individuals, with a resultant decrease in pH. An elevated anion gap is, therefore, expected for the NES group compared to the ES group. However, there was no difference between the NES and the ES group's anion gap values. The NES group's anion gap remained elevated at D0: 40 min compared to the ES group. The *P*-value (0.054) is higher at D0: 40 min than at D0: 0 min ($P = 0.264$) but was still not significant. The ES group also appeared to have a quicker decrease in the anion gap than the NES group. The NES group showed increased pH variance (Fig. 5.5), slight acidaemia (Fig. 5.5), higher lactate (Chapter 6, section 6.3), depleted buffering systems ($cHCO_3^-$ and BE_{ecf}) and an elevated anion gap. The NES group's homeostatic mechanisms were still able to maintain the acid-base balance and the capture stress was not adequate to be disruptive. Many of the correlations in

the acid base calculations are depended variables. For example, AGap to Na^+, Cl^- and $cHCO_3^-$ (Chapter 2, section 2.6) are dependent variables as they are used to calculate the anion gap, the same applies to $BE_{(ecf)}$, and pH and, $cHCO_3^-$ and $PaCO_2$.

ii. pH, $cHCO_3^-$ and BE_{ecf}

The pH data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected arterial pH significantly were time ($F_{(2,69, 95.54)} = 9.03$; $P < 0.0001$), and the interaction between time, exercise training and capture stress ($F_{(12, 142)} = 2.30$; $P = 0.01$). However, the exercise training and capture stress did not have a significant effect on pH ($F_{(3, 36)} = 1.23$; $P = 0.31$). There were only differences on D5 between the ENS and ES and ENS and NES groups (Fig. 5.5, Appendix: Table 9.5). The only difference occurred in the ENS group between Baseline and D5 ($P = 0.006$) and the NENS group between Baseline and D2 ($P = 0.03$). When the two stress groups variances were compared (NES vs ES, F-test), the NES group showed an increased pH variance (Chapter 4, section 4.4) compared to the ES group (NES: 7.32 ± 0.09, ES: 7.35 ± 0.04; $F_{(9, 9)} = 5.09$; $P = 0.02$). The pH for the capture stressed groups on D0: 0 min was positively correlated to $cHCO_3^-$ ($r = 0.83$, $R^2 = 0.68$, n = 19; $P < 0.001$) (Fig. 5.6). The pH for the capture stressed groups on D0: 0 min was negatively correlated to $BE_{(ecf)}$ ($r = -0.71$, $R^2 = 0.50$, n = 19; $P = 0.001$), AGap ($r = -0.75$, $R^2 = 0.56$, n = 20; $P < 0.001$) (Fig. 5.4) and lactate ($r = -0.72$, $R^2 = 0.51$, n = 20; $P < 0.001$) (Fig. 5.6).

The $cHCO_3^-$ data were found to have a normal distribution and did not require log transformation before further analysis. The factors that significantly affected arterial $cHCO_3^-$ were time ($F_{(2,62, 93.74)} = 65.54$; ($P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 15.19$; $P < 0.0001$), and the interaction between all these factors ($F_{(12, 143)} = 33.47$; $P < 0.0001$). The arterial $cHCO_3^-$ concentration were lower for the capture stressed groups compared to the non-capture stressed groups at D0: 0 min (Fig. 5.5, Appendix: Table 9.5). Within groups, the ES group differed at D0: 0 ($P = 0.0001$) and 40 min ($P = 0.0012$) and the NES group differed at D0: 0 ($P < 0.0001$) and 40 min ($P = 0.0004$), and D2 ($P = 0.002$) from their Baseline $cHCO_3^-$ concentration. When the two stressed groups were compared (NES vs ES, *t*-test) the NES group had a significantly lower $cHCO_3^-$ concentration at D0: 0 min ($P = 0.033$) and at D0: 40 min ($P = 0.030$) compared to the ES group.

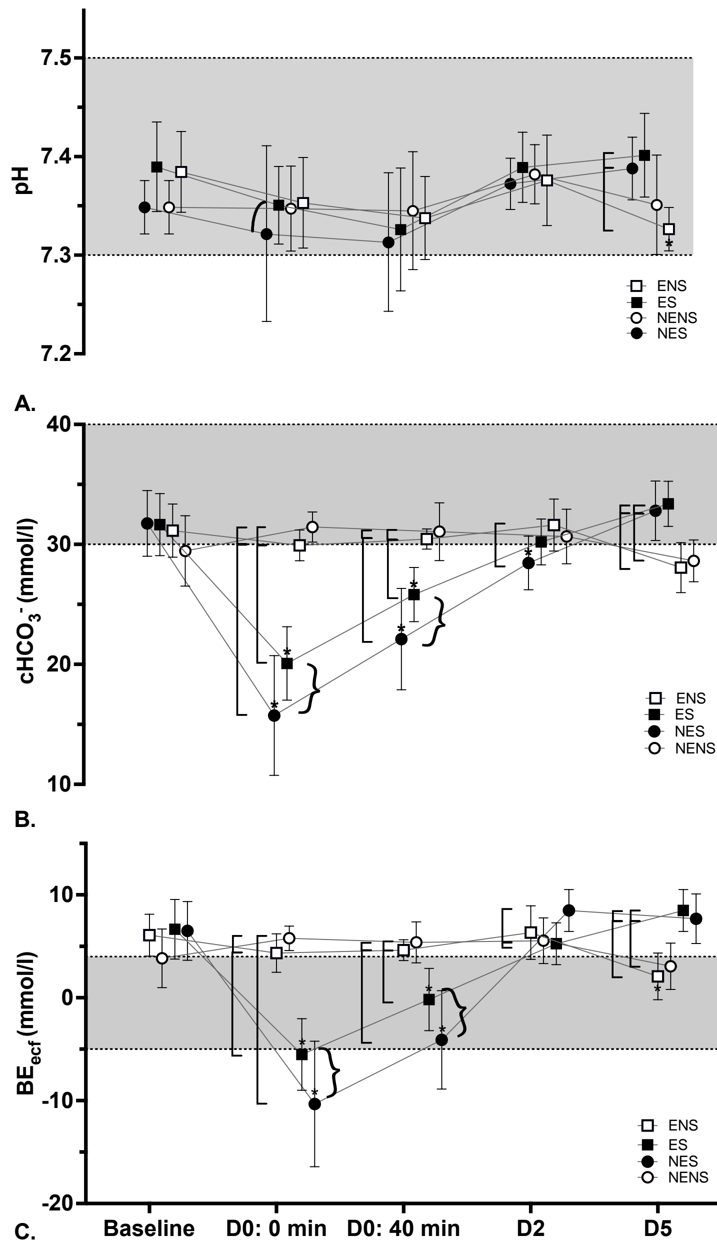


Figure 5.5 Arterial BE_{ecf}, $cHCO_3^-$ and pH

A. Back transformed mean ± SD of the pH for each group measured at D0:0 and 40 min, D2 and D5.

B. Mean ± SD of $cHCO_3^-$ (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

C. Mean ± SD of BE_{ecf} (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal $PaCO_2$ interval for blesbok (Stevens et al., 1994; Meyer et al., 2006; Pfitzer et al., 2020). Vertical brackets: different between groups ($P < 0.05$). * Different from D0: 0 min within group ($P < 0.05$). Curly bracket: difference between the NES and ES group (t-test, $P < 0.05$). Bow: different between ES and NES group (F-test, $P < 0.05$).

The $cHCO_3^-$ in the capture stressed groups on D0: 0 min had a positive correlation with pH (r = 0.55, R^2 = 0.31, n = 19; P = 0.01) (Fig. 5.6) and $PaCO_2$ (r = 0.83, R^2 = 0.68, n = 19; P < 0.001). The $cHCO_3^-$ in the capture stressed groups on D0: 0 min had a negative correlation to heart rate (r = - 0.54, R^2 = 0.29, n = 19; P = 0.02) (Fig. 4.4), PaO_2 (r = - 0.49, R^2 = 0.24, n = 19; P = 0.03), $BE_{(ecf)}$ (r = - 0.88, R^2 = 0.78, n = 18; P < 0.001), Cl^- (r = - 0.64, R^2 = 0.40, n = 19; P = 0.003), AGap (r = - 0.74, R^2 = 0.54, n = 19; P < 0.001) (Fig. 5.4), glucose (r = - 0.49, R^2 = 0.24, n = 19; P = 0.04), lactate (r = - 0.78, R^2 = 0.61, n = 19; P < 0.001) (Fig. 5.6), Hct (r = - 0.52, R^2 = 0.27, n = 19; P = 0.02), Hgb (r = - 0.45, R^2 = 0.20, n = 19; P = 0.05) and creatinine (r = - 0.72, R^2 = 0.52, n = 19; P < 0.001).

The BE_{ecf} data were found to have a normal distribution and did not require log transformation before further analysis. The factors that significantly affected arterial BE_{ecf}, were time ($F_{(2.47, 88.43)}$ = 70.22; P < 0.0001), exercise training and capture stress ($F_{(3, 36)}$ = 7.82; P = 0.0004), and the interaction of these factors ($F_{(12, 143)}$ = 32.18; P < 0.0001) on the groups. There were clear differences between the capture stressed and non-stressed groups at D0 (P < 0.05). BE_{ecf} followed similar trends to $cHCO_3^-$ in that there were no differences between groups at Baseline. The BE_{ecf} was lower for the capture stressed groups compared to the non-stressed groups at D0: 0 min and D0: 40 min (Fig. 5.5, Appendix: Table 9.5). The ES and NES groups differed at D0: 0 and 40 min were lower from Baseline concentration (P < 0.05). ES was lower at D5 from its Baseline concentration (P = 0.015). When the two stress groups were compared (NES vs ES, *t*-test), the NES group was lower than the ES group at D0: 0 min (P = 0.033) and at D0: 40 min (P = 0.048) (Fig. 5.5, Appendix: Table 9.5). The $BE_{(ecf)}$ in the capture stressed groups on D0: 0min had a positive correlation with heart rate (r = 0.55, R^2 = 0.30, n = 19; P = 0.02), PaO_2 (r = 0.52, R^2 = 0.27, n = 19; P = 0.02), Cl^- (r = 0.65, R^2 = 0.41, n = 19; P = 0.003), AGap (r = 0.78, R^2 = 0.61, n = 19; P < 0.001), lactate (r = 0.85, R^2 = 0.73, n = 19; P < 0.001), Hct (r = 0.49, R^2 = 0.24, n = 19; P = 0.03), creatinine (r = 0.59, R^2 = 0.23, n = 19; P = 0.01) and troponin I (r = 0.49, R^2 = 0.23, n = 19; P = 0.03). The $BE_{(ecf)}$ in the capture stressed groups on D0: 0 min had a negative correlation with heart rate (r = - 0.71, R^2 = 0.50, n = 19; P = 0.001), $PaCO_2$ (r = - 0.69, R^2 = 0.48, n = 19; P = 0.001) and $cHCO_3^-$ (r = - 0.88, R^2 = 0.78, n = 18; P < 0.001).

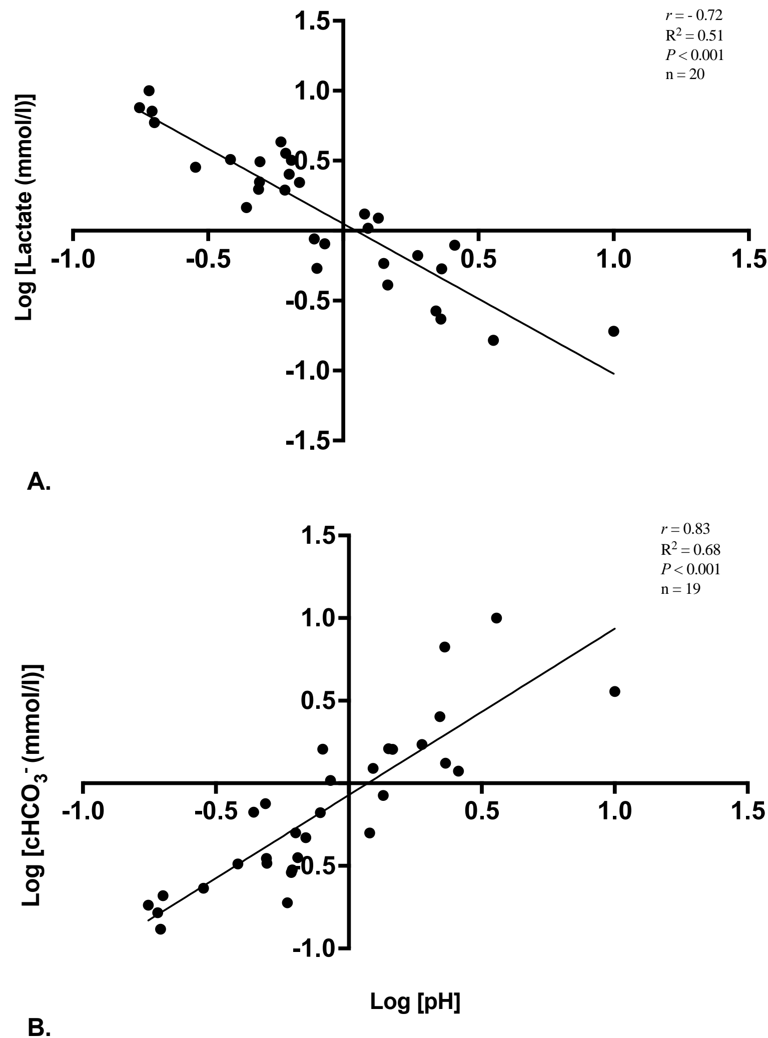


Figure 5.6 Correlation of $cHCO_3^-$ and lactate to pH.

A. Pearson's correlation plot of the blood pH to the lactate concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the blood pH to the $cHCO_3^-$ concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

Discussion

Metabolic and respiratory acidosis were expected in the capture stressed and immobilised groups. The respiratory and metabolic compensation that took place for the capture stressed groups were adequate as none of the groups were severely acidaemic. There was a decrease in pH over time during opioid immobilisation, that would have occurred due to hypoventilation

(Meyer et al., 2015; Zeiler & Meyer, 2017). The pH values within the D5 groups that differed were still within the reference interval limits and thus unlikely of clinical significance. The

differences in pH between the groups on D5 (Fig. 5.5, Appendix: Table 9.5) could be explained by a slight delay in sampling times, anticipatory stress or natural variation. At D0: 0 min (M ± SD = 7.32 ± 0.09; GM = 7.32, GSD factor = 1.012; Mdn = 7.34, IQR = 7.16 – 7.44), the NES group was the lowest mean value for all the groups at all the time points. This low pH for the NES group at D0: 0 min was also the only group and time point below the reference interval. The NES group contained the individual blesbok with the lowest arterial pH (7.156) for the whole study at D0: 0 min. Comparing the NES and the ES group, there was no significant difference. However, the NES group did show a significantly greater pH variance (Fig. 5.5, Appendix: Table 9.5) that indicates increased pH fluctuation for the NES group. The ES group comparatively showed a decreased pH variance after the capture stress event. The pH negatively correlated to lactate, meaning increased lactate produced during the capture stress increased H^+ production and lowered pH (Fig. 5.6).

Aerobically untrained animals will produce more lactate during exercise, and therefore a lower pH is to be expected for the NES group (Edwards et al., 1969; Messonnier et al., 2013). In this case, the NES group presented with an increased pH variance and slightly acidaemic, indicating adequate respiratory and metabolic compensation took place for the immobilisation and capture stress (Fig. 5.5). Some individual animals were, however, under physiological strain to maintain pH homeostasis. The increased respiratory rate (Fig 4.5) and the decreased CO_2, $cHCO_3^-$ and BE_{ecf} for the NES group compared to the ES group implies additional respiratory and metabolic compensatory mechanisms were required to maintain the blood pH in the NES group. The capture stress length and intensity may not have been adequate to disrupt the homeostasis in all the animals in the NES group. Notably, the NES group still presented with a slight acidaemia despite having low $PaCO_2$ due to hyperventilation. However, the ES group had a normal pH and was not hyperventilating (evident by the normal $PaCO_2$). The decreased pH variance seen in the ES group supports that the respiratory and metabolic compensation for the capture stress and immobilisation was adequate to maintain pH homeostasis.

Blood bicarbonate concentration is calculated by using the Henderson-Hasselbach equation as follows:

$$cHCO_3^- = 0.23 \times PaCO_2 \times \text{antilog}\ (pH – pKp^*)$$

$$^*\text{Where } pKp = 6.125 – \log\ (1 + \text{antilog}\ (pH – 8.7))$$

Studies in humans have shown that calculated HCO_3^- ($cHCO_3^-$) has a very close relationship to actual bicarbonate ($R^2 = 0.93$), and $cHCO_3^-$ provides a good estimate (Kumar & Karon, 2008).

An expected finding in capture stressed animals is a depletion of HCO_3^-. HCO_3^- is the primary buffering agent to maintain pH homeostasis and neutralises excess H^+ produced from skeletal muscles during exertion and consequent lactate production (McGinley & Bishop, 2016). Exercise untrained individuals that produce more lactate and H^+ would require increased buffering by compounds like HCO_3^-. Thus, a lower concentration is expected for HCO_3^- in untrained individuals (Stringer et al., 1992). The data supports this difference between trained and untrained blesbok on D0, and the NES group is significantly lower than the ES group at both D0: 0 min and D0: 40 min. Comparatively, the ES group was able to regulate its $cHCO_3^-$ for D0: 0 min (M ± SD = 20 ± 3 mmol/l) and D0: 40 min (M ± SD = 26 ± 4 mmol/l), which is also within the reference intervals for goats (20 - 30 mmol/l). However, the NES group decreased to substantially lower than the goat reference interval at D0: 0 min (M ± SD = 16 ± 5 mmol/ l) but increased at D0: 40 min (M ± SD = 22 ± 2 mmol/l).

The $cHCO_3^-$ concentration for the ES and NES group were lower or within low reference limits at D0: 0 min and 40 min compared to the Baseline concentration. The NES group's $cHCO_3^-$ concentration was still lower than the Baseline concentration at D2 but not at D5 (Fig. 5.5, Appendix: Table 9.5). Excluding the capture stress groups on D0, the rest of the groups and time points had $cHCO_3^-$ concentration within reference range limits, indicating no excess metabolic demand for buffering as there was no capture stress. The animals maintained a low normal blood pH despite the apparent hypoventilation (increased $PaCO_2$) during immobilisation indicating adequate respiratory compensation.

BE_{ecf} is like HCO_3^- in that it is derived from $PaCO_2$ and pH (Chapter 2, section 2.6). The capture stress event should result in a metabolic acidosis and a reduction in the BE_{ecf}. Increased buffering would be required for the exported lactate and H^+ from working muscles produced during exercise, this would reduce the BE_{ecf} for the NES group (McGinley & Bishop, 2016; Weston et al., 1996). The ES group maintained its BE_{ecf} values within the indicated goat reference interval ((-5) - (+4) mmol/l), whereas the NES group dropped significantly below the reference interval at D0: 0 min (M ± SD = -10 ± 6). The NES group had a higher plasma lactate concentration when compared to ES ($P < 0.05$), as seen later in Chapter 6 (Figure 6.1), which supports the increased H^+ production that would require buffering.

In the NES group, the hyperthermia (Chapter 4) and slight acidaemia likely caused hyperventilation and, consequentially, the arterial hypocapnia on D0. In addition to the respiratory compensation that took place to maintain blood pH, $cHCO_3^-$ and BE_{ecf} also decreased, indicating the need to address the metabolic acidosis caused by the capture stress.

The ES group at immobilisation had a respiratory rate, $PaCO_2$ and $cHCO_3^-$ and BE_{ecf} within the reference interval limits. The respiratory and metabolic compensation in the ES group was thus adequate, the HCO_3^- reserves not required and the BE_{ecf} within normal limits. The ES group may have developed an improved buffering capacity, a well-known adaptation in high-intensity interval training in humans and horses (McGinley & Bishop, 2016; Weston et al., 1996). Thus, due to metabolic adaptations (e.g. decreased lactate and thus H^+ production, increased buffering capacity) the ES group may have had less requirement for HCO_3^- or more HCO_3^- reserves available.

The correlations seen regarding $cHCO_3^-$ are all linked to the homeostatic mechanisms that occur in the body (Fig. 5.4, 5.6 & 5.10). The capture stressed groups responded to exercise with an increase in blood glucose (Chapter 6), Cl^- (due to the chloride shift that takes place) and lactate (Chapter 6), but at the same time, HCO_3^- was depleted. HCO_3^- buffers the increased H^+ production during exercise, H_2CO_3 is produced and dissociates to H_20 and CO_2. Ventilation removes excess CO_2, and it resulted in decreasing $PaCO_2$ to normal for the ES group and below the reference limit for the NES group.

5.3 Electrolytes

(i) Sodium (Na^+), potassium (K^+) , calcium (Ca^{2+}) and Chloride (Cl^-)

Results

The Na^+ data were found to have a normal distribution and did not require log transformation before further analysis. The only factor that affected blood Na^+ was time ($F_{(3.38,\ 120.8)} = 7.24$; $P < 0.0001$), but not the exercise training or capture stress ($F_{(3,\ 36)} = 1.70$; $P = 0.19$) or the interaction of time, training and capture stress had an influence on the blood Na^+ ($F_{(12,\ 143)} = 1.15$; $P = 0.32$). The ENS's Na^+ group concentration was significantly lower than the ES and NENS groups at D0: 0 min (Fig. 5.7, Appendix: Table 9.6). Within groups, only the ES group's D2 was higher than the Baseline Na^+ concentration ($P = 0.025$). When the two stress groups were compared (NES vs ES, F-test), the NES group's blood Na^+ ($M \pm SD = 136 \pm 5$ mmol/l) at D0: 0 min ($P = 0.027$) showed greater variance compared to the ES group ($M \pm SD = 138 \pm 2$ mmol/l). At D0: 40 min there was no difference. The Na^+ for the capture stressed groups on D0: 0 min correlated positively with K^+ ($r = 0.56$, $R^2 = 0.31$, n = 19; $P = 0.01$) (Fig. 5.8), Ca^{2+} ($r = 0.52$, $R^2 = 0.27$, n = 18; $P = 0.03$) (Fig. 5.8), Cl^- ($r = 0.80$, $R^2 = 0.64$, n = 20; $P < 0.001$) (Fig. 5.8) and the AGap ($r = 0.55$, $R^2 = 0.30$, n = 20; $P = 0.01$).

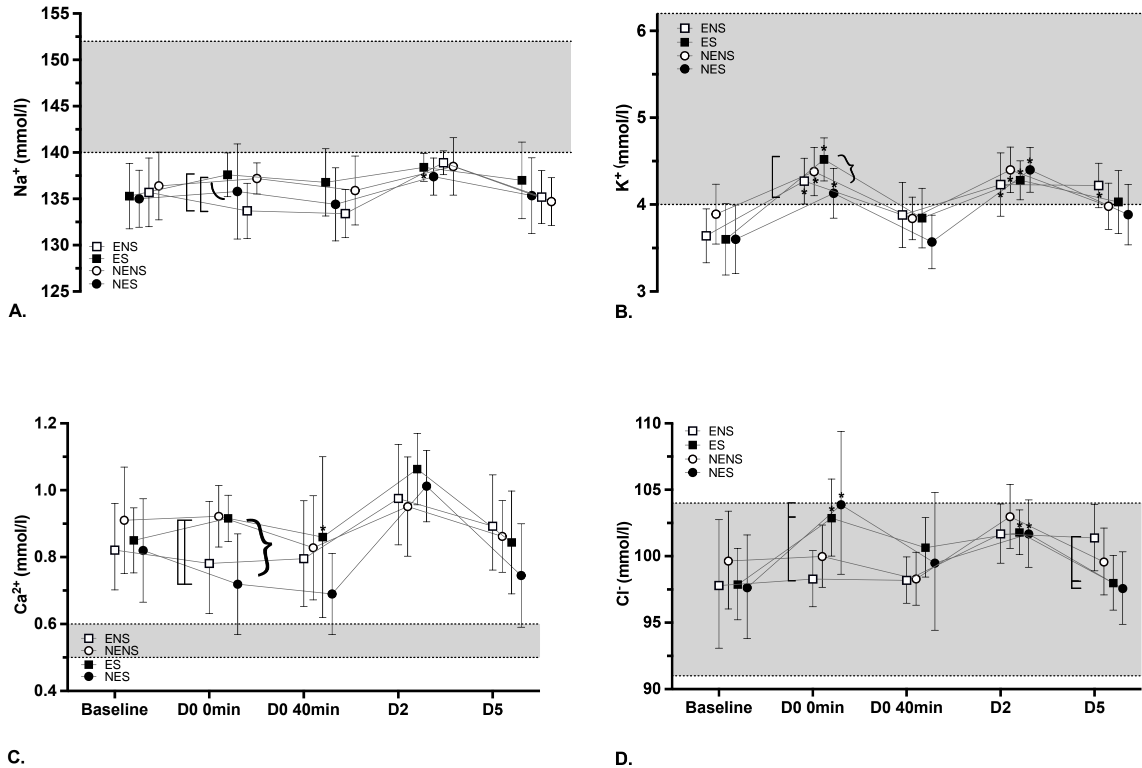


Figure 5.7 Na+, K^+, Ca^{2+} and Cl^- arterial blood concentrations

A. Mean ± SD of Na^+ (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

B. Mean ± SD of $cHCO_3^-$ (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

C. Back transformed mean ± SD of Ca^{2+} (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

D. Back transformed mean ± SD of Cl^- (mmol/l) for each group measured at D0:0 and 40 min, D2 and D5.

Shaded grey area and gridlines: normal $PaCO_2$ interval for blesbok (Stevens et al., 1994; Meyer et al., 2006; Pfitzer et al., 2020). Vertical bracket: different between groups ($P < 0.05$). * Different from D0: 0 min within group ($P < 0.05$). Curly bracket: difference between the NES and ES group (t-test, $P < 0.05$). Bow: different between ES and NES group (F-test, $P < 0.05$).

The K^+ data were found to have a normal distribution and did not require log transformation before further analysis. The only factor that significantly affected blood K^+ was time ($F_{(3.21, 117)} = 14.56$; $P < 0.0001$). Neither the training and capture stress ($F_{(3, 36)} = 2.20$; $P = 0.11$) nor the interaction of training, capture stress and time had an effect ($F_{(12, 143)} = 1.77$; $P = 0.06$). Between groups, the K^+ was higher for the ES group compared to the NES group at D0: 0 min ($P = 0.02$). Within all groups the blood K^+ increased at D0: 0 min and D2 ($P < 0.05$) compared

to Baseline concentration but was still within the reference intervals limits indicated. When the two stress groups were compared (NES vs ES, *t*-test), the ES group's blood K^+ was higher than the NES group at D0: 0 min ($P = 0.004$), but it was not significant at D0: 40 min ($P = 0.094$). The blood K^+ concentration for the ES and NES group at D0 was still within the indicated reference interval (Fig. 5.7, Appendix: Table 9.6). The K^+ for the capture stressed groups on D0: 0 min was positively correlated Na^+ ($r = 0.56$, $R^2 = 0.31$, n = 20; $P = 0.01$) (Fig. 5.8), Ca^{2+} ($r = 0.73$, $R^2 = 0.53$, n = 18; $P = 0.001$) (Fig. 5.9), GLDH ($r = 0.51$, $R^2 = 0.23$, n = 20; $P = 0.02$) (Fig. 5.9) and AST ($r = 0.54$, $R^2 = 0.29$, n = 20; $P = 0.01$) (Fig. 5.9).

The Ca^{2+} data were found to not have a normal distribution and required log transformation before further analysis. The factors that significantly affected blood Ca^{2+} was the time ($F_{(3.45, 122.6)} = 11.37$; $P < 0.0001$) and the exercise training and capture stress event ($F_{(3, 36)} = 5.68$; $P < 0.003$). The interaction of time, exercise training and capture stress was not significant ($F_{(12, 142)} = 1.43$; $P = 0.16$). NES had lower group concentration than NENS and ES at D0: 0 min. Within groups, only the ES group had higher blood Ca^{2+} at D2 than at Baseline ($P = 0.001$) (Fig. 5.7, Appendix: Table 9.6). When the two stress groups were compared (NES vs ES, *t*-test) at D0, the NES group was markedly lower than the ES group at D0: 0 min ($P = 0.006$), but there was no difference noted at D0: 40 min. The Ca^{2+} for the capture stressed groups on D0: 0 min was positively correlated to Na^+ ($r = 0.52$, $R^2 = 0.27$, n = 18; $P = 0.03$) (Fig. 5.8) and K^+ ($r = 0.73$, $R^2 = 0.53$, n = 18; $P = 0.001$) (Fig. 5.8).

The Cl^- data were found to have not have a normal distribution and required log transformation before further analysis. The factors that significantly affected blood Cl^- was time ($F_{(3.45, 123.4)} = 40.19$; $P < 0.0001$) and the interaction of time, exercise training and capture stress ($F_{(12, 143)} = 4.10$; $P < 0.0001$). The exercise training and capture stress ($F_{(3, 36)} = 0.29$; $P < 0.83$) did not affect blood Cl^- concentration. The Cl^- concentration of the ENS group was lower than the ES and NES groups at D0: 0 min (Fig. 5.7, Appendix: Table 9.6). When the two stress groups were compared (NES vs ES, *t*-test) at D0: 0 min and D0: 40 min, no differences were noted. Within the ES group the Baseline had lower blood Cl^- than D0: 0 min ($P = 0.027$) and D2 ($P = 0.002$). The NES group's Baseline blood Cl^- was lower compared to D0: 0 min ($P = 0.006$) and D2 ($P = 0.008$). The Cl^- for the capture stressed groups on D0: 0 min was positively correlated to Na^+ ($r = 0.80$, $R^2 = 0.64$, n = 20; $P < 0.001$) (Fig. 5.8), BE_{ecf} ($r = 0.64$, $R^2 = 0.41$, n = 19; $P = 0.003$), AGap ($r = 0.71$, $R^2 = 0.51$, n = 20; $P < 0.001$), glucose ($r = 0.46$, $R^2 = 0.22$, n = 20; $P = 0.04$) and lactate ($r = 0.45$, $R^2 = 0.20$, n = 20; $P = 0.05$). The Cl^- for the capture stressed groups on D0: 0 min was negatively correlated to pH ($r = - 0.55$, $R^2 = 0.30$, n = 20; $P = 0.01$) (Fig. 5.10),

$PaCO_2$ (r = - 0.58, R^2 = 0.34, n = 20; P = 0.01) (Fig. 5.10) and $cHCO_3^-$ (r = - 0.64, R^2 = 0.40, n = 19; P = 0.003) (Fig. 5.10).

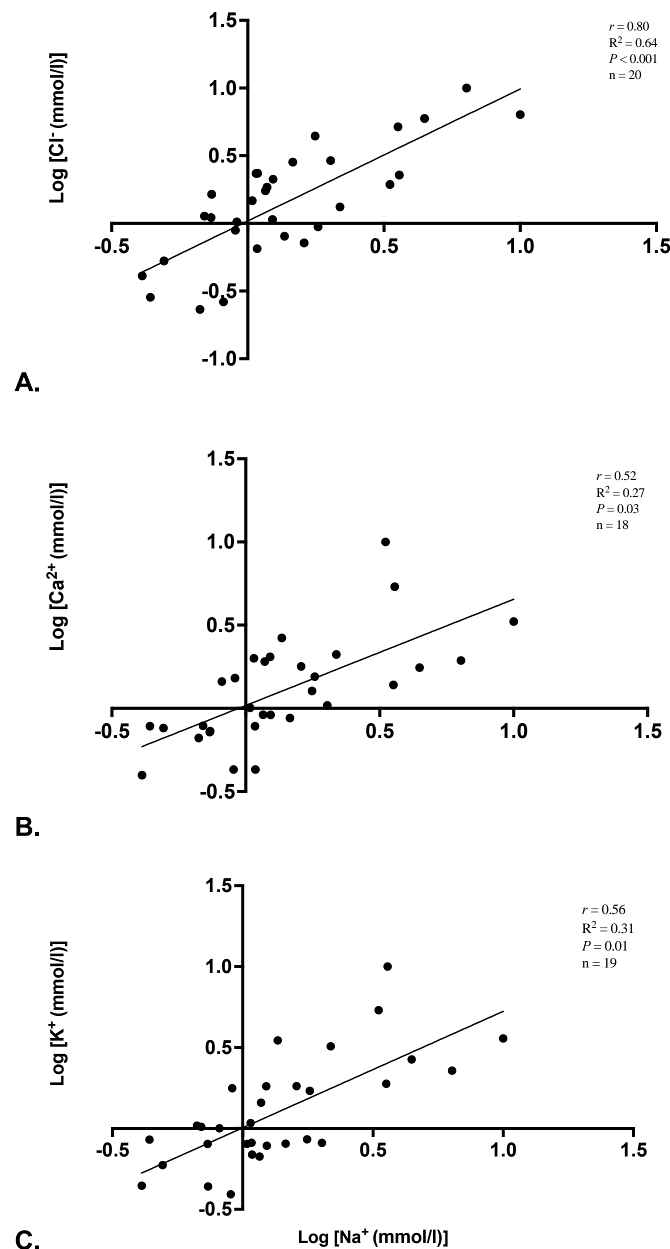


Figure 5.8 Correlation of Cl^-, Ca^{2+} and K^+ to Na+.

A. Pearson's correlation plot of blood Na^+ and Cl^- concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the blood Na^+ and Ca^{2+} concentration of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Pearson's correlation plot of the blood Na^+ and K^+ concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

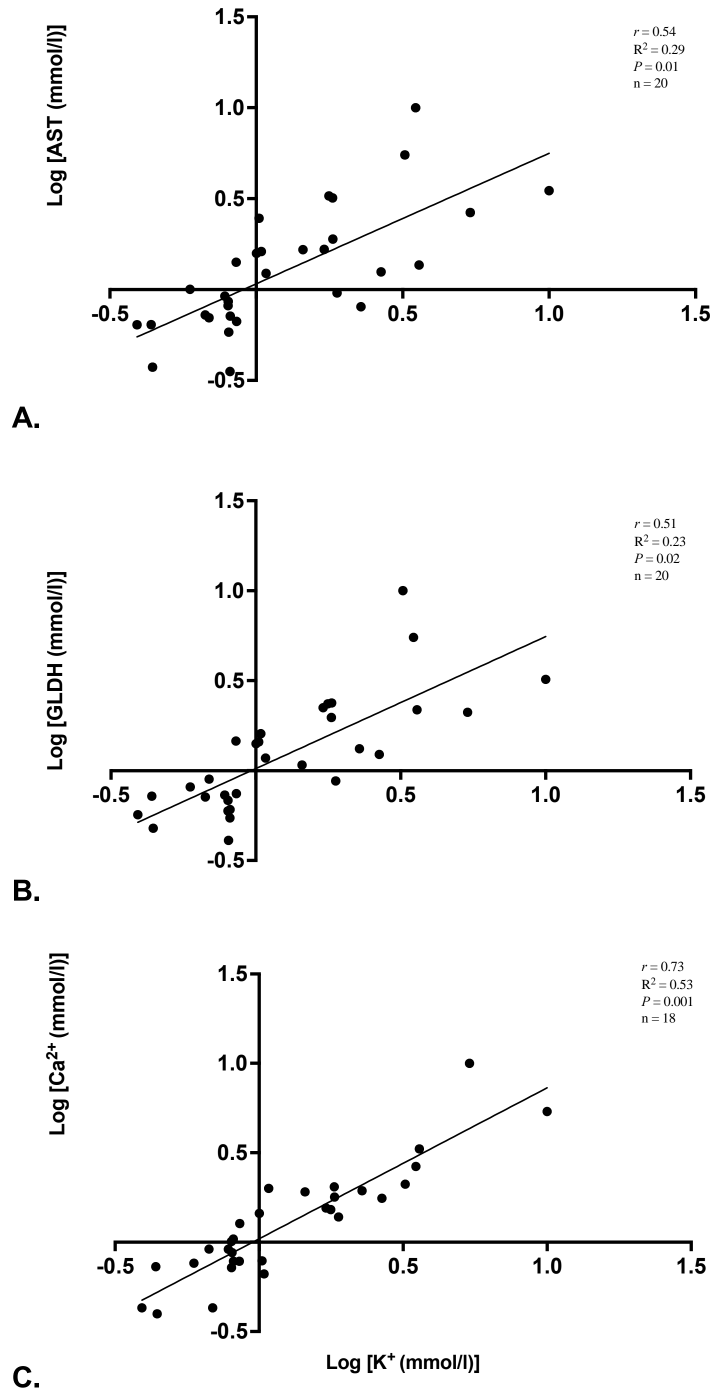


Figure 5.9 Correlation of AST, GLDH and Ca^{2+} to K^+.

A. Pearson's correlation plot of blood K^+ and AST concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the blood K^+ and GLDH concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Pearson's correlation plot of blood K^+ and Ca^{2+} concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

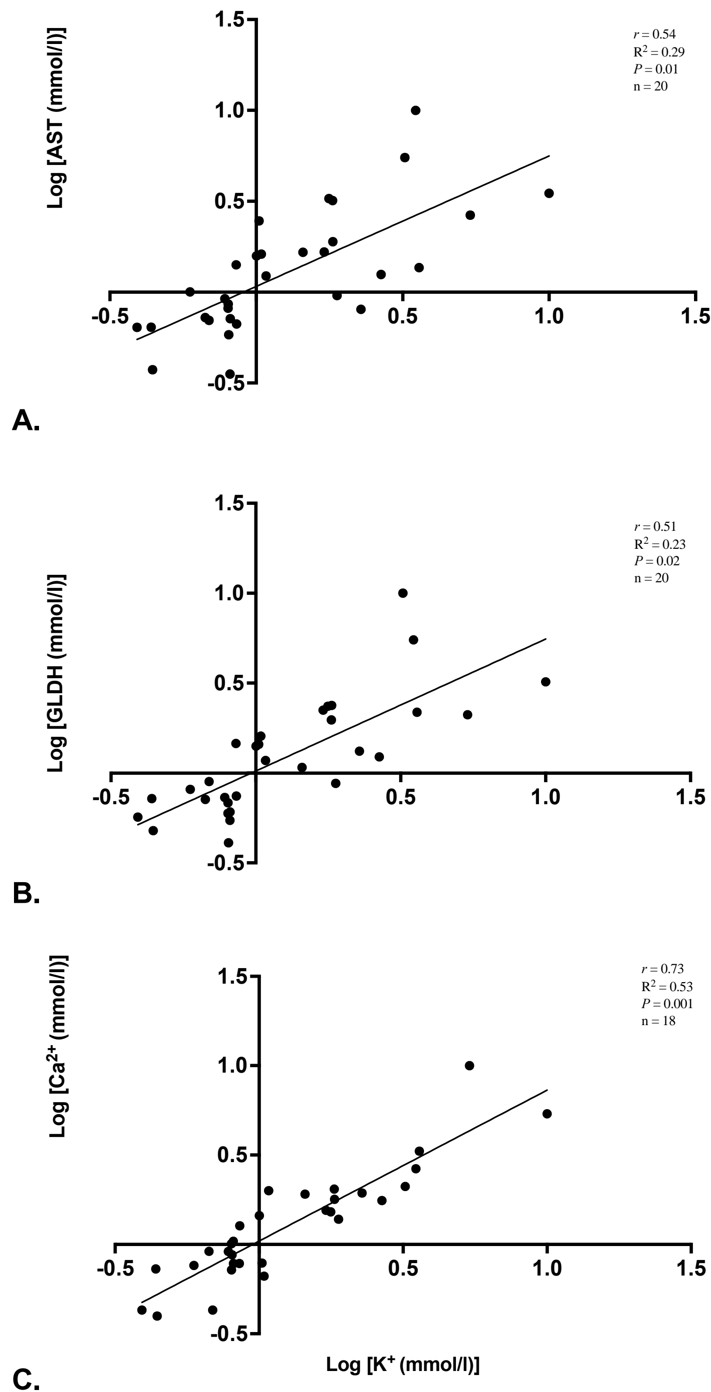


Figure 5.10 Correlation of AST, GLDH and Ca^{2+} to K^+.

A. Pearson's correlation plot of blood K^+ and AST concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the blood K^+ and GLDH concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Pearson's correlation plot of blood K^+ and Ca^{2+} concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

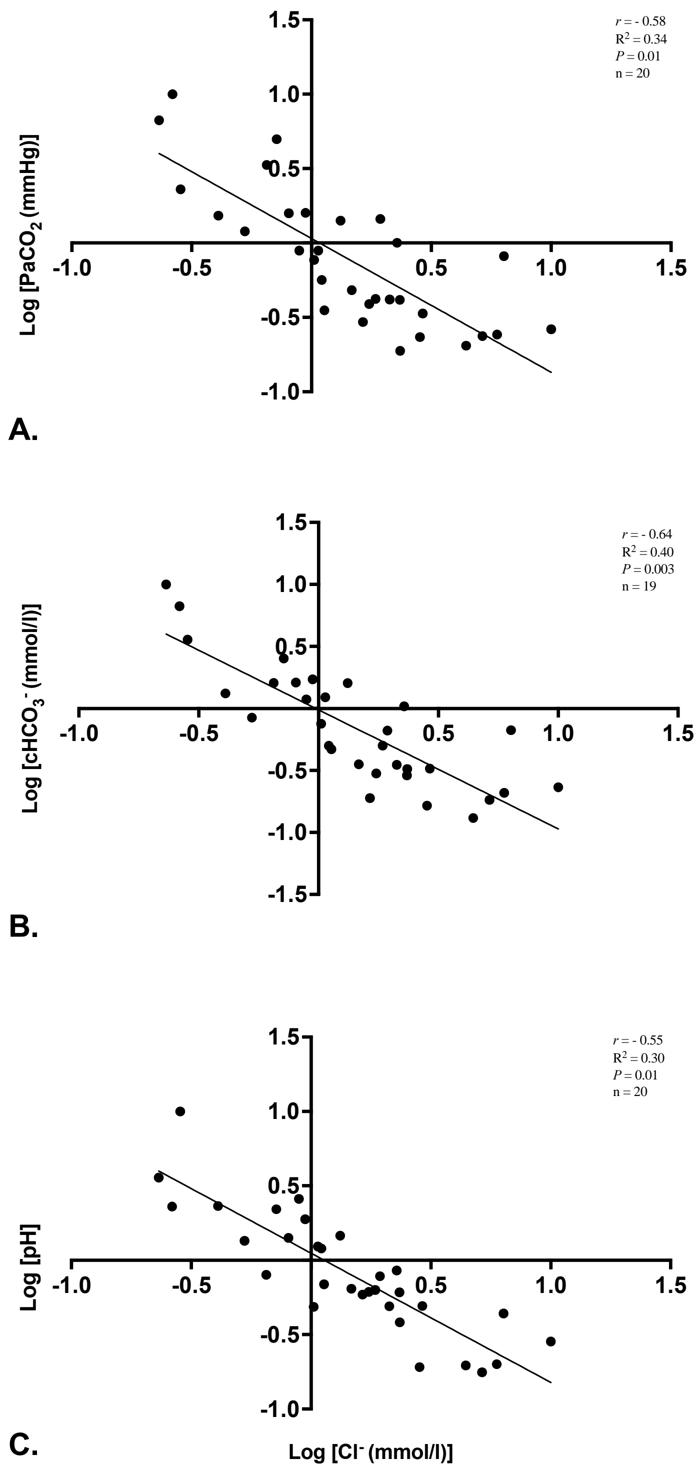


Figure 5.11 Correlation of $PaCO_2$, $cHCO_3^-$ and pH to Cl^-.

A. Pearson's correlation plot of blood Cl^- concentration and $PaCO_2$ of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Pearson's correlation plot of the blood Cl^- and $cHCO_3^-$ concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Pearson's correlation plot of blood Cl^- concentration and pH of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

Discussion

Na^+ accompanies fluid loss during exercise (Armstrong et al., 2006; Carter, 2008), but from previous exercise studies, no real definite trends were expected for Na^+ (Otto et al., 2017; Rovira et al., 2007). However, at different time points, all the blesbok appear to have lower Na^+ concentration than any of the reference intervals indicated. It may mean a hyponatraemic state for all animals, which, as previously described (Chapter 2, section 2.4), is possible with wild antelope. Wild antelope may be prone to hyponatraemia if the plants they consume have low Na^+ concentration and there are no natural forms of salt supplementation available. The blesbok in this study came from the veldt, and during the study period, supplemental feeding (lucerne and game pellets) was provided, but not specifically a salt lick. However, if the blesbok were truly hyponatraemic, more severe electrolyte disruptions would be expected in the groups exposed to the capture stress event. For one, the Na^+/Ca^{2+}-ATPase pump should become dysfunctional in response to the capture stress event (Otto et al., 2017; Scalco et al., 2016).

This dysfunction would lead to the activation of proteases and lipases and contribute to cell death (Scalco et al., 2016). There were no severe disruptions seen physiologically, and thus the Na^+ concentration obtained for the blesbok may likely be due to a different device and technique used to obtain Na^+ reference concentration. The study's blesbok may also have natural Na^+ variation, otherwise known as the 'herd effect'. The other possibility is that the capture stress event did not reach the point where it disrupted electrolyte homeostasis. The low Na^+ was just not low enough to be clinically significant yet. The increased variance of Na^+ concentrations in the NES group compared to the ES group may indicate increased fluctuations in response to exertion, but no significant physiological disruption occurred for either group.

Muscles during exercise release K^+ into the interstitial space, and the plasma K^+ concentration rises. The Na^+/K^+-ATPase pump retrieves the K^+ from the extracellular fluid in exchange for intracellular Na^+ to prevent K^+ from rising to a toxic concentration. Hypokalaemia may follow the initial hyperkalaemia due to the intracellular movement of K^+ through the Na^+/K^+-ATPase pump. Increased blood flow and possible intracellular acidosis may result in further movement of K^+ into cells (Warburton et al., 2002). The movement of K^+ intracellularly was confirmed in other studies (described in Chapter 2, section 2.5), where an increase in K^+ for animals exposed to exercise and capture was expected, but after the initial increase, a significant decrease in K^+ concentration is seen (Chalmers & Barrett, 1977; Jessup et al., 1987; Krustrup et al., 2015; Warburton et al., 2002). The hypokalaemia may be due to the reuptake of the K^+ in the muscle

post-exercise. Exercise causes an increased blood flow to the muscles and intracellular acidosis, resulting in an influx of K^+ in exchange for a H^+ efflux (Warburton et al., 2002). The relative increase in K^+ at D0: 0 min compared to Baseline K^+ concentration and the general decrease at D0: 40 min in the capture stressed groups confirm this movement of K^+ intracellularly. The K^+ fluctuations at D0 were still within the reference intervals indicated, with no severe disruptions. The Baseline blood K^+ that was lower than the blesbok reference interval concentration and like D0: 40 min could be due to a slight delay in obtaining samples resulting in a lower K^+ or could be due to a natural variation.

The loss of K^+ and gain of Na^+ in muscle is a significant contributor to fatigue and may contribute to the pain seen with prolonged exercise (Chapter 2, section 2.5) (Lindinger & Sjøgaard, 1991; McKenna et al., 2008). The increased K^+ can be due to electrolyte changes associated with metabolic acidosis caused by exertion. The K^+ will also increase during immobilisation due to respiratory acidosis (Harthoorn, 1976; Vanholder et al., 2000). The blood pH values were not severely disrupted for the capture stressed groups (Fig. 5.1) and remained within the indicated reference interval limits. The mild acidosis presented was likely not sufficient to cause severe changes in blood K^+ and why the K^+ concentration remained mostly within the reference interval limits. The higher K^+ value obtained (Fig. 5.7, Appendix: Table 9.6) for the ES group than the NES group at D0: 0 min is not expected. An expected outcome was to have improved K^+ regulation in the exercise-trained animals (McKenna, 1995). However, the ES group's K^+ increase is small and unlikely to be clinically relevant as the concentration was still within the indicated reference intervals.

The correlations indicated that K^+ had a positive correlation to Cl^- and Ca^{2+} and a weaker correlation to Na^+ (Fig. 5.8 & 5.9) Exercise associated haemolysis and rhabdomyolysis may increase blood K^+ and other electrolytes (Lindinger & Sjøgaard, 1991; McKenna, 1995). The positive correlation found to AST and GLDH could either be directly linked to K^+ release during liver damage (Fig. 5.9) (Reece et al., 2015; Vanholder et al., 2000) or possibly simultaneous changes in response to capture stress (Pettersson et al., 2008). The correlation of K^+ to pH was surprisingly not significant. Metabolic acidosis or acidaemia may result in an increase in blood K^+ due to the K^+/H^+ exchange across membranes (Aronson & Giebisch, 2011). The lack of an inverse correlation between pH and K^+ in this study is likely that none of the animals developed overt acidaemia that would result in arterial blood K^+ elevations.

The positive correlation of Na^+ to Cl^-, K^+ and Ca^{2+} (Fig. 5.7) in the capture stressed groups is unexpected as some Na^+ relationships to other electrolytes are expected to be inverse, e.g. as

Na^+ increases, K^+ decreases due to the Na^+/K^+-ATPase pump (Lindinger & Sjøgaard, 1991; McKenna, 1995). The positive correlation between Na^+ and the other electrolytes may be due to the capture stress that resulted in overall increases in blood electrolyte concentrations. Exercise results in increased tissue catabolism (haemolysis and rhabdomyolysis), and electrolytes are released from cells. In turn, this may have contributed to the overall increase of blood electrolytes (Vanholder et al., 2000).

The overall Ca^{2+} concentration appeared to be somewhat higher than the reference intervals for blesbok and goats obtained but lower than horses (Appendix: Table 9.6). Calcium is an important role player in muscle fatigue (Wan et al., 2017). The capture stress response of Ca^{2+} appeared to be in line with other studies in species that showed Ca^{2+} to decrease in response to exertion (Kohrt et al., 2018). The main finding was the lower blood Ca^{2+} for the NES group compared to the ES group. Blood Ca^{2+} may decrease with exercise (Kohrt et al., 2018), and the disruption is related to intensity and duration. This lower Ca^{2+} value supports the acid-base data. The capture stress experienced by the NES group resulted in the observed earlier onset of fatigue compared to the ES group. Notably, the decrease in Ca^{2+} may be related to the significant pH variation and increased presence of acidotic individuals in the NES group.

The blesbok responded similarly to humans and other wildlife at D0, where Cl^- increases with exertional stress (Foran et al., 2003; Jessup et al., 1987). The effect was not overt and did not cause a difference between all the non-capture and capture stressed groups at D0: 0 min. The lack of effect on Cl^- is dissimilar to the responses in dogs exposed to exertional stress that experienced severe Cl^- alterations (Alves & Santos, 2016). The positive correlation of Cl^- to glucose and lactate supports an increase in response to exercise, and as is seen Chapter 6, glucose and lactate increase in response to exercise. The negative correlation to pH, $PaCO_2$ and $cHCO_3^-$ can be explained by the chloride shift, where Cl^- facilitates the movement of HCO_3^- in and out of red blood cells (Fig. 5.10). The HCO_3^- intracellularly binds with H^+ obtained from the haemoglobin carriers to form CO_2 and H_2O (Reece et al., 2015; Westen & Prange, 2003). In turn CO_2 can move across the cell membrane into the plasma dissolved and be expelled during respiration (Reece et al., 2015; Westen & Prange, 2003).

5.4 Conclusion

The metabolic and respiratory acidosis was well compensated for in the capture stressed immobilised groups as none of the blesbok were severely acidaemic. However, physiological

deficits were becoming evident in the NES group by the increased pH variance, below normal $PaCO_2$ (hypocapnia), the significant decreased $cHCO_3^-$ and BE_{ecf} deficit on D0. The effects of the opioids were apparent in the in the non-stressed immobilised blesbok in the hypercapnia and low to normal pH they presented with. Also, except for D0: 40 min, all the blesbok groups were hypoxaemic,

The ES group managed to maintain all the variables within a normal physiological range, which supports an improved ability to compensate physiologically in response to the capture stress on D0. The electrolyte abnormalities in this study were mild in comparison with other capture stress studies and may indicate that the capture stress was not as severe as expected or that habituation played a significant role in reducing the stress experienced during capture stress (Chalmers & Barrett, 1977; Harthoorn, 1976; Harthoorn & Van der Walt, 1974; Jessup et al., 1987; Phillips et al., 2015).

To further the understanding of the physiological changes that occurred during this study the biochemistry and haematology results are reported and discussed in the following Chapter 6.

CHAPTER 6

RESULTS & DISCUSSION: BIOCHEMISTRY & HAEMATOLOGY

6.1 Introduction

In this chapter, the biochemistry and haematology values (see detail in Chapter 3, section 3.8) for the study's four groups are discussed. Only blood glucose and lactate had Baseline values collected. There were only D0: 0 min, D0: 40 min, D2 and D5 values for the other biochemical and haematological variables. The results and discussion focus on all significant biochemical and haematological values.

6.2 Biochemistry

(i) Exercise metabolites: glucose & lactate

Results

The blood glucose data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected blood glucose was time ($F_{(2.89, 102.6)} = 57.39$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 10.49$; $P < 0.0001$) and the interaction of all the factors ($F_{(12, 142)} = 12.07$; $P < 0.001$). The capture stressed groups had a high blood glucose from the non-stressed groups ($P < 0.0001$) at D0: 0 min (Fig 6.1). At D0: 40 min, the blood glucose only differed for the ENS and NES group ($P = 0.025$). Within groups, the blood glucose of the ENS group was lower at D5 ($P = 0.029$) than at Baseline. The blood glucose for the NENS group was lower at D0: 0 min ($P = 0.001$), D2 ($P < 0.001$) and D5 ($P = 0.001$) than at Baseline. The blood glucose of the ES group was higher at D0: 0 min ($P = 0.003$) than at Baseline (Appendix: Table 9.4). The glucose of the NES group was higher at D0: 0 min ($P = 0.011$), D2 ($P = 0.025$) and D5 ($P = 0.043$) than at Baseline. Comparing the animals from the capture stressed groups at D0: 0 min (ES vs NES, *t*-test), glucose was higher for the NES group ($P = 0.049$). There was no difference in blood glucose between the NES and ES group at D0: 40 min ($P > 0.05$).

The blood glucose for the stressed groups on D0: 0 min had a positive correlation to Cl^- ($r = 0.46$, $R^2 = 0.22$; n = 20; $P = 0.04$), AGap ($r =$ 051, $R^2 = 0.26$; n = 20; $P = 0.02$) and lactate ($r = 0.49$, $R^2 = 0.24$; n = 20; $P = 0.03$). The blood glucose for the stressed groups on D0: 0 min

had a negative correlation to $PaCO_2$ (r = - 0.48, R^2 = 0.22; n = 20; P = 0.04) , HCO_3^- (r = - 0.49, R^2 = 0.24; n = 19; P = 0.04) and AST (r = - 0.45, R^2 = 0.20; n = 20; P = 0.05).

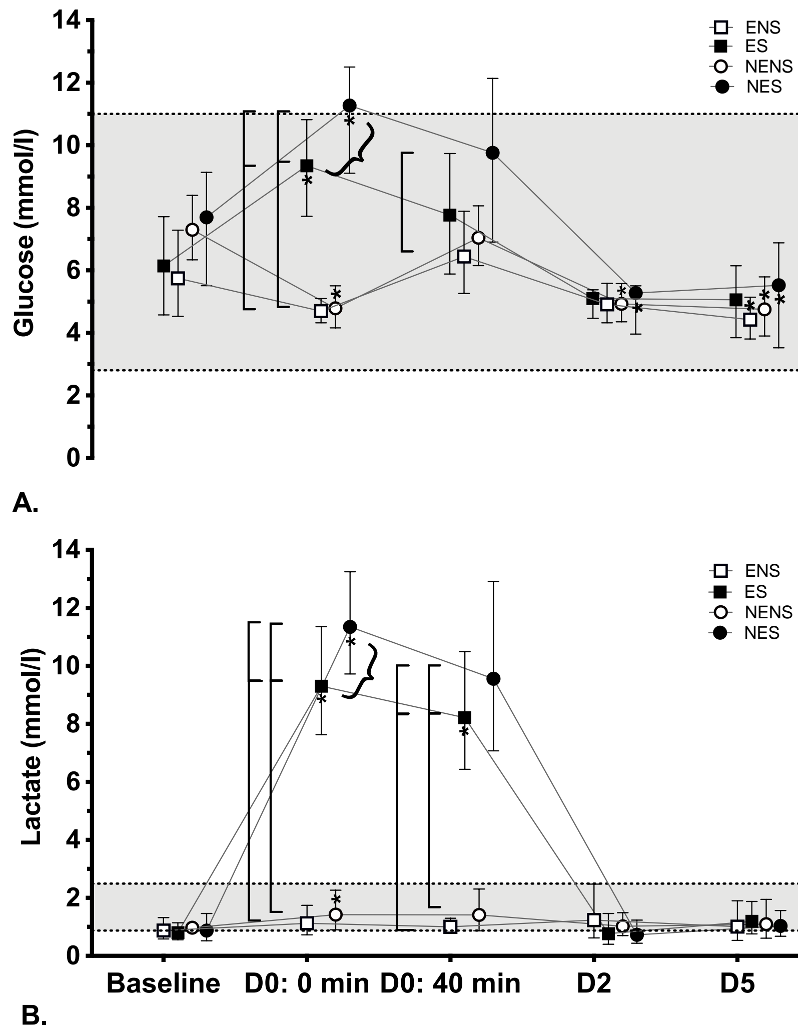


Figure 6.1 Arterial blood glucose and lactate concentration

A. Back transformed mean and SD of blood glucose (mmol/l) for each group measured at Baseline, D0: 0 and 40 min, D2 and D5.

B. Back transformed mean and SD of blood lactate (mmol/l) for each group measured at Baseline, D0: 0 and 40 min, D2 and D5.

Shaded grey area and gridlines: glucose reference interval for blesbok (Seal & Schobert, 1976). Vertical bars: different between groups ($P < 0.05$). * different from Baseline values within-group ($P < 0.05$). Curly bracket: different between NES and ES group (t-test, $P < 0.05$).

The blood lactate data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected blood lactate was time ($F_{(3.08, 109.4)}$ = 116.1; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)}$ = 28.48; $P < 0.0001$) and the interaction of all the factors ($F_{(12, 142)}$ = 29.62; $P < 0.001$). The blood lactate differed for the capture stressed groups from the non-stressed groups at D0: 0 and D0: 40 min ($P < 0.0001$) (Fig 6.1). When the capture stressed groups were compared (ES vs NES, *t*-test) at D0: 0 min and D0: 40 min, the blood lactate was higher for the NES group at D0: 0 min ($P = 0.023$) but not at D0: 40 min. Within groups, the lactate for the ES and NENS groups were higher at D0: 0 min and D0: 40 min than at Baseline ($P < 0.0001$). The lactate for the NENS group was higher ($P = 0.045$) at D0: 0 min than at Baseline but was still within the normal reference interval indicated (Fig. 6.1, Appendix: Table 9.4).

The blood lactate for the stressed groups on D0: 0 min had a positive correlation to PaO_2 (r = 0.51, R^2 = 0.23; n = 20; $P = 0.02$), BE_{ecf} (r = 0.85, R^2 = 0.72; n = 19; $P < 0.001$), AGap (r = 0.88, R^2 = 0.77; n = 20; $P < 0.001$), glucose (r = 0.49, R^2 = 0.24; n = 20; $P = 0.03$), Hct (r = 0.55, R^2 = 0.30; n = 20; $P = 0.02$), Hgb (r = 0.50, R^2 = 0.25; n = 20; $P = 0.01$) and creatinine (r = 0.63, R^2 = 0.40; n = 20; $P = 0.03$). The blood lactate for the stressed groups on D0: 0 min had a negative correlation to pH (r = - 0.72, R^2 = 0.52; n = 20; $P < 0.001$) (Fig. 5.6), $PaCO_2$ (r = - 0.63, R^2 = 0.51; n = 20; $P = 0.02$), HCO_3^- (r = - 0.78, R^2 = 0.61; n = 20; $P < 0.001$) (Fig. 5.6) and urea (r = - 0.57, R^2 = 0.33; n = 20; $P = 0.01$).

Discussion

Glucose initially decreases and then increases in response to continued exertion (Ferraz et al., 2008). In a study in horses, the plasma glucose decreases for the first 6 min after exercise, whereafter it increased for up to 30 min post-exercise (Ferraz et al., 2008). The sympathetic response during exercise increases the blood glucose due to adrenalin release results in glycolysis (Ferraz et al., 2008). The point at which glucose starts increasing post-exercise is known as the glucose threshold (Ferraz et al., 2008). The increased glucose post-exercise in the capture stressed blesbok in this study was an anticipated change in biochemistry.

Horses showed no remarkable difference between untrained and trained horses in their acute blood glucose changes post-exercise in a previous study (Ferraz et al., 2008). There was a more significant glucose increase for the NES group than the ES group in the current blesbok study. There is support for improved glucose regulation in trained animals compared to untrained (Chapter 2, section 2.8) (Adams, 2013). However, a few physiological factors influence the

glucose changes that occur during exercise. The concentration obtained for the ES and NES groups are still within the indicated reference interval range (Fig 6.1) obtained for blesbok (Seal & Schobert, 1976). However, the reference range shown of 2.8 - 11 mmol/l is substantially larger than for other species like the horse that is 3.3 – 6.4 mmol/l (Appendix).

The increased reference interval range indicated for blesbok from this study is likely from capture stress and not obtaining resting glucose concentration. In wild antelope, an acute stress response during capture results in cortisol release and stimulates rapid gluconeogenesis, with a resultant surge in blood glucose - with or without exertion (Jessup et al., 1987; Khani & Tayek, 2001). The blood cortisol was not significantly higher for the NES than the ES group, but as discussed later (section 7.2), cortisol changes need to be interpreted with caution. A further in-depth investigation is required to clarify the blood glucose changes during capture stress and whether training positively affects blood glucose regulation in blesbok.

As stated in Chapter 2 (section 2.6), blood lactate concentration indicates an individual's exertion level. Blood lactate is a better estimate of exertion than the heart rate or blood gas values (Goodwin et al., 2007; Messonnier et al., 2013). The ES group had a lower blood lactate value than the NES group at D0: 0 min (Fig. 6.1). The lower blood lactate in the trained groups supports effective aerobic training for this ES group, increasing the lactate threshold and resulting in a lower immediate blood lactate value measured (Goodwin et al., 2007; Messonnier et al., 2013; Swart & Jennings, 2004). Furthermore, as is seen in other result sections, variables like rectal temperature, PaO_2, $PaCO_2$, pH, $cHCO_3^-$, BE_{ecf}, Ca^{2+} and glucose are all supportive of improved physiological regulation in response to exercise for the ES group. The lactate is the most important biological marker that supports that the physically trained ES group experienced delayed onset in exceeding the lactate threshold (i.e., 'onset of fatigue') compared to the NES group. The positive physiological correlations of lactate to PaO_2 and glucose, indicates that the more oxygen and glucose that was consumed during exertion the more lactate was produced. The negative correlations of lactate to pH and $cHCO_3^-$ supports that during anaerobic metabolism and lactate production, the production of H^+ causes the pH to decrease, and HCO_3^- decreases as it is used as a buffer to normalise blood pH (Fig. 5.6).

(ii) Markers of muscle damage: AST, CK & troponin I

Results

The blood AST data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the AST blood concentration were time ($F_{(2.04, 73.29)} = 60.48$; $P < 0.0001$) and the interaction of time, exercise training and capture stress ($F_{(9, 108)} = 4.63$; $P < 0.0001$), but the exercise training and the capture stress event alone had no effect on blood AST concentration ($F_{(3, 36)} = 1.27$; $P = 0.30$). The blood AST concentration were higher for the ES group when compared to the NENS group at D2 ($P = 0.04$) (Fig. 6.2, Appendix: Table 9.4). Within groups, only ENS did not differ significantly between any time points. ES had higher blood AST at D2 ($P = 0.0002$) and D5 ($P = 0.003$) than at D0: 0 min. NENS and NES increased at D0: 40 min, D2 and D5 compared to D0: 0 min ($P < 0.05$). When the capture stressed groups were compared (ES vs NES, *t*-test) at D0:0 min and D0: 40 min, the blood AST was higher for the ES group at D0: 0 min ($P < 0.05$) but not at D0: 40 min. The blood AST for the stressed groups on D0: 0 min had a positive correlation to K^+ ($r = 0.54$, $R^2 = 0.30$; n = 20; $P = 0.01$) (Fig. 5.9), GGT ($r = 0.51$, $R^2 = 0.26$; n = 20; $P = 0.02$) (Fig. 6.3), GLDH ($r = 0.74$, $R^2 = 0.55$; n = 20; $P < 0.001$) (Fig. 6.3) and CK ($r = 0.50$, $R^2 = 0.25$; n = 20; $P = 0.02$) (Fig. 6.3).

The blood CK data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood CK concentration were time ($F_{(2.15, 77.37)} = 19.80$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 8.64$; $P < 0.0002$) and the interaction of all the factors ($F_{(9, 108)} = 2.341$; $P = 0.019$). CK increased for the capture stressed groups at D0: 0 min and D0: 40 min compared to the non-stressed groups ($P < 0.05$) (Fig 6.2). Within the capture stressed groups (ES and NES), CK at D0: 40 min was increased compared to D0: 0 min and D5 was decreased compared to D0: 0 min ($P < 0.05$). All the animals in the capture stressed groups returned to the indicated reference interval range at D5 (Fig. 6.2, Appendix: Table 9.4). Comparing the ES and NES groups at D0:0 min, D0: 40 min and D2 there were no differences (*t*-test, $P > 0.05$) noted. The blood CK for the stressed groups on D0: 0 min had a positive correlation to GGT ($r = 0.58$, $R^2 = 0.34$; n = 20; $P = 0.01$), AST ($r = 0.50$, $R^2 = 0.25$; n = 20; $P = 0.02$) and troponin I ($r = 0.47$, $R^2 = 0.22$; n = 20; $P = 0.04$). The blood CK for the stressed groups on D0: 0 min had a negative correlation to cortisol ($r = - 0.45$, $R^2 = 0.20$; n = 20; $P < 0.05$) and WBC ($r = - 0.62$, $R^2 = 0.38$; n = 20; $P = 0.004$)

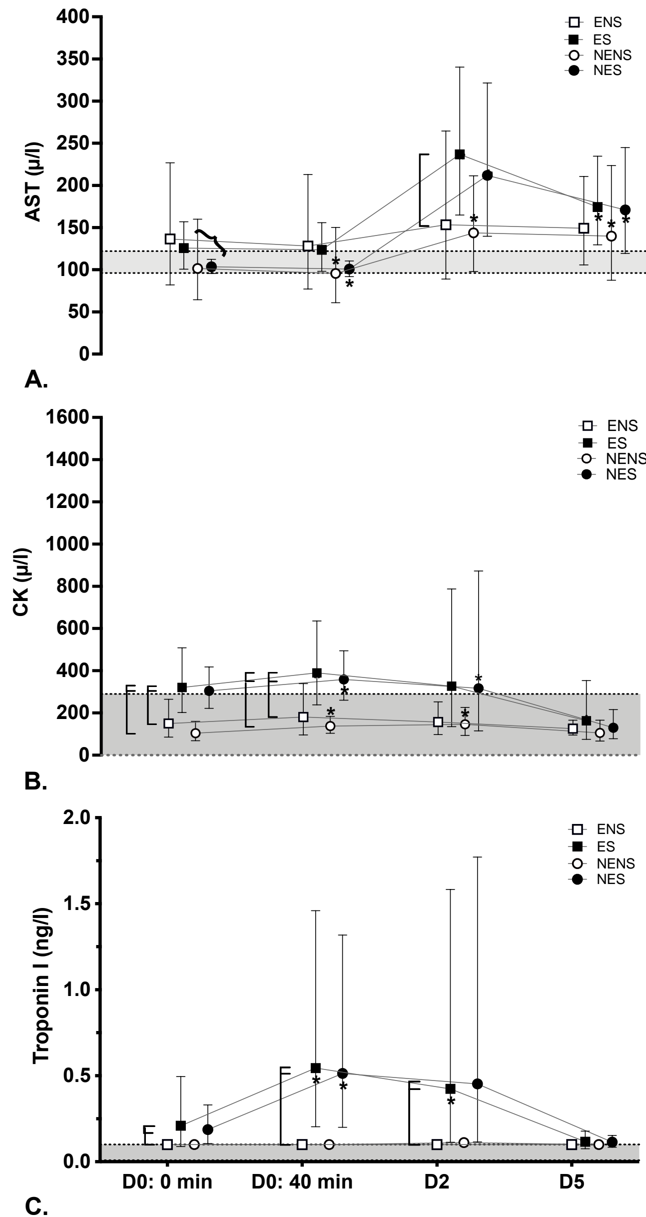


Figure 6.2 Venous blood AST, CK and troponin concentration

A. Back transformed mean and SD of blood AST (µ/l) for each group measured at D0: 0 min and 40 min, D2 and D5.

B. Back transformed mean and SD of blood CK (µ/l) for each group measured at D0: 0 min and 40 min, D2 and D5.

C. Back transformed mean and SD of blood troponin I (ng/l) for each group measured at D0: 0 min and 40 min, D2 and D5.

Shaded grey area and grid lines: reference interval in blesbok (Fitte, 2017; Seal & Schobert, 1976). Vertical brackets: different between groups (P < 0.05). * different from D0: 0 min within-group (P < 0.05). Curly bracket: different between NES and the ES group (t-test, P < 0.05). Bow: different between ES and NES group (F-test, *P* < 0.05).

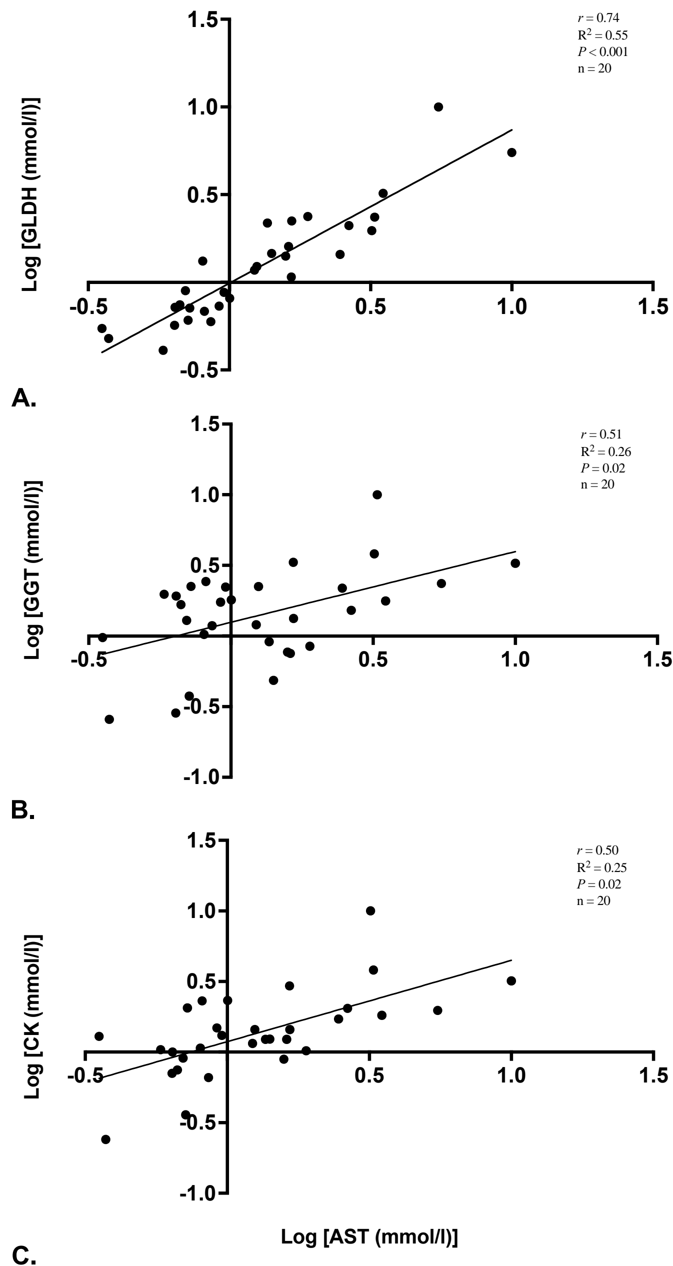


Figure 6.3 Correlation of CK, GGT and GLDH to AST

A. Linear correlation plot of blood AST and GLDH concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Linear correlation plot of the blood AST and GGT concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

C. Linear correlation plot of blood AST and CK concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

The blood troponin I data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood troponin I concentration were time ($F_{(2.86, 66.15)} = 30.6$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 10.5$; $P < 0.0001$) and the interaction of all of factors ($F_{(9, 107)} = 9.60$; $P < 0.0001$). The troponin I concentration were increased for the NES compared to the ENS and NENS groups at D0: 0 min and at D0: 40 min ($P < 0.05$). All the capture stressed groups had higher troponin I concentration than the non-stressed groups and this effect persisted till D2 ($P < 0.05$). At D5, the troponin I concentration of the stressed groups returned to indicated interval range

and had similar concentration to the non-stressed group (Fig. 6.2, Appendix: Table 9.4). The blood troponin I for the stressed groups on D0: 0 min had a positive correlation to BE_{ecf} (r = 0.49, $R^2 = 0.24$; n = 20; $P = 0.03$), Hct (r = 0.45, $R^2 = 0.20$; n = 20; $P < 0.05$), haemoglobin (r = 0.45, $R^2 = 0.20$; n = 19; $P < 0.05$), GGT (r = 0.52, $R^2 = 0.27$; n = 19; $P = 0.02$) and CK (r = 0.47, $R^2 = 0.21$; n = 20; $P = 0.04$) . The blood CK for the stressed groups on D0: 0 min had a negative correlation to cortisol (r = - 0.45, $R^2 = 0.20$; n = 20; $P < 0.05$) and WBC (r = - 0.77, $R^2 = 0.59$; n = 20; $P < 0.001$).

Discussion

AST is a marker for hepatocellular damage, and although it lacks specificity for the liver, it has shown to elevate with severe muscular damage (Hartup et al., 1999; Pettersson et al., 2008; Schomaker et al., 2020). However, AST in ruminants indicates muscular damage more than liver damage but is released slowly after an injury (Antonelli et al., 2007). All the groups were still within or near normal reference limits at D0: 0 min, and the differences between the ES and NES group unlikely to be of clinical value. The blood AST concentration appeared increased for the capture stressed groups compared to the non-stressed groups at D2 (Fig 6.2), but only the AST concentration for the ES compared to the NENS group were higher (P = 0.04). The delayed AST increase in the capture stressed blesbok supports muscular damage caused by the capture stress event. AST positively correlates to K^+ (Fig. 5.9), GGT (Fig. 6.3), GLDH (Fig. 6.3) and CK (Fig. 6.3), all markers of exertion, liver, and muscle damage that would all increase with exertion and the associated muscular damage.

CK activity increases when subjected to unaccustomed exercise or severe exertion (Vanholder et al., 2000). However, CK activity increases are variable in individuals, and some individuals may not show a rise in CK activity (Kindermann, 2016), the large SD (back transformed GSD in Fig. 6.2) support this variable response of CK to exertion. Blood CK activity significantly

increases with capture myopathy and as can be seen from the data (Fig. 6.2), some individuals had a significant increase in CK (Paterson, 2014). The capture stress event on D0 was adequate to result in an immediately increased CK activity. Although CK also appeared higher for D2, it was not significant. The D2 CK concentration for the blesbok reveal that the CK activity and return to the indicated reference interval concentration were highly variable (Fig. 6.2).

The blood CK values measured does not distinguish between CK from cardiac or skeletal muscle origin, and the exact source of the CK increases remains undetermined. In humans, strenuous exercise may peak in serum CK activity 48 hours post-exercise, with CK activities returning to normal within five days (Grobler et al., 2004). Horses serum CK peaks within 24 hours and returns to normal within 72 hours (Padilha et al., 2017). The blesbok in this study appeared to have had the most CK activity within the first two days and a return to normal by five days. The CK changes for the capture stressed blesbok appeared to be like the human changes seen in CK in response to strenuous exercise.

The blesbok in the stressed groups had increased troponin I post exercise on D0: 40 min and D2, that returned to the reference interval at D5. Healthy human individuals can have an elevated troponin I response post-exercise (refer to Chapter 2, section 2.8 (ii)). In human studies, troponin I post-exertion elevation and the return to normal troponin I serum concentration was highly variable (Gresslien & Agewall, 2016; Nie et al., 2018). Severe peaks in troponin I post-exercise are a prognostic indicator for cardiac stress susceptibility in humans (Nie et al., 2018; Rossi et al., 2015). Significant increases in troponin I in individuals are evident in the stressed blesbok groups post exercise (Fig. 6.2), but whether troponin I holds the same value as a prognostic indicator for cardiac stress in blesbok or wild antelope, in general, would have to be further researched. During the monitoring of animals, heart murmurs on auscultation were noted anecdotally but it was not specifically recorded so accurate figures of the blesbok that presented with heart murmurs cannot be given. Determining the correlation between troponin I elevations and heart murmurs would have been valuable. The individual blesbok response of troponin I and the correlation to subclinical heart pathology must be further investigated as previous human studies indicate that subclinical heart pathology may play a role in elevated troponin I response post-exercise.

In summary, a similar response in AST, CK and troponin I was seen in the capture stressed groups (ES and NES). There were no differences in the muscle enzyme changes between the exercise-trained and non-exercise trained blesbok in this study. Relating the AST, CK and

troponin I value to pathological findings may shed light on underlying pathological conditions that may predispose individuals to cardiac or muscular stress.

(iii) Renal function: Creatinine & urea

Results

The blood creatinine data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood urea were time ($F_{(2.16, 77.74)} = 29.61$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 3.33$; $P < 0.0001$) and the interaction of these factors ($F_{(9, 108)} = 3.46$; $P = 0.0009$). Between groups, the urea of the NES group was lower than the ES group at D0: 0 min and 40 min ($P < 0.05$), but there were no differences between the capture stressed and non-stressed groups (Fig. 6.4, Appendix: Table 9.4). Within groups, the ES, NES and NENS groups increased at D0: 40 min compared to D0: 0 min and the ES, NENS and ENS all decreased at D5 compared to D0: 0 min ($P < 0.05$). When the capture stressed groups were compared (ES vs NES, *t*-test) at D0:0 min and D0: 40 min, the ES group had higher urea concentration than the NES group at D0: 0 min ($P < 0.05$), but not at D0: 40 min. The blood urea for the stressed groups on D0: 0 min had a positive correlation to $PaCO_2$ ($r = 0.70$, $R^2 = 0.49$; n = 20; $P = 0.001$) (Fig. 6.5). The blood urea for the stressed groups on D0: 0 min had a negative correlation to PaO_2 ($r = -0.55$, $R^2 = 0.30$; n = 20; $P = 0.01$) (Fig. 6.5) and lactate ($r = -0.57$, $R^2 = 0.32$; n = 20; $P < 0.01$) .

The blood urea data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood creatinine concentration obtained were time ($F_{(2.68, 96.63)} = 117.3$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 14.47$; $P < 0.0001$) and the interaction of these factors ($F_{(9, 108)} = 25.74$; $P < 0.0001$). Between groups, the capture stressed groups had a higher creatinine blood concentration ($P < 0.05$) than the non-capture stressed groups at D0: 0 min and 40 min (Fig. 6.4, Appendix: Table 9.4). The elevated creatinine concentration for capture stressed groups at D0, ES and NES, return to similar serum concentrations as the non-capture stressed groups at D2. Within the capture stressed groups, blood creatinine decreased at D0: 40 min, D2 and D5 compared to D0: 0 min ($P < 0.05$). When the capture stressed groups were compared (ES vs NES, *t*-test) at D0: 0 min and D0: 40 min, the ES creatinine value was lower than the NES group at D0: 0 min ($P = 0.023$), but not at D0: 40 min.

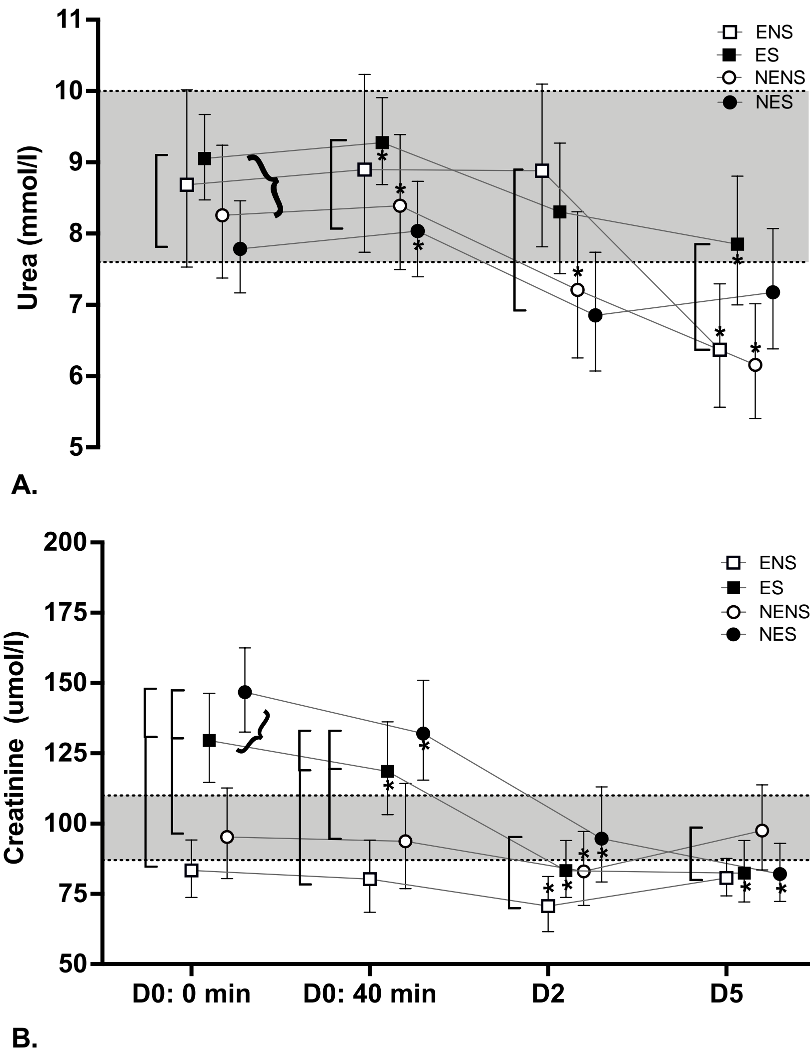


Figure 6.4 Venous blood urea and creatinine concentration

A. Back transformed mean and SD of blood urea (mmol/l) for each group measured at Baseline, D0: 0 and 40 min, D2 and D5.

B. Back transformed mean and SD of blood creatinine (μmol/l) for each group measured at Baseline, D0: 0 and 40 min, D2 and D5.

Shaded grey areas and gridlines: reference intervals for healthy blesbok (Fitte, 2017; Seal & Schobert, 1976).Vertical brackets: different between groups (*P* < 0.05). * different from D0: 0 min within-group (*P* < 0.05). Curly brackets: different between NES and ES group (*P* < 0.05).

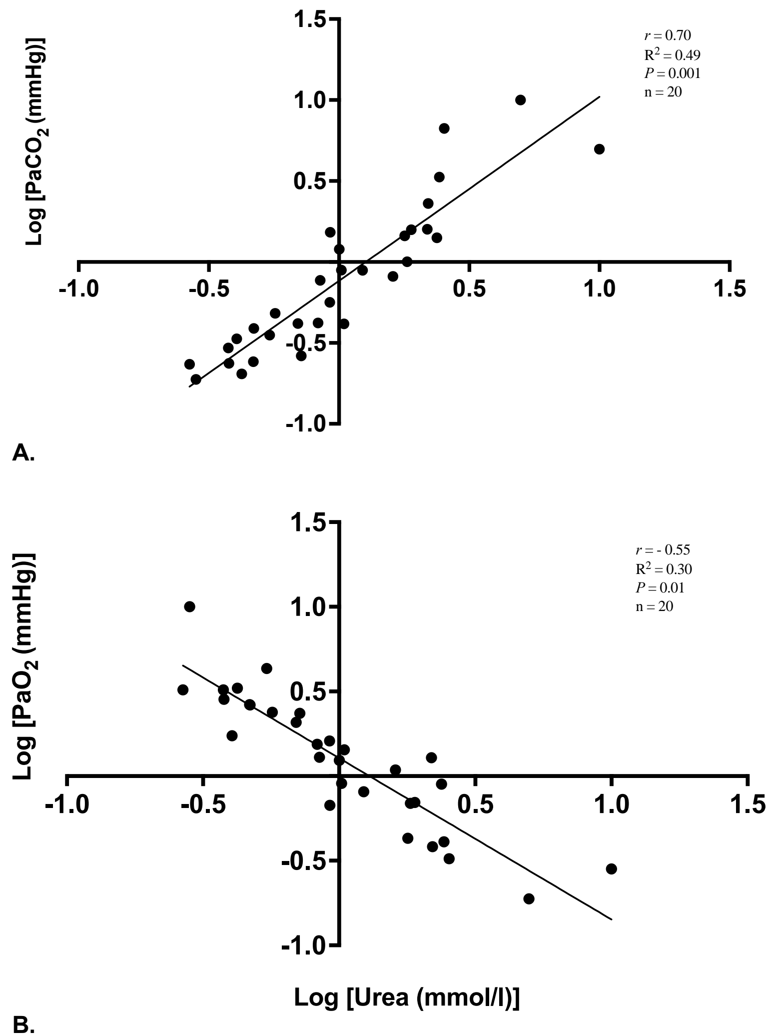


Figure 6.5 Correlation of $PaCO_2$ and PaO2 to urea

A. Linear correlation plot of $PaCO_2$ and urea concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

B. Linear correlation plot of the PaO_2 and urea concentrations of the stressed groups (NES and ES) on D0: 0 min using log transformed values.

The blood creatinine for the stressed groups on D0: 0 min had a positive correlation to respiratory rate ($r =$ 0.48, $R^2 = 0.23$; n = 20; $P = 0.03$), PaO_2 ($r =$ 0.51, $R^2 = 0.26$; n = 20; $P = 0.02$) (Fig. 6.5), BE_{ecf} ($r =$ 0.59, $R^2 = 0.35$; n = 19; $P = 0.01$), lactate ($r =$ 0.63, $R^2 = 0.40$; n = 20; $P = 0.003$), Hct ($r =$ 0.65, $R^2 = 0.42$; n = 20; $P = 0.002$) and Hgb ($r =$ 0.56, $R^2 = 0.32$; n = 20; P = 0.01). The blood creatinine for the stressed groups on D0: 0 min had a negative correlation to $PaCO_2$ ($r =$ - 0.53, $R^2 = 0.28$; n = 20; $P = 0.02$) (Fig. 6.5) and $cHCO_3^-$ ($r =$ - 0.72, $R^2 = 0.52$; n = 19; $P < 0.001$).

Discussion

Exercise stimulates protein catabolism, but research in humans indicates that lean muscle weight is maintained during exercise training and does not necessarily imply a negative nitrogen balance (Weiner et al., 2015). The positive correlation of blood urea to $PaCO_2$ and negative correlation to PaO_2 supports increased protein metabolism during exertion (Fig. 6.5). The increased urea concentration for the ES group may be due to training that activates protein metabolism, with a resultant increase of circulating urea. Athletes often show increased resting concentration for urea, which is likely due to the continuous strain and protein metabolism of exercise training (Warburton et al., 2002).

Most of the urea concentrations obtained were still within an expected reference interval range of urea concentration for blesbok (Fitte, 2017; Seal & Schobert, 1976). A few groups displayed concentration below the reference interval indicated at D2 and D5, but this is doubtful to be clinically significant. The non-capture stressed groups, NENS and ENS, experienced similarly increased urea concentration at D0, with a downward trend at D2 and D5 (Fig. 6.4). Urea is one of the metabolites known to elevate with capture stress (Harthoorn, 1976; Jessup et al., 1987; Spraker, 1993). The increase in urea may be due to acute dehydration leading to increased urea resorption by the kidneys (Mehta, 2008). Acute stress may also result in dehydration in ruminants due to cortisol's diuretic effect (Parker et al., 2003). However, no indicator for hydration status was directly measured. The blood cortisol was increased within groups at D0 compared to D5 (Fig. 6.7), which is indicative that acute stress was experienced and may have caused the increased urea concentration.

Renal failure and resultant creatinine increase are pivotal in the syndrome of capture myopathy (Bartsch et al., 1977; Herrá Ez et al., 2007; Jessup et al., 1987). Rhabdomyolysis during wildlife capture events may result in acute renal damage (Chapter 1, section 1.3). Capture stress results in catecholamine release resulting in renal vasoconstriction, with associated serum creatinine increases (López-Olvera et al., 2007). Also, myoglobin released from muscular breakdown and haem from haemoglobin breakdown are both nephrotoxic (Bartsch et al., 1977; Baxter & Moore, 2003; Herrá Ez et al., 2007; Vanholder et al., 2000).

The creatinine increases following exercise may suggest muscle injury and dehydration (Otto et al., 2017). The elevated serum creatinine initially and return to within the reference interval concentration post-exercise is expected. Serum creatinine may return to normal concentration if the acute increase were due to normal micro-tearing or dehydration during exercise, and the

renal function is not compromised (Padilha et al., 2017). In horses exposed to a short exertional event, creatinine increased and returned to normal within 24 hours (Özcan et al., 2013). There are numerous reasons that the NES group had an increased creatinine value compared to the ES group at D0: 0 min (Fig. 6.4). Firstly, muscular injury (evident by an increase in CK) may have increased the NES group's creatinine value. The CK concentration is not higher for the NES group than the ES group (Fig. 6.4). The apparent greater variation evident in the NES group on D2 indicates that muscular damage was variable in this group (Fig. 6.4). Plasma volume is also known to increase in exercise-trained groups, is more marked in those training in the heat (Geor & McCutcheon, 1998; Mairbäurl, 2013), which may have given rise to a lower creatinine value for the ES group. Lastly, there is some evidence that exercise training may provide improved glomerular filtration rates in humans with chronic kidney disease. The improved creatinine clearance rates will lower creatinine concentration for exercise-trained individuals in response to exercise (Wyngaert et al., 2018). Fluid balance and haemodynamic changes influence renal blood flow and may be favourably adapted in exercise-trained animals (Wyngaert et al., 2018). Increased renal blood flow to the kidneys during exercise may result in improved creatinine clearance.

Resting creatinine clearance is only affected when more than 75% of renal function is compromised and is only an accurate indication of severe kidney damage (Braun & Lefebvre, 2008). Additionally, a recent study in humans has shown that high-intensity interval resistance training may result in an acute creatinine increase and may result in subclinical renal injury. Individuals may be at a higher risk of developing renal failure later when subjected to other renal stressors (Spada et al., 2018). Hypothetically, this could happen for animals during capture stress, resulting in subclinical renal injury. Consequently, with repeated renal injuries like repeated captures, high salinity water, concurrent infections etc., it may turn into overt renal failure. Exercise training has shown to protect from renal insult by improving renal blood flow (Deferrari et al., 2018). Some evidence has indicated that the activation of heat shock proteins may assist with improved renal repair after a renal injury (Henstridge et al., 2016). To fully evaluate the renal response and subclinical injury in this study, if any, a histopathological examination of the kidneys would be required.

(iv) Liver function: GGT, GLDH & bilirubin

Results

The blood GGT data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood GGT concentration was only time ($F_{(1.458, 52.48)} = 4.75$; $P = 0.021$), but not exercise training and capture stress ($F_{(3, 36)} = 2.17$; $P = 0.108$) or the interaction of the factors had a significant effect on GGT concentration ($F_{(9, 108)} = 1.92$; $P = 0.057$) (Fig. 6.6, Appendix: Table 9.4). Between groups, NENS and NES differed at D0: 0 min and D0: 40 min. GGT decreased at D0: 40 min and D5 from D0: 0 min within the ES group. GGT decreased at D0: 40 min, D2 and D5 from D0: 0 min ($P < 0.05$) within the NES group. Within the NENS group, only D0: 40 min decreased from D0: 0 min ($P < 0.0001$). There were no differences within the ENS group. When the capture stressed groups were compared (ES vs NES, *t*-test) on D0, there were no differences in blood GGT at D0: 0 min and D0: 40 min. Comparing the capture stressed groups (ES vs NES, F-test), the ES group had a larger variance (Fig. 6.6). The blood GGT for the stressed groups on D0: 0 min had a positive correlation to AST ($r = 0.51$, $R^2 = 0.26$; n = 19; $P = 0.02$) (Fig. 6.3), CK ($r = 0.58$, $R^2 = 0.34$; n = 19; $P = 0.01$) and troponin I ($r = 0.52$, $R^2 = 0.27$; n = 19; $P = 0.02$). The blood GGT for the stressed groups on D0: 0 min had a negative correlation to WBC ($r = -0.59$, $R^2 = 0.34$; n = 19; $P = 0.01$) and Tbili ($r = -0.55$, $R^2 = 0.30$; n = 19; $P = 0.02$).

The blood GLDH data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood GLDH were time ($F_{(1.437, 51.72)} = 23.82$; $P < 0.0001$) and the interaction of the exercise training and capture stress over time affected GLDH ($F_{(9, 108)} = 16.36$; $P < 0.0001$), but not the exercise training and capture stress on its own ($F_{(3, 36)} = 0.95$; $P = 0.426$). The only difference was at D5 between the ENS and NES group ($P = 0.023$) groups. The ES group appeared elevated compared to the other groups at D0 (Fig. 6.6, Appendix: Table 9.4). When the capture stressed groups were compared (ES vs NES, *t*-test), the ES group was higher than the NES group at D0: 0 min and D0: 40 min ($P < 0.05$). The NES group's GLDH concentration were within the reference interval range and showed no difference from the NENS and ENS groups (Fig. 6.6). Within groups, the GLDH concentration decreased for ES and NES at D0: 40 min, D2 and D5 ($P < 0.05$). The blood GLDH for the stressed groups on D0: 0 min had a positive correlation to K^+ ($r = 0.51$, $R^2 = 0.26$; n = 20; $P = 0.02$) (Fig. 5.9) and AST ($r = 0.74$, $R^2 = 0.55$; n = 20; $P < 0.001$) (Fig. 6.3).

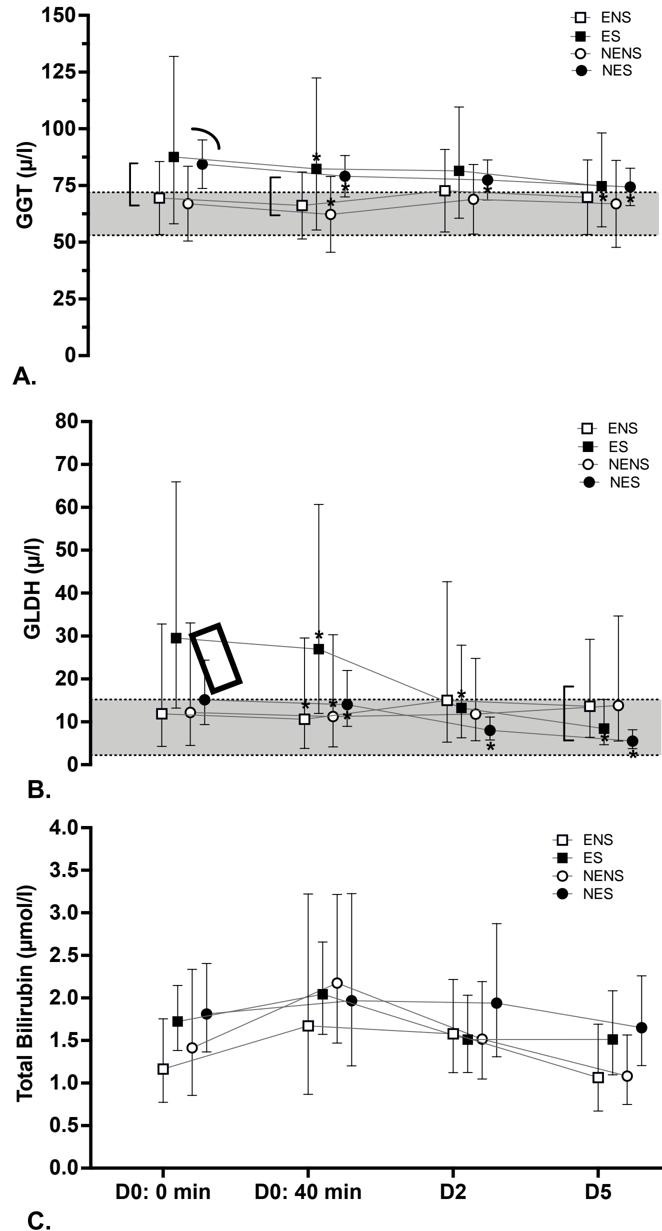


Figure 6.6 Venous blood GGT, GLDH and total bilirubin concentration

A. Back transformed mean and SD of blood GGT (µl/l) for each group measured at Baseline, D0: 0 and 40 min, D2 and D5.

B. Back transformed mean and SD of blood GLDH (µ/l) for each group measured at D0:0 and D0: 40 min, D2 and D5.

C. Back transformed mean and SD of blood total bilirubin (µ/l) for each group measured at D0:0 and D0: 40 min, D2 and D5.

Gridlines and grey shaded area: reference interval for healthy blesbok (Fitte, 2017; Seal & Schobert, 1976). Vertical bars: different between groups (P < 0.05). * different from D0: 0 min within-group (P < 0.05). Curly brackets: difference between NES and ES (t-test, P < 0.05). Bow: different between ES and NES group (F-test, *P* < 0.05).

The blood total bilirubin data were found to not have a normal distribution and required log transformation before further analysis. The blood total bilirubin concentration was not affected by time, exercise training and capture stress or the interaction of these factors ($P > 0.05$). Within and between groups, there were no differences noted for the bilirubin concentration (Fig. 6.6, Appendix: Table 9.4). The capture stressed groups and non-stressed or exercise-trained and non-trained groups did not differ ($P > 0.05$). The blood Tbili for the stressed groups on D0: 0 min had a negative correlation to GGT (r = - 0.55, $R^2 = 0.30$; n = 19; $P = 0.02$).

Discussion

GGT and GLDH are enzymes indicative of hepatic necrosis (Gowda et al., 2009; Reece et al., 2015). GGT is a non-specific enzyme that may originate from multiple liver sites (Gowda et al., 2009). GLDH is released in response to hypoxic hepatic injury (Schomaker et al., 2020). There were no changes noted or expected in GGT in the blesbok in this study, similar to a previous study in blesbok that were physically exerted (Fitte, 2017). GGT blood concentration in horses also showed no changes in response to training (Padilha et al., 2017).

Blood GLDH concentration did have an acute increase in the previous blesbok study that returned to pre-exertion concentration by day 2 (Fitte, 2017). This increase in GLDH at D0 was only evident in the ES group in the current study. The NES group did not significantly change its GLDH concentration after the capture stress event (Fig. 6.6). All the groups showed considerable variation in the GLDH concentration at all the time intervals (Fig. 6.6). The GLDH concentration of the ES group were elevated compared to the NES group on D0. However, as is reflected in the back transformed data in Fig. 6.6 the individual GLDH values varied substantially in each group at each time point, making identification of a pattern difficult. The increased GLDH concentration for the ES group may indicate training-related changes or prior injury due to training. No specific traumatic incidents were recorded in any of the blesbok, but micro injuries can easily occur between the animals or while training without it being observed. GLDH was positively correlated to K^+ (Fig. 5.9) and may be related to red blood cell damage (with the release of K^+) or pH changes during exertion. GLDH was also positively correlated to AST (Fig. 6.3), an indicator of muscle damage. These correlations are supportive that there might have been hypoxic damage to muscle and liver.

The findings for bilirubin do not align with results in humans where bilirubin increases due to increased haemolysis during exercise (Swift et al., 2012; Witek et al., 2017). Exercise trained athletes should also have elevated bilirubin concentration compared to non-exercise trained

individuals, which is not evident in the results (Witek et al., 2017). A study in horses that looked at the effect of exertion revealed no increases for bilirubin, similar to the current study's finding (Özcan et al., 2013). The level and length of exertion may have been inadequate to result in intravascular haemolysis and subsequent bilirubin increases. Bilirubin increases may become more evident if the animals with endurance type of exertion.

In summary, there were changes evident in the liver enzymes post the capture stress event, but no exact liver enzyme response with clear time frames could be identified in the stressed groups in response to the exertional event. Only the ES group had increased GLDH concentration that may indicate prior training-related changes or injury to the capture stress event.

6.3 Cortisol

Results

The blood cortisol data were found to not have a normal distribution and required log transformation before further analysis. The factors that affected the blood cortisol were time ($F_{(2.556, 92.01)} = 19.76$; $P < 0.0001$), but not exercise training and capture stress ($F_{(3, 36)} = 2.45$; $P < 0.081$) or the interaction of the factors ($F_{(9, 108)} = 0.64$; $P = 0.763$) had an effect. Between groups, none of the groups differed from each other at any time point (Fig. 6.7, Appendix: Table 9.4). Within the ES group, D2 ($P = 0.008$) and D5 ($P = 0.019$) was lower than D0: 0 min, but for the NES group, only D5 was lower than D0: 0 min ($P = 0.013$). Within the ENS and NENS groups, only D5 was lower than D0: 0 min ($P = < 0.05$). When the capture stressed groups were compared (ES vs NES, *t*-test), there was no difference at D0: 0 min or D0: 40 min. The blood cortisol for the stressed groups on D0: 0 min had a positive correlation to $PaCO_2$ ($r = 0.52$, $R^2 = 0.27$; n = 20; $P = 0.02$). The blood cortisol for the stressed groups on D0: 0 min had a negative correlation to Hct ($r = - 0.48$, $R^2 = 0.23$; n = 20; $P = 0.03$), Hgb ($r = - 0.49$, $R^2 = 0.24$; n = 20; $P = 0.03$), creatinine ($r = - 0.58$, $R^2 = 0.34$; n = 20; $P = 0.01$) and troponin I ($r = - 0.57$, $R^2 = 0.32$; n = 20; $P = 0.01$).

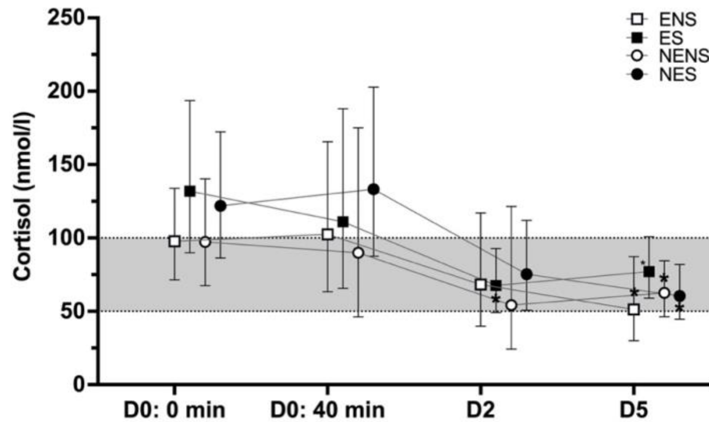


Figure 6.7 Venous blood cortisol concentration

Back transformed mean and SD of blood cortisol concentration (nmol/l) for each group measured at D0: 0 min and 40 min, D2 and D5.

Shaded grey area and gridlines: reference interval for healthy blesbok (Fitte, 2017; Seal & Schobert, 1976). * within groups different from D0: 0 min (*P* < 0.05).

Discussion

Cortisol is also a well-documented indicator of stress in many wildlife species - e.g., physical handling of wild animals results in increased cortisol concentrations (Kock *et al.*, 1987; Morton *et al.*, 1995). However, over the years it has become apparent that cortisol concentrations are not a reliable quantitative indicator of stress in wildlife and must be interpreted alongside other biological markers of stress. Blood cortisol concentration is increased with opioid administration, which is often used in wild animal immobilisation. Opioids result in the activation of the sympathoadrenal system and can result in drug-induced elevations in cortisol, which may overshadow the true level of stress the animal is experiencing (Seyfriedand Hester, 2012). Cortisol is interpreted alongside other stress markers like the neutrophil: lymphocyte ratio (Davis et al., 2008; Seyfried & Hester, 2012). All four groups had elevated cortisol concentration at D0: 0 min and D0: 40 min, compared to the reference interval concentration and D2 and D5. The increased cortisol in the non-stressed groups may be due to stress response to the novel human activity (approach of the boma by humans, talking etc.) before immobilisation on D0. Opioids may also affect the cortisol concentration measured and may have influenced the results on D0 of the capture stress groups (Fitte, 2017). However, the opioid dose used was consistent for all blesbok. The increased cortisol did show that there was stress related to novel activities with wild antelope and how habituation may reduce the stress response.

6.4 Haematology

(i) Red blood cells, haemoglobin and haematocrit

Results

The RBC data were found to have a normal distribution. The only significant changes in the red blood cell responses occurred for the RBC, Hgb and Hct when the haematological results were evaluated. Please refer to the Table 6.1 for the complete haematological results. The factors that affected the RBC count was time ($F_{(2.63, 93.91)} = 45.12$; $P < 0.0001$), but not exercise training and capture stress ($F_{(3, 36} = 2.08$; $P < 0.120$) or the interaction of the factors. Except for the ES and ENS groups, the capture stressed, and non-stressed groups differed ($P < 0.05$) at D0: 0 min. Only the NES and ENS groups differed at D0: 40 min. Within the capture stressed groups, the RBC decreased at D0: 40 min, D2 and D5 ($P < 0.05$) compared to D0: 0 min. The differences were variable within the non-stressed groups, and no trends could be identified (Fig. 6.8). When the capture stressed groups were compared (ES vs NES, *t*-test) at D0: 0 min and D0: 40 min, there were no differences noted ($P > 0.05$). The RBC ($P < 0.05$, n = 20) was positively correlated to Hct ($R^2 = 0.50$) and Hgb ($R^2 = 0.67$). The RBC for the stressed groups on D0: 0 min had a positive correlation to Hct ($r = 0.71$, $R^2 = 0.50$; n = 20; $P < 0.001$) and Hgb ($r = 0.82$, $R^2 = 0.67$; n = 20; $P < 0.001$).

The Hgb data were found to have a normal distribution. The factors that affected the Hgb concentration were time ($F_{(3, 36)} = 5.38$; $P = 0.004$), exercise training and capture stress ($F_{(1.56, 55.57)} = 50.06$; $P < 0.0001$) and the interaction of these factors ($F_{(9, 107)} = 5.09$; $P < 0.0001$). There were some differences between capture stressed and non-stressed groups at D0: 0 min and D0: 40 min. The capture stressed groups (ES and NES) also decreased ($P < 0.05$) at D0: 40 min, D2 and D5 (Fig. 6.8, Table 6.1). When the capture stressed groups were compared (ES vs NES, *t*-test), the NES group had a higher Hgb than the ES group at D0: 0 min ($P = 0.031$), but not at D0: 40 min.

The Hgb for the stressed groups on D0: 0 min had a positive correlation to muscle temperature ($r = 0.49$, $R^2 = 0.24$; n = 19; $P = 0.03$), respiratory rate ($r = 0.46$, $R^2 = 0.21$; n = 20; $P = 0.04$), lactate ($r = 0.50$, $R^2 = 0.25$; n = 20; $P = 0.02$), RBC ($r = 0.82$, $R^2 = 0.67$; n = 20; $P < 0.001$), Hct ($r = 0.96$, $R^2 = 0.91$; n = 20; $P < 0.001$), creatinine ($r = 0.56$, $R^2 = 0.32$; n = 20; $P = 0.01$) and troponin I ($r = 0.45$, $R^2 = 0.27$; n = 20; $P < 0.05$). The Hgb for the stressed groups on D0: 0 min had a negative correlation to cortisol ($r = - 0.49$, $R^2 = 0.24$; n = 20; $P = 0.01$).

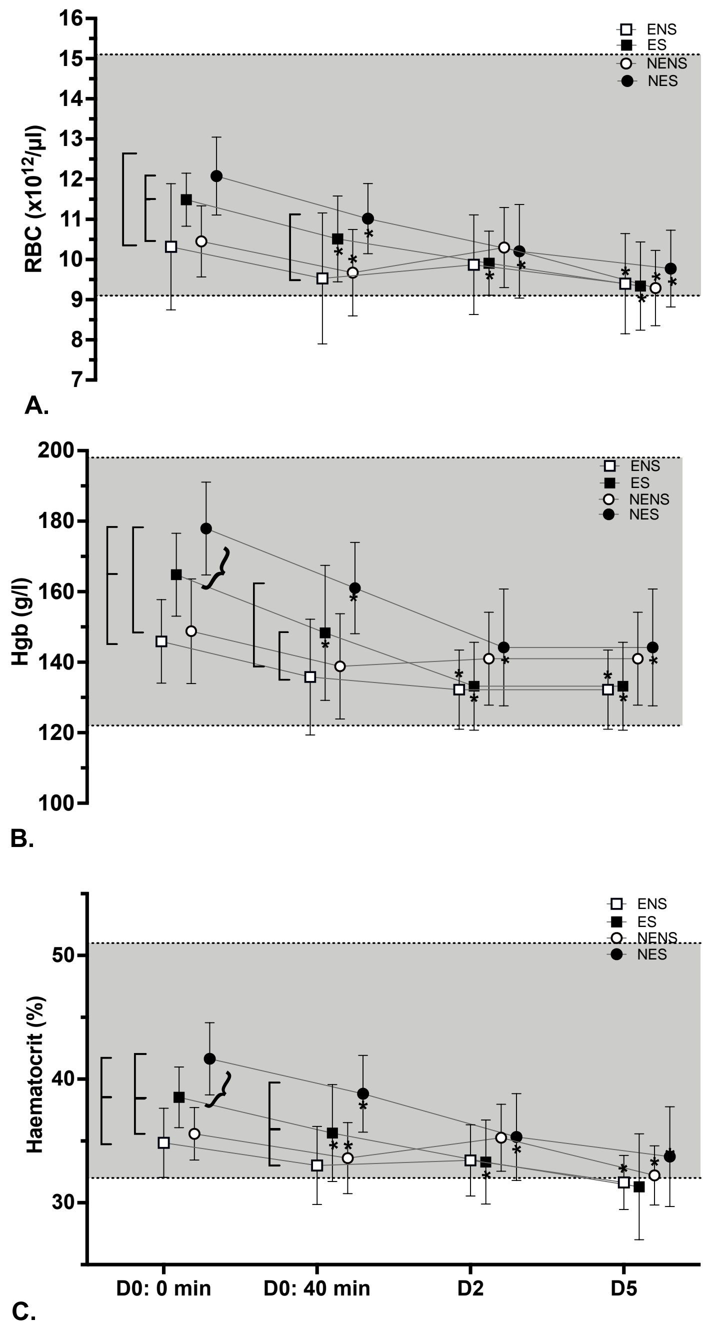


Figure 6.8 RBC, Hgb and Hct

Mean and SD of RBC (x1012/µl) for each group measured at D0:0 and D0: 40 min, D2 and D5.

Mean and SD of Hgb concentration (g/l) for each group measured at Baseline, D0:0 and D0: 40 min, D2 and D5.

Mean and SD of Hct (%) for each group measured at Baseline, D0:0 and 40 min, D2 and D5.

Gridlines and grey shaded area: reference range for healthy blesbok obtained from other studies (Seal & Schobert, 1976). Vertical brackets: different between groups ($P < 0.05$). * different from D0: 0 min within-group ($P < 0.05$). Curly bars: the difference between NES and NES groups at D0: 0 min (t-test, $P < 0.05$).

The Hct data were found to have a normal distribution. The factors that affected the Hct percentage was time ($F_{(2.49. 88.66)} = 51.13$; $P < 0.0001$), exercise training and capture stress ($F_{(3, 36)} = 4.71$; $P < 0.0001$), and the interaction of these factors ($F_{(9, 107)} = 5.21$; $P < 0.0001$). The capture stressed and non-stressed groups differed at D0: 0 min, but at 40 min only the ENS group differed from the capture stressed groups (Fig. 6.8). The concentration also decreased within the capture stressed groups at D0: 40 min, D2 and D5 compared to D0: 0 min (Fig. 6.8). When comparing ES and NES, the NES group was higher at D0: 0 min (*t*-test, $P = 0.019$), but not at D0: 40 min (Fig. 6.8). The Hct for the stressed groups on D0: 0 min had a positive correlation to muscle temperature ($r = 0.50$, $R^2 = 0.25$; n = 19; $P = 0.03$), BE_{ecf} ($r = 0.49$, $R^2 = 0.24$; n = 19; $P = 0.04$), AGap ($r = 0.47$, $R^2 = 0.22$; n = 20; $P = 0.04$), lactate ($r = 0.55$, $R^2 = 0.25$; n = 20; $P = 0.01$), RBC ($r = 0.71$, $R^2 = 0.50$; n = 20; $P < 0.001$), Hgb ($r = 0.96$, $R^2 = 0.91$; n = 20; $P < 0.001$) and creatinine ($r = 0.65$, $R^2 = 0.42$; n = 20; $P = 0.002$). The Hct for the stressed groups on D0: 0 min had a negative correlation to $cHCO_3^-$ ($r = - 0.52$, $R^2 = 0.27$; n = 19; $P = 0.02$).

Discussion

Horses, humans and dogs show increased plasma volume in response to training; this compensates for the high fluid loss during exercise (Rovira et al., 2007; Wan et al., 2017). RBC, Hct and Hgb were expected to increase post-exertion due to splenic contraction and haemoconcentration (Rovira et al., 2007; Wan et al., 2017). An increase is evident in the trained (ES) and non-trained (NES) capture stressed groups at D0. It is well established that exercise training increases red blood cell volume (Mairbäurl, 2013; Witek et al., 2017). However, there is no difference in the RBC between the ES, and NES group of blesbok (Fig 6.8). The increase in RBC in the ES group may not be apparent due to an increased plasma volume expansion. A high plasma volume is an exercise training adaptation in numerous species and may 'dilute' the RBC making the increase not apparent (Geor & McCutcheon, 1998; Mairbäurl, 2013; Rovira et al., 2007; Wan et al., 2017).

Haemoconcentration and possibly splenic contraction may increase Hct, and Hgb evident for the capture stressed groups (Rovira et al., 2007). The lesser increase of Hct and Hgb for the ES group at D0 compared to the NES group (Fig. 6.8, Table 6.1) could also be attributed to increased plasma volume seen in trained individuals mentioned above. Trained athletes have shown to have a decreased Hct which is known as 'sports anaemia' (Berg & Meyer, 2008; Foran et al., 2003). Sports anaemia is not anaemia in the traditional sense, as trained athletes have an increased total red blood cell mass and Hct. The same principle applies that an increased plasma

volume brings about the apparent decrease in Hct and Hgb in trained individuals (Berg & Meyer, 2008; Foran et al., 2003). The high plasma volume 'dilutes' the increases in Hct and, in turn, Hgb concentration. Other studies have shown variable changes in Hct to either decrease, stay the same or increase. (Berg & Meyer, 2008; Foran et al., 2003) Variations in fluid intake, fitness level, and environment may cause Hct values to fluctuate (Berg & Meyer, 2008; Foran et al., 2003). Many factors may influence RBC, Hct and Hgb fluctuations and, depending on the study, can decrease, stay the same or increase after exercise. Sample size, methodology or variations in fluid intake may account for some of the differences noted. Still, varying results may also be due to the timing of samples, training and environmental factors (Foran et al., 2003).

(ii) Leukogram

Results

The WBC data were found to have a normal distribution and did not require log transformation before further analysis. The factor that affected the WBC was time ($F_{(1.79, 63.90)} = 61.23$; $P < 0.05$), but not exercise training and capture stress ($F_{(3, 36)} = 2.16$; $P > 0.05$) or the interaction of the factors ($F_{(9, 107)} = 0.66$; $P > 0.05$) affected the WBC. There were no differences between groups at any time point (Fig. 6.9). Within groups, the WBC count on D0: 40 min was lower for the ENS, NENS and ES group ($P < 0.05$) (Fig 6.9). On D2, the WBC was higher than D0: 0 min for all groups ($P < 0.05$), and by D5, all groups did not differ from their D0: 0 min values. There were no differences between the capture stressed groups on D0: 0 min or D0: 40 min (ES vs NES, *t*-test). The WBC for the stressed groups on D0: 0 min had no positive correlations to any other variables in the correlation matrix. The WBC for the stressed groups on D0: 0 min had a negative correlation to GGT ($r = -0.59$, $R^2 = 0.34$; n = 19; $P = 0.01$), CK ($r = -0.62$, $R^2 = 0.38$; n = 20; $P = 0.004$) and troponin I ($r = -0.77$, $R^2 = 0.59$; n = 20; $P < 0.001$).

The neutrophil count data were found to have a normal distribution and did not require log transformation before further analysis. The factors that affected the neutrophil count was time ($F_{(2.173, 77.52)} = 56.78$; $P < 0.0001$), but not exercise training and capture stress ($F_{(3, 36)} = 2.01$; $P > 0.05$) or the interaction of all the factors ($F_{(9, 107)} = 1.4$; $P > 0.05$) affected the neutrophil count. The only difference was between the ENS and the ES group at D0: 0 min (Table 6.1).

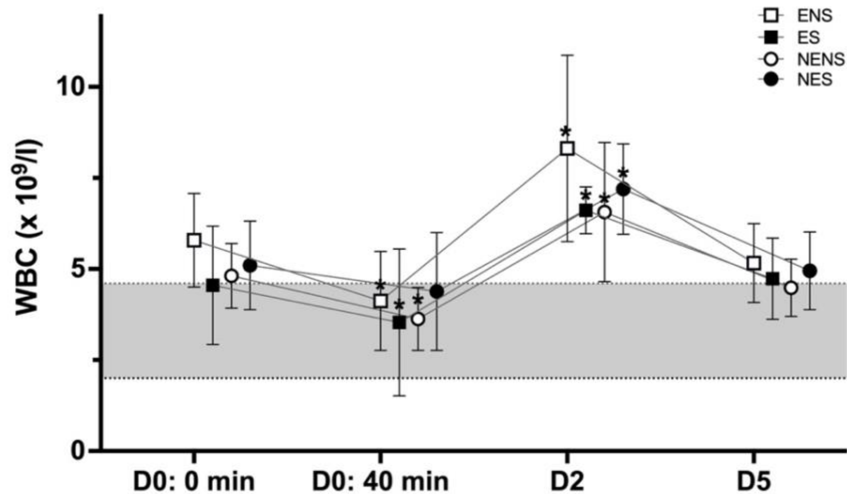


Figure 6.9 WBC

Mean and SD of the WBC ($x10^9/l$) for each group measured at Baseline, D0: 0 min, D0: 40 min, D2 and D5.

Gridlines and grey shaded area: reference range for healthy blesbok obtained from other studies (Seal & Schobert, 1976). * different from D0: 0 min within-group ($P < 0.05$).

The neutrophil count increased for all groups on D2 within groups compared to D0: 0 min. Within groups, the neutrophil count increased for the ENS group on D0: 40 min and the ES group on D5 compared to D0: 0 min ($P < 0.05$). The lymphocyte count data were found to have a normal distribution. The factors that affected the lymphocyte count was time ($F_{(1.68, 59.97)} = 20.0$; $P < 0.0001$) and the interaction of time, exercise training and the capture stress event ($F_{(9, 107)} = 2.43$; $P = 0.01$). However, the exercise training and capture stress ($F_{(3, 36)} = 0.61$; $P > 0.05$) factors on their own did not affect the lymphocyte count. Between groups, there were no differences (Table 6.1). Within groups, the lymphocyte count increased for all groups on D2 compared to D0: 0 min. The ENS group's lymphocyte count also increased on D0: 40 min, and the ES group on D5 compared to D0: 0 min.

No factors affected the immature neutrophil -, eosinophil – and basophil count. There was no difference between or within groups for these specific cell counts either. The monocyte count data were found to have a normal distribution and did not require log transformation before further analysis. The factor that affected the monocyte count was the exercise training and capture stress event ($F_{(3, 143)} = 1.85$; $P = 0.001$). Neither the time ($F_{(2.81, 133.7)} = 1.31$; $P = 0.27$) or the interaction ($F_{(9, 143)} = 1.85$; $P = 0.063$) of the factors had a significant effect on the monocyte count. The NENS and NES group differed at D0 and the ES and NENS at D5

Table 6.1 Differential white blood cell count

The differential white blood cell count values expressed as mean ± SD at D0: 0 min, D0: 40 min, D2 and D5.

The reference intervals provided are for goats (caprine = ca) (Cornell University, 2016; Rajion et al., 2001), horses (equine = eq) (Cornell University, 2016; Satué et al., 2014). Vertical bars: different between groups (*P* < 0.05). * different from D0: 0 min within-group (P < 0.05). Grey highlighted values: different between ES and NES (*t*-test, *P* < 0.05).

Parameter	Group	Reference range	Time Points			
			D0: 0 min	D0: 40 min	D2	D5
Neutrophil (mature) count	ENS	18 - 57 (ca)	64 ± 8	62 ± 13	64 ± 15	63 ± 14
	ES	22 - 72 (eq)	52 ± 7	61 ± 10*	65 ± 8*	64 ± 10*
	NENS	%	62 ± 10	64 ± 8	65 ± 6	55 ± 7
	NES		55 ± 6	61 ± 8	69 ± 10*	66 ± 7*
Neutrophil (immature) count	ENS	0 - 1 (ca)	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.6
	ES	0 – 8 (eq)	0 ± 0	0.1 ± 0.3	0 ± 0	0 ± 0
	NENS	%	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	NES		0 ± 0	0 ± 0	0.2 ± 0.6	0 ± 0
Lymphocyte count	ENS	35 - 80 (ca)	32 ± 9	33 ± 12	33 ± 14	31 ± 12
	ES	17 - 68 (eq)	43 ± 8	35 ± 10*	32 ± 8*	34 ± 9*
	NENS	%	32 ± 10	30 ± 6	31 ± 6	40 ± 8
	NES		42 ± 6	34 ± 9	26 ± 7*	30 ± 7*
Monocyte count	ENS	0 - 6 (ca)	3.1 ± 2.1	4.3 ± 2.4	2.6 ± 1.8	5.2 ± 3.7
	ES	0 - 14 (eq)	3.4 ± 1.8	3.9 ± 3.1	3.2 ± 1.5	2.2 ± 0.6
	NENS	%	5.2 ± 2.4	5.2 ± 3.1	3.2 ± 1.8	4.3 ± 1.9
	NES		2.0 ± 0.8	2.7 ± 1.3	3.5 ± 2.7	3.4 ± 1.5
Eosinophil count	ENS	0 - 6 (ca)	0.3 ± 0.5	0.4 ± 0.7	0.3 ± 0.7	1.1 ± 0.7
	ES	0 - 10 (eq)	0.8 ± 1.2	0.4 ± 0.8	0.5 ± 0.5	0.5 ± 1.0
	NENS	%	0.9 ± 1.1	0.8 ± 1.3	1.1 ± 1.3	1.1 ± 0.7
	NES		1.2 ± 1.2	2.1 ± 1.8	0.9 ± 1.4	0.8 ± 1.0
Basophil count	*ENS*	0 – 3 (ca)	0.1 ± 0.3	0 ± 0	0.1 ± 0.3	0.1 ± 0.3
	ES	0 – 4 (eq)	0.3 ± 0.7	0 ± 0	0 ± 0	0 ± 0
	NENS	%	0 ± 0	0 ± 0	0.2 ± 0.4	0.1 ± 0.3
	NES		0.3 ± 0.7	0.3 ± 0.5	0.1 ± 0.3	0 ± 0

Table 6.1 Differential white blood cell count

The differential white blood cell count values expressed as mean ± SD at D0: 0 min, D0: 40 min, D2 and D5.

The reference intervals provided are for goats (caprine = ca) (Cornell University, 2016; Rajion et al., 2001), horses (equine = eq) (Cornell University, 2016; Satué et al., 2014). Vertical bars: different between groups (*P* < 0.05). * different from D0: 0 min within-group (P < 0.05). Grey highlighted values: different between ES and NES (*t*-test, *P* < 0.05).

Parameter	Group	Reference range	Time Points			
Neutrophil:	ENS	0.5 – 0.8 (ca)	2.2 ± 0.9	2.2 ± 1.2	2.4 ± 1.5	2.5 ± 1.5
Lymphocyte Ratio	ES	1.5 – 2.5 (hu)	1.3 ± 0.4	1.9 ± 0.8	2.2 ± 0.7*	2.2 ± 1.1
	NENS		2.3 ± 1.1	2.3 ± 1.0	2.2 ± 0.5	1.5 ± 0.5
	NES		1.4 ± 0.4	1.9 ± 0.8	3.0 ± 1.3*	2.5 ± 1.0*

Within groups, there were no differences (Table 6.1). When the capture stressed groups were compared (ES vs NES, *t*-test), the ES group was higher at D0: 0 min ($P < 0.05$) but not at D0: 40 min for the monocyte count.

The N:L ratio data were found to have a normal distribution and did not require log transformation before further analysis. The factors that affected the neutrophil: lymphocyte ratio (N:L ratio) was time ($F_{(2.93,\ 104.4)} = 6.23$; $P = 0.0007$) and the interaction of the time, exercise training and the capture stress event ($F_{(9,\ 107)} = 20.0$; $P = 0.0005$). The exercise training and capture stress on their own did not affect the N:L ratio. Between groups, there were no differences in the N:L ratio. Within groups, the N:L ratio increased for the ES group at D2. The NES group increased at D2 and D5 compared to D0: 0 min.

Discussion

Possibly having Baseline haematological values available for all animals could have provided a clearer image of the stress response of the white blood cells within groups. However, control groups were available and could compare responses between groups exposed to the capture stress and exercise training. Previous studies on humans and animals show an immediate increase in white blood cells in response to acute exercise, which may likely be attributed to haemodynamic changes or possibly be a consequence of the adrenal response (Alves & Santos, 2016; Sand, 2013; Satué et al., 2014). The WBC at D0: 0 min were similar for all groups, but at D0: 40 min decreased. This decrease in WBC is likely due to fluid shifts post-exercise (McKeever & Lehnhard, 2014; Otto et al., 2017; Rovira et al., 2007). The WBC increased at

D2 in all groups; this may be due to the muscle biopsy and associated inflammatory response. An increase in the immature neutrophil count would be anticipated with an inflammatory response, but this was not apparent in the animals (van Grinsven et al., 2019). An increase in WBC in response to muscle repair after severe exertion could be expected (Alves & Santos, 2016; Satué et al., 2014) but the increased WBC on D2 was, for all, not only the capture stressed groups.

A typical stress leukogram presents with a normal or increased white blood cell count, right shift neutrophilia, eosinopaenia, lymphopaenia and a mild monocytosis (Grzelak et al., 2017). The stress leukogram is universal across species, especially the increase of neutrophils and decreased lymphocytes (Davis et al., 2008). The white blood cell count for all groups is slightly above the reference interval at D0: 0 min. The mature neutrophil count increased for the ES group at D0: 40 min and appeared raised for the NES group, but it was not significant. At D2 and D5, the neutrophil counts were increased for both capture stressed groups. Not typical of a stress leukogram, the lymphocyte count was higher at D0: 0 min than at D2 and D5. A possible explanation of the increase in the lymphocyte count is discussed below. The eosinophils and monocytes also did not change for any of the groups.

The N:L ratio is a sensitive indicator of stress and increases when animals are exposed to stress or cortisone treatment (Gross & Siegel, 1983; Rajion et al., 2001). Goats during transport have shown to have an immediately increased N:L ratio that decreases at 6 and 12 hours after transportation (Rajion et al., 2001). The capture stressed groups have an apparent lower N:L ratio at D0 than the-non stressed groups, but it was not significant. The N:L ratio increased within the capture stressed groups at D2 and D5 compared to D0. The increase in the lymphocyte counts on D0 in the capture stressed groups would have reduced the N:L ratio. The likely reason for the increased lymphocyte counts on D0 is the response of the blesbok to exercise during the capture stress event. Just the anticipation or stress of exercise results in an immediate lymphocytosis that will result in a reduced N:L ratio. A reduced N:L in response to exercise is evident in humans and horses (Sand, 2013; Snow et al., 1983). In horses the N:L ratio decreased 3-4 hours post-exercise due to decreased neutrophils and increased lymphocytes. At 6 hours post-exercise, the N:L ratio had returned to normal (Snow et al., 1983). The leukogram in the current study is difficult to analyse. The only effect apparent on the leukogram is the effect of exercise on the capture stressed groups, i.e., decreased N:L on D0. The direct impact of stress is more difficult to tease out and obtaining Baseline values might have provided more information.

6.5 Conclusion

An increased blood glucose and lactate was seen in response to exercise on D0 after the capture stress event. The ES group had lower lactate and glucose concentrations than the NES group at D0: 0 min. The reduced lactate increase in the ES group, is a typical expected fitness adaptation. The lesser increase in glucose concentration on D0 in the ES group as compared to the NES group, is not a typical fitness adaptation and may be indicative of a reduced stress response in the ES group. Muscle damage was evident by the increase in AST, CK and troponin I in response to the capture stress. The muscle enzyme concentration varied greatly, which may indicate individual susceptibility to muscle damage or cardiac stress. There was no hepatic injury evident in the blesbok in response to capture stress. Creatinine and urea increased in response to capture stress, which may be because of the stress response or acute renal injury inflicted by the capture stress. The increased cortisol for all groups on D0, including non-stressed, was similar to the finding in the study by Fitte (Fitte, 2017). Blood cortisol however is unreliable as a quantative marker of stress and should not be used on its own.

The N:L ratio appeared reduced for the capture stressed groups on D0 which is typical in humans where neutrophils decrease and lymphocytes increase in response to exercise resulting in a reduced N:L ratio (Sand, 2013; Snow et al., 1983). Increases in RBC, Hct and Hgb were also evident in the capture stressed groups at D0. RBC, Hct and Hgb were expected to increase post-exertion due to splenic contraction and haemoconcentration (Rovira et al., 2007; Wan et al., 2017).

This biochemistry and haematological results discussed in this chapter were the remaining analyses that contributed to the overall understanding of the physiological changes and adaptations in this blesbok in this study. The last chapter (Chapter 7) is an overarching discussion of the results obtained and the conclusions derived from this study's findings.

CHAPTER 7

DISCUSSION AND CONCLUSION

7.1 Summary of findings

Capture myopathy in wildlife resembles exertional myopathy in humans or malignant hyperthermia in pigs (Martucci et al., 1992). Apart from malignant hyperthermia, for which the cause is known, the causes for exertional and capture myopathy are multifactorial (Prelude and Chapter 1). Of these, physical inactivity may increase susceptibility to capture stress and the possible development of capture myopathy (Harthoorn, 1979). The current study aimed to investigate the physiological impact and potential benefit of exercise training blesbok before a capture stress event.

As discussed in the Prelude, the goals of this study were to determine if daily exercise training is feasible in wild antelope, if any physiological adaptations occurred and whether it would prove protective against the consequences of capture stress. The methodology and feasibility of the daily exercise training detailed in Chapter 3 showed that it is a feasible endeavour, albeit an expensive, laborious, and a logistical challenge. This study laid the foundation for future exercise training studies in wild animals, in particular wild antelope. Exercise training can be a feasible venture in some situations and can be introduced in simple ways to ensure adequate physical health. The additional inherent benefit of daily exposure to capture procedures allows for habituation and desensitisation to stress factors during future capture events. Exercise and habituation to capture procedures are essential aspects of wellbeing and welfare that should not be ignored in wildlife management.

The study aimed to physiologically challenge the animals to observe physiological differences in response to capture stress. The exercise training period was four weeks, and the exercise-trained capture stressed group (ES) showed specific exercise training adaptations, similar to previous studies on other species. The NES group had evidence of increased physiological deficits that likely would have become overt if the capture stress event was longer or more strenuous.

7.2 Physiological effects of the capture stress event

A typical physiological response to exertional capture stress is hyperthermia, tachycardia, hyperventilation, hyperlactaemia, hyperglycosaemia, a bicarbonate deficit, mild acidaemia and hyperkalaemia (Fitte, 2017; Harthoorn, 1976; Jessup et al., 1987; Kock et al., 1987; Mandelman & Skomal, 2009). Evidence of mild skeletal, cardiac, and liver insult may also present acutely (Fitte, 2017; Harthoorn & Van der Walt, 1974).

In the current study, the blesbok exposed to the capture stress event presented with typical hyperthermia, tachycardia, hyperventilation, hyperlactaemia, hyperglycosaemia, decreased HCO_3^-, mild acidaemia, a base deficit and a high anion gap (Chapter 4-6). The variables that changed in response to the capture stress event are normal responses to an acute physical exertion episode in mammals, including humans. Additionally, chronic endurance training resulted in some physiological responses to be blunted and seen as normal adaptations to training. However, some physiological responses were not anticipated.

The blesbok were not severely acidaemic, with blood pH ranging between normal to slight acidaemia. The ability to maintain pH within a normal physiological range indicates that the blesbok could physiologically compensate for the capture stress. Hyperkalaemia is a typical response to capture stress and was expected in the capture stressed groups at D0: 0 min (Linderman et al., 2008; Phillips et al., 2015). However, all the blesbok groups appeared to have increased blood K^+ at D0: 0 min, that decreased at D0: 40 min. The animals also appeared to be hyponatraemic but did not result in complications directly after the capture stress event or after immobilisation reversal, indicating that the low Na^+ concentrations may not have been clinically significant. The mild or lack of electrolyte imbalance in response to the capture stress event was not anticipated as in other capture stress studies where the electrolyte disruption was very evident (Chalmers & Barrett, 1977; Harthoorn, 1976; Harthoorn & Van der Walt, 1974; Jessup et al., 1987; Phillips et al., 2015). This relative electrolyte stability after the capture stress event on D0 may indicate that the capture stress event was not as severe as expected. The long habituation process (± 6 weeks) in the exercise trained and even non-exercise trained groups (through the indirect exposure to capture stressors, immobilisation and sorting of animals), may have played a significant role in reducing the psychological and consequently the physiological stress experienced.

The enzymes indicative of muscle damage, AST, CK and troponin I, expectedly increased in response to the capture stress. CK and troponin I appeared to follow a similar pattern, showing

a mild increase at D0: 40 min to D2 and returned to within normal reference range at D5. Blood AST concentrations had an expected delayed increase (typical of ruminants) at D2 and subsequently returned to normal at D5 (Antonelli et al., 2007). There was variability in muscle enzyme concentration obtained, with some individuals showing markedly higher concentration, which may indicate individual susceptibility to muscle damage or cardiac stress.

There was no hepatic injury evident in the blesbok in response to capture stress. Only an increase was noted in GLDH in the ES group (Fig 6.6). The total bilirubin did not increase in the blesbok on D0. In response to exertion, bilirubin increases in humans, whereas, in horses, it does not increase in response to exertion (Swift et al., 2012; Witek et al., 2017). Similarly, the capture stress may not have been exhaustive enough to elicit liver injury.

For the renal enzymes, only creatinine increased in response to the capture stress (Padilha et al., 2017), which was expected. Creatinine may increase due to capture stress due to catecholamine related renal vasoconstriction, but also possibly due to the nephrotoxic effects of myoglobin and haem from muscle and haemoglobin breakdown (Bartsch et al., 1977; Baxter & Moore, 2003; Herrá Ez et al., 2007; Vanholder et al., 2000). Creatinine increased and returned to normal by D2 after the capture stress event (Fig. 6.4).

Urea increased at D0 for all groups and decreased at D5 (Fig 6.4). Typically, urea would only increase in capture stressed groups (Harthoorn, 1976; Jessup et al., 1987). The increased cortisol for all groups on D0 (Fig. 6.7) and its diuretic effect may have resulted in acute dehydration and urea elevation. The cortisol for the capture stressed groups appeared higher than the non-stressed groups, but the difference was not statistically significant. The increased cortisol for all groups, including non-stressed, was similar to the finding in the study by Fitte (2017). Blood cortisol should not be used on its own as a marker of stress, as there are factors that could have impacted these results, including diurnal rhythms of cortisol and the effects of opioids (Arvidson et al., 2018; Grzelak et al., 2017).

The N:L ratio is a better marker of stress than cortisol. Stress on its own is typically associated with a high N:L ratio due to the increased neutrophils and decreased lymphocytes apparent in a stress leukogram (Davis et al., 2008; Grzelak et al., 2017). The N:L ratio appeared reduced for the capture stressed groups, being lower at D0 than at D2 and D5. Although the N:L ratio of the capture stressed groups appeared lower than the non-stressed groups, it was not statistically significant. Typically, neutrophils decrease, and lymphocytes increase in response to exercise in humans and horses, resulting in a reduced N:L ratio (Sand, 2013; Snow et al.,

1983). Increases in RBC, Hct and Hgb were also evident in the capture stressed groups at D0. RBC, Hct and Hgb were expected to increase post-exertion due to splenic contraction and haemoconcentration (Rovira et al., 2007; Wan et al., 2017).

In summary, the capture stress event appears to have induced most of the typical pathophysiological changes expected for exertional capture stress. The capture stress event was inadequate to overtly disrupt acid-base or electrolyte balance, or cause severe damage to skeletal and cardiac muscle, kidneys, or the liver. A study in blesbok, where animals were not exercised trained, but exposed to a similar capture stress protocol, elicited greater pathophysiological changes (Fitte, 2017). The exercise training and the habituation period of 6 weeks possibly assisted in desensitising all the blesbok in the study, albeit through indirect exposure to the stressors.

7.3 Physiological effects of immobilisation

Opioids are the preferred choice of drug to immobilise wild antelope. The use of opioids in animals are renowned to depress respiration rate and minute ventilation. Hypoventilation, hypoxaemia, hypercapnia and progressive acidaemia may result when opioids are administered (La Grange, 2006; Morkel et al., 2010). The use of opioids may also result in systemic and pulmonary hypertension, decreasing blood circulation to tissues and altered pulmonary perfusion resulting in ventilation-perfusion mismatch or alveoli membrane changes that lead to decreased O_2 diffusion across the alveolar membrane (increased A-a gradient) and ultimately hypoxaemia (Meyer, 2009; Zeiler & Meyer, 2017). The sympathetic effect of opioids can also result in an excitatory effect that may aggravate the stress response during capture (Fitte, 2017; Pfitzer et al., 2020; West et al., 2007).

Azaperone results in quicker recumbency and an overall smoother immobilisation and achieves this through reducing some of the side effects of opioids, particularly the excitatory effects (La Grange, 2006). The use of azaperone is thought to have minimal impact on respiration but does have an antagonist effect on α_1-adrenergic receptors that result in vasodilation, increasing heat loss during immobilisation (Hodgkinson, 2007; Sawicka et al., 2015). The α-adrenergic blocking effect may result in decreased stress-induced renal vasoconstriction during capture, which may have a renal protective function (López-Olvera et al., 2007).

The effects of the opioids were apparent in the changes to the vital signs and blood gas variables in the immobilised blesbok of the present study. Although a reduced resting heart rate is a well-known adaptation to exercise training, the cardiorespiratory effects of opioids may have directly influenced the heart rate data, making it difficult to conclude whether the effect was because of the capture stress event or the exercise training (Meyer, 2009; Pfitzer et al., 2020). It would only be possible to obtain the resting heart rates through remote logging devices that could not be used in this study due to cost. The blesbok groups exposed to capture stress were expected to be tachypnoeic on D0. During the capture stress event, some of the blesbok were observed to be breathing with open mouths, an indirect indicator of the level exertion animals might have experienced. During immobilisation on D0, the respiratory rates of all the capture stressed groups fell within the normal range of the non-stressed groups (Fig. 5.4), indicating the suppressive effect of the opioids were in effect (McKenzie, 1993; Meyer, 2009; Paterson, 2014).

Immobilisation may cause acidaemia due to hypoventilation resulting in a failure to expel excess CO_2 (Meyer et al., 2010). The immobilised animals that were not subjected to the capture stress event presented with a low normal to normal pH and an increased $PaCO_2$ (hypercapnia). The ES group presented with a normal $PaCO_2$ and the NES group with a decreased $PaCO_2$ (hypocapnia) on D0. $PaCO_2$ is an excellent indicator of ventilation, and the reduced $PaCO_2$ indicates increased ventilation in the capture stressed groups at D0: 0 min (Castro et al., 2021; Wagner, 2015). Reduced ventilation in the capture stressed groups because of opioids become apparent again at D0: 40 min, as the groups are hypercapnic. The non-stressed blesbok in the study generally had a low normal pH. Only the NES group had slightly acidaemic values on D0. The pH was adequately maintained through the study period, except for the slight acidaemia in the NES group on D0 despite variation in $PaCO_2$ from high, normal to low. This indicates adequate respiratory compensation took place for the blesbok overall to maintain the blood pH.

The animals in all the groups, at all the time points, except D0: 40 min, were hypoxaemic (some were severe < 60mmHg). The A-a gradients were higher for the capture-stressed groups compared to the non-stressed groups at D0: 0 min (Fig. 5.3) The combination of capture stress and etorphine may have increased pulmonary vascular pressure, causing congestion and possibly oedema, resulting in reduced oxygen diffusion and increased A-a gradients (Buss et al., 2016; Kim & Ha, 2016). The improved PaO_2 at D0: 40 min aligned with an improved A-a

gradient at D0: 40 min. The improved PaO_2 was likely due to the waning effect of etorphine resulting in a decreased pulmonary hypertension.

Interpretation of electrolyte changes is difficult when animals are immobilised after exertion. Immobilisation will result in acidaemia, hyperkalaemia and other electrolyte disturbances (Harthoorn, 1976; Vanholder et al., 2000). Therefore, it is difficult to distinguish what changes, especially if mild as in this study, were from immobilisation or from the capture stress. Except for blood Ca^{2+} (Fig. 5.7), none of the electrolytes showed a clear physiological change in response to the capture stress.

7.4 Exercise-induced physiological changes

Exercise training is expected to induce specific physical and physiological adaptations. Some of the typical physiological markers were difficult to evaluate due to the simultaneous immobilisation effects on the physiological responses of the immobilised animals. Control groups for immobilisation were included to evaluate the effect of the immobilisation on blesbok that were exercise trained and non-exercise trained (ENS and NENS) without the effect of the capture stress.

As a collective, weight loss occurred in the trained blesbok when compared to the untrained blesbok, which is one of the main outcomes of exercise training (Chapter 4). The reasons for the weight loss may have been a reduction in fat percentage or muscle fibre diameter, but these were not measured in the current study (Katch et al., 2010; Kohn et al., 2011; Swift et al., 2014; Trappe et al., 2006).

The magnitude by which body temperature increased was lower in the trained (ES) compared to the untrained blesbok (NES) exposed to capture stress (Fig. 4.1). Blesbok use both sweating and panting to allow for evaporative cooling when required (Fitte, 2017). Improved thermoregulation is an expected finding with exercise training due to an increased ability to dissipate heat mainly through sweat and improved metabolism (e.g. increased mitochondrial volume and oxidative enzyme to produce ATP) (Fortney & Vroman, 1985; Geor & McCutcheon, 1998; Périard et al., 2015). However, capture-induced hyperthermia can also be reduced by habituating the animals to capture stressors (Meyer et al., 2008). In the present study, the ES group might have been better habituated from their regular exercise training programme, which may explain the lower temperatures at D0: 0 min.

The respiratory rate of the NES group post-capture stress was higher than the ES group, despite the depressive effect of the opioids used (Fig. 4.5). This increased respiratory rate may be supportive of a delayed return to normal respiratory rate post exercise compared to the ES group, which is a well-known occurrence in untrained individuals (Depiazzi & Everard, 2016; Folinsbee et al., 1983). The increased respiratory rate was associated with increased ventilation in the NES group, as was evident by the decreased $PaCO_2$ and increased PaO_2 compared to the ES group (Fig. 5.1). The increased respiratory rate and ventilation of the NES group could indicate that animals in this group had a greater drive to increase ventilation. Elevations in plasma $PaCO_2$ and H^+ produced from energy metabolism or the increased hyperthermia in the NES group after the capture stress may have driven the increased ventilation (Delpierre et al., 1978; Robertshaw, 2006).

Aerobically untrained animals will produce more lactate during exercise, and therefore a lower pH is to be expected for the NES group (Edwards et al., 1969; Messonnier et al., 2013). The NES group was not more acidaemic compared to the ES groups after the capture stress. However, the pH for the NES group did display a more significant variance than the ES group on D0. The NES group presented with an increased number of severely acidotic animals, including the most acidotic blesbok in the whole study period (section 5.3 (ii), Fig. 5.5). The NES group was hypocapnic (due to the hyperventilation), and the $PaCO_2$ was significantly lower than the ES group (*t*-test). Additionally, the $cHCO_3^-$ and BE_{ecf} were lower for the NES group than the ES, indicating an increased buffering requirement to correct the metabolic acidosis for the NES group.

The near-normal pH for the NES group shows that both the respiratory and metabolic compensation in reaction to the capture stress and immobilisation was adequate. Increased demands to correct the expected respiratory acidosis in the NES group is evident by hypocapnia on D0, yet normal pH. Similarly, an increased demand to correct the metabolic acidosis is evident by the significant $cHCO_3^-$ and BE_{ecf} deficit. Perhaps if the capture stress event were of longer duration, a more overt difference in the acid-base balance would have been observed between the ES and NES groups on D0. In contrast to the NES group, the blood pH, $cHCO_3^-$, BE_{ecf} and $PaCO_2$ values for the ES group were all within normal physiological range on D0. The normal values obtained for the ES group supports an improved ability to maintain blood gas, acid-base homeostasis, and buffering capacity in response to the capture stress and immobilisation.

A typical spike in blood glucose and lactate was seen in response to exercise on D0 after the capture stress event (Adams, 2013; Ferraz et al., 2008). The ES group had a lower lactate and glucose concentration than the NES group at D0: 0 min. Lower blood lactate concentration indicate a higher lactate threshold and is a recognised training adaptation in numerous species (Ferraz et al., 2008; Messonnier et al., 2013). The lower increase of glucose in the ES group, than the NES group, was not an anticipated finding but may indicate improved glucose regulation post-capture stress or a decreased stress response.

The ES had a significantly lower creatinine concentration than the NES group on D0, possibly due to an improved creatinine clearance rate or increased plasma volume for this group (Wyngaert et al., 2018). The urea and creatinine concentration returned to normal for the blesbok by D2, but whether any subclinical damage occurred would need to be investigated on histological analysis of the kidneys.

Further possible evidence of exercise training adaptations was seen in the haematology results in that the ES group had a lower Hct and Hgb compared to the NES group on D0. A lower Hct and Hgb support a likely increased plasma volume, a well-known adaptation to exercise training (Geor & McCutcheon, 1998; Mairbäurl, 2013). Hct and Hgb would also be decreased in the ES group if there was a decreased capture stress response due to habituation in the ES, resulting in reduced splenic contraction and haemoconcentration (Rovira et al., 2007).

The immobilised capture stress groups experience typical metabolic and respiratory acidosis, that was according to the results, well compensated through respiratory and metabolic mechanisms. However, the capture stress was not severe enough to induce acidaemia, severe electrolyte imbalances or organ injury.

7.5 Limitations

The project was challenging due to the various locations, people and logistics required. Finding a suitable study site was onerous, and the blesbok had to be acquired from Limpopo and translocated to Wildlife Pharmaceutical research farm near Nelspruit in Mpumalanga (South Africa). Although the study site provided the necessary support and infrastructure required, the specific lab equipment and consumables had to be acquired and transported to the site from Pretoria and Cape Town. A large, refrigerated container had to be arranged on-site for the storage of samples and carcasses. The project was a logistical feat, and limitations are expected with this size and complexity of the project.

The blesbok obtained were not uniform in terms of sex, age and condition as would be ideal for a physiological study. The lack of uniformity of subjects used is a typical limitation of wildlife studies where it can be difficult to source subjects for a project. The sexes were allocated equally to the groups and any animals that appeared to not be in full normal health were removed.

There is an apparent overall lower or possibly different capture stress response induced in the blesbok in the current study compared to the blesbok in the Fitte (2017) study. The reduced lesser muscular injury and reduced maximum rectal and muscle temperatures in the present blesbok study compared to the Fitte (2017) blesbok study - possibly indicates a reduced stress response to capture. The prior exercise training intervention may have been the primary contributor to the level of habituation for all animals in this study, which may have reduced the capture stress response. The non-exercise trained groups of blesbok were housed with the exercise-trained groups. Thus, all groups received a certain level of unavoidable habituation by the procedures leading up to and during exercise training in the exercise-trained groups. In the setting of this study, habituation of all subjects to the capture stressors was inevitable, even by indirect exposure of the non-exercise trained groups. Any form of daily interaction (feeding, cleaning, monitoring) may decrease the stress response during other subsequent procedures.

The blesbok response to capture stress could be better evaluated if monitoring occurred during the capture stress event up to recumbent immobilisation. The implant of data loggers and remote blood sampling techniques can be employed to obtain this data without inducing stress on the animal (Hattingh et al., 1988; Whitford & Klimley, 2019). However, the size and cost of the equipment required, the collection method and storage of blood samples can all be problematic when using these techniques (Qvist et al., 1986; Whitford & Klimley, 2019).

Immobilisation with opioids causes significant physiological effects which can mask the physiological responses to exercise and stress and is an inherent limitation with studies in wildlife. Game capture utilises immobilisation with opioids frequently, thus the effect of drugs needs to also be accounted for in wildlife studies to evaluate the benefit that any interventions may have.

7.6 Recommendations

Capture myopathy is a multifactorial condition that appears to have many contributing factors, stress being the primary factor. It seems that lack of exercise training may play a role in

increased physiological stress for these proposed 'unfit' animals, which in turn, may predispose them to develop capture myopathy. Animals are often captured and kept as interim measure in boma housing, or kept in environments where limited movement is possible or required i.e. zoos and small wildlife camps. Animals in confined environments with stationary feeding points will result in a decrease in physical activity. Physical fitness adaptations may deteriorate quickly under such circumstances, and in humans, horses, fish and rats 'detraining' is already evident after 2 – 3 weeks of physical inactivity (Coyle et al., 1985; Craig et al., 1991; Harkins et al., 1993; Lu et al., 2020). Investigating the physiological and morphological changes due to boma housing or confinement of wild animals as a form of 'detraining' would provide information on the impact of such management interventions on animal health and well-being. However, animals may also be desensitised to many stressors during boma or other types of confinement, and the habituation may benefit the animals in that they are less stressed during handling. More research is required to see whether exercise training per se can prevent capture myopathy from developing in wild antelope.

Further investigation into individual predispositions to developing capture myopathy is an essential research area. There are inherent predispositions and external factors (outlined in Chapter 1) regarding species, skeletal muscle composition, muscle myopathies, nutritional factors etc. that may make animals more prone to capture myopathy. In this study blesbok were selected due to their calmer nature compared to for example impala and springbok (Chapter 3). Blesbok incur less injuries and die less during handling and capture operations and are reported to develop capture myopathy less frequently, which may have affected the clinical findings. Selecting another species that is reported to be more susceptible to capture myopathy for exercise training, may deliver increased clarity on the benefit of exercise to more susceptible species.

Elevated cardiac troponin I induced by exercise in certain individual blesbok may indicate possible underlying cardiac pathology. The variable creatine kinase response could also indicate an underlying predisposition in terms of muscle morphology or pathology. Evaluating animals for any subclinical injury, through biopsy and histological analysis, may also be helpful to detect subclinical renal and hepatic insult.

For a future exercise training study, a longer exercise training duration may result in even more overt exercise-induced adaptations in trained animals. A longer capture stress event may illicit clearer pathophysiological consequences in non-exercise trained animals. Capture operations are often under severe pressure to catch animals against a deadline and may continue to pursue

animals far beyond a 20-minute time limit. Housing the non-stressed control groups completely separately may also assist to prevent inadvertent habituation of all animals to the capture stressors. An important consideration is to ensure increased caloric intake in all animals when including increased daily activity for animals, as a loss of condition or weight loss is not always perceived positively in animals.

Continued research on how to alleviate the cardiopulmonary suppression of opioids or the use of alternative drug combinations is important. The immobilisation drugs may play a role in aggravating capture stress and the development of capture myopathy. Opioids profoundly affect the cardiorespiratory system, making it very difficult to study the effects of exercise training and capture stress. Remote monitoring and sampling techniques should also be explored to eliminate the impact of immobilisation on the animal's physiology.

In terms of wildlife management, the advantage of prior exposure to capture stressors and exercise training of animals is double fold. The animals develop increased physiological resilience to capture stress and have decreased sensitivity to developing the most common psychological stressors. The implementation of exercise training will be situationally dependent and more practical in captive or semi-captive situations. Many factors like the value of animals, threatened status, terrain and holding environment will need to be considered in implementing exercise training.

7.7 Conclusion

Physiological and psychological stress is an inevitable part of the capture and translocation process of wild animals. During capture, the inflicted stress and physical exertion would typically occur with long or short intense pursuit, capture, restraint, or transportation of wild animals. Capture myopathy may be a lethal consequence of the stress experienced during capture. Rhabdomyolysis is the lethal trademark of capture myopathy syndrome due to the severe impact on the kidney from the muscle breakdown products.

This study aimed to evaluate the differences between exercise-trained and non-exercise trained animals in response to a typical capture stress event. The exercise trained blesbok showed an improved physiological response to the capture stress, indicated by better thermoregulation, acid-base compensation, and lactate threshold. Additionally, there is evidence for possible improved glucose regulation, renal function and haemodynamic shifts that need to be further investigated to validate if these are also exercise-induced benefits. These adaptations contribute

to a delay in the onset of fatigue and increased physiological resilience to capture stress. The untrained capture stressed blesbok showed an earlier onset of fatigue, and specific physiological markers indicated that compensatory homeostatic mechanisms were placed under strain. The possible failure of physiological homeostasis could trigger the pathophysiological cascade that forms part of the capture myopathy syndrome. Therefore, the benefits of exercise training before capture will reduce the risks associated with capture and improve animal welfare, thus warranting the additional effort and costs that may incur to implementing training programmes before capture events.

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APPENDIX

Table 9.1 Sampling times and environmental conditions for the different groups on the different sampling days

T = Ambient temperature (°C); WBGT = wet bulb globe temperature (°C); Rel. H = relative humidity (%). Each group (n = 10) had been subdivided into smaller groups (n = 5) i.e., NENS Group 1 and NENS Group 2 to facilitate darting, immobilisation and sample collection. Due to the number of groups each time point had to be sampled over two days i.e., "D0" over day 1 of 2 and day 2 of 2. *On D0: Day 1 of 2 the animals in the groups NENS and ENS were wet due to the rain during the night. ** On D0: Day 2 of 2 the animals in groups NES and ES were doused post the capture stress intervention to simulate the rain from the groups on Day 1 of 2.

			Sampling Day					
			D0		D2		D5	
			Day 1 of 2*	Day 2 of 2**	Day 1 of 2	Day 2 of 2	Day 1 of 2	Day 2 of 2
Not-Exercised-Not-Stressed (NENS)	Group 1	Time	13:19		11:11		13:32	
		T (°C)	13.5		17.6		24.7	
		WBGT (°C)	13.4		17.2		21.7	
		Rel. H (%)	100		89.8		52.5	
	Group 2	Time	2:33		09:56		3:47	
		T (°C)	14.2		17.8		23.2	
		WBGT (°C)	14.3		17.4		16.7	
		Rel. H (%)	99.1		98.7		48.6	
Not-Exercised-Stressed (NES)	Group 1	Time		08:20		09:15		07:09
		T (°C)		14		19.7		14.8
		WBGT (°C)		16.4		17.8		13.5
		Rel. H (%)		77.5		71.2		93.5
	Group 2	Time		13:43		11:21		11:05
		T (°C)		21.5		21.4		22.8
		WBGT (°C)		17.4		19.8		22.7
		Rel. H (%)		47.9		59.1		65.8
Exercised-Not-Stressed (ENS)	Group 1	Time	08:55		08:50		07:52	
		T (°C)	18.4		17.1		14.2	
		WBGT (°C)	19.8		16.4		13.6	
		Rel. H (%)	75		88.5		79.5	

Table 9.1 Sampling times and environmental conditions for the different groups on the different sampling days

T = Ambient temperature (°C); WBGT = wet bulb globe temperature (°C); Rel. H = relative humidity (%). Each group (n = 10) had been subdivided into smaller groups (n = 5) i.e., NENS Group 1 and NENS Group 2 to facilitate darting, immobilisation and sample collection. Due to the number of groups each time point had to be sampled over two days i.e., "D0" over day 1 of 2 and day 2 of 2. *On D0: Day 1 of 2 the animals in the groups NENS and ENS were wet due to the rain during the night. ** On D0: Day 2 of 2 the animals in groups NES and ES were doused post the capture stress intervention to simulate the rain from the groups on Day 1 of 2.

			Sampling Day					
			D0		D2		D5	
			Day 1 of 2*	Day 2 of 2**	Day 1 of 2	Day 2 of 2	Day 1 of 2	Day 2 of 2
	Group 2	Time	10:22		09:58		10:33	
		T (°C)	14		17.8		19.9	
		WBGT (°C)	13.2		17.4		20.3	
		Rel. H (%)	89.5		87.7		69.0	
Exercised-Stressed (ES)	Group 1	Time		10:31		10:20		09:00
		T (°C)		18.2		21.5		18.8
		WBGT (°C)		15.3		20.8		18.1
		Rel. H (%)		57.3		64.0		79.6
	Group 2	Time		15:33		08:20		14:09
		T (°C)		21.1		23.1		25.5
		WBGT (°C)		17.1		19.9		17.8
		Rel. H (%)		50.7		58.6		40

Table 9.2 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured

Many physiological variables did not have a normal distribution and had to all be log transformed to perform a Pearson's correlation matrix analysis. R^2 was calculated from the *r*-value in the Pearson's correlation matrix (Hauke & Kossowski, 2011).

Grey highlighted values: significant ($P < 0.05$) correlation (*r*) and R^2 values between the physiological variables recorded within the stressed (NES and ES) groups (n = 20) on D0: 0 min directly after the capture stress event.

Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Rectal T	Muscle T	RR	HR	pH	$PaCO_2$	PaO_2	$cHCO_{3-}$	$BE_{(ecf)}$	Na^+	K^+	Ca^{2+}	Cl^-	AGap	Glu
Rectal T	***r***		0.48	0.25	0.001	0.08	-0.25	0.21	-0.30	0.24	-0.14	-0.10	-0.14	0.04	0.04	-0.03
	R^2		0.23	0.06	0.00	0.01	0.06	0.04	0.09	0.06	0.02	0.01	0.02	0.00	0.00	0.00
	P		0.04	0.29	1.00	0.74	0.29	0.38	0.21	0.32	0.57	0.69	0.57	0.86	0.87	0.89
	n		19	20	20	20	20	20	19	18	20	20	18	20	20	20
Muscle T	***r***	0.48**		0.48	0.01	-0.31	-0.38	0.32	-0.46	0.24	0.09	-0.11	-0.04	0.33	0.33	0.38
	R^2	0.23		0.23*	0.00	0.10	0.14	0.10	0.21	0.16	0.01	0.01	0.00	0.12	0.10	0.15
	P	0.04		0.04	0.96	0.20	0.12	0.19	0.06	0.10	0.71	0.66	0.88	0.17	0.18	0.10
	n	19		19	19	19	19	19	18	19	19	19	17	19	19	19
RR	***r***	0.25	0.48**		0.20	-0.16	-0.45**	0.64**	-0.33	0.33	-0.16	0.01	-0.11	0.05	0.28	-0.18
	R^2	0.06	0.23		0.04	0.03	0.20	0.40	0.11	0.11	0.03	0.00	0.01	0.00	0.08	0.03
	P	0.29	0.04		0.40	0.50	0.05	0.003	0.17	0.16	0.50	0.97	0.70	0.82	0.23	0.45
	n	20	19		20	20	20	20	19	19	20	20	18	20	20	20
HR	***r***	0.001	0.01	0.20		-0.22	-0.38	0.16	-0.54**	0.55**	0.03	0.03	0.37	0.37	0.24	0.14
	R^2	0.01	0.00	0.04		0.05	0.14	0.02	0.29	0.30	0.00	0.00	0.09	0.14	0.06	0.02
	P	1.00	0.96	0.40		0.36	0.10	0.52	0.02	0.02	0.90	0.89	0.23	0.11	0.30	0.56
	n	20	19	20		20	20	20	19	19	20	20	18	20	20	20

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Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Rectal T	Muscle T	RR	HR	pH	$PaCO_2$	PaO_2	$cHCO_3^-$	$BE_{(ecf)}$	Na^+	K^+	Ca^{2+}	Cl^-	AGap	Glu
pH	***r***	0.08	-0.31	-0.16	-0.22		0.36	-0.42	0.55**	-0.71***	-0.36	-0.07	-0.11	-0.55**	-0.75***	-0.31
	R^2	0.04	0.10	0.03	0.05		0.13	0.17	0.31	0.50	0.13	0.00	0.01	0.30	0.57	0.09
	P	0.74	0.20	0.50	0.36		0.12	0.07	0.01	0.001	0.12	0.77	0.67	0.01	<0.001	0.19
	n	20	19	20	20		20	20	19	19	20	20	18	20	20	20
$PaCO_2$	***r***	-0.25	-0.38	-0.45**	-0.38	0.36		-0.73***	0.83***	-0.69**	-0.09	0.001	0.16	-0.58**	-0.63**	-0.48**
	R^2	0.09	0.14	0.2	0.14	0.13		0.51	0.68	0.48	0.01	0.00	0.03	0.34	0.39	0.22
	P	0.29	0.11	0.05	0.10	0.12		<0.001	<0.001	0.001	0.71	1.00	0.52	0.01	0.003	0.04
	n	20	19	20	20	20		20	19	19	20	20	18	20	20	20
PaO_2	*r*	0.21	0.32	0.64**	0.16	-0.42	-0.73***		-0.49**	0.52**	0.04	-0.16	-0.37	0.37	0.45	0.24
	R^2	0.06	0.10	0.40	0.02	0.17	0.53		0.24	0.27	0.00	0.03	0.14	0.14	0.21	0.06
	P	0.38	0.19	0.003	0.52	0.07	<0.001		0.03	0.02	0.88	0.50	0.13	0.12	0.05	0.31
	n	20	19	20	20	20	20		19	19	20	20	18	20	20	20
$cHCO_3^-$	***r***	-0.30	-0.46		-0.54**	0.56**	0.83***	-0.49**		-0.88***	-0.18	0.02	-0.18	-0.64**	-0.74***	-0.49**
	R^2	0.02	0.21	0.11	0.29	0.31	0.68	0.23		0.78	0.03	0.00	0.03	0.40	0.54	0.24
	P	0.21	0.06	0.169	0.02	0.01	<0.001	0.03		<0.001	0.47	0.95	0.49	0.003	<0.001	0.04
	n	19	18	19	19	19	19	19		18	19	19	17	19	19	19
$BE_{(ecf)}$	***r***	0.24	0.41	0.33	0.55**	-0.71***	-0.69**	0.52**	-0.88***		0.25	-0.07	0.10	0.64**	0.78***	0.32
	R^2	0.01	0.16	0.11	0.30	0.50	0.48	0.27	0.78		0.06	0.00	0.01	0.41	0.61	0.10
	P	0.32	0.10	0.16	0.02	0.001	0.001	0.02	<0.001		0.31	0.78	0.69	0.003	<0.001	0.18
	n	19	18	19	19	19	19	19	18		19	19	17	19	19	19

Table 9.2 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured

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Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Rectal T	Muscle T	RR	HR	pH	$PaCO_2$	PaO_2	$cHCO_{3-}$	$BE_{(ecf)}$	Na^+	K^+	Ca^{2+}	Cl^-	AGap	Glu
Na^+	***r***	-0.14	0.09	-0.16	0.03	-0.36	-0.09	0.04	-0.18	0.25		0.56**	0.52**	0.80***	0.55***	0.30
	R^2	0.02	0.01	0.03	0.00	0.13	0.01	0.00	0.03	0.06		0.31	0.27	0.64	0.30	0.09
	P	0.57	0.71	0.50	0.90	0.12	0.71	0.88	0.47	0.31		0.01	0.03	<0.001	0.01	0.19
	n	20	19	20	20	20	20	20	19	19		20	18	20	20	20
K^+	***r***	-0.10	-0.12	0.01	0.03	-0.07	0.001	-0.16	0.02	-0.07	0.56**		0.73***	0.36	0.28	-0.09
	R^2	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.31		0.53	0.13	0.08	0.01
	P	0.69	0.66	0.97	0.89	0.77	1.00	0.50	0.94	0.78	0.01		0.001	0.12	0.24	0.71
	n	20	19	20	20	20	20	20	19	19	20		18	20	20	20
Ca^{2+}	***r***	-0.14	-0.04	-0.11	0.30	-0.12	0.16	-0.37	-0.18	0.10	0.52**	0.73***		0.29	0.14	0.02
	R^2	0.00	0.00	0.01	0.09	0.01	0.03	0.14	0.03	0.01	0.27	0.53		0.08	0.02	0.00
	P	0.57	0.88	0.68	0.23	0.67	0.52	0.13	0.49	0.69	0.03	0.001		0.25	0.58	0.95
	n	18	17	18	20	18	18	18	17	17	18	18		20	18	18
Cl^-	***r***	0.04	0.33	0.05	0.37	-0.55	-0.58	0.37	-0.64**	0.65	0.80***	0.36	0.29		0.71***	0.46**
	R^2	0.00	0.11	0.00	0.13	0.30	0.34	0.14	0.40	0.41**	0.64	0.13	0.08		0.51	0.22
	P	0.86	0.17	0.82	0.11	0.01	0.01	0.12	0.003	0.003	<0.001	0.12	0.25		<0.001	0.04
	n	20	19	20	18	20	20	20	19	19	20	20	18		20	20
AGap	***r***	0.04	0.32	0.28	0.24	-0.75***	-0.63**	0.45**	-0.74***	0.78***	0.55	0.73	0.14	0.71***		0.51**
	R^2	0.00	0.10	0.09	0.06	0.56	0.39	0.21	0.54	0.61	0.30*	0.08	0.02	0.51		0.26
	P	0.87	0.18	0.23	0.30	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	0.24	0.58	<0.001		0.02
	n	20	19	20	20	20	20	20	19	19	20	20	18	20		20

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		Rectal T	Muscle T	RR	HR	pH	$PaCO_2$	PaO_2	$cHCO_{3^-}$	$BE_{(ecf)}$	Na^+	K^+	Ca^{2+}	Cl^-	AGap	Glu
Glu	***r***	-0.03	0.38	-0.18	0.14	-0.31	-0.47**	0.24*	-0.49**	0.32	0.30	-0.09	0.02	0.46**	0.51**	
	R^2	0.00	0.15	0.03	0.02	0.09	0.22	0.06	0.24	0.10	0.09	0.01	0.00	0.20	0.30	
	P	0.89	0.20	0.46	0.56	0.19	0.04	0.03	0.04	0.18	0.19	0.71	0.95	0.04	0.02	
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	
Lactate	***r***	0.12	0.35	0.34	0.29	-0.72***	-0.63**	0.51**	-0.78***	0.85***	0.17	-0.09	-0.06	0.45	0.88***	0.49
	R^2	0.01	0.12	0.11	0.08	0.51	0.40	0.26	0.61	0.73	0.03	0.01	0.00	0.20	0.77	0.24*
	P	0.62	0.14	0.14	0.22	<0.001	0.003	<0.001	<0.001	<0.001	0.49	0.69	0.82	0.05	<0.001	0.03
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
WBC	***r***	0.26	-0.13	-0.42	-0.28	0.36	0.18	-0.17	0.12	-0.29	-0.39	-0.35	-0.40	-0.39	-0.46**	0.05
	R^2	0.07	0.02	0.18	0.09	0.13	0.03	0.03	0.01	0.08	0.15	0.13	0.16	0.15	0.21	0.00
	P	0.29	0.59	0.06	0.24	0.24	0.40	0.62	0.62	0.23	0.09	0.13	0.10	0.92	0.04	0.83
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
RBC	***r***	0.17	0.27	0.35	-0.18	0.13	0.08	0.09	-0.07	0.07	0.31	-0.23	-0.12	-0.28		-0.16
	R^2	0.02	0.07	0.12	0.03	0.02	0.01	0.01	0.01	0.00	0.09	0.05	0.01	0.08	0.01	0.03
	P	0.47	0.26	0.14	0.43	0.59	0.74	0.76	0.76	0.78	0.19	0.34	0.64	0.24	0.71	0.49
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
Ht	***r***	0.41	0.50**	0.43	-0.01	-0.21	-0.41	0.42**	-0.52**	0.49**	0.07	-0.17	-0.04	0.24	0.47	0.34
	R^2	0.16	0.25	0.18	0.00	0.05	0.17	0.18	0.27	0.24	0.00	0.03	0.00	0.06	0.21*	0.12
	P	0.08	0.03	0.06	0.96	0.37	0.07	0.02	0.02	0.03	0.79	0.46	0.88	0.30	0.04	0.14
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20

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		Rectal T	**Muscle T**	**RR**	**HR**	**pH**	**$PaCO_2$**	**PaO_2**	**$cHCO_3^-$**	**$BE_{(ecf)}$**	**Na^+**	**K^+**	**Ca^{2+}**	**Cl^-**	**AGap**	**Glu**
Hgb	***r***	0.33	0.49**	0.46**	-0.03	-0.19	-0.32	0.38*	-0.45	0.43	0.02	-0.09	0.003	0.17	0.39	0.22
	R^2	0.11	0.24	0.21	0.00	0.03	0.11	0.14	0.20	0.18	0.00	0.01	0.00	0.03	0.15	0.05
	P	0.16	0.03	0.04	0.92	0.42	0.17	0.05	0.05	0.07	0.95	0.69	0.99	0.48	0.09	0.35
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
GGT	***r***	0.07	0.24	0.34	0.08	-0.31	-0.11	0.11	-0.12	0.22	-0.04	0.25	0.18	0.01	0.34	-0.01
	R^2	0.01	0.06	0.11	0.01	0.10	0.01	0.01	0.02	0.05	0.00	0.06	0.03	0.00	0.12	0.00
	P	0.76	0.34	0.16	0.75	0.19	0.64	0.63	0.63	0.38	0.87	0.31	0.49	0.96	0.15	0.96
	n	19	18	19	19	19	19	19	18	18	19	19	17	19	19	19
GLDH	***r***	-0.22	-0.14	0.16	0.07	0.17	0.15	-0.05	0.21	-0.13	0.39	0.51**	0.32	0.12	-0.06	-0.26
	R^2	0.05	0.02	0.03	0.00	0.03	0.02	0.00	0.04	0.02	0.11	0.23	0.10	0.01	0.00	0.07
	P	0.34	0.58	0.50	0.77	0.49	0.53	0.84	0.40	0.60	0.15	0.02	0.19	0.61	0.81	0.26
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
AST	***r***	-0.06	-0.04	0.39	0.09	0.15	0.20	-0.16	0.21	-0.18	0.13	0.54	0.42	-0.10	-0.02	-0.45
	R^2	0.00	0.00	0.15	0.01	0.02	0.04	0.02	0.04	0.03	0.02	0.07	0.10	0.00	0.00	0.20
	P	0.78	0.88	0.09	0.71	0.53	0.40	0.51	0.39	0.47	0.57	0.01	0.08	0.69	0.94	0.05
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
CK	***r***	-0.18	0.17	0.24	0.06	0.09	-0.05	-0.04	0.09	-0.13	0.09	0.26	0.31	0.03	0.12	0.11
	R^2	0.03	0.03	0.06	0.00	0.01	0.00	0.00	0.01	0.02	0.01	0.07	0.10	0.00	0.01	0.01
	P	0.45	0.49	0.32	0.80	0.70	0.83	0.86	0.72	0.61	0.70	0.27	0.21	0.91	0.62	0.64
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20

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		Rectal T	Muscle T	RR	HR	pH	$PaCO_2$	PaO_2	$cHCO_{3^-}$	$BE_{(ecf)}$	Na^+	K^+	Ca^{2+}	Cl^-	AGap	Glu
Tbili	***r***	0.40	0.02	-0.08	0.06	0.41	-0.05	-0.09	0.07	-0.01	-0.05	-0.41	-0.37	-0.05	-0.16	0.05
	R^2	0.16	0.00	0.01	0.00	0.12	0.00	0.01	0.01	0.00	0.00	0.17	0.13	0.00	0.03	0.00
	P	0.08	0.95	0.72	0.79	0.07	0.83	0.70	0.77	0.96	0.85	0.08	0.13	0.83	0.49	0.83
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
Urea	***r***	-0.04	-0.16	-0.27	0.02	0.34	-0.70***	-0.55**	0.40	-0.37	0.21	0.26	0.25	-0.15	-0.42	-0.39
	R^2	0.00	0.03	0.07	0.00	0.05	0.49	0.26	0.52	0.34	0.04	0.07	0.06	0.02	0.18	0.15
	P	0.88	0.52	0.26	0.93	0.14	0.001	0.01	0.09	0.12	0.38	0.27	0.31	0.54	0.06	0.09
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
Crea	***r***	0.27	0.42	0.48**	0.28	-0.23	-0.53**	0.51**	-0.72***	0.59**	-0.13	-0.36	-0.14	0.21	0.42	0.34
	R^2	0.02	0.17	0.23	0.08	0.04	0.28	0.26	0.52	0.23	0.02	0.13	0.02	0.05	0.18	0.12
	P	0.26	0.08	0.03	0.23	0.33	0.02	0.02	<0.001	0.01	0.58	0.12	0.59	0.36	0.06	0.14
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
Troponin	***r***	0.13	0.05	0.39	0.42	-0.2	-0.38	0.19	-0.30	0.49**	0.03	0.16	0.28	0.27	0.41	-0.09
	R^2	0.02	0.00	0.15	0.17	0.04	0.14	0.03	0.09	0.23	0.00	0.03	0.08	0.07	0.17	0.01
	P	0.58	0.85	0.09	0.07	0.40	0.10	0.43	0.21	0.03	0.77	0.50	0.26	0.25	0.07	0.70
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20
Cortisol	***r***	0.04	-0.10	-0.44	-0.17	-0.10	0.52**	-0.39	-0.21	-0.27	0.03	-0.09	-0.12	-0.19	-0.25	-0.12
	R^2	0.00	0.01	0.19	0.03	0.01	0.28	0.15	0.04	0.07	0.00	0.01	0.01	0.03	0.06	0.01
	P	0.87	0.70	0.06	0.48	0.48	0.02	0.09	0.40	0.27	0.89	0.71	0.67	0.43	0.29	0.63
	n	20	19	20	20	20	20	20	19	19	20	20	18	20	20	20

Table 9.3 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured continued

Many physiological variables did not have a normal distribution and were all log transformed prior to the Pearson's correlation matrix analysis. R^2 was calculated from the *r*-value in the Pearson's correlation matrix (Hauke & Kossowski, 2011).

Grey highlighted values: significant ($P < 0.05$) correlation (*r*) and R^2 values between the physiological variables recorded within the stressed (NES and ES) groups (n = 20) on D0: 0 min directly after the capture stress event.

Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
Rectal T	***r***	0.12	0.26	0.17	0.41	0.33	0.07	-0.22	-0.07	-0.18	0.40	-0.04	0.27	0.13	0.04
	R^2	0.01	0.07	0.03	0.16	0.11	0.01	0.05	0.00	0.03	0.16	0.00	0.07	0.02	0.00
	P	0.62	0.28	0.47	0.08	0.16	0.77	0.34	0.78	0.45	0.08	0.88	0.26	0.58	0.87
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
Muscle T	***r***	0.35	-0.13	0.27	0.50**	0.49**	0.24	-0.14	-0.04	0.17	0.02	-0.16	0.42	0.05	-0.10
	R^2	0.12	0.02	0.08	0.25	0.24	0.06	0.02	0.00	0.03	0.00	0.03	0.17	0.00	0.01
	P	0.14	0.59	0.26	0.03	0.03	0.34	0.58	0.88	0.49	0.95	0.52	0.08	0.85	0.70
	n	19	19	19	19	19	18	19	19	19	19	19	19	19	19
RR	***r***	0.34	-0.42	0.35	0.43	0.46**	0.34	0.16	0.39	0.24	-0.08	-0.27	0.48**	0.39	-0.44
	R^2	0.12	0.18	0.12	0.18	0.21	0.11	0.03	0.15	0.06	0.01	0.07	0.23	0.15	0.19
	P	0.14	0.06	0.14	0.06	0.04	0.16	0.50	0.09	0.32	0.72	0.23	0.03	0.09	0.06
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
HR	***r***	0.29	-0.28	-0.18	-0.12	-0.03	0.08	0.07	0.09	0.06	0.06	0.02	0.28	0.42	-0.17
	R^2	0.08	0.08	0.03	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.08	0.17	0.03
	P	0.22	0.24	0.44	0.96	0.92	0.75	0.77	0.71	0.80	0.79	0.94	0.23	0.07	0.48
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20

Table 9.3 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured continued

Many physiological variables did not have a normal distribution and were all log transformed prior to the Pearson's correlation matrix analysis. R^2 was calculated from the *r*-value in the Pearson's correlation matrix (Hauke & Kossowski, 2011).

Grey highlighted values: significant ($P < 0.05$) correlation (*r*) and R^2 values between the physiological variables recorded within the stressed (NES and ES) groups (n = 20) on D0: 0 min directly after the capture stress event.

Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
pH	***r***	-0.72***	0.36	0.13	-0.21	-0.19	-0.31	0.17	0.15	0.09	0.41	0.34	-0.23	-0.2	-0.10
	R^2	0.52	0.13	0.02	0.05	0.04	0.10	0.03	0.02	0.01	0.17	0.11	0.05	0.04	0.01
	P	<0.001	0.12	0.56	0.34	0.42	0.19	0.49	0.53	0.70	0.07	0.14	0.34	0.40	0.68
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
$PaCO_2$	***r***	-0.63**	0.18	0.08	-0.41	-0.32	-0.11	0.15	0.20	-0.05	-0.05	0.70***	-0.53**	-0.38	
	R^2	0.40	0.03	0.01	0.17	0.10	0.01	0.02	0.04	0.00	0.00	0.49	0.28	0.14	0.28*
	P	0.003	0.44	0.74	0.08	0.17	0.64	0.53	0.40	0.83	0.83	0.001	0.02	0.10	0.02
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
PaO_2	***r***	0.51**	-0.17	0.09	0.42	0.38	0.11	-0.05	-0.16	-0.04	-0.09	-0.55**	0.51**	0.20	-0.39
	R^2	0.23	0.03	0.01	0.18	0.14	0.01	0.00	0.02	0.00	0.01	0.30	0.26	0.04	0.15
	P	0.02	0.46	0.70	0.07	0.10	0.65	0.84	0.51	0.86	0.70	0.01	0.02	0.43	0.09
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
$cHCO_3^-$	***r***	-0.78***	0.12	-0.07	-0.52**	-0.45	-0.12	0.21	0.21	0.09	0.07	0.40	-0.72***	-0.30	0.21
	R^2	0.61	0.01	0.01	0.27	0.20	0.02	0.04	0.04	0.01	0.01	0.16	0.52	0.09	0.04
	P	<0.001	0.62	0.76	0.02	0.05	0.63	0.40	0.39	0.72	0.77	0.09	<0.001	0.21	0.40
	n	19	19	19	19	19	18	19	19	19	19	19	19	19	19
$BE_{(ecf)}$	***r***	0.85***	-0.29	0.07	0.49**	0.43	0.22	-0.13	-0.18	-0.13	-0.01	-0.37	0.59**	0.49**	-0.27
	R^2	0.72	0.08	0.00	0.24	0.18	0.05	0.02	0.03	0.02	0.00	0.14	0.35	0.24	0.07
	P	<0.001	0.23	0.78	0.03	0.07	0.38	0.60	0.47	0.61	0.96	0.12	0.01	0.03	0.27
	n	19	19	19	19	19	18	19	19	19	19	19	19	19	19

Table 9.3 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured continued

Many physiological variables did not have a normal distribution and were all log transformed prior to the Pearson's correlation matrix analysis. R^2 was calculated from the *r*-value in the Pearson's correlation matrix (Hauke & Kossowski, 2011).

Grey highlighted values: significant ($P < 0.05$) correlation (*r*) and R^2 values between the physiological variables recorded within the stressed (NES and ES) groups (n = 20) on D0: 0 min directly after the capture stress event.

Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
Na^+	***r***	0.17	-0.39	-0.31	0.07	0.02	-0.04	0.34	0.13	0.09	-0.05	0.21	-0.13	0.07	0.03
	R^2	0.03	0.15	0.09	0.00	0.00	0.00	0.11	0.02	0.01	0.00	0.04	0.02	0.00	0.00
	P	0.49	0.09	0.19	0.79	0.95	0.87	0.15	0.57	0.70	0.85	0.38	0.58	0.77	0.89
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
K+	***r***	-0.09	-0.35	-0.23	-0.17	-0.10	0.25	0.51**	0.54**	0.26	-0.41	0.26	-0.36	0.16	-0.09
	R^2	0.01	0.13	0.05	0.03	0.01	0.06	0.26	0.30	0.07	0.17	0.07	0.13	0.03	0.01
	P	0.69	0.13	0.34	0.46	0.69	0.31	0.02	0.01	0.27	0.08	0.27	0.12	0.50	0.71
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
Ca^{2+}	***r***	-0.06	-0.40	-0.12	-0.04	0.003	0.18	0.32	0.42	0.31	-0.37	0.25	-0.14	0.28	-0.12
	R^2	0.00	0.16	0.01	0.00	0.00	0.03	0.10	0.18	0.10	0.14	0.06	0.02	0.08	0.01
	P	0.82	0.10	0.64	0.88	0.99	0.49	0.19	0.08	0.21	0.13	0.31	0.59	0.26	0.67
	n	20	18	18	18	18	17	18	18	18	18	18	18	18	18
Cl^-	***r***	0.45	-0.39	-0.28	0.24	0.17	0.01	0.12	-0.10	0.03	-0.05	-0.15	0.21	0.27	-0.19
	R^2	0.20	0.00	0.15	0.08	0.06	0.03	0.00	0.01	0.01	0.00	0.00	0.02	0.05	0.07
	P	0.45	0.92	0.24	0.31	0.48	0.96	0.61	0.69	0.91	0.83	0.54	0.36	0.25	0.43
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
AGap	***r***	0.88***	-0.46	-0.13	0.47**	0.39	0.35	-0.06	-0.02	0.12	-0.16	-0.42	0.42	0.41	-0.25
	R^2	0.77	0.21	0.08	0.22	0.15	0.12	0.00	0.00	0.03	0.03	0.12	0.18	0.17	0.06
	P	<0.001	0.09	0.23	0.04	0.09	0.15	0.81	0.93	0.62	0.49	0.06	0.06	0.07	0.29
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20

Table 9.3 Correlation (*r*), coefficient of determination (R^2), *P*-value and sample size (n) for physiological variables measured continued

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Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
Glu	***r***	0.49**	0.05	-0.16	0.34	0.22	-0.01	-0.26	-0.45	0.11	0.05	-0.39	0.34	-0.09	-0.12
	R^2	0.60	0.27	0.02	0.12	0.05	0.00	0.07	0.20	0.01	0.00	0.15	0.12	0.01	0.01
	P	0.03	0.83	0.49	0.14	0.35	0.96	0.26	0.05	0.64	0.83	0.09	0.14	0.70	0.63
	n	20	20	20	20	20	19	20	20	20	20	20	20	20	20
Lactate	***r***		-0.27	0.09	0.55**	0.50**	0.30	-0.39	-0.24	0.02	-0.10	-0.57**	0.63**	0.40	-0.27
	R^2		0.07	0.01	0.31	0.25	0.09	0.15	0.06	0.00	0.01	0.32	0.40	0.16	0.07
	P		0.24	0.71	0.01	0.02	0.22	0.09	0.32	0.94	0.66	0.01	0.003	0.08	0.25
	n		20	20	20	20	19	20	20	20	20	20	20	20	20
WBC	***r***	-0.27		-0.15	-0.31	-0.34	-0.59**	-0.32	-0.43	-0.62**	0.34	-0.03	-0.13	-0.77***	0.44
	R^2	0.07		0.02	0.09	0.11	0.34	0.1	0.18	0.38	0.11	0.00	0.02	0.59	0.19
	P	0.24		0.54	0.19	0.15	0.01	0.17	0.35	0.004	0.15	0.89	0.60	<0.001	0.05
	n	20		20	20	20	19	20	20	20	20	20	20	20	20
RBC	***r***	0.09	-0.15		0.71**	0.82***	0.26	-0.09	0.001	0.37	-0.02	<0.001	0.27	0.25	-0.30
	R^2	0.01	0.02		0.50	0.67	0.07	0.01	0.00	0.13	0.00	0.00	0.08	0.06	0.09
	P	0.71	0.54		<0.001	<0.001	0.29	0.70	1.00	0.11	0.93	1.00	0.25	0.29	0.19
	n	20	20		20	20	19	20	20	20	20	20	20	20	20
Ht	***r***	0.55**	-0.31	0.71***		0.96****	0.35	-0.15	-0.14	0.31	0.05	-0.33	0.65***	0.45	-0.48**
	R^2	0.30	0.09	0.50		0.91	0.12	0.02	0.02	0.10	0.00	0.11	0.42	0.20	0.23
	P	0.01	0.19	<0.001		<0.001	0.14	0.54	0.56	0.18	0.84	0.16	0.002	0.05	0.03
	n	20	20	20		20	19	20	20	20	20	20	20	20	20

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Negligible *r* (0.00 – 0.10); * weak *r* (0.10 – 0.39); ** moderate *r* (0.40 – 0.69); *** strong *r* (0.70 – 0.89); **** Very strong *r* (0.9 – 1.00) (Schober *et al.*, 2018).

		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
Hgb	***r***	0.50**	-0.34	0.82***	0.96****		0.39	-0.17	-0.09	0.36	-0.08	-0.25	0.56**	0.45	-0.49**
	R^2	0.25	0.11	0.67	0.91		0.15	0.03	0.01	0.13	0.01	0.06	0.32	0.20	0.24
	P	0.02	0.15	<0.001	<0.001		0.10	0.49	0.71	0.12	0.74	0.30	0.01	0.05	0.03
	n	20	20	20	20		19	20	20	20	20	20	20	19	20
GGT	***r***	0.30	-0.59**	0.26	0.35	0.39		0.37	0.51**	0.58**	-0.55**	-0.08	0.28	0.52**	-0.43
	R^2	0.09	0.34	0.07	0.12	0.15		0.14	0.26	0.34	0.30	0.01	0.08	0.27	0.18
	P	0.22	0.01	0.29	0.14	0.10		0.12	0.02	0.01	0.02	0.77	0.24	0.02	0.07
	n	19	19	19	19	19		19	19	19	19	19	19	19	19
GLDH	***r***	-0.39	-0.32	-0.09	-0.15	-0.17	0.37		0.74**	0.30	-0.25	0.38	-0.14	0.03	-0.22
	R^2	0.15	0.10	0.01	0.02	0.03	0.14		0.55	0.09	0.06	0.14	0.02	0.00	0.05
	P	0.09	0.17	0.70	0.54	0.49	0.12		<0.001	0.21	0.30	0.10	0.55	0.90	0.36
	n	20	20	20	20	20	19		20	20	20	20	20	20	20
AST	***r***	-0.24	-0.43	0.001	-0.14	-0.09	0.51**	0.74***		0.50**	-0.19	0.28	-0.19	0.22	-0.15
	R^2	0.06	0.18	0.00	0.02	0.01	0.26	0.55		0.25	0.04	0.08	0.04	0.05	0.02
	P	0.32	0.06	1.00	0.56	0.71	0.02	<0.001		0.02	0.41	0.24	0.42	0.35	0.54
	n	20	20	20	20	20	19	20		20	20	20	20	20	20
CK	***r***	0.02	-0.62**	0.37	0.31	0.36	0.58**	0.30	0.50**		-0.15	0.01	0.001	0.47**	-0.45
	R^2	0.00	0.38	0.13	0.10	0.13	0.34	0.09	0.25		0.02	0.00	0.00	0.21	0.20
	P	0.94	0.004	0.11	0.18	0.12	0.01	0.21	0.02		0.53	0.97	1.0	0.04	0.05
	n	20	20	20	20	20	19	20	20		20	20	20	20	20

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		Lactate	WBC	RBC	Ht	Hgb	GGT	GLDH	AST	CK	Tbili	Urea	Crea	Troponin	Cortisol
Tbili	***r***	-0.10	0.34	-0.02	0.05	-0.08	-0.55**	-0.25	-0.19	-0.15		0.09	-0.07	-0.09	0.23
	R^2	0.01	0.11	0.00	0.00	0.01	0.30	0.06	0.04	0.02		0.01	0.01	0.12	0.32
	P	0.66	0.15	0.93	0.002	0.74	0.02	0.30	0.41	0.53		0.71	0.76	0.72	0.33
	n	20	20	20	20	20	19	20	20	20		20	20	20	20
Urea	***r***	-0.57**	-0.03	<0.001	-0.33	-0.25	-0.07	0.38	0.28	0.01	0.09		-0.43	-0.08	0.39
	R^2	0.33	0.00	0.00	0.11	0.06	0.01	0.144	0.08	0.00	0.01		0.18	0.01	0.15
	P	0.01	0.89	1.00	0.16	0.30	0.77	0.10	0.24	0.97	0.71		0.06	0.74	0.09
	n	20	20	20	20	20	19	20	20	20	20		20	20	20
Crea	***r***	0.63**	-0.13	0.27	0.65**	0.56**	0.28	-0.14	-0.19	0.001	-0.08	-0.43		0.35	-0.58**
	R^2	0.40	0.02	0.07	0.42	0.32	0.08	0.02	0.04	0.00	0.01	0.18		0.12	0.34
	P	0.003	0.60	0.25	0.002	0.01	0.24	0.55	0.42	1.00	0.76	0.06		0.13	0.01
	n	20	20	20	20	20	19	20	20	20	20	20		20	20
Troponin	***r***	0.40	-0.77	0.25	0.45	0.45	0.52**	0.03	0.22	0.47**	-0.09	-0.08	0.35		-0.57**
	R^2	0.16	0.59**	0.06	0.20	0.20	0.27	0.00	0.05	0.22	0.01	0.01	0.12		0.32
	P	0.08	<0.001	0.29	0.05	0.05	0.02	0.90	0.35	0.04	0.72	0.74	0.13		0.01
	n	20	20	20	20	20	19	20	20	20	20	20	20		20

Cortisol	***r***	-0.27	0.44	-0.30	-0.48	-0.49**	-0.43	-0.22	-0.15	-0.45	0.23	0.39	-0.58**	-0.57**
	R^2	0.07	0.19	0.09	0.23	0.24	0.18	0.05	0.02	0.20	0.05	0.15	0.33	0.32
	P	0.25	0.05	0.19	0.32	0.03	0.07	0.36	0.54	0.05	0.33	0.09	0.01	0.01
	n	20	20	20	20	20	19	20	20	20	20	20	20	20

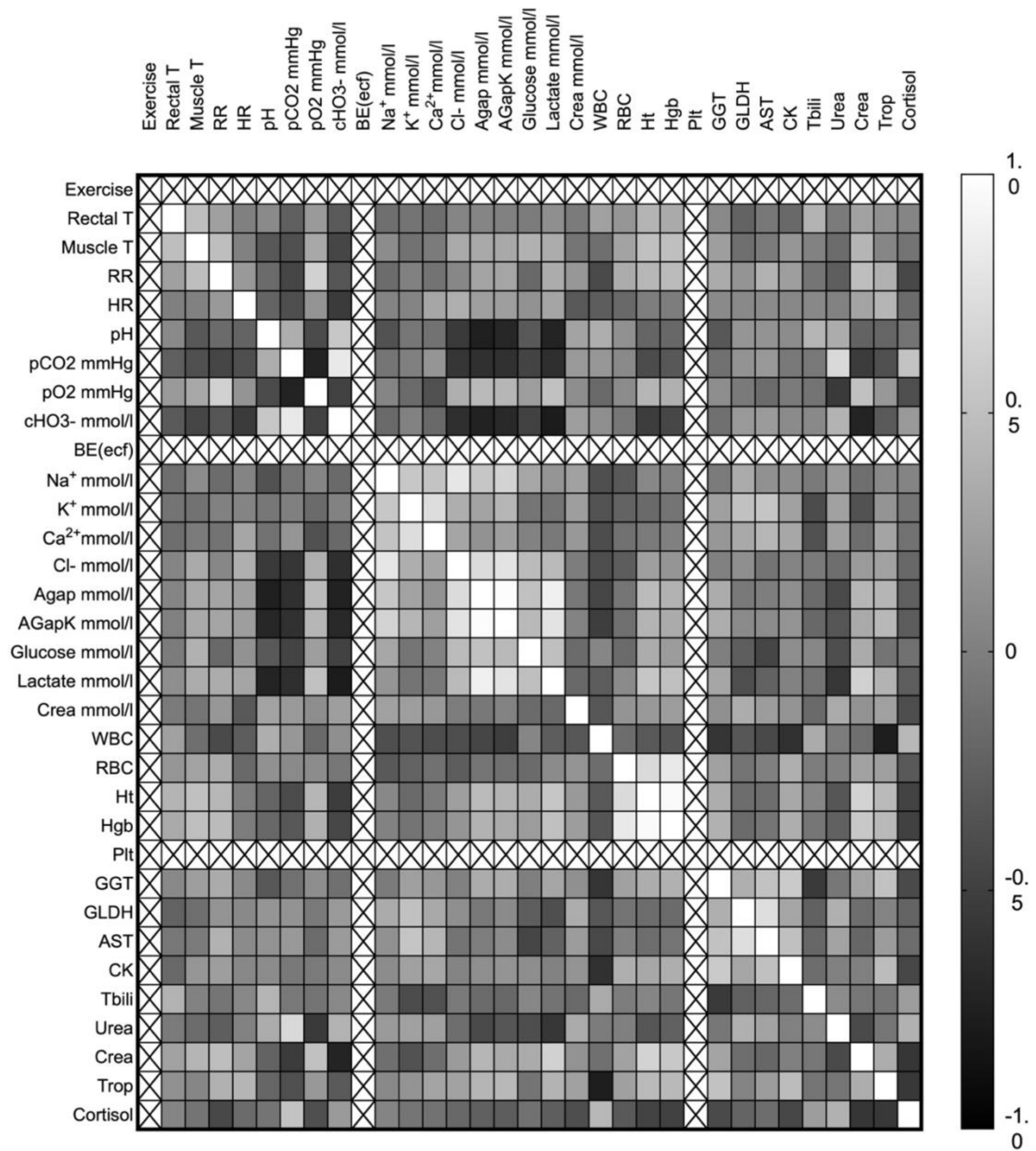

Figure 9.1 **Pearson's correlation matrix heat map of physiological variables measured.**

The Pearson's correlation coefficients for multiple physiological variables were measured in the stressed groups (NES and ES, n = 20) on D0: 0 min. All original values were log transformed for this analysis.

Right hand side: Scale indicating -1 as black and +1 as white and in-between as a grey scale from black to white. A negative value would indicate a negative correlation or association, whereas a positive value would indicate a positive correlation or association.

Table 9.4 Blood biochemistry and haematology values

Untransformed blood biochemistry and haematology values expressed as mean ± SD for blesbok groups at Baseline, D0: 0 min, D0: 40 min, D2 and D5.

Normal reference range intervals were obtained for multiple species: ble1 = blesbok (Seal and Schobert, 1976), ble2 = blesbok (Fitte, 2017), ca = caprine/goats (Stevens et al., 1994) and eq = equine (Epocal Inc., 2012).

Bars: different between groups ($P < 0.05$). * different within a group from Baseline or D0: 0 min ($P < 0.05$). Grey highlighted values: different between NES and ES on D0 (*t*-test, $P < 0.05$).

Parameter	Reference range	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
Glucose	2.8–11 (ble1)	ENS	5.9±1.5	4.7±0.4	6.6±1.2	5.0±0.6	4.5±0.7*
	2.5-4.7 (ca)	ES	6.1±1.6	9.3±1.6*	7.8±1.7	4.9±0.5	5.0±1.3
	3.3-6.4 (eq)	NENS	7.4±1.0	4.8±0.7*	7.1±1.0	5.0±0.6*	4.8±1.0*
	mmol/l	NES	7.3±1.7	10.8±1.7*	9.5±2.5	4.7±0.8*	5.2±1.8*
Lactate	0.87-2.49 (ble2)	ENS	0.9±0.4	1.2±0.6	1.0±0.3	1.6±1.2	1.3±1.2
	0.6-2.9 (eq)	ES	0.9±0.3	9.5±1.7*	8.4±2.0*	1.0±1.2	1.3±0.8
	mmol/l	NENS	1.0±0.1	1.6±0.8*	1.6±0.8	1.1±0.4	1.3±1.0
		NES	1.0±0.7	11.5±1.6*	10.0±1.0*	0.9±0.7	1.1±0.5
AST	96-122 (ble2)	ENS		154±83	145±81	178±113*	157±55*
	35-72 (ca)	ES		129±30	127±31	251±91*	182±54*
	u/l	NENS		114±76	108±71*	156±82*	159±112*
		NES		104±8	101±9*	231±109*	182±74*
CK	0-290 (ble1)	ENS		183±63	226±201	178±114	130±34
	24-98 (ble2)	ES		351±150	433±208*	476±479	218±188*
	89-182u/l	NENS		116±67	144±43*	160±76*	119±83
		NES		319±32	376±43*	581±892	149±102*
Troponin I	0.008-0.036 (ble2)	ENS		0.1±0.0	0.1±0	0.1±0.0	0.1±0.0
	ng/ml	ES		0.3±0.3	0.9±0.9*	1.1±1.8*	0.1±0.1
		NENS		0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
		NES		0.2±0.1	0.7±0.5*	0.9±1.0	0.1±0.0
Creatinine	87–110 (ble2)	ENS		84±10	81±13	71±10*	81±7
	mmol/l	ES		130±16	120±17*	84±10*	83±10*
		NENS		96±15	95±17	84±12*	99±14
		NES		148±16	133±17*	96±17*	83±9*
Urea	7.6-10 (ble2)	ENS		8.8±1.7	9.0±1.7	9.0±1.3	6.5±1.2*
	mmol/l	ES		9.1±0.8	9.3±0.9*	8.4±1.3	7.9±1.2*
		NENS		8.3±1.3	8.5±1.3*	7.3±1.4*	6.3±1.2*
		NES		7.8±0.9	9.1±0.9*	7.0±1.2	7.3±1.1

Table 9.4 Blood biochemistry and haematology values

Untransformed blood biochemistry and haematology values expressed as mean ± SD for blesbok groups at Baseline, D0: 0 min, D0: 40 min, D2 and D5.

Normal reference range intervals were obtained for multiple species: ble1 = blesbok (Seal and Schobert, 1976), ble2 = blesbok (Fitte, 2017), ca = caprine/goats (Stevens et al., 1994) and eq = equine (Epocal Inc., 2012).

Bars: different between groups ($P < 0.05$). * different within a group from Baseline or D0: 0 min ($P < 0.05$). Grey highlighted values: different between NES and ES on D0 (*t*-test, $P < 0.05$).

Parameter	Reference range	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
GGT	53-75 (ble2)	ENS		69±16	66±15	73±18	70±16
	16-45 (ca)	ES		95±46	89±41*	85±26	77±22*
	u/l	NENS		67±17	62±17*	69±15	67±19
		NES		84±11	79±9*	77±9*	74±8*
GLDH	2.2-15.2 (ble2)	ENS		21±29	19±26*	27±41	17±3
	u/l	ES		39±28	35±26*	17±13*	10±7*
		NENS		21±29	19±26*	16±14	24±38
		NES		17±8	15±7*	8±3*	6±3*
Total bilirubin	3.42-5.13 (ca)	ENS		1.3±0.5	2.0±1.1	1.7±0.6	1.2±0.6
	umol/l	ES		1.8±0.4	2.1±0.5	1.6±0.6	1.6±0.5
		NENS		1.6±0.7	2.3±0.8	1.6±0.6	1.2±0.4
		NES		1.9±0.6	2.2±0.3	2.1±0.7	1.7±0.5
Cortisol	50-100 (ble2) nmol/l	ENS		102±28	113±51	78±44	57±26*
		ES		140±45	125±63	71±21*	80±22*
		NENS		103±35	105±49	68±46	65±18*
		NES		128±40	144±60	81±31	63±19*
WBC	2.0-4.6 (ble1)	ENS		5.8±1.3	4.1±1.4*	8.3±2.6*	5.1±1.1
	5.1-17.2 (ca)	ES		4.6±1.6	3.5±2.0*	6.6±0.6*	4.7±1.1
	$x10^9/\mu l$	NENS		4.8±0.9	3.6±0.9*	6.6±1.9*	4.5±0.8
		NES		5.2±1.2	4.4±1.6	7.1±1.2*	4.9±1.1
RBC	9.1-15.1 (ble1)	ENS		10.3±1.6	9.5±1.6	9.9±1.2	9.4±1.2*
	11-21.7 (ca)	ES		11.5±0.7	10.5±1.1*	9.9±0.8*	9.3±1.1*
	$x10^{12}/\mu l$	NENS		10.5±0.9	9.7±1.1*	10.3±1.0	9.3±0.9*
		NES		12.1±1.0	11.0±0.9*	10.2±1.2*	9.8±1.0*
Hgb concentration	122-198 (ble1)	ENS		146±12	136±16	132±11*	131±13*
	84-129 (ca)	ES		165±12	148±19*	133±12*	125±16*
	g/l	NENS		149±15	139±15	141±13	127±11
		NES		178±13	161±13*	144±17*	137±15*
Haemato-crit (Hct)	32-51 (ble1)	ENS		35±3	33±3	33±3	32±2*
	22-42 (ca)	ES		39±2	36±4*	33±3*	31±4*
	%	NENS		36±2	34±3*	35±3	32±2*
		NES		42±3	39±3*	35±4*	34±4*

Table 9.4 Blood biochemistry and haematology values

Untransformed blood biochemistry and haematology values expressed as mean ± SD for blesbok groups at Baseline, D0: 0 min, D0: 40 min, D2 and D5.

Normal reference range intervals were obtained for multiple species: ble1 = blesbok (Seal and Schobert, 1976), ble2 = blesbok (Fitte, 2017), ca = caprine/goats (Stevens et al., 1994) and eq = equine (Epocal Inc., 2012).

Bars: different between groups (*P* < 0.05). * different within a group from Baseline or D0: 0 min (*P* < 0.05). Grey highlighted values: different between NES and ES on D0 (*t*-test, *P* < 0.05).

Parameter	Reference range	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
Mean cell volume (MCV)	31-39 (ble1)	ENS		34±2	35±4	34±4	34±4
	16-24 (ca)	ES		34±1	34±2	34±2	33±2
	fl	NENS		34±2	35±2*	34±2	35±2*
		NES		35±2	35±2*	35±3	35±3
Mean cell Hgb level (MCH)	5.4-7.9 (ca)	ENS		14.3±1.3	14.4±1.2	13.5±1.4	14.1±1.2*
	pg	ES		14.3±0.5	14.1±0.8	13.4±0.6*	13.4±0.6*
		NENS		14.5±0.7	14.3±0.8	13.7±0.8*	13.7±0.7*
		NES		14.8±0.8	14.6±0.8	14.1±0.7*	14.0±0.5*
Mean cell haemoglobin-bin concentration (MCHC)	351-431 (ble2)	ENS		419±16	411±16	396±17*	415±30
	300-370 (ca)	ES		427±11	415±17*	401±14*	399±14*
	g/l	NENS		426±12	413±14*	400±15	395±13*
		NES		427±10	415±16	409±26	407±24
Red blood cell distribution width (RDW)	%	ENS		23.0±1.1	22.4±1.4	21.9±0.9*	22.0±0.9
		ES		24.1±0.9	23.7±0.6	22.0±0.6*	22.9±0.4*
		NENS		23.1±0.9	22.4±0.6*	22.3±0.8	21.9±0.8*
		NES		23.8±1.2	23.2±0.8	22.3±1.0*	22.3±0.9*
Platelet count	x10^9/µl	ENS		156±46	142±33	158±53	146±43
		ES		187±52	131±32*	135±32*	136±26*
		NENS		157±30	142±49	149±40	130±39
		NES		203±66	164±45	135±52*	136±36
Mean platelet volume (MPV)	Fl	ENS		5.0±0.4	5.0±0.3	6.0±0.4*	6.0±0.5*
		ES		5.2±0.5	4.8±0.3	6.0±0.1*	5.8±0.2*
		NENS		5.1±0.4	5.0±0.6	6.0±0.3*	5.9±0.2*
		NES		5.1±0.4	5.0±0.5	5.8±0.4*	5.8±0.4*

Table 9.5 Arterial blood gas and acid-base values

Untransformed arterial blood gas and acid-base values expressed as mean ± SD at different time points in the study. The reference intervals provided are for sheep (ovine = ov), goats (caprine = ca) (Meyer et al., 2006; Pfitzer et al., 2020; Stevens et al., 1994), horses (equine = eq) (Epocal Inc., 2012) and humans (humans = hu) (Castro et al., 2021; Peake & White, 2002). Vertical bars: different between groups (*P* < 0.05). * different from Baseline within-group (*P* < 0.05). Grey highlighted values: different between ES and NES group (*t*-test, P < 0.05). Underlined values: different between ES and NES group (F-test, P < 0.05).

Variable	Reference interval	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
$PaCO_2$	35-49 (ca)	ENS	54±6	54±4	57±6	55±6	54±3
	36-46 (eq)	ES	52±6	36±4*	50±6	50±5	55±4
	35-45 (hu)	NENS	54±7	58±7	58±12	51±6	52±5
	mmHg	NES	55±6	30±7*	43±9*	49±4*	56±7
PaO_2	90-100 (eq)	ENS	50±11	50±6	84±22*	58±10*	43±7
	75 -100 (hu)	ES	41±11	46±9	72±14*	51±15	39±12
	mmHg	NENS	44±14	53±10	81±21*	53±11	43±9
		NES	41±9	61±15*	78±12*	52±10*	42±8
A-a gradient	20-25 (ov, ca)	ENS	34±9	32±6	11±8*	23±10*	39±8
	2-15 (hu)	ES	42±10	52±9*	17±13*	32±13	39±10
	mmHg	NENS	38±11	25±10*	11±12*	31±7	40±6
		NES	39±8	43±10	19±10*	32±8	46±32
pH	7.3-7.5 (ca)	ENS	7.36±0.04	7.35±0.05	7.34±0.04	7.38±0.05	7.33±0.02*
	7.35–7.45	ES	7.39±0.05	7.35±0.04	7.33±0.06	7.39±0.04	7.40±0.04
	(eq & hu)	NENS	7.35±0.03	7.35±0.04	7.35±0.06	7.38±0.03*	7.35±0.05
		NES	7.34±0.03	7.32±0.09	7.31±0.07	7.37±0.03	7.34±0.03
$cHCO_3^-$	20-30 (ca)	ENS	31±2	30±1	30±1	32±2	28±2
	24–30 (eq)	ES	32±3	20±3*	26±2*	20±2	33±2
	22-26 (hu)	NENS	29±3	31±1	31±2	31±2	29±2
	mmol/l	NES	32±3	16±5*	22±4*	28±2*	33±2

Table 9.5 Arterial blood gas and acid-base values

Untransformed arterial blood gas and acid-base values expressed as mean ± SD at different time points in the study. The reference intervals provided are for sheep (ovine = ov), goats (caprine = ca) (Meyer et al., 2006; Pfitzer et al., 2020; Stevens et al., 1994), horses (equine = eq) (Epocal Inc., 2012) and humans (humans = hu) (Castro et al., 2021; Peake & White, 2002). Vertical bars: different between groups (*P* < 0.05). * different from Baseline within-group (*P* < 0.05). Grey highlighted values: different between ES and NES group (*t*-test, P < 0.05). Underlined values: different between ES and NES group (F-test, P < 0.05).

Variable	Reference interval	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
BE(ecf9	(-5)-(+4) (ca)	ENS	+6±2	+4±2	+5±1	+6±3	+2±2*
	(-5)-(+5) (eq)	ES	+7±3	-5±3*	0±3*	+5±2	+8±2
	(-4)-(+2) (hu)	NENS	+4±3	+6±1	+5±2	+6±2	+3±2
	mEq/L	NES	+6±3	-10±6*	-4±5*	+3±2	+8±2
Anion Gap	10-26 (ca)	ENS	8±3	6±3	5±3	6±1	6±2
	5–15 (eq)	ES	6±3	15±4*	10±2*	6±2	6±1
	mmol/l	NENS	7±2	6±2	7±2*	5±2	7±1
		NES	6±1	16±2*	13±3*	7±2	5±2

Table 9.6 Electrolyte concentrations

The untransformed electrolyte concentrations recorded as mean ± SD at different time points in the study.

The reference interval given is for immobilised blesbok (ble) from a previous study (Seal and Schobert, 1976), healthy non-immobilised goats (ca) (Stevens *et al.*, 1994) and horses(eq) as per the e*P*OC™ veterinary user guide (Epocal Inc., 2012). Vertical bars: different between groups ($P < 0.05$). * different from Baseline within-group ($P < 0.05$). Grey highlighted values: different between ES and NES group (*t*-test, $P < 0.05$). Underlined values: different between ES and NES group (F-test, $P < 0.05$).

Variable	Reference interval	Group	Time Points				
			Baseline	D0: 0 min	D0: 40 min	D2	D5
Na^+	140-152 (ble)	ENS	136±4	134±3	133±3	139±1	135±3
	144-156 (ca)	ES	135±4	<u>138±2</u>	137±4	139±2*	137±4
	139-142 (eq)	NENS	137±4	137±2	136±4	139±3	135±3
	mmol/l	NES	135±3	<u>136±5</u>	134±4	137±2	135±4
K^+	4.0-6.2 (ble)	ENS	3.6±0.3	4.3±0.3*	3.9±0.4	4.2±0.4*	4.2±0.3*
	3.9-5.2 (ca)	ES	3.6±0.4	4.5±0.2*	3.8±0.3	4.3±0.2*	4.0±0.4
	3.4-4.9 (eq)	NENS	3.9±0.3	4.4±0.2*	3.8±0.2	4.4±0.3*	4.0±0.3
	mmol/l	NES	3.6±0.4	4.1±0.3*	3.6±0.3	4.4±0.3*	3.9±0.3
Ca^{2+}	0.5-0.6 (ble)	ENS	0.8±0.1	0.8±0.1	0.8±0.2	1.0±0.1	0.9±0.1
	0.5-0.6 (ca)	ES	0.9±0.1	0.9±0.1	0.9±0.2*	1.1±0.1	0.8±0.2
	1.12-1.4 (eq)	NENS	0.9±0.2	0.9±0.1	0.8±0.2	1.0±0.1	0.9±0.1
	mmol/l	NES	0.8±0.2	0.7±0.2	0.7±0.1	1.0±0.1	0.74±0.2
Cl-	91-104 (ble)	ENS	98±5	98±2	98±2	102±2	101±3
	107-121 (ca)	ES	98±3	103±3*	101±2	102±2*	98±2
	106-127 (eq)	NENS	100±4	100±2	98±2	103±2	100±3
	mmol/l	NES	98±4	104±5*	100±5	102±3*	98±3