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COOLING BY DOUSING WITH COLD WATER DOES NOT ALTER THE PATHOPHYSIOLOGICAL BIOCHEMICAL CHANGES INDUCED BY CAPTURE IN BLESBOK (*Damaliscus pygargus phillipsi).*

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ABSTRACT

Wild animals are commonly captured for conservation, research and wildlife management purposes. However, capture is associated with a high risk of morbidity or mortality. Capture-induced hyperthermia is a commonly encountered complication believed to contribute significantly to morbidity and mortality. Active cooling of hyperthermic animals by dousing with water is believed to treat capture-induced pathophysiological effects, but remains untested. This study aimed to determine the pathophysiological effects of capture, and whether cooling by dousing with cold water effectively reduces these effects in blesbok (*Damaliscus pygargus phillipsi)*. Thirty-eight blesbok were randomly allocated into three groups: a control group which was not chased (Ct, n = 12), a chased not cooled (CNC, n = 14) and chased plus cooled group (C+C, n = 12). The CNC and C+C groups were chased for 15 minutes prior to chemical immobilization on day 0. Animals in the C+C group were cooled with 10 L of cold water (4°C) for 10 minutes during immobilization. All animals were immobilised on day 0, 3, 16 and 30. During each immobilization, rectal and muscle temperatures were recorded, and arterial and venous blood samples collected. Blesbok in the CNC and C+C groups presented with capture-induced pathophysiological changes characterised by hyperthermia, hyperlactatemia, increased markers of liver, skeletal and cardiac muscle damage, hypoxaemia and hypocapnia. Cooling effectively returned body temperatures to normothermic levels, but neither the magnitude nor the duration of the pathophysiological changes differed between the CNC and C+C groups. Therefore, at least in blesbok, capture-induced hyperthermia appears not to be the primary cause of the pathophysiological changes, but is more likely a clinical sign of the hypermetabolism resulting from capture-induced physical and psychological stress. Thus, while cooling is still recommended

to prevent the compounding cytotoxic effects of persistent hyperthermia, it unlikely prevents stress- and hypoxia-induced damage caused by the capture procedure.

Keywords: blesbok, capture-induced hyperthermia, capture myopathy, cooling, stress,

INTRODUCTION

The capture and handling of wildlife is an important tool required for various conservation, research and wildlife management programs (Dickens et al. 2010; Brivio et al. 2015; Breed et al. 2019) and is widely used for disease testing, veterinary treatment of individuals and translocation of animals. Despite its common application, the risk of complications is high, even in healthy animals (Arnemo et al. 2006; Dickens et al. 2010; Brivio et al. 2015). Capture and handling are believed to be one of the most stressful occurrences for wild animals and result in capture-related deaths above the generally accepted 2% natural mortality rate (Gericke et al. 1978; Jacques et al. 2009; Dickens et al. 2010; Zeiler et al. 2017; Hampton et al. 2021). The pathophysiological processes responsible for capture-related mortalities are often unknown (Breed et al. 2019). However, hyperthermia, which is commonly observed during capture, is thought to be one of the main contributors (Gericke et al. 1978; Kock et al. 1987; 1990; Montané et al. 2003; Arnemo et al. 2006; Meyer et al. 2008a; Sawicka et al. 2015; Breed et al. 2019). Capture-induced hyperthermia is defined by a rise in body temperature above normal physiological values, caused by the capture procedure mainly in response to capture-induced stress, and has been reported in various species (Meyer et al. 2008a; Ozeki et al. 2015; Sawicka et al. 2015; Braud et al. 2019; Breed et al. 2019).

Hyperthermia can cause cellular injury (Sakaguchi et al. 1995; Bouchama and Knochel 2002; Epstein and Roberts 2011) and lead to organ damage, rhabdomyolysis, alterations in electrolyte

balance and possible death (Fajardo 1984; Kosaka et al. 2004). Exposure to excessive environmental heat loads, especially during exercise, can result in heatstroke in humans and animals. The pathophysiology of heatstroke results from the interplay between changes in physiology caused by hyperthermia (i.e. circulatory failure, hypoxia, increase in metabolic demand) and the damaging effects of heat on tissues (Bouchama and Knochel 2002). Cooling has been recommended to treat or prevent the pathophysiology seen in heatstroke in humans (Kosaka et al. 2004; Sawicka et al. 2015; Breed et al. 2019) and since it has been largely effective (Bouchama and Knochel 2002) it has been assumed to be of value in capture-induced hyperthermia in wildlife (Ozeki et al. 2015; Sawicka et al. 2015).

The efficacy of different cooling methods to reduce capture-induced hyperthermia have been studied (Sawicka et al. 2015). However, the effect of cooling on preventing or reducing the possible pathophysiological effects of capture-induced hyperthermia has not been determined.

In this prospective, controlled, randomised study using blesbok (*Damaliscus pygargus phillipsi*), we aimed to determine the pathophysiological effects induced by a mimicked field-capture procedure that is known to cause capture-induced hyperthermia, and whether cooling could reduce or prevent these pathophysiological effects from occurring.

MATERIALS AND METHODS

The study was approved by the Animal Ethics Committee at the University of Pretoria (clearance number V047-14). The study was conducted at Groenkloof Nature Reserve (25.7934° S, 28.2039° E), Pretoria, Gauteng, South Africa, 1300m above sea level.

Environmental conditions during captures

The study was conducted during the winter and spring months in the southern hemisphere; ambient temperatures ranged from 3.8 to 31.8°C (wet bulb globe temperatures range: 12.6 - 24.9°C) on the days of the experiments.

Animals

A group of 38 free-living blesbok were mass-captured from the wild (26 adults and 12 sub-adults (approximately 1-year-old), 22 males and 16 females) and housed in a holding facility (boma complex) divided into 10 small housing enclosures (bomas 5 x 5 m). The blesbok were allotted into groups of four animals per boma, with only one adult male and an approximate balanced mix of females and sub-adults. The blesbok received hay (*Eragrostis tef*), lucerne (*Medicago sativa)*, water *ad libitum* and antelope pellets (150 g/animal, Voermol Game Pellets, Maidstone, RSA) daily. The animals were given six weeks to adapt to confinement and boma management procedures before the trials commenced.

Experimental procedure

The blesbok were randomly allocated into three experimental groups: a control (Ct, n = 12), a chased not cooled (CNC, n = 14) and a chased plus cooled (C+C, n = 12) group. During the experimental trial period, the blesbok were immobilized four times: on Day 0 (experimental intervention day, D0), and on Days 3, 16 and 30 (sampling days, D3, D16, D30) thereafter. The sampling times were chosen based on the time key biochemical markers were expected to be elevated and when significant changes were anticipated (Ward et al. 2020, Moxnes and Sandbakk 2014; Hopkins et al. 2021). Furthermore, the sampling intervals were limited based on ethical

considerations regarding repetitive immobilizations and could not be performed sooner based on the duration of the antagonist effects of naltrexone.

On D0, blesbok from the CNC and C+C groups were vigorously chased for 15 minutes by people in an adjoining large boma compartment (50m x 50m), after which they were returned to their boma and immediately darted (immobilized). The blesbok in the Ct-group were not chased, and disturbance was kept to a minimum when they were immobilized in their bomas. Immobilization was achieved using etorphine (Captivon®, 9.8 mg/ml, Wildlife Pharmaceuticals, White River, South Africa, 3 mg sub-adults and 3.5 mg adults) intramuscular (IM) combined with azaperone (100 mg/ml, Kyron, Johannesburg, South Africa, 40 mg sub-adults and 60 mg adults) IM. The drugs were administered using a projectile dart (Dan-Inject International, Skukuza, South Africa) consisting of a 3 ml syringe and a 1.5 x 25 mm collared needle. After sample collection, etorphine's immobilizing effects were antagonised with naltrexone (Trexinol®, 50 mg/ml, Wildlife Pharmaceuticals, White River, South Africa, 20 mg per mg of etorphine) IM.

In all three groups, biological samples were taken and rectal and muscle temperature were recorded immediately after the blesbok became recumbent (D0-1/2). Animals in the C+C group, at approximately 10 minutes into the immobilization, were doused with 10 L of cold water (4°C) over a period of 10 minutes. The water was poured over the body surface and rubbed into the hair of the blesbok. In all animals, data was collected again 40 minutes after recumbency (D0-2/2) before the immobilization was antagonized. On D3, D16, D30 animals from the C+C and CNC groups were immobilized in the same way as the animals in the Ct group. On these days, no additional interventions occurred (no chasing or cooling) and only one set of samples was collected directly after the animals became recumbent.

For post-mortem evaluation and organ sample collection for an additional research study (data not reported here), six blesbok randomly selected from each group were humanely euthanized with a pentobarbitone (Eutha-Naze®, 200mg/ml, Bayer, Johannesburg, South Arica) overdose on D16, and the remainder on D30.

Blood samples and analysis

At each sampling time, arterial blood (0.5 ml) was drawn into a 1 ml heparinized syringe from an auricular artery. The samples were collected anaerobically, immediately placed on ice and analyzed within 10 min of sampling using pre-calibrated EPOC BGEM blood test cards and an EPOC Reader (Epocal, Inc. Ottawa, Ontario, Canada) to determine blood lactate concentrations, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), pH and bicarbonate (HCO_3^-).

Venous blood samples were collected into vacutainer tubes from the cephalic vein for biochemistry analysis. These samples were left to clot for 30 minutes and then centrifuged at 2000g for 8 minutes. Serum was aliquoted into 2 mL cryovials, snap-frozen in liquid nitrogen, and then stored in a -80°C freezer until analysis (within three months). A Cobas Integra 400 plus device (Roche, Johannesburg, South Africa) was used for biochemistry analysis. The concentrations of the following variables were determined: gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), aspartate transaminase (AST), creatine kinase (CK), blood urea nitrogen (UREA), creatinine (CREA). Cardiac troponin-I (cTnI) concentrations were determined using an Immulite 1000 immunoassay analyser (Siemens, Johannesburg, South Africa).

The Immulite 1000 cTnI assay has not been validated for the use in blesbok yet. However, within the artiodactyla family cTnI measurements have been validated in white tailed deer (Boesch et al.

2015) using a VetScan i-STAT 1 Handheld Analyzer and cTnI cartridges (i-STAT cTnI assay) (Abbott Point of Care Inc., Princeton, New Jersey 08540, USA). From a subset of 113 samples cTnI concentrations were measured both by the i-STAT and Immulite 1000 analysers and correlated to determine if these devices measured similar changes in the blesbok.

Body temperature

Rectal temperature was measured using a thermocouple probe (ESO-1 Oesophageal, Physitemp Instruments Inc, NJ, USA) connected to a digital thermometer (BAT-12, Physitemp Instruments, Clifton, NJ, USA) that was inserted approximately 7 cm into the rectum. Muscle temperature was measured using a fine thermocouple probe (IT-18, Physitemp Instruments, Clifton, NJ, USA) inserted sterilely ±20 mm into the gluteus muscle through an 18G needle, and connected to the same digital thermometer. All the probes and thermometers were calibrated individually in an insulated water bath against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany).

Data Analysis

Data were analysed using Graph Pad Prism 9 (GraphPad Software, San Diego, USA). The data was treated as parametric or non-parametric based on data distribution of variables in a normal antelope population. For non-parametric data (cardiac troponin I, lactate, CK and GLDH), the data was $\log^{10}$ transformed. Transformed data was used to calculate mean and standard deviations and this data was transformed back so that mean and standard deviations of the actual data could be depicted in all the figures. All measured variables and log transformed data were compared over time in each group and between the groups using a mixed effects model analysis. A post-hoc Tukey-Kramer multiple comparison test identified where differences occurred between the three groups. A post-hoc Dunnett's multiple comparison test was used to identify differences between

the first sample on Day 0-1/2 and all other sampling times within each group. Cardiac troponin I concentrations measured by the i-STAT and Immulite 1000 analysers were correlated using a Spearman's rank correlation coefficient test. A p value < 0.05 was considered significant.

RESULTS

Rectal and muscle temperatures

At D0-1/2 the rectal temperatures in the chased blesbok groups (CNC and C+C) were on average 3.8°C higher than in the Ct group (38.12 ± 0.74°C), with no difference between the two chased groups (Figure 1A). Dousing with cold water for 10 minutes reduced the rectal temperature of the blesbok in the C+C group from 41.9°C to 37.9°C ± 1.0°C, a temperature similar to that of the Ct group and lower than that of the CNC group ($p < 0.001$). Changes in muscle temperature, although on average 1°C higher, followed a similar pattern to that observed for rectal temperatures (Figure 1B). Mean (SD) rectal and muscle temperatures as well as mean (SD) blood biochemical variables from the Ct, CNC and C+C group on D0, D3, D16 and D30 are listed in the supplementary material, table 1.

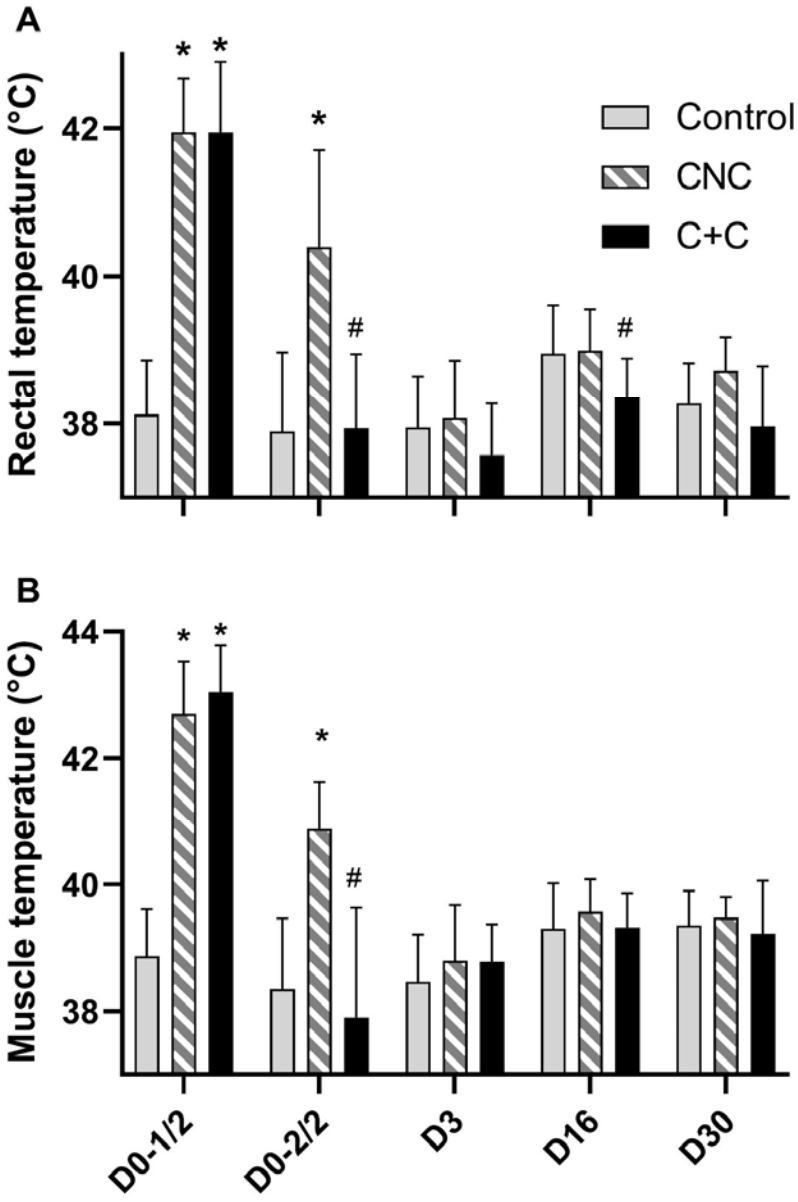


Figure 1: Rectal (A) and muscle (B) temperatures (mean ± SD) of immobilized blesbok (*Damaliscus pygargus phillipsi)* in the control (Ct), the chased not cooled (CNC) and the chased plus cooled (C+C) groups on the day of the intervention (D0–1/2 and D0-2/2) and the three data collection days (D3, D16 & D30). Cooling (C+C group) for 10 minutes by dousing with cold water was started 10 minutes after D0-1/2 and 30 minutes prior to D0-2/2). The * indicates where the temperatures in the CNC and C+C groups were significantly different compared to the Ct group and the # indicates where the temperatures in the CNC and C+C were significantly different to each other (Mixed effects model, Tukey-Kramer post hoc test, $p < 0.05$).

Markers of potential skeletal muscle damage

Creatine kinase concentrations in the two chased groups were higher than those in the Ct group at D0-1/2 (CNC: $p < 0.001$; C+C: $p < 0.001$) and D0-2/2 (CNC: $p < 0.001$; C+C: $p < 0.001$, Figure 2A). No differences at any time points were found between the CNC and C+C groups.

At D0-1/2 and D0-2/2, compared to the Ct group, the AST concentrations were higher in the CNC group (D0-1/2, $p = 0.009$; D0-2/2, $p = 0.005$) and C+C group (D0-1/2, $p = 0.017$; D0-2/2, $p = 0.014$), but there were no differences between the CNC and C+C groups at any of the sampling times ($p > 0.05$, Figure 2B).

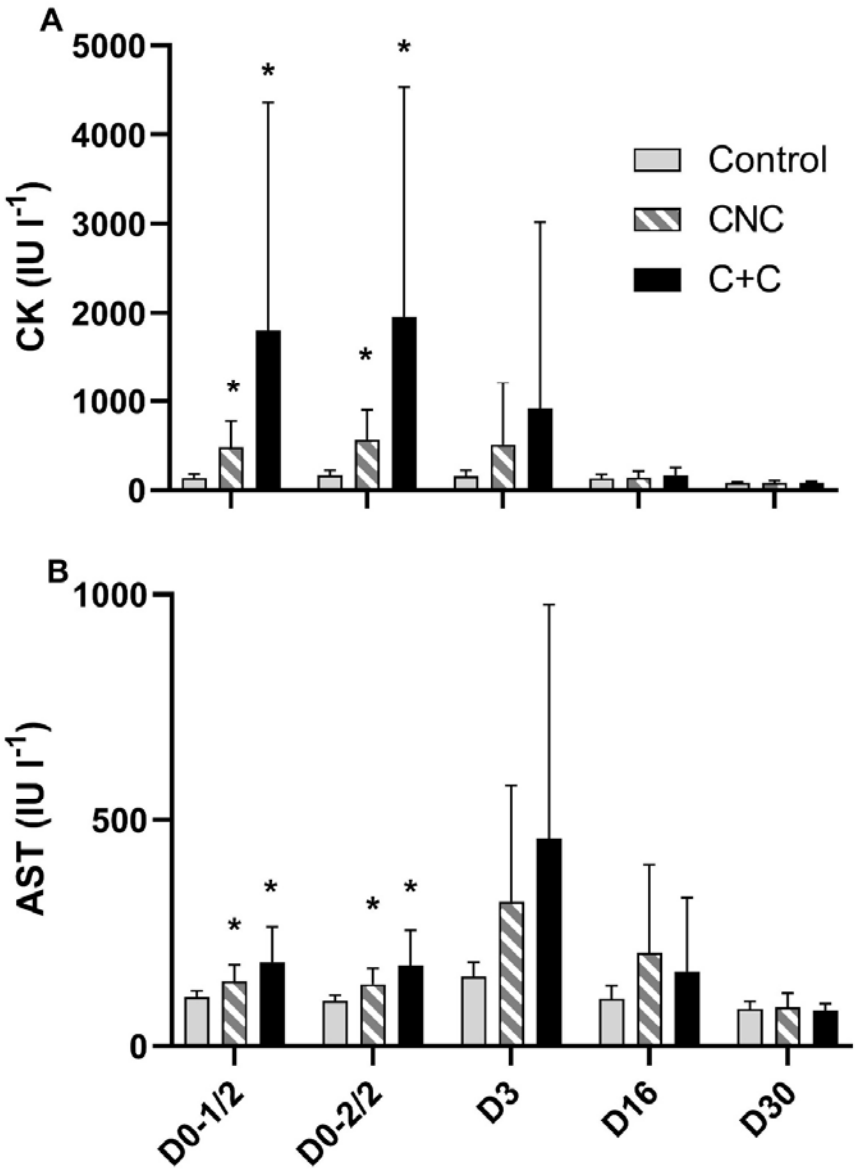


Figure 2: Creatine kinase (CK; A) and Aspartate transaminase (AST; B) concentrations (mean ± SD) of immobilized blesbok (*Damaliscus pygargus phillipsi)* in the control (Ct), chased not cooled (CNC) and the chased plus cooled (C+C) groups on the day of the intervention (D0–1/2 and D0-2/2) and the three data collection days (D3, D16 & D30). Cooling (C+C group) by dousing with cold water for 10 minutes was started 10 minutes after D0-1/2 and 30 minutes prior to D0-2/2. The * indicates where the concentrations in the CNC and the C+C groups were significantly different compared to the Ct group (Mixed effects model, Tukey-Kramer post hoc test, $p < 0.05$).

Markers of potential hepatic damage

The concentrations of hepatic enzyme GGT did not differ between the groups at the various sampling times (Figure 3A). However, on D0, compared to the Ct group, the liver specific enzyme GLDH was higher in the CNC group (D0-1/2, $p = 0.019$; D0-2/2, $p = 0.018$) and C+C group (D0-1/2, $p = 0.018$; D0-2/2, $p = 0.018$, Figure 3B). No differences were seen in the GLDH concentrations between the CNC and C+C groups. AST, which is a marker of both muscle and hepatic damage, has been reported above (Figure 2B).

Markers of potential kidney damage

On D0, compared to the Ct group, the CREA concentrations (Figure 3C) were higher in the CNC group (D0-1/2, $p < 0.001$; D0-2/2, $p < 0.001$) and C+C group (D0-1/2, $p < 0.001$; D0-2/2, $p = 0.003$), but returned to baseline on D3. However, on D16 and D30, the CNC and C+C group had lower creatinine concentrations compared to the Ct group (D16: CNC $p = 0.014$, C+C $p = 0.046$; D30: CNC $p = 0.003$, C+C $p = 0.001$). There were no differences between the two chased groups at any of the sampling points.

Blood urea concentrations did not differ between the groups except on D16 where the concentrations in CNC group were elevated compared to the Ct ($p = 0.007$) and C+C ($p = 0.007$) groups (Figure 3D).

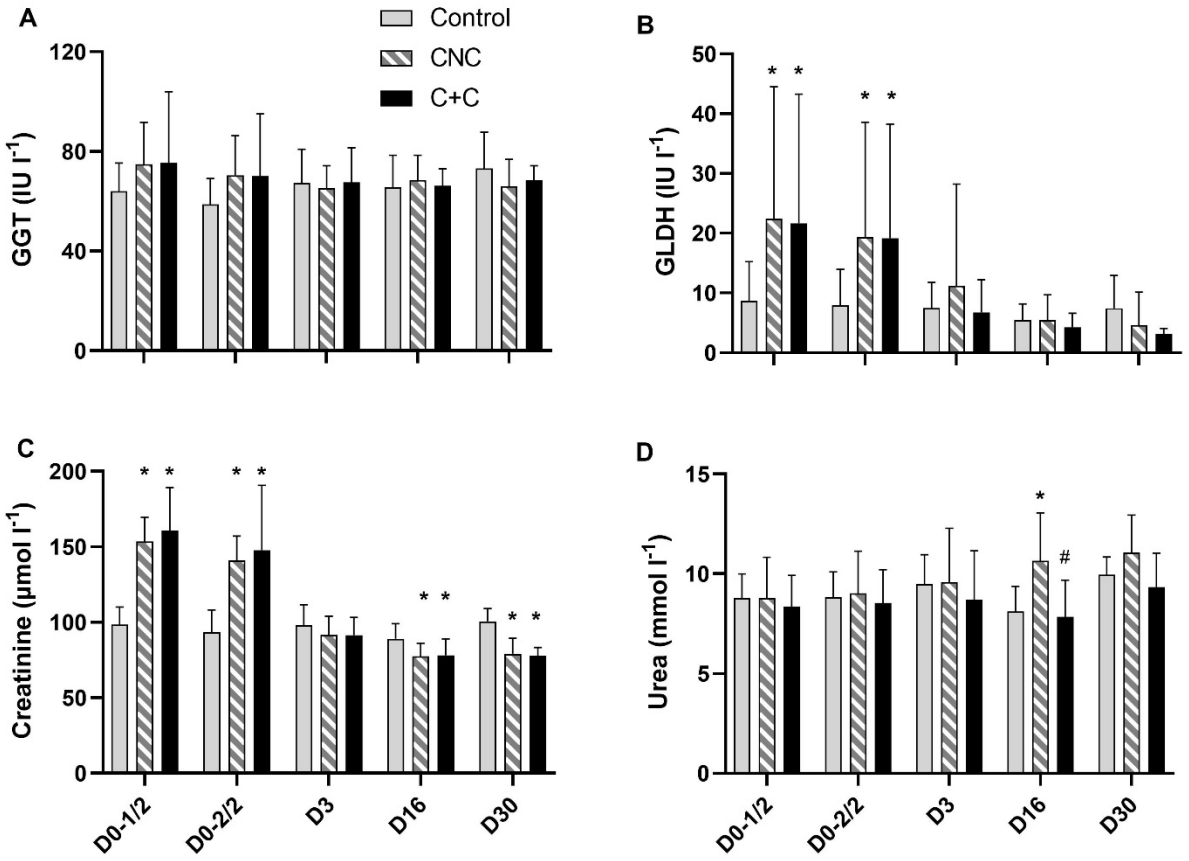


Figure 3: Gamma-glutamyl transferase (GGT; A) and Glutamate dehydrogenase (GLDH; B), Creatinine (C) and Blood Urea Nitrogen (Urea, D) concentrations (mean ± SD) of immobilized blesbok (*Damaliscus pygargus phillipsi)* in the control (Ct), the chased not cooled (CNC) and the chased plus cooled (C+C) groups on the day of the intervention (D0–1/2 and D0-2/2) and the three data collection days (D3, D16 & D30). Cooling (C+C group) by dousing with cold water for 10 minutes was started 10 minutes after D0-1/2 and 30 minutes prior to D0-2/2. The * indicates where the concentrations in the CNC and C+C groups were significantly different compared to the Ct group and the # indicates where the temperatures in the CNC and C+C were significantly different to each other (Mixed effects model, Tukey-Kramer post hoc test, $p < 0.05$).

Lactate concentration

On D0 lactate concentrations in the CNC and C+C groups were on average 8 to 10 times higher ($p < 0.001$) than those in the Ct group (Figure 4A). There were no differences in lactate concentrations between the two chased groups at any sampling time.

Marker of potential cardiac muscle damage

The comparison of cTnI concentrations measured by the i-STAT and Immulite 1000 analysers showed a positive correlation ($r = 0.89$, $p < 0.0001$). Compared to the Ct group, the chased groups had cTnI concentrations that were 10 (CNC, $p < 0.001$) and 16 (C+C, $p < 0.001$) times higher at D0-1/2 and 26 (CNC, $p < 0.001$) and 28 (C+C, $p < 0.001$) times higher at D0-2/2 (Figure 4B). On D3, although cTnI concentrations were visibly lower than at D0-2/2, cTnI concentrations were 51 (CNC, $p < 0.001$) and 40 (C+C, $p < 0.001$) times higher than in the Ct group. On D16, cTnI concentrations were back to baseline in the C+C group, but were still elevated in the CNC group compared to the Ct group ($p = 0.022$). There were no differences in cTnI concentrations between the two chased groups at any of the sampling times.

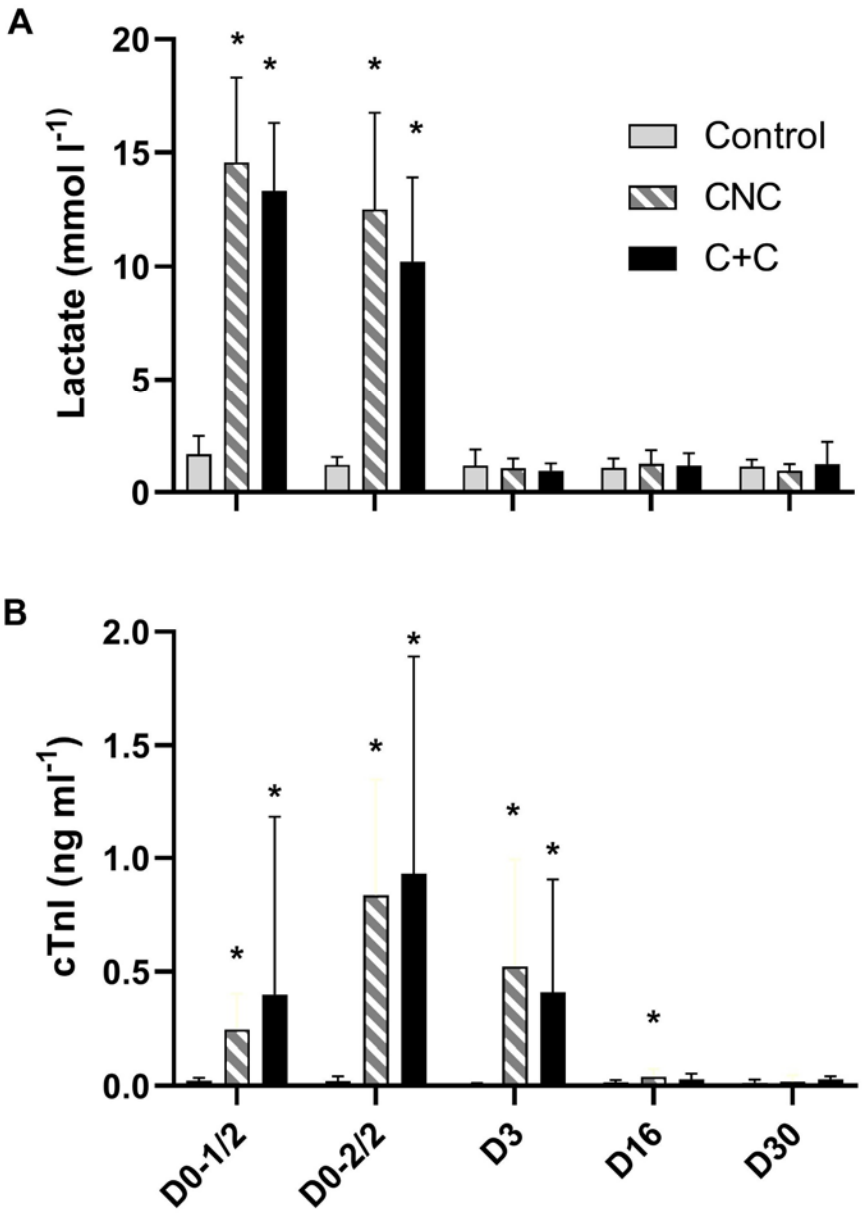


Figure 4: Lactate (A) and cardiac Troponin I (cTnI) concentrations (mean ± SD) of immobilized blesbok (*Damaliscus pygargus phillipsi)* in the control (Ct), the chased not cooled (CNC) and the chased plus cooled (C+C) groups on the day of the intervention (D0–1/2 and D0-2/2) and the three data collection days (D3, D16 & D30). Cooling (C+C group) by dousing with cold water for 10 minutes was started 10 minutes after D0-1/2 and 30 minutes prior to D0-2/2. The * indicates where the values in the CNC and C+C groups were significantly different compared to the Ct group (Mixed effects model, Tukey-Kramer post hoc test, $p < 0.05$).

Arterial blood gases

Partial pressure of oxygen did not differ between the groups except at D0-2/2 where it was higher in the C+C group than in the Ct group ($p = 0.018$, Figure 5A). No differences were recorded between the CNC and C+C group at any sampling time.

On D0, $PaCO_2$ (Figure 5B) was lower in the CNC (D0-1/2 and D0-2/2, $p < 0.001$) and C+C (D0-1/2 and D0-2/2, $p < 0.001$) groups compared to the Ct group. No differences were recorded between the CNC and C+C group at any sampling time.

Markers of acid-base disturbances

At D0-1/2 and D0-2/2, compared to the Ct group, the HCO_3^- concentrations (Figure 5C) were lower in the CNC (D0-1/2, $p < 0.001$; D0-2/2, $p < 0.001$) and C+C (D0-1/2, $p < 0.001$; D0-2/2 $p = 0.001$) groups. No differences were found between the two chased groups at any sampling time.

The pH in the CNC group was lower than in the Ct group ($p = 0.046$, Figure 5D) at D0-1/2, but did not differ between the chased groups and the Ct group at any other sampling time.

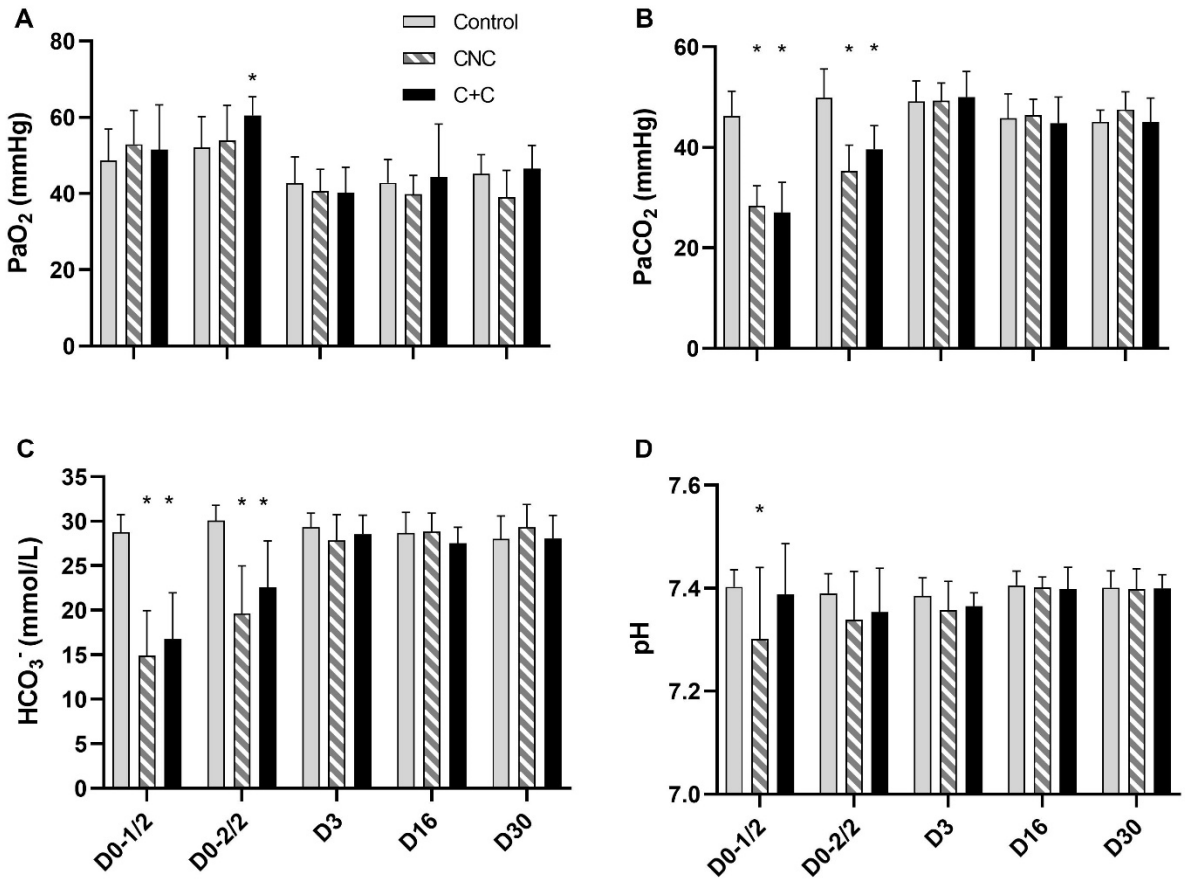


Figure 5: Partial pressure of oxygen (PaO_2, A), partial pressure of carbon dioxide ($PaCO_2$, B), bicarbonate (HCO_3^-, C) and pH (D, mean ± SD), of immobilized blesbok (*Damaliscus pygargus phillipsi)* in the control (Ct), chased not cooled (CNC) and chased plus cooled (C+C) groups on the day of the intervention (D0–1/2 and D0-2/2) and the three data collection days (D3, D16 & D30). Cooling (C+C group) by dousing with cold water for 10 minutes was started 10 minutes after D0-1/2 and 30 minutes prior to D0-2/2. The * indicates where concentrations in the CNC and C+C groups were significantly different compared to the Ct group (Mixed effects model, Tukey-Kramer post hoc test, $p < 0.05$).

DISCUSSION

The capture procedure used in the CNC and C+C groups mimicked a field-capture procedure in that both groups developed capture-induced hyperthermia (approximately 42°C). Furthermore,

cold water dousing in the C+C group was effective at treating this capture-induced hyperthermia and normalised the animal's body temperatures within 30 minutes from the start of cooling. The blesbok in the Ct group, which were not chased but immobilized with minimal disturbance, appeared to serve as good controls. They did not develop hyperthermia, changes in their serum biochemistry were minimal and within the reference ranges reported for blesbok in the Species360 Zoological Information Management System (ZIMS, n.d.).

In contrast to the Ct group, the CNC and C+C groups initially developed hyperlactatemia and elevated CK, AST, and GLDH concentrations, capture-induced pathophysiological changes often seen when capture myopathy occurs (Breed et al. 2019). Increases in cTnI concentrations, up to 51 (CNC group) and 40 (C+C group) times compared to the Ct group, likely indicate capture-induced cardiac muscle damage. These pathophysiological changes in both chased groups were mostly apparent only up to day 3, suggesting that these were short-term, likely reversible changes.

Despite cooling correcting the hyperthermia in the C+C group, neither the magnitude nor the duration of the pathophysiological changes differed between the two chased groups. Therefore, cooling did not appear to prevent or treat any pathophysiological effects induced by the simulated capture.

Cooling also did not appear to affect changes seen in the blood gases and acid-base balance. During the immobilizations the animals all developed hypoxaemia, and mostly hypercapnia, but on the day of capture the chased animals initially had normo- or hypocapnia, indicating normal- or hyperventilation. The animals also mostly had normal pH values, except the animals in the CNC group at D0-1/2 that had mild acidaemia. On the day of capture, the animals in the CNC and C+C group had low bicarbonate values, and with their elevated lactate levels, had a metabolic acidosis,

but since they mostly were hyperventilating, this acidosis was corrected by the resultant respiratory alkalosis.

Although we could not show how quickly body temperature started to drop after cooling, by 40-minutes into the immobilization the body temperatures in the blesbok in the C+C group had normalized when compared to the control group. This finding is similar to that of Sawicka et al. (2015), who showed that cooling by dousing with cold water led to a significant reduction of body temperature within 30 minutes.

In various animal species, hyperthermia is believed to play a role in the pathogenesis of capture myopathy, a complex, often fatal exertion- and stress-induced muscle degenerative condition affecting captured wild animals (Gericke et al. 1978; Drew 1996; Breed et al. 2019). The precise mechanistic aetiology of this condition, and the reasons why certain species, and even individuals within a species, are more susceptible remains unsolved (Breed et al. 2019).

Because hyperthermia is believed to play a role in the pathogenesis of capture myopathy and capture-related deaths, research on the cause and occurrence of capture-induced hyperthermia, and on measures to effectively reduce its severity and incidence, are ongoing (Dickens et al. 2010; Ozeki et al. 2015; Sawicka et al. 2015). One of the main questions revolves around the possible pathogenesis of capture-induced hyperthermia. Drugs used for chemical immobilization, tranquilization and sedation of wildlife can alter thermoregulation. However, the disruption of thermoregulation, particularly by opioid drugs, is further influenced by species, environmental conditions at the time of use, stress, the specific drug used, its dose as well as route of administration (Schönbaum and Lomax 1991). Meyer at al. (2008a and b) showed that the extent of capture-induced hyperthermia is not correlated to the physical activity and environmental conditions at capture and that the capture-induced hyperthermia is not primarily caused by the

pharmacological properties of different capture drugs in impala. However, although not the driving factor of capture-induced hyperthermia, capture-drugs may result in body temperature lability during immobilization and environmental conditions can affect body temperature during- and post-immobilization, which can worsen capture-induced hyperthermia if environmental heat loads are high (i.e. heat stress). Furthermore, if the immobilizing drugs were responsible for the hyperthermia observed in the blesbok of the CNC and C+C group, we would expect similarly elevated temperatures in the control group, which were immobilized with the same drug combination. In impala, the magnitude of capture-induced hyperthermia is primarily associated with the magnitude of stress (predominantly psychological stress) induced by the capture procedures (Meyer et al. 2008a), and while this has not yet been specifically assessed in blesbok, we assume a similar effect to occur in related species. The exact mechanisms causing the development of capture-, or, stress-induced hyperthermia remain unknown. Possible metabolic dysfunction, such as in porcine stress syndrome, or malignant hyperthermia or central activation of metabolic pathways are plausible but unconfirmed causes (Mitchell and Heffron 1980; Meyer et al. 2008a; Oka 2018; Breed et al. 2019).

The findings from our study suggest that hyperthermia was unlikely the primary cause of the pathophysiology observed in the blesbok from the study. Thus, capture-induced hyperthermia is more likely an outcome, representing a clinical sign, rather than the cause of the observed changes. Instead, tissue hypoxia is a more plausible cause of the pathophysiological changes. Although the animals that were chased in the CNC and C+C groups did not have lower PaO_2 compared to the animals in the Ct group, all animals were hypoxaemic. This hypoxaemia was likely mild as there was no indication that it caused hypoxia associated effects in the control animals. However, in the chased animals the PaO_2 levels did not meet the metabolic demands of the body, as is indicated by

the hyperlactaemia that they developed at capture. During the chase the animals were likely also hypermetabolic, and it is possible that at this time, tissue oxygen requirements outweighed supply.

The psychological and physical stress induced by the chase and capture likely led to aerobic and metabolic capacities being exceeded. Elevated metabolic requirements during stressful and exhaustive capture procedures, such as those experienced by the chased blesbok, may lead to an increased demand for ATP and depletion of oxygen, which results in activation of anaerobic pathways for alternate energy synthesis, causing hyperlactatemia (Breed et al. 2019). During hypoxia, and when metabolic demands exceed alternative energy supplies, organ damage will occur, particularly in organs that have limited anaerobic capacity or are very metabolically active, like the brain, heart, liver, kidneys and exercising skeletal muscles (Beringer et al. 1996; Leach and Treacher 1998; Schober and Schwarte 2012). We believe that these effects resulted in the markedly elevated cTnI concentrations, indicating probable myocardial injury (Wu 2017), and in elevation of CK and AST concentrations in the overexerted skeletal muscles (Keenan 1967; Valberg et al. 1989; Khan 2009). Elevated AST concentrations may however also result from hepatocellular damage (Brunner 2009). Furthermore, the elevation in GLDH concentrations, which are usually seen when centrilobular hepatocellular hypoxia occurs (Dancygier 2009), also probably occurred from these effects.

Creatinine is a waste product from energy producing processes of the skeletal muscles (Hosten 1990). As creatinine levels are used as an indicator of the glomerular filtration rate and, hence kidney function, the mildly elevated creatinine concentrations may indicate some kidney injury, however further urine analysis would be required to diagnose any kidney damage.

Although the analysis techniques used to evaluate many of the variables we measured have not been validated in blesbok, we believe that most of these variables are ubiquitous across mammalian

species, especially artiodactyls, and that the analysis reflect actual physiological changes. However, cTnI is measured by immunoassay techniques with assays that do not always cross-react between species (Rossi et al. 2018). The correlation of the cTnI concentrations measured by the i-STAT assay, which has been validated for the use in another artiodactyla species, namely white-tailed deer (Boesch et al. 2015), and the Immulite 1000 assay was strong ($r = 0.89$, $p < 0.0001$). Although this finding suggests that our measures of cTnI concentrations by the Immulite 1000 likely reflect cardiac damage, further validation of this technique in blesbok may be necessary.

Furthermore, additional research is required to confirm that hypermetabolism, resulting from capture-induced physical and psychological stress, leads to an oxygen supply-and-demand imbalance which results in tissue hypoxia and damage during the chase and capture procedures. However, based on the findings, we believe that an important shift in thinking is required, particularly away from the belief that cooling hyperthermia will treat the pathophysiological effects caused by capture. The data also suggests that capture-induced hyperthermia is not the primary cause of organ damage and its associated pathophysiological changes in blesbok, but a clinical sign of the hypermetabolism resulting from physical and psychological stress. Therefore, during capture procedures, minimizing physical and psychological stress should be a priority to prevent capture-induced pathophysiological changes and hyperthermia in antelope such as impala and blesbok (Meyer et al. 2008a; Sawicka et al. 2015). This approach is particularly important, considering that harmful and possibly lethal capture-induced complications may not be overtly evident, such as severe capture-induced cardiomyopathy, which we potentially identified. However, even if capture-induced hyperthermia is not the cause of the pathophysiological changes observed during capture, its damaging impact should not be underestimated. Untreated and prolonged hyperthermia will likely worsen capture-induced pathophysiology as it may cause

cellular injury and increased metabolic demand (Kosaka et al. 2004; Epstein et al. 2011), thereby worsening tissue hypoxia and damage. Cooling is therefore still recommended to prevent the capture-induced pathophysiology from worsening due to the chronic effects of persistent or increasing hyperthermia post capture, but importantly, as we have shown in blesbok, it is unlikely to prevent the stress- and hypoxic-induced damage that already results from the capture procedures. Additionally, oxygen administration should also be considered to treat hypoxaemia, and to correct oxygen supply-and-demand imbalance, in order to prevent further hypoxic damage from occurring.

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LITERATURE CITED

Arnemo JM, Ahlqvist P, Andersen R, Berntsen F, Ericsson G, Odden J, Brunberg S, Segerström P, Swenson JE. 2006. Risk of capture-related mortality in large free-ranging mammals: Experiences from Scandinavia. *Wildlife Biol* 12:109–113.

Beringer J, Hansen LP, Wilding W, Fischer J, Steven L. 1996. Factors Affecting Capture Myopathy in White-Tailed Deer. *J Wildl Manage* 60:373–380.

Boesch JM, Gleed RD, Erb HN, Solter PF. 2015. Laboratory validation of a point-of-care cardiac troponin I assay for use in white-tailed deer (*Odocoileus virginianus*). *J Zoo Wildl Med* 46:468–475.

Bouchama A, Knochel J. 2002. Heat Stroke. *N Engl J Med* 346:1978–1988.

Braud C, Mitchell EP, van der Merwe V, Tordiffe ASW. 2019. A veterinary survey of factors associated with capture-related mortalities in cheetahs (*Acinonyx jubatus*). *J S Afr Vet Assoc* 90:1–7.

Breed D, Meyer LCR, Steyl JCA, Goddard A, Burroughs R, Kohn TA. 2019. Conserving wildlife in a changing world: Understanding capture myopathy - A malignant outcome of stress during capture and translocation. *Conserv Physiol* 7:1–21.

Brivio F, Grignolio S, Sica N, Cerise S, Bassano B. 2015. Assessing the impact of capture on wild animals: The case study of chemical immobilisation on Alpine ibex. *PLoS One* 10:1–18.

Brunner LS. 2009. Aspartate Aminotransferase. In: *Brunner and Suddarth's Handbook of Laboratory and Diagnostic Tests,* Wolters Kluver Health/ Lippincott Williams & Wilkins, Philadelphia, Pennsylvania, 68 pp.

Dickens MJ, Delehanty DJ, Michael Romero L. 2010. Stress: An inevitable component of animal translocation. *Biol Conserv* 143:1329–1341.

Drew ML. 1996. The use of a tympanic membrane thermometer for assessing hyperthermia in bighorn sheep. *J Wildl Dis* 32:512–516.

Epstein Y, Roberts WO. 2011. The pathopysiology of heat stroke: an integrative view of the final common pathway. *Scand J Med Sci Sport* 21:742–748.

Fajardo LF. 1984. Pathological effects of hyperthermia in normal tissues. *Cancer Res* 44:4826–4835.

Gericke MD, Hofmeyr JM, Louw GN. 1978. The effect of capture stress and haloperidol therapy on the physiology and blood chemistry of springbok, *Antidorcas marsupialis*. *Madoqua* 11:1–18.

Hampton JO, Amos M, Pople A, Brennan, M, Forsyth DM. 2021. Minimising mortalities in capturing wildlife: refinement of helicopter darting of chital deer (*Axis axis*) in Australia. *Wildl Res* 48:304–313

Hopkins E, Sanvictores T, Sharma S. 2022. Physiology, Acid Base Balance. In: *StatPearls*. StatPearls Publishing, Treasure Island, Florida. https://www.ncbi.nlm.nih.gov/books/NBK507807/

Hosten AO. 1990. BUN and creatinine, 3rd edition. In: *Clinical Methods: The History, Physical, and Laboratory Examinations*, Walker HK, Hall WD, and Hurst JW, editors. Butterworths, Boston. pp. 874 - 878.

Jacques, CN, Jenks JA, DePerno CS, Sievers JD, Grovenburg TW, Brinkman TJ, Swanson CC, Stillings BA. 2009. Evaluating ungulate mortality associated with helicopter net-gun captures in the Northern Great Plains. *J Wildl Manag 73*(8):1282–1291

Keenan DM. 1967. Changes of blood metabolites in horses after racing, with particular reference to uric acid. *Aust Vet J* 55:54–57.

Khan FY. 2009. Rhabdomyolysis: Review of the literature. *Neth J Med* 67:272–283.

Kock MD, Clark RK, Franti CE, Jessup DA, Wehausen JD. 1987. Effects of capture on biological parameters in free-ranging bighorn sheep (*Ovis canadensis*): evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. *J Wildl Dis* 23:652–662.

Kock MD, du Toit R, Kock N, Morton D, Foggin C, Paul B. 1990. Effects of Capture and Translocation on Biological Parameters in Free-Ranging Black Rhinoceroses (*Diceros bicornis*) in Zimbabwe. *J Zoo Wildl Med 21*(4): 414–424.

Kosaka M, Yamane M, Ogai R, Kato T, Ohnishi N, Simon E. 2004. Human body temperature regulation in extremely stressful environment: Epidemiology and pathophysiology of heat stroke. *J Therm Biol* 29:495–501.

Leach RM, Treacher DF. 1998. Oxygen transport — 2 . Tissue hypoxia. *Br Med J* 317, pp. 1370-1373.

Meyer LCR, Fick L, Matthee A, Mitchell D, Fuller A. 2008 a. Hyperthermia in captured impala (*Aepyceros melampus*): A fright not flight response. *J Wildl Dis* 44:404–416.

Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D, Fuller A. 2008 b. Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. *J S Afr Vet Assoc* 79(3):121-9.

Mitchell G, Heffron JJ. 1980. Porcine stress syndromes: A mitochondrial defect? *S Afr J Sci* 76:546–551.

Montané J, Marco I, López-Olvera J, Perpinán D, Manteca X, Lavín S. 2003. Effects of acepromazine on capture stress in roe deer (*Capreolus capreolus*). *J Wildl Dis* 39:375–386.

Moxnes JF, Sandbakk Ø. 2012. The kinetics of lactate production and removal during whole-body exercise. *Theor Biol Med Model* 9(7):1-14.

Oka T. 2018. Stress-induced hyperthermia and hypothermia. In: *Handbook of clinical neurology*, Romanovsky AA, editor. Elsevier, Amsterdam, Netherlands, pp. 599-621.

Ozeki LM, Caulkett N, Stenhouse G, Arnemo JM, Fahlman Å. 2015. Effect of active cooling and α-2 adrenoceptor antagonism on core temperature in anesthetized brown bears (*Ursus arctos*). *J Zoo Wildl Med* 46:279–285.

Rossi TM, Kavsak PA, Maxie MG, Pearl DL, Pyle WG, Physick-Sheard PW. 2018. Analytical validation of cardiac troponin I assays in horses. *J Vet Diagn Invest* 30(2):226–232.

Sakaguchi Y, Stephens C, Makino M, Kaneko T, Strebel F, Danhauser LL, Jenkins GN, Bull J. 1995. Apoptosis in tumors and normal tissues induced by whole body hyperthermia in rats. *Cancer Res* 55:5459–5464.

Sawicka J, Fuller A, Fick LG, Hetem RS, Meyer LCR. 2015. Efficacy of different cooling methods for capture-induced hyperthermia in antelope. *African J Wildl Res* 45:100–110.

Schober P, Schwarte LA. 2012. From system to organ to cell: Oxygenation and perfusion measurement in anesthesia and critical care. *J Clin Monit Comput* 26:255–265.

Schönbaum E and Lomax P. 1991. Opioids and opioid receptors in thermoregulation. In: *Thermoregulation: Pathology, Pharmacology and Therapy*, Burkes TF, editor. Pergamon Press, Oxford, UK, pp. 489-508.

Species360 Zoological Information Management System (ZIMS). n.d. zims.Species360.org. Accessed October 2021.

Valberg S, Gustavson BE, Lindholm A, Persson SGB. 1989. Blood chemistry and skeletal muscle metabolic responses during and after different speeds and durations of trotting. *Equine Vet J* 21:91–95.

Ward MD, King MA, Gabrial C, Kenefick RW, Leon LR. 2020. Biochemical recovery from

exertional heat stroke follows a 16-day time course. *PLoS One* 15(3):e0229616.

Wu AHB. 2017. Release of cardiac troponin from healthy and damaged myocardium. *Front Lab Med* 1:144–150.

Zeiler GE, Meyer LCR. 2017. Chemical capture of impala (*Aepyceros melampus*): A review of factors contributing to morbidity and mortality. *Vet Anaesth Analg* 44(5):991-1006.

Supplementary material

Table 1: Mean (SD) rectal and muscle temperatures, concentrations of blood clinical chemistry analytes, arterial blood gas variables and markers of acid- base disturbances from immobilized blesbok (*Damaliscus pygargus phillipsi*) in the control (Ct). the chased non-cooling (CNC) and the chased plus cooled (C+C) groups on the day of the intervention (D0 – 1/2 and D0 - 2/2) and the three data collection days (D3, 16 & 30). The * indicates significant differences within the groups between the values measured directly after immobilization at D0 - 1/2 and D0 - 2/2, D3, D16 & D30. The † indicates where the CNC and C+C groups differed significantly from the Ct group and the # indicates where the CNC and C+C groups differed significantly between each other.

Variable	Unit	Group	D0 (1/2)	D0 (2/2)	D3	D16	D30
Rectal temperature	°C	Ct	38.12 (0.74)	37.89 (1.08)	37.95 (0.70)	38.96 (0.65) *	38.27 (0.55)
		CNC	41.93 (0.73) †	40.40 (1.32) *†	38.08 (0.78) *	39.00 (0.56) *	38.72 (0.45) *
		C+C	41.95 (0.95) †	37.94 (1.01) *#	37.57 (0.70) *	38.35 (0.54) *#	37.96 (0.82) *
Muscle temperature	°C	Ct	38.87 (0.74)	38.35 (1.11)	38.47 (0.74)	39.30 (0.72)	39.35 (0.54)
		CNC	42.70 (0.83) †	40.88 (0.75) *†	38.80 (0.87) *	39.57 (0.51) *	39.48 (0.33) *
		C+C	43.21 (0.73) †	37.90 (1.73)*#	38.78 (0.59) *	39.32 (0.54) *	39.22 (0.84) *
Gamma-glutamyl transferase (GGT)	IU/L	Ct	64.08 (11.23)	58.75 (10.41)*	67.33 (13.47)	65.50 (12.92)	73.17 (14.61) *
		CNC	74.86 (16.82)	70.43 (15.88) *	65.29 (8.93) *	68.57 (9.84)	66.00 (10.81)
		C+C	75.42 (28.51)	70.17 (24.98) *	67.67 (13.75)	66.25 (6.81)	68.50 (5.79)
Glutamate dehydrogenase (GLDH)	IU/L	Ct	8.70 (6.54)	7.94 (6.02) *	7.46 (4.30)	5.48 (2.69) *	7.43 (5.51)
		CNC	22.45 (22.07) †	19.36 (19.20) †	11.24 (16.98) *	5.48 (4.22) *	4.64 (5.49) *
		C+C	21.66 (21.63) †	19.16 (19.08) *†	6.72 (5.45) *	4.28 (2.31) *	3.13 (0.94) *
Creatinine	µmol/L	Ct	98.42 (11.72)	93.33 (14.83) *	97.83 (13.78)	89.00 (10.13) *	100.50 (8.67)
		CNC	153.64 (15.95) †	140.86 (16.29) *†	91.57 (12.36) *	77.43 (8.68) *†	79.13 (10.26) *†
		C+C	160.67 (28.54) †	147.75 (43.06) †	91.33 (11.95) *	78.08 (10.79) *†	77.83 (5.46) *†
Urea	mmol/L	Ct	8.80 (1.20)	8.83 (1.27)	9.49 (1.46)	8.12 (1.25)	9.95 (0.89)
		CNC	8.79 (2.03)	9.01 (2.12) *	9.58 (2.70)	10.65 (2.40) *†	11.05 (1.90) *
		C+C	8.36 (1.56)	8.54 (1.66) *	8.71 (2.45)	7.84 (1.84) #	9.33 (1.70)
Variable	Unit	Group	D0 (1/2)	D0 (2/2)	D3	D16	D30

Creatine Kinase (CK)	IU/L	Ct	135.42 (46.53)	168.42 (53.34) *	156.67 (67.16)	132.17 (46.61)	80.00 (10.73) *
		CNC	481.86 (296.87) †	563.64 (339.37) *†	509.50 (699.30)	140.71 (74.51) *	81.50 (23.02) *
		C+C	1807.00 (2551.89) †	1956.75 (2576.73) *†	917.00 (2100.69)	166.67 (90.01) *	86.50 (11.43) *
Aspartate transaminase (AST)	IU/L	Ct	109.08 (13.16)	100.67 (11.86) *	154.08 (31.28) *	104.33 (29.43)	83.00 (15.53) *
		CNC	143.79 (35.51) †	137.43 (34.79) *†	319.36 (258.65) *	206.86 (194.68)	86.63 (30.53) *
		C+C	185.08 (79.02) †	178.50 (77.97) *†	459.08 (518.07)	164.58 (163.17)	78.50 (15.08)
Lactate	mmol/L	Ct	1.68 (0.81)	1.21 (0.35) *	1.17 (0.73)	1.07 (0.42) *	1.14 (0.30)
		CNC	14.58 (3.73) †	12.53 (4.24) *†	1.06 (0.44) *	1.25 (0.60) *	0.94 (0.29) *
		C+C	13.34 (2.98) †	10.23 (3.68) *†	0.94 (0.33) *	1.17 (0.56) *	1.24 (0.98) *
Cardiac troponin I (cTnI)	ng/ml	Ct	0.03 (0.01)	0.03 (0.02)	0.01 (0.01) *	0.02 (0.01)	0.02 (0.01)
		CNC	0.25 (0.16) †	0.84 (0.51) *†	0.52 (0.47) *†	0.04 (0.03) *†	0.03 (0.03) *
		C+C	0.40 (0.79) †	0.93 (0.96) *†	0.41 (0.50) †	0.03 (0.02) *	0.03 (0.01) *
Partial pressure of oxygen (PaO_2)	mmHg	Ct	48.73 (8.26)	52.13 (8.12)	42.79 (6.81)*	42.81 (6.18)	45.18 (5.04)
		CNC	52.95 (8.87)	53.99 (9.16)	40.72 (5.65)*	39.81 (4.93)*	39.04 (7.08)*
		C+C	51.53 (11.76)	60.48 (4.94)* †	40.23 (6.70)*	44.33 (13.87)	46.55 (6.07)
Partial pressure of carbon dioxide ($PaCO_2$)	mmHg	Ct	46.24 (4.95)	49.86 (5.76)*	49.13 (4.09)*	45.84 (4.84)	45.08 (2.32)
		CNC	28.38 (3.99) †	35.25 (5.22)* †	49.34 (3.48)*	46.42 (3.14)*	47.51 (3.57)*
		C+C	27.07 (5.99) †	39.61 (4.70)* †	50.03 (5.10)*	44.81 (5.24)*	45.07 (4.74)*
pH		Ct	7.40 (0.03)	7.39 (0.04)	7.39 (0.03)	7.41 (0.03)	7.40 (0.03)
		CNC	7.30 (0.14) †	7.34 (0.09)	7.36 (0.06)	7.40 (0.02)*	7.40 (0.04)
		C+C	7.39 (0.10)	7.35 (0.09)*	7.37 (0.03)	7.39 (0.04)	7.40 (0.03)
Base excess (BE)	mmol/L	Ct	4.00 (2.00)	5.11 (1.65)*	4.33 (1.81)	4.00 (2.30)	3.27 (3.01)
		CNC	-11.48 (7.20) †	-6.14 (6.78)*†	3.56 (2.40)*	4.10 (2.20)*	4.54 (2.98)*
		C+C	-8.21 (6.34) †	-2.98 (6.49)*†	3.18 (2.06)*	2.72 (1.78)*	3.05 (2.39)*

Variable	Unit	Group	D0 (1/2)	D0 (2/2)	D3	D16	D30

Bicarbonate (HCO_3^-)	mmol/L	Ct	28.76 (1.98)	30.08 (1.72)*	29.35 (1.58)	28.70 (2.29)	28.03 (2.55)
		CNC	14.92 (5.05) †	19.64 (5.35)*†	27.85 (2.88)*	28.86 (2.05)*	29.35 (2.51)*
		C+C	16.79 (5.16) †	22.58 (5.23)*†	28.57 (2.12)*	27.55 (1.76)*	28.06 (2.58)*