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Does cooling affect skeletal muscle glycogen replenishment after an acute bout of fear-induced exertional hyperthermia in blesbok (*Damaliscus pygargus phillipsi*)?

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ABSTRACT

Rhabdomyolyses is a clinical sign of capture myopathy in wild animals and may be linked to glycogen metabolism. To study potential mechanisms, 26 wild blesbok were chased for 15 min and immobilised, whereafter 12 of these blesbok were doused with ice-water (n = 14 chased only group; n = 12 chased + cooled group). An additional 12 blesbok served as resting (not chased) uncooled controls. *Vastus lateralis* biopsies were obtained after immobilisation for biochemical analyses. Biopsies obtained at initial capture, 3- and 16-days post exercise were analysed for glycogen content. Blesbok muscles contained predominantly myosin heavy chain (MHC) IIA (~50 ± 9 %), followed by IIX (32 ± 10 %) and MHC I (18 ± 5 %), with no difference between groups. Citrate synthase (mean: 87 ± 48), 3-hydroxyacetyl co A dehydrogenase (47 ± 17), lactate dehydrogenase (1567 ± 654), phosphorylase (162 ± 94), phosphofructokinase (250 ± 123) and creatine kinase (12,455 ± 6372) activities (in μmol/min/g prot) were not different between groups. Similarly, superoxide dismutase (7.9 ± 7 U/mg prot), catalase (8.8 ± 5.8 mmol/min/g prot), and overall antioxidant capacity (ORAC: 23055 ± 18,460 μmol/g prot) were not different between groups. Glycogen content was reduced in both chased groups and not replenished by day 3. Glycogen supercompensation was observed on day 16 in both chased groups (~33 % higher than resting control group). The results confirm that blesbok have high muscle metabolic capacities, and that glycogen resynthesis is slow, which could lead to metabolite deficiency during prolonged chase events (>15 min).

1. Introduction

Muscle glycogen is an important fuel for the rapid resynthesis of ATP to maintain muscle function during high intensity exercise (Ørtenblad et al., 2013). When glycogen stores become critically low after exercise, it results in muscle fatigue and a concomitant decrease in force output, therefore necessitating rapid repletion during rest periods (Debold and Westerblad, 2024). Similarly, muscle diseases that impair glycogen accessibility (e.g. polysaccharide storage myopathy in horses or McArdle's disease in humans) or from acute glycogen depletion due to vigorous exercise, result in exercise intolerance and muscle weakness, and combined with physical activity, results in extensive muscle fibre damage (rhabdomyolysis) leading to myoglobinuria (Debold and Westerblad, 2024; Henning et al., 2017; McCue et al., 2008).

Mammalian skeletal muscle is comprised of three distinct fibre types, each differing in kinetic and metabolic properties (as reviewed by

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Mallett (2025)). Type I fibres have large mitochondrial numbers and can generate ATP from fat and glycogen through the Krebs cycle and electron transport system, which gives this fibre type a high capacity to withstand fatigue. On the other hand, type IIX fibres, usually recruited during intense exercise (e.g. sprinting), have low mitochondrial numbers, and ATP replenishment is primarily achieved through anaerobic glycolysis, metabolising blood glucose and muscle glycogen, which leads to lactate accumulation and fatigues quickly. Type IIA fibres resemble a combination of the two, having contractile properties of type IIX fibres, but metabolic properties resembling those of type I fibres, thus also having a capacity to resist fatigue for longer periods. Varying proportions of these fibres will therefore affect the contractile and metabolic properties of the overall muscle. In all fibre types, muscle glycogen serves as the most abundant energy source, critical for quick ATP resynthesis during contraction (Hargreaves and Spriet, 2020).

Human muscles comprise of high proportions of type I and IIA fibres (Esbjornsson et al., 2021), whereas muscles from wild animals (e.g. lion, cheetah and antelope) contain predominantly type IIA and IIX fibres (Curry et al., 2012; Hohl et al., 2020; Kohn, 2014; Kohn et al., 2024; Pösö et al., 1996), offering an explanation for their exceptional strength and speed compared to human performance.

Apart from glycogen that is used during exercise, psychological stress from fear (e.g. animal restraint, confined spaces) with or without physical exertion triggers significant physiological responses through the release of catecholamines (epinephrine and norepinephrine) that results in increased heart rate, body temperature, and metabolism via increased glycogenolysis and glycolysis (Breed et al., 2019; Dickens et al., 2010; McVeigh et al., 1982). In particular, McVeigh and Tarrant (1982) showed that 6 h of psychological stress combined with uncontrolled physical activity in bulls resulted in increased plasma creatine kinase (CK) activity (a marker of muscle damage) and a 44 % decrease in muscle glycogen content. The increase in metabolism also results in a concomitant increase in reactive oxygen species (ROS) production (indicating increased oxidative stress) and can lead to tissue damage. However, these radicals are neutralised by endogenous antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase and catalase (CAT), with the activities of SOD and glutathione peroxidase increasing with endurance type exercise (Breed et al., 2019; Hohl et al., 2020; Powers et al., 1999).

Wild animals being chased, especially by humans during wild animal translocation operations (an event where one to many animals are captured, relocated using motorised transport and released), induces a strong psychological fear response combined with intense exertion (typical flight response), with physiological consequences, including catecholamine surge and hyperthermia (Breed et al., 2019). These operations may result in animals developing a condition known as capture myopathy that presents with hyperthermia (core temperature $> 42\ ^{\circ}C$), stiff limbs, extensive rhabdomyolysis and myoglobinuria (Breed et al., 2019). The outcome of this condition is unpredictable, but once myoglobinuria presents, the prognosis is poor. Although the exact causes of capture myopathy are not yet confirmed, it is known to be a complex condition and could have one or multiple causes, ranging from genetic predispositions to nutritional deficiencies (Breed et al., 2019). The human equivalent of this condition, known as exertional heatstroke, is effectively treated by active cooling until temperatures return to normal (Demartini et al., 2015; Rae et al., 2008). Indeed, treating hyperthermic animals at capture is considered standard practice, but cooled animals can still develop rhabdomyolysis and capture myopathy, likely due to the condition that has progressed significantly before it is recognised by clinicians (Leiberich et al., 2023; Sawicka et al., 2015).

As acute muscle glycogen depletion from intense muscle activity was hypothesised as a contributor towards developing capture myopathy (Breed et al., 2019), the aim of this study was to evaluate muscle glycogen depletion and repletion of wild blesbok subjected to a chase event, with or without post-exertion cooling. To our knowledge, muscle glycogen depletion and concomitant repletion, and the effect of cooling have not yet been investigated in fear-induced exertional hyperthermia of wild animals. Previous research has shown that cooling of the muscles directly after exercise may delay glycogen repletion (Cheng et al., 2017; Tucker et al., 2012). Our hypothesis is that cooling will influence the glycogen repletion in blesbok post-exertion.

2. Methods

2.1. Ethical approval

Ethical approval was obtained from the University of Pretoria (V047–14), and reciprocal ethical approval obtained from the University of Cape Town (AREC 015/033) and University of the Western Cape (AR20/3/2).

2.2. Animals and intervention

Fig. 1 depicts blesbok during the exercise-induced hyperthermia (chase) event. The detailed methods on animal handling and the intervention have been described elsewhere (Leiberich et al., 2023), and an overview of the study is presented in Fig. 1B. Briefly, 38 wild blesbok were caught, habituated for 2 weeks (to allow for acclimatisation to confined spaces and unfamiliar smells to minimise psychological stress and its physiological consequences at the time of the study interventions) and then randomly assigned to control ($n = 12$), chased only ($n = 14$) and chased + cooled ($n = 12$) groups. Each day, all animals received the same feed in the form of hay, lucerne, water ad libitum, and approximately 150 g of pellets per animal (Voermol Game Pellets, Maidstone, RSA). The latter contains primarily protein, fibre and carbohydrate in the form of molasses. Both chased groups were subjected to 15 min of intense physical exertion combined with psychological fear through human presence and actions (Fig. 1A), returned to their bomas and chemically immobilised. The chased + cooled group was actively cooled for 10 min by dousing the hides of the animals with 4 °C water. The control group was neither chased nor cooled, and only immobilised. Blood samples and physiological variables (e.g. rectal temperature, muscle temperature) were collected immediately after immobilisation and 40 min thereafter. A muscle biopsy from the *vastus lateralis* muscle was obtained approximately 15 min after immobilisation using the suction assisted biopsy technique described by Tarnopolsky et al. (2011). These biopsies were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Additional biopsies were obtained 3 and 16 days after the chase event from all animals using alternating hind legs. These intervals were based on previous research on maximum skeletal muscle repair activity (day 3) and complete recovery (day 14) after severe muscle damage (Ciciliot and Schiaffino, 2010). In addition, ethical aspects were also considered due to the harmful nature of repetitive drug immobilisations and the duration of effect of the antagonist drug (Leiberich et al., 2023).

2.3. Sample preparation for assays

Except for the glycogen assay (see below), a small piece of muscle tissue, obtained on day 0 weighing between 50 and 90 mg wet weight (ww) was homogenised in 0.1 M potassium phosphate buffer, pH 7.40 in a ratio of 1:5 for every 1 mg muscle, 5 µl buffer was added) using a glass homogeniser, after which it was sonicated on ice. From this original stock homogenate, the following were determined (see specific details below): protein content of the whole homogenate and fractionated supernatant using the assay developed by Bradford (1976), enzyme activities, antioxidant capacity and the myosin heavy chain (MHC) isoform content. The latter technique provides a good estimation of the fibre type composition of a muscle (Andersen et al., 1994; Kohn et al., 2007a).

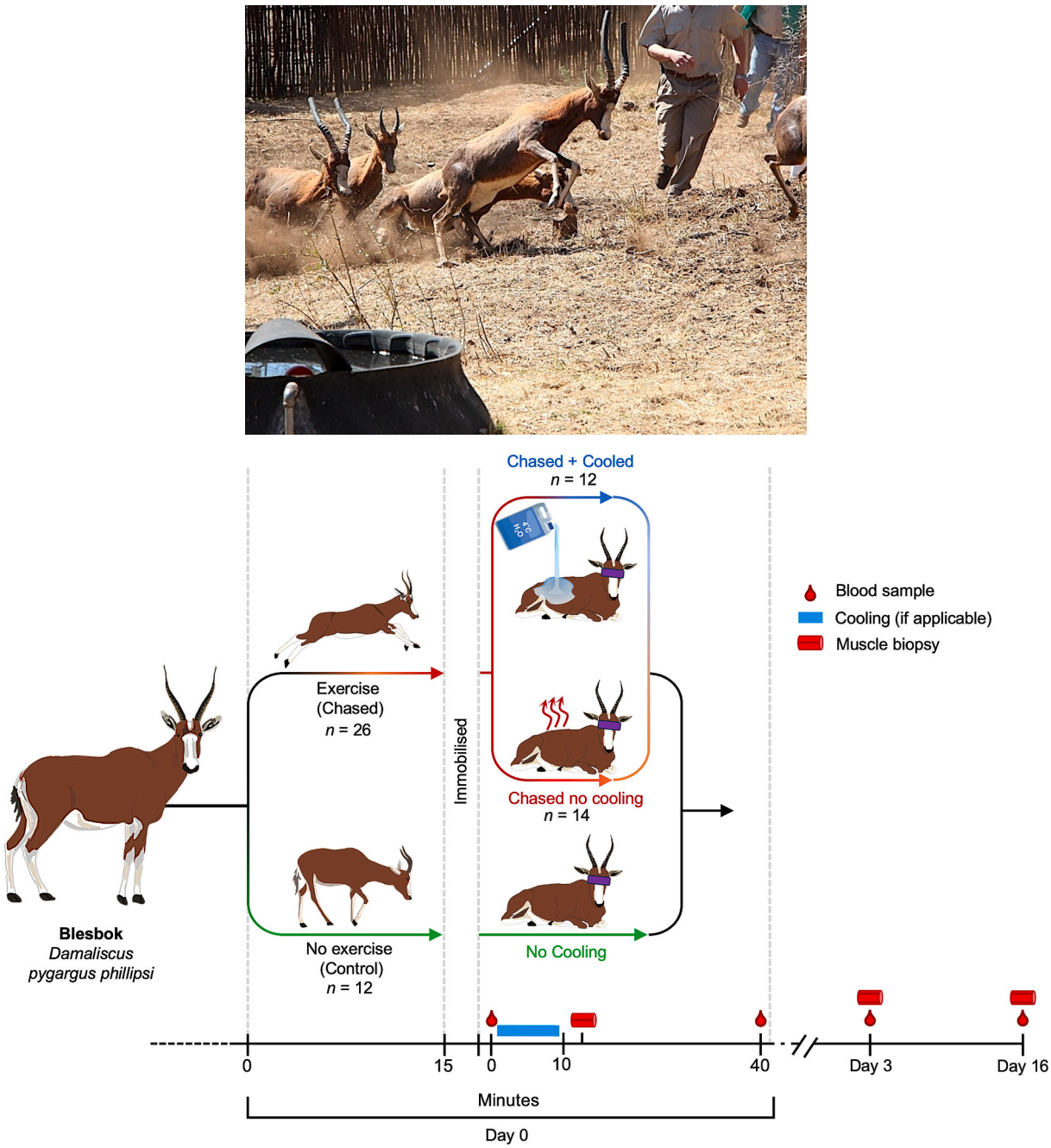

Fig. 1. A - Photograph of blesbok (*Damaliscus pygargus phillipsi*) antelopes being chased to simulate a capture- stress event to induce hyperthermia. B - Schematic diagram depicting the experimental design of the study.

2.4. Muscle glycogen content

The glycogen content of muscle samples obtained from all three sampling days were determined as previously described by Kohn et al. (2023). Briefly, muscle samples were freeze-dried overnight, followed by isolation of glycogen, digestion to glucose, and the glucose concentration was determined fluorometrically. The glycogen content was calculated and expressed as mmol glucose equivalents/kg dry weight.

2.5. Enzyme activities

Enzyme activities, serving as specific markers of muscle metabolism, were determined fluorometrically from the initial post-capture samples (day 0). The maximum capacity of the enzymes of interest were assessed by providing the respective substrates and enzymes in abundance. All assays were coupled to the appearance or disappearance of NADH and assessed at an excitation and emission wavelength of 340 nm and 460 nm, respectively, as previously described (Hohl et al., 2020; Kohn et al., 2011a). Citrate synthase (CS) and 3-hydroxyacyl Co enzyme A dehydrogenase (3HAD), markers of the Krebs cycle and β-oxidation, respectively, represented the oxidative capacity of the muscle. Markers of oxygen independent metabolism and rapid energy production via the high energy phosphagen system included lactate dehydrogenase (LDH), phosphofructokinase (PFK), creatine kinase (CK) and glycogen phosphorylase (PHOS). Maximum enzyme activities were expressed as μmol/min/g protein.

2.6. MHC isoform content

A small part of the stock homogenate from day 0 samples was diluted with SDS sample buffer (5 % β-MEtOH, 2.5 % SDS, 10 % glycerol, 62.5 mM Tris, pH 6.8 with 0.1 % bromophenol blue) to a concentration of 0.1 g/l for MHC isoform content determination, as previously described (Kohn et al., 2011a). The migration pattern of the isoforms was confirmed using antibodies reacting to specific isoforms as described in a previous study (Kohn et al., 2011a; Kohn et al., 2007b). The samples were loaded onto SDS-PAGE gels containing 7 % glycerol and resolved overnight at constant 275 V in a cold room. After electrophoresis, the gels were silver stained and digitised using a scanner (Epson Perfection V850). The relative MHC isoform proportions were determined using the Un-Scan-It Software package (Silk Scientific Corporation, Utah).

2.7. Antioxidant capacity of muscle

The stock homogenate samples from day 0 was centrifuged at 1000 $\times g$ for 20 min and the supernatant removed for use in determining the protein content, SOD and CAT activities, and the oxygen radical absorbance capacity assay (ORAC), as previously described (Hohl et al., 2020; Huang et al., 2002; Kohn et al., 2024). Briefly, undiluted supernatant sample was used to determine SOD activity. The working solution comprised 210 mM potassium phosphate buffer, pH 7.80, 110 μM xanthine, 1.1 mM cytochrome C, 10 mM EDTA, and 0.25 U/ml xanthine oxidase. To generate a standard curve, pure SOD at various concentrations were assayed using the same working solution. Absorbance was measured at 550 nm (SpectraMax, Molecular Devices, San Jose, CA) over 5 min and activity expressed as U/mg protein, where one unit of SOD (U) was defined as the activity that inhibits the rate of cytochrome *c* reduction by 50 %.

The CAT assay was performed according to Aebi (1984). Diluted supernatant was added to the catalase working solution, comprising 11 mM H_2O_2 and 50 mM potassium phosphate buffer, pH 7.00. Absorbance was measured at 240 nm over 5 min at 25 °C. CAT activity was expressed as μmol/min/g protein.

The ORAC assay, that provides a measure of overall antioxidant capacity, was determined fluorometrically at an emission and excitation wavelength of 485 nm and 520 nm, respectively (Huang et al., 2002). The working solution contained (final concentration): 75 mM phosphate buffer, pH 7.40, 96 nM fluorescein, and 19.1 mM 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). Various concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analogue, were subjected to the same working solution and served to construct a standard curve. Fluorescence was recorded over a period of 60 min every 5 min, and the area under the curve was plotted against known Trolox concentrations. ORAC values were expressed as μmol Trolox equivalents/mg protein.

2.8. Statistical analyses

All statistical analyses were performed using GraphPad Prism for Mac (version 10.4.2 GraphPad Software, Boston, Massachusetts, USA). Data are expressed as mean ± standard deviation (SD). The D'Agostino & Pearson test was used to test for normality and data was confirmed as normally distributed. Except for the glycogen data, biopsies taken from D0 only were compared using a one-way analysis of variance (ANOVA). For the glycogen content over multiple days, a two-way mixed-effects repeated measures ANOVA with a Tukey post hoc test was performed. Where only two groups were compared, an unpaired *t*-test was performed. Correlation coefficients were determined using a multiple linear regression model. Where applicable, effect size and power were calculated using G*Power for Mac version 3.1.9.4 (Faul et al., 2007). Significance was set at $P < 0.05$.

3. Results

The physiological and blood biomarkers for this study have been published elsewhere (Leiberich et al., 2023). To provide context for the findings of the present study, rectal and muscle temperature, blood lactate and pH measured directly after the chase event (day 0), 40 min later (day 0) and on days 3 and 16 post chase were extracted from the supplementary table and plotted in Fig. 2. These variables were used to identify relationships with the muscle findings described in the present study. Briefly, immediately after the chase event, rectal and muscle temperature were elevated (Figs. 2A and B). Ice water cooling for 10 min decreased body temperature to that of the control values. All three groups had similar rectal and muscle temperatures at days 3 and 16. Blood lactate concentrations were elevated immediately after the chase, and remained elevated above control values at 40 min, but were normal between all three groups on days 3 and 16 (Fig. 2C). Blood pH immediately after the chase was lower in the chased group only, but within physiological range (Fig. 2D).

3.1. Glycogen content

Results of the glycogen content of the *vastus lateralis* muscle at day 0, 3 and 16 days after the chase event are depicted in Fig. 3. The control group showed no change in glycogen content, confirming that this group was not under metabolic stress throughout the study (days 0 to 16). The glycogen concentrations of the chased only (D0: 48 ± 60 mmol/kg, $P < 0.001$) and chased + cooled (D0: 73 ± 75 mmol/kg, $P < 0.001$) groups, ranging from undetectable (a value of 1 assigned to these animals) to a maximum glycogen content of 216 mmol/kg, were significantly lower after the chase events compared to the control group for the same day (D0: 396 ± 195 mmol/kg). On day 3, the glycogen concentrations of the chased only (296 ± 99 mmol/kg, $P = 0.027$) and chased + cooled (210 ± 113 mmol/kg, $P = 0.0004$) groups were still lower compared to the control values (i.e. Control D3: 417 ± 161 mmol/kg). On day 16, the glycogen concentrations of both the chased only (578 ± 162 mmol/kg, $P = 0.032$) and chased + cooled (552 ± 181 mmol/kg, $P = 0.049$) groups were higher than the control value on the same sampling day (i.e. Control D16: 420 ± 205 mmol/kg), with no difference found between the chased only and chased + cooled groups.

3.2. Enzyme assays

Only day 0 muscle samples were analysed for the purpose of determining maximum capacity of the various metabolic pathways and to confirm that the muscles from the three groups were similar in metabolic capacity. The activities of the various metabolic enzymes are presented in Table 1. There was no difference between the three groups with respect to their enzyme activities, indicating that the muscles sampled were similar at the start of the study.

3.3. Antioxidant capacity

The activities of the antioxidant enzymes are presented in Table 1. Neither SOD nor CAT activities were different between the three groups. Similarly, the overall antioxidant capacity in the muscles (ORAC) was also not different between the groups.

3.4. Myosin heavy chain isoform content

There was a predominance of MHC IIA isoforms in the blesbok (±50 %), followed by MHC IIX (±32 %) and MHC I (±18 %) as shown in Table 1. There was no difference between the three groups with regards to their MHC isoform content, which indicates similar fibre type proportions between the groups.

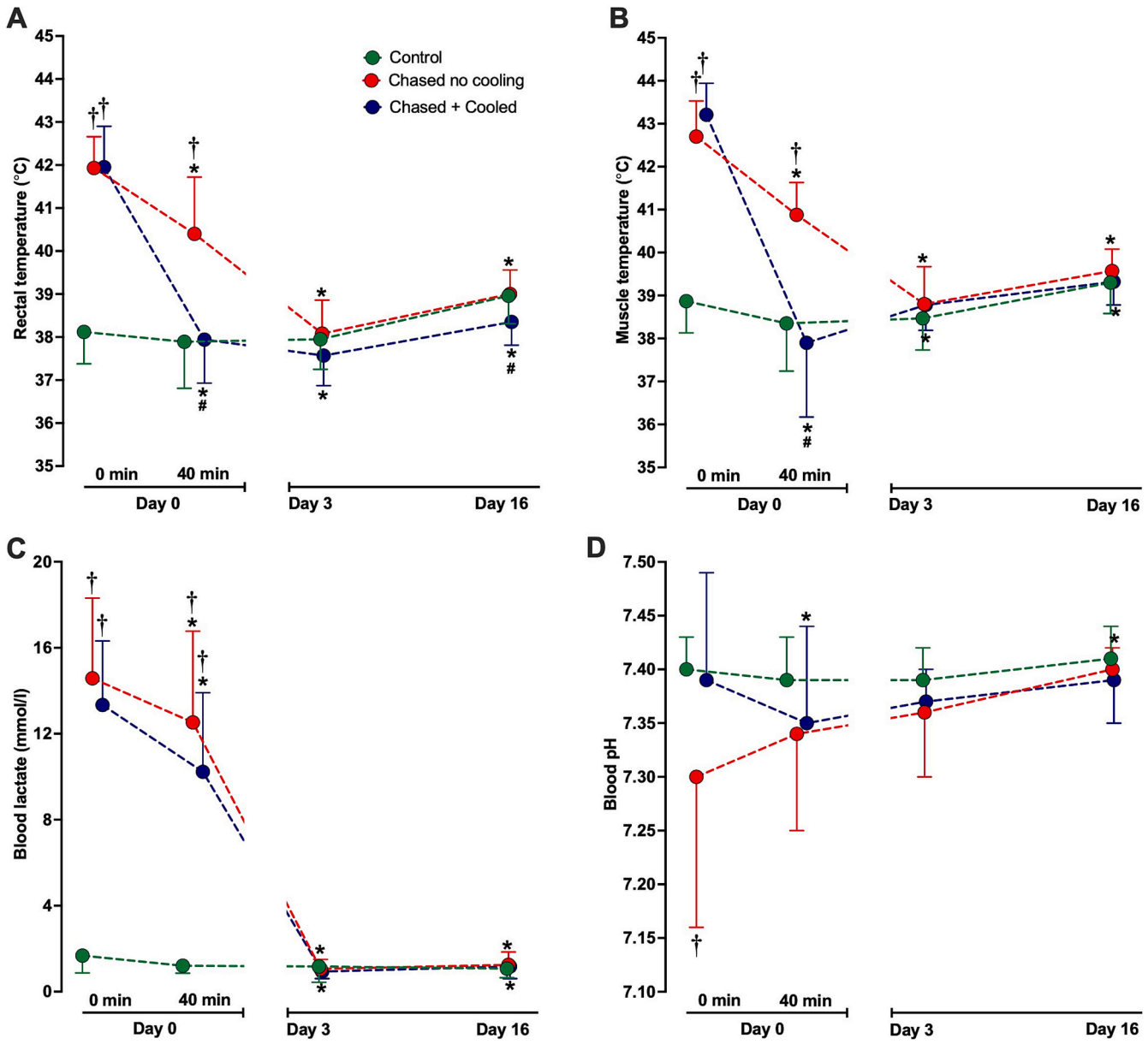

Fig. 2. Rectal (A) and muscle temperatures (B), blood lactate (C) and pH (D) obtained after blesbok were subjected to a chase event with cooling (Chased + Cooled) or without cooling (Chased no cooling) compared to controls. Data extracted from Leiberich et al. (2023). * - indicates difference between initial measurement at 0 min and sequential measurements within a group; † – indicates difference between the control and chased animals at the same time interval; # - indicates difference between the two chased groups at the same time interval. Note that the left and right X-axis represent minutes and days, respectively.

4. Discussion

In this study, a simulated capture-stress event (animals being chased) was performed to better understand the physiological consequences to chase-induced hyperthermia and subsequent cooling. Wild caught blesbok were subjected to 15 min of intense exertion, then immobilised for muscle and blood sample collection (Leiberich et al., 2023). It is standard practice in all game translocation operations that stress and physical exertion be kept to a minimum, and therefore highly recommended that capture events last for short periods at low intensities (Breed et al., 2019). Whenever an animal is suspected of being hyperthermic, cooling is administered (Sawicka et al., 2015). Since the conditions and circumstances for a particular capture event may vary, longer chase events at higher intensities may be unavoidable. The chase event in our study lasted for 15 min, which would be considered relatively extended in the field. Moreover, based on the lactate response after the chase (D0: control: 1.68 ± 0.81 mmol/l; chased: 14.6 ± 3.7 mmol/l; chased + cooled: 13.3 ± 3.0 mmol/l - Fig. 2C), the muscles of the blesbok also relied extensively on anaerobic metabolism of carbohydrate, and thus the vigour of the chase event was of medium to high intensity. Based on previously published data on the same group of blesbok (Fig. 2) (Leiberich et al., 2023), it is clear that the chase event resulted in hyperthermia (e.g. increased rectal and muscle temperature), and that the intensity resulted in blood lactate accumulation (via the conversion of pyruvate to lactate) above the lactate threshold (currently the exact threshold for these specific antelope species is unknown) (Poole et al., 2021). As it was near impossible to determine the fitness level of the blesbok, the present study relied on indirect measurements to ensure that the groups were similar in physical exertional capacity (Gollnick et al., 1972). These included assessing muscle fibre type and enzyme activities, which showed that the groups were at least matched for these variables.

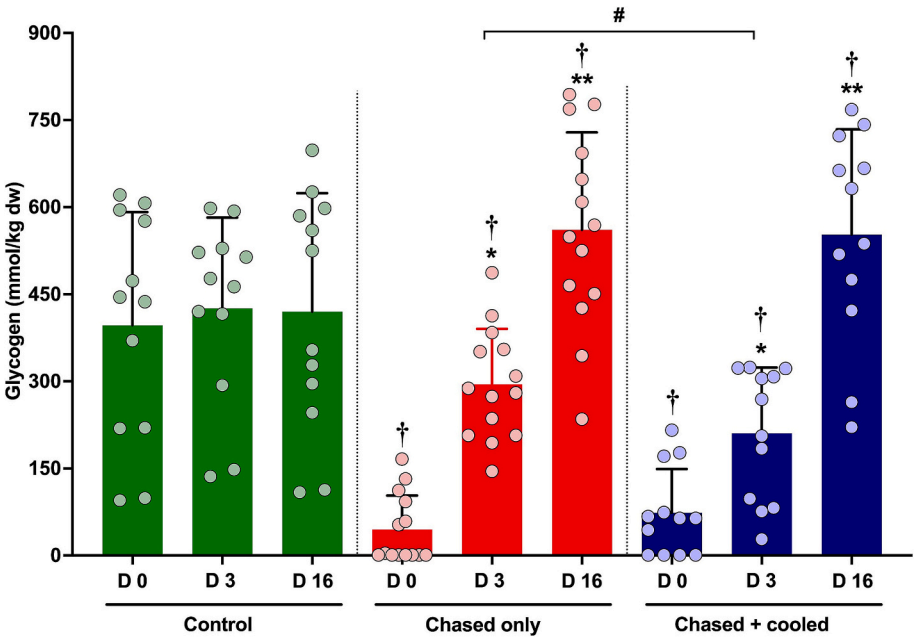

Fig. 3. Glycogen content of the *vastus lateralis* muscle from blesbok not chased (Control, $n = 12$, green), subjected to a 15 min chase with no cooling (Chased only, $n = 14$, red), and chased followed by cooling (Chased + cooled, n = 12, blue): (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
* different from day 0 ($P < 0.05$); ** different from days 0 and 3 ($P < 0.05$); † different from the control on the same day ($P < 0.05$); # a tendency to be different between chased only and chased + cool ($P = 0.097$, effect size 0.81, power 63 %). Refer to the text for exact *P*-values.

Table 1
The aerobic and anaerobic enzyme activity, antioxidant capacity and myosin heavy chain (MHC) content in the *vastus lateralis* of 38 blesbok in the control, chased only and chased + cooled groups on day 0.

	Control (n = 12)	Chased only (n = 14)	Chased + Cooled (n = 12)
Enzyme activities			
Oxidative pathway		µmol/min/g prot	
3HAD	51 ± 18	48 ± 11	40 ± 13
CS	88 ± 34	93 ± 38	86 ± 63
Glycolytic pathway		µmol/min/g prot	
CK	11,785 ± 4414	13,214 ± 3644	13,099 ± 8891
LDH	1614 ± 703	1684 ± 576	1470 ± 713
PHOS	172 ± 85	157 ± 65	168 ± 119
PFK	262 ± 120	258 ± 121	218 ± 120
Antioxidant capacity			
SOD (U/mg prot)	10.2 ± 11.9	6.5 ± 3.4	7.1 ± 4.1
Catalase (µmol/min/g prot)	8.4 ± 5.7	10.9 ± 7.4	7.2 ± 3.7
ORAC (µmol Trolox equivalents/mg prot)	26.1 ± 26.0	23.0 ± 11.6	20.4 ± 16.4
Myosin heavy chain content		Percentage (%)	
MHC I	20 ± 5	19 ± 4	16 ± 6
MHC IIA	50 ± 7	47 ± 8	53 ± 10
MHC IIX	30 ± 7	34 ± 8	31 ± 13

Values are expressed as mean ± SD. 3HAD, 3-hydroxyacyl acetyl Co enzyme A dehydrogenase; CS, citrate synthase; CK, creatine kinase; LDH, lactate dehydrogenase; MHC, myosin heavy chain; ORAC, oxygen radical absorbance capacity; PFK, phosphofructokinase; PHOS, glycogen phosphorylase; prot, protein; SOD, superoxide dismutase.

Sustained muscle contraction during high intensity exercise greatly relies on the adequate and immediate supply of ATP via the phosphagen, glycolytic and oxidative metabolic pathways. The fuel preference during periods of ATP demand depends on blood glucose and muscle glycogen content. Various studies have shown a direct link between glycogen content and muscle fatigue in humans and animal models (Hargreaves and Spriet, 2020; Ørtenblad et al., 2013). Similarly, the restoration of depleted muscle glycogen stores after such heavy bouts of exercise are also crucial to ensure optimal muscle function. Previous research has shown that the muscles of southern African antelope species (including blesbok) have high mitochondrial numbers and, thus, high oxidative capacity in the form of CS activity, compared to humans (Curry et al., 2012; Kohn, 2014; Kohn et al., 2011b). Additionally, their muscles also have LDH activities that are three to four times higher than their human counterparts (Table 1). This difference suggests that blesbok can utilise their glycogen at a much quicker rate than humans (Curry et al., 2012; Kohn, 2014).

Our study was the first to determine glycogen stores in a wild Southern African antelope (i.e. blesbok) after a high intensity simulated capture event. The glycogen content of the *vastus lateralis* from the blesbok in the control group was similar in range to that reported for horses and reindeer at rest, ranging from 300 to 600 mmol/kg dw (Essén-Gustavsson et al., 1984; Essén-Gustavsson and Rehbinder, 1985; Serrano et al., 2000). Overall, the chase event induced a significant reduction in muscle glycogen in both the chased only and chased + cooling groups (Fig. 3). The present study further showed that the muscle biopsies from 10 of the 26 chased blesbok had undetectable glycogen content after the 15 min chase (Fig. 3). It is well known that skeletal muscle is dependent on muscle glycogen to sustain high intensity muscle contraction, as was shown in *in vitro* and *in vivo* studies using isolated muscle and intact muscle, respectively (Nielsen et al., 2014; Ørtenblad et al., 2013). Depletion of muscle glycogen results in a rapid decline in the ability to generate force, and fatigue follows shortly afterwards. It therefore raises the question whether a longer chase event (e.g. 20–25 min) would have resulted in undetectable glycogen in the muscles of the remaining blesbok and whether it would have ultimately resulted in exhaustion, rhabdomyolysis and myoglobinuria in chased animals.

Notably, our study showed slow repletion of glycogen after the chase event for the blesbok. After 3 days, the muscle glycogen of the chased only and chased + cooled groups had not yet returned to the concentrations determined in the control group ($P < 0.05$), even though animals were fed a carbohydraterich diet (Leiberich et al., 2023). Similar

slow repletion was observed in horses after a glycogen depleting exercise bout where 24 h after exercise, even on a high starch diet, glycogen content was still well below pre-exercise values (Lacombe et al., 2004; Valberg et al., 2023). According to Lacombe et al. (2004), it appears that muscle glycogen will be fully repleted after 3 days in the horse when fed a high carbohydrate diet, whereas a high fat diet will result in delayed repletion. In humans, complete glycogen repletion, after a depleting bout of exercise, takes approximately 24 h (Hingst et al., 2018). It must be noted that blesbok are ruminants, and humans are single stomach mammals. Ruminants rely on bacteria to assist digestion of cellulose (a carbohydrate) into primarily volatile fatty acids, from which repletion of glycogen will occur. Similarly, horses are hindgut fermenters and have similar limitations with glycogen repletion after exercise (Lacombe et al., 2004; Valberg et al., 2023). Too much easily fermentable carbohydrate (e.g. starch) in the diet could cause detrimental effects to the animal, such as rumen acidosis that inhibits fibre digestion and can lead to various digestive complications (Zebeli and Metzler-Zebeli, 2012). Finally, 16 days after the chase event, muscle glycogen content was significantly higher in the chased groups compared to the control group (Fig. 3). This phenomenon, known as supercompensation of muscle glycogen, has been shown in rats 8 h after a depleting bout of exercise, whereafter the glycogen content gradually decreased to baseline values (Sano et al., 2012). Similarly, the same supercompensation was shown 5 days after a single bout of exercise in humans, resulting from the exercise and was not related to a high carbohydrate diet (80 % carbohydrate) (Hingst et al., 2018). Unfortunately, glycogen synthase activity was not measured in the blesbok samples and would have aided in better understanding the slow glycogen repletion.

Another objective of this study was to investigate the effect of cooling on glycogen repletion. Previous studies, using human and rodent muscles have shown that cooling negatively impacts the resynthesis of muscle glycogen (Cheng et al., 2017; Tucker et al., 2012). Tucker et al. (2012) was able to show that after glycogen depleting exercise in humans, applying ice to one leg significantly reduced the repletion rate of glycogen after 4 h compared to the contralateral leg. Similar findings were reported in mice where cooling decreased, and heating increased the repletion rate of glycogen (Cheng et al., 2017). Based on these studies, closer inspection of the day 3 glycogen concentrations of the two chased groups at day 3 showed that there was a large difference between the two means, but with large standard deviations. An unpaired *t*-test of the glycogen content at this timepoint between the chased only and chased + cooled groups resulted in a non-significant *P*-value of 0.097, and a calculated effect size of 0.81 (large effect), but with a power of only 63 %. Nevertheless, biologically, this finding in blesbok corroborated by previous research on humans and rodents, would suggest that muscle cooling could slow down glycogen repletion, and subsequently influence muscle function (Cheng et al., 2017; Tucker et al., 2012).

Various intrinsic factors, either directly or indirectly, can result in rhabdomyolysis. These include genetic mutations of metabolism (e.g. Ca^{2+} regulation by ryanodine receptor 1 mutations causing malignant hyperthermia), to extreme exertion leading to severe muscle tears and necrosis (Bagley et al., 2007; van Adel and Tarnopolsky, 2009). Glycogen depletion, inaccessible glycogen (e.g. polysaccharide storage myopathy in horses) or muscle phosphorylase deficiency (e.g. glycogen storage myopathy V - McArdle's disease), will lead to compromised ATP resynthesis to fuel muscle contraction, and can result in a type of muscle rigor which often results in muscle rhabdomyolysis (Debold and Westerblad, 2024; Henning et al., 2017; McCue et al., 2009). Previous studies on the muscles of antelope, such as springbok, blesbok, mountain reedbuck and reindeer, have all shown that, despite having large numbers of type IIX fibres, their muscle are both highly glycolytic and oxidative i.e. having high mitochondrial enzyme activities equivalent to that found in trained horses and highly trained endurance athletes (Curry et al., 2012; Essén-Gustavsson et al., 1984; Essén-Gustavsson and Rehbinder, 1985; Hohl et al., 2020; Kohn, 2014; Kohn et al., 2011b). Furthermore, Kohn and co-workers have shown that antelope muscles contain type IIX fibres that can have large numbers of mitochondria - a fibre that would thus be highly fatigue resistant compared to e.g. type IIX fibres from lions that only have low mitochondrial numbers (Curry et al., 2012; Kohn, 2014; Kohn et al., 2011a; Kohn et al., 2011b). The muscles of the blesbok from the present study had similarly high oxidative and glycolytic enzyme activities, which can add to the rapid depletion of glycogen during high intensity physical exertion, and if glycogen is depleted, could result in fibre rigor. Various studies have shown that human patients with glycogen storage diseases subjected to physical activity, present with stiff muscles followed by myoglobinuria, the latter being indicative of rhabdomyolysis (reviewed in Breed et al. (2019)). Thus, it is highly likely that capture myopathy in wild antelope, which presents with these signs, may be caused by exertional muscle glycogen depletion and poor repletion, which is likely aggravated by fear. Further studies are warranted to investigate whether glycogen depletion and its repletion are involved in the primary mechanisms causing the rhabdomyolysis observed in capture myopathy.

5. Limitations and future directions

Being the first study to investigate glycogen replenishment over multiple days in blesbok, this study was not without its limitations. Considering the large standard deviations observed in the glycogen content, and the lack of statistical significance ($P = 0.097$) of the glycogen content between the chased only and chased + cooling groups on D3, despite showing a large effect size (0.81), a larger sample size may confirm whether a type II error (false negative) may have occurred. The muscle glycogen concentrations of the present study were also comparable to those obtained from biopsied reindeer (Pösö et al., 1996), and from various muscle groups of reindeer obtained postmortem (Essén-Gustavsson and Rehbinder, 1985).

It is also important to note that the physiology of blesbok is not well known as is the case for humans or horses. Chemical immobilisation of the blesbok is necessary to acquire blood and muscle biopsies, but the drugs used cause respiratory depression, which directly affects blood PaO_2 and $PaCO_2$ (refer to Leiberich et al. (2023)). Immobilising drugs potentially also cause body temperature to rise and because of these physiological effects from the drugs, a control (resting) blesbok group not subjected to exertion and cooling was included to account for any drug induced effects on the muscle glycogen.

Another limitation of this study is that only one muscle group was biopsied. As this was the first study of its kind using wild habituated blesbok and biopsying over multiple days, only one biopsy per immobilisation was performed. From human participant feedback, a biopsy is associated with some pain, discomfort and inflammatory response. It is, however, acknowledged, that the various muscles of the limbs vary in fibre type composition and metabolism, as was shown in lion, caracal, black wildebeest, fallow deer, springbok and reindeer (Curry et al., 2012; Essén-Gustavsson and Rehbinder, 1985; Kohn et al., 2011a; Kohn et al., 2011b). However, in these studies, animals were killed before muscles were sampled, whereas for the present study, wild blesbok had to be immobilised for each sampling interval. In addition, the muscle biopsies were not the only samples that were acquired during immobilisations, and the frequency of immobilisations were not dictated by the present research question. Biopsying multiple muscles also increases the risk to the animals for infection, and, as this was a novel study in open veld conditions, the study was limited to one biopsy per immobilisation.

Taken together, although there were limitations that cannot be avoided, future studies should focus on a larger sample size basing the power analyses on the data obtained in the present study. In addition, biopsying animals earlier after the chase event and cooling (e.g. 1- or 2-days post chase), may provide a better window to confirm the effect of cooling on glycogen repletion.

6. Conclusion

This is the first study to evaluate glycogen content in skeletal muscle biopsies from blesbok subjected to a simulated 15 min chase event to activate the flight response, inducing psychological fear, physical exertion and hyperthermia. The main findings were that after the chase event, glycogen content was significantly reduced and undetectable in the *vastus lateralis* of some animals. After 3 days, glycogen concentrations in both chased groups were still below resting control values, indicating slow glycogen resynthesis rates in the skeletal muscle of blesbok. Cooling for 10 min after the chase event resulted in a non-significant reduction in glycogen repletion, but after 16 days of rest, glycogen supercompensation was observed in both chased groups. A change in glycogen concentration, triggered by the consequences of the flight response (i.e. fear and exertion), may have important clinical implications for understanding and preventing rhabdomyolysis and capture myopathy in wild antelope/ungulates.

CRediT authorship contribution statement

Tertius A. Kohn: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Mahmooda Martin:** Writing – review & editing, Methodology, Formal analysis. **Kathryn M. van Boom:** Writing – review & editing, Methodology, Formal analysis. **Byron Donaldson:** Writing – review & editing, Methodology, Formal analysis. **Dee M. Blackhurst:** Writing – review & editing, Validation, Methodology, Formal analysis. **Agustina Fitte:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Richard Burroughs:** Writing – review & editing, Resources, Methodology, Investigation. **Johan C.A. Steyl:** Writing – review & editing, Supervision, Methodology, Investigation. **Amelia Goddard:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Leith C.R. Meyer:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Andersen, J.L., Klitgaard, H., Bangsbo, J., Saltin, B., 1994. Myosin heavy chain isoforms in single fibres from m. vastus lateralis of soccer players: effects of strength-training. Acta Physiol. Scand. 150, 21–26.
Bagley, W.H., Yang, H., Shah, K.H., 2007. Rhabdomyolysis. Intern. Emerg. Med. 2, 210–218.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Breed, D., Meyer, L.C.R., Steyl, J.C.A., Goddard, A., Burroughs, R., Kohn, T.A., 2019. Conserving wildlife in a changing world: understanding capture myopathy—a malignant outcome of stress during capture and translocation. Conserv. Physiol. 7, 559–621.
Cheng, A.J., Willis, S.J., Zinner, C., Chaillou, T., Ivarsson, N., Ortenblad, N., Lanner, J.T., Holmberg, H.C., Westerblad, H., 2017. Post-exercise recovery of contractile function and endurance in humans and mice is accelerated by heating and slowed by cooling skeletal muscle. J. Physiol. 595, 7413–7426.
Ciciliot, S., Schiaffino, S., 2010. Regeneration of mammalian skeletal muscle. Basic mechanisms and clinical implications. Curr. Pharm. Des. 16, 906–914.
Curry, J.W., Hohl, R., Noakes, T.D., Kohn, T.A., 2012. High oxidative capacity and type IIx fibre content in springbok and fallow deer skeletal muscle suggest fast sprinters with a resistance to fatigue. J. Exp. Biol. 215, 3997–4005.
Debold, E.P., Westerblad, H., 2024. New insights into the cellular and molecular mechanisms of skeletal muscle fatigue: the Marion J. Siegman award lectureships. Am. J. Physiol. Cell Physiol. 327, C946–C958.
Demartini, J.K., Casa, D.J., Stearns, R., Belval, L., Crago, A., Davis, R., Jardine, J., 2015. Effectiveness of cold water immersion in the treatment of exertional heat stroke at the Falmouth road race. Med. Sci. Sports Exerc. 47, 240–245.
Dickens, M.J., Delehanty, D.J., Romero, L.M., 2010. Stress: an inevitable component of animal translocation. Biol. Conserv. 143, 1329–1341.
Esbjornsson, M.E., Dahlstrom, M.S., Gierup, J.W., Jansson, E.C., 2021. Muscle fiber size in healthy children and adults in relation to sex and fiber types. Muscle Nerve 63, 586–592.
Essén-Gustavsson, B., Rehbinder, C., 1985. Skeletal muscle characteristics of reindeer (Rangifer tarandus L.). Comp. Biochem. Physiol. 82, 675–679.
Essén-Gustavsson, B., Karlström, K., Lindholm, A., 1984. Fibre types, enzyme activities and substrate utilisation in skeletal muscles of horses competing in endurance rides. Equine Vet. J. 16, 197–202.
Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39, 175–191.
Gollnick, P.D., Armstrong, R.B., Saubert, C.W., Piehl, K., Saltin, B., 1972. Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J. Appl. Physiol. 33, 312–319.
Hargreaves, M., Spriet, L.L., 2020. Skeletal muscle energy metabolism during exercise. Nat. Metab. 2, 817–828.
Henning, F., Cunninghame, C.A., Martín, M.A., Rubio, J.C., Arenas, J., Lucia, A., HernáNdez-Laín, A., Kohn, T.A., 2017. Muscle fiber type proportion and size is not altered in McArdle disease. Muscle Nerve 55, 916–918.
Hingst, J.R., Bruhn, L., Hansen, M.B., Rosschou, M.F., Birk, J.B., Fentz, J., Foretz, M., Viollet, B., Sakamoto, K., Faergeman, N.J., Havelund, J.F., Parker, B.L., James, D.E., Kiens, B., Richter, E.A., Jensen, J., Wojtaszewski, J.F.P., 2018. Exercise-induced molecular mechanisms promoting glycogen supercompensation in human skeletal muscle. Mol. Metabol. 16, 24–34.
Hohl, R., Blackhurst, D.M., Donaldson, B., van Boom, K.M., Kohn, T.A., 2020. Wild antelope skeletal muscle antioxidant enzyme activities do not correlate with muscle fibre type or oxidative metabolism. Comp. Biochem. Physiol. 242, 110638.
Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., Prior, R.L., 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J. Agric. Food Chem. 50, 4437–4444.
Kohn, T.A., 2014. Insights into the skeletal muscle characteristics of three southern African antelope species. Biol. Open 3, 1037–1044.
Kohn, T.A., Essen-Gustavsson, B., Myburgh, K.H., 2007a. Do skeletal muscle phenotypic characteristics of Xhosa and Caucasian endurance runners differ when matched for training and racing distances? J. Appl. Physiol. 103, 932–940.
Kohn, T.A., Hoffman, L.C., Myburgh, K.H., 2007b. Identification of myosin heavy chain isoforms in skeletal muscle of four southern African wild ruminants. Comp. Biochem. Physiol. A 148, 399–407.
Kohn, T.A., Burroughs, R., Hartman, M.J., Noakes, T.D., 2011a. Fiber type and metabolic characteristics of lion (Panthera leo), caracal (Caracal caracal) and human skeletal muscle. Comp. Biochem. Physiol. 159, 125–133.
Kohn, T.A., Curry, J.W., Noakes, T.D., 2011b. Black wildebeest skeletal muscle exhibits high oxidative capacity and a high proportion of type IIx fibres. J. Exp. Biol. 214, 4041–4047.
Kohn, T.A., Anley, M.J., Magwaza, S.t.N., Adamson, L., Hoffman, L.C., Brand, T.S., 2023. Muscle fiber type and metabolic profiles of four muscles from the African black ostrich. Meat Sci 200, 109156.
Kohn, T.A., Knobel, S., Donaldson, B., van Boom, K.M., Blackhurst, D.M., Peart, J.M., Jensen, J., Tordiffe, A.S.W., 2024. Does sex matter in the cheetah? Insights into the skeletal muscle of the fastest land animal. J. Exp. Biol. 227, jeb247284.
Lacombe, V.A., Hinchcliff, K.W., Kohn, C.W., Devor, S.T., Taylor, L.E., 2004. Effects of feeding meals with various soluble-carbohydrate content on muscle glycogen synthesis after exercise in horses. Am. J. Vet. Res. 65, 916–923.
Leiberich, M., Fitte, A., Burroughs, R., Steyl, J., Goddard, A., Haw, A., Boesch, J.M., Kohn, T.A., Meyer, L.C.R., 2023. Cooling by dousing with cold water does not alter the pathophysiological biochemical changes induced by capture in blesbok (*Damaliscus pygargus phillipsi*). J. Wildl. Dis. 59, 288–298.
Mallett, G., 2025. The effect of exercise and physical activity on skeletal muscle epigenetics and metabolic adaptations. Eur. J. Appl. Physiol. 125, 611–627.

McCue, M.E., Valberg, S.J., Miller, M.B., Wade, C., DiMauro, S., Akman, H.O., Mickelson, J.R., 2008. Glycogen synthase (GYS1) mutation causes a novel skeletal muscle glycogenosis. Genomics 91, 458–466.
McCue, M.E., Valberg, S.J., Jackson, M., Borgia, L., Lucio, M., Mickelson, J.R., 2009. Polysaccharide storage myopathy phenotype in quarter horse-related breeds is modified by the presence of an RYR1 mutation. Neuromuscul. Disord. 19, 37–43.
McVeigh, J.M., Tarrant, P.V., 1982. Glycogen content and repletion rates in beef muscle, effect of feeding and fasting. J. Nutr. 112, 1306–1314.
McVeigh, J.M., Tarrant, P.V., Harrington, M.G., 1982. Behavioral stress and skeletal muscle glycogen metabolism in young bulls. J. Anim. Sci. 54, 790–795.
Nielsen, J., Cheng, A.J., Ørtenblad, N., Westerblad, H., 2014. Subcellular distribution of glycogen and decreased tetanic Ca2+ in fatigued single intact mouse muscle fibres. J. Physiol. 592, 2003–2012.
Ørtenblad, N., Westerblad, H., Nielsen, J., 2013. Muscle glycogen stores and fatigue. J. Physiol. 591, 4405–4413.
Poole, D.C., Rossiter, H.B., Brooks, G.A., Gladden, L.B., 2021. The anaerobic threshold: 50+ years of controversy. J. Physiol. 599, 737–767.
Pösö, A.R., Nieminen, M., Raulio, J., Räsänen, L.A., Soveri, T., 1996. Skeletal muscle characteristics of racing reindeer (Rangifer tarandus). Comp. Biochem. Physiol. 114, 277–281.
Powers, S.K., Ji, L.L., Leeuwenburgh, C., 1999. Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med. Sci. Sports Exerc. 31, 987–997.
Rae, D.E., Knobel, G.J., Mann, T., Swart, J., Tucker, R., Noakes, T.D., 2008. Heatstroke during endurance exercise: is there evidence for excessive endothermy? Med. Sci. Sports Exerc. 40, 1193–1204.
Sano, A., Koshinaka, K., Abe, N., Morifuji, M., Koga, J., Kawasaki, E., Kawanaka, K., 2012. The effect of high-intensity intermittent swimming on post-exercise glycogen supercompensation in rat skeletal muscle. J. Physiol. Sci. 62, 1–9.
Sawicka, J., Fuller, A., Fick, L.G., Hetem, R.S., Meyer, L.C.R., 2015. Efficacy of different cooling methods for capture-induced hyperthermia in antelope. Afr. J. Wildl. Res. 45, 100–110.
Serrano, A.L., Quiroz-Rothe, E., Rivero, J.L.L., 2000. Early and long-term changes of equine skeletal muscle in response to endurance training and detraining. Pflugers Arch. 441, 263–274.
Tarnopolsky, M.A., Pearce, E., Smith, K., Lach, B., 2011. Suction-modified Bergström muscle biopsy technique: experience with 13,500 procedures. Muscle Nerve. 43, 717–725.
Tucker, T.J., Slivka, D.R., Cuddy, J.S., Hailes, W.S., Ruby, B.C., 2012. Effect of local cold application on glycogen recovery. J. Sports Med. Phys. Fitness 52, 158–164.
Valberg, S.J., Velez-Irizarry, D., Williams, Z.J., Pagan, J.D., Mesquita, V., Waldridge, B., Maresca-Fichter, H., 2023. Novel expression of GLUT3, GLUT6 and GLUT10 in equine gluteal muscle following glycogen-depleting exercise: impact of dietary starch and fat. Metabolites 13.
van Adel, B.A., Tarnopolsky, M.A., 2009. Metabolic myopathies: update 2009. J. Clin. Neuromuscul. Dis. 10, 97–121.
Zebeli, Q., Metzler-Zebeli, B.U., 2012. Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle. Res. Vet. Sci. 93, 1099–1108.