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RESEARCH ARTICLE

Black wildebeest skeletal muscle exhibits high oxidative capacity and a high proportion of type IIx fibres

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SUMMARY

The aim of the study was to investigate the skeletal muscle characteristics of black wildebeest (*Connochaetes gnou*) in terms of fibre type and metabolism. Samples were obtained *post mortem* from the vastus lateralis and longissimus lumborum muscles and analysed for myosin heavy chain (MHC) content. Citrate synthase (CS), 3-hydroxyacyl co A dehydrogenase (3HAD), phosphofructokinase (PFK), lactate dehydrogenase (LDH) and creatine kinase (CK) activities were measured spectrophotometrically to represent the major metabolic pathways in these muscles. Both muscles had less than 20% MHC I, whereas MHC IIa and MHC IIx were expressed in excess of 50% in the vastus lateralis and longissimus lumborum muscles, respectively. Overall fibre size was 2675±1034 μm^2, which is small compared with other species. Oxidative capacity (CS and 3HAD) in both muscles was high and did not differ from one another, but the longissimus lumborum had significantly (P<0.05) higher PFK, LDH and CK activities. No relationships were observed between fibre type and the oxidative and oxygen-independent metabolic capacity as measured by specific enzyme activities. This study confirms the presence of both fast-twitch fibres and high oxidative capacity in black wildebeest, indicating an animal that can run very fast but is also fatigue resistant.

Key words: fibre type, enzyme activity, *Connochaetes gnou*.

INTRODUCTION

Wild animals are remarkable athletes. Being a predator requires physiological attributes that allow for the effective capture and killing of prey almost on a daily basis. However, comparable physiology is essential for prey to escape capture. It could therefore be argued that the skeletal muscles of each wild animal play a vital role in ensuring its survival. A number of superior performing animals exist but there are few studies of the skeletal muscle characteristics of these animals.

The southern African continent has a vast number of wild animals, particularly antelope and bovine species. One such species, the black wildebeest (*Connochaetes gnou*), is indigenous to South Africa. These animals are approximately half the size of their cousins, the blue wildebeest (*Connochaetes taurinus*), with body masses ranging from 130 to 160 kg (Skinner and Chimimba, 2005). They are known to be fast runners, reaching speeds of up to 70 km h^{-1}. The blue wildebeest, like the zebra, migrates long distances for better vegetation and therefore requires muscles that also have a high resistance to fatigue (Skinner and Chimimba, 2005). Thus, it is possible that both the black and blue wildebeest (and zebras) have muscles that are adapted for both high speed (to escape predation) and endurance (to allow migration over very long distances).

The locomotor muscles from large mammals (e.g. humans, horses, lions and black bears) are primarily composed of three fibre types, namely types I, IIa and IIx (Gollnick et al., 1972; Rivero et al., 2007; Smerdu et al., 2009; Kohn et al., 2011). They derive their individual characteristics from the type of myosin heavy chain (MHC) isoform each expresses. MHC I results in fibres that have the slowest contraction speed, but are rich in mitochondria and myoglobin, rendering them highly fatigue resistant. In contrast, fibres expressing MHC IIx have the fastest contraction speed of the three types but are usually described as having low mitochondrial numbers and myoglobin concentrations; as a result, these fibres fatigue rapidly. Type IIa fibres, which express the MHC IIa isoform, are a combination of both, with fast contraction speeds and sufficient mitochondria and myoglobin to ensure fatigue resistance (Essén-Gustavsson and Henriksson, 1984; Bottinelli, 2001). The very fast MHC IIb isoform has been detected in cheetah, pig and llama skeletal muscle, with its expression in other larger animals (bovine, human and horse) absent and restricted to specialized muscles (e.g. eye muscles) (Quiroz-Rothe and Rivero, 2001; Lefaucheur et al., 2002; Toniolo et al., 2005; Stirn Kranjc et al., 2009; Hyatt et al., 2010).

Previous research from our laboratory has shown that feline predators (lion and caracal) have an abundance of fast-twitch type IIx fibres (≥60%), which correlated well with their maximum running capability (Kohn et al., 2011). In addition, their enzyme activities indicate poor oxidative capacity and hence an absence of fatigue resistance, as is clearly apparent in their chase techniques, which involve short sprints usually lasting less than 60 s.

At present, very little is known about the skeletal muscle fibre characteristics of African mammals such as the wildebeest, which have the capacity to both run at high speeds to avoid predation and migrate over very long distances to source optimum grazing. Their behaviour suggests that their skeletal muscle fibres must have the capacity for both speed and endurance. Although a few comparative studies exist on skeletal muscle from blue wildebeest (Stickland, 1979; Hoppeler et al., 1981; Spurway et al., 1996), to our knowledge, only

one study has examined the relative fibre type proportions in the longissimus lumborum muscle from black wildebeest (Kohn et al., 2007a). Conventional thinking would expect their muscle fibre composition to resemble that of predators (e.g. lion, cheetah and caracal) that are able to run at great speeds but which have poor fatigue resistance (Williams et al., 1997; Kohn et al., 2011). But the ability of wildebeest to withstand fatigue for long periods of time during their annual migration or when chased by predators (Skinner and Chimimba, 2005) suggests that they must possess fast-twitch muscle fibres that are also fatigue resistant. This combination of features has not previously been described in an African mammal.

Therefore, the aim of this study was to investigate muscle fibre type and metabolism in two muscle groups from the black wildebeest to determine whether the metabolic profile could explain the potential co-existence of high speed and significant fatigue resistance in the same muscles. For this study, the vastus lateralis and longissimus lumborum muscles were analysed because of their pivotal role during running in these mammals. Particularly, the former is known to be essential for forward propulsion and jumping ability, whereas the latter aids in jumping and stabilising the back when the animal runs. We hypothesise that the skeletal muscles of black wildebeest have large proportions of MHC IIx fibres, but that these fibres also demonstrate metabolic characteristics that allow for superior fatigue resistance.

MATERIALS AND METHODS

Animals and tissue sampling

Professional hunters randomly shot four male and six female adult black wildebeest, *Connochaetes gnou* (Zimmermann 1780), on game farms during the annual cropping season in the Pearston-Somerset East area, Eastern Cape province, South Africa. These animals were classified as middle to elderly adults on the basis that all had fully developed horns. Although physical activity was not recorded, these animals roam freely on the game farm (10,000 hectares), and are considered wild as they flee from human presence and were never manhandled. All samples were collected *post mortem* from the longissimus lumborum and vastus lateralis muscles within 2 h after death.

It is well known that superficial parts of animal muscle contain an abundance of type IIx (e.g. cheetah and tiger) or IIb fibres (e.g. rat) (Delp and Duan, 1996; Kohn and Myburgh, 2007; Hyatt et al., 2010). The portion of tissue processed for this study was intended to represent the middle portion of the muscle as a whole.

All sampling sites were standardised. A portion of the hide was removed and the muscle of interest was identified using anatomical markers and then exposed. The sample site for the vastus lateralis was calculated as half the distance between the knee and the hip joint. For the longissimus lumborum, a 1-cm-long incision was made approximately 5 mm parallel to the spinous process between L3 and L4 of the lumbar spine. For both muscles, a block of tissue (sampling depth of 1 cm) was removed and the superficial part of approximately 5 mm was discarded. The sample was divided into smaller parts, rapidly frozen in liquid nitrogen and stored in a cryo-preservation tank (–200°C). After harvesting, samples were transported in liquid nitrogen to the laboratory and stored at –87°C until subsequent analyses.

Unless otherwise stated, all chemicals were from Sigma (St Louis, MO, USA).

Homogenisation of tissue

Samples were prepared for enzyme analyses as described by Kohn et al. (Kohn et al., 2011). Briefly, each sample was weighed and a volume of 100 mmol l^{-1} potassium phosphate buffer, pH 7.30, was added (ratio of 1:19). After the tissue was thoroughly homogenised on ice using a Teflon tip, samples were sonicated twice for 10 s using a micro sonication probe (VirSonic, Virtis Co., Gardiner, NY, USA). The homogenate was briefly centrifuged for 5 min at 1700 ***g*** (4°C) and the supernatant was transferred to a new microtube. Protein concentration of the latter was measured using the Bradford method (Bradford, 1976). Enzyme assays were performed using the supernatant, whereas the pellet was diluted with sample buffer (5% β-MEtOH, 2.5% SDS, 10% glycerol, 62.5 mmol l^{-1} Tris, pH 6.8 and 0.1% Bromophenol Blue), heated to 95°C for 5 min and used to determine the MHC isoform content and western blots.

Enzyme analyses

The activities of citrate synthase (CS; EC 4.1.3.7), 3-hydroxyacyl co A dehydrogenase (3HAD; EC 1.1.1.35), phosphofructokinase (PFK; EC 2.7.1.11), lactate dehydrogenase (LDH; EC 1.1.1.27) and creatine kinase (CK; EC 2.7.3.2) were measured spectrophotometrically (Beckman Coulter, Johannesburg, RSA) at 25°C as previously described [for a detailed explanation, refer to Kohn et al. (Kohn et al., 2011)]. Activities are expressed as μmol min^{-1} g^{-1} protein.

MHC isoform content and western blots

The method to separate the MHC isoforms was based on that developed by Talmadge and Roy (Talmadge and Roy, 1993) and adapted by Kohn et al. (Kohn et al., 2007a). Briefly, a volume of sample containing approximately 0.5 μg total protein was loaded on 7% polyacrylamide gels containing 30% glycerol (Hoefer SE600, Holliston, MA, USA). Gels were packed in ice and run in the cold at a constant 70 V for 4 h, followed at 275 V for 20 h. After silver staining, the gels were scanned and the density of the separated bands was quantified using the Un-Scan-It software package (Silk Scientific Corporation, Orem, UT, USA). Three bands were identified and each band was expressed as a percentage of the total densitometric profile of the three bands.

Western blots were also performed to validate the migration pattern of the separated isoforms; this has been described elsewhere (Kohn et al., 2011). Monoclonal primary antibodies, previously validated for specific isoform reactivity in various species, were used to identify the bands (Lucas et al., 2000; Acevedo and Rivero, 2006; Hyatt et al., 2010; Kohn et al., 2011). Specifically, in skeletal muscle, BAD5 reacts only with MHC I, 2F7 only with MHC IIa, BF35 with all except MHC IIx, and 6H1 with only MHC IIx.

Histochemical and immunohistochemical analyses

Serial cross-sections (10 μm thick) were stained for ATPase activity (pre-incubated at pH 10.3) or with NADH-tetrazolium stain to distinguish between type I and II (IIa and IIx) fibres or determine oxidative capacity of the muscle fibres, respectively (Novikoff et al., 1961; Brooke and Kaiser, 1970).

Immunohistochemistry was performed by incubating sections with the specific MHC antibodies (listed above), as previously described (Kohn et al., 2011). Anti-mouse HRP-conjugated secondary antibodies and a DAB stain kit (DAKO Laboratories, Glostrup, Denmark) were used to visualise the sections.

All sections were photographed at a 10× magnification and the fibres were classified as either pure type I, IIa or IIx, or hybrid fibres Ia or IIa/IIx. All fibres in view were included, except those that were not completely in the view-field (i.e. half fibres). A total of 199±32 fibres per animal muscle group were typed. The cross-sectional areas (CSA) of each fibre type were determined from

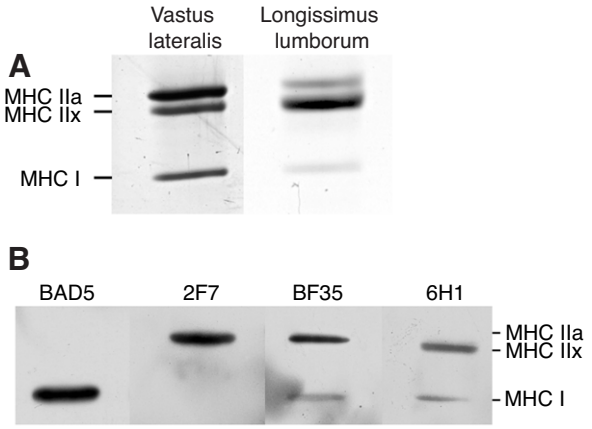

Fig. 1. (A) Myosin heavy chain (MHC) isoform separation and content of vastus lateralis and longissimus lumborum muscles from black wildebeest using SDS-PAGE. (B) Identification of separated bands using antibodies specific to the MHC isoforms. Antibodies recognised the bands as follow: BAD5, MHC I; 2F7, MHC IIa; BF35, MHC I and MHC Iia; 6H1, MHC I and MHC IIx.

pH 10.3 slides using pre-calibrated software (AxioVision, Carl Zeiss, Germany) and are expressed as μm^2.

Statistical analyses

Values are expressed as means ± s.d. Data from both muscle groups were compared using an unpaired *t*-test and significance was set at $P<0.05$ (GraphPad Prism, GraphPad Software, La Jolla, CA, USA). CSA of the fibre types from the same muscle group were compared using a one-way ANOVA with a Tukey's *post hoc* test. Relationships between fibre type and enzyme activities were determined using Pearson's correlation coefficient.

RESULTS

MHC isoform identification

SDS-PAGE was able to successfully separate the MHC isoforms into three distinct bands (Fig. 1A). The MHC I specific antibody BAD5 only reacted with the bottom band, whereas the MHC IIa antibody, 2F7, only reacted with the top band (Fig. 1B). BF35, which does not recognize the MHC IIx isoform but all the other isoforms, reacted with the bottom and top bands, confirming their characteristics as MHC I and MHC IIa, respectively. The antibody specific to MHC IIx (6H1) reacted with both the middle and bottom bands. This confirms that the middle band could be classified as MHC IIx, but that the same antibody had cross-reactivity with the MHC I isoform.

Immunohistochemistry in conjunction with the pH 10.3 ATPase stain of vastus lateralis samples confirmed that BAD5 only reacted with type I fibres (Fig. 2B,C). Antibody 2F7 only reacted with type IIa fibres (Fig. 2D), whereas antibody 6H1 reacted with type I and IIx fibres (Fig. 2E). To confirm that those fibres earmarked as type IIx fibres were indeed type IIx fibres, BF35 was included in the analyses, which only reacted with type I and IIa fibres (Fig. 2F). A few fibres were typed as hybrid IIax fibres as they showed reactivity with antibodies 2F7, 6H1 and BF35.

MHC isoform content and muscle fibre type

The MHC isoform content of the two muscle groups of black wildebeest are shown in Table 1. The vastus lateralis had significantly more MHC IIa and less MHC IIx than the longissimus lumborum muscle, whereas the amount of MHC I was similar for both groups. Immunohistochemical fibre type classification revealed a similar profile as that derived from the isoform analyses. The total number of hybrid fibres amounted to less than 6% for both muscle groups. Nevertheless, both methods confirmed more type IIa and lower type IIx fibres in the vastus lateralis compared with the longissimus lumborum, whereas the type I fibres were much fewer in number, yet similar in both muscles.

Muscle morphology

The muscle groups of these animals were dark red–purple in colour on gross examination. NADH staining revealed that all fibres had a relatively high oxidative capacity, with type I and IIa fibres being more oxidative than type IIx fibres (Fig. 2A). A few fibres classified as pure type IIx are shown in Fig. 3. Although each fibre expressed only MHC IIx, the fibres labelled with an arrow had a greater oxidative capacity than their neighbouring type IIx fibres. ATPase staining at pH 10.3, which reveals type I fibres, showed a low proportion of type I fibres, confirming the results from overall MHC isoform content (Fig. 2B, Table 1).

The type IIx fibres in vastus lateralis muscle were significantly larger than the type I and IIa fibres, with the latter two fibre types similar in size (Table 1). In contrast, all three fibre types from the longissimus lumborum muscle differed in size, with type IIa being the smallest and IIx the largest.

Enzyme activities

Overall, oxidative capacity and oxygen-independent (anaerobic) metabolism were high in both muscle groups (Table 1). CS and

Table 1. Enzyme activity, myosin heavy chain (MHC) isoform content and fibre cross-sectional areas of the vastus lateralis and longissimus lumborum muscles from black wildebeest

	Vastus lateralis	Longissimus lumborum
Protein/wet tissue	Protein (g kg^{-1} wet mass)	
	82±8	87±21
	Enzyme activity (μmol min^{-1} g^{-1} protein)	
CS	315±32	304±84
3HAD	56±18	64±27
PFK	349±71	745±229*
LDH	2518±883	3300±686*
CK	4036±1021	4304±1303
	MHC isoform content (%)	
MHC I	13±5	10±5
MHC IIa	55±11	38±13*
MHC IIx	32±9	52±15*
	Muscle fibre type (%)	
Type I	15±11	9±4
Type Ia	2±1	1±1
Type IIa	49±7	37±5*
Type IIax	4±3	3±3
Type IIx	30±9	51±9*
	Cross-sectional area (μm^2)	
Type I	2245±554	2235±775
Type Ia	2373±320	1943±289
Type IIa	3155±930†	1740±400*
Type IIax	3455±807†	2254±470*
Type IIx	4145±1156†	4079±1210††

Data are means ± s.d.

*Significantly different ($P<0.05$) from vastus lateralis. Within-muscle comparison: significantly different ($P<0.05$) from: †type I fibres; ††all fibre types.

CS, citrate synthase; 3HAD, 3-hydroxyacyl co A dehydrogenase; PFK, phosphofructokinase; LDH, lactate dehydrogenase; CK, creatine kinase.

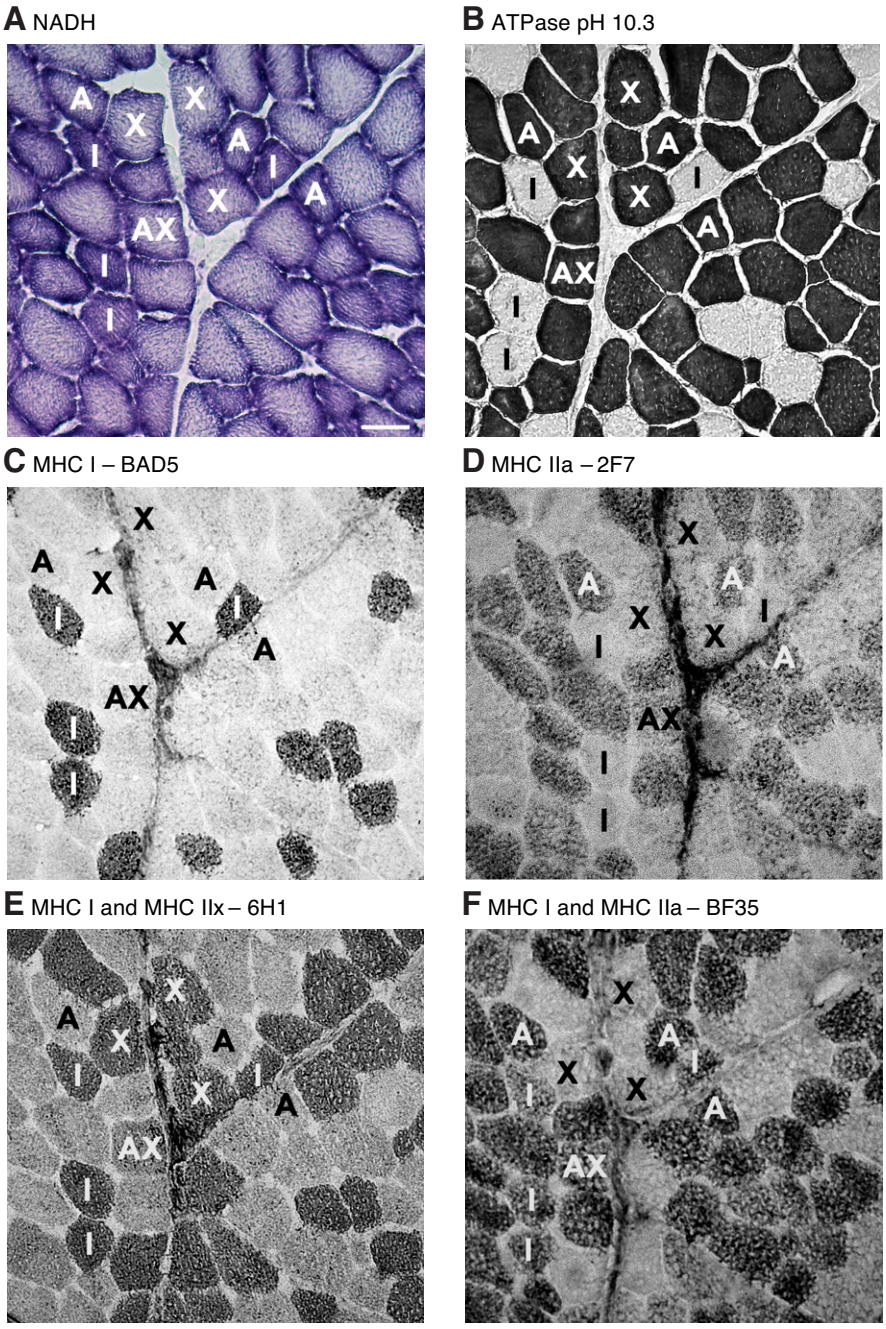

Fig. 2. Histochemistry and immunohistochemistry of the vastus lateralis muscle from black wildebeest. Letters within figures represent fibre types (I, type I; A, type IIa; X, type IIx; AX, type IIax hybrid). (A) NADH stain depicting oxidative capacity of fibres. Scale bar (applies to all panels), 50 μm. (B) ATPase ph 10.3. Unstained fibres represent type I fibres. (C) Anti-myosin heavy chain I (BAD5). (D) Anti-myosin heavy chain IIa (2F7). (E) Anti-myosin heavy chain I and IIx (6H1). (F) Anti-myosin heavy chain I and IIa (BF35).

3HAD activities, representing oxidative metabolism, were not different between the two muscle groups, neither was ATP generation from phosphocreatine stores (CK). In contrast, pathways representing the flux through glycolysis (PFK) and oxygen-independent metabolism of pyruvate (LDH) were all significantly higher in the longissimus lumborum compared with the vastus lateralis.

Correlations

Pearson's *r*-values between MHC isoform content and enzyme activities are presented in Table 2. Strong relationships were observed between PFK and MHC IIa (r=–0.674, P<0.01) and MHC IIx (r=0.75, P<0.001) content when the two muscles were grouped. However, this relationship was lost when individual muscles were analysed and was attributed to data clustering because of the muscle groups. No relationships between any of the remaining enzymes and MHC content were found.

DISCUSSION

This is the first study to investigate the skeletal muscle fibre type, morphology and energy metabolism in an African wild mammalian species that demonstrates both high speed and superior fatigue resistance. The main finding of this study was that the skeletal muscle fibres of black wildebeest exhibit a high proportion of fast-contracting type IIx fibres (vastus lateralis: ~30%, longissumus lumborum: ~51%), in combination with both a high oxidative and high glycolytic capacity, presumably providing the animal with fatigue resistance and high speed capability (Table 1). A similar observation was made by Essén-Gustavsson and Rehbinder (Essén-Gustavsson and Rehbinder, 1985), who studied the oxidative

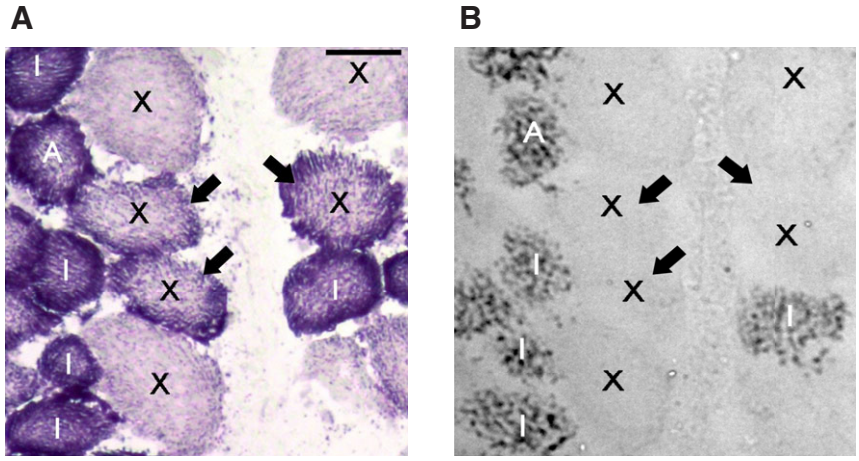

Fig. 3. Sections of vastus lateralis muscles from black wildebeest indicating type IIx muscle fibres with different staining intensities for oxidative capacities. Arrows indicate type IIx fibres with high oxidative capacity. (A) NADH stain depicting oxidative capacity. Scale bar (applies to both panels), 50 μm. (B) Anti-myosin heavy chain I and IIa (BF35), indicating the absence of both MHC I and MHC IIa in type IIx fibres. I, type I; A, type IIa; X, type IIx.

capacity and fibre type content of reindeer. These authors commented that the reindeer muscle possessed high quantities of type IIx fibres but also showed high oxidative capacity.

This finding is different to what has been described in the muscle of the major predators of black wildebeest, primarily the lion. Lions appear to have a muscle fibre composition designed purely for speed, with an even higher proportion of type IIx fibres (~60%), but with the expected lower oxidative and similar glycolytic capacity, providing a lesser fatigue resistance (Kohn et al., 2011). Thus, this study establishes that the pressures of predation on the wildebeest, along with the need to migrate for better vegetation, has produced a specific skeletal muscle fibre phenotype designed for both speed and fatigue resistance.

MHC IIx isoform identification

Previously, using antibodies against MHC I (N2.261) and MHC IIa (A4.74), Kohn et al. (Kohn et al., 2007a) showed that, of the three separated MHC isoforms from black wildebeest, the top and bottom bands corresponded to the MHC I and IIa isoforms, respectively (Fig. 1A). Only recently has an antibody become available that reacts with the MHC IIx isoform. Therefore, a secondary aim of this study was to validate the previous findings and to confirm that the second migratory band was indeed MHC IIx.

Western blotting and immunohistochemistry using previously validated antibodies (for both isoform specificity and species reactivity) showed that the black wildebeest expressed only MHC IIa, MHC IIx and MHC I (corresponding to the migratory pattern observed in Fig. 1A) (Smerdu et al., 2005; Acevedo and Rivero, 2006; Kohn et al., 2011). This migratory profile is consistent with that from a number of large domestic and wild species, including cattle, horses, lions, dogs, tigers and cheetahs, whereas human MHC IIx migrates slower than its MHC IIa isoform (Toniolo et al., 2005; Acevedo and Rivero, 2006; Kohn et al., 2007a; Rivero et al., 2007; Hyatt et al., 2010). BAD5 and 2F7 reacted with only one band each, corresponding to MHC I and MHC IIa, respectively. BF35 was able to recognize both these isoforms and validated their migratory pattern (Fig. 1A,B). These antibodies also reacted only with their respective isoforms when used in immunohistochemistry (Fig. 2C,D,F).

Antibody 6H1 was shown to exclusively react with the MHC IIx isoform from rat, mouse, rabbit, guinea pig, lion, human and caracal muscle (Lucas et al., 2000; Kohn et al., 2011). Although this antibody was able to correctly identify the MHC IIx band in black wildebeest, it also showed cross-reactivity with the MHC I band (Fig. 1B). This cross-reactivity was confirmed using immunohistochemical staining (Fig. 2E). No trace of the MHC IIb isoform was found when probed with the MHC IIb specific antibody, BFF3 (data not shown). Nevertheless, using these four antibodies (BAD5, 2F7, BF35 and 6H1) allowed us to successfully identify the isoforms expressed in black wildebeest muscle as MHC I, MHC IIa and MHC IIx.

Muscle morphology

Muscle fibre size is directly related to its capacity to generate force. The average CSA of human muscle fibres ranges between 4000 and 5000 μm^2. Endurance runners tend to lie towards the top end of this range [5614±862 μm^2; values averaged from Kohn et al. (Kohn et al., 2007b; Kohn et al., 2010)], whereas strength-trained body builders can have CSA in excess of 11,000 μm^2 (D'Antona et al., 2006). On average, the CSA of black wildebeest fibres from the present study was 2675±1034 μm^2, rendering these fibres small in comparison to human fibres. However, the findings are in agreement with CSA from other species, such as horses, reindeer, dogs and other wild antelope and felids (Stickland, 1979; Essén-Gustavsson and Rehbinder, 1985; Karlström et al., 1994; Spurway et al., 1996; Acevedo and Rivero, 2006; Hyatt et al., 2010; Kohn et al., 2011). The significance of this finding may not yet be fully understood. Smaller fibre diameter is a typical trait of human endurance-trained athletes, which may improve the overall economy of the fibre (e.g. reducing the diffusion distance of O_2 and improving lactate clearance) (Kohn et al., 2010). In contrast, and purely speculative, a smaller diameter may provide space for a greater number of muscle fibres per area, which may result in greater force production capability of the whole muscle. However, future research on contractile properties and muscle volume is required to elucidate the role of fibre size in whole muscle kinetics.

MHC isoform content, fibre type and metabolism

Human endurance athletes are known to have an abundance of type I fibres in their vastus lateralis muscles. These fibres are highly oxidative, having an abundant capillary supply, large mitochondrial volume and high oxidative enzyme capacities, allowing the muscle to rely on fat as an energy source for greater fatigue resistance during exercise (Gollnick et al., 1972; Kohn et al., 2007b). On the contrary, humans and animals (particularly cheetahs and lions) that are sprinters have poor oxidative capacity and rely primarily on the oxygen-independent supply of ATP *via* phosphocreatine stores and carbohydrate, with resulting high blood lactate concentrations during vigorous exercise (Essén-Gustavsson and Henriksson, 1984; Williams et al., 1997; Kohn et al., 2011). It does seem that metabolism is more adaptive than fibre type, as there is a large

Table 2. Relationships between muscle fibre type composition and enzyme activities in black wildebeest

	r				
Muscle fibre type	CS	3HAD	PFK	LDH	CK
MHC I	–0.120	0.155	–0.449	–0.385	–0.024
MHC IIa	–0.008	–0.295	–0.674**	–0.199	–0.073
MHC IIx	0.044	0.214	0.746**	0.297	0.073

Muscles were grouped for Pearson's correlation analyses (refer to Results for additional explanation). ** $P<0.01$.

difference in the amount of type I fibres from human sprinters and animals that are considered sprinters (see below) (Andersen et al., 1994; Hyatt et al., 2010).

On gross examination, the black wildebeest muscles appeared dark red in colour, suggesting the presence of a large proportion of type I fibres. Instead, both muscle groups contained only approximately 5–18% type I fibres, with an abundance of type II fibres (Table 1). The longissimus lumborum had the largest proportion of type IIx fibres (>50%), whereas the vastus lateralis had more than 50% type IIa fibres. These findings are consistent with previous findings on wild animals and cattle (Stickland, 1979; Hoppeler et al., 1981; Karlström et al., 1994; Spurway et al., 1996; Kohn et al., 2007a). Dog muscles, in contrast, contain a high proportion of pure type IIa and type IIax hybrid fibres, with few type IIx fibres (Acevedo and Rivero, 2006), whereas the fibre type profile from endurance runners appears to harbour primarily type I and IIa fibres, and few (if any) IIx fibres (Gollnick et al., 1972; Kohn et al., 2007b; Kohn et al., 2007c).

The longissimus lumborum of the black wildebeest had a higher oxygen-independent metabolic component (CK, PFK and LDH) than the vastus lateralis muscle, which could be explained by the higher proportion of type IIx fibres in this muscle group (Table 1). However, both muscle groups showed high and similar oxidative capacities, despite a very low type I fibre content. Collectively, the oxidative capacities were similar to those of human endurance runners, horses and reindeer (Essén-Gustavsson and Rehbinder, 1985; Karlström et al., 1994; Pösö et al., 1996; Kohn et al., 2011). The oxygen-independent component (especially that of PFK and LDH activities) was much higher when compared with that from humans and also higher, but to a lesser extent, than activities in horses and reindeer (Chi et al., 1983; Essén-Gustavsson and Rehbinder, 1985; Karlström et al., 1994) but similar to values measured in lion, caracal and domestic cat (van Lunteren and Brass, 1996; Kohn et al., 2011). The latter three species are known to be sprinters and have poor fatigue resistance, as would be predicted from their respective muscle metabolic profiles. These data clearly show that the muscle of the black wildebeest has the potential to withstand fatigue for long periods of time and is also capable of generating great force.

The lack of a relationship between muscle fibre type and its metabolism (Table 2) in this study was an interesting find. It is generally accepted that high oxidative metabolism and poor glycolytic capacity coincides with the percentage of type I fibres and that the regulatory mechanisms are linked (Essén-Gustavsson and Henriksson, 1984; Karlström et al., 1994; Schiaffino et al., 2007). However, the lack thereof for the black wildebeest may suggest that genetic factors or muscle innervation, rather than an adaptation to actual physical training, is the primary cause of the high type IIx fibre content and oxidative capacity.

To partially support the above, pure type IIx fibres were found that presented with a higher oxidative capacity than similar classified fibre types (Fig. 3). This is, however, not an unusual finding, although it is possibly underreported in the current literature. Specifically, a fibre type was identified in the dog (termed type II-Dog) with type IIb characteristics but unusually high oxidative capacity (Latorre et al., 1993). Dog muscle is also higher in oxidative capacity, despite having a low type I fibre content (Acevedo and Rivero, 2006). Essén-Gustavsson and Rehbinder (Essén-Gustavsson and Rehbinder, 1985) reported surprisingly high oxidative capacities and a high type IIx fibre content in reindeer muscle. This would suggest that the particular muscle profiles (high speed generation capacity and fatigue resistance) observed in these animals would most likely be from inherent factors rather than exercise. Although the natural predator of black wildebeest is the lion, the animals in this study were born and bred on game farms with no natural predators.

Nevertheless, more research is required to determine the metabolic profile of each individual muscle fibre type (which is only semi-quantifiable from the NADH stains) (Fig. 2A, Fig. 3A) and their contribution to the overall metabolic profile of the muscle. Furthermore, inclusion of the superficial and deep parts in the analyses may reveal a different metabolic and fibre type profile in this species.

Relationship between sprinting speed and muscle fibre composition

Our recent paper showed a positive relationship between maximum sprinting speed and MHC IIx content of nine different species (Kohn et al., 2011). From this graph, the conclusion was drawn that an abundance of type IIx fibres is required to achieve high

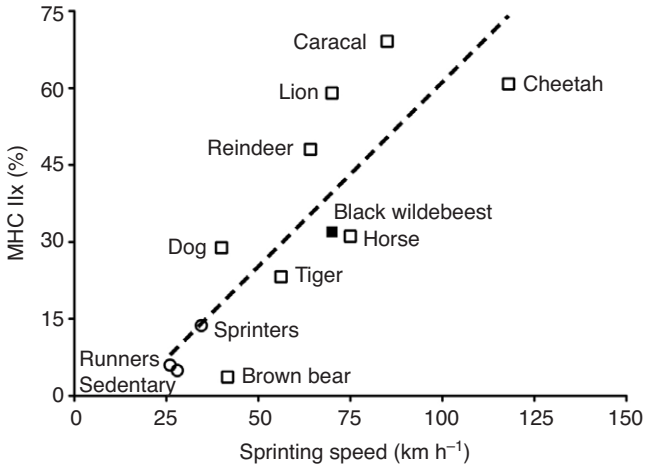

Fig. 4. Correlation between MHC IIx and maximal sprinting speed (km h^{-1}). This figure was adapted from Kohn et al. (Kohn et al., 2011). Open circles, previous human data; open squares, previous data on wild animal muscle; filled square, black wildebeest from the present study. r=0.84, P<0.001.

speeds. Fig. 4 is an adaptation of the previously published graph, but with the black wildebeest added. It was assumed that the maximum sprinting speed of this animal was 70 km h^{-1}. It is emphasised that the speed reported here is from non-scientific sources and is merely an estimation. The addition did not alter the Pearson's coefficient (from 0.85 to 0.84), but strengthens the proof that speed is dependent on the amount of type IIx fibres in the muscles used for running and/or sprinting.

Conclusion

This study investigated fibre type and metabolism in skeletal muscle from black wildebeest, an animal adapted for sprinting and endurance. Type IIa and IIx fibres were primarily expressed in the two muscles used for locomotion, with high oxidative and oxygen-independent capacities, suggesting an animal that may run at great speeds and withstand fatigue. It would also be interesting to compare the muscle profiles of other antelope and the pronghorn (*Antilocapra americana*) – the latter is claimed to reach speeds of 100 km h^{-1} for long periods of time (Lindstedt et al., 1991) – to see whether these species also contain high numbers of type IIx fibres with high oxidative capacity.

LIST OF ABBREVIATIONS

3HAD	3-hydroxyacyl co A dehydrogenase
CK	creatine kinase
CS	citrate synthase
CSA	cross-sectional area
LDH	lactate dehydrogenase
MHC	myosin heavy chain
PFK	phosphofructokinase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis

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