Title

High oxidative capacity and type IIx fibre content in springbok and fallow deer skeletal muscle suggest fast sprinters with a resistance to fatigue.

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SHORT TITLE

Wild antelope skeletal muscle

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SUMMARY

Some wild antelopes are fast sprinters and more resistant to fatigue than others. This study therefore investigated two wild antelope species to better understand their reported performance capability. Muscle samples collected *post mortem* from the *Vastus lateralis* and *Longissimus lumborum* of fallow deer (*Dama dama*) and springbok (*Antidorcas marsupialis*) were analysed for myosin heavy chain isoform content, citrate synthase (CS), 3-hydroxyacyl Co A dehydrogenase, phopshofructokinase, lactate dehydrogenase and creatine kinase activities. Cross-sectional areas, fibre type and oxidative capacities of each fibre type were determined in the *Vastus lateralis* only. The predominant fibre type in both muscle groups and species were type IIX (>50%), with springbok having more type IIX fibres than fallow deer ($P < 0.05$). Overall cross-sectional area was not different between the two species. The metabolic pathway analyses showed high glycolytic and oxidative capacities for both species, but springbok had significantly higher CS activities than fallow deer. Large variation and overlap in oxidative capacities existed within and between the fibre types. Some type IIX fibres presented with similar oxidative capacities as that from type I and IIA fibres. The data suggest that springbok and fallow deer could sprint at >90 $km \cdot h^{-1}$ and 46 $km \cdot h^{-1}$, respectively, partly from having large type IIX fibre contents and high glycolytic capacities. The high oxidative capacities also suggest animals that could withstand fatigue for long periods of time.

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
OD	optical density
PFK	phosphofructokinase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis

INTRODUCTION

Southern Africa has a vast number of large mammalian species that present with superior exercise performance capability, either sprinting (e.g. cheetah, lion) or endurance, or both (wildebeest). However, only a few studies exist on skeletal muscle properties of selected species to explain their performance capabilities.

Skeletal muscle of particularly large mammals (including human) generally contains three fibre types (type I, IIA and IIX fibres), with each of these fibre types differing in fuel preference and contractile properties. Varying the composition of these three fibre types give rise to the unique functional properties of a particular muscle (e.g. soleus vs. biceps). Type I (slow oxidative) fibres express the myosin heavy chain (MHC) I isoform, which gives rise to a relatively slow contraction speed (Schiaffino and Reggiani, 1996; Bottinelli, 2001). They contain large numbers of mitochondria, are efficient in using fat, glucose and glycogen aerobically (high activities of citrate synthase (CS), 3-hydroxyacyl Co A dehydrogenase (3HAD), but low activities of lactate dehydrogenase (LDH), phosphofructokinase (PFK) and creatine kinase (CK)) to produce the required ATP and are considered highly fatigue resistant (Pette, 1985). Type IIX (fast glycolytic) fibres express the MHC IIx isoform. This isoform allows the fibre to contract fast relative to type I fibres (Bottinelli, 2001). Their primary source of ATP generation is derived from the anaerobic metabolism of glucose and glycogen *via* glycolysis, and hence they contain very few mitochondria (low CS and 3HAD activities), have a high glycolytic capacity (high activities of LDH, PFK and CK) and fatigue quickly (Pette, 1985). Lastly, type IIA (fast oxidative) fibres are fast contracting fibres (less so than the type IIX fibres), and they derive their contractile properties from the expression of the MHC IIa isoform. This fibre type contains large numbers of mitochondria and can produce ATP from both aerobic and anaerobic metabolism, rendering this fibre type more resistant to fatigue (Pette, 1985; Schiaffino and Reggiani, 1996). All three fibre types also differ in the amount of maximum force and power generation capability, with type I fibres being poor at both and type IIX fibres the best (Chi et al., 1983; Essén-Gustavsson and Henriksson, 1984; Bottinelli, 2001). A fourth fibre type (type IIB expressing MHC IIb), fast twitch glycolytic, is primarily abundant in limb muscles of rodents (Pette and Staron, 1993; Delp and Duan, 1996; Kohn and Myburgh, 2007). Although small quantities of this fibre type was detected in cheetah, llama and pig limb muscles, it seems that this fibre type is reserved for more specialised muscles, such as that in the eye, and undetectable in horse, cattle, black and blue wildebeest, blesbuck, kudu, lion, caracal and brown bear (Quiroz-Rothe and Rivero, 2001; Toniolo et al., 2005; Kohn et al., 2007; Smerdu et al., 2009; Hyatt et al., 2010; Kohn et al., 2011b; Kohn et al., 2011a).

Recent investigations showed that the *Vastus lateralis* and *Longissimus lumborum* muscles of feline predators (lion and caracal) exhibit a predominance of type IIX muscle fibres (>50%), high glycolytic but relatively poor oxidative capacity (as revealed by their oxidative capacities - i.e. NADH stain, CS and 3HAD activities) (Kohn et al., 2011b). Similar large quantities of type IIX fibres were found in tiger and cheetah muscle (Williams et al., 1997; Hyatt et al., 2010). On the other hand, the same muscle groups from black wildebeest, impala and reindeer were found to contain high proportions of type IIX fibres (30 – 60%), high glycolytic but also high oxidative capacities (Essén-Gustavsson and Rehbinder, 1985; Kohn et al., 2005;

Kohn et al., 2011a). Thus, the muscle metabolic and fibre type profiles observed in these species closely resemble their physical activity behaviour. For example, felids are fast sprinters reaching speeds of up to 120 $km.h^{-1}$ but lack endurance, whereas black wildebeest and other antelopes can maintain a relatively high running intensity for long periods of time (Skinner and Chimimba, 2005). Additionally, Kohn et al., (2011a) recently showed that black wildebeest muscle harbour type IIX muscle fibres that either contained low or high oxidative capacities in muscle sections stained for oxidative capacity. This is however not an uncommon finding. Others have shown that type IIX fibres from rat, mouse, reindeer and horse vary significantly in oxidative capacity having these fibres with low and high capacities (Essén-Gustavsson and Rehbinder, 1985; Pette, 1985; Pösö et al., 1996; Linnane et al., 1999; Smerdu et al., 2009). These findings are in contrast to human muscle as historically, only type I and type IIA fibres are considered oxidative in nature (Essén-Gustavsson and Henriksson, 1984). However, the presence of high oxidative type IIX fibres in the black wildebeest was argued to sustain fast running speeds for prolonged periods of time in these animals, especially to escape predation (Kohn et al., 2011a).

Many antelope species with varying sizes roam the savannahs of the southern African content. Very few have been investigated on muscular level, but yet, each species presents with their own unique sprinting and endurance capabilities. The national mammal of South Africa is the springbok (*Antidorcas marsupialis)* and is indigenous to large parts of South Africa, Namibia and Botswana. They are some of the few antelope species where both males and females have horns. With its small body mass (males range between 31 to 46 kg), springbok can gallop at an unconfirmed 88 – 97 $km.h^{-1}$ and is well known for their stotting ability - the latter being periodic jump-like actions during the gallop (Garland, 1983; Skinner and Chimimba, 2005). Although not scientifically measured, the lay press have reported that they can leap more than 3 meters high and reach a distance of 14 meters from the point of take off. Fallow deer (*Dama dama*) were introduced by the British in the late 19^{th} centuray to South Africa, primarily for hunting. These animals adapted well to the South African climate and their population grew ever since. Where springbok (males and females) usually roam in herds, the fallow deer males are solitary animals. Males average approximately 67 kg and carry antlers (ranging from 50 – 70 cm), while females are smaller (44 kg on average) and carry no antlers (Dharmani, 2000). Fallow deer are not particularly fast animals (reported maximum sprinting speed of 45 $km.h^{-1}$) and despite their size, can only jump approximately 1.75 meters high (Garland, 1983).

Therefore, the first aim of this study was to investigate the skeletal muscle properties of springbok and fallow deer, focussing on muscle fibre type and metabolism. Muscle groups harvested and analysed were the *Vastus lateralis* (aids in forward propulsion and jumping), and the *Longissimus lumborum* (aids in stabilising the back during running and jumping). Due to the ability of the springbok to run fast, and being a high jumper and migrator, it is hypothesised that their muscle contain large numbers of type IIX fibres, with high oxidative and glycolytic capacities and large fibre cross-sectional areas (CSA), similar to that found in the black wildebeest. However, it is hypothesised that fallow deer muscle would contain large numbers of type I and IIA fibres, high oxidative but poor glycolytic capacity, giving rise to their poor sprinting ability.

METHODS

All the methods (except the histological intensity analyses) reported below, were previously described in detail by Kohn et al. (2011a). A brief summation of each analysis will follow.

Animals and tissue sampling

Adult wild animals were randomly shot during the annual cropping season by professional hunters. The cropping occurred on private game farms in the Eastern Cape province, South Africa. Muscle samples from 7 female fallow deer and 12 springbok (7 male and 5 female) were collected post mortem from the *Longissimus lumborum* and *Vastus lateralis* within 4 hours after death.

The muscle of interest was identified using anatomical markers. Once identified, an incision was made through the hide and fascia, and the muscle of interest exposed. For the *Vastus lateralis*, the sample site was determined as half the distance between the knee and hip joint, whereas the sampling site for the *Longissimus lumborum* was between 2-3 mm parallel to the spinous process between L3 and L4 of the lumbar spine. Using a scalpel blade, a block of tissue (±1 cm^3) was removed from both muscle groups and the superficial part thereof discarded. The remaining piece was divided into smaller parts, rapidly frozen in liquid nitrogen and stored in a cryo-preservation tank (-200 °C). After harvesting, samples were transported to the laboratory in liquid nitrogen and stored at -87 °C until subsequent analyses.

Homogenisation of tissue

Samples were prepared for enzyme and myosin heavy chain (MHC) isoform analyses as described by Kohn et al. (2011a). Briefly, frozen tissue was weighed and 100 mM potassium phosphate buffer, pH 7.30, added to a ratio of 1:19. Homogenisation was performed on ice using a Teflon tip, and sonicated twice for 10 seconds at 6 W (Virtis Virsonic Ultrasonic Cell Disrupter 100). The suspension was centrifuged at 1700xg for 5 minutes (4 °C) and the protein concentration of the supernatant determined (Bradford, 1976). The supernatant was used for enzyme activity measurements and the pellet for MHC isoform content.

Enzyme analyses

Enzyme activities were determined spectrophotometrically at 25 °C as described and modified by Kohn et al. (2011b). Maximum CS (EC 4.1.3.7) and 3HAD (EC 1.1.1.35) activities represented the flux through the Kreb's cycle and β-oxidation, respectively, whereas PFK (EC 2.7.1.11) and LDH (EC 1.1.1.27) activities represented flux through the glycolytic pathway. CK (EC 2.7.3.2) activity represented rapid ATP replenishment from phosphocreatine stores. All activities are expressed as $\mu mol.min^{-1}.g^{-1}$ protein.

MHC isoform separation, Western blots and relative fibre type

All methods to identify the MHC isoforms of springbok and fallow deer were previously described (Kohn et al., 2011a). Briefly, MHC isoforms were separated using 7% SDS-polyacrylamide gels ran for 24 hours. For MHC migratory identification, a human sample from previously published research was used to serve as control (Kohn et al., 2011b). Separated isoforms were transferred to PVDF membranes and probed with antibodies specific to MHC I (BAD5), MHC I and IIa (BF35) and MHC IIx (6H1) (Lucas et al., 2000; Kohn

et al., 2011b; Kohn et al., 2011a). The migration patterns were then used to identify the location of the specific MHC isoform in question. Relative MHC isoform content of the three isoforms for each muscle group was calculated as a percentage of the total from silver stained gels using the Un-Scan-It software package (Silk Scientific Corporation, Orem, UT, USA).

Histochemical and immunohistochemistry

Histological procedures were performed as described by Kohn et al. (2011a). Briefly, 10 µm serial cross-sections were cut from the *Vastus lateralis* muscle only in a cryostat at -25 ˚C. Sections were stained for ATPase activity (pH 10.3) to aid in fibre boundary identification during subsequent immunohistochemical stains and analyses. Oxidative capacity of the fibres was achieved by staining the mitochondria with an NADH-nitro blue tertrazolium reaction for 30 minutes at 37 ˚C (Novikoff et al., 1961). In order to ensure that the NADH reaction was in the linear phase at the end of 30 minutes and that the incubation time was adequate, a series of incubation times were performed for 10, 20 and 30 minutes, respectively. The optical densities (OD) of the fibres were determined (see below) and fibres divided into dark, medium and light staining intensities, based on values obtained at the 30 minute time point.

Additional sections were used for immunohistochemistry using the three antibodies listed under Western blot. Immunoreactivity was visualised using a DAB staining kit (DAKO, Denmark). Sections were viewed under a light microscope at 10x magnification and photographed (AxioVision, Zeiss, Germany). Fibres were typed according to their reactivity to the specific antibodies and identified as either type I, IIA, IIAX and IIX. Once identified, fibre CSAs were determined using the ImageJ for Mac software package (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011). In order to quantify differences in oxidative capacity between fibre types, OD analysis of each fibre type was performed with pre-calibrated software (ImageJ). Frequency (percentage) distribution curves were generated for each fibre type and species. This was achieved by counting the number of fibres in a specific range of OD values (e.g. the number of fibres between 0.35 – 0.39 OD expressed as a percentage of the total number of fibres) (see Fig. 5 for clarity).

Statistical analyses

Values are expressed as mean ± standard deviation. Inter- and intra-species differences were analysed using a one-way analyses of variance with a Tukey post hoc test. Whenever the variances differed (tested by the Bartlett's test for equal variance), the non-parametric Kruskal-Wallis ANOVA with a Dunn's post hoc test was implemented. Where only two groups were compared, an unpaired t-test (Mann-Whitney) was used. Relationships were determined using the Pearson's correlation coefficient. For all statistical analyses, significance was set at $P < 0.05$. Clarification comment: Uppercase letters are used for fibre types derived from histology, whereas lower case lettering refers to fibre types derived from the MHC isoform analyses using SDS-PAGE.

RESULTS

Grouping of animals

Of the 12 springbok sampled, 5 were female. Statistical analyses of the data showed no significant difference between male and female springbok, and therefore data were pooled.

MHC isoform identification

Three MHC isoforms were separated for each species using SDS-PAGE (Figs. 1A & 1B). Western blot analyses using anti-myosin I (BAD5) recognized the bottom band in all three species, confirming its location to be similar to that of the human MHC I isoform (Fig. 2). BF35 (specific to MHC I and MHC IIa) was able to recognise two bands in all the species. These bands corresponded to the top (MHC IIa) and bottom (MHC I) isoforms of human. Anti-MHC IIx (6H1) recognized only the top band for human, whereas this antibody recognized the middle band in fallow deer and springbok. It also showed cross-reactivity with the bottom band (MHC I) in these antelope species. The location of the identified MHC isoforms confirms the migratory profile separated in Figure 1A and 1B.

To confirm the specificity of the antibodies and hence, muscle fibre type of the antelope species, serial cross-sections from the *Vastus lateralis* muscle pre-incubated at pH 10.3 and stained for ATPase activity (Fig. 4B), and immunohistochemistry using the above mentioned antibodies (Figs. 4C, 4D & 4E) were included. BAD5 only reacted with springbok MHC I fibres, whereas this antibody reacted with MHC I fibres and also showed slight cross-reactivity with MHC IIX fibres in fallow deer (Fig. 4C). BF35 reacted with only MHC I and IIA fibres (including hybrids) in both species and showed no cross-reactivity with pure MHC IIX fibres (Fig. 4D). 6H1 reacted with pure MHC IIX fibres and showed strong cross-reactivity with pure MHC I fibres in both species, but not with pure type IIA fibres (Fig. 4E).

Myosin heavy chain isoform content and muscle fibre type

Both animals expressed an abundance of the MHC IIx isoforms in the two muscle groups, with springbok muscles containing significantly more of this isoform than fallow deer (Table 1). Fallow deer, on the other hand, expressed more MHC IIa in both muscle groups. Both muscle groups from both species expressed very little MHC I.

The isoform proportions were further confirmed using histological techniques on *Vastus lateralis* sections only, using the ATPase (pH 10.3) and immunohistochemistry (Table 2). Using these techniques, the fibres were subdivided into pure type I, IIA and IIX, and one hybrid fibre category (type IIAX). Both species had approximately equal amounts of type I fibres, with springbok having significantly less and more type IIA and type IIX fibres, respectively, than fallow deer ($P < 0.05$). Although not statistically significant, springbok tended to have more hybrid type IIAX fibres than fallow deer ($P = 0.08$).

Muscle morphology

The fibre types of *Vastus lateralis* from both species showed a continuous increase in fibre size from type I, IIA, IIAX and IIX (Table 2). Type IIA fibres were smaller in springbok, whereas their type IIX fibres were

significantly larger than that of fallow deer. Overall, when the CSA of the fibre types were pooled, no significant difference was found between the fibre size of fallow deer (2731 ± 613 μm^2) and springbok (2726 ± 970 μm^2).

Enzyme activities

The oxidative enzyme capacities (CS and 3HAD) and anaerobic metabolic pathway capacities were high in both species (Table 1). CS activities of *Vastus lateralis* and *Longissimus lumborum* muscles were significantly higher in springbok compared to fallow deer, whereas the capacity to utilise fat (3HAD) was similar in both muscle groups and species. With the exception of CK in the *Longissimus lumborum* muscle (which was significantly lower in springbok), PFK, LDH and CK (*Vastus lateralis*) activities were not different between springbok and fallow deer. On the other hand, LDH and CK activities of springbok *Vastus lateralis* were significantly lower and higher, respectively, compared to its respective *Longissimus lumborum* activities. These differences were not apparent in the fallow deer.

Oxidative capacity quantification of muscle fibres

To ensure that the NADH reaction was in the linear phase after 30 minutes of incubation, three incubation time points (10, 20 and 30 minutes) were evaluated and OD measurements performed (Fig. 3A & 3B). As incubation time increased, so did the OD values increase in a linear fashion (R^2: white fibres, 0.76; medium fibres, 0.90; dark fibres, 0.85). The rates at which the intensity increased (slope of the lines) were faster in fibres that contained more mitochondria (dark fibres > medium fibres > light fibres). At 30 minutes the reaction was still linear, but with clearer distinction between the OD of the three groups. Based on these findings, all NADH incubations were performed for 30 minutes.

The NADH staining of springbok and fallow deer *Vastus lateralis* muscle are shown in figure 4A. As expected, type I and IIA fibres had the highest oxidative capacities in both animal species, followed by type IIAX and finally type IIX. However, most notably was the presence of type IIX fibres that stained light or dark (Fig. 4A: X vs. X_O), indicating fibres with low or high oxidative capacities in both species.

Figure 5 depicts fibre type frequency distributions (in percentage) of the number of fibres having the same range of OD values. The lowest and highest OD values for this analysis were 0.40 and 2.29, respectively. Most notably from the graphs is the large range in oxidative capacities within a specific fibre type. Furthermore, there is large overlap in oxidative capacities between the four fibre types. Comparing the species, springbok type IIAX and IIX fibres had a greater oxidative capacity distribution than fallow deer, whereas type I and IIA seemed similar in distribution.

Correlations

Pooled data of the two species and their muscle groups revealed a positive relationship between CS activity and MHC IIx only (Pearson's r = 0.45, $P < 0.01$). However, this relationship was lost once the data was separated into muscle groups and species, suggesting that this relationship was an artefact of the pooling of the data sets. No other relationships were observed between any of the data sets analysed.

DISCUSSION

This is the first study to characterise skeletal muscle properties (fibre type and metabolism) of fallow deer and springbok. Both species had high oxidative and glycolytic capacities, and high type IIX (MHC IIx) fibre content. There were also large variations in oxidative capacity within a specific fibre type, but less so between fibre types.

MHC isoform identification and antibody specificity

Both species expressed three isoforms in the muscle groups analysed, and corresponded to (in order of migration) MHC IIa, MHC IIx and MHC I (Fig. 1 & 2). MHCs I and IIa were located on a similar migratory level as the human isoforms, whereas their MHC IIx migrated slightly below the MHC IIa isoforms. The size and pattern correspond to the MHC isoform migratory profile observed for blue and black wildebeest, blesbuck, kudu and bovine (Kohn et al., 2007; Kohn et al., 2011a). Although the migration of MHC I and IIa of human, lion and caracal are similar to those above, their MHC IIx isoform shows the most diversity in size (Kohn et al., 2011b; Kohn et al., 2011a). It would therefore seem that there exist large homology between the MHC I and MHC IIa isoform structures in mammals, whereas the MHC IIx is the least conserved in structure. Further research is required, particularly for the fibres expressing MHC IIx, in order to elucidate whether the functional properties of the fibre is different (e.g. maximum force production, shortening velocity, etc.).

The MHC I antibody BAD5 was shown to only react with the MHC I isoform in human, lion, caracal, dog, rat and black wildebeest (Acevedo and Rivero, 2006; Kohn et al., 2011b; Kohn et al., 2011a). The current study showed similar results for the springbok on both western blot and immunohistochemistry. However, this antibody showed slight cross-reactivity with type IIX fibres from fallow deer (Fig. 4C). The explanation for this is not clear, but could be related to these animals having a different gene sequence compared to the indigenous southern African antelopes.

Antibody BF35 are specific to MHC I and IIa, respectfully. Its reactivity and specificity were confirmed in various animal species, including human, dog, llama, felines and black wildebeest (Graziotti et al., 2001; Acevedo and Rivero, 2006; Hyatt et al., 2010; Kohn et al., 2011a). Some species that do express the MHC IIb isoform in their muscle also showed reactivity with this antibody (e.g. llama and rodent)(Graziotti et al., 2001). In fallow deer and springbok, this specificity was confirmed using Western blots and immunohistochemistry, showing no cross-reactivity with pure type IIX (MHC IIx) fibres. To confirm the absence of the MHC IIb isoform in springbok and fallow deer, the antibody specific to the IIb isoform (10F5) were included in Western blots and immunohistochemistry (data not shown). All these were negative for the presence of the MHC IIb isoform or type IIB fibres, thus confirming only three fibre types for these two species. This is not an unusual find as no MHC IIb was found in lion, caracal and black wildebeest using the same antibody (Kohn et al., 2011b; Kohn et al., 2011a).

Muscle fibre size

On average, there was no difference between fibre CSA from fallow deer (2731 ± 613 μm^2) and springbok (2726 ± 970 μm^2), whereas the fibre types tended to increase in size from type I to type IIX. The average fibre size and that of the different fibre types are in accordance to that reported for black bear, blue and black wildebeest, bovine, caracal, cat, dog, horse, lion, llama and reindeer (Young, 1982; Essén-Gustavsson and Rehbinder, 1985; Pösö et al., 1996; Spurway et al., 1996; Williams et al., 1997; Serrano et al., 2000; Graziotti et al., 2001; Acevedo and Rivero, 2006; Marx et al., 2006; Toniolo et al., 2008; Smerdu et al., 2009; Kohn et al., 2011b; Kohn et al., 2011a). It is well known that, on average, human fibres tend to be larger than animals (exception is the rhinoceros), and may increase in size as a result of training. Particularly for humans, the type of training may affect to what extent hypertrophy occurs (i.e. endurance vs. resistance training). However, it seems that the small CSA of the fibres from these animals may largely be genetically determined. The main hurdle currently is the lack of understanding what the functional significance would be for mammals to have small fibre CSA. Kohn et al. (2011a) recently speculated that it could improve overall fibre economy (e.g. smaller O_2 diffusion distance). Additionally, these authors also suggested that the smaller fibres may allow for a greater number of fibres present per muscle area that could allow for a greater force output of the muscles. Springbok type IIX fibres were on average ~16% larger than fallow deer, whereas their type IIA fibres were 21% smaller. Giving the anecdotal evidence that springbok are able to sprint at speeds greater than 88 $km \cdot h^{-1}$ and able to leap more than 3 meters high during locomotion, it would seem that the larger CSA and shear abundance of this fibre type may significantly contribute to these physical attributes. Therefore, future studies on muscle contractile properties in the various fibre types may shed light on the role of fibre size.

Fibre type and metabolism of the muscles

Examination on a gross level revealed that both fallow deer and springbok muscles were dark red in colour, an observation also evident of muscles from black wildebeest (Kohn et al., 2011a). This red colour is historically associated with an abundance of myoglobin present in the fibres. Furthermore, it is well known that rat muscle presenting dark in colour contain large numbers of type I and IIA fibres (Delp and Duan, 1996; Kohn and Myburgh, 2007). On the contrary, both muscle groups from the two species in this study contained more than 50% type IIX fibres, with less than 15% type I fibres. This finding seems to be a recurring observation for these types of wild animals. Previous research on black and blue wildebeest, blesbuck, kudu, reindeer, topi, hartebeest and waterbuck all reported high proportions of type IIX fibres, indicating a predominant genetic component affecting muscle fibre type (Essén-Gustavsson and Rehbinder, 1985; Spurway et al., 1996; Kohn et al., 2007; Kohn et al., 2011a).

Although the muscle groups serve different functions during locomotion, no difference in relative fibre type distribution (MHC isoforms) was found between *Vastus lateralis* and *Longissimus lumborum* of fallow deer and springbok (Table 1). The same muscle groups analysed from lion and caracal also showed no difference in muscle fibre type composition, but was evident in black wildebeest (Kohn et al., 2011b; Kohn et al., 2011a). There is no doubt that large differences in muscle fibre type exist between muscle groups, but highlights the point that not all species are the same, thus suggesting a greater genetic contribution towards

fibre type distribution in muscle groups. The fibre type profile observed for the two species analysed confirms their physical performance capacity.

Glycolytic enzyme activities (PFK, LDH and CK) were all exceptionally high and similar to that found in other antelopes (black wildebeest, reindeer), horse and felines (lion and caracal) (Essén-Gustavsson and Rehbinder, 1985; Karlström et al., 1994; Kohn et al., 2011b; Kohn et al., 2011a). The findings suggest that these animals have adequate capacity to anaerobically supply sufficient ATP for activities involving sprinting and jumping.

In comparison to lion and caracal, the enzyme activities in the fallow deer and springbok responsible for aerobic (oxidative) energy production were all high (CS and 3HAD) (Kohn et al., 2011b). The activities were similar to that found for black wildebeest, reindeer, endurance trained human athletes and the horse (Essén-Gustavsson and Rehbinder, 1985; Karlström et al., 1994; Pösö et al., 1996; Kohn et al., 2011b; Kohn et al., 2011a). CS activities in springbok muscles are significantly higher than that from fallow deer, but fat metabolism is similar in both species (Table 1). It is interesting to note that fallow deer and springbok had predominantly type IIX fibres, thus refuting the evidence that a high oxidative capacity of muscle results from type I and IIA fibres (Essén-Gustavsson and Henriksson, 1984). Supporting this statement would be the lack of relationships between any of the muscle fibre types and enzyme activities. This is, however, not a novel finding, as similar observations were made in reindeer, black wildebeest, horse and black bear (Essén-Gustavsson and Rehbinder, 1985; Pösö et al., 1996; Linnane et al., 1999; Smerdu et al., 2009). The statement is further strengthened by the presence of type IIX fibres with a range of low and high oxidative capacities in both species (Fig. 5). Indeed, Kohn et al. (2011a) and Linnane et al. (1999) previously showed the presence of highly oxidative type IIX muscle fibres in black wildebeest and horses, respectively. Similarly, hartebeest and topi (both antelope species) also contained type IIX fibres with large variations in oxidative capacities (Spurway et al., 1996). However, it seems that the range of oxidative capacity within a fibre type, as well as the overlap between fibre types may differ substantially between species and could be related to genetic factors. Unfortunately, it was not reported whether brown bear or reindeer contained type IIX fibres with high oxidative capacities. Nevertheless, the springbok muscle had more type IIX and IIAX fibres that were oxidative in nature and could explain the significantly higher CS activities in springbok.

Performance and muscle characteristics – do they concur?

Anecdotal performance characteristics for the animals investigated here suggests that springbok are able to sprint twice as fast as fallow deer, with both species seen as possessing the ability to withstand fatigue for long periods of time, but at much lower speeds. Kohn et al. (2011a) recently showed a strong positive linear relationship between maximum sprinting speed and the MHC IIx content in various mammalian species. An adaptation of this plot to include the two species investigated (Fig. 6) strengthened this relationship, but fallow deer seemed to be the greater outlier, emphasising the other factors that could influence running speed (e.g. biomechanics, genetics, etc.). Additionally, both animals had high glycolytic capacities in their muscle, suggesting that sufficient energy could be provided to attain their respective sprinting speeds. The added high oxidative capacities (Table 1), as well as the range in oxidative type IIX fibres (Fig. 5D) would also

suggest that these animals could withstand fatigue at high intensities for longer periods of time. The latter is of course purely speculative. Whether the supply of ATP *via* the oxidative pathways could sustain the ATP demand by the muscle at the reported high running speeds would require further investigation (e.g. lactate kinetics and oxygen and carbon dioxide kinetics during running).

Limitations of study

On a methodological level, this study confirms the importance of using different techniques (e.g. metabolic vs. antibodies vs. SDS-PAGE) to classify muscle fibres into their respective types. There are many studies that have used only one such technique, resulting in the misclassification of fibres (Linnane et al., 1999). This also sheds doubt on the accuracy of assessing solely the fibre type composition of muscles in order to better understand their function, especially when comparing various species to one another.

A final point that requires attention is the lack of studies that accurately measured sprinting speeds and endurance capability of wild animals. Performance data are readily available for horses, dogs and humans as these frequently compete in races where time keeping is considered accurate. Data for wild animals, on the other hand, are scarce and to a large extent anecdotal (Garland, 1983). Therefore, the need for accurate and reliable measurements of their sprinting speeds and endurance capability is essential to draw concrete conclusions on the functional characteristics of wild animal skeletal muscle.

CONCLUSION

This is the first study to investigated skeletal muscle fibre type and metabolism of two muscle groups from fallow deer and springbok. The main findings were the presence of high proportions of type IIX fibres in the *Vastus lateralis* and *Longissimus lumborum* (MHC isoforms and histologically types), and containing both high glycolytic and oxidative metabolism. Additionally, this study confirmed the presence of type IIX muscle fibres that are highly oxidative, which could aid in maintaining a high running speed for long periods of time.

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TABLES

Table 1 – Enzyme activities and myosin heavy chain isoform content in *Vastus lateralis* and *Longissimus lumborum* muscles from fallow deer and springbok. * Different from fallow deer ($P < 0.05$); † Different from *Vastus lateralis* ($P < 0.05$).

	Vastus lateralis		Longissimus lumborum	
	Fallow deer	Springbok	Fallow deer	Springbok
	Enzyme activities (μmol.min^{-1}.g^{-1} protein)			
CS	258 ± 30	382 ± 87*	309 ± 34	435 ± 147*
3HAD	60 ± 33	57 ± 28	63 ± 40	50 ± 27
PFK	373 ± 70	391 ± 111	552 ± 200	478 ± 158
LDH	3169 ± 491	2623 ± 602	4029 ± 730	3799 ±1161†
CK	3651 ± 1104	5220 ± 1341	4603 ± 1266	2798 ± 1421†*
	Myosin heavy chain content (%)			
MHC I	8 ± 3	5 ± 3	7 ± 2	6 ± 3
MHC IIa	38 ± 7	27 ± 5*	42 ± 9	22 ± 7*
MHC IIx	53 ± 8	68 ± 7*	51 ± 10	72 ± 8*

Table 2 – Muscle fibre type and fibre cross-sectional areas of *Vastus lateralis* muscle from fallow deer and springbok. * Different from fallow deer ($P < 0.05$)

	Fallow deer	Springbok
	Fibre type (%)	
Type I	11 ± 2	7 ± 1
Type IIA	32 ± 5	19 ± 7*
Type IIAX	8 ± 4	16 ± 8
Type IIX	48 ± 5	58 ± 12
	Cross-sectional area (μm^2)	
Type I	2063 ± 710	1714 ± 257
Type IIA	2564 ± 546	2033 ± 411*
Type IIAX	2925 ± 695	2598 ± 386
Type IIX	3064 ± 563	3643 ± 780*

FIGURE LEGENDS

Figure 1 – Myosin heavy chain isoforms from human, fallow deer and springbok muscles separated by SDS-PAGE. A: Fallow deer isoforms compared to human. B: Springbok isoforms compared to human.

Figure 2 – Identification of the separated MHC isoforms of human (H), fallow deer (FD) and springbok (SB) using antibodies specific to the three isoforms. See text for detail.

Figure 3 – NADH stain intensities as a function of incubation time.

A: Serial cross-sections incubated for 10, 20 or 30 minutes in NADH-nitroblue tetrazolium solution.

B: Relationship between optical density and incubation time in white ($R^2 = 0.76$), medium ($R^2 = 0.90$) and dark fibres ($R^2 = 0.85$).

Figure 4 – Histology of fallow deer and springbok *Vastus lateralis* muscles. Fibres were classified (and labelled) as types I (I), IIA (A), IIAX (AX) and IIX (X). Type IIX fibres presenting with high oxidative capacity are labelled as X_O.

A: NADH stain showing oxidative capacity of muscle fibres

B: ATPase stain at pH 10.3. Type I fibres are clear. No type IC (grey) fibres are visible. All other fibres are stained dark.

C: Immunohistochemistry using MHC I specific antibody (BAD5). Note cross-reactivity with type IIX fibres in fallow deer.

D: Immunohistochemistry using MHC I and IIa specific antibody (BF35).

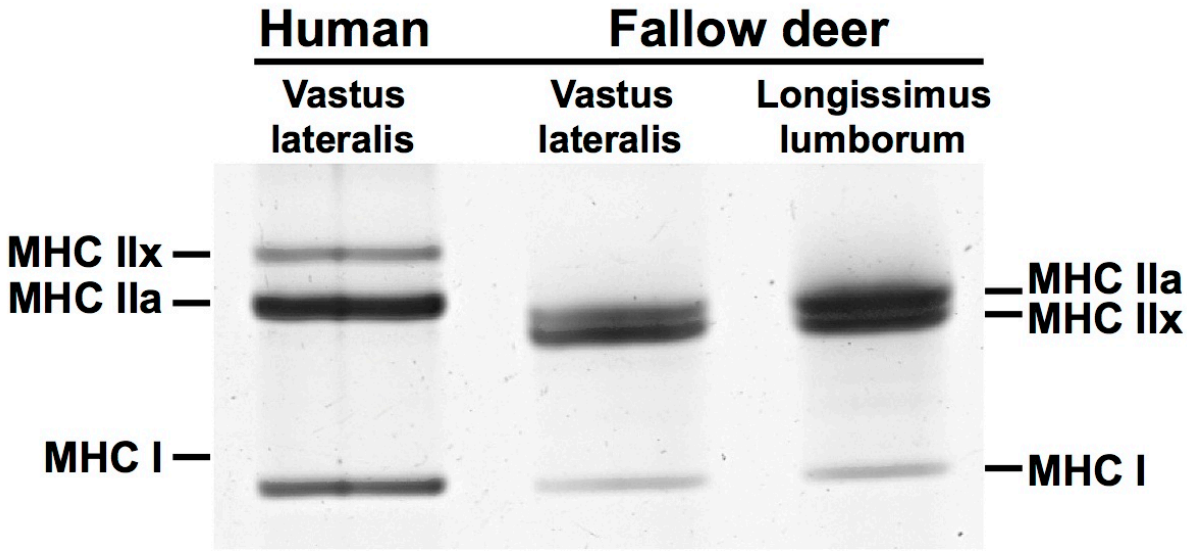
E: Immunohistochemistry using MHC IIx specific antibody (6H1). Note cross-reactivity with type I fibres in both species.

Figure 5 – Frequency distribution plots (expressed as a percentage of total number of fibres) of the oxidative capacity (OD in arbitrary units (AU)) from springbok and fallow deer *Vastus lateralis* muscle. Each bar was calculated as the number of fibres that obtained OD values in a specific range (e.g. from 0.60 to 0.69 OD).

Figure 6 – The relationship between MHC IIx isoform content in muscle and reported maximal sprinting speeds. Adapted from Kohn et al. (2011b). Additional species added are indicated with a filled circle. Pearson's r: 0.80, $P < 0.001$

FIGURE 1

A



B

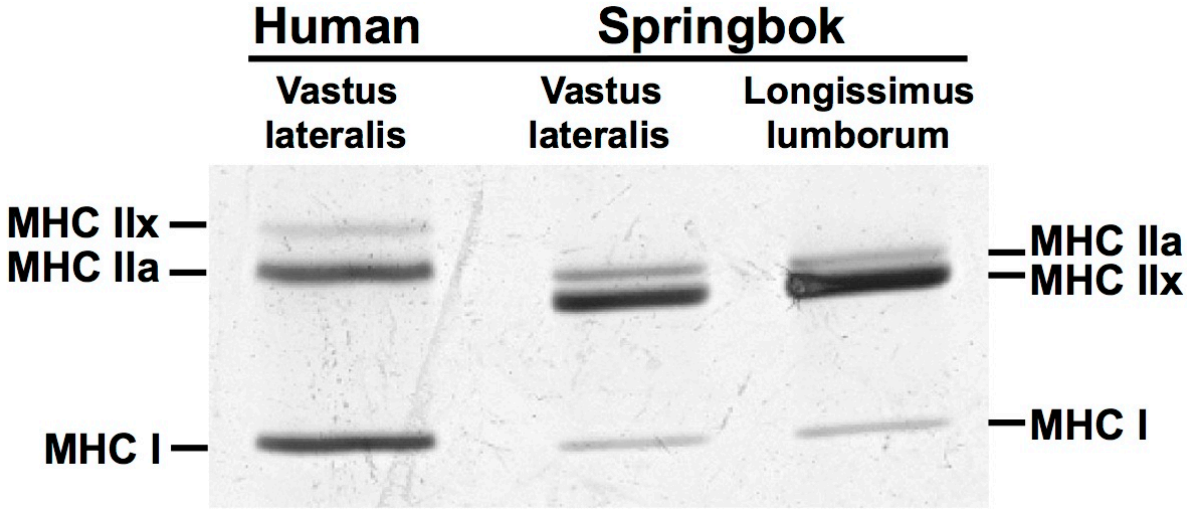


FIGURE 2

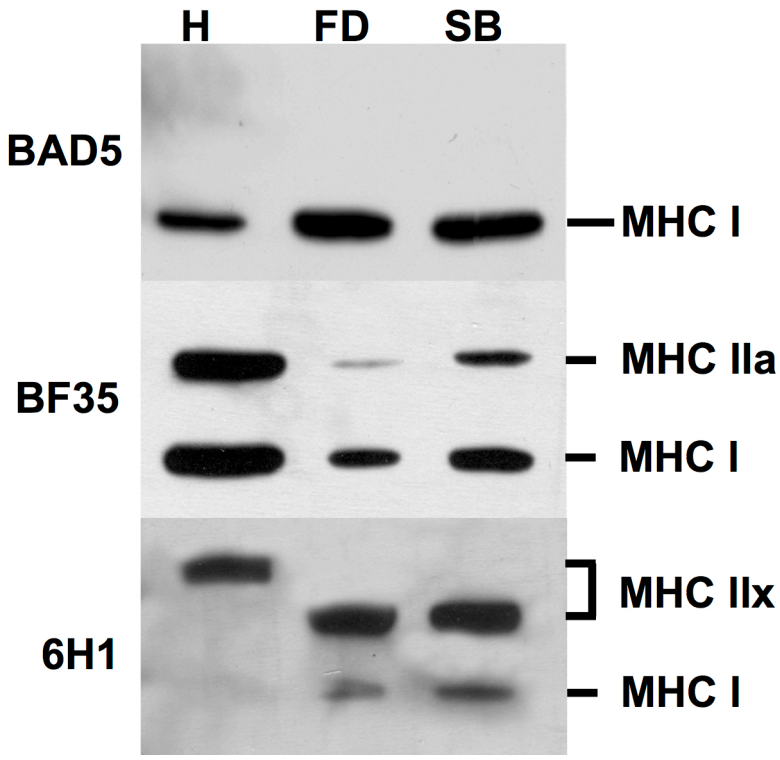


FIGURE 3

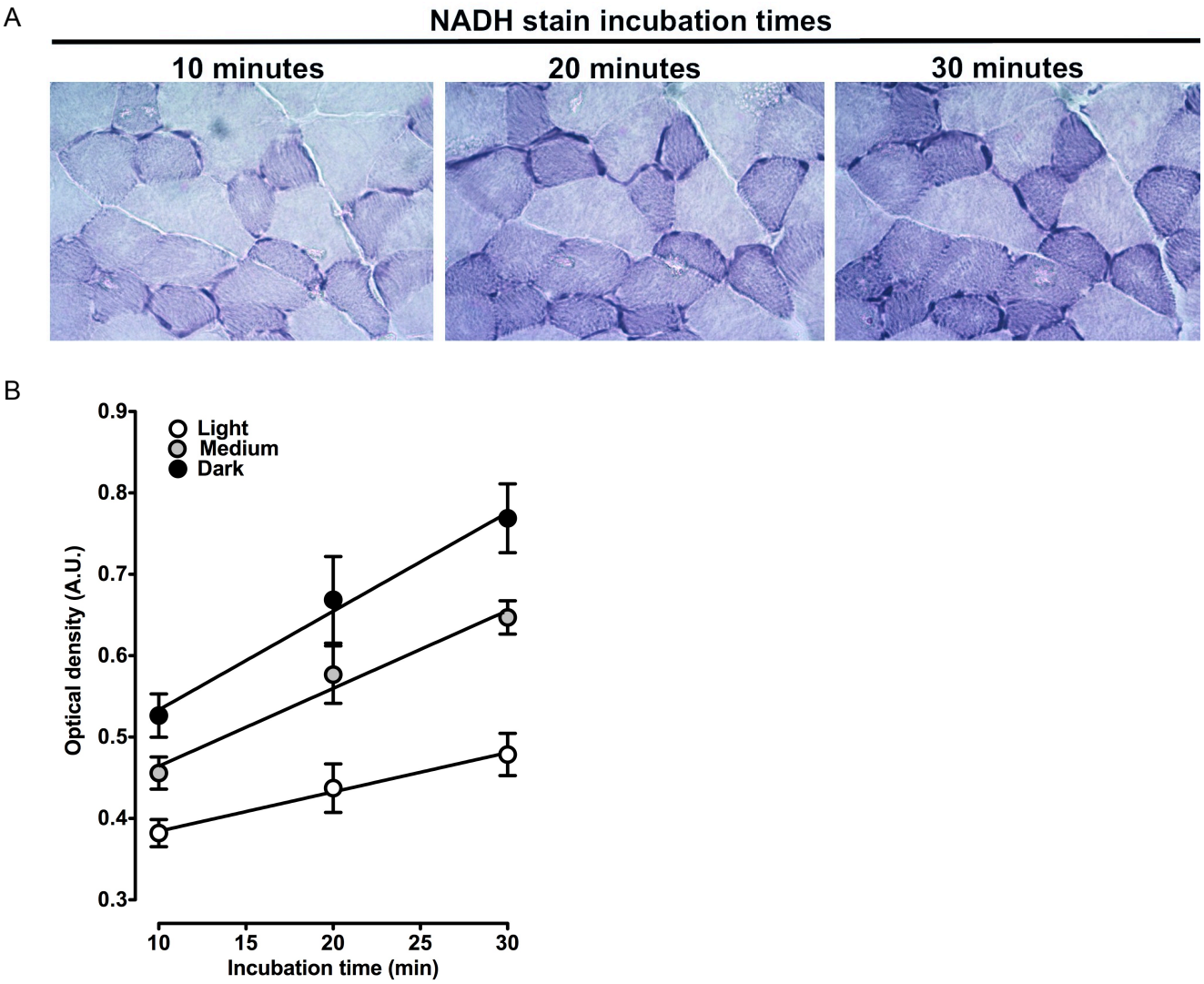


FIGURE 4

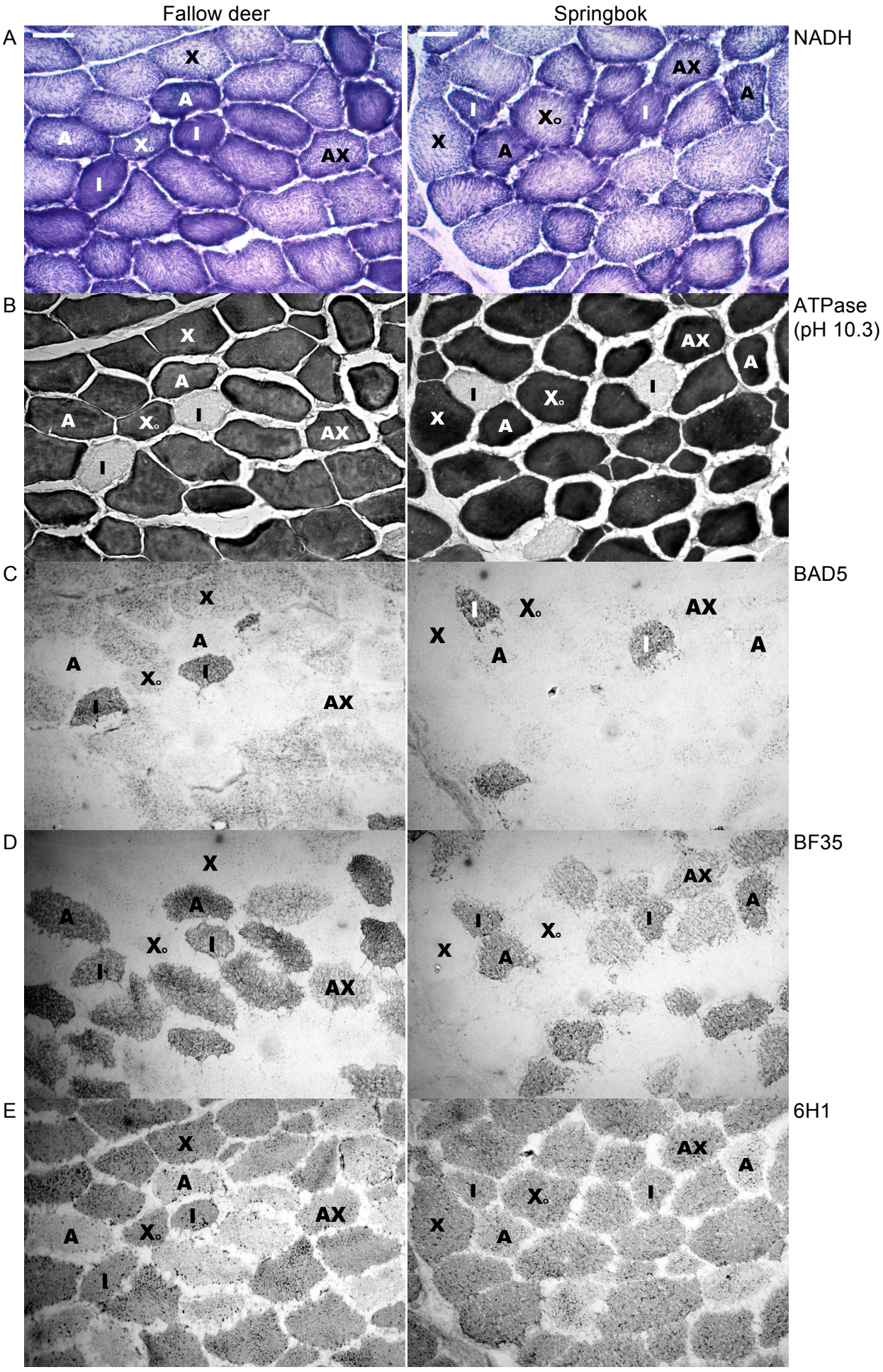


FIGURE 5

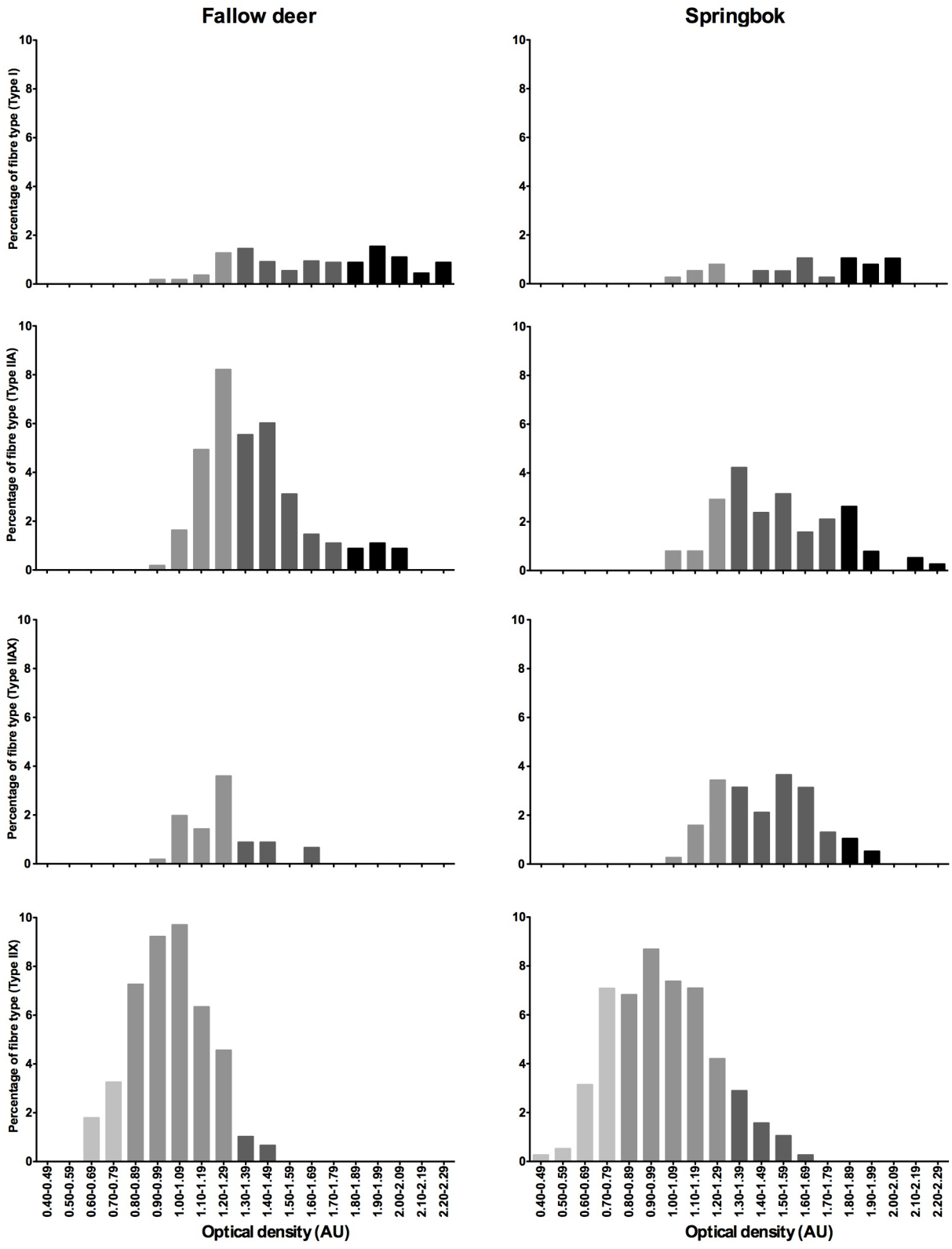


Figure 6