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RESEARCH ARTICLE

Lion (*Panthera leo*) and caracal (*Caracal caracal*) type IIx single muscle fibre force and power exceed that of trained humans

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SUMMARY

This study investigated for the first time maximum force production, shortening velocity (V_{max}) and power output in permeabilised single muscle fibres at 12°C from lion, *Panthera leo* (Linnaeus 1758), and caracal, *Caracal caracal* (Schreber 1776), and compared the values with those from human cyclists. Additionally, the use and validation of previously frozen tissue for contractile experiments is reported. Only type IIx muscle fibres were identified in the caracal sample, whereas type IIx and only two type I fibres were found in the lion sample. Only pure type I and IIa, and hybrid type IIax fibres were identified in the human samples – there were no pure type IIx fibres. Nevertheless, compared with all the human fibre types, the lion and caracal fibres were smaller (P<0.01) in cross-sectional area (human: 6194±230 μm^2, lion: 3008±151 μm^2, caracal: 2583±221 μm^2). On average, the felid type IIx fibres produced significantly greater force (191–211 kN m^{-2}) and ~3 times more power (29.0–30.3 kN m^{-2} fibre lengths s^{-1}) than the human IIax fibres (100–150 kN m^{-2}, 4–11 kN m^{-2} fibre lengths s^{-1}). V_{max} values of the lion type IIx fibres were also higher than those of human type IIax fibres. The findings suggest that the same fibre type may differ substantially between species and potential explanations are discussed.

Key words: skeletal muscle, single fibre, wild animals.

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INTRODUCTION

The wild animal kingdom has a wide array of remarkable animals that show great performance capabilities, such as speed, strength and endurance. Very little is known about the skeletal muscle characteristics of these animals and how these might contribute to their exceptional physical abilities. Species such as the cheetah and lion are considered some of the fastest and strongest animals, whereas animals from the family Bovidae can also reach great speeds but exhibit superior endurance (Skinner and Chimimba, 2005).

Being in the fortunate position of having ready access to the array of wild animal species in South Africa, our laboratory have been able to acquire, analyse and publish a number of morphological studies on the skeletal muscle of antelopes and felids (Kohn et al., 2011a; Kohn et al., 2011c; Curry et al., 2012). The present study therefore expands on the muscle characteristics of samples harvested from the lion and caracal.

Recent studies have established that wild animal species, ranging from predators to prey, all contain large numbers of type IIx muscle fibres (Williams et al., 1997; Kohn et al., 2007a; Hyatt et al., 2010; Kohn et al., 2011a; Kohn et al., 2011c; Curry et al., 2012). Morphologically, their fibres are significantly smaller in cross-sectional area (CSA) than human fibres (Kohn et al., 2011a; Kohn et al., 2011c; Curry et al., 2012). Different mammalian species also vary significantly in their ability to derive ATP from the metabolic pathways to fuel muscle contraction. For example, black wildebeest, springbok and fallow deer, all prey animals, contain skeletal muscle fibres that possess both high oxidative and high glycolytic capacities, suggesting that both types of metabolism could efficiently provide ATP during endurance or high intensity exercise. Compared with endurance-trained humans, these antelope species have a similar oxidative capacity, but their ability to metabolise carbohydrate and fat aerobically (as suggested by their respective enzyme activities) is significantly elevated (Kohn et al., 2011a; Kohn et al., 2011c). Additionally, it has been shown (Kohn et al., 2011a; Curry et al., 2012) that these three species also contain highly oxidative type IIx fibres, suggesting that this fibre type can resist fatigue, despite being a fast twitch fibre that requires a high ATP production rate (Essén-Gustavsson and Henriksson, 1984; Pette, 1985; Kohn et al., 2011a; Kohn et al., 2011c; Curry et al., 2012).

In contrast, the vastus lateralis muscle of predators contains more than 55% type IIx fibres, with the caracal and cheetah possessing ~70% type IIx fibres (Williams et al., 1997; Kohn et al., 2011c). The proportion of type IIx fibres correlates well with the maximum sprinting capability of these species (Kohn et al., 2011a). Thus, for mammals to achieve speeds in excess of 80 km h^{-1} they require large numbers of type IIx fibres. Metabolically, these fibres have a lesser capacity to metabolise fat and carbohydrate aerobically but similar glycolytic capacities to those found in antelope.

The myosin heavy chain (MHC) isoform was shown to be the major factor influencing the contractile properties of the single fibre, with the myosin light chains playing a less crucial role (Bottinelli, 2001). Previous work on humans and rats showed that force and shortening velocity are primarily determined by these isoforms. Humans and larger mammals primarily express three isoforms, namely MHC I, IIa and IIx (Rivero et al., 1997; Kohn et al., 2007a; Kohn et al., 2011a; Kohn et al., 2011b; Kohn et al., 2011c). Fibres expressing MHC I have slow shortening velocities and produce less force and power compared with fibres expressing MHC IIa.

Similarly, fibres containing the MHC IIx isoform contract more rapidly and produce more force and power than type I and IIa fibres (Bottinelli, 2001).

Only a handful of laboratories across the world have studied the contractile properties of individual single fibres from a few species. From these analyses, it would appear that the same fibre type from different species may possess different contractile properties. For example, force production and unloaded shortening velocity at 12°C of type I, IIa and IIx fibres from cat, rat and dog were found to be higher than those from humans (Bottinelli et al., 1994; Bottinelli, 2001; D'Antona et al., 2006; Toniolo et al., 2007; Toniolo et al., 2008). However, very little is known about the contractile properties of the muscle fibres of wild animal species. It is reasonable to presume that the great metabolic capacity for ATP supply of these athletic mammals is driven by a greater capacity to consume ATP as a result of a greater skeletal muscle fibre contractile capacity. The amount of absolute force a fibre can produce is directly proportional to its CSA (Woledge et al., 1985; Bottinelli, 2001; Krivickas et al., 2011). Thus, as the majority of morphological studies on the skeletal muscle of these wild animals have reported significantly smaller CSA of the fibres compared with that of humans, our hypothesis was that their fibres would produce less absolute force, but greater specific force.

Therefore, the aim of this study was to investigate the contractile properties of permeabilised single fibres from human, caracal and lion, and deduce whether differences exist in force, shortening velocity (V_{max}) and power-generating capability between the same muscle fibres in these different animals.

MATERIALS AND METHODS

Ethical approval

Samples from the animals used in this study formed part of previously published work (Kohn et al., 2011c). The lion (Animal Use and Care Committee, University of Pretoria) and human subjects (Human Research Ethics Committee, University of Cape Town) formed part of earlier studies for which ethical approval was already obtained. The lion was killed at the Onderstepoort Veterinary Faculty, University of Pretoria, and muscle samples obtained within 10 min post-mortem. The caracal carcass was donated to the authors, and the Departmental Research Committee (University of Cape Town) was informed about the intended research. This particular animal was killed on a game farm by a dog in the early hours of the morning (between 04:00 h and 07:00 h), where temperatures averaged between 4 and 7°C. The researcher was fortunate to be stationed on the farm at that particular time and, once notified, samples were collected.

Sample collection and preparation

Vastus lateralis muscle samples were collected post-mortem from one caracal and one lion, rapidly frozen in liquid nitrogen and stored at −87°C for less than 1 month before single fibre preparation. Three human biopsies from the same muscle group were generously donated by A. N. Bosch (University of Cape Town) and were used for validation and comparative aspects of the study. These three biopsies were collected from three trained cyclists using the percutaneous biopsy technique (Bergstrom, 1962), rapidly frozen in liquid nitrogen and stored at −87°C.

Solutions for single fibre experiments

The concentrations of the solutions were based on those reported previously (Pansarasa et al., 2009; Bottinelli et al., 1996; Widrick et al., 1996). Table 1 lists the composition and concentrations of the skinning, relaxing, pre-activating and activating solutions. The free ions (Ca^{2+}, Mg^{2+}, etc.) were calculated using the software program developed by Dweck et al. (Dweck et al., 2005), based on the calculations reported by Fabiato and Fabiato (Fabiato and Fabiato, 1979). Stock solutions without creatine kinase were prepared, aliquoted and stored at −87°C until use. A pre-determined volume of a creatine kinase stock solution was added on the day of the experiments.

Single fibre preparation and experimental protocols

The protocols explained below were based on those reported previously (Malisoux et al., 2006; Ottenheijm et al., 2011; Gilliver et al., 2009; Bottinelli et al., 1999). Muscle samples were rapidly thawed at 35°C in saline (pH 7.4) for not longer than 1 min. These samples were then placed in ice-cold skinning solution and cut into smaller longitudinal bundles, after which they were incubated in skinning solution for 24 h at 4°C. The skinning solution was then replaced with fresh solution and the samples stored at −20°C until analyses were performed.

Table 1. Summary of solution concentrations used for the single fibre experiments

	Skinning	Relaxing	Pre-activating	Activating
Potassium propionate	150			
Glycerol	50%			
KH_2PO_4	5			
Magnesium acetate	5			
EGTA	5	7	0.5	7
$CaCl_2$	1	0.02		7.03
Imidazole		20	20	20
ATP	3	5.1	5.1	2.7
$MgCl_2$		5.5	5.3	5.3
Creatine		14.5	14.5	14.5
Creatine kinase			200 U ml^{-1}	200 U ml^{-1}
KCl		Amount added to raise ionic strength to 180		
Ionic strength		180	180	180
pH	7.0	7.0	7.0	7.0
pCa		9.0	9.0	4.5
pMg		3.0	3.0	3.0
pMg-ATP		2.4	4.0	2.4

Values are in mmol l^{-1}, unless otherwise indicated. Concentrations were based on those reported elsewhere (Bottinelli et al., 1996; Widrick et al., 1996; Pansarasa et al., 2009).

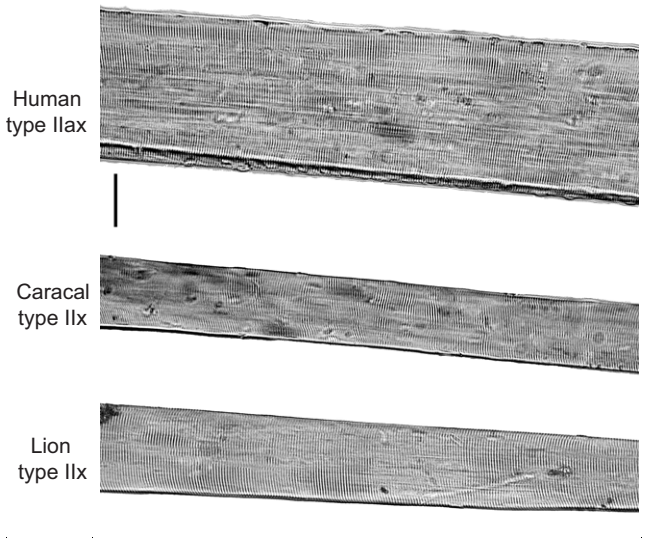

Fig. 1. Photomicrographs of skinned single muscle fibres from human, caracal and lion samples. Images were acquired before submersion of the fibre in activating solution. Scale bar, 50 μm. Note the large difference in diameter between the human and felid fibres.

Single muscle fibres were dissected under a stereomicroscope using fine stainless steel tweezers. The ends of the fibre were secured using aluminium clips. The integrity of each fibre was then determined by briefly investigating the striations of the fibre under a light microscope. Once satisfied, the fibre was then transferred to the single fibre apparatus (1400A, Aurora Scientific, Aurora, ON, Canada). The single fibre apparatus consists of a force transducer, motor lever arm and eight temperature-controlled baths. Hooks attached to the force transducer and lever arm allow the single fibre to be attached using the clips. Once attached, the fibre always remained stationary with only the baths moving up/down and forward/backward. The single fibre unit was mounted on an inverted microscope (Olympus, Tokyo, Japan) fitted with a 5 megapixel pre-calibrated digital camera (AxioCam, Carl Zeiss, Jena, Germany) to assist in fibre dimension measurements (Fig. 1). Fibre length, diameter and sarcomere spacing were measured using still photographs. Fibre length averaged between 1.9 and 2.4 mm, whereas sarcomere spacing was set between 2.4 and 2.6 μm. The latter was based on optimal values reported by a meta-analysis of sarcomere lengths from human and cat skeletal muscle (Burkholder and Lieber, 2001). A minimum of five measurements across the length of each fibre was performed and averaged. Fibre diameter was then corrected by 20% for swelling before calculation of fibre CSA (Godt and Maughan, 1977). CSA was determined from the diameter of the fibre, assuming that fibres have a circular shape, using the equation $\pi\,[(0.8\times\text{fibre diameter})/2]^2$, where 0.8 is to correct for fibre swelling.

During all the contraction experiments, force and length were recorded at 20 Hz until the cessation of the test protocol. The fibre was allowed to equilibrate in the relaxing solution for ~1 min. During this time the temperature of the baths was decreased to 12°C. The fibre was then incubated in the second bath containing the pre-activating solution for 30 s, after which it was moved to the bath containing the activating solution. The fibre was allowed to contract until a plateau in force was reached, and then rapidly moved back to the relaxing solution until the force decreased to zero (Fig. 2A). This protocol was repeated to ascertain fibre integrity and whether the maximum force was repeatable.

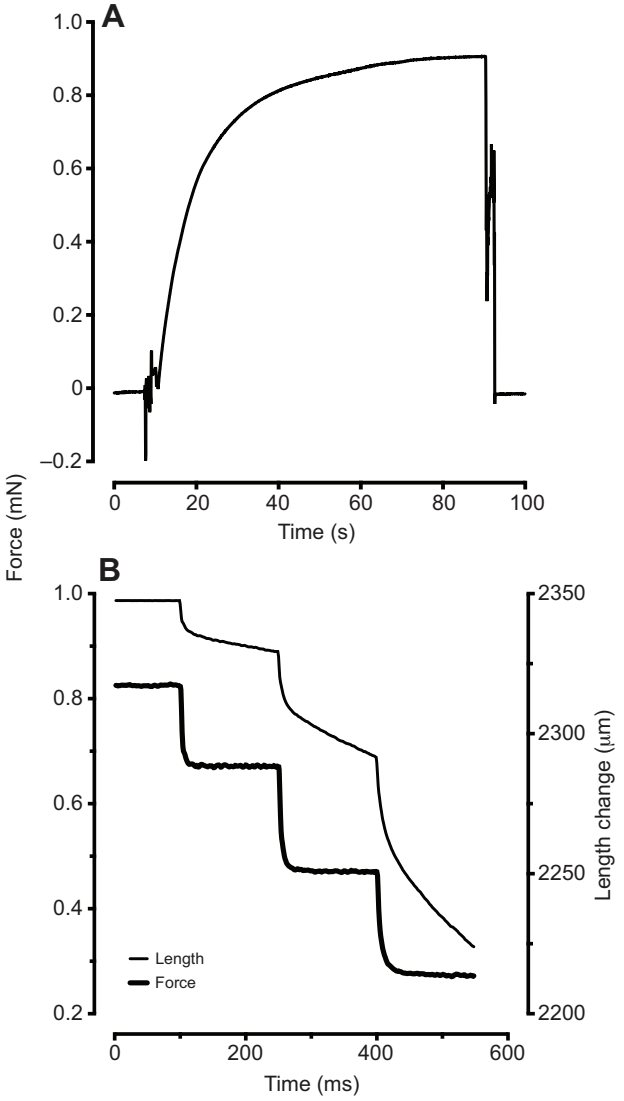

Fig. 2. (A) Maximum absolute force production curve of a human type IIax fibre. After submerging the fibre in activating solution (pCa 4.50), the fibre was allowed to contract isometrically until a clear plateau in force was reached. Once achieved, the fibre was transferred back to relaxing solution. (B) Force–length traces of the same maximally activated muscle fibre. After attaining maximum force, the lever motor was controlled by the software to shorten the length of the fibre (right axis) to yield a predetermined percentage of absolute maximum force isotonically (force clamp, left axis). For this fibre, the series of force clamps corresponded to 81%, 57% and 33% of absolute maximum force, each lasting 150 ms. After the last force clamp, the fibre was briefly shortened for 2 ms to 50% of its initial length, and rapidly re-stretched back to its original length. Two to three force clamp series followed, each series consisting of different percentages of maximum force. Once all the series were completed, the fibre was returned to the relaxing solution.

After the maximum contractile force experiments, the absolute maximum force (in mN) was entered into the software program. The fibre was then subjected to the same series of bath moves (relaxing solution, 30 s pre-activating solution followed by submersion in activating solution) to attain maximum force. After maximum force was achieved in the activating solution, the fibre was subjected to four series of force clamps, each series comprising three isotonic force clamps, each force clamp lasting for 150 ms (refer to Fig. 2B) to produce the force–velocity curve for each fibre.

For example, in Fig. 2B, the first force clamp series was held constant at 81% of the maximum absolute force. This was achieved by control of the motor to which the fibre was attached, allowing the fibre to continually shorten at a pace determined by its own ability *via* continuous feedback from the force transducer (thus reducing the length readout) in real time. After 150 ms of the fibre shortening against this constant load (i.e. 81% of maximum force), the motor allowed the fibre to shorten to the next pre-programmed isotonic load (e.g. 57% of maximum force), and the process and data recording was repeated. Once the last load in the series (e.g. 33% of maximum force) was completed, the fibre was subjected to a 2 ms shortening to 50% of its original fibre length, after which it was immediately re-stretched within 1 ms to its original length whilst in activating solution. This method of re-stretching the fibre whilst it is still submerged in activating solution aids in preserving the fibre and sarcomere integrity when subjected to the next series of contractions and re-stretches (Brenner, 1983; Gilliver et al., 2009). The researcher ensured that the force returned to maximum, whereafter the second series containing three different pre-programmed force clamp loads was initiated. A third and fourth series of force clamps was also performed, bringing the total number of isotonic loads to 12. Time, force and length were recorded at 1000 Hz.

Fibre typing of single fibres

After the contractility tests, the clips were removed and the fibre transferred to 100 µl solution containing 5% β-mercaptoethanol, 2.5% SDS, 10% glycerol, 62.5 mmol l^{-1} Tris, pH 6.8, and 0.1% Bromophenol Blue. The samples were heated to 95°C for 5 min and used to determine the MHC isoform content by electrophoresis (18×16 cm, SE 600 system, Hoefer, Holliston, MA, USA). The electrophoresis protocol was based on that previously published (Talmadge and Roy, 1993) and subsequently modified (Kohn and Myburgh, 2006). The separating gel contained 7% acrylamide (ratio of 50 acrylamide:1 bis-acrylamide), 30% glycerol, 0.2 mol l^{-1} Tris buffer, pH 8.80, 0.1 mol l^{-1} glycine and 0.4% SDS. The stacking gel contained 4% acrylamide (50:1), 30% glycerol, 70 mmol l^{-1} Tris buffer, pH 6.80, 0.4% SDS and 4 mmol l^{-1} EDTA. Polymerisation was initiated using ammonium persulphate and TEMED. An approximately 25 µl sample was loaded into each well. A loading control for each species containing all three MHC isoforms was included in each electrophoretic run to ensure accurate identification of the separated bands. The outer running (anode) buffer contained 50 mmol l^{-1} Tris, 75 mmol l^{-1} glycine and 0.05% SDS, whereas the inner running (cathode) buffer contained twice the concentrations mentioned above. Prior to the electrophoretic run, 0.12% β-mercaptoethanol was added to the inner running buffer (Kohn and Myburgh, 2006). Gels were run for 4 h at a constant 70 V, whereafter the voltage was increased to 275 V and electrophoresis was carried out for an additional 20 h. The gels were subsequently silver stained, scanned and the fibre type of each individual fibre determined using the loaded control as reference.

Single fibre inclusion criteria

Fibres were rejected for the following reasons: (i) visual sarcomere disarray and non-uniformity across the length of the fibre, (ii) not achieving at least 90% of maximum force after the second contraction effort and (iii) force dropped below 80% during the force-clamp protocol.

Analyses of single fibre data

Maximum force of each single fibre was derived from the plateau, averaging the last 1 s at maximum contraction. Force was corrected where the initial start of contraction was less or greater than 0 mN. The same correction was applied for the force clamp protocol. To determine the velocity of shortening for each force clamp, the slope was determined over the last 50 ms of the linear part of the change in length. This yielded 12 velocity points for the 12 force-clamp loads performed. Each velocity point was normalised to the initial sarcomere and fibre length and expressed as fibre lengths (FL) s^{-1} (Woledge et al., 1985). Velocity and force (expressed as a percentage of the maximum force) were plotted and the hyperbolic Hill equation $(\mathbf{P}+a)(V+b)=(\mathbf{P}_o+a)b$ was fitted to the data using a least-square regression analysis (GraphPad Prism for Mac version 5.0c, GraphPad Software, La Jolla, CA, USA), where **P** is force, V is shortening velocity, $\mathbf{P}_o$ is maximum isometric force attained before the initiation of the force clamp, and a and b are constants (Hill, 1938). V_{max} of each fibre was calculated by extrapolating the data back to 0% force. Fibre power was calculated from the velocity–force parameters (expressed as kN m^{-2} FL s^{-1}), by multiplying force (in kN m^{-2}) by velocity (FL s^{-1}). Using these values, composite velocity–force and power–force curves were constructed, to ultimately determine the maximum power output of each fibre. The velocity and power outputs for the same fibre type obtained from the Hill equation were averaged and plotted against the percentage of maximum force.

Contractile properties of fresh *versus* frozen tissue

To ensure the validity of the measurements performed on the frozen tissue, a second set of contractile experiments was performed using a small human muscle sample (destined for another study of which ethical approval was obtained), generously donated by Prof. Wayne

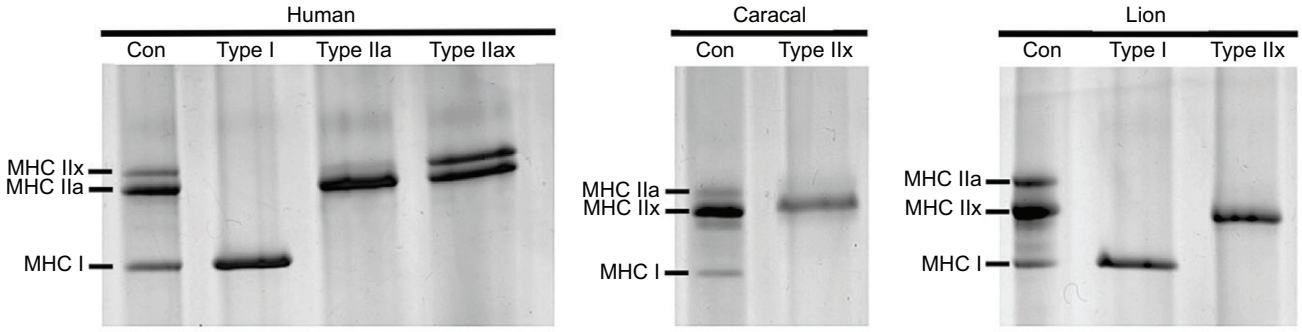

Fig. 3. Electrophoretic separation of myosin heavy chain (MHC) isoforms of human, caracal and lion single fibres. A control sample (Con) for each species containing all three isoforms is included, which assisted in the identification of the single fibre. Note the difference in migration of the MHC IIx isoform in the three species.

Table 2. Comparison of single fibre contractile properties between a fresh and frozen tissue sample obtained from the same human individual

	CSA (μm^2)	Maximum absolute force (mN)	Maximum specific force ($kN\,m^{-2}$)	V_{max} ($FL\,s^{-1}$)	Maximum power ($kN\,m^{-2}\,FL\,s^{-1}$)
Type IIa					
Fresh (*N*=21)	7485±366	0.83±0.06	112±7	0.85±0.07	5.9±0.5
Frozen (*N*=16)	7063±534	0.76±0.05	116±10	0.89±0.09	7.6±1.1
Type IIax					
Fresh (*N*=9)	5816±551	0.86±0.06	151±8	0.98±0.14	10.4±1.0
Frozen (*N*=3)	6458±252	1.10±0.06	170±8	1.26±0.08	19.1±0.9*

Values are means ± s.e.m.
CSA, cross-sectional area; V_{max}, maximum loaded shortening velocity.
*Significantly different from fresh (P<0.05) – see Results for additional explanations.

Derman (University of Cape Town). This sample was divided into two pieces; the first was directly submerged into skinning solution and stored at 4°C (as described under 'Single fibre preparation and experimental protocols', above), whereas the remaining piece was rapidly frozen in liquid nitrogen and stored at −87°C for 2 months. This latter piece of frozen tissue was thawed, processed for contractile measurements as described above (Single fibre preparation and experimental protocols) and subjected to the same contractile tests (maximum force, force clamps, etc.). The fibres were typed as described under 'Fibre typing of single fibres', above. The data from this experiment were not included in the comparison of the felids and humans.

Statistical analyses

Unless otherwise stated, all values are expressed as means ± s.e.m. A one-way ANOVA with a Bonferroni correction was applied to determine significant differences between the human groups, as well as between human, caracal and lion data. Whenever fewer than three groups were compared, Student's *t*-test for unpaired data were used. Significance was set at P<0.05. Note, only two type I fibres could be found in the lion sample and the data were added to the figures. Similarly, only two type IIx fibres from the caracal were successfully analysed to obtain V_{max}, $a/\mathbf{P}_o$ and maximum power. No statistics were performed on these two fibre types as the fibre numbers were too low. The data were included purely as observations and for interest to the reader.

RESULTS

Single fibres per fibre type

Human, lion and caracal MHC I and IIa migrated to a similar distance on SDS-PAGE gels, whereas there was a large difference in size between the three species for MHC IIx, as previously demonstrated (Kohn et al., 2011c) (Fig. 3). In humans, the MHC IIx isoform seems larger than its IIa isoform, whereas the IIx isoform of caracal and lion is smaller than the IIa isoform. Additionally, the IIx isoform of lion also seems smaller than the caracal IIx isoform. The migration pattern of the MHC isoforms from the two wild felids was in accordance with previous results using monoclonal antibodies directed against these isoforms (Kohn et al., 2011c).

Based on this separation profile for the three species, the isoform content and its migration for each single fibre was determined. A total of 151 fibres were dissected (89 human, 21 caracal and 41 lion). The samples from the felids were small and only a few viable fibres could be successfully dissected (see Fig. 1). Of the 151 fibres, 64 human, 8 caracal and 26 lion fibres were included in the analyses. No pure type IIx fibres were identified in the human samples, whereas no type IIa or IIax fibres were found in the lion and caracal samples. Only two type I lion fibres could be identified and these are included for descriptive purposes. No type I fibres could be identified in the caracal sample.

Contractile properties of fresh *versus* frozen tissue

Table 2 reports the CSA and contractile properties of fresh *versus* frozen human type IIa (N=37) and IIax (N=12) single fibres from the same sample. No type I fibre could be identified from this sample. Apart from the maximum power of frozen type IIax fibres being significantly higher than that of fresh muscle, no differences were found between CSA, absolute force, specific force, V_{max} and maximum power. The range of values for the type IIa fibres from the frozen sample was also well within the range obtained for the fresh fibres. Frozen type IIax fibres had slightly higher absolute force, specific force and V_{max} values (not significantly different).

CSA of single fibres from human, caracal and lion

Fig. 4A depicts the CSA of human type I, IIa and IIax fibres, and that of type IIx fibres from caracal and lion (type I fibres of lion are included as observational data). No difference was found between the CSA of the three human single fibre type groups, averaging a fibre size of 6194±230 μm^2 (N=64), in accordance with previous results on humans. The two type I fibres identified in the lion sample had a CSA of 1257±63 μm^2, five times smaller than the human type I fibres. Lion (N=24) and caracal (N=8) type IIx fibres were 1.6 and 2.0 times smaller, respectively, than human type IIax fibres.

Maximum force of single fibres from human, caracal and lion

Absolute force (Fig. 4B) produced by the human type I fibres (N=9) averaged 0.41±0.03 mN, whereas absolute force for type IIa (N=36) and IIax (N=19) fibres was exactly the same (0.76±0.05 mN). Corrected for fibre CSA, the three fibre types differed significantly from each other in specific force (P<0.01), with type IIax fibres producing the greatest force, followed by type IIa and type I.

Absolute force produced by lion and caracal type IIx fibres *was* lower (P<0.05) than that of human type IIax fibres, but once corrected for CSA, the muscle fibres of both wild animal species produced significantly more specific force than human fibres (Fig. 4B,C). The two type I fibres produced an absolute force of 0.21±0.02 mN and a specific force of 162±9.9 $kN\,m^{-2}$. Although statistical power is weak (N=2 for lion), the specific force of lion type I fibres appeared to be ~2.5-fold greater than their human equivalent.

V_{max}, $a/\mathbf{P}_o$ and maximum power of human, caracal and lion fibres

Note that only two type IIx fibres could be analysed to obtain values for V_{max}, $a/\mathbf{P}_o$ and power of caracal fibres; these values are

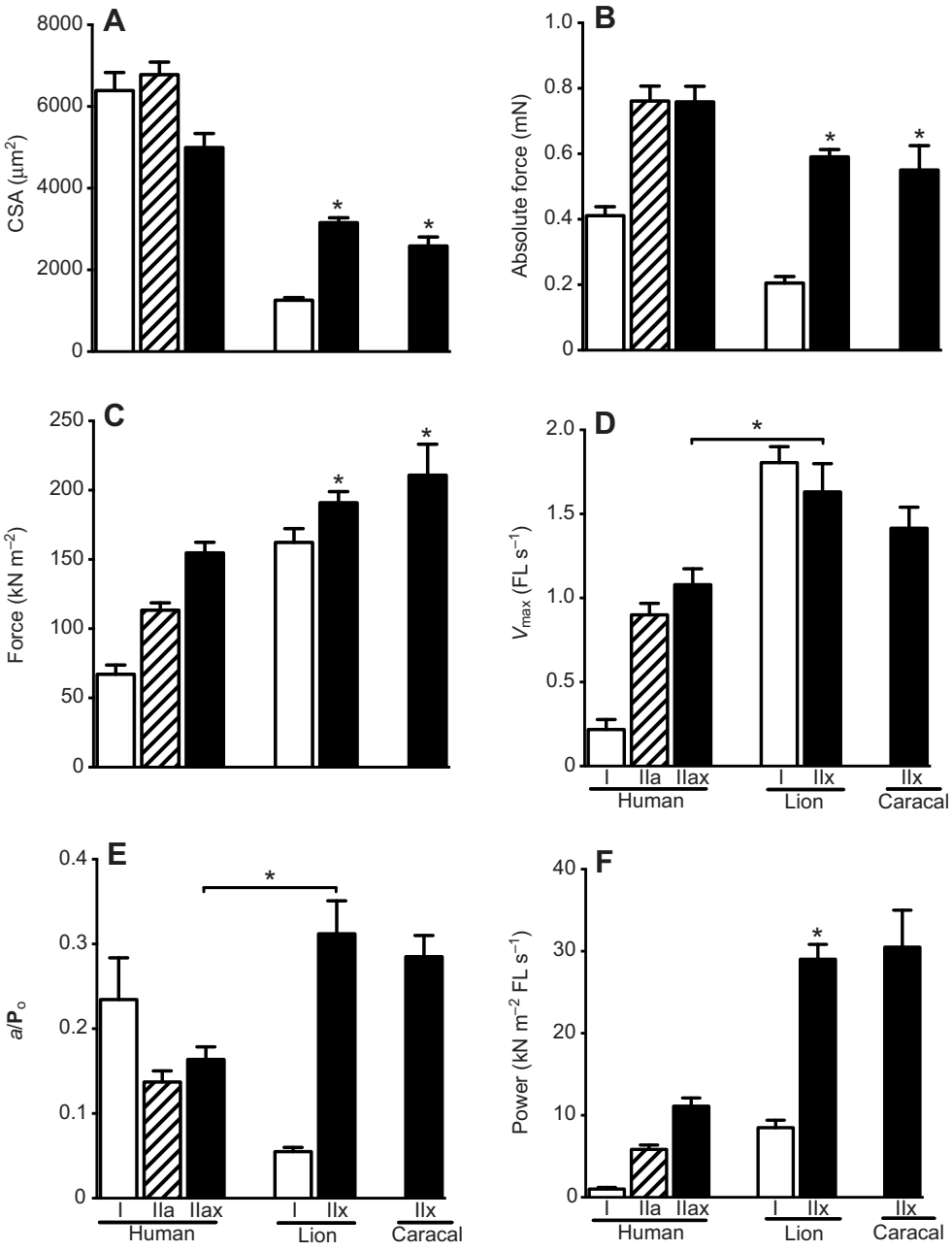

Fig. 4. Contractile properties and muscle morphology of human type IIax and caracal and lion type IIx fibres. Values are means and s.e.m. *Significantly different from human (P<0.05). (A) Cross-sectional area (CSA) of fibres. (B) Maximum absolute force. (C) Maximum specific force. (D) Maximum shortening velocity (V_{max}). (E) $a/\mathbf{P}_o$ ratio (the constant that defines the shape of the force–velocity curve). (F) Maximum power output (FL, fibre length).

included merely for interest. V_{max} values for human type I (N=9), IIa (N=35) and IIax (N=19) fibres, and V_{max} for type IIx fibres from caracal and lion are presented in Fig. 4D. The V_{max} of human type I fibres was significantly slower compared with that of their type IIa (4.0 times) and type IIax (4.9 times) fibres, whereas their IIa and IIax fibres appeared to have a similar contraction speed (Fig. 4D, Fig. 5A). V_{max} for the two type I lion fibres was 1.81±0.1 FL s^{-1}, far greater than that obtained for the human type I fibres. Lion type IIx fibres (N=15) had a greater V_{max} compared with human type IIax fibres (P<0.05). Caracal type IIx fibres appeared similar in shortening velocity to lion type IIx fibres (N=2, not significant).

The constant that defines the shape of the force–velocity curve ($a/\mathbf{P}_o$) was not different between the three human fibre types. In contrast, this value was significantly higher in lion type IIx fibres than in the human type IIax fibres, with a similar observation made for the two type IIx fibres from the caracal sample (Fig. 4E). The two lion type I fibres appeared to have the lowest $a/\mathbf{P}_o$ values.

The amount of power each human fibre type could produce increased from type I, to IIa to IIax fibres (P<0.01; Fig. 4F and Fig. 5B). These type IIa and IIax fibres produced 6 times and 11 times more power than type I fibres. Most significantly, the maximum power of lion type IIx fibres was 2.6 times greater than that of human type IIax fibres (with caracal IIx fibres unofficially producing 2.7 times greater power than human type IIax fibres). Maximum power obtained by the two lion type I fibres amounted to 8.48±0.92 kN m^{-2} FL s^{-1}, which also seems well above the values obtained for the human type I and IIa fibres (not analysed statistically).

From the composite power–force curves (Fig. 5B), it was deduced that maximum power was generated between 30% and 50% of maximum force for the wild animals, whereas human type IIax fibres

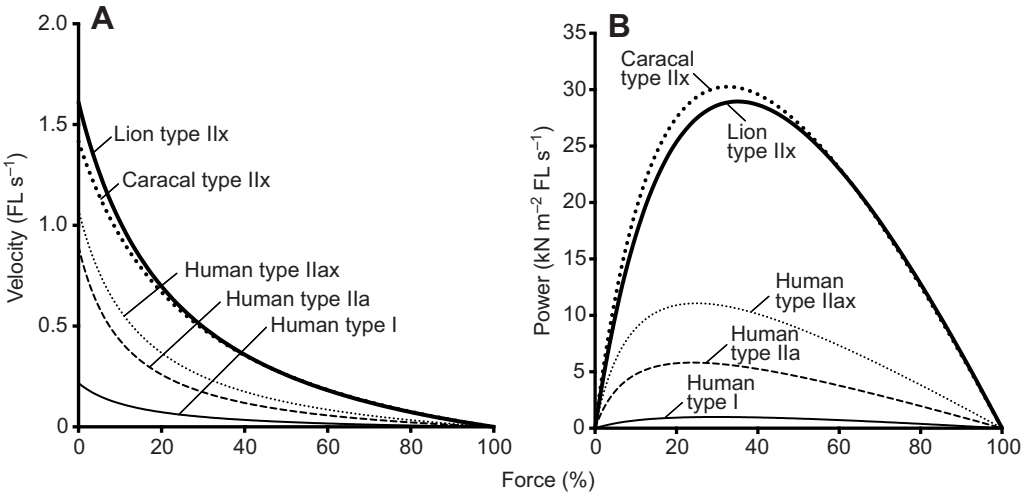

Fig. 5. Composite velocity–force (A) and power–force (B) curves of human type I, IIa and IIax fibres and type IIx fibres of caracal and lion.

reached their maximum power between 10% and 30% of maximum force.

DISCUSSION

This is the first study to investigate the contractile properties of single muscle fibres from lion and caracal. The main findings were that the type IIx fibres of the felids are able to produce between 1.2 and 1.4 times more force per area, have similar V_{max} values, and produce ~3 times more power than human fibres (type IIax and IIx).

Integrity of frozen *versus* fresh tissue and effect of time delay before preservation on contractile properties of single fibres

Tissue harvesting from wild animals is difficult as most of the collection is performed in rural areas where proper storage facilities (and even the supply of electricity) are limited. For this reason, we primarily relied on a mobile liquid nitrogen canister that does not require an electricity source, in which samples can be rapidly frozen and stored during the collection process (Kohn et al., 2011a; Kohn et al., 2011c; Curry et al., 2012). However, the primary problem associated with biomechanical experiments on single fibres is the effect of tissue storage. The efficacy of various methods of muscle tissue preservation has previously been tested. These methods include freeze-drying or storing of the tissue in a high sucrose solution (Frontera and Larsson, 1997). Recently, it was shown that muscle tissue rapidly frozen in liquid nitrogen can be successfully used in single fibre contractile experiments (Ottenheijm et al., 2011). As a result of that publication, this approach was implemented in the present study. To confirm that the freezing process did not affect the contractile properties of the fibres, a small sample from human muscle was obtained, split into two and processed (see Materials and methods and Table 2). The results were similar to those from the literature for human single fibres at 12°C (Bottinelli et al., 1996; Bottinelli et al., 1999; D'Antona et al., 2006). No difference in contractile properties was found between fresh and frozen type IIa fibres. Interestingly, it seemed that the force and shortening velocities of the frozen type IIax fibres were higher than those of fresh fibres. However, the low fibre number for this group of fibres may have made the average values appear higher, when actually there would be no difference. Thus, it was concluded that freezing muscle tissue in liquid nitrogen and storing it for up to 2 months at −87°C did not affect the contractile properties of single fibres.

The opportunity sometimes arises to collect tissue from an exotic animal that has been killed, such as the case for the caracal. While the lion and human samples were frozen immediately post-extraction, a few hours passed before the caracal sample could be frozen. However, this was shown to have had no effect on the integrity of the muscle (histologically or enzymatically) in a previous publication (Kohn et al., 2011c). As mentioned, the environmental temperature when the animal was killed up to when samples were harvested could also have served in preserving the tissue. Furthermore, for the present study, the utmost care was taken to ensure that only fibres that were visually unaffected and did not show any disarray in sarcomere spacing were included. Additionally, the contractile properties did not differ between the lion and caracal fibres, indicating that the caracal sample had been well preserved.

Comparing the contractile properties of human single fibres with those of lion and caracal

A major problem in comparing the human single fibre data with those from lion and caracal was the absence of pure type IIx fibres in the human cyclists. By analysing the relative MHC isoform content in the whole muscle sample, we previously reported (Kohn et al., 2011c) that the vastus lateralis muscle of the lion and caracal contains ~17% and 18% type I fibres, 28% and 15% type IIa fibres, and 55% and 68% type IIx fibres, respectively. This technique was shown to accurately reflect the fibre type composition determined from either histological sections or dissection of single fibres (Andersen et al., 1994; Kohn et al., 2007b; Kohn et al., 2011b). The samples from the three human cyclists were also too small to measure relative fibre type distribution, but it is well known that endurance-trained cyclists have large numbers of type I and IIa fibres, occasional hybrid type IIax fibres but very few to no pure type IIx fibres (Saltin and Gollnick, 1983).

This study therefore had to rely on the data obtained from the human hybrid type IIax fibres to compare with the results from the felids. Considering the other fibre types, only two type I and no type IIa fibres were found in the lion sample. The results obtained for these fibres (force, V_{max} and power) exceeded all the values obtained for human type I and IIa fibres. Except for their V_{max} values, which seemed higher than those of all the human fibres (Fig. 4D), the force and power outputs from the lion type I fibres were comparable to those of human type IIax fibres. It is acknowledged that these values should only be viewed as an observation because of the low fibre number (N=2), and more research is required to fully understand the results obtained for these type I fibres. Similarly, no type I or IIa fibres could be isolated from the caracal sample. Explanations for this may be that the samples used for the single fibres of both lion and caracal contained very few type I and IIa

fibres [as reported previously for the vastus lateralis muscle (Kohn et al., 2011c)] and that the sample itself may have been too small.

The type IIx fibres from both caracal and lion produced less absolute force compared with human type IIax and type IIx fibres from body builders (D'Antona et al., 2006) (Fig. 4B). However, the absolute force tended to be the same when compared with type IIx fibres from sedentary human individuals, cats and dogs, although the sedentary humans had larger fibre sizes (Bottinelli et al., 1996; D'Antona et al., 2006; Toniolo et al., 2008).

It is well known that the CSA plays an important role in the maximum capacity of single fibres to produce force; thus, the amount of force generated by a fibre is related to the CSA of the fibre, and not its length (Woledge et al., 1985; Bottinelli, 2001; Krivickas et al., 2011). The highest specific force in human muscle was achieved by the type IIax fibres (155±8 $kN\,m^{-2}$), whereas the lion and caracal type IIx fibres reached values of 191±8 and 211±22 $kN\,m^{-2}$, respectively (Fig. 4C). These values were similar to those measured in dog and cat IIx fibres, whereas the absolute force and specific force were lower than those of rat type IIx fibres (Bottinelli et al., 1991; Toniolo et al., 2008). As both absolute force and the CSA of type IIx fibres from cat and dog were similar to those of the lion and caracal, this may suggest a genetic conservation in the MHC IIx isoform functionality. Whether there exists structural homology of this isoform between the four species would require further research.

Type IIx fibre V_{max} derived from extrapolation of the force–velocity curves produced values comparable to those in the published literature. Values reported in the literature for rat and human IIx were 1.45±0.07 and 1.29±0.11 $FL\,s^{-1}$, respectively, and fell well within the range of values obtained for the V_{max} values from the present study (Bottinelli et al., 1991; Bottinelli et al., 1996). The literature is clear that the MHC isoform determines the speed of contraction, increasing in velocity from MHC I to IIa to IIx to IIb. The significant difference shown between human type IIax and lion IIx fibres was therefore not surprising, as the former expresses two different isoforms, each of these contributing to the overall velocity of contraction (Fig. 4D, Fig. 5A). In contrast, the two type I fibres appeared to have V_{max} values similar to those of the IIx fibres. This was a finding that did not follow the conventional trend and requires further investigation.

Although the V_{max} of lion and caracal was not different from reported values for human and rat IIx values, the maximum power output of the felids was still substantially higher than values in the literature (Bottinelli et al., 1991; Bottinelli et al., 1996). Human type IIax on average produced power of between 4 and 11 $kN\,m^{-2}\,FL\,s^{-1}$, whereas the values for the felids ranged between 28 and 30 $kN\,m^{-2}\,FL\,s^{-1}$ (Fig. 4F, Fig. 5B). Thus, this would equate to a threefold higher power output for the felids, suggesting that the specific force produced by the fibres contributed more than shortening velocity (V_{max}). Additionally, the felid type IIx fibres had higher $a/\mathbf{P}_o$ values compared with humans, which would suggest that these fibres could sustain a greater force at a particular velocity and, thus, produce more power (Woledge et al., 1985; Gilliver et al., 2009).

In summary, this would therefore imply that the MHC IIx isoform is not similar between the species, or that other factors may play a determining factor. Although the mechanisms are not fully understood, the nature of contributing factors could be qualitative or quantitative. The former would indicate that proteins other than the MHC contribute to the generation of absolute force and the velocity of shortening. The alkali myosin light chains, for example, have been implicated in explaining the differences in shortening velocities within the same fibre type of rats, but not in humans (Bottinelli, 2001). A quantitave factor associated with effects on primarily specific force production is the myosin concentration within a fibre. A number of studies (reviewed by Canepari et al., 2010) have shown that the concentration of myosin within a fibre is also a determining factor of force production. Given the differences in the MHC IIx isoform protein sizes and the small diameters of the felid fibres compared with those of humans, there is no doubt that these factors do indeed influence the overall power production of the fibre, but would require future studies.

In support of the potential functional differences between the MHC IIx isoforms, recent studies (Kohn et al., 2011a; Kohn et al., 2011c) have shown that, although these isoforms still reacted with the MHC IIx specific antibody, there are protein migratory differences on SDS-PAGE gels, suggesting differences in the actual size of the human, black wildebeest, springbok, fallow deer, lion and caracal MHC IIx isoform (Lucas et al., 2000). This would suggest that amino acid sequence differences exist that could potentially influence the functionality of this isoform. Indeed, the antibody used for IIx fibres does show cross-reactivity with type I fibres from caracal and lion, but not human, thus supporting the notion of structural differences in the protein (Kohn et al., 2011a; Kohn et al., 2011c). It also suggests that the felid MHC IIx may indeed be another type of isoform, only showing some similarity to the rat and human type IIx. This would require more research on the functionality of the MHC IIx protein in these different species.

Finally, skeletal muscle is the only tissue that generates locomotion, generating forces and, hence, power to allow animals and humans to travel from point A to point B. It is of course not the only determinant of overall power and performance. Many biomechanical factors falling outside the scope of this study (each contributing to a different degree and related to species) could potentially affect power output and are worth mentioning. These include the mass of the animal, being quadrupedal or bipedal, lever arm angles and length of the limbs (affecting stride length), muscle and tendon lengths, and the ability to store elastic energy, to name but a few (Alexander, 2006; Hudson et al., 2011a; Hudson et al., 2011b).

Technicalities: bath temperatures of experiments and normalisation of force

There are many studies of the contractile function of single muscle fibres performed at a range of temperatures. There are multiple reasons for choosing different temperatures, but primarily they revolve around the stability of the fibres. Bottinelli and colleagues showed that, compared with experiments at higher temperatures, experiments performed at 12°C allowed for more repetitions of maximum activation and stretching of the fibre without disrupting the sarcomeric architecture (Bottinelli et al., 1996). For the present study, experiments were initially performed at 15°C and were successful in terms of performing multiple maximum contractions with force clamps using a single human muscle fibre. However, most fibres from caracal and lion ruptured during the plateau phase of the first or second maximum activation. This might be because the fibres themselves are of small CSA relative to human fibres, but produce relatively more force. We speculate that the sarcolemma and connective tissue surrounding the fibre assist in strengthening the fibre's overall resistance to tear. Performing the experiments at 12°C was more successful, allowing multiple activations and stretches to be performed. Unfortunately, very few laboratories have investigated single fibre contractions at 12°C. Furthermore, it seems that there are no conversion factors to accurately convert values

obtained at 12°C to higher temperatures. For the present study, this may be even more problematic as different species were compared and each may vary in the response to different temperatures. Nevertheless, at 12°C, maximum activation of fibres and force clamps yielded accurate and reproducible results when compared with data in the literature measured at the same temperature (Bottinelli et al., 1996; Bottinelli et al., 1999).

An important parameter that is currently receiving attention in the literature is the manner in which force is normalised. Most studies, including the present one, normalised force to CSA assuming the fibre being circular, yielding the specific force (Bottinelli et al., 1996; Widrick et al., 1996), whereas others have assumed the fibre to be elliptical in shape (Frontera and Larsson, 1997). The latter requires equipment that can additionally calculate the depth of a fibre, or measuring diameters in two planes, resulting in two values for diameter that could yield a more accurate measure of CSA. Recently, Krivickas and colleagues (Krivickas et al., 2011) proposed normalising force to fibre diameter, as a better relationship was found using this parameter compared with CSA, suggesting a new normalisation method that would allow for values obtained from different laboratories to be more easily compared. However, it requires a separate investigation to determine whether this would be true across species.

The limitations of the present study were the lack of type I and IIa fibres from both felids, and the lack of optimal sarcomere spacing data for the felids. We (Kohn et al., 2011c) clearly showed their presence in histological sections, but during the present investigation they were virtually absent. Therefore, utilising samples obtained from deeper regions known to be of greater fibre type mixture would aid in future investigations. Additionally, because of the small size of the muscle samples from both felids, optimal sarcomere spacing determinations could not be performed and were therefore based on those obtained from cats (range ~2.2 to ~2.6 μm) (Burkholder and Lieber, 2001). Future studies need to determine whether the range used in this study (2.4–2.6 μm) for the felids hampered the data presented here. If this range is indeed incorrect, it is hypothesised that the contractile properties would only improve at values outside this chosen range.

CONCLUSION

This is the first study to investigate the contractile properties of single muscle fibres from lion and caracal. Compared with human type IIax fibres, their type IIx fibres were smaller in size, produced more specific force and had greater shortening velocities and power outputs. Therefore, their muscle is well adapted and may partly explain the physical capabilities in their running speed, jumping and strength. Additionally, this study also confirms that frozen tissue can be successfully employed to perform single fibre contractile experiments.

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