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Skeletal muscle histology and biochemistry of an elite sprinter, the African cheetah

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Abstract To establish a skeletal muscle profile for elite sprinters, we obtained muscle biopsy samples from the vastus lateralis, gastrocnemius and soleus of African cheetahs (*Acinonyx jubatus*). Muscle ultrastructure was characterized by the fiber type composition and mitochondrial volume density of each sample. Maximum enzyme activity, myoglobin content and mixed fiber metabolite content were used to assess the major biochemical pathways. The results demonstrate a preponderance of fast-twitch fibers in the locomotor muscles of cheetahs; 83% of the total number of fibers examined in the vastus lateralis and nearly 61% of the gastrocnemius were comprised of fast-twitch fibers. The total mitochondrial volume density of the limb muscles ranged from 2.0 to 3.9% for two wild cheetahs. Enzyme activities reflected the sprinting capability of the cheetah. Maximum activities for pyruvate kinase and lactate dehydrogenase in the vastus lateralis were 1519.00 $\pm$ 203.60 and 1929.25 $\pm$ 482.35 μmol $min^{-1} \cdot g$ wet wt^{-1}, respectively, and indicated a high capacity for glycolysis. This study demonstrates that the locomotor muscles of cheetahs are poised for anaerobically based exercise. Fiber type composition, mitochondrial content and glycolytic enzyme capacities in the locomotor muscles of these sprinting cats are at the extreme range of values for other sprinters bred or trained for this activity including greyhounds, thoroughbred horses and elite human athletes.

Key words Cheetah · Fiber type · Mitochondrial volume density · Skeletal muscle · Sprinting

Abbreviations *CS* citrate synthase · *CTP* creatine triphosphate · *EDTA* ethylenediaminetetra-acetic acid · *FG* fast-twitch glycolytic · *FOG* fast-twitch oxidative and glycolytic · *GDP* guanidine diphosphate · *GTP* guanidine triphosphate · *HOAD* 3-hydroxyacyl-CoA dehydrogenase · *IMP* inosine monophosphate · LDH lactate dehydrogenase · *Mb* myoglobin · *MET* Ministry of Environment and Tourism, Namibia · *NAD, NADH* nicotinamide adenine dinucleotide, reduced · *PCr* phosphocreatine · *Pi* inorganic phosphate · *PK* pyruvate kinase · *SO* slow-twitch oxidative · *UTP* uridine triphosphate

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Introduction

The African cheetah (*Acinonyx jubatus*), long considered the quintessential sprinting athlete, has fascinated humans for many centuries. Cheetahs are one of the fastest terrestrial vertebrates and can attain running speeds in excess of 60 mph (27 m s^{-1}) in less than 3 s. Over short distances these cats will reach 64 mph (29 m s^{-1}) (Sharp 1997), nearly three times the maximum speeds of Olympic human sprinters. Searching entirely by sight, the cheetah may approach its prey in full view by walking and slowly trotting. Alternatively, these cats may stalk prey under the camouflage of natural vegetation (Moss 1982; Caro 1994). Once the cheetah is within 50–60 m of its prey, it begins a high-speed chase

that generally only lasts a few seconds. This extraordinary locomotor performance often leads to fatigue and a prolonged recovery period (Schaller 1972; Eaton 1974). In addition to sprinting activity, some African cheetah populations show long-duration, large-scale movements. Radiotelemetry studies of cheetahs in Namibia indicate that this cat will occupy home ranges in excess of 1500 km^2. Individuals may move 14–26 km per day (Marker-Kraus et al. 1996).

Studies on human athletes have shown that sprinters place a greater demand on anaerobic metabolic pathways and demonstrate a greater reliance on muscle energy stores than endurance athletes (Brooks et al. 1996). Routine use of these energy sources results in a characteristic skeletal muscle profile; thus, human athletes trained for sprinting events have skeletal muscle fiber and enzyme profiles that differ from marathoners. Muscles trained for intense short-term exercise demonstrate a greater cross-sectional area of fast-twitch fibers (Tesch et al. 1983) which coincides with elevated glycolytic enzyme activities (Essén-Gustavsson and Henriksson 1983). These characteristics support a comparatively higher ATP turnover in the skeletal muscles of the sprinter.

Similar results are observed for dogs selectively bred for sprinting activity. In comparing the morphology and histochemistry of the cranial tibial muscle of different breeds of domestic dogs, Rodríguez-Barbudo et al. (1984) found a greater cross-sectional area and higher proportion of fast-twitch fibers in the greyhound than for other breeds of endurance and sprinting dogs. Morphological specialization in greyhounds also includes a higher muscle mass to total body weight ratio.

To date, it has been difficult to distinguish between the genetic and environmental factors that dictate the fiber type profile of a sprinter (Allemeier et al. 1994). Furthermore, there have been few studies that have examined the skeletal muscle profile of a mammalian predator that routinely engages in and relies on sprinting activity. The purpose of this study was to develop such a profile for one elite sprinter, the wild African cheetah. The cheetah provided a unique subject because of its evolutionary and behavioral predisposition for sprinting. In view of this, the skeletal muscle profile of this animal provides insights regarding the biochemical and morphological adaptations necessary for supporting elite sprinting performance. To assess the capacity for aerobic or anaerobic metabolism, we determined the fiber-type profiles, metabolic enzyme capacities, myoglobin (Mb) content and mitochondrial volume density of three major limb muscles: vastus lateralis, gastrocnemius, and soleus. In an additional pilot study using an isolated muscle preparation, we examined muscle metabolites, high-energy phosphates and nucleoside phosphates for the resting and stimulated gastrocnemius. The results of this study indicate that the skeletal muscles of African cheetahs show extreme adaptations for anaerobically based performance when compared to other mammalian sprinters.

Materials and methods

Animals

Six adult cheetahs (three wild, three captive) were used for these studies (Table 1). Muscle samples for fiber typing and fiber diameter assessment were obtained from all of the animals. Two of the wild cheetahs were used for complete muscle profiles including determination of mitochondrial volume density, maximum enzyme activity, and Mb content of skeletal muscle. A pilot study concerning metabolite turnover was conducted on one wild cheetah before euthanasia. As might be expected for an endangered species, the number of animals and muscle samples available for study was limited. The results are presented and interpreted in the context of these limitations.

Wild cheetahs were captured on ranches throughout Namibia and examined under permits from the Ministry of Environment and Tourism (MET). Animals were sedated by intramuscular injection of ketamine (5 mg kg^{-1}) and rompin (0.5 mg kg^{-1}) administered with a blowdart. Muscle samples were obtained either from sedated animals by biopsy needle introduced through a 0.5-cm incision in the skin overlying the muscles or during necropsy from animals that had been euthanized in a MET program. Biopsy sites were sterilized and injected with a local anesthetic (2% xylocaine) before sampling, and treated with antibiotics afterwards. Total time for the biopsy procedure was less than 10 min and was followed by morphometric measurements to assess general body condition. Behavioral observations made for each animal following recovery from anesthesia were continued until the animal returned to routine activities. One cheetah, designated a "problem" animal by local ranchers, was euthanized. Prior to this we conducted a muscle stimulation test to assess maximum metabolite turnover in the gastrocnemius (see below).

In addition to the wild animals, three captive, adult cheetahs were examined at the San Diego Wild Animal Park (Zoological Society of San Diego). These animals were housed in 1-acre pens for more than 5 years and maintained sedentary lifestyles. Muscle samples from these animals were obtained during necropsy immediately following euthanasia for age-related health problems.

Histochemistry

Skeletal muscle samples for histochemistry were obtained from the gastrocnemius, vastus lateralis and soleus according to Dubowitz (1985). Briefly, samples from the belly of each muscle were isolated, trimmed, mounted on cork, and immediately frozen in isopentane cooled with liquid N_2. For two animals additional samples from adjacent sites in each muscle were taken for determination of mitochondrial volume density and enzyme activity (see below). Once obtained, these supplemental samples were either frozen immediately in liquid N_2 (enzyme analysis) or placed in glutaraldehyde

Table 1 Morphological characteristics of wild and captive cheetahs examined in this study. Wild animals were from ranches in Namibia. Captive animals were from the San Diego Wild Animal Park (Zoological Society of San Diego). Body length represents curvilinear length from nose to tail tip. Values are means ± 1 SD. Approximate age of the subjects are shown in parentheses

	n	Weight (kg)	Body length (cm)
Captive			
Adult (> 7 years)	3	46.5 ± 7.35	205.8 ± 9.5
Wild			
Adult (2.5–5 years)	3	36.7 ± 7.0	184.3 ± 6.4

fixative (mitochondrial volume density assessment). Frozen muscle samples were stored at −70 °C until assayed.

Serial sections (10 μm) were stained for fiber-type analysis following procedures modified from Dubowitz (1985). Myofibrillar ATPase (Barbitol method) and nicotinamide adenine dinucleotide (NADH) diaphorase stains have been described in Barnard et al. (1971). ATPase pre-incubation was for 10 min at 37 °C and pH 9.0; incubation was for 30 min at the same temperature and pH 9.7. Visualization used a 2% (w/v) cobalt chloride solution followed by fresh 1% (w/v) ammonium sulphide solution. Glycerophosphate dehydrogenase was stained according to the methods of Wattenberg and Leong (1960). Fiber types were determined from the guidelines of Peter et al. (1972). Classifications were limited to fast-twitch oxidative and glycolytic (FOG), fast-twitch glycolytic (FG), and slow-twitch oxidative (SO) fibers. Assessment of fiber size, and evidence of hypertrophy or atrophy were according to Dubowitz (1985). Fiber type proportions are presented as a numerical proportion (the number of a specific fiber type/total number of fibers counted).

Mb concentration

Skeletal muscle Mb was determined for two adult cheetahs using the techniques of Reynafarje (1963) as modified by Castellini (1981). Tissue samples were collected from fresh carcasses, immediately frozen in liquid N_2 and stored at −70 °C until analysis. Approximately 1 g of each sample was sonicated (Branson Sonifier Cell Disrupter 185) in 19.25 ml low ionic strength buffer (40 mmol·l^{-1} phosphate, pH 6.6). The solutions were centrifuged (13 000 g, 1.5 h, 4 °C), and the supernatant retained for analyses. Supernatants were split into two separate samples, and bubbled with CO before and after the addition of sodium dithionite. Absorbance was determined at 538 nm and 568 nm. The spectrophotometer (Beckman) was calibrated with blank solutions containing buffer, CO, and sodium dithionite, and with span solutions of known Mb content from terrestrial and marine mammals (Castellini 1981).

Electron microscopy and mitochondrial density

Samples for electron microscopy were taken from two wild cheetahs. Medial portions of the gastrocnemius and soleus (cheetahs 1 and 2), and from the vastus lateralis (cheetah 2) were fixed by immersion in fixative (6.25% glutaraldehyde in 0.1 mol·l^{-1} sodium cacodylate buffer; total osmolarity 1100 mosmol·l^{-1}; pH 7.4). Fixed samples were processed according to Mathieu-Costello (1987). Ultrathin sections (50–70 nm) cut transversely to the muscle fiber axis in each sample were contrasted with uranyl acetate and bismuth subnitrate (Riva 1974). Electron micrographs of each were taken on 70-mm films with a Zeiss 10 electron microscope.

The volume density of mitochondria, myofibrils and lipid droplets per volume of fiber was estimated by point-counting as described previously (Mathieu-Costello et al. 1992). Twenty micrographs were obtained by systematic sampling in one ultrathin section from each of four blocks in each sample (total of 80 micrographs per sample). Contact prints of EM films were projected on a 144-point square grid at a final magnification of × 49 000, using a microfilm reader (Documator DL 2, Jenoptic, Jena). All volume density values are reported as percentages.

Maximum enzyme activity

Frozen samples of the gastrocnemius, vastus lateralis and soleus from wild cheetahs were minced with scissors and subsequently homogenized (Ultra-Turrax with a 10N shaft) in 9 vols of 20 mmol·l^{-1} Hepes (pH 7.4), 1 mmol·l^{-1} EDTA, 1% Triton X-100 + 10 mmol·l^{-1} B-mercaptoethanol. Homogenization was at 30–40% of maximum speed for three sessions of 10 s each. The homogenate was sonicated (Kontes Micro-Ultrasonic Cell Disrupter) three times for 10 s each. Samples of the homogenate were spun at 10 000 *g* for 5 min in a Micro Centaur microcentrifuge.

Enzyme assays were conducted at 25 °C for comparison with other studies. Maximum enzyme activity for citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (HOAD), lactate dehydrogenase (LDH) and pyruvate kinase (PK) were determined spectrophotometrically (Pye-Unicam SP8-400) according to Suarez et al. (1986). All results are presented in μmol min^{-1} g wet wt^{-1}.

Pilot study – in situ muscle stimulation and muscle metabolite turnover

An examination of maximal skeletal muscle fuel utilization was conducted on one wild adult cheetah prior to euthanasia. The cheetah was sedated (ketamine/rompin) and supplemented with ketamine by intravenous injection at approximately 20% of the initial dose as needed. Stimulation protocols were modified from Hogan and Welch (1986). The gastrocnemius was carefully isolated by a medial incision from the knee to calcaneum, leaving the blood supply intact. Once the skin was retracted, the medial surface of the gastrocnemius was freed from adjacent musculature and connective tissue. The hind limb was stabilized at a 90-degree angle at the knee and ankle. A warm saline drip maintained the exposed muscle temperature at 36.8 °C. Electrode needles connected to a Grass (SD5) stimulator were placed on either end of the muscle belly. Following preliminary sampling, the muscle was maximally stimulated (5 pulses s^{-1}, 200 ms duration, 40 volts/pulse) for 5-s, 10-s, and 30-s bouts. Muscles samples were taken with liquid N_2 cooled clamps during 10-s "rest" periods after each stimulation period. Frozen samples were cleared from the surrounding muscle and placed immediately in liquid N_2. Samples were transported from the field in a dry cryogenic shipping container and stored at −70 °C.

The muscle tissue was ground under liquid N_2 and stored in sealed 50-ml test tubes at −80 °C until use. Powdered tissue (80–100 mg) was homogenized in 5 vols of 3.6% (w/v) perchloric acid at 0 °C. Homogenization was carried out in 1.5-ml Eppendorf tubes containing small spherical glass beads and a dismembrator (Braun, Germany). After sitting on ice for 15 min, the tube was centrifuged at 15 000 *g* for 30 s and the supernatant removed.

A portion (350 μl) of this acid abstract was neutralized by the addition of 2 vols of alamine chloroform (Reiss et al. 1984). After vortexing for 30 s the contents were centrifuged for 1 min, and the upper phase layer removed and discarded. The neutralized sample in the lower phase was used for HPLC analysis of the nucleotides (Reiss et al. 1984).

Pyruvate, lactate, inorganic phosphate (P_i), and phosphocreatine (PCr) were measured enzymatically by fluorometry on small aliquots of the initial perchloric acid extract (Lowry and Passonneau 1972). Muscle glycogen was measured by the following procedure: powdered tissue (50 mg) was transferred to 10-ml Pyrex glass tubes pre-cooled in liquid N_2 to which 1.0 ml 0.5 mol·l^{-1} NaOH was added. Each tube was sealed and then placed in near-boiling water for 20–25 min to destroy the free glucose. The sample was then neutralized with the addition of 50 μl of 12 mol·l^{-1} HCl. An aliquot (50 μl) of the neutralized sample was transferred and digested for 1 h at room temperature in 1 ml 200 mmol·l^{-1} acetate buffer (pH 4–5) containing 0.5 mg lyophilized amyloglucosidase (Boehringer, Mannheim). The free glucose was then enzymatically determined using fluorometry (Lowry and Passonneau 1972). This method of enzymatically cleaving glycogen to free glucose was shown to be 98–100% complete in 50 mmol·l^{-1} standard solutions.

Statistical analysis

Statistical comparison of different muscle fiber populations, and pre- and post-activity metabolite levels was by Student's paired *t*-tests (Zar 1974). Values in the text, tables and figures are presented as means ± 1 SEM unless otherwise indicated.

Results

Histochemistry

Skeletal muscle fibers of the cheetah showed discrete reactions to ATPase activity (Fig. 1). Based on this the fibers were classified as SO or FOG and FG according to Peter et al. (1972) and Dubowitz (1985). We found that fast-twitch fibers made up more than 61% of the fiber population in the gastrocnemius and nearly 83% of the vastus lateralis of the cheetahs (Table 2). The reaction of the fast-twitch fibers to NADH tetrazolium reductase (NADH-TR) was relatively homogeneous with few exceptions. Although a small population of FOG fibers were found, these represented approximately 10% of the total fibers in the vastus lateralis, and were detected in only one of the gastrocnemius samples. Few fast-twitch fibers of either muscle showed intense reaction intensities for NADH-TR activity, and therefore, the majority were classified as FG fibers.

The diameter of SO and FG fibers were not significantly different (at $P < 0.05$) for individual muscles. There was also a marked difference in the range of fiber diameters for captive and wild cheetahs (Fig. 2). SO and FG fiber diameters in the vastus lateralis ranged from approximately 40 μm to 80 μm for the wild cheetahs. In comparison, the range for captive cheetahs spanned 20–100 μm.

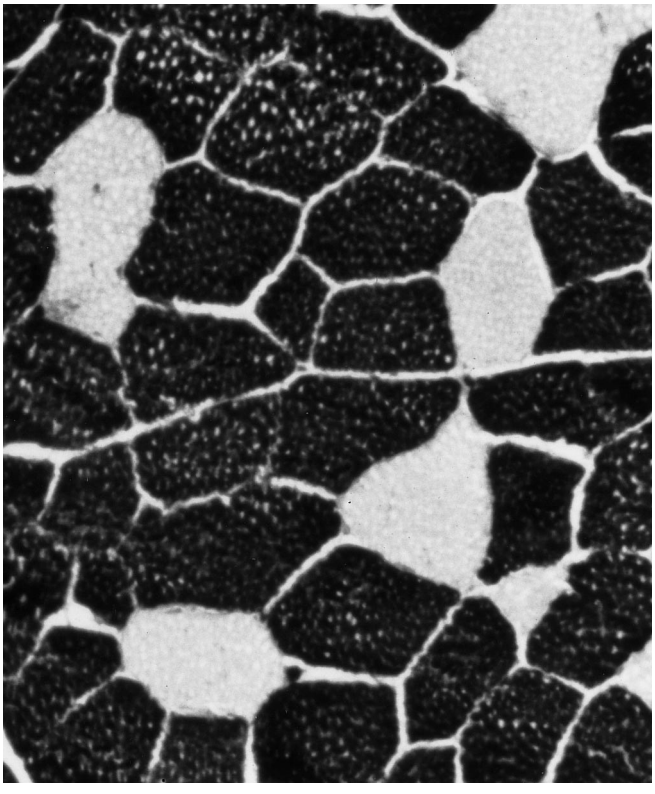

Fig. 1 Photomicrograph of a thin section (10 μm thick) of the wild cheetah vastus lateralis stained by the myosin ATPase method for fiber type. Pre-incubation was at 37 °C and pH 9.0; incubation was at pH 9.7. *Light areas* denote SO fibers; *dark areas* are FG and FOG fibers. The oxidative potential of these fiber types was further differentiated by histochemical tests for NADH diaphorase and glycerophosphate dehydrogenase

Mb concentration

The concentration of Mb in the vastus lateralis of the cheetah was 0.50 g 100 g tissue^{-1}. This level was within the range found for other terrestrial sprinters and endurance runners (Fig. 3).

Mitochondrial volume density

The volume of mitochondria per volume of fiber in the gastrocnemius, soleus and vastus lateralis of two wild cheetahs are presented in Table 3. Total mitochondrial volume density ranged from 2.0% to 3.9% in the samples, a small portion of which was subsarcolemmal (5–14%). There was no detectable accumulation of lipid droplets in the muscle fibers.

Enzyme activity

Table 4 summarizes the enzyme activities for the skeletal muscles of two wild cheetahs. Maximum activity for LDH and PK, enzymes along anaerobic pathways, indicate a high capacity for glycolysis in the cheetah skeletal muscle. The activities of these enzymes were considerably higher than for enzymes typically associated with aerobic metabolic pathways (CS and 3-hydroxyacyl-CoA dehydrogenase). The PK:LDH activity ratio, an indicator of biochemical specialization for carbohydrate oxidation (Suarez et al. 1986), ranged from 0.8 to 1.0 for the three limb muscles of the cheetah and were similar to values for other nonspecialized vertebrate muscles. In contrast, LDH:HOAD activity ratios for the three muscles of the cheetah ranged from approximately 300 to 490, indicating a metabolic focus on glycogenolytic pathways.

Muscle metabolites and nucleoside phosphates

Except for lactate and PCr, the total tissue contents of selected metabolites, high-energy phosphates and nucleoside phosphates of the gastrocnemius showed little change following 5–15 s of electrical stimulation. Therefore, the results for muscle samples obtained at rest and following 5 s and 10 s of stimulation are pooled where appropriate (Table 5). It was not until a further 30 s of stimulation that the glycogen content decreased by 65% to 14 μmol g wet wt^{-1}. ATP and ADP each decreased by 50% from resting although the initial levels of these high-energy phosphates were relatively low.

Table 2 Fiber-type composition of the vastus lateralis and gastrocnemius of adult African cheetahs. Data for wild and captive cheetahs are compared. n = number of animals in each group. A population of 106–206 fibers were counted for each animal depending on the size of the muscle sample. Percentages represent the number of each fiber type/total number of fibers counted. Numbers are mean ± 1 SEM

	SO	FOG	FG
Wild cheetah			
Vastus lateralis ($n = 2$)			
% population	15.5 ± 3. 4	8.6 ± 3.0	75.9 ± 0.4
Gastrocnemius ($n = 3$)			
% population	40.4 ± 3.4	3.1 ($n = 1$)	58.5 ± 3.0
Captive cheetah			
Vastus lateralis ($n = 3$)			
% population	18.5 ± 5.0	12.3 ± 2.0	69.1 ± 6.5
Combined			
Vastus lateralis ($n = 5$)			
% population	17.3 ± 3.0	10.9 ± 1.7	71.8 ± 3.9

Both PCr and lactate contents of the gastrocnemius changed immediately upon stimulation. PCr content in the resting muscle was 25.8 μmol g wet wt^{-1} and decreased linearly with increased duration of stimulation. The PCr content after a total of 45 s of stimulation was 4.7 μmol g wet wt^{-1}. The resting level of lactate was 2.19 μmol g wet wt^{-1}. Following 5 s of electrical stimulation lactate content increased to 2.5 times resting; 45 s of stimulation resulted in a 4.3-fold increase over the resting value. The lactate:pyruvate ratio was 41 in the resting state and 195 after 45 s of electrical stimulation.

Tissue contents of uridine triphosphate, guanidine triphosphate and creatine triphosphate were all maintained near resting values after 15 s of stimulation (Table 5). However, a 50% decrease in content occurred in each after an additional 30 s of electrical stimulation. No change occurred in uridine diphosphate or guanidine diphosphate content during the experimental period. In contrast, a pronounced eight fold change in IMP from 0.018 to 0.113 μmol g wet wt^{-1} was found after 45 s of

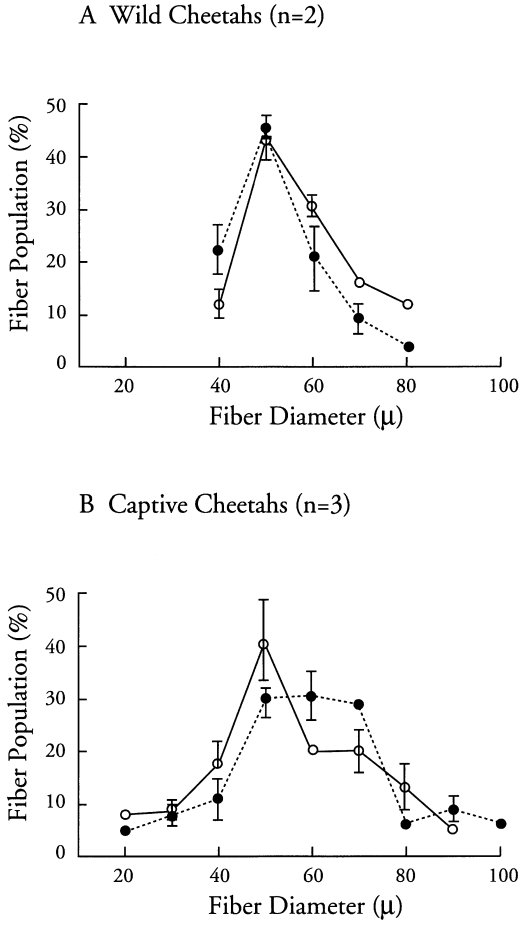

Fig. 2A, B Percentage population of fast-twitch (●) and slow-twitch (○) muscle fibers in relation to fiber diameter for the vastus lateralis of cheetahs. Values for wild (**A**) and captive (**B**) cheetahs are presented. *Circles and vertical lines* are means ± 1 SEM

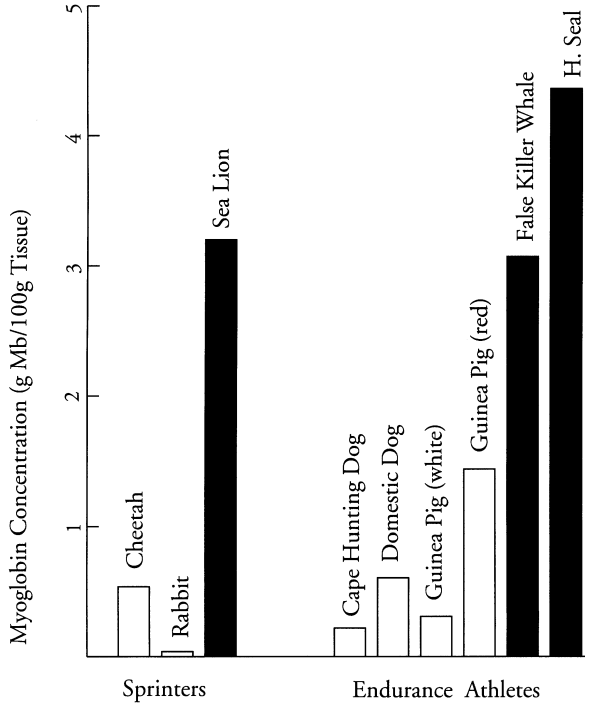

Fig. 3 Mb concentration in the primary locomotor muscles of sprinting and endurance mammals. Note the comparatively higher values for the marine mammals (*filled bars*) in comparison to terrestrial mammals (*open bars*). Values are from the present study (cheetah vastus lateralis); Castellini 1981 (rabbit, sea lion and dog longissimus dorsi); Reed et al. 1994 (harbor seal longissimus dorsi); Peter et al. 1972 (guinea pig vastus lateralis); and T.M. Williams, unpublished data (Cape hunting dog vastus lateralis; false killer whale longissimus dorsi)

Table 3 Mitochondrial volume density and lipid droplet accumulation in the major hind limb muscles of two adult, wild African cheetahs. Volume density values for mitochondria are reported as a percentage of the unit volume of muscle fibers. Eighty micrographs were examined for each sample with results presented as mean ± SEM. Numbers in parentheses are RSE in %

Muscle site	Volume density of mitochondria[a]			Volume[b] density of myofibrils	Volume[b] density of lipid droplets
	Total	Subsarcolemmal	Interfibrillar		
Gastrocnemius					
Cheetah 1	3.9 ± 0.4 (9)	0.3 ± 0.1 (37)	3.7 ± 0.3 (9)	93.3 ± 0.5 (1)	0
Cheetah 2	3.2 ± 0.4 (12)	0.2 ± 0.1 (42)	3.0 ± 0.4 (13)	89.1 ± 0.7 (1)	0
Soleus					
Cheetah 1	2.2 ± 0.2 (10)	0.1 ± 0.03 (62)	2.1 ± 0.2 (10)	93.1 ± 0.6 (1)	0
Cheetah 2	2.0 ± 0.2 (10)	0.1 ± 0.1 (58)	1.9 ± 0.2 (10)	91.5 ± 0.6 (1)	0
Vastus lateralis					
Cheetah 2	3.7 ± 0.4 (11)	0.5 ± 0.2 (45)	3.3 ± 0.3 (10)	86.0 ± 0.8 (1)	0

[a] Per volume of muscle fiber
[b] Per volume of fiber

Table 4 Maximum enzyme activities in the skeletal muscle of wild African cheetahs. Samples from only two cheetahs were available for these analyses. Units are μmol min^{-1} g wet wt^{-1} for all enzymes

	CS	HOAD	LDH	PK
Vastus lateralis	7.31 ± 2.01	4.83 ± 1.20	1929.25 ± 482.35	1519.00 ± 203.60
Gastrocnemius	6.18 ± 1.66	5.00 ± 0.36	1507.25 ± 301.44	1291.90 ± 86.09
Soleus	5.41 ± 0.21	3.07 ± 0.38	1461.55 ± 146.15	1406.75 ± 200.95

Table 5 Total tissue contents of selected metabolites, phosphagens, and nucleoside phosphates in the gastrocnemius of the wild African cheetah at rest and following electrical stimulation. Except for lactate and PCr, pre-active values represent the mean ± 1 SEM for three samples taken following 0–15 s of stimulation. The pre-active lactate and PCr contents were determined from muscle samples taken prior to stimulation. Active values represent total tissue contents determined for muscle samples obtained following 45 s of electrical stimulation

	Pre-active	Active
Metabolites (μmoles g wet wt^{-1})		
Glycogen	39.7 ± 1.5	14.4
Lactate	2.19 (0 s)	9.36
Pyruvate	0.053 ± 0.005	0.048
High-energy phosphates and adenylates (μmoles g wet wt^{-1})		
NAD	0.82 ± 0.03	0.38
PCr	25.8 (0 s)	4.71
ATP	6.59 ± 0.25	2.94
P_i	9.23 ± 0.71	10.00
ADP	0.97 ± 0.05	0.55
IMP	0.018 ± 0.003	0.113
AMP	0.019 ± 0.002	0.020
Nucleoside phosphates (μmoles g wet wt^{-1})		
UTP	0.109 ± 0.005	0.056
GTP	0.072 ± 0.004	0.040
CTP	0.023 ± 0.001	0.009
UDP	0.011 ± 0.001	0.012
GDP	0.005 ± 0.000	0.005

stimulation. The total acid-labile NAD content remained relatively constant (range 0.76–0.85 μmol g wet wt^{-1}) during the first 15 s of stimulation; this level decreased by over 50% after an additional 30 s of stimulation.

Discussion

Skeletal muscle characteristics of an elite sprinter

The skeletal muscles of the cheetah showed an extreme profile for the support of anaerobic-based metabolism that was consistent with the animal's hunting behavior. FOG and FG fibers comprised nearly 83% of the total population of fibers in the vastus lateralis, and approximately 61% of the gastrocnemius muscle (Table 2). This compares well with muscle profile of other sprinting animals including domestic cats (Henneman and Olson 1965), mountain hares (Pyörnilä et al. 1992), horses (Lindholm 1974; Kayar et al. 1988), rabbits and frogs (Arora and Talesara 1991) as well as human elite sprinting athletes. In human sprinters, the proportion of fast-twitch fibers in locomotor muscles shows a positive correlation with acceleration and maximum constant speed (review: Mero et al. 1992). Likewise, Schéle and Kaiser (1982) found a positive correlation between sprinting speed and fast-twitch fiber composition in the

vastus lateralis of humans. Fast-twitch fibers in the humans ranged from 10% to almost 70% of the muscle fiber population, with the greatest proportion found in faster athletes. Maximum speed of the human was approximately 9.0 m s^{-1}. By comparison, the population of fast-twitch fibers in the vastus lateralis of cheetahs (over 82% of the total number of fibers counted, Table 2) supports sprinting speeds of 29 m s^{-1}.

A sedentary lifestyle appeared to affect the fiber size distribution (Fig. 2) but not fiber type (Table 2) in the cheetah. Wild African cheetahs showed a fiber diameter histogram for the vastus lateralis similar to those of healthy human males, i.e., 40–80 μm (Dubowitz 1985). In contrast, the same muscle from captive cheetahs showed a much wider distribution, 20–100 μm. Possible explanations for the differences in muscle fiber diameters for wild and captive cheetahs include the age and activity levels of the subjects. Wild subjects in this study were younger and considerably more active than captive counterparts. The random distribution and increased variation in fiber diameter for captive cheetahs is consistent in pattern to that of a general myopathy rather than a disuse atrophy per se (Dubowitz 1985).

Maximum enzyme activity (Table 4) also distinguishes cheetah locomotor muscles from those of other athletes. Key enzymes in the glycolytic pathway, LDH and PK, are elevated in cheetah skeletal muscle in comparison to many other animals. For example, PK activity of the cheetah gastrocnemius is 50% higher than predicted for mammals of similar body mass (Hochachka et al. 1988). The activity of this enzyme is similar for the cheetah and another sprinter, the rabbit (Hochachka et al. 1988), when differences in body mass are taken into account. Activity levels of the glycolytic enzyme LDH were similar for the cheetah gastrocnemius and predicted values for the same muscle in running mammals of similar body mass. However, the activity of this enzyme can be considerably higher for the muscles of cheetahs than for the primary locomotor muscles of other athletic species. LDH activity of the cheetah vastus lateralis is more than two times higher than levels for the vastus medialis of the horse assayed at 25 °C (Kayar et al. 1988), two times higher than in the longissimus dorsi of phocid seals measured at 37 °C (Reed et al. 1994), and nine times higher than the value reported for the vastus lateralis of the human at 25 °C (Saltin and Gollnick 1983).

In contrast, enzymes that support aerobic processes, CS and HOAD, show lower capacities in the muscles of cheetahs than in mammals trained or bred for sprinting activity, i.e., humans (Saltin and Gollnick 1983), and greyhounds and race horses (Essén-Gustavsson 1986). Many of these sprinters possess locomotor muscles high in both glycolytic and oxidative enzyme capacities. Conversely, cheetah locomotor muscles were characterized by high glycolytic capacities only despite reported long-range, extended-duration movements on territories (Marker-Kraus et al. 1996). Enzyme activities for CS and HOAD of the cheetah gastrocnemius were only 19.4% and 20.6% of predicted values for similarly sized runners (Hochachka et al. 1988). This metabolic focus on anaerobic pathways is evident from the LDH:HOAD activity ratios calculated for cheetah muscles. LDH:HOAD is approximately 300 for the gastrocnemius of the cheetah (Table 4), a value that is approximately double the level expected based on body mass (Hochachka et al. 1988) or fiber-type content (Reed et al. 1994).

As might be expected from this fiber-type and enzyme profile, mitochondrial volume density, a descriptor of the muscle cell's oxidative capacity (Hoppeler et al. 1987), was lower in the locomotor muscles of the cheetah than in comparable muscles of more aerobic athletes. The total volume density of mitochondria in the vastus medialis of dogs and ponies is 6.5–10.7% (Hoppeler et al. 1987). In comparison, the volume density of mitochondria in the cheetah vastus lateralis is 3.7% (Table 3). This value is almost 16% lower than predicted the mitochondrial volume density for the vastus medialis of wild and domestic mammals of similar body mass (Mathieu et al. 1981).

Mb content is an important O_2 store within mammalian skeletal muscle and was two times higher in the cheetah vastus lateralis than in the Cape hunting dog, an endurance specialist (Fig. 3). However, the Mb content of cheetah locomotor muscle was within the range of values determined for a variety of sprinting and endurance runners. When compared to swimming specialists, we find that the Mb content of the cheetah vastus lateralis is only 10–16% of the values for marine mammals regardless of their classification as sprint or endurance swimmers. Thus, it appears that adaptations for diving, rather than sprinting or endurance ability, are the dominant factors in predicting Mb concentration in mammals (Fig. 3; Castellini 1981).

Metabolite and nucleoside phosphate turnover

Evidence from the stimulated gastrocnemius of the cheetah supports a hierarchy of metabolite and nucleoside phosphate turnover that is consistent with the low aerobic and high anaerobic enzyme profile. The rate of glycogen use in the gastrocnemius of the cheetah was similar to levels reported for the muscles of animals bred for sprinting, and considerably greater than the utilization rate in human athletes. The amount of glycogen used by the cheetah gastrocnemius in 30 s of constant stimulation was 24.5 μmol g wet wt^{-1} or 49.0 μmol g wet $wt^{-1}min^{-1}$. This turnover rate was 10% lower than the level observed for the gastrocnemius, vastus lateralis and biceps femoris of greyhounds following an 800-m sprint (Dobson et al. 1988) and 21% higher than levels for the gluteal muscle of the thoroughbred race horse following an 800-m maximal gallop (reported in Dobson et al. 1988). In comparison, the rate of glycogen breakdown in the vastus lateralis of humans performing a 30 s sprint on a nonmotorized treadmill was less than half of the value for the cheetah or 24.1 μmol g wet $wt^{-1}min^{-1}$,

assuming 75% of the muscle is water (Cheetham et al. 1986).

In the present study, we found that glycogen utilization in the electrically stimulated gastrocnemius of the cheetah was delayed for 15 s (Table 5). As a result, glycogen did not appear to be the primary fuel in regenerating ATP during the early phases of electrical stimulation; presumably, this role was taken over by PCr. That PCr hydrolysis precedes the activation of anaerobic glycogenolysis agrees with a study on fish white locomotory muscle during a 10 s sprint (Dobson et al. 1987), and is consistent with the work of Danforth (1965) on isolated frog skeletal muscle. It was not until after an additional 30 s of electrical stimulation that PCr was reduced by 80% and became limited as the principal fuel. At this time, glycogen was mobilized to support the muscle cell's ATP requirement. ATP subsequently decreased by 50%, IMP increased eight fold, and P_i and AMP remained unchanged over control values. These preliminary results present a biochemical profile complementary to a lifestyle requiring high-intensity performance. How this pattern of metabolite and nucleoside phosphate utilization is regulated and how the muscle is buffered during periods of maximal sprint performance by cheetahs remain to be answered and warrant further investigation.

In conclusion, histological and biochemical evidence demonstrate that the primary adaptation for sprinting in the skeletal muscles of cheetahs is at the level of the glycolytic pathway. Although the fiber-type composition showed a predominance of fast-twitch fibers it was not extraordinarily higher than humans trained for sprinting. Rather, the distinguishing factors were high anaerobic (LDH and PK) and low aerobic (CS and HOAD) enzyme capacities of the cheetah locomotor muscle. These enzymes may support comparatively higher glycogen utilization rates in the cheetah than for human athletes trained for sprinting. Endogenous glycogen was the principal fuel and anaerobic glycogenolysis the preferred pathway in the active muscle of the cheetah.

Our study supports Allemeier et al. (1994) who suggest that training to improve sprinting performance should concentrate on adaptations at the level of the biochemical pathways within the major locomotor muscles. In the cheetah, biochemical specialization within the working muscles in addition to mechanical (Hildebrand 1959, 1961, 1984) and physiological (Taylor and Rowntree 1973; Taylor et al. 1974) specialization at the whole-animal level undoubtedly contribute to the extraordinary speed of this animal.

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