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Original Investigation

Distribution of muscle fibers in skeletal muscles of the cheetah (*Acinonyx jubatus*)

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ABSTRACT

We examine the muscle fiber population of skeletal muscles from whole body in the cheetah (*Acinonyx jubatus*). In the present experiments, we showed the characteristics of fiber composition in the cheetah by comparative studies among the cheetah, domestic cat, and the beagle dog. Fiber population was determined on muscle fibers stained with monoclonal antibody to each myosin heavy chain isoform. Histochemical analysis demonstrated that many muscles in the cheetah and domestic cat had a low percentage of Type I fibers and a high percentage of Type IIx fibers, while those in the beagle dog showed a high percentage of Type IIa. The hindlimb muscles in the cheetah had a higher percentage of Type II (Type IIa + IIx) fiber than the forelimb muscles. This fact suggests that the propulsive role of the hindlimb is greater than the forelimb in the cheetah. The longissimus in the cheetah had a high percentage of Type IIx fibers over a wide range from the thoracic to lumbar parts, while the population of muscle fibers in this muscle was different depending on the parts in the domestic cat and beagle dog. This indicates that the cheetah can produce a strong and quick extension of the spinal column and increase its stiffness during locomotion. Furthermore, we found the notable difference of muscle fiber type population between flexors and extensors of digits in the cheetah. The present experiments show the characteristics of muscle fibers in the cheetah, corresponded to its ability to perform high-speed running.

Introduction

The cheetah (*Acinonyx jubatus*) is the best sprinter on earth (Alexander, 2003). It has been reported that its running speed reaches at around 29 m/s (Sharp, 1997) and mainly depends on prolongation of the stride length (Alexander, 2003). The cheetah uses large angular movements of the limb joints and bending of the spine to prolong the stride length (Gambaryan, 1974; Hildebrand, 1959, 1961). The movements are produced by activation of the muscles. To understand the movements during running in the cheetah, the studies of muscles in the cheetah are indispensable. Hudson et al. extensively studied the skeletal muscles in the cheetah. They calculated the physiological cross-sectional area (PCSA) and moment arm, and showed that the cheetah exhibits several unique skeletal adaptations that mostly act to increase muscle moment arm (Hudson et al., 2011a,b). PCSA was calculated as the muscle volume/fascicle length. Maximum isometric contraction force (F_{max}) was estimated by PCSA (Wells, 1965; Medler, 2002), and PCSA is useful to understand the balance of F_{max} among many kinds of skeletal muscle.

It is well known that the skeletal muscles contain different types of muscle fiber (Burke, 1981). The muscle fibers can be classified into Type I, Type IIa, Type IIb and Type IIx by stainimg with monoclonal antibody for each myosin heavy chain isoform and metabolic enzyme activities (Pette and Staron, 1993, 1997). Type I is a muscle fiber with a high metabolic cost of maintenance and a small force output, Type IIa is a muscle fiber with high metabolic cost of maintenance and larger force output, and Type IIb is a muscle fiber with a low metabolic cost for maintenance and a largest force output. Type IIx has intermediate characteristics between Type IIa and IIb. A single motor neuron and the muscle fibers that it innervates comprise a motor unit. The motoneuron properties are exquisitely matched to the properties of the motor units supplying the muscles and the properties of the muscles themselves (Burke, 1991). There are systematic differences in the size, excitability, and corresponding variation of speed, power, and endurance in different types of motor unit. Motor units are classified into S, FR, FI, and FF types (Burke, 1991). The muscle fibers in S, FR, FI, and FF types

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correspond to Type I, Type IIa, Type IIx, and Type IIb, respectively. Henneman (1981) showed the existence of a recruitment order among different types of motor unit on the activation of muscle. The recruitment of motor units is very important for motor performance. On the progression from standing to walking, trotting, and galloping, it was reported that the motor units were recruited from S type to FR type and then FF type (Burke, 1990). It could be considered that the FF or FR type was required to perform high-speed running. Studies of the distribution of different types of muscle fiber are indispensable to understand locomotion in animals. However, studies on muscle fiber compositions are limited to humans and experimental or domestic animals (rat: Ariano et al., 1973; Hintz. et al., 1980; cat: Reichmann and Pette, 1982; Ariano et al., 1973; dog: Tonilo et al., 2007; horse: Kawai et al., 2009; van den Hoven et al., 1985; human: Essen et al., 1975; Johnson et al., 1973) and studies on the muscle fiber composition of skeletal muscles of the cheetah have not been done. To study the muscle fiber composition, sampling fresh muscles is required. We had a chance to sample muscles from the cheetah within 24 h after death. In the present experiments, we showed the characteristics of the fiber composition of skeletal muscles of two cheetahs by comparative studies among the cheetah, domestic cat, and beagle dog.

Material and methods

All experimental procedures were reviewed and approved by the Animal Welfare and Ethics Committee of Yamaguchi University.

Animals

Samples were taken from two adult Cheetahs (male: 37 kg, female: 35 kg) that were obtained from Akiyoshidai Wild Animal Park (Japan). The cheetahs that we studied did not have any disorder of movements. The animals were stored in a freezer at 4 °C just after death. Within 24 h after death, samples of 50 muscles (43 muscles from a male and 7 muscles from a female, Fig. 1, Tables 1–3) were taken from whole parts of the body. We studied the distribution of different muscle fiber types in a beagle dog (*Canis lupus familiaris*, female: 7.5 kg) and domestic cat (*Felis silvestris catus*, male: 3.8 kg) to indicate the characteristics of muscles in the cheetah. The domestic cat and beagle dog were obtained from a laboratory animal supplier. They were healthy and without disorder of movements. After death by an overdose of pentobarbital sodium (60–75 mg/kg), 32 and 31 muscles were taken from the domestic cat and beagle dog, respectively (Figs. 2–4). Each whole muscle was isolated, and then a 0.25–0.70-cm^3 block was taken from the center of the superficial part of each muscle. The samples of m. longissimus were taken from the 8–9th thoracic (T8–9) and the 4–5th lumbar vertebrate (L4–5) levels. The blocks were frozen in liquid nitrogen and stored at −80 °C until analysis. The most important point when making a muscle preparation is the condition of muscles. We confirmed that the muscles sampled within 48 h after death from the animal body maintained at around 4 °C were useful for staining by monoclonal antibody using rats.

Histochemical analysis

Four to eight cross-sections of 10 μm thickness were obtained from each block of frozen muscles using a cryostat (Leica, Nusslock, Germany) at −20 °C. The sections were allowed to warm to room temperature and then preincubated in goat normal serum in 0.2 M phosphate buffer (pH 7.6) at 25 °C for 10 min. Primary monoclonal antibody was then applied: (1) fast myosin, which specifically reacts with myosin heavy chain (MHC)-IIa and -IIx (Schiaffino and Reggiani, 1994); (2) BA-D58, which specifically reacts with MHC-I; and (3) SC-71, which specifically reacts with MHC-IIa. An antibody that specifically reacts with MHC-IIx was not used to identify Type IIx fibers. The sections were incubated at 25 °C for 180 min, then washed with phosphate buffer and reacts with a secondary antibody conjugated with horseradish peroxidase at 25 °C for 180 min, and then washed with phosphate buffer again. Diaminobenzidine tetrahydrochloride was used as a chromogen to localize peroxidase in secondary antibodies (Kawai et al., 2009). Images of the stained muscle fibers were obtained by microscopy (Nikon E600, Tokyo Japan) and an image-processing system (Nikon DS-U1, Tokyo, Japan). On the basis of immunohistochemical staining images, the fibers were classified as Type I, IIa, and IIx fibers (Fig. 2), and then the population (as a percentage) of each muscle fiber type were calculated in 500 muscle fibers. After sampling the block from each muscle, the muscle weight (MW), fascicle length (FL) and resting pennation angle (α) were measured to calculate PCSA, ($PCSA = MW \times \cos\alpha / 1.059 \times FL$ following Medler, 2002; Mendez and Keys, 1960; Wells, 1965).

Statistical analysis

To determine the level of significance among muscle fiber population of three types of muscle fiber, one-way ANOVA and post hoc analysis (*t*-test with Bonferroni adjustment) were used. Significance was set at $P < 0.05$.

Results

Population of muscle fiber types in the cheetah

Hind limb muscles (23 hind limb muscles: Table 1)

The mean percentages of Type I, Type IIa and Type IIx fibers were 20.7, 29.3 and 50.1%, respectively. Eighteen muscles out of studied 23 muscles showed the highest percentage of Type IIx fibers. The vastus intermedius, soleus, and flexor digitorum profundus had the highest percentage of Type I fibers (63.0, 53.1, and 56.2%, respectively) and had no or very few Type IIx fibers. The extensor digitorum lateralis and flexor digitorum superficialis had the highest percentage of Type IIa fibers (53.3 and 68.1%, respectively).

Neck/trunk muscles (13 neck and trunk muscles: Table 2)

The mean percentages of Type I, IIa and IIx were 28.0, 31.6 and 40.0%, respectively. The splenius, brachiocephalicus, latissimus dorsi, pectoralis profundus, longissimus thoracis (T9), longissimus lumborum (L4), and obliquus extensor abdominis had the highest percentage of Type IIx fibers (37.2, 37.2, 70.3, 84.1, 66.1, 84.1, and 51.1%, respectively), while the trapezius cervicis, rhomboideus, and rectus abdominis had the highest percentage of Type I fibers (48.3, 58.6, 39.1%, respectively). Eleven muscles out of 13 muscles included three types of muscle fiber. The rhomboideus and pectoralis superficialis only consisted of Type I and Type IIa fibers.

Forelimb muscles (14 forelimb muscles: Table 3)

The mean percentages of Type I, IIa and IIx were 34.1, 29.9 and 36.0%, respectively. The triceps brachii caput mediale, deltoideus scapular part, supraspinatus, infraspinatus, and teres major had the highest percentage of Type I (68.7, 51.0, 48.9, 65.1, and 37.3%, respectively). The triceps brachii caput longum, biceps brachii, deltoideus acromial part, extensor digitorum communis, extensor digitorum lateralis, and flexor digitorum profundus had a relatively high percentage of Type IIx fibers (49.3, 58.8, 58.0, 41.9, 51.8, and 57.4%, respectively). The triceps brachii caput mediale did not include Type IIx fiber.

PCSAs (Tables 1 and 3) of forelimb and hind limb muscles shown in the present experiment were similar to those reported by Hudson et al. (2011a,b). This fact indicates that our studied