<Short communication>

(Title)

Distribution of Muscle Fibers in Skeletal Muscles of the African Elephant (Loxodonta africana africana)

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Abstract

We examined the muscle fiber population of skeletal muscles from the whole body in the African elephant. The fiber population was determined in muscle fibers stained with monoclonal antibody to each myosin heavy chain isoform. Histochemical analysis demonstrated that many muscles in the elephant had high percentages of Type I and IIa fibers and a low percentage of hybrid fibers between Type IIa and IIx fibers (Type II-h). The cross-sectional area of Type I fibers, with a high metabolic cost of maintenance was significantly larger than in Type IIa and II-h. This indicates that the type I muscle fibers of the skeletal muscles in the elephant can produce marked and lasting muscle tension. The forelimb muscles in the elephant had a higher percentage of Type II-h fibers than the hind limb muscles. This fact might indicate that forelimb muscles corresponded to the propulsive role of the forelimb and quick fore foot movements in the elephant. It could be considered that the studied muscle fiber population indicates the characteristics of skeletal muscles in the elephant to support and move such a heavy body weight.

Keywords

Elephant, muscle fiber type, myosin heavy chain, skeletal muscles
The African elephant (*Loxodonta africana africana*) is the largest land animals on the earth, today. The structure and motion of the elephant are unusual compared with those of other animals. The most significant characteristic regarding the frame of the elephant is the legs, shaped like columns or pillars. The elephant supports its bulk with its straight legs and padded feet. It could be considered that the elephant needs less power to support the body. The elephant walk and swim, but cannot run (Gambaryan, 1974; Genin et al. 2010; Hutchinson et al. 2006; Ren et al. 2008). Elephants move with straighter limbs, and have limited speed and gait. The elephant cannot perform locomotion with an aerial phase. However, elephants have been reported to reach speeds of up to 40 km/h using a fast walk (Hutchinson et al., 2006). The elephant performs fast walking mainly by increasing the stride frequency (Biewener, 2003; Hutchinson et al., 2006). The movements are produced by activation of the muscles. To understand movements during the locomotion of animals, studies of muscles are indispensable. In the elephant, the placement, origin, and insertion of skeletal muscles throughout the body have been well studied (Gambaryan, 1974; Mariappa, 1986; Shindo and Mori, 1956a, 1956b, 1956c). However, to understand the function of skeletal muscles, we must studied the force and action produced by muscle. The skeletal muscles contain different types of muscle fiber (Burke, 1981). Muscle fibers can be classified into Type I, Type IIa, Type IIb, and Type IIx by staining with monoclonal antibody for each myosin heavy chain (MHC) 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 a high metabolic cost of maintenance and larger force output, and Type IIb is a muscle fiber with a low metabolic cost for maintenance and the 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 properties of the motor units supplying the muscles and 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 correspond to Type I, IIa, IIx, and 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. 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).

In our previous study, we showed the muscle fiber composition in skeletal muscles from
the body of the cheetah, domestic cat, and beagle dog (Goto et al., 2012). Our results
indicate that the distribution of different types of muscle fiber reflects the kinematic
characteristics of each animal, and studies of muscle fiber distribution are very
important to understand animal locomotion. The most important point for studies of
muscle fiber composition is the condition of muscles. We had a chance to sample
muscles from the African elephant within 24 hours after death. We measured
cross-sectional area (CSA) of muscle fibers of muscle fibers in addition to rates of each
muscle fiber type.

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

Samples were taken from one adult female African elephant (BW: 3,800kg, age: 32
years) obtained from Tokuyama Zoo (Shunan City, Japan). The elephant that we studied
did not have any disorder of movements. Within 24 hours after death, samples of 42
muscles (Tables 1-3) were taken from whole parts of the body. Each whole muscle was
isolated, and then a 1 cm³ block was taken from the center of the superficial part of each
muscle. Samples of m. longissimus were taken from the 8-9th thoracic (T10) and 3rd
lumbar vertebrate (L3) levels. The blocks were frozen in liquid nitrogen and stored at
-80°C until analysis.

Four to eight cross-sections of a 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 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 reacted 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 (Goto et al., 2012).

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 hybrid II fibers (Type II-h: Fig.1), and then the populations (as a percentage) of each muscle fiber type were calculated in 500 muscle fibers. Type I has a low staining property using both anti-MHC fast and SC-71, and a high staining property using ant-MHC fast. Type IIa has a high staining property using both anti-MHC fast and SC-71. There was a fiber with high staining property by anti-MHC fast and medium staining property by SC-71. It could be considered that this fiber is a hybrid fiber between Type IIa and IIx. In the present experiments, this muscle fiber type was indicated as Type II-h. CSA of each type of muscle fiber was measured in 25 muscle fibers in each type.

To determine the level significance among CSA of three types of muscle fiber, one-way ANOVA were used. Significance was set at P<0.05.

Forelimb muscles (18 muscles: Table 1)

The mean percentages of Type I, IIa, and II-h fibers were 37.9, 41.2, and 20.8%, respectively. Eight muscles out of the 18 studied muscles showed the highest percentage of Type IIa fibers. The deltoideus acrominal part, biceps brachii, and extensor digitorum quanti had the highest percentage of Type I fibers (74.1, 95.8, and 64.2%, respectively) and had no or very few Type II-h fibers. The infraspinatus, deltoideus scapular part, triceps brachii caput magnus, and flexor digitorum communis had the highest percentage of Type II-h fibers (41.2, 47.1, 41.8, and 65.1%, respectively). The average CSA of Type I, IIa, and II-h were 7,765, 6,498, and 6,139 μm², respectively. In thirteen out of the 18 muscles studied, CSA of Type I was the largest among the three types of muscle fibers.

Hindlimb muscles (15 forelimb muscles: Table 2)

The mean percentages of Type I, IIa, and II-h were 48.4, 40.2, and 11.4%, respectively. The rectus femoris, vastus externus and semitendinousus had the highest percentage of Type II-h (51.4, 53.3, and 42.9%, respectively). The tensor faciae latae, biceps femoris, semimembranosus, and flexor digitorum profundus had the highest
percentage of Type IIa fibers (81.3, 48.0, 79.4, and 63.6%, respectively). The vastus internus, semimembranosus, tibialis anticus, extensor digitorum communis, extensor digitorum brevis, and flexor digitorum profundus consisted of Type I and IIa. The adductor had only Type I. Eight out of the 15 muscles had the highest percentage of Type I. The average CSA of Type I, IIa and II-h were 8,948, 5,860, and 6,535 $\mu m^2$, respectively. In eight out of 15 muscles studied, the CSA of Type I was the largest among the three types of muscle fiber.

The mean percentages of Type I, IIa and II-h were 39.6, 49.0 and 11.4%, respectively. The trapezius cervicis, trapezius thoracis, pectoralis superficialis, longissimus dorsi thoracis, longissimus dorsi lumborum, and rectus abdominis had the highest percentage of Type IIa fibers (53.3, 52.7, 61.5, 65.2, 65.8 and 67.7% respectively), while the rhomboideus and pectoralis profundus had the highest percentage of Type I fibers (73.5 and 61.6%, respectively). Four muscles out of 9 muscles did not included Type II-h. The averaged CSA of Type I, IIa and II-h were 6764, 5117 and 5920 $\mu m^2$, respectively. In six muscles out of studied 9 muscles, the CSA of Type I is the largest among three types of muscle fiber.

This is the first report to show the muscle fiber population of skeletal muscles obtained from the whole body excluding the head in the African elephant. In the present experiments, we classified muscle fibers into three kinds: Type I, IIa, and II-h. In our previous report, we could divide muscle fibers in the domestic cat, beagle dog, and cheetah into Type I, IIa, and IIx using the present method (Goto et al., 2012). However, in the elephant, muscle fibers could not be clearly divided into two groups using SC-71. There are muscle fibers with medium and unstable staining properties using SC-71. It could be considered that this type of muscle fiber has characteristics between Type IIa and IIx. In the present experiments, the samples were cut from the surface of each muscle. A previous study (van den Hoven et al., 1985) reported differences in the muscle fiber population between the middle part and surface of large and thick muscles. However, we determined that the functional properties could be represented by the three types of muscle fiber population of superficial portions in most muscles in this study.

The characteristic of the muscle fiber population in the elephant skeletal muscles was
that many muscles had a high percentage of Type I and IIa fibers. In general, the skeletal muscles in heavy-weight animals tend to have more slow fibers (Type I) and fewer fast fibers (Type IIx and IIb: Ariano et al., 1973; Goto et al., 2012; Hintz et al., 1980; Kawai et al., 2009; Reichmann and Pette, 1982; Tonilo et al., 2007; van den Hoven et al., 1985). Many muscles in the elephant include Type II-h fibers. Type II-h is a muscle fiber with a lower metabolic cost for maintenance and a larger force output than Type I and IIa, and a higher metabolic cost for maintenance and a smaller force than type IIx and IIb. In our previous paper (Goto et al. 2012), we described, the skeletal muscle in Felidae, which excels in instantaneous force but exhibits no stamina, mainly consisting of Type IIa and IIx, while those in Canidae, which excel in endurance and allow long-distance running, mainly consisted of Type I and IIa. The muscle fiber population of the different fiber types is closely related to characteristics of locomotion. It should be noted that the CSA of Type I muscle fibers was significantly larger than that of Type IIa and Type II-h in the elephant. The CSA related to magnitude of muscle tension (El-Khoury et al., 2012). This fact indicates that Type I fibers in the elephant can produce continuous and strong tension. These characteristics of muscle fibers in the elephant adjust to support the huge body weight and perform fast walking.

We should note that the some distal muscles of the forelimb (extensor digitorum communis, flexor digitorum communis) have many type II-h fibers (41.2% and 65.1 %, respectively), while those of the hind limb have no or very few type II-h fibers. Type II-h fibers are muscle fibers which produce fast and strong muscle tension. It might be considered that the distribution of Type II-h fibers in the forelimb corresponds to the skillful and quick movements for digging soil and rolling the articles using forelimbs. In the horse and cheetah, the forelimb muscle has a higher percentage of Type IIa and a lower percentage of Type IIx than the hind limb muscles, suggesting that the forelimb plays a less propulsive role than the hind limb (Merkens et al., 1993; Niki et al., 1984; Payne et al., 2004, Goto et al., 2012). The present result might indicate that the elephant produces a large proceeding force using the forelimb. Ren et al. (2010) showed that the proceeding force produced by the forelimb was greater than that by the hindlimb by analyzing the ground reaction force in the Asian elephant.

The results of the present experiments show that the elephant has muscle fiber populations corresponding to its huge body weight.

Acknowledgement
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References


Figure Legends

Fig. 1

Serial transverse sections of the latissimus dorsi muscle from an elephant. The sections were stained with monoclonal antibody against myosin heavy chain (MHC) isoforms. Muscle fibers were classified into Type I, IIa, and II-h. A: anti-fast myosin (anti-MHC) B: SC-71 (anti-MHC-IIa). Bar=200 μm.

Table I

Muscle fiber population (%) and the cross-sectional area of each type of muscle fiber (μm²) of skeletal muscles of the forelimb.*1, *2, and *3 indicate significant differences between CSA of Type I and Type IIa, Type I and II-h, and Type IIa and II-h, respectively.

<table>
<thead>
<tr>
<th>muscle name</th>
<th>%</th>
<th>CSA mean (SD) μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Type</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>IIa</td>
</tr>
<tr>
<td>supraspinatus</td>
<td>37.5</td>
<td>25.0</td>
</tr>
<tr>
<td>infraspinatus</td>
<td>35.3</td>
<td>23.5</td>
</tr>
<tr>
<td>deltoideus acrominal part</td>
<td>74.1</td>
<td>25.9</td>
</tr>
<tr>
<td>deltoideus scapular part</td>
<td>23.5</td>
<td>29.4</td>
</tr>
<tr>
<td>teres major</td>
<td>47.8</td>
<td>52.2</td>
</tr>
<tr>
<td>subscapularis</td>
<td>46.3</td>
<td>53.7</td>
</tr>
<tr>
<td>brachiocephalicus</td>
<td>42.6</td>
<td>51.9</td>
</tr>
<tr>
<td>serratus vertralis</td>
<td>40.0</td>
<td>34.3</td>
</tr>
<tr>
<td>coracobrachialis</td>
<td>26.7</td>
<td>64.4</td>
</tr>
<tr>
<td>triceps brachii caput magnus</td>
<td>21.8</td>
<td>36.4</td>
</tr>
<tr>
<td>triceps brachii caput parvum</td>
<td>10.3</td>
<td>58.6</td>
</tr>
<tr>
<td>triceps brachii caput medialis</td>
<td>43.7</td>
<td>56.3</td>
</tr>
<tr>
<td>brachialis</td>
<td>10.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>
Table 2

Muscle fiber population (%) and the cross-sectional area of each type of muscle fiber ($\mu m^2$) of skeletal muscles of the hind limb. *1, *2, and *3 indicate significant differences between CSA of Type I and IIa, Type I and II-h, and Type IIa and II-h, respectively.

<table>
<thead>
<tr>
<th>Muscle name</th>
<th>Type I %</th>
<th>Type IIa %</th>
<th>Type II-h %</th>
<th>Type I CSA (μm²)</th>
<th>Type IIa CSA (μm²)</th>
<th>Type II-h CSA (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>biceps brachii *1</td>
<td>95.8</td>
<td>4.2</td>
<td>0.0</td>
<td>11,028(3,691)</td>
<td>12,357(1,870)</td>
<td></td>
</tr>
<tr>
<td>extensor digitorum communis</td>
<td>17.6</td>
<td>41.2</td>
<td>41.2</td>
<td>16,159(4,487)</td>
<td>14,941(1,934)</td>
<td>12,498(2,427)</td>
</tr>
<tr>
<td>extensor digitorum quanti *1</td>
<td>64.2</td>
<td>35.8</td>
<td>0.0</td>
<td>4,707(1,167)</td>
<td>3,569(1,605)</td>
<td></td>
</tr>
<tr>
<td>flexor digitorum communis *2, *3</td>
<td>11.1</td>
<td>23.8</td>
<td>65.1</td>
<td>4,696(1,284)</td>
<td>4,501(794)</td>
<td>5,056(1,232)</td>
</tr>
<tr>
<td>flexor digitorum quanti *1</td>
<td>34.1</td>
<td>65.9</td>
<td>0.0</td>
<td>8,737(1,581)</td>
<td>5,563(607)</td>
<td></td>
</tr>
<tr>
<td>average</td>
<td>37.9</td>
<td>41.2</td>
<td>20.8</td>
<td>7,764(3,849)</td>
<td>6,498(3,950)</td>
<td>6,139(2,913)</td>
</tr>
</tbody>
</table>

Note: *1, *2, and *3 indicate significant differences between CSA of Type I and IIa, Type I and II-h, and Type IIa and II-h, respectively.
Table 3

Muscle fiber population (%) and the cross-sectional area of each type of muscle fiber (μm²) of skeletal muscles of the trunk.*1, *2, and *3 indicate significant differences between CSA of Type I and IIa, Type I and II-h, and Type IIa and II-h, respectively.

<table>
<thead>
<tr>
<th>muscle name</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type II-h</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type II-h</th>
</tr>
</thead>
<tbody>
<tr>
<td>trapezius cervicis *1, *2</td>
<td>24.4</td>
<td>53.3</td>
<td>22.2</td>
<td>5,951(1,191)</td>
<td>6,609(1,408)</td>
<td>6,703(1,500)</td>
</tr>
<tr>
<td>trapezius thoracis *1, *2</td>
<td>31.2</td>
<td>52.7</td>
<td>16.1</td>
<td>35,666(1,180)</td>
<td>2,414(732)</td>
<td>2,349(688)</td>
</tr>
<tr>
<td>rhomboideus</td>
<td>73.5</td>
<td>26.5</td>
<td>0.0</td>
<td>4,910(1,114)</td>
<td>5,529(2,137)</td>
<td></td>
</tr>
<tr>
<td>latissimus dorsi</td>
<td>32.4</td>
<td>23.5</td>
<td>44.1</td>
<td>7,907(3,134)</td>
<td>8,110(3,726)</td>
<td>8,175(2,282)</td>
</tr>
<tr>
<td>pectalis superficialis *1</td>
<td>38.5</td>
<td>61.5</td>
<td>0.0</td>
<td>5,621(2,100)</td>
<td>3,005(1,191)</td>
<td></td>
</tr>
<tr>
<td>pectalis profundus *1, *2, *3</td>
<td>61.6</td>
<td>24.7</td>
<td>13.7</td>
<td>8,384(2,317)</td>
<td>5,693(1,125)</td>
<td>6,209(1,421)</td>
</tr>
<tr>
<td>longissimus dorsi thoracic part *1</td>
<td>34.8</td>
<td>65.2</td>
<td>0.0</td>
<td>3,463(712)</td>
<td>2,714(808)</td>
<td></td>
</tr>
<tr>
<td>longissimus dorsi lumbar part</td>
<td>34.2</td>
<td>65.8</td>
<td>0.0</td>
<td>6,296(2,318)</td>
<td>5,019(1,404)</td>
<td></td>
</tr>
<tr>
<td>rectus abdominus *1, *2</td>
<td>25.8</td>
<td>67.7</td>
<td>6.5</td>
<td>14,776(5,822)</td>
<td>6,962(2,881)</td>
<td>5,117(2,020)</td>
</tr>
<tr>
<td>average</td>
<td>39.6</td>
<td>49.0</td>
<td>11.4</td>
<td>6,764(3,444)</td>
<td>5,117(2,020)</td>
<td>5,920(2,156)</td>
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</table>