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Cell Size and Oxidative Enzyme Activity of Rat Biceps Brachii and Triceps Brachii Muscles

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Abstract: Fiber-type distributions, cross-sectional areas, and oxidative enzyme activities of type-identified fibers in the biceps brachii and triceps brachii muscles of 10-week-old male Wistar rats were determined and compared with those in the soleus and plantaris muscles. The soleus and plantaris muscles consisted of two (I and IIA) and three (I, IIA, and IIB) types of fibers, respectively. The deep regions of the biceps brachii and triceps brachii muscles consisted of three types of fibers, while the surface regions of those muscles consisted only of type IIB fibers. The cross-sectional areas of fibers in the deep and surface regions of the plantaris muscle and in the deep regions of the biceps brachii and triceps brachii muscles were in the rank order of type I = type IIA < type IIB, while the oxidative enzyme activities of fibers in the deep and surface regions of the plantaris muscle and in the deep region of the triceps brachii muscle were in the rank order of type IIB < type I = type IIA. These results indicate that fiber-type distributions, cross-sectional areas, and oxidative enzyme activities are muscle type- and region-specific. Therefore, the metabolic and functional significance of the biceps brachii and triceps brachii muscles, especially in the surface regions, where only type IIB fibers are located, in those muscles, appears to be determined by their fibers having larger cells and lower oxidative enzyme activity.

Key words: biceps brachii muscle, cell size, oxidative enzyme activity, rat, triceps brachii muscle.

Skeletal muscle fibers are classified into several types (I, IIA, and IIB) based on their differences in the activity of myosin adenosine triphosphatase (ATPase) following preincubation at pH 4.3, 4.5, or 10.4 [1]. The oxidative enzyme activities of fibers correspond well with their types in that type I and IIA fibers show higher oxidative enzyme activities than type IIB fibers [2–4]. Furthermore, type I and IIA fibers have lower maximum tension and slower contraction speed than type IIB fibers [5–7]. Therefore, fiber-type distribution in the skeletal muscle reflects its functional capacity (see Ref. [8] for review).

An inverse relationship between cross-sectional areas and oxidative enzyme activities of fibers in hindlimb muscles, including the soleus, plantaris, extensor digitorum longus, and tibialis anterior muscles in rats, was observed in previous studies [2, 3, 9]. This indicates that smaller fibers have higher oxidative enzyme activity than larger fibers in hindlimb muscles.

The differences in fiber properties were examined among hindlimb muscles in the same [10–13] or different species [14], and among forelimb muscles in monkeys [15, 16]. Hindlimb muscles, especially the soleus muscle, presumably work as anti-gravity muscles to maintain posture and walking in relatively low-intensity and long-duration activities, while forelimb muscles do not need to work as anti-gravity muscles. It is suggested that there are muscle type-specific patterns in the fiber-type distribution, cross-sectional area, and oxidative enzyme activity in forelimb muscles, as well as hindlimb muscles. However, no data are available about fiber properties in the biceps brachii and triceps brachii muscles of rats, which are located in the upper part of forelimb. Therefore, this study compared the fiber-type distributions, cross-sectional areas, and oxidative enzyme activities of fibers in the biceps brachii and triceps brachii (forelimb) muscles of rats with those in the soleus and plantaris (hindlimb) muscles.

METHODS

All experimental procedures and animal care conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Japanese and American Physiological Society. This study was also approved by the Institutional Animal Care Committee at Kyoto University.

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Animals and tissue processing. Eight 10-week-old male Wistar rats weighing 287 ± 11 g (mean ± SD) were used in this study. The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The soleus, plantaris, biceps brachii, and triceps brachii muscles were removed from the left limb and cleaned of excess fat and connective tissue. Thereafter, the rats were sacrificed by an overdose of sodium pentobarbital. The muscles were pinned on a cork at their *in vivo* length, quickly frozen in isopentane cooled with liquid nitrogen, and stored at –80°C until analyses. The mid-portion of the muscle was mounted on a specimen chuck by compound. Serial transverse sections, 10 μm thick, of the muscle on a chuck were cut in a cryostat maintained at –20°C. The sections were brought to room temperature, air-dried for 30 min, and incubated for ATPase activity following acid (pH 4.5) or alkaline (pH 10.4) pre-incubation [2, 3].

The same areas of each section were digitized as gray-level pictures using a computer image processing system (Neuroimaging System, Kyoto, Japan) [2, 3]. The fibers in each section were matched with those in other sections. The soleus muscle fibers were classified into type I (high and low intensity in response to pre-incubation at pH 4.5 and pH 10.4, respectively) and type IIA (low and high intensity in response to pre-incubation at pH 4.5 and pH 10.4, respectively) (Fig. 1). The plantaris, biceps brachii, and triceps brachii muscle fibers were classified into type I (high intensity in response to pre-incubation at pH 4.5), type IIA (low intensity in response to pre-incubation at pH 4.5), and type IIB (medium intensity in response to pre-incubation at pH 4.5) (Fig. 2). The fiber cross-sectional area was measured and stored in a computer image processing system by tracing the outline of each fiber in the section. The fiber-type distribution and cross-sectional area of the soleus muscle were determined from approximately 100 fibers in the central region of the section in the muscle, while those of the plantaris, biceps brachii, and triceps brachii muscles were determined from approximately 50 fibers in the deep (close to the bone) and surface (away from the bone) regions of the section in the muscles.

The sections were also stained for succinate dehydrogenase (SDH) activity, an indicator of mitochondrial oxidative potential [17, 18] (Figs. 1 and 2). SDH activities of 50–100 fibers, matched with those analyzed for fiber-type distribution and cross-sectional area, from each muscle were determined using a computer-assisted image processing system. The sections were digitized as gray-scale images. Each pixel was quantified as one of 256 gray levels. A gray level value of zero was equivalent to 100% transmission of light and that of 255 was equivalent to 0% transmission of light. The mean optical density (OD) value of all pixels within a fiber was determined using a calibration tablet, which has 21-step gradient density ranges and corresponding diffused density values.

Statistics. Means, standard deviations, and correlation coefficients were calculated from individual values using standard procedures. Student's *t*-test or one-way analysis of variance was used to test for overall regional differences. A 0.05 level of probability was established for statistical significance.

RESULTS

Fiber-type distribution

The soleus and plantaris muscles consisted of two (I and IIA) and three (I, IIA, and IIB) types of fibers, respectively (Figs. 1 and 2). The percentage of type I fibers in the soleus muscle was higher than that of type IIA fibers (Fig. 3). The fiber-type percentages in the deep and surface regions of the plantaris muscle were in the rank order of type I < type IIA < type IIB.

The deep regions of the biceps brachii and triceps brachii muscles consisted of three (I, IIA, and IIB) types of fibers, while the surface regions of those muscles consisted only of type IIB fibers (Fig. 2). The fiber-type percentages in the deep region of the biceps brachii muscle were in the rank order of type I < type IIA = type IIB, while those in the deep region of the triceps brachii muscle were in the rank order of type I < type IIA < type IIB (Fig. 3).

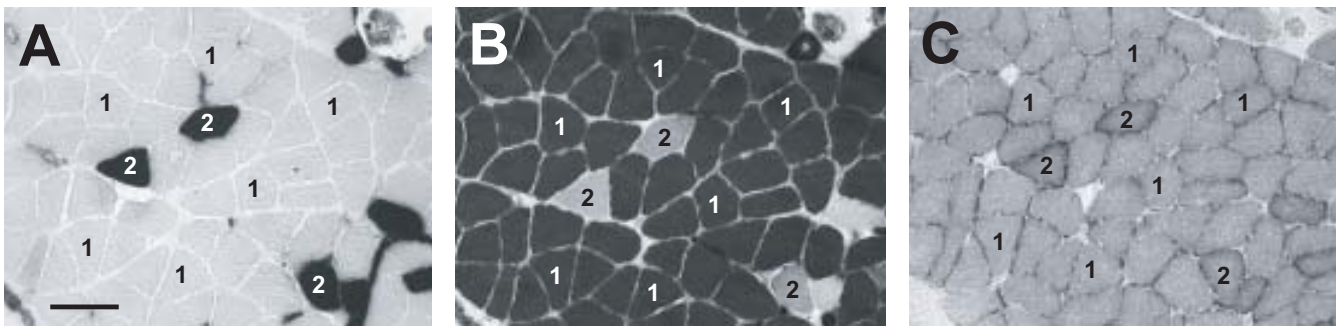

Fig. 1. Serial transverse sections of the rat soleus muscle. Sections were stained for adenosine triphosphatase activity following pre-incubation at pH 10.4 (**A**) and 4.5 (**B**) and for succinate dehydrogenase activity (**C**). 1. type I; 2, type IIA. Scale bar in A indicates 50 μm.

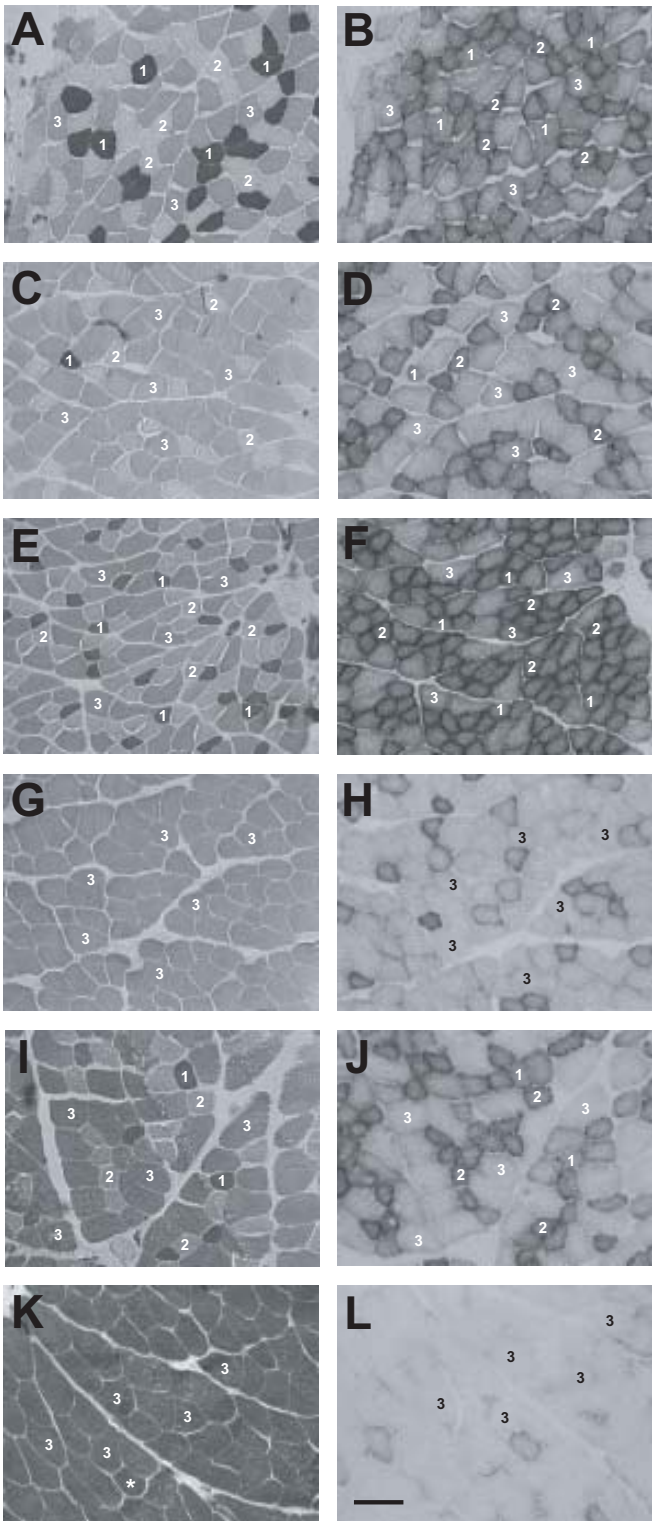

Fig. 2. Serial transverse sections of the rat plantaris, biceps brachii, and triceps brachii muscles. Sections were stained for adenosine triphosphatase activity following pre-incubation at pH 4.5 (left) and succinate dehydrogen activity (right). **A** and **B**, deep region of plantaris; **C** and **D**, surface region of plantaris; **E** and **F**, deep region of biceps brachii; **G** and **H**, surface region of biceps brachii; **I** and **J**, deep region of triceps brachii; **K** and **L**, surface region of triceps brachii; 1. type I; 2, type IIA; 3, type IIB. Scale bar in L indicates 50 μm.

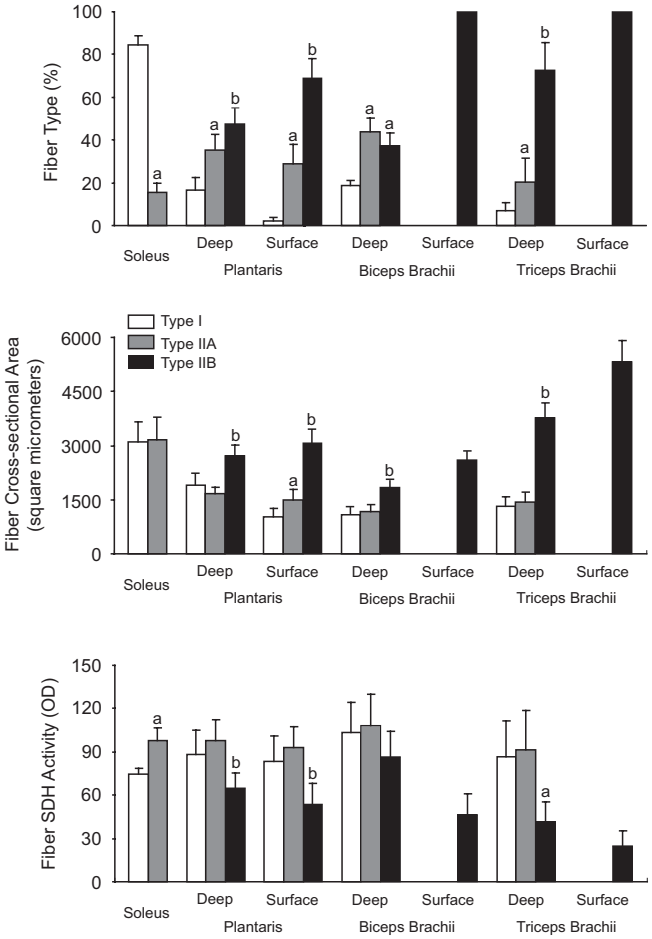

Fig. 3. Fiber-type distributions (top), cross-sectional areas (middle), and succinate dehydrogenase activities (bottom) of the rat soleus, plantaris, biceps brachii, and triceps brachii muscles. Data indicate the means and standard deviations (n = 8). SDH, succinate dehydrogenase; OD, optical density. $^{a}p < 0.05$ compared with type I and $^{b}p < 0.05$ compared with type I and type IIA when comparing values between two types of fibers in the soleus muscle and among three types of fibers in the plantaris, biceps brachii, and triceps brachii muscles.

Fiber cross-sectional area

In the soleus muscle, there were no differences in the cross-sectional area of fibers between type I and type IIA (Fig. 3). The cross-sectional areas of fibers in the deep region of the plantaris muscle were in the rank order of type I = type IIA < type IIB, while those in the surface region of the muscle were in the rank order of type I < type IIA < type IIB.

The cross-sectional areas of fibers in the deep regions of the biceps brachii and triceps brachii muscles were in the rank order of type I = type IIA < type IIB (Fig. 3).

Fiber oxidative enzyme activity

In the soleus muscle, type IIA fibers had higher oxidative enzyme activities than type I fibers (Fig. 3). The oxidative enzyme activities of fibers in the deep and surface

regions of the plantaris muscle were in the rank order of type IIB < type I = type IIA.

In the deep region of the biceps brachii muscle, there were no differences in the oxidative enzyme activity of fibers among any types of fibers (Fig. 3). The oxidative enzyme activities of fibers in the deep region of the triceps brachii muscle were in the rank order of type IIB < type I = type IIA.

Cross-sectional area within the same fiber type

In type I, cross-sectional areas of fibers were in the rank order: surface of plantaris = deep of biceps brachii = deep of triceps brachii < deep of plantaris < soleus (Fig. 3). In type IIA, cross-sectional areas of fibers were in the rank order: deep of biceps brachii < deep of plantaris = surface of plantaris = deep of triceps brachii < soleus. In type IIB, cross-sectional areas of fibers were in the rank order: deep of biceps brachii < deep of plantaris = surface of plantaris = surface of biceps brachii < deep of triceps brachii < surface of triceps brachii.

Oxidative enzyme activity within the same fiber type

In type I, there were no differences in the oxidative enzyme activity of fibers among deep of plantaris, surface of plantaris, deep of biceps brachii, or deep of triceps brachii, while deep of biceps brachii had higher oxidative enzyme activity than soleus (Fig. 3). In type IIA, there were no differences in the oxidative enzyme activity of fibers among soleus, deep of plantaris, surface of plantaris, deep of biceps brachii, or deep of triceps brachii. In type IIB, there were no differences in the oxidative enzyme activity of fibers among deep of plantaris, surface of plantaris, surface of biceps barchii, or deep of triceps brachii. Deep of biceps brachii had higher oxidative enzyme activity than surface of biceps brachii, deep of triceps brachii, and surface of triceps brachii, while deep and surface of plantaris had higher oxidative enzyme activity than surface of triceps brachii.

Relationship between fiber cross-sectional area and oxidative enzyme activity

An inverse relationship between cross-sectional areas and oxidative enzyme activities of fibers in the soleus, plantaris, biceps brachii, and triceps brachii muscles was observed (Fig. 4).

DISCUSSION

In this study, an inverse relationship between cross-sectional areas and oxidative enzyme activities of fibers in the soleus and plantaris (hindlimb) muscles and biceps brachii and triceps brachii (forelimb) muscles of rats was observed (Fig. 4). This indicates that smaller fibers have higher oxidative enzyme activities than larger fibers in the muscles, regardless of the muscle type. This result was consistent with our previous studies using hindlimb muscles, including the soleus, plantaris, extensor digitorum longus, and tibialis anterior muscles in rats [2, 3, 9].

The biceps brachii and triceps brachii muscles are unique because the fibers in the surface regions, where only type IIB fibers were located, of those muscles had large cross-sectional areas while low oxidative enzyme activity. It is considered that the low oxidative enzyme activity of fibers in the surface region of the biceps brachii and triceps brachii muscles is because they do not need to work as anti-gravity muscles.

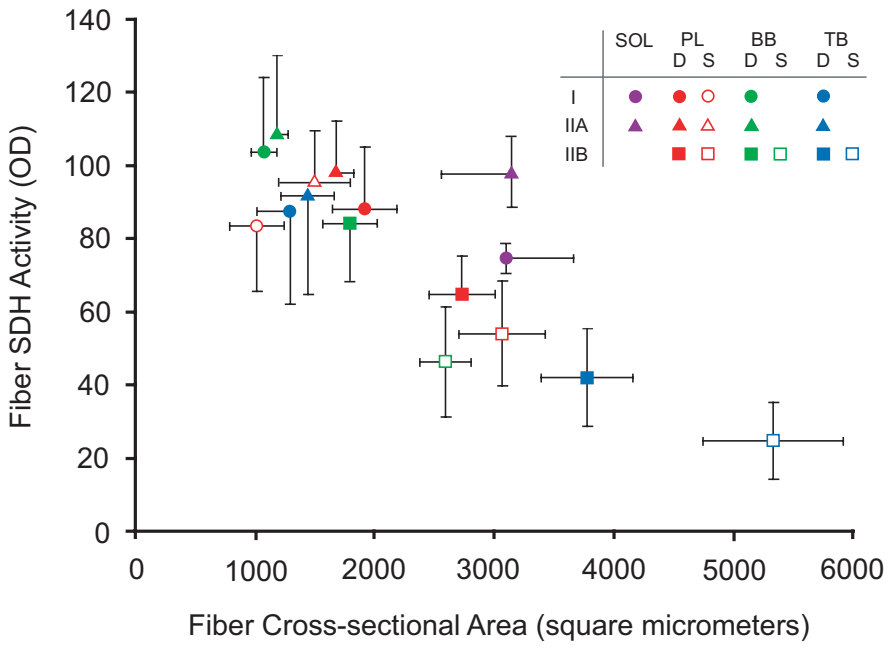

Fig. 4. Relationship between cross-sectional areas and oxidative enzyme activities of type-identified fibers in the rat soleus, plantaris, biceps brachii, and triceps brachii muscles. Data indicate the means and standard deviations (n = 8). SDH, succinate dehydrogenase; OD, optical density; SOL, soleus; PL, plantaris; BB, biceps brachii; TB, triceps brachii; D, deep region; S, surface region. An inverse relationship is observed between cross-sectional areas and oxidative enzyme activities of fibers in the muscles (r = –0.825, $p < 0.05$, n = 16).

Type IIB fibers in the deep regions of the biceps brachii and triceps brachii muscles showed higher oxidative enzyme activity than the same type of fibers in the surface regions of the corresponding muscles. This indicates a differing functional and metabolic demand for type IIB fibers between the deep and surface regions of those muscles. This may be because supplies of oxygen and substrates for oxidative metabolism from capillaries, which are located close to the membrane and are highly dense in the deep region of the muscle, are more plentiful in smaller fibers in the deep region.

Muscle fiber type-specific adaptations under a variety of conditions, such as hypobaric [19] or hyperbaric [20, 21] exposure and increased [22, 23] or decreased [24] neuromuscular activity, are observed. Furthermore, patients with peripheral arterial disease [25] and animal models with mutation in hemoglobin [26, 27], hypertension [28], or diabetes mellitus [29, 30] have different patterns of fiber-type distribution compared with controls. Type shifts of fibers from high-oxidative type I to low-oxidative type II were observed in those patients and animal models. However, the mechanisms for metabolic and functional changes of fibers in the muscles are not clear. Exposure to microgravity causes fiber atrophy and type shift of fibers from high-oxidative type I to low-oxidative type II in the muscles, especially in antigravity muscles, e.g., the rat soleus muscle [31]. Decreased oxidative enzyme activity of spinal motoneurons innervating high-oxidative fibers in the muscles was observed following exposure to microgravity [32, 33]. It is of interest to note that the oxidative enzyme activity of alpha motoneurons innervating extrafusal fibers in hindlimb muscles decreased after exposure to microgravity, whereas there was no change in the oxidative enzyme activity of alpha motoneurons innervating extrafusal fibers in forelimb muscles after exposure to microgravity [34]. In contrast, no data are available comparing the fiber properties of fore- and hind-limb muscles following exposure to microgravity. It is expected that forelimb muscles do not have severe responses to microgravity and unloading in the fiber-type distribution, cross-sectional area, or oxidative enzyme activity compared with hindlimb muscles based on the findings observed in this study.

In summary, this study combined with our previous studies [2, 3, 5] observed that fiber-type distributions, cross-sectional areas, and oxidative enzyme activities are muscle type- and region-specific. It is concluded that there are metabolic and functional significances of the biceps brachii and triceps brachii muscles, especially in the surface regions, where only type IIB fibers having larger cells and lower oxidative enzyme activity are located.

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