Individual Variation in Myofiber Type Composition in the Triceps Surae and Flexor Digitorum Superficialis Muscles of Japanese Macaques

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Abstract Individual variation in composition of myofiber types (SO, FOG, and FG) was examined in the m. triceps surae and m. flexor digitorum superficialis of male and female Japanese macaques. The m. soleus generally had more SO myofibers than FOG and FG myofibers. The m. gastrocnemius and m. flexor digitorum superficialis had more FG myofibers than the other myofiber types. No sex-related differences in composition of myofiber types were noted in the muscles. Great differences in myofiber type percentages existed among the animals. The proportion of SO myofibers ranged from 32.8 to 95.9% in the m. soleus, from 8.1 to 50.5% in the m. gastrocnemius caput medialis, from 6.5 to 43.8% in the caput laterale, and from 3.2 to 44.7% in the m. flexor digitorum superficialis. Interindividual differences in myofiber type composition may be determined partially by genetic factors and be due partially to differences in capacity for changing myofiber types to meet postural or locomotory requirements.

Key Words: individual variation, Japanese macaque, leg muscle, myofiber type, myofiber type composition

INTRODUCTION

Skeletal muscles of primates are composed mainly of three myofiber types that differ in enzyme-histochemical characteristics (Sickles and Pinkstaff, 1981a; Edgerton et al., 1975; McIntosh et al., 1985; Acosta and Roy, 1987; Suzuki and Hayama, 1991). Myofibers are classified into slow-twitch/oxidative (SO) myofibers (Edgerton et al., 1975; Sickles and Pinkstaff, 1981a; Acosta and Roy, 1987; Suzuki and Hayama, 1991) or type I myofibers (McIntosh et al., 1985), fast-twitch/oxidative/glycolytic (FOG) myofibers or type IIA myofibers, and fast-twitch/glycolytic (FG) myofibers or type IIB myofibers by differences in histochemical reactivity for myosin ATPase and dehydrogenases.

A proportion of myofiber types in muscles varies from muscle to muscle. The soleus muscles of tree shrews, lesser bushbabies, slow lorises (Sickles and Pinkstaff, 1981b), cynomologi (Acosta and Roy, 1987), and Japanese macaques (Suzuki and
Hayama, 1991) have numerous SO myofibers, whereas the gastrocnemius and flexor digitorum superficialis muscles contain many fast-twitch myofibers (FOG plus FG). In humans, the soleus muscles have more SO myofibers than do the gastrocnemius muscles (Johnson et al., 1973). Postural or antigravity muscles have many SO myofibers, and propulsive or locomotory muscles have numerous fast-twitch myofibers (Armstrong et al., 1982; Suzuki and Tamate, 1988; Smith et al., 1977; Walmsley et al., 1978; Burke, 1981). The composition of myofiber types in muscle reflects an aspect of function that a muscle has.

Interindividual differences in proportion of myofiber types have been reported to exist in the triceps surae and flexor digitorum superficialis muscles of female Japanese macaques (Suzuki and Hayama, 1991) as well as in human muscles (Johnson et al., 1973). The muscles generally give larger variations of myofiber type percentages than do hindlimb muscles of rats (Armstrong and Phelps, 1984) and thigh muscles of sheep (Suzuki and Tamate, 1988). The purpose of the present study was to determine whether an interindividual difference in composition of myofiber type exists in muscles of male Japanese macaques. Also, sex-related differences in composition of myofiber types were examined by comparing the data from males with those of females (Suzuki and Hayama, 1991).

MATERIALS AND METHODS

Muscle samples were obtained from four male and four female Japanese macaques (Macaca fuscata) kept at the Primate Research Institute of Kyoto University. The ages and liveweight of the animals used were shown in Table 1. A small piece of paper as a marker was attached to superficial, deep, cranial, or caudal portion in samples for identification when they were sectioned. Whole transverse sections (1 cm thick) were removed from the belly of the m. gastrocnemius caput laterale and caput mediale, m. soleus, and m. flexor digitorum superficialis (m. plantaris). They

<table>
<thead>
<tr>
<th>No. of Animal</th>
<th>Sex</th>
<th>Age (year)</th>
<th>Liveweight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Male</td>
<td>7</td>
<td>10.1</td>
</tr>
<tr>
<td>M2</td>
<td>Male</td>
<td>10</td>
<td>10.2</td>
</tr>
<tr>
<td>M3</td>
<td>Male</td>
<td>7</td>
<td>11.6</td>
</tr>
<tr>
<td>M4</td>
<td>Male</td>
<td>7</td>
<td>8.6</td>
</tr>
<tr>
<td>F1</td>
<td>Female</td>
<td>7</td>
<td>6.9</td>
</tr>
<tr>
<td>F2</td>
<td>Female</td>
<td>6</td>
<td>6.3</td>
</tr>
<tr>
<td>F3</td>
<td>Female</td>
<td>23</td>
<td>8.3</td>
</tr>
<tr>
<td>F4</td>
<td>Female</td>
<td>8</td>
<td>7.1</td>
</tr>
</tbody>
</table>
were frozen in a mixture of acetone and dry ice, and cut on a cryostat. The whole cross sections (10 μm thick) were used to observe the distribution pattern of myofiber types throughout the muscles and to determine the exact sites for measurements on muscle sections (Figs. 1 and 2).

Unfixed cross sections were stained with myosin ATPase (Padykula and Herman, 1955) after preincubation at pH 4.3 and 10.5 (Suzuki and Cassens, 1980a) and with NADH dehydrogenase (NADH-D) and menadione-linked glycerol-3-phosphate dehydrogenase (3-GPD) (Lojda et al., 1979). Photomicrographs were taken of muscle sections stained with myosin ATPase after acid and alkaline preincubation; they were used for classifying and counting myofiber types. The middle portion of the flexor digitorum superficialis and soleus muscles and the cranial and caudal portions of the gastrocnemius muscle were photographed for measurement. A total of 910 to 1,210 myofibers were examined in each section. Means of percentages between myofiber types were compared by student’s t-test. Differences between means were considered significant P<0.05.

RESULTS

1. Classification of myofiber types

Myofibers that reacted strongly for acid-stable myosin ATPase and were weakly reactive or unreactive for alkali-stable myosin ATPase stained strongly with NADH-D and weakly with 3-GPD; those were classified as slow-twitch/oxidative (SO) myofibers (Peter et al., 1972; Suzuki and Hayama, 1991, Figs. 3–6). They correspond to type I myofibers (Brooke and Kaiser, 1970). Myofibers that were unreactive or weakly reactive for myosin ATPase after preincubation at pH 4.3 and strongly reactive for alkali-stable myosin ATPase were classified as fast-twitch myofibers: those are type II myofibers. The fast-twitch myofibers were classified into fast-twitch/oxidative/glycolytic (FOG) myofibers, which stained moderately to strongly with NADH-D and 3-GPD, and into fast-twitch/glycolytic (FG) myofibers, which stained weakly with NADH-D and strongly with 3-GPD. Myofibers that reacted moderately to strongly for both acid-stable and alkali-stable myosin ATPase were categorized as type IIC (Brooke and Kaiser, 1970; Figs. 7 and 8), which stained strongly with NADH-D and moderately to strongly with 3-GPD.

2. Composition of myofiber types

In the male, the soleus muscles tended to have larger percentages of SO myofibers than FOG myofibers, and smallest percentages of FG myofibers (Table 2). The soleus muscles of females had more numerous SO myofibers than FOG and FG myofibers (P<0.05). In the males and females, the m. gastrocnemius and m. flexor digitorum superficialis generally had larger percentages of FG myofibers than those
Figs. 1 and 2. Whole cross sections of the soleus muscle of Japanese macaques. Myosin ATPase reaction after preincubation at pH 4.3. Fig. 1. Animal M1. Dark dots show a distribution of SO (type I) myofibers. Fig. 2. Animal M3. White spots dispersed in the darkly stained cross section occupied by SO myofibers show a distribution of fast-twitch (type II) myofibers. Note great differences in distribution of SO myofibers between the two animals. ×2.

Figs. 3-6. Histochemical profiles of myofiber types in the gastrocnemius muscle of Japanese macaques. Serial cross sections stained with myosin ATPase after preincubation at pH 4.3 (Fig. 3) and at pH 10.5 (Fig. 4), with NADH dehydrogenase (Fig. 5), and with menadione-linked glycerol-3-phosphate dehydrogenase (Fig. 6). F: FOG myofiber; G: FG myofiber; S: SO myofiber. ×100.

Figs. 7 and 8. Type IIC myofibers in the soleus muscle of animal M4. Serial cross sections stained with myosin ATPase after preincubation at pH 4.3 (Fig. 7) and at pH 10.5 (Fig. 8). I: SO (type I) myofibers; II: fast-twitch (type II) myofibers; C: type IIC or intermediate type myofiber. ×100.

Figs. 9 and 10. Atrophic myofibers in the flexor digitorum superficialis muscle. Serial cross sections stained with myosin ATPase after preincubation at pH 10.5 (Fig. 9) and with NADH dehydrogenase (Fig. 10). Atrophic angulated (large arrow) and elongated (small arrow) myofibers are distributed among normal myofibers, and show histochemical profiles similar to those of FG myofibers (G). ×130.

Table 2. Mean proportion (%) of myofiber types in the triceps surae and flexor digitorum superficialis muscle of male and female Japanese macaques

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Portion</th>
<th>Sex</th>
<th>SO</th>
<th>FOG</th>
<th>FG</th>
<th>IIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>M</td>
<td>male</td>
<td>58.7±29.1</td>
<td>25.7±22.2</td>
<td>7.0± 8.6</td>
<td>8.6±10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>81.8±14.9</td>
<td>15.5±12.2</td>
<td>2.7± 2.9</td>
<td>0</td>
</tr>
<tr>
<td>Gastrocnemius caput mediale</td>
<td>Cr</td>
<td>male</td>
<td>24.6±14.5</td>
<td>22.1±13.6</td>
<td>45.3±10.3</td>
<td>8.0±12.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>33.0±13.0</td>
<td>24.6± 7.6</td>
<td>42.2±10.3</td>
<td>0.2± 0.6</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>male</td>
<td>12.2± 9.5</td>
<td>30.6±14.7</td>
<td>52.8± 2.2</td>
<td>4.4± 5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>13.7± 9.3</td>
<td>27.0±15.5</td>
<td>58.9±16.1</td>
<td>0.4± 0.8</td>
</tr>
<tr>
<td>Caput laterale</td>
<td>Cr</td>
<td>male</td>
<td>22.4±16.0</td>
<td>33.5±11.9</td>
<td>40.2±19.2</td>
<td>3.9± 5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>25.5± 9.2</td>
<td>25.3± 7.3</td>
<td>49.2±13.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>male</td>
<td>8.8±10.0</td>
<td>35.0± 5.1</td>
<td>55.1±10.9</td>
<td>1.1± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>13.7± 5.9</td>
<td>31.7±10.8</td>
<td>53.0±14.2</td>
<td>1.6± 0.7</td>
</tr>
<tr>
<td>Flexor digitorum superficialis</td>
<td>M</td>
<td>male</td>
<td>21.8±21.3</td>
<td>34.7±14.7</td>
<td>41.3±11.8</td>
<td>2.2± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>female</td>
<td>21.9±11.2</td>
<td>30.7± 6.5</td>
<td>47.1±16.7</td>
<td>0.3± 0.6</td>
</tr>
</tbody>
</table>

a Ca: caudal; Cr: cranial; M: middle
b Mean ±S.D. (n=4)
of FOG myofibers. In these muscles, percentages of SO myofibers were similar to or smaller than those of FOG myofibers. All the muscles had smaller percentages of type IIC myofibers than of the other three myofiber types.

3. Comparison of myofiber type percentages between males and females

The mean percentages of SO myofibers in the soleus muscle of the females were larger than those in the males, but no significant differences in percentage existed between males and females (Table 2). Similarly, no significant differences in percentages of FOG and FG myofibers existed between males and females. No sex-related differences in myofiber type percentages were observed in other muscles examined. The m. triceps surae and m. flexor digitorum superficialis gave large values of standard deviation of samples in males and females. The distribution patterns of myofiber types in male’s muscles were similar to those in female’s muscles.

4. Differences in percentages of myofiber types among individuals

The percentages of SO myofibers in the m. soleus differed most greatly among the individuals of both sexes (Table 3). The proportion of SO myofibers in the m. soleus ranged from 32.8 to 95.9%, from 8.1 to 50.5% in the m. gastrocnemius caput mediale, from 6.5 to 43.8% in the caput laterale, and from 3.2 to 44.7% in the m. flexor digitorum superficialis. The m. soleus of animals M3, F2, and F4 had about more than 90% SO myofibers. The m. soleus of animals M1 and M2 had smaller percentages of SO myofibers than did that of the other males and females. Similarly, the gastrocnemius and flexor digitorum superficialis muscles of M1 and M2 had smaller percentages of SO myofibers than did those of all other animals. The male’s muscles tended to have larger variations in SO myofiber percentages than did the female’s muscle. The proportions of SO myofibers in the muscles were not related with liveweight and aging (Tables 1 and 3).

The muscles with smaller percentages of SO myofibers generally had larger percentages of FG myofibers than those with larger percentages of SO myofibers in each animal. Variations in percentages of FOG myofibers were generally smaller than those of FG and SO myofibers. The muscles of animal M4 had larger percentages of type IIC myofibers than did those of the other animals. The type IIC myofibers were distributed more in the m. soleus and m. gastrocnemius caput mediale than in the caput laterale and m. flexor digitorum superficialis.

5. Atrophic myofibers

Atrophic myofibers assuming a compressed angular or elongated configuration were found in the muscles of animals F3 and M4 (Figs. 9 and 10). Almost all of the myofibers showing the angulated or elongated configuration were of FG myofibers.
They were distributed more in the m. gastrocnemius and m. flexor digitorum superficialis than in the m. soleus.

**DISCUSSION**

Differences in percentages of myofiber types among individuals existed in the muscles of the males and females. Variations in myofiber type percentages in the muscles of Japanese macaques are larger than those in muscles of sheep (Suzuki and Tamate, 1988) and rats (Armstrong and Phelps, 1984). A great difference in myofiber type percentages among individuals has been reported in muscles of humans (Johnson et al., 1973; Doriguzzi et al., 1984; Mahon et al., 1984; Lindman et al., 1990, 1991) and house shrews (Suzuki, 1990). The interindividual difference in the muscles of Japanese macaques is not necessarily larger than that of humans.

No sex-related differences in composition of myofiber types were noted in the Japanese macaques. The proportion of SO myofibers in the soleus muscle has been reported to be greater in female and castrated mice than in male mice (Vaughan et al., 1974). In humans, the distribution pattern of SO myofibers differs slightly between the male and female in the m. tibialis anterior (Henriksson-Larsén, 1985), but not in the m. trapezius (Lindman et al., 1991). The leg muscles of Japanese macaques showed no differences in distribution patterns of myofiber types between the males

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Myofiber type</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>Soleus</td>
<td>SO</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>49.6</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>7.1</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>SO</td>
<td>8.1</td>
</tr>
<tr>
<td>caput mediale cranial portion</td>
<td>FOG</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>1.3</td>
</tr>
<tr>
<td>Caput laterale cranial portion</td>
<td>SO</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>39.4</td>
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<tr>
<td></td>
<td>FG</td>
<td>54.1</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>0</td>
</tr>
<tr>
<td>Flexor digitorum superficialis</td>
<td>SO</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>54.9</td>
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<td>FG</td>
<td>39.6</td>
</tr>
<tr>
<td></td>
<td>IIC</td>
<td>2.3</td>
</tr>
</tbody>
</table>
and females.

Fast-twitch myofibers (type II) have been shown to be transformed into slow-twitch myofibers (type I) in prenatal development (Beermann et al., 1978; Schloon et al., 1979) and postnatal growth (Kugelberg, 1976). In growing pigs and sheep (Suzuki and Cassens, 1980b, 1983), the transformation ends from 8 to 16 weeks of age. The transformation of myofiber types by growth is presumed to be completed in the muscles of Japanese macaques aged over 6 years.

Increases in postural activity and in liveweight during growth have been considered to induce an transformation from fast-twitch myofibers to slow-twitch myofibers (Kugelberg, 1976; Suzuki and Cassens, 1980b, 1983). Although differences in liveweight among individuals may influence a variation in percentages of myofiber types in muscle, no relationship of liveweight to myofiber type percentages was recognized in leg muscles of Japanese macaques.

The muscles of animal M4 had many type TIC myofibers, which have been regarded as a transitional form in transformation of fast-twitch myofibers into slow-twitch myofibers (Kugelberg, 1976; Suzuki and Cassens, 1980b, 1983). Hence, changes in myofiber types may occur in the muscles of animal M4. The sum (90.3%) of percentages of SO and type IIC myofibers in the soleus muscle of this animal corresponds to the mean values (90.8%) of percentages of SO myofibers in animals M3, F1, F2, and F4, which had large percentages of SO myofibers.

Long-term endurance exercise induces increases in SO myofibers (Luginbuhl et al., 1984; Kovanen and Suominen, 1987) and causes a transformation from FG myofibers into FOG myofibers and from FOG myofibers to SO myofibers (Baumann et al., 1987; Staron et al., 1987). On the other hand, a decrease in percentage of SO myofibers occurs in hindlimb immobilization (Edgerton et al., 1975) or suspension (Desplanches et al., 1987).

The presence of atrophic angulated myofibers in animals F3 and M4 is not related with occurrence of type IIC myofibers because the muscles of F3 have very few type IIC myofibers only in the flexor digitorum superficialis muscle. The configuration of the angular myofibers resembles that of angulated myofibers observed in the denervated muscles (Jennekens, 1982) but is dissimilar to that of atrophied myofibers in disused muscle (Suzuki, 1988). Therefore, the disuse of muscle is not assumed to cause an occurrence of many type IIC myofibers in M4, although the intermediate type of myofibers occurs in the experimentally induced disuse of muscle in rats (Desplanches et al., 1987).

The distribution patterns of atrophic angulated myofibers in muscles of animals F3 and M4 resemble those observed in early denervation of muscle (Jennekens, 1982). Partial denervation brings about collateral sprouting of nearly intact axons and leads to reinnervation with transformation of myofiber types (Jennekens, 1982). The atrophic angulated myofibers have been noted in the gastrocnemius muscle of
old rats, in which the total number of myofibers decreases and total slow-twitch myofiber number increases (Kanda and Hashizume, 1989). This phenomena are interpreted as to indicate that some myofibers denervated by motoneuronal death are reinnervated by other motoneuron (Foehring et al., 1986; Kanda and Hashizume, 1989).

The angulated myofibers in muscles of animals F3 and M4 are probably deprived of nerves that innervated FG myofibers, because almost all atrophic angulated myofibers were FG myofibers. Type IIC myofibers in the muscles of M4 may indicate an intermediate stage during transformation from denervated fast-twitch myofibers into SO myofibers by reinnervation of SO motoneurons. However, it is unclear why some type II myofibers are denervated in Japanese macaque’s muscles.

In this study, the muscles of the oldest female animal had small percentage of SO myofibers than those of younger female animals. Great differences in percentages of SO myofibers among the individuals in the males and females are not attributable to aging. The percentages of slow-twitch myofibers increase in human muscles (Larsson et al., 1978) and in rat muscles (Kovanen and Suominen, 1987; Kanda and Hashizume, 1989). The proportions of slow-twitch myofibers have been reported to increase in the soleus and diaphragm muscles, but are unchanged in the extensor digitorum longus and tibialis anterior muscles in aged rats; a total number of myofibers is unchanged in the soleus and extensor digitorum longus muscles (Eddinger et al., 1985; Larsson and Edström, 1986). An increase in the total number of slow-twitch myofibers has been shown in the gastrocnemius caput mediale muscle of old rats (Kanda and Hashizume, 1989). Changes or no changes in myofiber type percentages in aging vary among muscles.

The presence of many type IIC myofibers in animal M4 indicates a possibility of changing myofiber types in a middle age in Japanese macaques. Muscle activities of Japanese macaques living in forests and fields may differ from those of macaques kept in a cage or pen because of differences in postural and locomotory requirements: sitting, standing, walking, running, jumping, and climbing.

Japanese macaques living in forests and fields move freely on the ground and in the trees. Their muscles need to generate a large propulsive force for running, jumping, climbing, and leaping. The motor units composed of FG myofibers must be recruited for running, jumping, climbing, and leaping in addition to recruitments of the motor units of SO and FOG myofibers (Smith et al., 1977; Walmsley et al., 1978; Burke et al., 1973, 1974; Burke, 1981). The motor units composed of SO myofibers are recruited for standing and walking, and motor units of FOG myofibers for running and jumping in addition to recruitments of motor unit of SO myofibers. Changes in life modes for animals may give rise to differences in postural or locomotory requirements and influence recruitments of motor units of SO, FOG,
and FG myofibers. A possible explanation for co-existence of type IIC myofibers and angulated myofibers in M4 is that parts of fast-twitch myofibers reinnervated by SO motoneurons after denervation consequently show an intermediate stage during transformation into SO myofibers to meet functional requirements for maintaining sitting and standing positions. No data on frequencies and duration of sitting, standing, walking, running, etc. were obtained in the animals examined.

The gastrocnemius and flexor digitorum superficialis muscles of the slow loris have more SO myofibers than do those of the tree shrew and lesser bushbaby (Sickles and Pinkstaff, 1981b) and the macaques (Acosta and Roy, 1987; Suzuki and Hayama, 1991). The triceps surae and flexor digitorum superficialis muscles of the house shrew have no SO myofibers (Suzuki, 1990). It is considered that the differences in composition of myofiber types in analogous muscles are correlated with locomotory patterns of animals (Sickles and Pinkstaff, 1981b; Suzuki, 1990), but not with the phylogenetic classification.

The great differences in myofiber type percentage in leg muscles among Japanese macaques are considered to show individual variation and may be determined partially by genetic factors. Lindman et al. (1990) have suggested that interindividual differences in myofiber type composition in human muscles are due, at least in part, to genetic factors. The muscle of Japanese macaques is assumed to have a capacity for transforming myofiber types to meet postural or locomotory requirements and the difference of the capacity to be a cause of individual variation in composition of myofiber types in leg muscles.

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REFERENCES


