Specific Mitochondrial Responses to Running Training are Induced in Each Type of Rat Single Muscle Fibers

Hiroaki TAKEKURA* and Toshitada YOSHIOKA**

* Department of Physiology and Biomechanics, National Institute of Fitness and Sports, Kanoya, 891-23 Japan
** Department of Physiology, St. Marianna University School of Medicine, Miyamae-ku, Kawasaki, 213 Japan

Abstract The effects of training by running (sprint or endurance) on the mitochondrial volume, number, and succinate dehydrogenase (SDH) activity of different types of single muscle fibers, and on the elemental composition of soleus and extensor digitorum longus muscles were studied employing histochemical, electron microscopic, and electron probe-micro analysis (EPMA). Newly weaned male Wistar rats were trained on a motor-driven treadmill endurance exercise for 14 weeks. The relative mitochondrial volume per single muscle fiber of slow-twitch oxidative (SO) fiber was significantly increased following endurance training ($p < 0.01$). There was no significant correlation between mitochondrial volume and number of SO fibers following endurance exercise training. Following sprint training, both mitochondrial volume and number of fast-twitch oxidative glycolytic (FOG) fibers increased significantly ($p < 0.01$), with significant correlation ($r = 0.69$) between these parameters. SDH activity was higher in the order of SO, FOG, and fast-twitch glycolytic (FG) fibers following endurance training; however, the activity was higher in the order FOG, SO, and FG fibers following sprint training. The potassium concentration in cytoplasm of the soleus muscle showed a tendency to decrease following both types of training. These results suggest that the oxidative capacity of each type of muscle fiber does not always increase equally following training. Changes in mitochondrial number and volume in response to the two different types of training differed according to the type of fiber.

Key words: single muscle fiber, fiber type, mitochondria, elemental composition, running training.
It has been reported that histochemical and biochemical changes are induced in skeletal muscle as a result of regular endurance and sprint exercise training (Benzi et al., 1975). Glycolytic and oxidative enzyme activities were increased following sprint (Saubert et al., 1973; Staudte and Exner, 1973) and endurance exercise training (Riedy et al., 1985), respectively. Moreover, high-intensity interval training caused an increase in oxidative enzyme activities associated with structural changes in the skeletal muscle (Hickson et al., 1976). Increases in the activity of oxidative enzymes following exercise training accompanied by increased mitochondrial number and/or volume in skeletal muscle have been reported by Holloszy and Booth (1970). However, the authors recently reported that the metabolic profiles of a given type of muscle fiber were not always influenced in the same way by exercise training (Takekura and Yoshioka, 1987, 1989). It has been reported that the characteristics of skeletal muscle mitochondria are fiber type specific, that is, the relative mitochondrial volume per muscle fiber of slow-twitch fiber is significantly greater than that of fast-twitch fiber (Eisenberg and Salmon, 1980, 1981). It has also been reported that the mitochondria were swollen in response to hypoxia (Pelosi and Agliati, 1968), lower pH (Cereijo-Santalo, 1966), and changes in elemental composition (Lehninger, 1962) of muscle cells; however, it is not clear how exercise training affects mitochondrial number and volume in different types of muscle fibers. It is not clear either whether the elemental composition in muscle cell is changed by exercise training. Therefore, the present study was designed to investigate the effects of exercise training on the mitochondrial structure and activity of succinate dehydrogenase (SDH) in various type of rat single skeletal muscle fiber and elemental composition.

**MATERIALS AND METHODS**

*Animal and training procedure.* Male Wistar rats (3 weeks old, weighing 40–45 g, n=15) were used. They were divided into the following three groups: sedentary control (C), sprint exercise training (S), and endurance exercise training (E). For grouping, the animals were matched according to their body weight (50–52 g) after preliminary training (at 30 m/min, 0 degree, 10 min/day for 1 week) using motor-driven treadmill for rodents. The rats in exercise training groups (S and E) were given a program of treadmill running for 14 weeks in the same manner as described in our previous report (Takekura et al., 1985). The exercise training was performed once a day, 5 days per week. Finally, the rats in group S were loaded with interval running for 45 s at a speed of 85 m/min, 10 times with 2.75-min intervals each day. The rats in group E were run continuously for 120 min daily at a speed of 40 m/min. Water and food were supplied to both groups ad libitum for 24 h.

*Muscle sampling.* The enzyme activity of the skeletal muscle increased following acute exhaustive exercise and normalized after 48 h (Takekura and Yoshioka, 1988a). Furthermore, Gollnick and King (1969) reported that swelling and/or enlargement of mitochondria in the muscle cells occurred following acute
exhaustive exercise. Therefore, all rats in both training groups were sacrificed 48 h after the last training program in the present study. Rats were anesthetized with i.p. injections of sodium pentobarbital (40–42 mg/kg body weight); soleus (SOL) and extensor digitorum longus (EDL) muscles were sampled from both legs as quickly as possible, and adipose and connective tissues were removed.

Ultrastructural study in single muscle fiber. Each type of single muscle fiber was dissected from both SOL and EDL in ice-cold relaxing solution for mammals (120 mM KCl, 0.5 mM EGTA, 4 mM MgCl₂, 10 mM PIPES, 10 mM ATP, pH 6.8). Several fragments were cut off from the fiber ends, and were separated longitudinally into two. Then they were placed on glass slides with the intact portion of the cell membrane facing the glass, and stained by two methods in order to classify the fiber type. Histochemical staining was done for actomyosin ATPase (GUTH and SAMARA, 1970; both at pH 4.35 and 10.4 for pre-incubation) and SDH (NACHLAS et al., 1957). Based on the staining characteristics, all of the single muscle fibers were classified into the following three types: SO, slow-twitch oxidative; FOG, fast-twitch oxidative glycolytic; FG, fast-twitch glycolytic (PETER et al., 1972). The remaining portion of the single fiber was used for biochemical and electron microscopical analyses. Fiber was fixed with cold Ringer's solution containing 2.5% glutaraldehyde (GA) for 30–60 min and then submerged in cold 0.05 M phosphate buffer solution with 2.5% GA for 30 min. After rinsing in the buffer solution, each muscle fiber was fixed further in 1% osmium tetroxide in the phosphate buffer solution at 4°C. Each fiber was then dehydrated with graded (80, 90, 95, and 100%) ethanol, embedded with Epon or Spurr resin mixture, and sectioned. Ultrathin sections were cut longitudinally and transversely using a microtome (LKB Co. Ltd., Bromma, Sweden), and were observed by an electron microscope (JEOL-100C, Nihon Denshi Co. Ltd., Tokyo, Japan) with a calibration grid (No. 6002; 54.864 per inch; Ernest F Fullan Inc., New York, U.S.A.). Random photographs were taken at magnifications of x 5,000 and x 10,000. Stereological measurements were performed using a point-counting method (WEIBEI, 1969) for quantitative analysis of the mitochondrial volume (%) per muscle fiber. The point-counting method that was used for estimating the relative mitochondrial volume in each type of single muscle fiber was performed using electron micrographs (x 5,000 and x 10,000). Section paper was put on the electron micrographs, and enumerated the points those were put on the mitochondria. Finally, relative mitochondrial volume (%) was calculated using the following equation:

\[
\text{Mitochondrial volume (\%)} = \frac{\text{enumerated points (on the mitochondria)}}{\text{total number of points in section paper}}
\]

Mitochondrial number and Z-line width were also analyzed in the same fiber. Mitochondrial number was analyzed in a certain area (168 \(\mu\)m²) of each type of single muscle fiber.

Analysis of succinate dehydrogenase. A portion of the fiber, typed histochemically using a corresponding portion, was fully homogenized using a micro-glass
homogenizer in an ice-cold phosphate buffer (0.1 M, pH 7.4) for measurement of SDH (EC:1.3.99.1) according to the method of Cooperstein et al. (1950). Enzyme activity was expressed as \( \mu \text{mol/(min \cdot mg)} \) protein. Total protein was measured by the method reported by Goldberg (1973).

**Electron probe X-ray microanalysis.** For electron probe X-ray microanalysis (EPMA), both muscles (SOL and EDL) were frozen in liquid freon (\(-165 \pm 5, \text{ mean} \pm \text{S.D.}, \text{ C}\)) super-cooled by liquid nitrogen, and frozen ultrathin sections were cut. The methods for quantitative analysis of the elemental compositions (Na, Mg, P, S, Cl, K, and Ca) have been published elsewhere (Shuman et al., 1976; Yoshioka and Somlyo, 1984, 1987). In the present study, elemental compositions were analyzed in cytoplasm of muscle fiber in each group.

### RESULTS

**Mitochondrial response**

The histogram of the relative mitochondrial volume in each type of fiber of group C is shown in Fig. 1. The volume was analyzed by stereological analysis. It was recognized that the mitochondrial volume in each type of fiber overlaps over a considerable range. Mitochondrial volume in SO fiber of group E was significantly \((p < 0.01)\) greater than those of groups C and S (Figs. 2 and 3). In FOG fiber, mitochondrial volume was significantly larger in group S than in the other two groups \((p < 0.01)\). In FG fiber, mitochondrial volume was unchanged by exercise training.

Mitochondrial numbers of SO fibers in group S were significantly less than in group C \((p < 0.05, \text{ Fig. 4})\). Significantly more mitochondria were observed in FOG fibers of group S than group C \((p < 0.01)\) and E \((p < 0.01)\). There was no significant difference among these groups in terms of FG fibers (Fig. 4).

Relationships between mitochondrial volume and Z-line width are shown in Fig. 2. In group C, there was no significant overlap in Z-line width. The Z-line width in SO fiber was reduced following sprint exercise training \((p < 0.01)\), and was increased following endurance training \((p < 0.01)\). Significant increase of Z-line width occurred \((p < 0.01)\) in FOG fiber following sprint training. In FG fiber, there was no significant change following exercise training.

The SDH activity was increased in all fiber types by endurance training (Figs. 3 and 4, \(p < 0.01)\). Sprint training caused an elevation of SDH activity only in SO and FOG fibers \((p < 0.01)\).

**Elemental composition**

The concentrations of P, S, and K in skeletal muscle cell tended to be higher, in general, and those of Na, Mg, and Ca were lower (Table 1). There was no significant difference between each group for any ionic concentration of SOL and EDL, although K concentration in SOL showed a tendency to decrease following both types of exercise training.
The relative mitochondrial volume and SDH activity in FOG fiber were increased following sprint training (Figs. 2 and 3). These results indicate that the resistance to fatigue in FOG fiber was increased following short-term high-intensity running. Such results may have been obtained because the sprint running was repeated 10 times at intervals of 2.75 min. This training protocol might stimulate
mitochondrial bioenergetics as well as speed-related factors. Increase of relative mitochondrial volume in SO fiber following endurance training and in FOG fiber following sprint training may be caused by a selective recruitment by motor units depending on the exercise intensities, as was reported by Edgerton et al. (1969) and Edgerton (1970). Positive recruiting of SO fiber following endurance and of FOG fiber following sprint training varied according to exercise intensity.

The Z-line of the skeletal muscle fibers is reported to be composed of α-actinin which is about 100 kD in molecular weight (Ebashi et al., 1964). Actinin possesses a three-dimensional structure binding with thin filaments (Goldstein et al., 1980).

Fig. 2. Relationship between mitochondrial volume (%) and Z-line width (nm) in each type of muscle fiber of sedentary control, sprint training, and endurance training groups. Sedentary control group was analyzed at 17 weeks after birth. Abbreviations are the same as in Fig. 1.

Japanese Journal of Physiology
The Z-line has a role in the cytoskeleton (Kelly and Chahill, 1972) and participates in development of tension at the muscle contraction (Elliott, 1973). Because the width of the Z-line varies according to type, it is used as a marker for fiber type classification on electron micrographs. In the present study, the histochemical method was employed in addition to the currently used morphological method. The results suggested that the method was appropriate for classification of fiber type on the electron micrographs, and revealed that the Z-line width was related to contraction speed and metabolic profiles. It has been reported that the Z-line width was altered by tenotomy (Pesnick et al., 1968) or by exercise (Takekura and Yoshioka, 1987, 1988b). In the present study, the Z-line width was changed following both sprint and endurance trainings. However, it is unclear whether or not the alterations in the Z-line width were accompanied by receipts and disbursements of α-actinin molecules.

Although an increased mitochondrial volume was observed in SO and FOG fiber following endurance or sprint training, the mitochondrial number did not always increase following the same exercise training. The relationship between

Vol. 39, No. 4, 1989
mitochondrial number and volume is shown in Table 2. Significantly positive (p < 0.05) correlations were observed in FOG fibers of groups S and E, but not in SO fiber of group E. These results suggest that an elevation of oxidative capacity of muscle fiber may be caused by an increased mitochondrial number in FOG fiber and by increased mitochondrial volume in SO fiber following exercise training.

It was reported that swelling or enlargement of mitochondrial volume in muscle cells was induced by hypoxia (PELOSI and AGLIATI, 1968), anoxia (OUDEA, 1963), higher temperature (BROOKS et al., 1971), lower pH (CEREJO-SANTALO, 1966), increased DNA (LAUGENS and GONEZ-DUMM, 1968), and changes in elemental composition (LEHNINGER, 1962; LYMM and BROWN, 1965). Since blood flow in skeletal muscle is increased during endurance running (LAUGHLIN and ARMSTRONG, 1983), the cause of mitochondrial swelling in endurance training may not be hypoxia or anoxia. However, increased temperature must not be ignored as a possible factor. On the other hand, hypoxia, and/or lowered pH could be the cause of enlargement of mitochondria following sprint training. It is also possible that the mechanisms of swelling and/or enlargement of mitochondria in skeletal muscle...
SPECIFIC CHANGES IN SINGLE MUSCLE FIBER

Table 1. Elemental compositions of soleus (SOL) and extensor digitorum longus (EDL) muscles in sedentary control, sprint training, and endurance training groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Na</th>
<th>Mg</th>
<th>P</th>
<th>S</th>
<th>Cl</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>M</td>
<td>S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>76.0</td>
<td>23.0</td>
<td>550.0</td>
<td>423.0</td>
<td>65.0</td>
<td>880.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Sprint training</td>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>63.0</td>
<td>11.0</td>
<td>520.0</td>
<td>469.0</td>
<td>65.0</td>
<td>714.0</td>
<td>3.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>35.0</td>
<td>11.0</td>
<td>115.0</td>
<td>79.0</td>
<td>19.0</td>
<td>196.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Endurance</td>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>training</td>
<td>M</td>
<td>18.0</td>
<td>87.0</td>
<td>401.0</td>
<td>75.0</td>
<td>690.0</td>
<td>3.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>33.0</td>
<td>15.0</td>
<td>90.0</td>
<td>85.0</td>
<td>25.0</td>
<td>130.0</td>
<td>4.0</td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>M</td>
<td>S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>80.0</td>
<td>9.0</td>
<td>24.0</td>
<td>520.0</td>
<td>450.0</td>
<td>55.0</td>
<td>750.0</td>
</tr>
<tr>
<td>Sprint training</td>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>63.0</td>
<td>27.0</td>
<td>495.0</td>
<td>446.0</td>
<td>72.0</td>
<td>764.0</td>
<td>4.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>16.0</td>
<td>12.0</td>
<td>120.0</td>
<td>96.0</td>
<td>21.0</td>
<td>133.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Endurance</td>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>training</td>
<td>M</td>
<td>25.0</td>
<td>510.0</td>
<td>475.0</td>
<td>69.0</td>
<td>863.0</td>
<td>5.0</td>
</tr>
<tr>
<td>S.D.</td>
<td>23.0</td>
<td>9.0</td>
<td>109.0</td>
<td>83.0</td>
<td>27.0</td>
<td>159.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Values are expressed by mmol/kg dry weight of muscle. M, mean; S.D., standard deviation. Sedentary control group was analyzed at 17 weeks after birth.

Table 2. Relationship between mitochondrial volume (%) and mitochondrial number in each type of muscle fiber in sedentary control, sprint training, and endurance training groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber type</th>
<th>N</th>
<th>r</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>SO</td>
<td>9</td>
<td>0.1252</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>13</td>
<td>0.4060</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>5</td>
<td>0.5557</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sprint training</td>
<td>SO</td>
<td>10</td>
<td>0.4947</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>8</td>
<td>0.6921</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>9</td>
<td>0.2526</td>
<td>N.S.</td>
</tr>
<tr>
<td>Endurance</td>
<td>SO</td>
<td>13</td>
<td>0.0089</td>
<td>N.S.</td>
</tr>
<tr>
<td>training</td>
<td>FOG</td>
<td>12</td>
<td>0.6911</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>11</td>
<td>0.1717</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

SO, slow-twitch oxidative; FOG, fast-twitch oxidative glycolytic; FG, fast-twitch glycolytic fiber; N, number of muscle fibers measured; r, rank correlation. Sedentary control group was analyzed at 17 weeks after birth.

fibers following sprint and endurance exercise training might be different. HOLLOSZY and BOOTH (1970) reported that endurance training caused the increases of both mitochondrial volume and number. In general, it is thought that the
increase of both mitochondrial volume and number in skeletal muscle fiber following endurance training is parallel. In the present study, the adaptive patterns of mitochondrial volume and number in each type of single muscle fiber following running training are not completely equal. However, the different mechanism(s) of adaptive pattern of mitochondrial volume and number in skeletal muscle fiber to running training is not clear in the present study.

There was no significant difference in elemental composition of SOL and EDL muscles between the three groups. The concentration was measured in whole muscle, because it is difficult to judge the fiber type by EMPA analysis due to methodological problems. Elemental composition in skeletal muscle was maintained in dynamic equilibrium and the composition was not damaged by various physiological conditions (SOMLYO et al., 1981).

A slight decrease of K concentration in cytoplasm of SOL muscle (not statistically significant) following both types of exercise training might suggest that the mitochondrial swelling and/or enlargement was caused by Na-K transport through the cell membrane. If K were taken into mitochondria, H2O would also enter and swelling and/or enlargement would occur as a result (LEHNINGER, 1962). The concentration of P in mitochondria is higher than in other organs, because of the high amount of phospholipid that constitutes the mitochondrial membrane, and the K concentration is lower (PACKER et al., 1968). This study does not present data concerning elemental composition in mitochondria; thus, further studies are necessary to analyze small organelles of different types of single muscle fibers.

Oxidative capacities in skeletal muscle fiber following exercise training did not always increase equally in all types of muscle fibers. For example, endurance exercise training affected only SO fiber and sprint training increased SDH activity only in FOG fiber.

No relationship between mitochondrial volume and the Z-line width in group C (Fig. 2) was observed. Mitochondrial volume was increased but the Z-line width did not always increase in SO fiber following endurance exercise training. On the other hand, sprint training caused a reduction of the Z-line width without any change in mitochondrial volume in these fibers. These results suggest that the effects of exercise training on the mitochondrial alteration might differ depending upon the fiber type and the type of exercise.

The relationship between SDH activity and number of mitochondria of each type of fiber is shown in Fig. 4. The SDH activity was increased in all of the three types of fibers following endurance training. However, the mitochondrial number in all of the three types of fibers tended to decrease following endurance training. SDH activity was also increased following sprint training in SO and FOG fibers.

In SO fiber, SDH activity and relative mitochondrial volume increased following endurance training (Fig. 3). The activity of SDH in FOG fibers, but not mitochondrial volume, was elevated following endurance training. A significant difference (p < 0.05) was observed in relative mitochondrial volume, but not in SDH activity, between SO and FOG fibers following sprint training.
These results may suggest that the increase of oxidative capacity in skeletal muscle fiber following exercise training is not always due to the increase in mitochondrial volume. There are various types of adaptation patterns related to exercise intensity and fiber type.

The authors sincerely appreciate the many invaluable comments and encouragement given us by Associate Professor J. Patrick Barron, St. Marianna University School of Medicine, during his revision of the English manuscript.

REFERENCES


TAKEKURA, H. and YOSHIOKA, T. (1988b) Running training produces the metabolic and


