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The relationship of voluntary running to fibre type composition, fibre area and capillary supply in rat soleus and plantaris muscles

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Summary. Twenty 4-week-old Wistar rats exercised voluntarily in running wheels each day for 45 days. Fibre type composition, fibre cross-sectional area and the number of capillaries around a fibre of the slow-twitch soleus and fast-twitch plantaris muscles were examined and compared with animals which had no access to running wheels. The exercise group had a higher percentage of fast-twitch oxidative glycolytic (FOG) fibres and a lower percentage of fast-twitch glycolytic (FG) fibres in the deep portion of the plantaris muscle. The area of FOG fibres in the surface portion of the plantaris muscle was also greater in the exercise group. In the exercised animals, there was a positive relationship between the running distance and the area of FOG fibres in both the deep and surface portions of the plantaris muscle. In addition, the running distance correlated positively with the percentage of FOG fibres and negatively with that of FG fibres in the deep portion of the plantaris muscle. There were no relationships between the running distance and fibre type composition, or fibre area and capillary supply in the soleus muscle. These results suggested that the increase in the percentage and area of FOG fibres in the fast-twitch muscle was closely related to voluntary running.

Key words: Voluntary running - Soleus muscle - Plantaris muscle - Fibre type composition - Fibre area - Capillary supply - Rat

Introduction

Exercise training changes the metabolic and contractile properties of muscle as well as other organs. The skeletal muscles of animals trained over long periods have an increased proportion of high-oxidative fibres, higher oxidative capacity, more myoglobin and mitochondria, and more capillaries (Holloszy 1967; Pattengale and Holloszy 1967; Gollnick and King 1969; Holloszy et al. 1970; Molé et al. 1971; Baldwin et al. 1972). On the other hand, high intensity, repetitive training causes muscle fibre hypertrophy and an increase in the muscle's glycolytic capacity (Staudte et al. 1973; Gillespie et al. 1982). Although these adaptations have been described, their relationships to the frequency, intensity (speed) and duration of exercise are only partly understood. Hickson et al. (1976) and Dohm et al. (1977) examined the effects of training at varying intensities and durations of exercise on the histochemical properties of muscle. These studies indicated that when exercise training increased the oxidative capacity of the muscle, it did so irrespective of the intensity or duration of exercise.

However, it is likely that skeletal muscle fibres adapt differently depending on the type of exercise. While maximal training effects may not be induced by voluntary running, there may be relationships between voluntary running and fibre type composition and fibre area. Though there are many histochemical studies of the effects of forced exercise (treadmill running or forced swimming, which are undoubtedly stressful), there are no reports of the influence of voluntary running on the histochemical properties of muscle. Therefore, in this study we investigated the influence of voluntary running exercise on the fibre type composition, fibre area and capillary supply of the rat slow-twitch soleus and fast-twitch plantaris muscles.

Methods

In the investigations 25 4-week-old male Wistar rats were used; 20 animals (exercise group) were housed, 1 per cage, with a freely rotating wheel (CL-4579, Clea Industries, Japan) in a controlled environment of 12-h daylight and 12-h darkness for 45 days. A wheel was attached to one side of a standard rat cage and the animal had free access to the wheel. The animals were allowed to run voluntarily in the wheel for 12 h each day, while they were awake. The distance the animals ran was measured each day by a counter attached to the wheel. It was expressed as $m \cdot day^{-1}$ and was used to calculate the mean of the total running distance for 45 days. The 5 animals which served as a control group were housed

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as described above, but without a running wheel. Food and water were provided ad libitum and the room temperature was maintained at 22±2° C. The body mass of each animal was recorded each week.

After 45 days the animals were anaesthetized with ether, and the soleus and plantaris muscles of one hindlimb were removed and weighed. The muscles of the midbelly region were sectioned, and then mounted and frozen rapidly in isopentane precooled with liquid nitrogen. Transverse serial sections, 10 μm thick, were cut in a cryostat at approximately −20° C, and placed on cover glasses for histochemical staining. The sections were processed alternately for myosin adenosine triphosphatase after pre-incubation at pH 10.3 (Padykula and Herman 1955) and succinate dehydrogenase (SDH, Nachlas et al. 1957).

The muscle fibres were classified as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), or fast-twitch glycolytic (FG) (Peter et al. 1972). The plantaris muscle was divided from the deep to surface axes throughout its cross-section. To ensure adequate sampling, fibre type composition was determined by directly counting at least 200 fibres in each portion. In the soleus muscle, the middle portion of the cross-section was used. The fibre area on SDH-stained slides was measured from the photomicrographs using a digitizer connected to a personal computer. Areas of more than 20 fibres of each type were measured in each portion of the muscle. The capillaries were identified with amylase and the periodic acid-Schiff reaction procedure (Andersen 1975). The number of capillaries for each fibre type was counted directly from the photomicrographs of the muscle cross-section.

Results

Comparison between control and exercise groups

Body mass. The masses of the control group in the 3rd to 6th week of exercise were significantly greater than those of the exercise group. During the last week of exercise, however, no significant difference was observed between the two groups (Fig. 1).

Muscle mass. No significant differences were observed in the masses of the soleus and plantaris muscles between the two groups. The relative masses (muscle mass per body mass) of the two muscles of the exercise group were significantly greater than those of the control group (Table 1).

Muscle fibre type. There was a significant difference between the two groups in fibre type composition in the deep portion of the plantaris muscle. The percentage of FOG fibres was larger in the exercise group, and the percentage of FG fibres was lower (Table 2).

Muscle fibre area. A significant difference between the two groups was observed only in the area of FOG fibres in the surface portion of the plantaris muscle (Table 2).

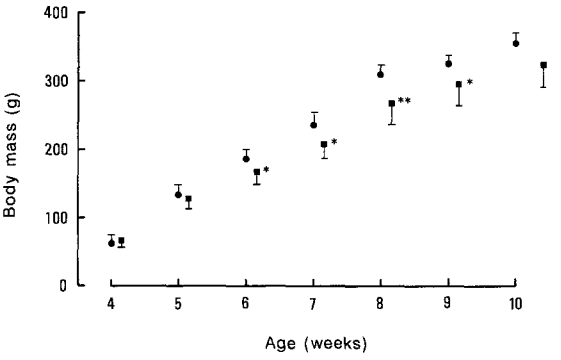

Fig. 1. Changes in body mass with time in the control and exercise groups (means and standard deviation). ●, control group; ■, exercise group. * $P<0.05$, ** $P<0.01$

Table 1. Muscle masses in the control (C) and exercise (E) groups

Group	Soleus muscle				Plantaris muscle			
	MM (mg)		RMM (mg·100 g body mass^{-1})		MM (mg)		RMM (mg·100 g body mass^{-1})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	121	11.5	33	3.7	331	21.3	91	2.9
E	131	18.2	39*	4.8	326	33.9	98*	5.8

MM, muscle mass; RMM, relative muscle mass; * $P<0.05$ (C vs E)

Table 2. Fibre type compositions (%) and cross-sectional areas (μm^2) in the control (C) and exercise (E) groups

Muscle	Fibre type composition						Fibre area					
	SO		FOG		FG		SO		FOG		FG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Soleus												
C	91.1	5.09	8.9	5.09			3668	609.3	2012	188.0		
E	91.0	4.77	9.0	4.77			3397	930.2	2228	522.7		
Plantaris (S)												
C	5.8	2.47	41.7	6.76	52.6	8.57	1847	242.7	2351	255.6	3581	735.5
E	6.4	1.81	47.8	7.36	45.8	7.39	2543	704.3	3066*	696.4	3766	1064.8
Plantaris (D)												
C	9.5	1.93	57.5	3.01	33.0	2.60	1902	319.2	2219	303.8	2866	352.4
E	11.8	3.89	63.7*	5.64	24.5**	5.86	2599	807.4	3024	859.1	3499	713.1

SO, slow-twitch oxidative fibre; FOG, fast-twitch oxidative glycolytic fibre; FG, fast-twitch glycolytic fibre; S, surface portion; D, deep portion; * $P<0.05$, ** $P<0.01$ (C vs E)

Capillary supply. The exercise group had more capillaries around each SO fibre in the surface portion of the plantaris muscle, and fewer capillaries around each FG fibre in the surface and deep portions of the plantaris muscle (Table 3). On the other hand, the exercise group had fewer capillaries around SO and FG fibre per fibre type area ($10^{-3} \cdot \mu m^{-2}$) in the deep portion of the plantaris muscle (Table 3).

Relationships between running and physiological parameters

Running distance. The running distance per day increased progressively. During the 1st week of exercise the mean was 1194 $m \cdot day^{-1}$, SD 626.4, whereas in the last week of exercise the mean was 3168 $m \cdot day^{-1}$, SD 2655.6 (Fig. 2).

Body mass and muscle mass. The running distance per day did not correlate with body mass ($r = 0.187$, $n = 20$) or relative mass of either soleus ($r = 0.119$, $n = 20$) or plantaris ($r = -0.124$, $n = 20$) muscles.

Muscle fibre type. There was a negative relationship between the running distance per day and the percentage of SO fibres in the surface portion of the plantaris muscle ($r = -0.495$, $n = 18$, $P < 0.05$). On the other hand, in the deep portion of the plantaris muscle, there was a positive relationship between the running distance per day and the percentage of FOG fibres, and a negative relationship between the running distance per day and the percentage of FG fibres (Fig. 3).

Muscle fibre area. There was a positive relationship between the running distance per day and the area of FOG fibres in both the deep and surface portions of the plantaris muscle (Fig. 4).

Capillary supply. There was a negative relationship between the running distance per day and the number of capillaries around a FOG fibre in the surface portion of the plantaris muscle ($r = -0.483$, $n = 18$, $P < 0.05$). Furthermore, there was a negative relationship between the running distance per day and the number of capillaries around a FOG fibre per fibre type area in the surface ($r = -0.657$, $n = 16$, $P < 0.01$) and deep ($r = -0.699$, $n = 16$, $P < 0.01$) portions of the plantaris muscle.

Table 3. Numbers of capillaries around each fibre and around fibre type per fibre type area ($10^{-3} \cdot \mu m^{-2}$) in the control (C) and exercise (E) groups

Muscle	Number of capillaries around fibre type						Number of capillaries around fibre type per fibre type area					
	SO		FOG		FG		SO		FOG		FG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Soleus												
C	3.4	0.15	2.9	0.48			0.94	0.144	1.43	0.246		
E	3.5	0.22	2.5	0.35			1.10	0.310	1.17	0.392		
Plantaris (S)												
C	3.2	0.21	2.8	0.26	2.2	0.05	1.73	0.315	1.19	0.181	0.62	0.113
E	3.7**	0.31	2.8	0.26	2.0**	0.17	1.58	0.486	0.94	0.278	0.59	0.238
Plantaris (D)												
C	3.7	0.15	2.7	0.38	2.2	0.19	2.01	0.338	1.23	0.222	0.76	0.096
E	3.6	0.23	2.7	0.25	1.9**	0.16	1.48*	0.443	0.92	0.225	0.55**	0.122

For definitions see Table 2
* $P < 0.05$, ** $P < 0.01$ (C vs E)

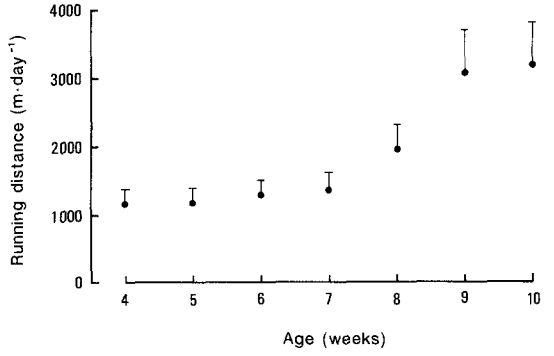

Fig. 2. Changes in running distance with time in the exercise group (means and standard error)

Discussion

Voluntary running distances varied widely among the exercised animals. The running distance during the last week of exercise ranged from 10634 $m \cdot day^{-1}$ to 343 $m \cdot day^{-1}$. In addition, of the total of 20 animals allowed to use running wheels over 45 days, 13 of them showed an increase of daily running distance with time. The most marked change was a fourteen-fold increase, from 741 $m \cdot day^{-1}$ during the 1st week of exercise to 10634 $m \cdot day^{-1}$ during the last week. The mechanism responsible for this variability could not be determined from these data.

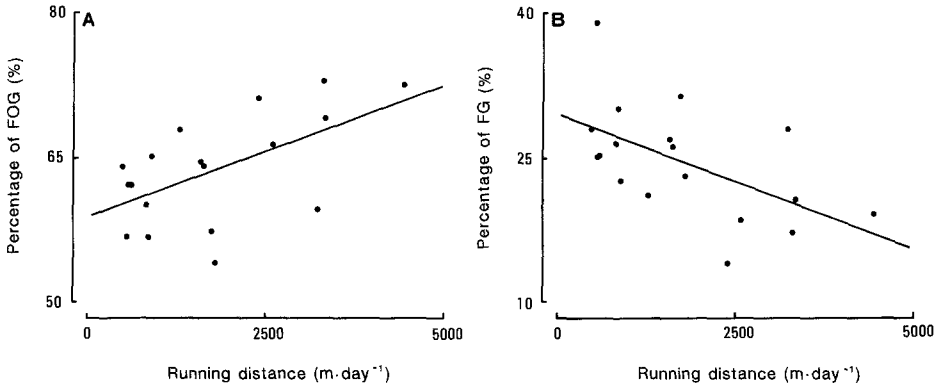

Fig. 3. Percentages of muscle fibre types in the deep portion of the plantaris muscle plotted against running distance per day. In **A**, $r=0.573$ ($n=18$, $P<0.05$) and in **B**, $r=-0.561$ ($n=18$, $P<0.05$). *FOG*, fast-twitch oxidative glycolytic fibre; *FG*, fast-twitch glycolytic fibre

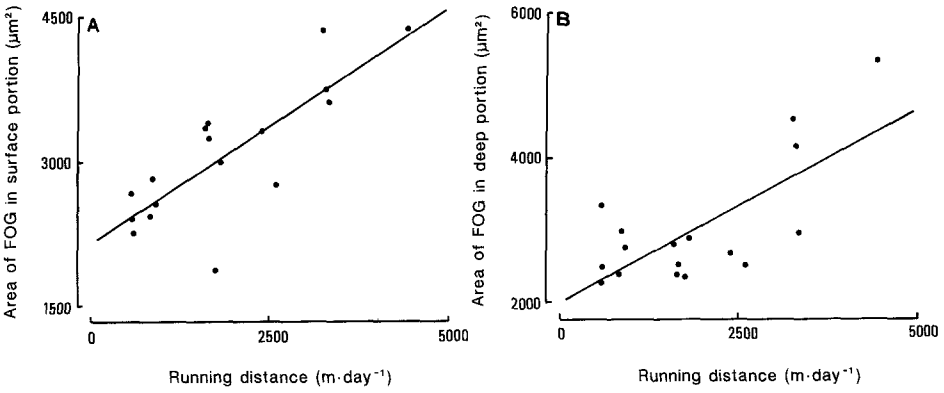

Fig. 4. Cross-sectional areas of fast-twitch oxidative glycolytic (*FOG*) fibres in the plantaris muscle plotted against running distance per day. In **A**, $r=0.807$ ($n=17$, $P<0.001$) and in **B**, $r=0.723$ ($n=17$, $P<0.01$)

In this study, although the exercise and control groups had similar percentages of the major fibre types (fast-twitch and slow-twitch), a higher percentage and area of FOG fibres was observed in the plantaris muscle of the exercise group. An increase in the percentage of high-oxidative fibres has already been reported in previous studies (Barnard et al. 1970; Faulkner et al. 1971; Edgerton 1978; Green et al. 1983) using endurance type training. It has also been observed that endurance training induces an increase in the capillary density of skeletal muscle (Carrow et al. 1967; Mai et al. 1970; Adolfsson et al. 1981). On the other hand, other studies (Ljungqvist and Unge 1977; Banchero et al. 1979) have failed to confirm such changes. In general, the increase in the capillary supply, expressed as the number of capillary contacts around specific fibre types, is an early adaptative response to increased muscle contraction. In this study, however, capillarization varied little with exercise in the plantaris muscle.

In the soleus muscle, there were no exercise-related changes in fibre type composition, fibre area or capillary supply. This is in agreement with the finding (Edgerton et al. 1969) that endurance training had no effect on the proportion of fibres in the soleus muscle. The rat soleus muscle fibres probably have sufficient aerobic capacity to meet the increased energy demand needed for running. Thus it appears that voluntary running exercise requires recruitment of FOG fibres in the plantaris muscle and alters the activity pattern of a relatively large population of these fibres. However, when the running distance increases dramatically, a selective energy depletion of FOG fibres will occur. Because the energy source for this exercise is mainly oxidative phosphorylation, activation of different motor units, such as recruitment of FG or SO fibres, may be needed during severe prolonged exercise (Terjung 1976; Dudley et al. 1982).

Although the frequency, intensity (speed) and duration of exercise were not measured in this study, voluntary running is an aerobic type of exercise, judging from the histochemical results. Furthermore, because it has been reported that it is difficult to induce muscle hypertrophy by endurance training (Müller 1974), it is likely that voluntary running, which resulted in hypertrophy of the fast-twitch fibres in this study, entails an adequate rest interval between exercise bouts, and is a highly repetitive and more intense exercise than the forced endurance training used earlier (Faulkner et al. 1971; Bagby et al. 1972).

Previous studies (Hickson et al. 1976; Dohm et al. 1977) have investigated how different types of training affect enzyme activities in skeletal muscle. Those studies have shown that certain enzyme adaptations occur in exercised muscle, irrespective of the intensity and duration of exercise. On the other hand, Fitts et al. (1975) trained rats using regimens of different durations and found that the change in the oxidative enzyme activity of the muscle was positively correlated with the total amount of work performed. The major purpose of this study was to determine whether adaptation of the three skeletal muscle fibre types in the rat was further enhanced by increased voluntary running distance.

It is interesting to note that there was a positive relationship between the running distance and the percentage and area of FOG fibres in the plantaris muscle. The FOG fibres probably make a major contribution to voluntary, prolonged, and relatively intensive exercise. This response of FOG fibres is reasonable since FOG fibres are easily activated, relatively resistant to fatigue, and they have a high oxidative capacity and rich blood supply. In this regard, running activity of individual animals may be related to the percentage and area of FOG fibres in the plantaris muscle. This indicates that voluntary running is an important cause of aerobic adaptations to chronic exercise, especially in fast-twitch muscle.

Although the progressive increase of running distance was closely related to an increased percentage and area of FOG fibres in the fast-twitch muscle in this study, the question arises as to whether these results also apply to humans. It would not be surprising if such relationships were also found in humans. However, further detailed study is needed to elucidate the relationship between voluntary activity and histochemical and morphological properties of human skeletal muscle.

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