

A histochemical study on the influence of high-gradient treadmill endurance running on the skeletal muscles in rats

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ラットの骨格筋に及ぼす急勾配トレッドミル走の影響に関する組織科学的研究

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Abstract

Sixteen female rats of the Fischer line were administered a series of treadmill running for eight weeks (5 times a week, 60 minutes a session a day) at a 15-degree gradient and at the speed of 23 m/min. Then their leg skeleton's soleus muscle and plantaris muscle were extracted. Dyeing methods like myosin ATPase, SDH and Amylase PAS were employed on a microscopic basis in order to determine the composition, transection volume, and capillary development of SO (superb activation of oxidizing enzymes), FG (excellent activation of sugar enzymes), and FOG (mediating muscles) fibers. The analytic results are as follows;

1. Body weight significantly increased ($p < 0.001$) in the training group as compared with the control group.
2. Both m. soleus and m. plantaris increased with significance ($p < 0.05 \sim p < 0.001$) in both absolute and relative muscle weight as well.
3. Even though m. soleus didn't have any changes of fiber composition in the two groups, m. plantaris was noteworthy. In its deep region, SO fibers were not affected, however, FOG fibers increased ($p < 0.05$) and FG fibers decreased ($p < 0.01$). In the peripheral region, the two groups had few SO fibers, while the training group's FOG fibers grew ($p < 0.01$) and FG fibers lowered ($p < 0.01$). This phenomenon may be accounted for by FG fibers' transfer to FOG fibers.
4. A significant ($p < 0.05 \sim p < 0.01$) increase of the transection volume was noticed in SO and FOG fibers of m. soleus as well as SO, FOG, and FG fibers of m. plantaris. The difference among the fibers was seen in the peripheral region of m. plantaris only.
5. As to the barometer of the muscle fiber capillary, the ratio between CD and CF was not affected by the endurance training. Excepting FG fibers of m. soleus, CC increased ($p < 0.01 \sim 0.001$), which implies the increase in oxidizing capacity of muscular fibers.
6. Since the effect of the training in capillary development was clearly identified by the values

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of muscle fibers transection volume divided into CC, any type of muscle fibers discussed here seems to have been influenced by the endurance treadmill running.

Introduction

Along with nerves, skeletal muscles are best fitted to the body's efficient movement, getting fat or lean according to the degree of the use and exercise. In spite of much research on the motor-physiological phenomena caused by the increase of age or the lack of exercise, most of the mechanism resulting in a change of kinematic function is still unknown.

The basic function of skeletal muscles is to change chemical energy into mechanical energy, accompanying ATP's consumption or mutual mechanical flection aided by nerves. This kind of mechanism involved in a muscle's exercise effect is thought important. The influences of training on skeletal muscles have been studied in various viewpoints. Recently, in particular, we can see many papers on the effects of training on fiber composition of skeletal muscles in the wake of the development of histochemical method.

Armstrong et al⁶⁾ administered rats running and swimming of different intensities, and found that low-load exercise required oxidative muscular fibers and high-load training accompanied glycolytic fibers. Gillespie et al²⁰⁾ used guinea pigs and reported that glycogen was exhausted in SO(slow-twitch oxidative) and FOG(with superb oxidative enzyme activity) fibers for running and in FG(fast-twitch glycolytic) fiber for jumping. KATSUDA et al³⁾ experimented intermittent sprint and endurance running with rats and concluded that different exercises tended to employ different fibers, antagonists, etc. As for man, Gollnick et al^{21) 22)} acknowledged the use of different fibers according to the kind of exercise.

The different participation of muscular fibers according to the degree of work intensity represents, according to ISHIHARA et al,¹⁾ the characteristic of kinematic neurons governing these muscle units. Also, the different modes of participation are thought to be resulted from training^{8) 42), 10) 37)}

In 1873 Ranvier¹¹⁾ first distinguished skeletal muscles into red ones and white ones. In addition, though the training effects on muscles have mostly been for animals, man's training influence has recently become possible by way of the needle biopsy technique.¹¹⁾ According to histochemistry, skeletal muscle can be classified into Type I (high oxidative enzyme activity or high OEA, and low glycolytic enzyme activity or low GEA) fibers and Type II (low OEA and high GEA) fibers. Type I muscular fibers have high neutral lipids and low glycogen and Type II fibers vice versa. The former hardly feels fatigue, while the latter easily gets fatigued.

This classification can be made by Myosin ATPase and SDH(Succinate Dehydrogenase) dyeing methods. Under the SDH dyeing, SO fibers have slow flection speed and high OEA, FG fibers have fast flection speed and high GEA, while FOG(fast-twitch oxidative glycolytic) fibers slow fast flection speed along with high OEA and GEA. But this method of distinction applies to animals and man's fibers are generally called Type I, Type II_A, and Type II_B.^{12) 18) 30)} The dyeing of skeletal muscles after endurance training is generally know to result in the increase in capillaries, mitochondrias, fiber's cutting area, OEA, glycogen in cells, and metabolic enzyme activity.^{31) 7)} But such researches were mostly done with level treadmill runing or

weight-loaded swimming.

Based on the preceding background, this study uses Fischer-line rats for endurance running on the treadmill with 15° slope. After extracting soleus muscles and plantaris muscle from among their skeletal muscles, the weight of muscles, the fiber composition, the transection volume, and the change of capillaries were studied to determine the characteristics of the muscles before and after the training session.

Methods

Sixteen female rats of the Fischer-line(aged 4 weeks) were given a preparatory training for a week in a small cage, 42, 24.5, and 17.5 cm in dimension. They were divided two groups: Group C (control: n=8) and Group T(trained: n=8). They were reared with the temperature of 22 ± 2°C and the relative humidity of 60 ± 5%, while resting from 7 p.m. through 7 a.m. for their nocturnal habits. They were free to absorb water and the specially prepared foods.

After nurturing for one week, a week preparatory training was given Group T on the special treadmill for small animals. (See Fig.1.) In the first 4-week training session, the running time and slope were gradually increased, while the slope of 15° and the speed of 23 m/min were maintained in the second 4-week session (5 times a week and 60 minutes a day).

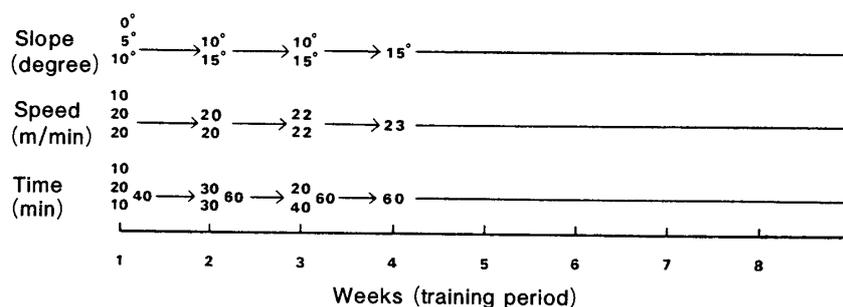


Fig 1. Progressive control and trained group programs.

The body weight was measured before training once a week. 24 hours after the final training, under abdominal anesthesia with urethan (2 mm per 100 g of body weight), soleus muscle (*m.sol.*) and plantaris muscle (*m.plan.*) were extracted for measurement on an electronic balance. The extracted muscles of 5 mm or so were made into slices 8 μm by way of rapid freezing and storing in very low temperatures.

For the histochemical analysis, three kinds of dyeing were held: Myosin ATPase method by Padykula et al.³⁹⁾ SDH method introduced by Nachlas et al.³⁵⁾ and Amylase PAS method⁸⁾. Thus dyed slices of *m.plan.* were sectioned into deep and peripheral regions and each part's picture was enlarged before classifying into SO, FOG and FG fibers.

Along with the aforementioned data, the following were analyzed: %fiber type or the ratio of fiber composition, fiber area or transection volume, CD(capillary density), CF ratio or capillaries per fiber, CC or CA(mean number of capillaries in contact with fiber), and DD(diffusion distance). After all, the mean values and standard deviations for each group were calculated tes-

tifying difference by t-test and choosing significant risk ratio as below 5%

Results

1. Body Weight & Muscle Weight

The change of body weight accompanied by the training are shown in Table 1, Fig 2 and Fig 3. Though both groups showed a similar increase in weight until the third week of the exercise, Group T has ever since maintained a great margin of increase as compared with Group C. After

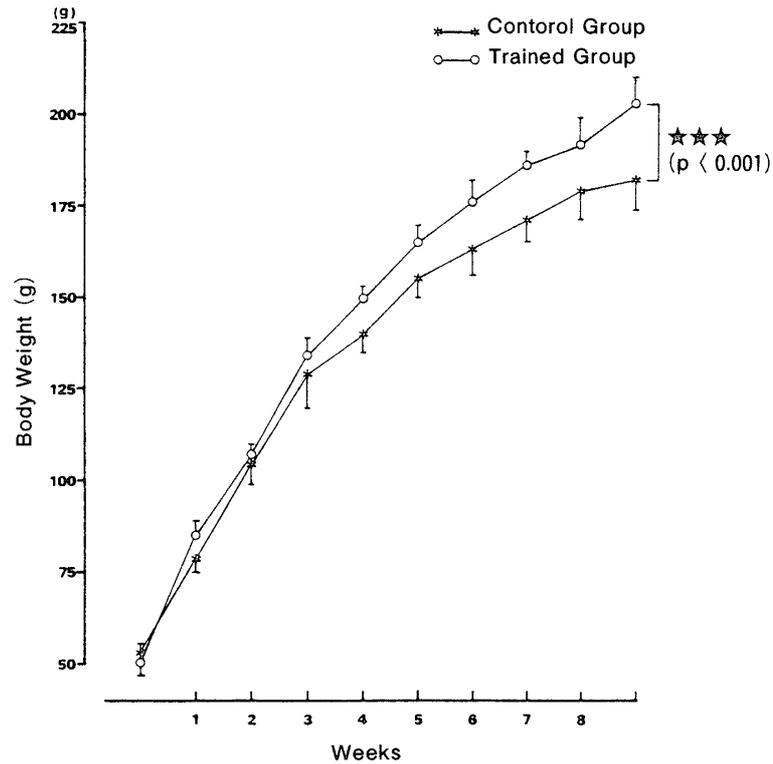


Fig 2 Change of body weight in control and trained groups

Table 1 Body weight, muscle weight, and relative muscle weight (mean \pm 50) in control and trained groups

Item		Control (n=8)	Trained (n=8)
Body Weight	(g)	181.9 \pm 7.2	202.3 \pm 6.1***
m. soleus	(mg)	75.5 \pm 6.5	98.5 \pm 9.4**
	(mg/g B.W.)	0.415 \pm 0.030	0.487 \pm 0.045*
m. plantaris	(mg)	178.6 \pm 9.2	214.6 \pm 9.5***
	(mg/g B.W.)	0.982 \pm 0.036	1.061 \pm 0.035**

* Denotes a significant difference, $p < 0.05$.

** Denotes a significant difference, $p < 0.01$.

*** Denotes a significant difference, $p < 0.001$.

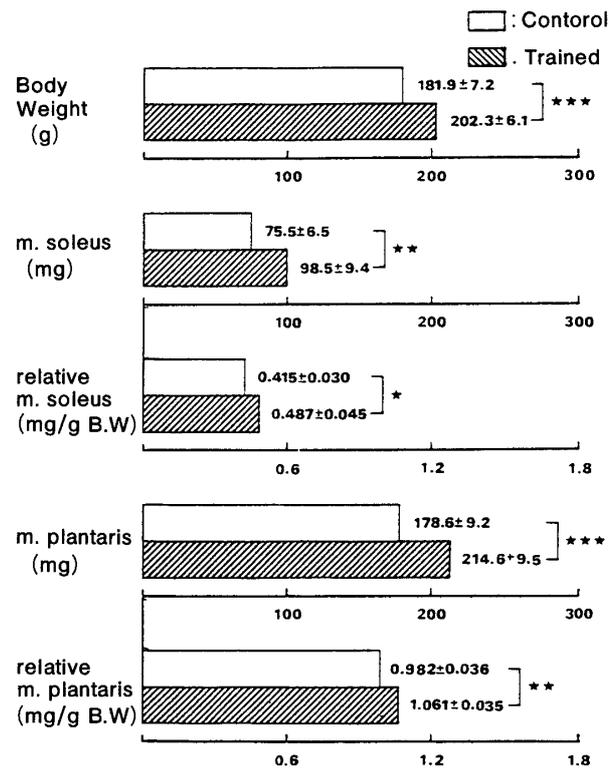


Fig 3. Comparison of body weight muscle weight and relative muscle weight in control and trained groups.
 (★,P<0.05, ★★;P<0.01, ★★★P<0.001)

the training, Group T averaged 202.3 ± 6.1 g while Group C recorded 181.9 ± 7.2 g, the difference of 20.4 g being significant at the level of 1%.

The comparison of absolute and relative muscle weights is also given in Table 1 and Fig.3. Concerning *m.sol*, the absolute muscle weights were 98.5 ± 9.4 mg and 75.5 ± 6.5 mg for Group T and Group C, respectively (significant at the 1% level). The difference in relative muscle weight between Group T and Group C was also significant at the 0.1% level (Group T: 214.6 ± 9.5 mg, Group C: 178.6 ± 9.2 mg).

2.Muscular Fiber Composition

Fig.4 depicts the composition ratio of muscular fibers. The muscular fiber type of *m.sol* consisted of FOG and SO fibers. Group T had 89.0% of SO and 11.0% of FOG, while Group C held 85.0% of SO and 15.0% of FOG. Thus, by way of the training, SO fibers increased by 4% and FOG decreased by 4%. However, the difference was insignificant.

In the deep region of *m.plan.*, Group T's composition was 17.0%(SO), 70.0%(FOG) and 13.0%(FG), while that of Group C was 16.0%(SO), 63.0%(FOG) and 21.0%(FG). Though an increase of SO fibers by 1% was not significant, the 7% increase in FOG fibers and 8% decrease in FG fibers were significant at the 5% level. In the peripheral region, SO fibers were hardly seen. But the 14% increase in FOG and the 14% decrease in FG after the training were significant at the level of 1%.

3.Fiber Area

The mean values of the transection volume and the % fiber area in SO, FOG, and FG fibers are

depicted in Figs 5 and 6 About fiber area in *m.sol.*, Group T increase SO and FOG by $642 \mu\text{m}^2$ (at the 1% level) and by $344 \mu\text{m}^2$ (at 5%), respectively. In *m.plan.*, a significant increase (at 5%) was seen in SO ($276 \mu\text{m}^2$), FOG($462 \mu\text{m}^2$) and FG($653 \mu\text{m}^2$)

Regarding the % fiber area, SO of *m.sol.* had an insignificant 2% increase and FOG a 2% decrease. In the deep region of *m.plan.*, SO merely increased by 1%. But FOG significantly increased by 11% (at 1%) and FG significantly decreased by 12% (at 5%) In the peripheral region, SO fibers were rarely noticed, while FOG showed a significant (at 5%) increase by 14%

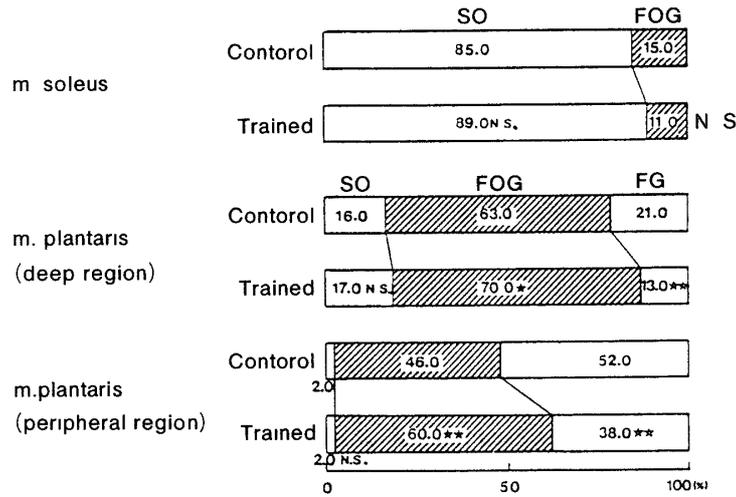


Fig 4 Percentage of fiber type composition in m soleus and m. plantaris for % fiber

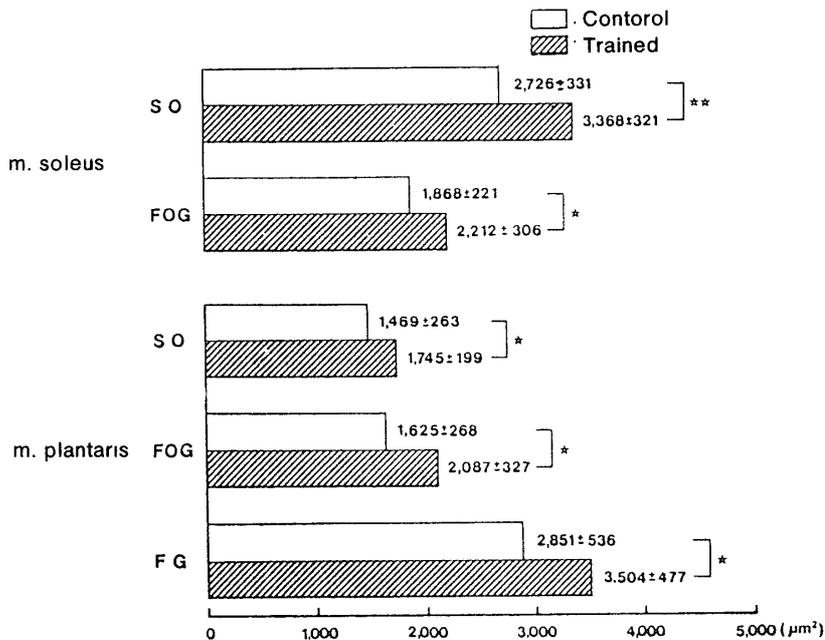


Fig 5 Comparison of fiber area (μm^2) in cross sections of m soleus and m plantaris.

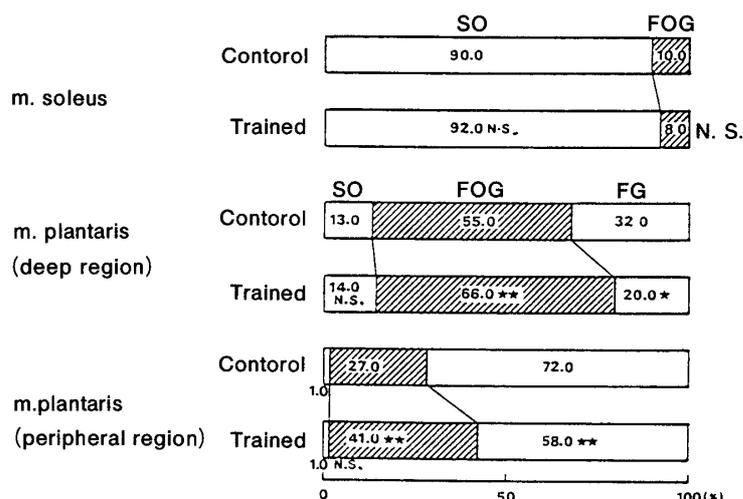


Fig 6. Percentage of fiber type composition in m.soleus and m.plantaris for % fiber area.

and FG showed a significant decrease by 14% (at 1%).

(1) CD & CF Ratio

In Fig.7, CD (capillary density, or capillaries per mm²) and CF ratio (capillaries per fiber, or CD/muscle fibers) are given with Group C's scores as 100. In both muscles, the effect of the training could not be found.

(2) CC

Fig.8 shows CC (mean number of capillaries in contact with fiber) Here again, Group C is given as 100. In *m.sol.*, SO had an increase of 13% (at 1%) and FOG a 16% increase (at 0.1%). In *m.plan.*, significant increases of 14% and 15% were seen in SO (at 1%) and FOG (at 0.1%), respectively, while an increase of 9% in FG showed no significance.

(3) DD

The values of fiber area/capillary (μm²/CC), or DD (diffusion distance) are presented in Table 2. SO's increase (52 μm²/capillary) and FOG's decrease (58 μm²/capillary). In *m.sol.* had no significance. In *m.plan.*, an increase by 21, 34, and 97 μm²/capillary was seen for SO, FOG, and FG fibers, respectively. However those increases were not statistically significant

4.Capillaries

The examined results are shown in Figs.7 and 8, and Table 2.

Discussion

The research on a rat's weight change by way of training has so far concluded on the hindrance of weight increase^{9),23),4)} because of such factors as exercise controlling the formation of fat,^{16),36)} stress checking the desire for eating,⁴³⁾ and the decrease of eating.^{24),2)} In this study, however, a steady increase in Group T appeared from early in the training and the increment was 20.4 g when the training terminated.

As for endurance running training, the speed of 1 mile/hour (26.7 m/min) was used by Gollnick and King,²⁴⁾ while 30~32 m/min was employed by Itoh,²⁾ Hickson et al²⁹⁾ and Gillespie et al.²⁵⁾ Since Shepherd and Gollnick⁴⁴⁾ reported that 30.0 m/min equaled to the intensity of

Table 2 Distance of disfusion in m soleus and m.plantararis in control and hvained groups

Item		Control (n=8)	Trained (n=8)
m. soleus	SO	774 ± 135	826 ± 78 NS
	FOG	655 ± 117	597 ± 102 NS
m. plantaris	SO	410 ± 46	431 ± 53 NS
	FOG	438 ± 75	472 ± 76 NS
	FG	801 ± 97	898 ± 150 NS

Distance of diffusion : Fiber area/cc ($\mu\text{m}^2/\text{capillary}$)

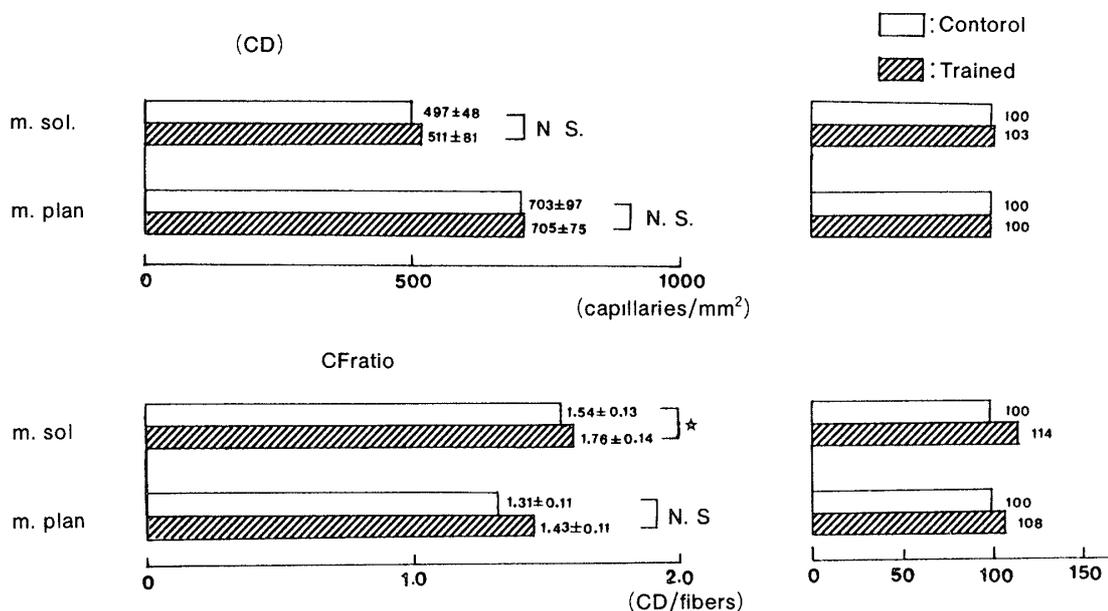


Fig 7 Comparison of capillary, CF ratio and percentage in m plantaris

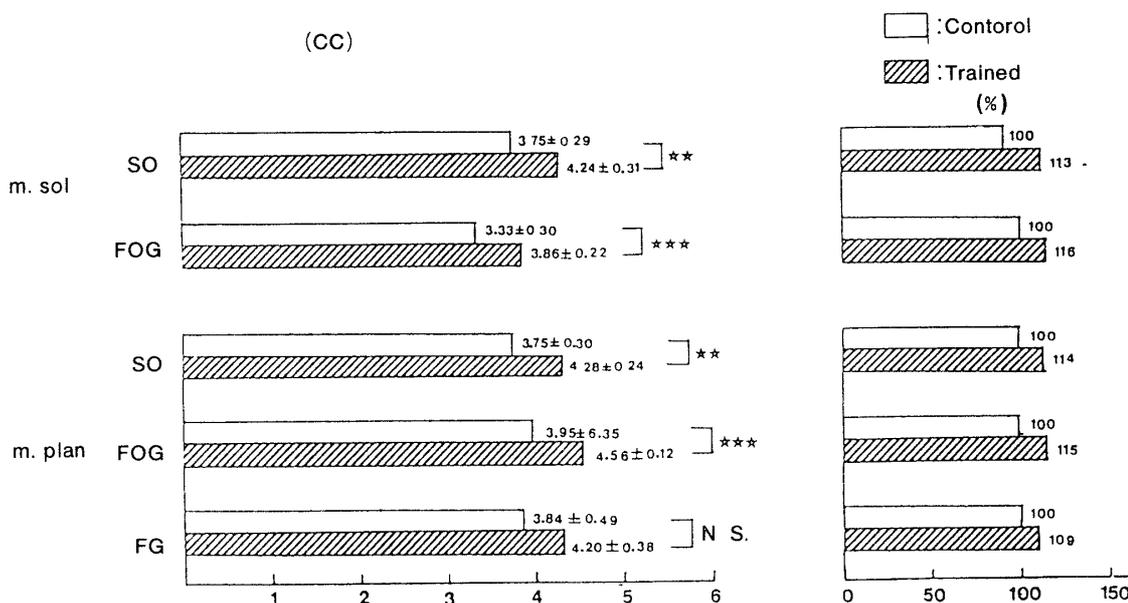


Fig 8 Comparison of mean number of capillary in contact with fiber(CC) and percentage in m soleus and m plantaris

84%VO² max, 23 m/min and 15°-slope used in this research (that is, a very high work intensity) are thought to have resulted in the weight gain in Group T.

In agreement with previous studies (Itoh,² Kanao et al,⁵ and Sillau et al,¹⁵) the present investigation demonstrates that endurance trained group exceeded control group in absolute and relative values of muscle weight (in that the gap in body weight has a great influence on muscle weight).

Many studies on the transformation of muscular fibers demonstrated that the fiber quality never change.^{14),26)} In this study, SO and FG of *m.plan.* had no difference in the deep region and, like the precedent research, the peripheral region had no SO fibers. The effects of training happened in metabolic quality. Namely, as reported in the studies of Hickson et al,²⁹⁾ Bagby et al,¹³⁾ Itoh,²⁾ and Barnard et al,¹⁴⁾ Group T's FOG had a high composition ratio, FG's ratio was low, and FG transformed into FOG. The size of fiber area has a lot to do with fattening muscles.

A slim muscle is efficiently enduring and specifically adapted to training. The *m.plan.* of Group T had more fatness in the fibers than in the fibers of Group C (deep region: 11%, $P < 0.01$; peripheral region: 14%, $P < 0.01$). In that FG transformed into FOG in the peripheral region, it might be right that there was no difference of fatness rate among the fibers.

Gollnick et al^{27),28)} noticed the 23% increase in SO's fiber area when men were given bicycle pedaling for 5 months. They also concluded that a player of endurance exercise has a rather high rate of SO as compared with Group C. Faulkner et al¹⁹⁾ observed, similarly to our results, a 19% increase in the red fibers of *m.plan.* in guinea pigs after endurance training.

As shown in these cited studies, different load conditions on the same treadmill can cause different fatness of fibers. However, additional research is needed to reveal the exact relationship between work load and fatness ratio.

The change in capillaries of skeletal muscles by way of training can be different according to the mode of exercise, sex, age, and the section of the muscle. In the case of evaluating training effects, to examine the change of capillaries is important because they can tell us the growth of muscular fibers and the recovery of muscular fatigue. After Krogh's study³⁴⁾ on the exercise and muscular capillaries, a number of studies have been conducted in the field. Mostly done horizontally on both non-athletes and athletes, and it is well agreed that the capillaries in endurance athletes are well developed.^{25),32),33)} One study found no difference in CD between control and trained groups when muscles get fat by way of training.⁴⁶⁾

The present study witnessed a high value (14%, $P < 0.05$) in *m.sol.*'s CF ratio though no great increase was seen in Group T. It is particularly interesting to note that CD had no change in spite of the growth of FOG and FG. In that CC had significantly high values in *m.sol.* and *m.plan.*, the effects of endurance running training were found in both FOG and SO fibers.

Therefore, the effect of increasing muscular oxidation ability can be brought up at the high-intensity training on the high-gradient treadmill. DD also increased greatly in *m.plan.*'s fibers. As a result, in the case of evaluating training effect by way of capillaries, many barometers including CD, CC, and CF ratio are required.

Conclusion

- The results concerning body weight, muscle weight, fiber area, and capillaries are as follows:
- 1 The body weight in Group T significantly increased ($P < 0.01$) as compared with Group C
 - 2 Both in *m. soleus* and *m. plantaris*, absolute and relative muscle weights increased significantly ($P < 0.05 \sim P < 0.001$)
 3. The two groups showed no changes in the fiber composition of *m. sol.* In the deep region of *m. plan.*, SO did not change, FOG increased ($P < 0.05$) and FG decreased ($P < 0.01$). In its peripheral region, SO was hardly noticed, while FOG increased ($P < 0.01$) and FG decreased ($P < 0.01$). This might be due to the transformation of FG into FOG fibers.
 4. Significant ($P < 0.05 \sim P < 0.01$) increases were obtained in SO and FOG fibers of *m. sol.* and in SO, FOG and FG fibers of *m. plan.* The difference of fatness rate among the fibers appeared only in the peripheral region of *m. plan.*
 5. The endurance training did not affect CD or CF ratio. Excepting FG fibers of *m. plan.*, CC increased significantly ($P < 0.01 \sim P < 0.001$). This implies the increase of muscular oxidative ability.
 - 6 The training effect was evident in the values of fiber area divided into CC. Therefore, the influence of the endurance running discussed here upon muscular fibers was clearly witnessed.

要 約

16匹のフィッシャー系ラットを8週間にわたって、傾斜15度、スピード23m/分のトレッドミル上を週5回（1回のトレーニング時間は60分）走らせた。SO線維(superd activation of oxidizing enzymes), FG線維(excellent activation of sugar enzymes), FOG線維(mediating muscles)の組成、横断面積、それに毛管形成に及ぼすトレーニング効果を検討するためにマイオシンATPエース、SDH、アミラーゼPASのような染色技法を用いてラットのひらめ筋と足底筋に対して組織学的研究を行った。その結果は下記の如くである。

- 1, コントロール群に比較して、トレーニング群に有意に体重増が見られた。
- 2, ひらめ筋、足底筋の両方に、絶対量においても相対量においても有意な増加が見られた ($P < 0.0 \sim P < 0.001$)。
- 3, 足底筋深部では、SO線の組成に変化は見られなかったが、FOG線維は有意に増加 ($P < 0.05$) し、逆にFG線は減少した ($P < 0.01$)。これに対して、ひらめ筋には何らの変化を観察することはできなかった。
一方、末梢部においては、トレーニング群のFOG線維は増加 ($P < 0.01$) し、FG線維は減少した ($P < 0.01$)。このことはFG線維からFOG線維への移行が行われたことを示唆するものであろう。
- 4, ひらめ筋のSO線維、FOG線維、それに足底筋のSO線維、FOG線維、FG線維に横断面積の有意な増加 ($P < 0.05 \sim P < 0.01$) が見られた。
- 5, 筋線維毛細血管のバロメーターとして、CD/CF比が用いられるが、その比は持久性トレ

ニングの影響を受けなかった。ひらめ筋のFG線維を除いて、他の線維にCCの増加が見られた ($P < 0.01 \sim P < 0.001$)。そのことは筋線維の酸化能力の増加を意味するのである。

- 6, 毛細血管形成と筋線維横断面積に及ぼすトレーニング効果が類似することから、本論文に検討されたどの筋線維もトレッドミル持久走に影響を受けたものと思われる。

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