Skeletal Muscle Phenotype, Fat Content and Voluntary Running Activity in the Fast-Twitch Fibre Dominant and Control Rats

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Skeletal Muscle Phenotype, Fat Content and Voluntary Running Activity in the Fast-Twitch Fibre Dominant and Control Rats

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Abstract

The purposes of the present study were to investigate various muscle characteristics including the fibre composition and capillary supply, abdominal fat accumulation, and the distance for voluntary wheel running. Fast−twitch fibre dominant rats (FFDR) obtained by selection breeding for higher percentage of type II fibres and control rats (CR) obtained by random breeding were used in this study. FFDR had lower percentage of type I (%type I) fibres in M. gastrocnemius and soleus than those of CR, and higher percentage of type IID/B (%type IID/B) fibres in M. gastrocnemius and percentage of type IIA (%type IIA) fibres in M. soleus than those of CR. In the fibre compositions of plantaris muscle, no significant differences were observed between FFDR and CR. The body weight, abdominal fat content, and capillary supply in FFDR were identical with CR. Another groups of animals aged 4wks were subjected to voluntary wheel running for 5 weeks. The running distance in FFDR during the last week and the total running distance were both significantly higher than those in CR. Based on these observations, it is suggested that the muscle fibre composition is not a single determinant of body fat accumulation and influences on physical activity level.

Key words: abdominal fat content, capillary supply, muscle fibre composition, voluntary wheel running
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Introduction

Skeletal muscle fibres are categorized as slow-twitch (type I) and fast-twitch (type II) fibres. It was pointed out that the muscle fibre composition was one of the risk factors of hypertension, insulin resistance, and obesity[2)10)]. Some studies have found that the percentage of type I (%type I) fibres is negatively correlated with the percentage of body fat (%fat) in men[11)20)], and corresponding findings have been reported in rats[12)18)]. In addition, a significantly positive correlation has also been observed between the percentage of type IIB (%type IIB) fibres and %fat in men[11)]. In contrast to this, no such correlations have been observed in humans[15)] and rats[5)]. From these studies, it is not clear whether or not the muscle fibre composition is related to

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fat accumulation. The physical activity level is also related the fat accumulation[7], but there have been no reports of the influence of hereditary determined muscle fibre composition on the voluntary activity level.

Suwa et al.[17] developed fast-twitch fibre dominant rats (FFDR) by selection breeding and control rats (CR) by random breeding. The percentage of type II (%type II) fibres of FFDR in the gastrocnemius, soleus, vastus intermedius, and biceps brachii muscles were higher than those of CR[16]. These rats are thus considered to be a good model to examine the relation between muscle fibre composition and either the fat accumulation level or the physical activity level. The main purpose of the present study was thus to compare the muscle fibre composition, abdominal fat content, and voluntary wheel running distance in FFDR with those in CR.

Materials and Methods

Animal

All experiments and procedures were approved by the Saga Medical School Animal Experiment Committee. To obtain the FFDR and CR, we used both selective and random mating. The methods of breeding have been previously described by Suwa et al.[17]. The FFDR were bred by selective mating while the CR were bred randomly. Two sets of animals were used for these studies. The first was composed of CR (n=8) and FFDR (n=6) aged 8-9 weeks to compare muscle fibre composition, capillary network, and abdominal fat content. The other was composed of 4-week-old CR (n=7) and FFDR (n=7) to compare the wheel running activity. All animals were housed in a temperature (22±2℃) and humidity-controlled (60±5℃) room with a 12-hour light (8.30 a.m. to 8.30 p.m.), 12-hour dark (8.30 p.m. to 8.30 a.m.) cycle. Food and water were provided ad libitum.

Wheel running

The experiment was started at 4 weeks of age and continued until 9 weeks of age. The distance covered by wheel running was recorded in both groups. 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. All animals were housed one per cage. The distance the animals ran was measured each day by a counter attached to the wheel. The body mass of each animal was recorded each week.

Muscle preparation and histochemical analysis

The rats were anaesthetized with pentobarbital sodium (50 mg/kg body weight i.p.). The muscles were rapidly dissected and about 2–3 mm cross-sectional segments were cut at the muscle belly. Each muscle piece was mounted on a specimen holder in an OCT embedding medium (Miles Tissue-Tek L) and frozen in isopentane previously cooled to a viscous fluid with liquid nitrogen. Transverse sections (10 μm) were then cut from each muscle in a cryostat maintained at −20°C and mounted on cover glasses. Each cryosection was processed to determine the myosin adenosine triphosphatase activity after preincu bation at pH 4.3, 4.6 and 10.3 using the procedures described by Gollnick et al.[6]. A composite photomontage of each preparation was made using micrographs, and then each fibre was identified as type I, type IIA, type IID/B and type IIC based on the nomenclature system of Brooke and Kaiser[4]. The muscle fibre composition was determined by evaluating more than 600 M. gastrocnemius fibres, 500 M. soleus fibres, and 3500 M. plantaris fibres in each section. Deep portions were used for the M. gastrocnemius analysis. In order to visualize the capillaries, transverse sections (16 μm) were cut and then subjected to a two−step histochemical method[3]. The stained sections were photographed (magnification, 200). In these photographs, artifact-free 0.35 mm^2 areas were analyzed and the capillary density (capillaries/mm^2), capillary to fibre ratio (capillaries/muscle fibre), and mean number of capillaries around the fibre were determined. Deep portions were used in the M. gastrocnemius, the muscle core was used in the M. soleus, and deep and superficial portions were used in the M. plantaris for the capillary analyses. All procedures were performed by one researcher blinded to the group.

Statistical analysis

All data are presented as the mean±SD. To determine any differences between the 2 means in all parameters, except for the running distance per week, the data were analyzed by Student's t test. To determine any differences in the running distance per week, we used the two-way ANOVA (week and group). Scheffe's post hoc test was conducted if the ANOVA indicated a significant difference. $P < 0.05$ was considered to indicate significance.

Results

In the rats subjected to the non-running experiments, the body weight, fat contents, and individual muscle weight of the FFDR were not observed to be significantly different from the CR, except for a higher weight of the M. soleus in the FFDR (Table 1). The percentage of type I fibres of M. gastrocnemius and soleus were lower than CR, and percentage of type IID/B (%type IID/B) fibres in M. gastrocnemius and percentage of type IIA (%type IIA) fibres in M. soleus were higher than CR. On the other hand, no significant differences were observed between the two groups regarding the percentage of fibre types of M. plantaris (Fig.2). These results regarding the body and muscle fibre composition corresponded to the rats subjected to voluntary running (data not shown). Table 2 showed the capillary network in CR and FFDR. No significant differences were observed in any muscles between two groups.

Fig. 2 showed the voluntary wheel running distances in the CR and FFDR. The distance per week in FFDR from 1 to 4 weeks did not significantly differ from that in CR. On the

Table 1. Morphometric measurements in the CR and FFDR.

	CR (n=8)	FFDR (n=6)
Body mass (g)	247±17	257±13
Abdominal fat mass (g)	9.16±1.51	7.67±2.19
Gastrocnemius mass (mg)	1258±87	1293±76
Soleus mass (mg)	110±11	123±10*
Plantaris mass (mg)	263±19	238±23

Data are expressed as mean±SD.
* $P < 0.05$ vs. CR.

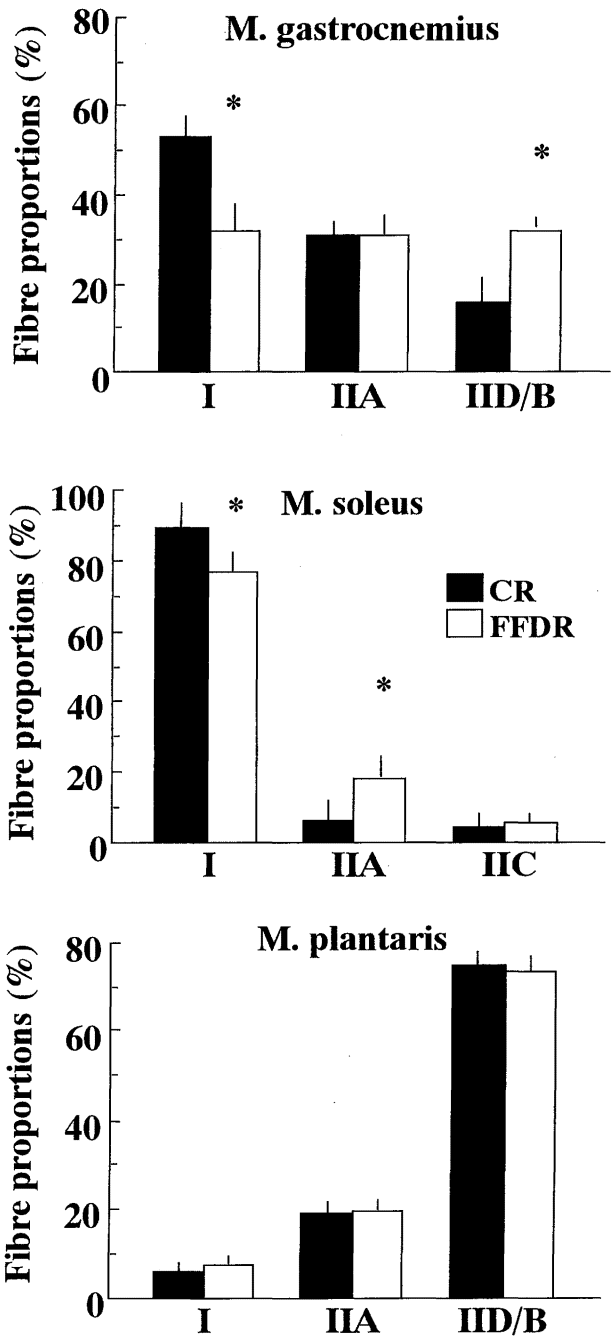

Fig. 1. Muscle fibre compositions in CR and FFDR. A: M. gastrocnemius. B: M. soleus. C: M plantaris. Value are the means ± SD. *:Significantly different from CR ($p < 0.05$).

Table 2. Capillary network in the CR and FFDR.

	CR	FFDR
Deep portion of M. gastrocnemius		
Capillaries/mm^2	736.4±131.6	786.2±123.3
Capillaries/muscle fibre	1.77±0.31	1.63±0.18
No. capillaries around fibre	4.56±0.61	4.52±0.63
M. soleus		
Capillaries/mm^2	486.3±49.4	523.8±49.9
Capillaries/muscle fibre	1.53±0.27	1.6±0.24
No. capillaries around fibre	4.26±0.63	4.31±0.68
Deep portion of M. plantaris		
Capillaries/mm^2	654.2±106.3	607.8±94.8
Capillaries/muscle fibre	1.57±0.13	1.43±0.16
No. capillaries around fibre	4.75±0.42	4.32±0.30
Superficial portion of M. plantaris		
Capillaries/mm^2	400.6±42.7	427.8±89.7
Capillaries/muscle fibre	1.27±0.12	1.19±0.13
No. capillaries around fibre	3.53±0.50	3.56±0.51

Data are expressed as mean±SD.

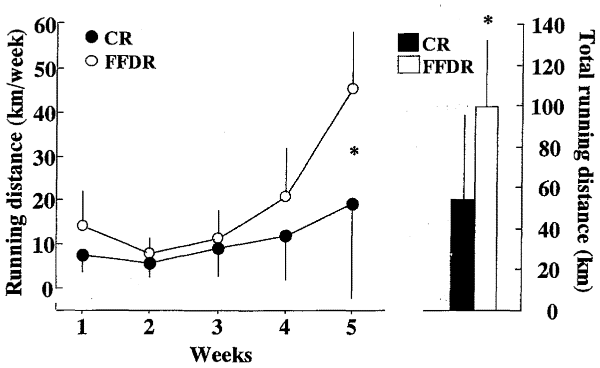

Fig. 2. *Left* : Weekly progression of voluntary running activity in the CR and FFDR. Values are the mean±SD. *:Significantly differences between CR and FFDR ($p < 0.05$).
Right : Total running distance for 5 weeks. Values are the mean±SD. *: Significantly different from CR ($p < 0.05$).

other hand, the running distance during last week and the total running distance for 5 weeks were significantly higher than that in CR.

Discussion

Interestingly, this study showed voluntary running to be related to the muscle fibre composition. Ishihara et al.[9] indicated that the percentage of slow-twitch oxidative (%SO) fibres, which was corresponded to type I fibres, was negatively correlated with voluntary running distance and the percentage of fast-twitch oxidative glycolytic (%FOG) fibres, which roughly corresponded to type IIA or type IID fibres, was positively correlated with it. All type IIA and IID/B fibres in the deep portion of M. gastrocnemius and soleus of FFDR and CR were FOG fibres (Suwa et al., unpublished observation), therefore, it is suggested that the %FOG fibres of M. gastrocnemius and soleus in FFDR are higher than those of CR. It is thus considered that the results in this study are therefore consistent with those of Ishihara et al.[9]. Although the intensity or speed of exercise were not recorded in this study, the voluntary wheel running might be a relatively high intensity exercise[14]. From these data, it appears that the voluntary wheel running exercise requires the recruitment of FOG fibres and the distribution of FOG fibres affects the activity pattern. Further experiments concerning the relation between muscle fibre composition and the characteristics of spontaneous physical activity are thus still required.

There have been several reports concerning the relationship between muscle fibre composition and fat accumulation in humans[11)20)], and rats[12)18)]. On the other hand, interestingly, our study did not show any difference in the abdominal fat content between CR and FFDR while FFDR had lower %type I fibres than CR. The data in this study conflict with the results of Mrad et al.[12], who recorded the weight gain in normal young rats having free access to food. The upper and lower quartile with respect to gain in body mass were classified as obesity-prone and obesity-resistant rats, respectively. The authors found that the obesity-prone rats had 16% fewer %type I fibres in the M. gastrocnemius than in the obesity-resistant rats. Significant genetic effects on the muscle fibre composition of rats were reported[13)17)]. In addition, no change in the muscle fibre composition due to diet was observed[19]. Therefore, it was speculated that the genetic effects mainly induced the difference of muscle fibre composition between obesity-prone and obesity-resistant rats. However, in the study of Mrad et al.[12], the rats were given a high-fat diet (60% calories from fat) whereas the FFDR and CR were fed a normal diet (17% calories from fat). The muscle fibre composition therefore might not be a single determinant of fat accumulation and thus the muscle fibre composition plays a role in fat accumulation when combined with other factors, such as food intake. In this context, we recently demonstrated that FFDR with high-fat diet was more obesity-resisitant as compared with CR with high-fat diet (Kumagai et. al, unpublished observation). Further studies focused on biochemical characteristics of skeketal muscle in FFDR.

In the present study, the running distance in the FFDR was significantly longer than in the CR. Obese animals and humans tend to be less active than lean individuals[7]. It is possible that the effect of high %type II fibres in FFDR on fat accumulation is compensated by higher physical activity.

The oxidative enzyme activity of skeletal muscle was a important determinant of fat content[15]. The oxidative capacity of skeletal muscle was associated with the supply of capillaries[1][8]. Indeed, Capillary density was negatively correlated with %fat[11]. In this study, no significant differences between FFDR and CR were observed in capillary density, the capillary to fibre ratio, and the mean number of capillaries around the muscle fibre of all muscles. This finding may thus be one possible reason why the abdominal fat content in FFDR was identical to CR.

In conclusion, the muscle fibre composition is thus not considered to be a single determinant of body fat accumulation and it influences on physical activity level. Further studies focused on aging, a high-fat diet and the amount of physical activity regarding body fat accumulation and insulin sensitivity using this animal model are thus called for.

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要約

本研究の目的は、骨格筋線維組成、毛細血管供給などの骨格筋特性、腹腔内脂肪蓄積、および自発運動量を調査することであった。選択交配によって作成された速筋優位モデルラット（fast－twitch fibre dominant rats; FFDR）とランダム交配によって作成されたコントロールラット（CR）が本研究に用いられた。いずれも、被検動物は8週令の雄ラットである。FFDRの腓腹筋およびヒラメ筋のtype I 線維の割合（%type I）はCRに比べ有意に低値であり、一方腓腹筋のtype IID/B線維の割合（%type IID/B）とヒラメ筋の%type IIA線維の割合（%type IIA）は、CRに比べ有意に高値を示した。足底筋の筋線維組成には、FFDRおよびCR間に有意差を認めなかった。FFDRの体重、腹腔内脂肪量、および毛細血管供給は、CRとの間に有意差を認めなかった。他のグループを用い、4週令で自発運動量を開始し、5週間継続する研究を行った。FFDRの8週令目の走行距離と全走行距離は、CRに比べ有意に高値であった。これらの観察から、骨格筋線維組成は腹腔内脂肪蓄積に関する単一の要因ではないが、身体活動水準には影響していることが示唆された。