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Original Article

Effect of Short Duration Stretching for Prevention of Disuse Muscle Atrophy in Mature Rats*

Toshiaki YAMAZAKI[1], Katsuhiko TACHINO[1]
Nobuhide HAIDA[1], and Susumu MUSHA[2]

Abstract

The purpose of this study was to investigate the effect of short duration stretching for prevention of disuse muscle atrophy under anesthesia in mature rats. Fourteen male Wistar rats (weight: 522±62g) were divided into experimental and control groups. Disuse muscle atrophy in experimental group was induced by hindlimb suspension (HS) for two weeks. Non-elastic tape was used to maximally stretch the right soleus muscle (SOL) by dorsiflexing the ankle joint under anesthesia for 20 min a day, 5 times a week, during the period of HS. The left SOL was left unstretched. Control rats were only anesthetized without HS.

Muscle adenosine triphosphatase (ATPase) histochemical staining, followed by morphometric analysis, demonstrated that mean cross-sectional area of muscle fiber in stretched SOL was significantly greater than that in unstretched SOL (both type I and II). No differences in muscle weight and the percentage distribution of muscle fiber type were observed between stretched and unstretched SOL. The results suggest that progression of disuse muscle atrophy in mature rats can be attenuated, but not completely prevented, by maintaining stretched position for 20 min a day.

Key words: Stretching, Disuse muscle atrophy, Mature rat

Introduction

Disuse muscle atrophy has been reported in many studies[1-5], but these contain few data of use to a physical therapist. In recent years, hindlimb suspension (HS) has been developed as a model for the effects of long-term bed rest[6)7]. We have used HS for animal experiments to study prevention of disuse muscle atrophy[8-12].

For prevention of disuse muscle atrophy in the lower extremities, weight-bearing is an important factor[9) 13]. However, clinically weight-bearing can't be applied to patients of

* 短時間筋伸張位保持が成熟ラットの廃用性筋萎縮予防に及ぼす効果

1) School of Health Sciences, Faculty of Medicine, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 920, Japan
2) Kanazawa Municipal Hospital

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long-term bed rest or soon after an operation. Instead, electrical stimulation and isometrical contraction have been used[8]. For this reason, we were particularly interested in "stretching", an important factor in atrophy prevention for skeletal muscle.

Previous findings from animal experiments indicate that skeletal muscle hypertrophies during immobilization in lengthened position, and atrophies when shortened[14)15)]. However, because lengthened immobilization can induce muscle atrophy of the antagonist, we thought it might be possible to prevent atrophy clinically by short duration stretching and have examined the effect of this procedure[11)12)]. A previous study confirmed the positive effect in the absence of anesthesia in growing rats[11)]. To exclude the influence of resistive exercise, the effect of the procedure under anesthesia is reported here[12)].

The purpose of this investigation then was to examine the effect of short duration stretching under anesthesia to prevent disuse muscle atrophy in mature rats, and to exclude the influence of growth. In addition, the results were compared with those of the previous study.

Materials and Methods

Experiments were performed on 14 mature male Wistar rats (age, 6 months; weight, 522 ±62g). Soleus muscle (SOL) atrophy was induced by HS with the aid of a jacket[12)]. Weight-bearing was the only restriction on hindlimb activity, while the forelimb maintained contact with the floor, thus allowing the rats free access to food and water. The rats were divided into an experimental and a control group.

In the experimental group, non-elastic tape (100×25mm) was used to maximally stretch the right SOL (n=7; STR) by dorsiflexing the ankle joint under ether anesthesia for 20 min a day, 5 times a week, during the period of HS[11)]. The left SOL (n=7; SUS) was left unstretched. Control rats (n=7; CON) were only anesthetized for 20 min a day without HS.

The experiment lasted two weeks. Rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital. After body weight was measured, the SOL was dissected, weighed, and prepared for histochemical study. Serial transverse sections of 10 μm were cut with a cryostat and stained for myofibrillar adenosine triphosphatase (ATPase) to determine the fiber type (Ⅰ・Ⅱ). A minimum of 200 fibers in each muscle was used to determine the percentage distribution of fiber types and the cross-sectional area of the fibers[10)].

Results were tested with a one-way analysis of variance (ANOVA). If significance was achieved ($p<0.05$), paired comparisons were performed with Scheffe's method.

Results

Summaries of the results are presented in Tables 1 and 2. Muscle wet weight and muscle-to-body weight ratio were significantly decreased in the experimental group as compared with CON, but differences between SUS and STR did not reach statistical significance. A trend toward a higher percentage of type Ⅱ fiber and less type Ⅰ fiber was noted in the experimental group, but again differences between SUS and STR were not significant.

The mean cross-sectional area of type Ⅰ fiber in SUS and STR decreased significantly

Table 1. Muscle wet weight and muscle-to-body weight ratio

(n)	SUS (7)	STR (7)	CON (7)
Muscle wet wt (mg)	143.7±23.5	163.6±22.1	232.6±34.4*
Muscle wet wt (mg) / Body wt (g)	0.33±0.03	0.38±0.03	0.45±0.04*

Values are means±SD. *: $p<0.05$ (compared with SUS).

Table 2. Percentage and cross-sectional area of SOL fibers

		SUS	STR	CON
Percentage (%)	Type I	77.6±4.6	75.1±5.3	85.0±4.5*
	Type II	22.4±4.6	24.9±5.3	15.0±4.5*
Cross-sectional area (μm^2)	Type I	1938±645	2586±748*	3784±1067*
	Type II	1603±527	2031±527*	3293±844*

Values are means±SD. *: $p<0.05$ (compared with SUS).

to 51% and 68% of CON, respectively. Similarly, the type II fiber area in SUS and STR decreased significantly to 49% and 62% of CON, respectively. In both the type I and II fiber areas, STR was significantly larger than SUS (Fig. 1).

Figure 2 shows the distribution of type I and II fiber population for each group. For both types, STR was situated between SUS and CON. The results indicate that disuse muscle atrophy could not be completely prevented by stretching, but it was possible to delay the progression in mature rats.

Discussion

Differentiation of SOL (slow muscle) in rat is slower than that of the extensor digitorum longus muscle (fast muscle), which is almost completed by the 90th day after birth[16]. Also, as muscle weight and diameter of muscle fiber grow the greatest in the first 20–30 weeks after birth[17], the rats (24 weeks) used for this study can be regarded as being in the mature phase. Further, because type I fibers of SOL in mature rats account for 85–90% of all fibers[18], it is thought that the results for CON (85%) in this study are appropriate.

No differences in muscle weight or percentage distribution of muscle fiber type were observed between STR and SUS, whereas the cross-sectional area of muscle fiber in STR was significantly greater than in SUS (both type I and II), and retardation of atrophy progress was seen. Table 3 shows a comparison of the results of our previous study and this one. In studies of growing rats, the effect of resistive exercise without anesthesia was confirmed on the basis of muscle weight and the cross-sectional area of type II fiber[11]. Furthermore, because the effect of temporary stagnation on growth by HS had to be estimated[12], this study was done with matured rats. The results show that retardation of atrophy progress was also confirmed under anesthesia. Therefore, our results suggest that progression of disuse muscle atrophy can be attenuated, but not completely prevented, by maintaining a stretched position for 20 min per day.

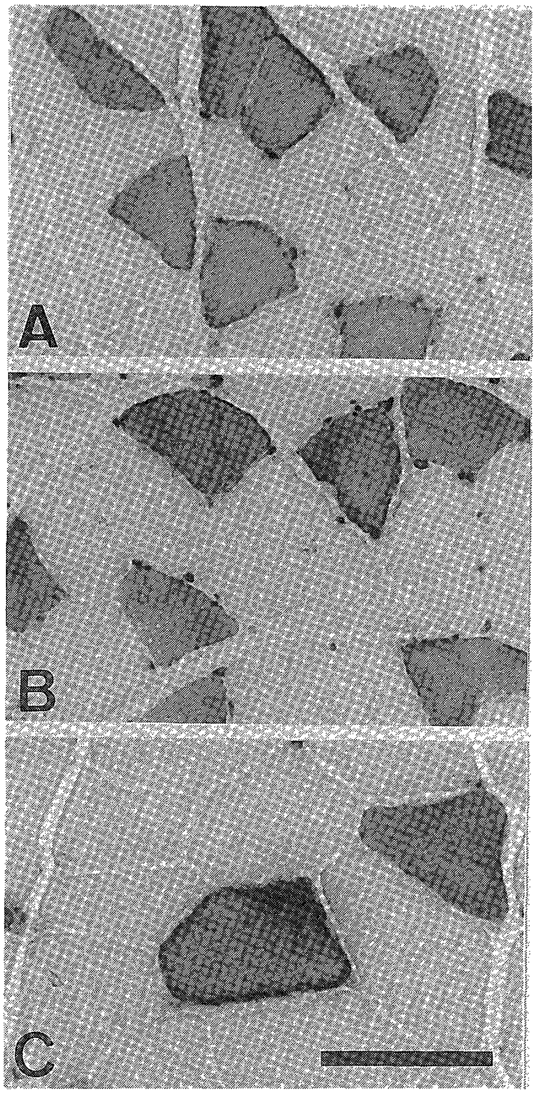

Fig. 1. Cross-sections of soleus muscle is shown. Alkaline ATPase activity was used to discriminate type I (light stain) and type II (dark stain) fibers. A: SUS, B: STR, C: CON. The scale bar represents 100 μm.

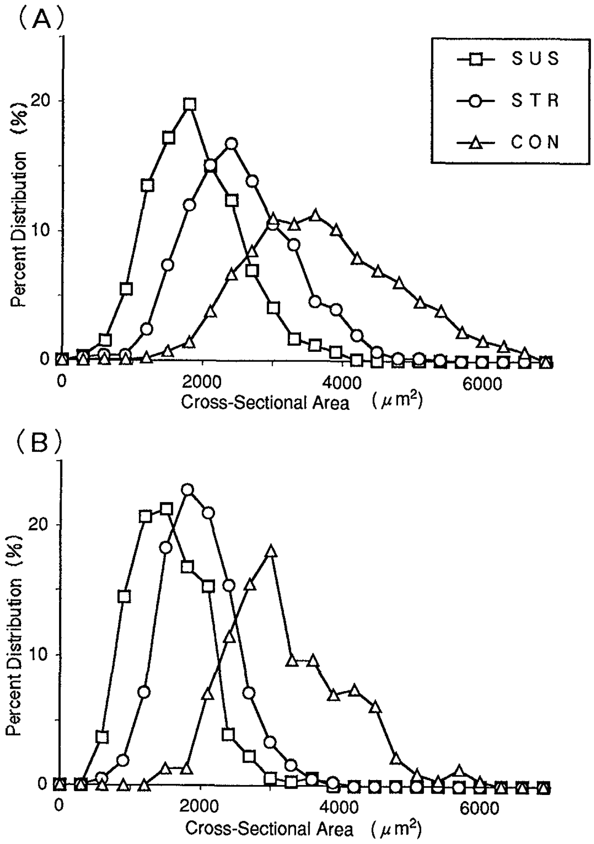

Fig. 2. Percent distribution in cross-sectional area of muscle fiber. (A) Type I fiber. (B) Type II fiber.

As for the degree of attenuation, although the two studies show few difference in regard to type I fiber, consideration that the percentage of type I is higher in the mature than in the growing phase would lead one to expect, stretching would be more effective for the entire SOL in the mature phase. Reversely, although differences in the relative values for type II fiber were seen in the growing and mature phases, namely 8% and 13%, respectively, almost similar effect can be expected because the actual percentage, even in the mature phase, is relatively low.

Chino[19] has reported that atrophy as a result of aging is more remarkable in type II than type I. It is suggested that muscle power decreases remarkably, but that muscle strength is comparatively maintained in older people. Furthermore, Haida *et al.*[20] have shown in animal experiments that atrophy in the early stage of the aging process is produced selectively in fast muscle. Consequently, in terms of retarding the progression of disuse atrophy as a result of aging, inhibition of atrophy in type II fiber is regarded as important. As the effect of short duration stretching was recognized in the mature phase for both type I and II fiber, further studies of the effect in older rats is needed.

Table 3. Comparison of stretching effect by difference in experimental method

Experimental method	Phase of rat	Relative wt ratio	Percentage of fiber types	Cross-sectional area type I	Cross-sectional area type II
Without anesthesia (Ref. 11)	Growth	STR>SUS	NS	STR>SUS (19%)	STR>SUS (17%)
Under anesthesia (Ref. 12)	Growth	NS	NS	STR>SUS (16%)	STR>SUS (8%)
Under anesthesia (This study)	Maturity	NS	NS	STR>SUS (17%)	STR>SUS (13%)

NS: Not significant. >: Significant. STR: Stretch group. SUS: Suspension group. Parcentage in parenthese shows difference in relative value compared with control.

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<要　　旨>

短時間筋伸張位保持が成熟ラットの廃用性筋萎縮予防に及ぼす効果

山崎　俊明，立野　勝彦，灰田　信英
金沢大学医学部保健学科

武舎　　進
金沢市立病院

成熟ラットヒラメ筋の廃用性萎縮予防に及ぼす，短時間筋伸張位保持の効果を麻酔下で検索した。

14匹の成熟ウィスター系雄ラット（24週齢）を，実験群と対照群に分けた。実験群は2週間の後肢懸垂法にて筋萎縮を惹起した。その間，右足関節を一日20分間（5日／週），麻酔下で最大背屈位に保持し，ヒラメ筋を伸張した（伸張群)。左側は懸垂のみとした（懸垂群)。対照群は懸垂せずに，麻酔のみ実施した。実験終了時にヒラメ筋重量を測定後，ATP染色を行い組織化学的に分析した。

その結果，筋湿重量および筋線維タイプ構成比率は，実験群間に差はなかった。伸張群の筋線維断面積の平均値は，タイプI・IIともに対照群より萎縮を示したが，懸垂群より有意に大きかった。以上より，短時間筋伸張位保持は，成熟ラットヒラメ筋の廃用性萎縮による断面積の減少を，抑制できることが示唆された。