Age-related differences in the effect of weight bearing on the rat soleus muscle subjected to hindlimb suspension

Original Article

Journal of the Tsuruma HEALTH SCI. MED. KANAZAWA UNIV. Vol. 29(1)
35~50 2005

Age-related differences in the effect of weight bearing on the rat soleus muscle subjected to hindlimb suspension

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ABSTRACT

OBJECTIVE : The purpose of this study was to explore differences in the effects of weight bearing (WB) on the soleus muscle associated with aging among rats reared with their hindlimbs suspended. METHODS : A total of 34 3- and 9-month-old male rats were assigned to two experimental groups and one control group for a 3-week experiment. The rats in one experimental group (HS) had their hindlimbs kept in suspension and the other experimental groups' (HS+) hindlimbs were also suspended but subjected to loading of the soleus through WB for one hour per day that began one week after the commencement of the experiment. The control group was subjected to no suspension. The control group had four 3-month-old and four 9-month-old rats and the HS and HS+ groups seven 3-month-old and six 9-month-old rats each. ANALYSIS : To examine whether or not the effect of WB would differ depending on age, the difference in CSA between the HS+ and HS groups was compared between the two age groups using the Student's t test. The mean CSA of each soleus fiber was analyzed, as well as a comparison of the distribution pattern of the muscle fibers using the Kolmogorov-Smirnov test. Each histogram was constructed from 50 randomly selected fibers from all of the samples. RESULTS : The wet weight of the soleus relative to the body weight of the 3-month-old rats was significantly lower in the HS and HS+ groups than that in the control group. Among the 9-month-old rats, there were no significant inter-group differences in the relative weight of the soleus. Nor were there significant inter-group differences among any of the two age groups in terms of the percentage of fiber type of the soleus. The analyses by means of the mean cross-sectional area and the histogram revealed no effect of WB on type Ⅱ fibers for the two age groups. The WB-related change in the mean cross-sectional area of type Ⅰ fibers was also not significantly different between the two age groups. But the histogram analysis revealed that the effect of WB on the soleus for the 9-month-old rats was greater than that observed for the 3-month-old rats. CONCLUSION : The findings suggest that WB is more effective for attenuating progression of muscle atrophy in 9-month-old rats than in 3-month-old rats.

KEY WORDS

Disuse atrophy, Hindlimb suspension, Weight bearing, Aging, Soleus

Introduction

Disuse syndrome is a collective term used for reduced physical strength and physical and mental symptoms caused by prolonged inactivity or bed rest[1], symptoms of which can appear in various organs throughout the entire body[2]. Healthcare professionals have been paying close attention in recent years to the complications caused by inactivity or bed rest and the benefits of exercise in dealing with these problems. Disuse atrophy of the skeletal muscles can be roughly divided into two types : localized atrophy due to immobilization of joints and systemic atrophy due to reduced activity[3]. Both types of disuse atrophy cause a reduction in muscle strength. The magnitude of reduction in strength due to systemic disuse muscle atrophy is 10-15% after one week of bed rest and

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50% after 3-5 weeks of bed rest. The effect is particularly great on the anti-gravity muscles of the lower limbs and the trunk[2].

The importance of weight bearing (WB) on the lower limbs has been pointed out in past studies in the prevention and treatment of disuse muscle atrophy[4, 5]. In a clinical situation, walking and intense muscle strengthening exercise are not possible for every patient because of his or her physical or mental condition. The WB exercise of standing is effective as a progressive loading after a bed-ridden period or prior to the recommencement of walking. In one study, WB while standing practiced by inactive patients was more useful than muscle strengthening engaged in lying[6].

The salient characteristic of aging musculoskeletal change is a loss of muscle mass. Muscle mass begins to decrease at around 25 years of age, which cannot be prevented even by regular exercises[7]. After age 50, the muscle mass decreases by 1% each year, and this accelerates after age 65. Eventually, by age 80, 20-30% of the total muscle mass is lost[8]. Factors associated with age-related decrease in muscle mass include attenuation of peripheral nerve innervations to muscles, reduction in myoprotein synthesis, the effects of hormones and a reduction in the supply of substrates for myoprotein synthesis[8, 9]. The decrease in muscle strength observed in the elderly can be largely attributable to a reduction in muscle mass. In this connection, it has recently been pointed out that exogenous factors such as inactivity and reduced activity during daily life have a greater impact than the endogenous factors cited above[8]. Aging, thus, seems to intensify muscle atrophy, so that the length of time required for recovery from disuse atrophy resulting from prolonged bed rest will be longer as individuals age. Furthermore, the response to WB may be slower in the elderly than in younger patients. On the contrary, it is also known that individual differences in physiological function are broadened by aging[10]. The age-related decrease in muscle mass and increase in body fat ratio[11] will, therefore, lead to a relative increase in the load on the muscles of the lower limbs during WB. Consequently, with advancing age, it is likely that individual differences in the effect of WB may become greater, or the effect of WB may become greater.

In clinical situations, the majority of disuse muscle atrophy is secondary to underlying conditions. Accordingly, animal models have been used to study the actual pathophysiology of disuse atrophy[12]. Several animal models are available for this purpose, including models of disuse atrophy induced by immobilization with casts, denervation, hindlimb suspension, to name a few. Of these techniques to induce disuse atrophy, hindlimb suspension has been used to create systemic disuse atrophy such as that resulting from prolonged bed rest.

The author does not consider that past studies using animal models have adequately clarified the relationship between aging and changes that occur in skeletal muscles due to disuse or the effect of WB on atrophic muscles. The contractility of the soleus has been found to be unrelated to age, but the degree of atrophy assessed morphologically and histochemically was greater among younger rats than aged rats subjected to hindlimb suspension[13]. Regarding the effect of WB for preventing muscle atrophy, some investigators have reported that this effect has been confirmed even among aged rats[14], while others have found that intervention by WB can alter the function of the satellite cells of aged rats, leading to exacerbation of disturbed muscle responses[15]. In all of these previous studies, the rats were subjected to hindlimb suspension alone or to hindlimb suspension immediately following WB. In the present study, one week of hindlimb suspension preceded WB. This experimental design reflected the course found in many clinical situations of prolonged bed rest leading to the onset of disuse atrophy, and consequently followed by mobilization in standing. Morphological and histochemical analyses of the soleus of each mature rat were conducted to determine whether or not the effect of WB on disuse atrophy would differ with advancing age. Specifically, histograms of cross-sectional areas (CSA) of the muscle fibers were constructed, followed by statistical comparisons.

Methods

1. Animals and Protocol

Thirty-four male Wistar rats (Charles River Japan, Atsugi, Japan) were used for a 3-week experiment. Of these, 18 were 3 months old with their body weight ranging from 380 to 420 g and 16 were 9 months old with their body weight ranging from 572 to 670 g. The author randomly divided the rats in each age group into three subgroups. The rats in the WB (HS+) group were reared with their hindlimbs in suspension and subjected to a load on the soleus for one hour every day that began one week after the commencement of the experiment. In the suspension (HS) group the rats were reared with the hindlimbs in suspension during the whole experimental period and, in the control group, they were reared with no suspension of the hindlimbs. Table 1 shows the number of randomly allocated rats to the three subgroups of the two age groups. The rats were kept in individual cages measuring 280 × 440 × 180mm, placed in an ambient room temperature of 21° to 26° Celsius and a 12-hour cycle of light and dark periods to simulate a 24-hour day. The light period commenced at 0800 hrs and terminated at 2000 hrs and the dark period lasted from 2001 to 0759 hrs. The experiment was carried out under the authorization of the Kanazawa University Takaramachi Campus Animal Experiment Committee, and the rats were treated in accordance with the Guidelines for Experiments prepared by the said committee (Approval No. 041781).

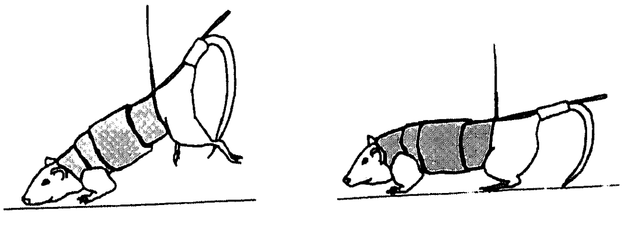
2. Hindlimb suspension and weight bearing

The hindlimbs of each rat in HS+ and HS groups were suspended in a jacket developed by Musacchia et al.[16] with modification by Yamazaki et al.[17] A jacket for immobilization of the rats, linked to a metal post and a ring, was fastened to the forelimbs and trunk of each rat, and it was adjusted with Velcro tape. The proximal end of the tail was immobilized on the metal post, using adhesive tape. The hindlimbs were then suspended in the jacket using a ring. The jacket was applied to each rat while under general anesthesia with diethyl ether. The rats in the control group were also administered anesthesia, but they were not required to wear the jacket. A stainless steel pipe was placed along the major axis of the cage at a point above the center. It was connected with a swivel hook to the suspension ring. The height of suspension was adjusted so that the hindlimbs of the rat would not touch the cage floor (Fig. 1). During suspension, each rat was free to move, but was unable to place its hindfeet on the floor or the side of the cage. The rat used its forelimbs to move about while the hindlimbs were suspended in space. Each rat was able to move in an anteroposterior direction along the stainless steel pipe assembled along the major axis of the cage and to fully rotate themselves 360 degrees. All of the rats were allowed free

Table 1. Age, number and three subgroups of rats

	CON	HS+	HS
3 month	4	7	7
9 month	4	6	6

CON: Control; HS+: Hindlimb suspended with weight bearing; HS: Hindlimb suspended.


Suspended

Weight bearing

Fig. 1. Schematic representation of the hindlimb suspension.

access to food and water.

Daily WB on the hindlimbs for one hour was conducted from 0930 to 1030 hrs of the daylight period. The timing for this loading was selected because it was during this period of the day that the rats that are of a nocturnal nature were relatively inactive[17] so that over-activity could be avoided as much as possible during intervention. The loading was carried out on the rats in a conscious state with the jacket *in situ* by releasing them from the suspension (Fig. 1). In both age groups, a few rats moved about the cage immediately after weight application, but the majority of them remained motionless within the cage. The suspension was resumed at the termination of the one-hour intervention.

3. Preparation of the soleus

Each rat was weighed at the termination of the experiment. The right soleus of each rat was subjected to testing. The soleus is an anti-gravity muscle prone to the influence of non-WB[18-22]. The muscle was removed while under anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg body weight). The wet weight of the soleus was measured immediately after isolation. Because the wet weight of the muscle is affected by the body weight, the relative weight of the soleus was calculated using the following equation :

Relative weight=wet weight (mg)/body weight (g)

The muscle was then cut into approximately 5mm pieces including the center of the venter and fixed on a corkboard. Then, the fixed muscle pieces were immediately frozen with liquid nitrogen in cooled isopentane and stored at $-70°$ Celsius until analysis took place.

4. Myosin adenosine triphosphatase staining

The frozen fiber section was subjected to myosin adenosine triphosphatase (ATPase) staining. This method is regarded as an excellent means of typing muscle fibers[23]. Red muscle corresponds to type I fibers, and white muscle to type II fibers. In routine ATPase staining, the type I fibers lose their activity following pretreatment with alkali. Therefore, the type I fibers become stained white, while the type II fibers that retain activity become stained black[23].

Transverse sections were prepared from each frozen section. Within a cryostat with a temperature of $-25°$ Celsius, each section was then cut into 10μm-thick slices and dried on a glass slide. Thereafter, the following consecutive steps were carried out[24] : a) preincubation for 15 min in 0.1M sodium barbital and 0.18M $CaCl_2$ in distilled water, the pH was adjusted to 10.7 or 10.8 with NaOH ; b) incubation for 45 min at 37℃ in ATP disodium salt, 0.1M sodium barbital and 0.18M $CaCl_2$ in distilled water, the pH was adjusted to $9.4-9.7$ with NaOH ; c) washed for 3 minutes with 3 changes of 1% $CaCl_2$; d) immersed for 3 min in 2% $CoCl_2$; e) washed in 8 changes of 0.01M sodium barbital ; f) washed in 3 changes of distilled water ; g) immersed for 1 min in 1% yellow ammonium sulfide ; h) washed in distilled water ; i) dehydrated in graded series of ethanol, passed through xylene and then cover slipped.

5. Percentage of each type of muscle fiber and measurement of the cross-sectional area

The ATPase-stained section was observed under a light microscope (BX50, Olympus) and a photographic image was taken with a digital camera, followed by feeding it into a computer. Using NIH Image 1.62 (a public domain NIH image program), more than 200 muscle fibers constituting each section were classified into type I and II fibers, and the percentage distribution of each type was calculated. Furthermore, the cross-sectional area (CSA) of the muscle fiber was measured.

6. Statistical analysis

1) Comparison of the mean of each parameter

For each group, the mean±SD of each parameter was obtained. In each group, pre- and post-experiment body weight was compared using the Student's *t* test.

A two-way analysis of variance (ANOVA) was employed to determine interactions between age and WB. The mean of each parameter was compared among the three groups for each age and between the two age groups. The Bonferroni's method was employed for all of the multiple comparisons.

In order to examine whether or not the effect of WB would differ depending on age, the difference in CSA between the HS+ and HS groups was compared between the two age groups using the Student's *t*-test.

Table 2. Changes in the body weight of the rats during the experiment

	3 month			9 month		
	CON	HS+	HS	CON	HS+	HS
Initial (g)	407.5±5.3	393.1± 8.6	399.7±15.6	645.5±26.5	619.7±29.0	624.7±19.1
Terminal (g)	449.5±1.0*	293.1±29.6*	290.0±14.5*	623.0±28.5	433.3±35.0*	469.8±24.8*
Rate of change (%)	110±2	74±7	73±5	97±1	70±6	75±3

CON: control; HS+: hindlimbs suspended with weight bearing; HS: hindlimbs suspended.

Values are mean±standard deviation.

Rate of change (%) =(Terminal value/Initial value) × 100.

* p<.0001 (vs. Initial).

Table 3. Comparison of the parameters for the soleus muscle

		CON	HS+	HS
Wet weight (mg)				
	3 month	236.5±28.8	111.3±15.1‡	102.0±14.1‡
	9 month	328.5±19.7†	215.8±21.2‡,§	202.5±34.2‡,§
Relative weight				
	3 month	0.53±0.06	0.38±0.05*	0.35±0.04‡
	9 month	0.53±0.04	0.50±0.07¶	0.43±0.08
CSA of the type I fibers (μ㎡)				
	3 month	4688.11±689.95	1721.49±266.98*	1401.29±129.19*
	9 month	5413.26±607.28	2874.64±765.74*,†	2236.21±312.79*,§
CSA of the type II fibers (μ㎡)				
	3 month	3547.11±643.33	1492.47±408.39*	1419.38±237.31*
	9 month	4941.48±166.92¶	2755.38±542.44*,†	2335.44±363.68*,§

CON: control; HS+: hindlimbs suspended with weight bearing; HS: hindlimbs suspended.

CSA: cross-sectional area.

Values are mean±standard deviation.

¶: p<.05 (vs. 3 month); †: p<.01 (vs. 3 month); § : p<.001 (vs. 3 month)

* : p<.001 (vs. CON); ‡ : p<.0001 (vs. CON)

2) Analysis of histograms of CSA

The mean CSA of each soleus fiber was analyzed, as well as a comparison of the distribution pattern of the muscle fibers. Fifty samples were randomly selected from each group followed by the computation of the descriptive statistics. A histogram was constructed at intervals of 700 μm^2 for type I fibers and 500 μm^2 for type II fibers in each group. A two-sample Kolmogorov-Smirnov test was employed to compare the histogram patterns.

Results

1. Changes in the body weight and wet weight of the soleus in the rats

Table 2 shows the time course of the rats' body weight. On commencement of the experiment, there was no significant difference in the body weight between any two of the three groups of 3-month-old or 9-month-old rats. At the termination of the experiment, the 3-month-old rats in the control group significantly increased their body weight, while it decreased in HS+ and HS· groups. Among the 9-month-old group, the body weight did not change in the control group, while it significantly decreased in HS+ and HS groups.

Table 3 shows the wet weight and relative weight of the soleus. The two-way ANOVA revealed no interaction between the age of the rats and WB in the absolute wet weight and relative weight of the soleus. In the control group, the wet weight of the soleus was significantly heavier for the 9-month-old rats than for the 3-month-old rats. The relative weight of

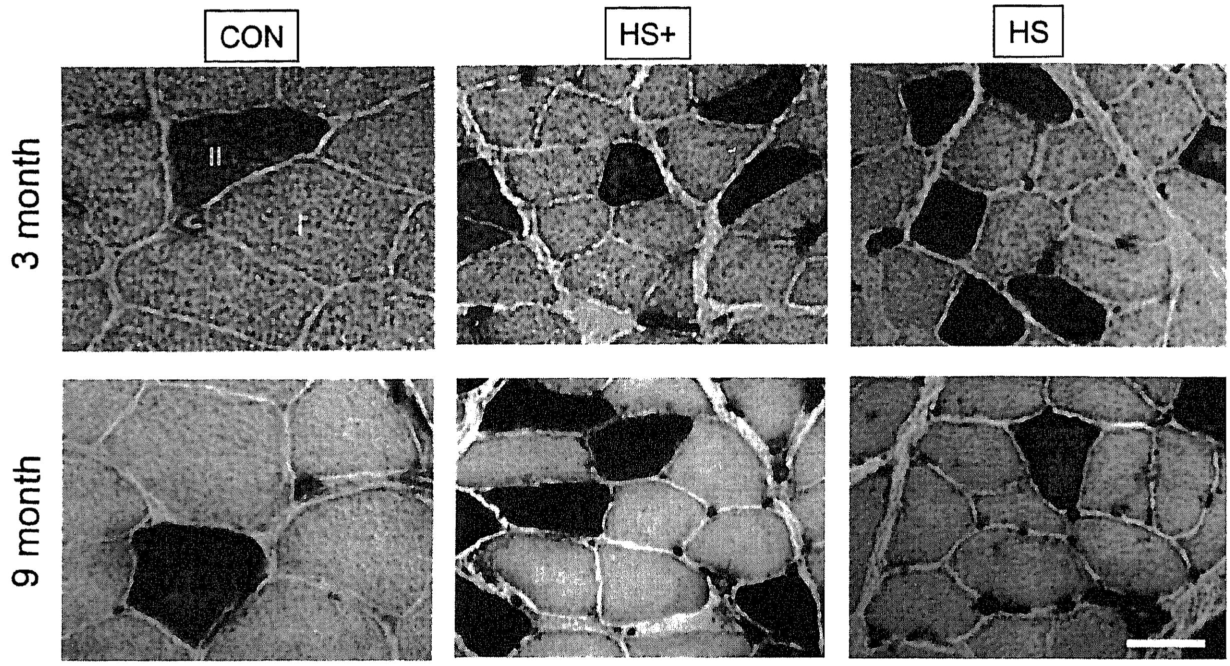


Fig. 2. Histological examination of the soleus fibers with ATPase staining. The upper section represents the 3-month-old rats and the lower section the 9-month-old rats in each group.
CON : control ; HS+ : hindlimbs suspended with weight bearing ; HS : hindlimbs suspended.
I : Type I fiber ; II : Type II fiber.
Bar : 50μm.

soleus in the control group showed no significant age-related difference. When the wet weight of soleus was analyzed for each age, it showed a significantly greater decrease in the HS+ and HS groups than in the control group. Among the 3-month-old rats, the relative weight of soleus was significantly smaller in HS+ and HS groups than in the control group ($p<.001$), but among the 9-month-old rats, this parameter showed no significant difference among any two of the three groups.

2. Histological findings following ATP staining (Fig. 2)

Type I fibers were stained white, while type II fibers were stained black. For both age groups, a decrease in the CSA was noted in HS+ and HS groups.

3. Percentage distribution of the fiber type of the soleus (Table 4)

The type I and II fibers in the control group accounted for 89.4% and 10.6% for the 3 month-old rats and 88.4% and 11.6% for the 9 month-old rats. The 2-way ANOVA revealed no interaction between age and loading. When the percentage distribution of the type I and II fibers was analyzed for each age group, there was no significant difference between any two of the three groups.

4. Comparison of CSA (Table 3)

The 2-way ANOVA revealed no interaction between age and loading. In the control group, the CSA of type I fibers did not differ significantly according to age, while that of the type II fibers was significantly greater for the 9-month-old rats than the 3-month-old rats. When the types I and II fibers were analyzed for each age, the CSA was significantly smaller for the HS+ and HS groups than for the control group of both the 3- and 9-month-old rats ($p<.001$). The CSA of type I fibers tended to be greater in HS+ group than in HS group ($p=.06$ for 3 months of age and $p=.08$ for 9 months of age), although no such tendency was apparent for type II

Table 4. Percentage distribution of the fiber type of the soleus

	Type I fibers (%)	Type II fibers (%)
3 month		
CON	89.4±2.3	10.6±2.3
HS+	85.3±6.1	14.7±6.1
HS	86.3±5.6	13.7±5.6
9 month		
CON	88.4±5.8	11.6±5.8
HS+	85.5±8.1	14.5±8.1
HS	84.9±5.7	15.1±5.7

CON: control; HS+: hindlimbs suspended with weight bearing; HS: hindlimbs suspended. Values are mean±standard deviation.

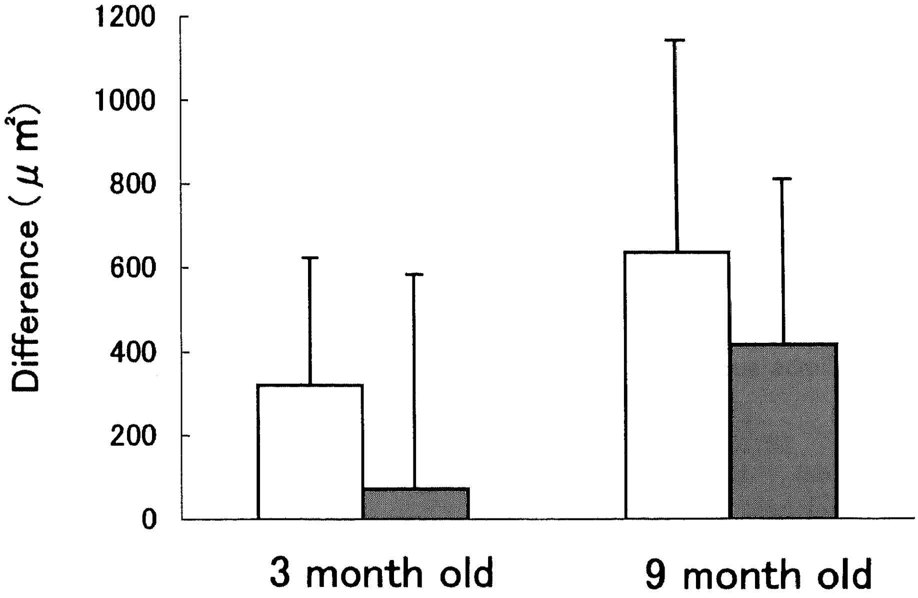


Fig. 3. Difference in the CSA between the two experimental groups comparing the effect of weight bearing.
□: Type I fibers ; ■: Type II fibers.
Error bars denote standard deviation.

fibers.

There was no significant difference in the effect of WB on type I or II fibers between the two age groups (Fig. 3).

5. Histogram for CSA

In Figures 4 and 5, the vertical axis of the histogram indicates the number of fibers included in the class, while the horizontal axis indicates the upper limit of CSA for each class. Table 6 shows the result of the Kolmogorov-Smirnov test for the pattern of the histogram.

Table 5. Basic statistics for the histogram (μm^2)

	3 month			9 month		
	CON	HS+	HS	CON	HS+	HS
Type I fibers						
Mean	4866.2	1758.3	1460.4	5190.1	2912.6	2171.0
SD	1633.6	581.7	374.5	1252.2	1040.0	632.7
Minimum	1719.8	1003.9	611.2	2776.0	891.4	873.7
Maximum	8895.7	3297.6	2314.0	8700.2	5813.3	3516.5
Range	7175.9	2293.7	1702.8	5924.2	4921.9	2642.8
Type II fibers						
Mean	3509.3	1523.3	1414.9	4794.3	2662.8	2366.0
SD	811.0	524.0	341.0	1099.6	968.1	631.5
Minimum	1899.4	478.7	791.8	1650.9	1069.6	517.9
Maximum	4907.3	2679.4	2476.3	7397.9	6144.1	3688.9
Range	3007.9	2200.7	1684.5	5747.0	5074.5	3171.0

CON: control; HS+: hindlimbs suspended with weight bearing; HS: hindlimbs suspended.
SD: standard deviation.

Table 6. Comparison of histogram's patterns

	Type I fibers		Type II fibers	
	3 month	9 month	3 month	9 month
CON				*
HS+	†	*,†,§	†	†
HS	†	*,†	†	†

CON: control; HS+: hindlimbs suspended with weight bearing; HS: hindlimbs suspended.
*: p<.001 (vs. 3 month)
†: p<.001 (vs. CON)
§: p<.001 (vs. HS)

1) Comparison of the type I fibers in the histograms

For the type I fibers, both the range of distribution and the mean CSA were smaller in the HS+ and HS groups than in the control group for both age groups (Table 5, Fig. 4). In other words, the distribution of the CSA in the HS+ and HS groups was biased toward the smaller side. The result of the Kolmogorov-Smirnov test revealed no significant difference in the pattern on the histogram between the two ages in the control group (Table 6). But, there were significant differences in the pattern between the ages in the HS+ and HS groups, indicating that it differed between the two age groups. The inter-group comparison for each age resulted in a significant difference in the pattern between the control and HS+ groups for both age groups and also between the control and HS groups. There was a significant difference in the pattern between the HS+ and HS groups of the 9-month-old rats, but the 3-month-old rats did not yield any statistical significance.

2) Comparison of the type II fibers in the histograms

For the type II fibers, both the range of distribution

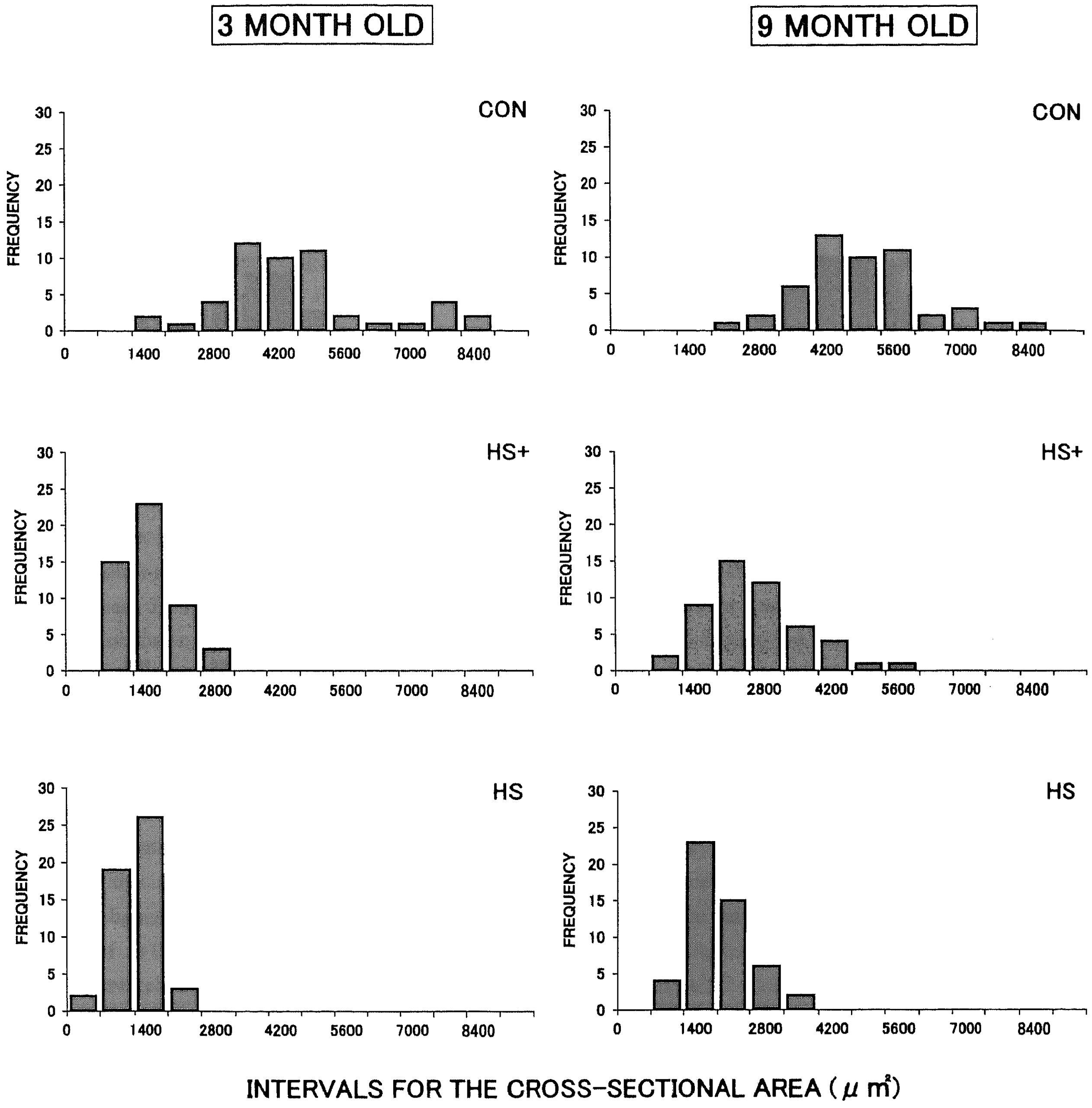


Fig. 4. Frequency distribution of the cross-sectional area for the type I fibers of soleus of the three groups.
CON : control ; HS+ : hindlimbs suspended with weight bearing ; HS : hindlimbs suspended.

and the mean CSA were smaller in the HS+ and HS groups than in the control group for both age groups (Table 5, Fig. 5). In the HS+ and HS groups, the distribution of the CSA was biased to the smaller side as in the type I fibers. The result of the Kolmogorov-Smirnov test revealed a significant difference in the pattern for age in the control group (Table 6), precluding the author's ability to compare the HS+ group with the HS group. The result of the inter-group comparison for each age was similar to that for the control groups for both age groups. There was a significant difference in the histogram patterns between the control and HS+ groups and between the control and HS groups, but there was no significant

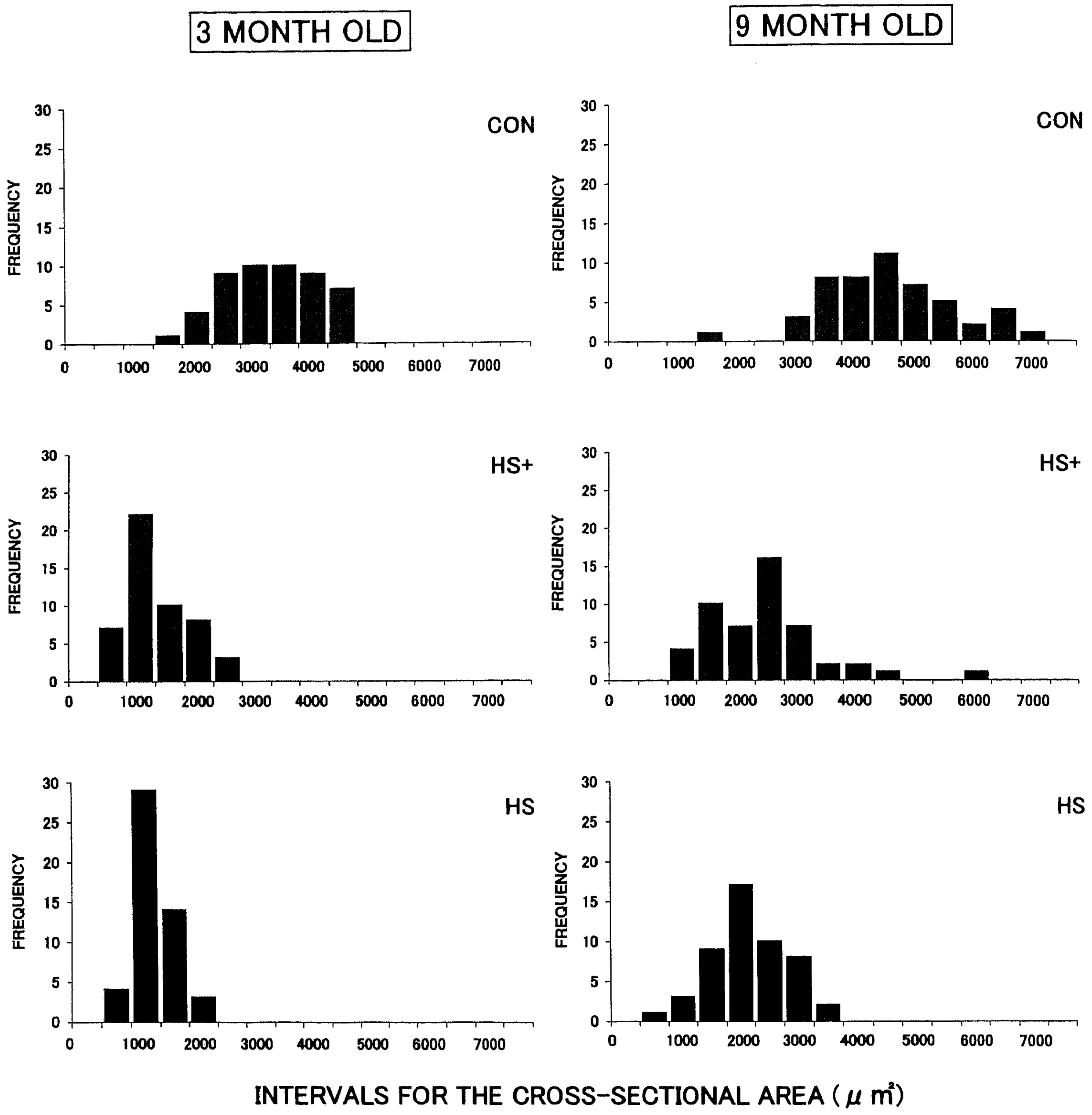


Fig. 5. Frequency distribution of the cross-sectional area for the type II fibers of soleus of the three groups.
CON : control ; HS+ : hindlimbs suspended with weight bearing ; HS : hindlimbs suspended.

difference between the HS+ and HS groups.

Discussion

The discussion will be focused on the age, lifespan and physical characteristics of rats tested in this study. The mortality doubling time shown by Finch et al. differs from humans to experimental rats ; i.e., 8.9 vs. 0.3 years[25]. The mortality doubling time is defined as the interval of time needed for the probability of death to be doubled. The body weight of male Wistar rats continues to increase during the first 52 weeks of rearing, but it begins to slow down at about 20 weeks[26]. Differentiation of the soleus of rodents is found to be completed in 8-14 weeks after birth, and that the weight of the hindlimb muscles and the diameter of muscle fibers reaches its maximum at 20 to 30 weeks of age[27]. Among the rats used in this study, the 3-month-old rats had a percentage distribution of a fiber type of the soleus comparable to that of adult rats, and 9-month-old rats had a diameter of the hindlimb soleus fibers equivalent to the diameter seen some time after it has reached its maximum. With regard to the fiber type percentage distribution of the soleus in mature rats, the type I fibers are found to account for more than 90%[28]. In fact, the percentage of type I fibers in the present study was 89.4% for the 3-month-old rats and 88.4% for the 9-month-old rats. The wet weight of the soleus was greater for the 9-month-old rats than for the 3-month-old rats, but its relative weight was similar for both age groups. The mean CSA was greater for the 9-month-old rats than for the 3-month-old rats for both the type I and II fibers. However, the type I fibers showed no significant difference in the mean CSA between the two age groups. It has been shown that the percentage of the type I fibers of the rats' skeletal muscle increases and that of the type II fibers decreases with age[29, 30]. In the present study, the percentage of the type II fibers for the 9-month-old rats was not markedly lower than that for the 3-month-old rats. It may, therefore, be stated that the present study has examined disuse-associated changes and an aging-related difference in the effect of WB for skeletal muscles of mature rats before fast twitch fibers begin to decrease.

The wet weight of the soleus in the HS group was approximately 40% of that in the control group for the 3-month-old rats and approximately 60% of that of the control group for the 9-month-old rats, thus, showing atrophy of the soleus in the HS group. In the HS+ group, WB was performed for one hour each day after one week of hindlimb suspension. Except for the time of the WB period, the rats were kept load-free, like those allocated to the HS group. Although the hindlimb of the HS+ group showed some atrophy, it was less severe than that observed in the HS group. When changes in the body weight during the experiment were analyzed, the 3-month-old rats in the control group gained 10% weight on average, while the 9-month-old rats in the same group showed little change in their body weight ; approximately 3% of their weight was lost. In the HS+ and HS groups, a 25%-to-30% weight loss was noted for both the 3-month-old and the 9-month-old rats.

Both hypertrophy and atrophy of muscles are reversible[31]. The decrease in skeletal muscle mass (i.e., muscle atrophy) seen while in prolonged bed rest is more likely to occur in anti-gravity muscles[32]. Rats whose hindlimbs have been kept load-free show a similar pattern to the one seen in patients following prolonged bed rest. Jaspers et al. found in their analysis by the fiber type composition, that muscle atrophy was more marked in the ankle plantar-flexors (slow muscles containing more type I fibers) than the dorsiflexors (fast muscles containing more type II fibers)[33]. Thomason et al. and Thompson, in their reviews of the fiber type of the soleus in the absence of load, found changes in both the type I and II fibers occurred rapidly during the first 1 to 2 weeks[34, 35]. During the author's 3-week experiment, the CSA in the HS+ and HS groups decreased, similar to the results shown in the aforementioned reports. It has also been reported that suspension of the hindlimb results in a shift of the type I fibers to type II fibers, i.e., a shift to fast twitch fibers[34, 36-39]. On the contrary, it has also been pointed out that change in the percentage of fiber type or the contraction time in load-free animals is small depending on the strain and age of the rats, or the duration of the experiment[5, 13, 40]. In the present study, the change in the percentage distribution of fiber type observed in the HS+ and HS groups for both the 3- and 9-month-old rats resembled those shown by Brown, M. e al.[5], Simard, C. et al.[13], and Simard, C. et al.[40]. Tha is, the percentage of the type II fibers increased slightly, but no significant change was observed. The rats used in previous studies showing a shift in the fiber type were either 6 to 8 weeks old corresponding to the soleus muscle growth and differentiation period[37, 36]. Also mature rats were used for studies tha found no significant shift in the fiber type[5, 13]. It is therefore, likely that the degree of shift in the fibe type in load-free animals can vary depending on th growth stage of the muscle and the age of the ani mal.

In the present study, WB was used as a method o loading. The skeletal muscles of the rats were loaded by their own body weight, and this load also caused stretching of the soleus. Fujiwara et al. investigated the load on human leg muscles relative to that during maximum voluntary contraction (%max)[41]. They reported that the relative muscle load for the soleus was 4.9% max in the quiet standing position and 28.4% max during maximum forward bending. It has been found that the strength of the skeletal muscles is maintained if they contract up to 20-30% of their maximum strength and will increase if they contract up to 30% or more of their maximum strength[42]. It may, therefore, be stated that WB in standing exerts sufficient load on the muscle to retain strength, therefore, retaining muscle mass. In fact, in a clinical situation, WB has been reported to be useful because it increases muscle strength[6]. The mechanism of this effect is probably explained by the fact that WB is likely to achieve a high degree of muscle contraction compared to concentric contraction in positions other than standing[43] and as well as by the specificity of muscular activity in standing[44]. Another possible factor explaining such a mechanism is task specificity that is known as one of the mechanisms for neural adaptation in muscle strengthening[45]. More than 18 hours per day were required for the duration of loading to prevent changes in the CSA of soleus fibers of rats in hindlimb suspension[36]. In a 2-week experiment conducted by Brown et al., the unweighted rats showed a 37% reduction in wet weight of the soleus as compared to the control rats, but the decrease was smaller (22%) for the rats loaded for one hour per day[5]. Brown et al. reported that WB that was commenced early during a period of prolonged bed rest could slow down a decrease in muscle mass and strength of postural muscles such as the soleus.

When multiple comparison of the mean CSA was carried out to analyze the effect of WB in the present study, the CSA for the type I fibers tended to be greater in the HS+ group than in the HS group for both age groups. No such tendency was observed for the type II fibers, demonstrating that the effect of WB was more apparent for the type I fibers. This finding agrees with that reported by other investigators[46, 47]. Generally, muscle strengthening is carried out either statically or dynamically. It has been shown that static contraction of muscles leads to a greater increase in the CSA of red muscle fibers than that of the white muscle fibers[48]. The method of WB in this study can be classified as static exercise, and this probably explains why it was primarily effective on the type I fibers. Factors that caused changes in the CSA in the HS+ group included not only attenuation of the progression of atrophy, but also possible hypertrophy effected by WB. However, it is known that the nervous system adapts itself faster than the muscles to strengthening exercises, and that muscular adaptation observed in the early stages of training are a neural factor in nature[49]. Fukunaga et al. conducted an experiment involving 60 days of static exercise and reported that the strength increased from the beginning of the training, but the CSA showed little change during the first 20 days of training[50]. In view of the findings of these past studies, the difference in the CSA between the HS+ and HS groups after the 2-week loading in this study is attributable to suppressed progression of atrophy of the soleus in the HS+ group. Meanwhile, two-way analysis of variance revealed no interaction between the age of the rats and WB.

With regard to the effect of WB by analysis of the difference in CSA between the two experimental groups, it did not differ vis-à-vis the age of the rats. The muscle mass that is lost by prolonged immobilization with casts or external fixation is recovered in juvenile animals, but it was not so in the aged animals even after a long period of recovery[51, 52]. These findings imply that the underlying mechanisms regulating the size of muscles are likely to change with ageing. In a study conducted by Gallegly et al., WB for one hour per day was associated with an attenuation of atrophy in the soleus during the disuse period for young rats, but not of old rats[15]. They demonstrated that the ability to maintain muscle mass had been lost in the aged rats, and that maintenance of CSA as a factor determining muscle mass, capacity for cell proliferation, expression of MyoD and myogemin genes, and the regulation of myonuclear domain had been disrupted at an earlier age.

Histograms for the transverse diameter and CSA of each type of muscle fiber have been used for quantitative evaluation of the degree and extent of atrophy or hypertrophy[53]. In the present study, comparison of the pattern of the histograms revealed age-dependent differences. In general, evaluation by the use of histograms involves descriptions of the pattern, together with mean and range of CSA[53-55]. In addition, a method of calculation of factors regarding atrophy and hypertrophy has been devised as a means of quantification of changes in fiber size[53, 54]. To date, however, no detailed statistical analysis of histograms related to muscle fibers has been reported. As shown in Table 5, the CSA ranged widely, which indicates that combined use of histograms for comparison of means is useful when analyzing changes in CSA due to atrophy or WB. According to Dubowitz, distribution of the dilated or atrophic muscle fibers caused by myopathy is random, and atrophic fibers grow in clusters in a case of denervation[54]. In the present study, the distribution of the CSA in the two experimental groups was biased to the smaller side for both age groups. However, all of these changes were monophasic, and no biphasic change was apparent in any of the three groups. These findings suggest that the fiber size in all groups changed uniformly in the direction to the smaller side.

Of the past studies regarding atrophy induced by an unweighted soleus, some investigators demonstrated that a similar degree of atrophy occurred in both the young and aged animals[56, 57], while others reported more atrophy in the young than in the aged animal s[13, 58]. Simard et al. investigated the effect of suspension on the soleus and gastrocnemius of 3- and 22-month-old rats[13, 40]. They reported that contractility was not associated with age, but that atrophy, as evaluated at a morphological or histochemical level, was severe in the 3-month-old group. In the present study, the pattern of histogram for the type I fibers did not differ significantly between the age groups in the control group as was demonstrated by the Kolmogorov-Smirnov test (Table 6). But the pattern was significantly different in the two experimental groups between the two age groups. The range and mean shown on the histogram for the 3-month-old rats were smaller than that for the 9-month-old rats in all of the groups (Table 5). Consequently, the CSA of the 3-month-old rats in the two experimental groups became uniform and biased to the smaller side compared to that of the 9-month-old rats.

As for the effect of WB for each age group, there was no significant difference in the pattern of the histogram of the type I fibers between the two experimental groups for the 3-month-old rats (Table 6). However, the soleus of the 9-month-old rats showed atrophy when unweighted, but the effect of the 1-hour WB was shown to be positive. This finding suggests that WB is effective for the aged in attenuating the progression of atrophy due to unweighting. Steffen et al. carried out an experiment involving two weeks of hindlimb suspension in juvenile and mature rats and analyzed changes in the wet weight of leg muscles and biochemical parameters[59]. They reported that the degree of atrophy one week after the commencement of the experiment differed between the juvenile and aged groups, but the difference disappeared at the end of the experiment. Further, the changes in biochemical parameters began to be noted after two weeks of suspension in the mature rat group, while marked changes in biochemical parameters were already apparent within one week of suspension in the juvenile rat group[59]. It was thus shown that the effect in response to suspension appears earlier in juvenile rats during the growth period than in mature rats. The results from the present study concerning the relative weight of the soleus and analysis of the histograms do not contradict the findings reported by Steffen et al.[59]. It is possible that one week of suspension prior to WB on the soleus had a greater effect on the 3-month-old rats than the 9-month-old rats, resulting in a lesser effect of loading in suppressing atrophy in the 3-month-old rats. Therefore, in order to achieve adequate suppression of atrophy in younger rats WB should begin early, and consideration should also be given to optimum duration and intensity of WB.

The pattern of the histograms for the type II fibers of the soleus in the control group showed a significant difference between the two age groups. As for each age group, there was no significant difference between the two experimental groups. WB had no effect on both the CSA of the type II fibers and distribution pattern of the histogram regardless of age groups.

Conclusions

From the findings of this study, WB is more effective for attenuating disuse atrophy of the soleus in the aged rats than the young ones. This result can be applied to the daily practice of physical therapy by prescribing a regimen of a routine standing exercise for bed-ridden patients, especially the elderly, for it is a simple and effective tool to minimize disuse atrophy.

Acknowledgements

The author is indebted to Prof. Katsuhiko Tachino, MD, DMSc, for his guidance for this study and Mr. Katsumi Inoue, BSc, MSc, for his advice with the statistical analyses. The author's appreciation is also addressed to Prof. Shimpachiro Ogiwara, RPT, SRP (UK), ONC (UK), MCPA (C), BPT (C), MA (USA), PhD (USA) and Mrs. Sandra M. Ogiwara, CSP (UK), HT (UK), CPA (C), BScPT (C), for their review and advice on the manuscript, as well as the members of the Division of Rehabilitation Science, Department of Health Sciences, Graduate School of Medical Science, Kanazawa University for their encouragement and cooperation in this study.

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後肢懸垂ラットヒラメ筋における荷重負荷効果の加齢による差異

横川　正美

要　旨

【目的】後肢懸垂ラットヒラメ筋における荷重負荷効果の加齢による差異を検討した。【方法】対象は３ヶ月齢と９ヶ月齢の雄ラットで、実験期間は３週間とした。月齢ごとに通常飼育の対照群、後肢懸垂下で飼育した懸垂群、後肢懸垂下で飼育し、１週間後から毎日１時間の荷重負荷を行った荷重群の３群を設定した。対照群は各月齢４匹、荷重群と懸垂群は３ヶ月齢が各７匹、９ヶ月齢が各６匹であった。被験筋は右ヒラメ筋とし、筋湿重量および筋線維タイプ別の筋線維横断面積の平均値を月齢間と月齢内の各群間で比較した。測定項目のうち、筋線維横断面積は各群50本の筋線維からヒストグラムを作成し、分布の形状についても比較した。【結果】体重に対するヒラメ筋湿重量の比は、３ヶ月齢の荷重群、懸垂群は対照群に比べて減少していたが、９ヶ月齢では３群間に有意差は認められなかった。筋線維タイプ構成比率は両月齢とも３群間に有意な差異は認められなかった。筋線維横断面積における荷重負荷効果は、タイプⅡ線維では両月齢ともに示されなかった。タイプⅠ線維は平均値の比較では月齢間の差異は認められなかったが、ヒストグラム分析において９ヶ月齢に有意な荷重効果が示された。【結論】荷重負荷による筋萎縮の進行抑制は、９ヶ月齢のほうが３ヶ月齢に比べてより有効であると考えられた。