Influence of the Time When Weight Bearing Is Started on Disuse Atrophy in Rat Soleus Muscle

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Abstract. Prevention of disuse muscle atrophy is an important problem in physical therapy. Although several reports have been published concerning the effect of weight bearing on the prevention of disuse atrophy of lower extremity muscles, few basic clinical data are available. The present study was undertaken to examine differences in the progression of disuse muscle atrophy in terms of the time when weight bearing is started. The experimental materials consisted of the soleus muscles of 30 male Wistar rats (weight: 220 ± 6 g). The rats were divided into the control group (CON) and the experimental group, and disuse muscle atrophy caused by non-weight bearing was induced by hindlimb suspension for two weeks in the latter group. The experimental group was subdivided into four groups: no weight bearing permitted (SUS), and daily weight bearing permitted from 1, 4 and 7 days after the start of suspension (1-D, 4-D and 7-D). The relative weight of the soleus muscles did not differ for CON and 1-D or for 7-D and SUS. The mean cross-sectional area of type I fibers was 68% of that of CON for 1-D, 61% for 4-D, 52% for 7-D and 52% for SUS. This parameter showed significant differences in any two groups, except for between 7-D and SUS. The results were regarded as normal in view of differences in the total time of weight bearing. The data also suggested that disuse muscle atrophy can be prevented quantitatively if weight bearing is initiated from the day following the start of suspension, and that early start of weight bearing is more effective for controlling atrophy progression.

Key words: atrophy prevention, disuse atrophy, rat, soleus muscle, weight bearing

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A reduction in muscle volume is known as atrophy, and muscle weakness is generally observed in atrophied muscle. The influence of muscle atrophy accompanying aging and non-activity (disuse) on activities of daily living is serious in the elderly who otherwise show no signs of morbidity. Disuse muscle atrophy is frequently seen in clinical situations, but many cases are reversible if the cause is removed and muscle strengthening exercise is enforced. Furthermore, even for the elderly, it has been suggested that more muscle mass can be retained by training and reducing non-activity\textsuperscript{12).} The establishment of effective prevention of disuse muscle atrophy is therefore regarded as an important problem in physical therapy.

To understand the mechanism of disuse muscle atrophy, studies of muscle atrophy resulting from non-weight bearing have proved useful. Although there are many reports\textsuperscript{4–7) about adaptation to microgravity in space, little basic research has been conducted based on clinical situations. Gravity is effective so long as physical therapy is performed on the ground and it should be utilized. In cases of disuse atrophy of skeletal muscles in the lower extremities, weight bearing is an important factor\textsuperscript{8–10).} An increase in the total time of weight bearing is effective for atrophy prevention, except, of course, when weight bearing is not permitted postoperatively. On muscle atrophy caused by non-weight bearing, Someya \textit{et al.}\textsuperscript{11) reported that the cross-sectional area of type I fibers decreased less with weight bearing of up to 18h/day and even weight bearing for at least 6h/day also limited the reduction in the proportion of type I fibers. However, there are many clinical cases in which it is difficult to impose weight bearing for a long time. In order to restrict the progression...
of atrophy under such circumstances, effect of intermittent short-term weight bearing was investigated\(12-14\). Although the results are not entirely satisfactory, some limitation of atrophy was recognized. We adopted a method of weight bearing that could be enforced in clinical situations, and investigated in animal experiments\(15-18\). Those results indicate that atrophy progression restriction by weight bearing for 1h/day is possible, although not always entirely effective, and also that weight bearing frequency and intervals are important factors.

The purpose of this study was to examine differences in the progression of disuse muscle atrophy in terms of the time when weight bearing is started. The effect of early weight bearing was examined in order to establish basic data for a clinical index. This study was subject to two conditions: 1) The investigation was limited to two weeks. 2) The total weight bearing time differed depending on the time of initiation because weight bearing was imposed for 1h/day. The hypothesis was that early start of weight bearing is more effective for controlling atrophy progression.

**Methods**

The experimental materials consisted of 30 Wistar male rats (age; 8 weeks, weight; 220 ± 6 g). The soleus muscle was selected as the anti-gravity muscle because it is easily affected by non-weight bearing\(19\). At first, the rats were divided into the control group (CON) and the experimental group (6 rats/group). The experimental group was subjected to hindlimb suspension for two weeks, and disuse muscle atrophy by non-weight bearing was induced in the lower extremity muscles. Hindlimb suspension was non-invasive, consisting of the application of a simplified jacket as described in previous study\(20\). The hindlimb was suspended so that it did not touch the floor and the lower extremity muscles were non-weight bearing. During suspension, rats could move their forelimbs freely for the intake of food and water. All rats were exposed to reverse 12:12-h dark-light cycle. This study was undertaken pursuant to the guidelines for the care and use of laboratory animals in Takara-machi Campus of Kanazawa University.

The experimental group was subdivided into four groups: no weight bearing permitted (SUS), daily weight bearing permitted from 1, 4 and 7 days after the start of suspension (1-D, 4-D and 7-D). Weight bearing was induced by temporarily removing the suspension so that the rats supported their body weight on all four limbs and took place during the light cycle when rats are relatively inactive\(15\). On the last day of the experimental period (two weeks), body weight was measured, the bilateral soleus muscles were extracted under anesthesia (pentobarbital sodium, 50mg/kg body weight, ip) and the muscle wet weight was measured. The muscles were then rapidly frozen in isopentane cooled by liquid nitrogen and stored at −80 °C until histochemical analysis. Later, transverse sections (10 µm) were prepared and classified as to muscle fiber type (I and II) by ATPase staining (pH 10.6). This analysis used the microscopic images (×216) as described in a previous study\(16\). The cross-sectional area of more than 200 muscle fibers in each muscle as quantitative index of muscle atrophy was measured with a digitizer (KD4030, Graphtec Corp., Japan) using a three-dimensional image analysis system (COSMO ZONE 98, Nippon Kogaku K.K., Japan). The proportion of each type in number of muscle fiber was also calculated as qualitative analysis.

Results

The wet weight of the soleus muscle in the experimental group was significantly smaller than that in CON (124 mg), indicating that non-weight bearing caused marked atrophy. In the experimental group, soleus muscle weight decreased in the order of 1-D (77 mg), 4-D (69 mg), 7-D (62 mg) and SUS (58 mg), which is the equivalent of 62 %, 56 %, 50 % and 47 % of CON, respectively (Table 1). The muscle-to-body weight ratio (relative weight) was the highest for CON (0.42) and the lowest for SUS (0.30). In the experimental group, the ratio tended to decrease as the starting time of weight bearing was delayed. No statistically significant difference in relative weight was observed between CON and 1-D or between 7-D and SUS (Table 1).

The proportion of type I fiber was the highest in CON (76.6%). In the experimental group, it tended to decrease the more the time of weight bearing initiation was delayed: 1-D (71.9%), 4-D (69.4%), 7-D (68.9%) and SUS (67.4%). The ratio of type II fibers showed the opposite tendency. A statistically significant difference was recognized between any two groups, except between 4-D and 7-D or between 7-D and SUS (Table 2, Fig. 1).

The mean cross-sectional area of type I fibers was equivalent to 68% of CON for 1-D, 61% for 4-D, 52% for 7-D and 52% for SUS. A statistically significant difference was recognized between any two groups, except between 7-D and SUS. The mean cross-sectional area of type II fibers decreased to 67% of CON for 1-D, 63% for 4-D, 52% for 7-D and 48% for SUS. A statistically significant difference was recognized between any two groups (Table 2, Fig. 1).

Figures 2 and 3 show in graph form the distribution of the cross-sectional area of muscle fibers of all measured fibers. For type I and II fibers, the distribution in the experimental group was shifted to the left, of that in CON.
**Table 1** Wet weight of soleus muscle

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>1-D</th>
<th>4-D</th>
<th>7-D</th>
<th>SUS</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>MW(mg)</td>
<td>124 ± 6†</td>
<td>77 ± 6*†</td>
<td>69 ± 4*†</td>
<td>62 ± 8*</td>
<td>58 ± 10*</td>
</tr>
<tr>
<td>MW/BW</td>
<td>0.42 ± 0.02†</td>
<td>0.40 ± 0.02†</td>
<td>0.36 ± 0.02*†</td>
<td>0.32 ± 0.04*</td>
<td>0.30 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SD. n: number of muscles. MW: muscle wet weight. BW: body weight (g). Significantly different (p<0.05) compared with: *CON, † SUS.

**Table 2** Proportion and size of muscle fibers

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>1-D</th>
<th>4-D</th>
<th>7-D</th>
<th>SUS</th>
</tr>
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<tbody>
<tr>
<td>Proportion (%)</td>
<td></td>
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<tr>
<td>Type I</td>
<td>76.6 ± 1.0†</td>
<td>71.9 ± 1.6*†</td>
<td>69.4 ± 1.8*†</td>
<td>68.9 ± 1.6*</td>
<td>67.4 ± 1.5*</td>
</tr>
<tr>
<td>Type II</td>
<td>23.4 ± 1.0†</td>
<td>28.1 ± 1.6*†</td>
<td>30.6 ± 1.8*†</td>
<td>31.1 ± 1.6*</td>
<td>32.6 ± 1.5*</td>
</tr>
<tr>
<td>Size(μm²)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Type I</td>
<td>2166 ± 553†</td>
<td>1477 ± 481*†</td>
<td>1328 ± 365*†</td>
<td>1133 ± 404*</td>
<td>1125 ± 406*</td>
</tr>
<tr>
<td>Type II</td>
<td>1817 ± 399†</td>
<td>1213 ± 333*†</td>
<td>1148 ± 336*†</td>
<td>950 ± 324*†</td>
<td>877 ± 265*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Type I: type I fiber. Type II: type II fiber. Significantly different (p<0.05) compared with: *CON, † SUS.

**Fig. 1** Cross section of soleus muscle. Alkaline ATPase activity was used to discriminate type I (light stain) and type II (dark stain) fibers. The scale bar represents 100 μm.
The range of distribution was particularly narrow for SUS. 7-D and SUS showed a similar distribution of type I fibers. On the other hand, 1-D and 4-D were located between SUS and CON, and that 1-D was shifted to the right, compared to 4-D. 1-D and 4-D showed a similar distribution of type II fibers, which was slightly different from that of type I fibers.

**Discussion**

Brown et al.\(^{13}\) examined the effect of weight bearing of 1h/day from the day following the start of suspension, and suggested that this treatment was useful. The results of this study support their findings. The main reason that weight bearing stimulation under general non-weight bearing conditions has a positive effect on the soleus muscle is thought to be that the muscle is stretched and contractile activity increases by weight bearing\(^{15,21}\). Krippendorf et al.\(^{22}\) reported that re-weight bearing after non-weight bearing causes a pathological change different from that seen with only non-weight bearing, and that the degree of change depends on re-weight bearing time. When weight bearing is repeated during non-weight bearing as in this study, the mechanism is assumed to be more complex. St-Pierre et al.\(^{23}\) reported that the soleus muscle subjected to re-weight bearing after non-weight bearing shows specific macrophage reactions (necrosis and regeneration). We also observed a marked accumulation of macrophages in muscle tissue under certain weight bearing conditions in a previous study\(^{17}\). In other words, some methods of weight bearing are thought to be capable of inducing muscle injury. However, because macrophage reactions may be necessary for recovery, effective weight bearing should be explored.

As for the effect of the time of weight bearing initiation, the results of relative weight suggest that atrophy can be prevented quantitatively if weight bearing is initiated.
from the day following the start of suspension. All parameters measured indicate that the extent of atrophy tended to decrease as weight bearing was started earlier, so that the beneficial effect of early weight bearing was confirmed. But the effect was not clear qualitatively in this study, because no significant difference in proportion of muscle fiber types was observed between 4-D and 7-D or between 7-D and SUS. There were no differences in muscle wet weight, relative weight, proportion of muscle fiber type and cross-sectional area of type I fiber between the suspension only group and the group which started weight bearing 7 days after the start of suspension. However, movement and activity accompanying weight bearing can be expected to have a clinical effect on the condition of the body as a whole.

The distribution of the cross-sectional area showed a slightly different reaction to weight bearing in terms of muscle fiber type. Sandmann et al. described the effect of intermittent weight bearing during non-weight bearing on the gastrocnemius muscle of aged rat. They found that weight bearing restrained atrophy of type I fibers, but was ineffective for type II fibers. In other words, type I fiber reacted relatively strongly to weight bearing, so that weight bearing can be considered to be more effective for muscles with predominantly type I fiber, such as the soleus muscle. However, further examination is needed of the effect of different methods of weight bearing in terms of muscle fiber type.

The subjects after this study is thought to be the following. First, one period of weight bearing of 1h/day was enforced in this study, but various other methods are possible. For example, Alley et al. enforced four periods of weight bearing of 15 minutes (total 1 hour) every day when investigating the effect of intermittent weight bearing on aged rat, and reported that atrophy was limited. Second, the effect of the total time of weight bearing on atrophy restraint should be further examined. Third, this study was conducted for two weeks, but long-term examination is also thought to be clinically significant. Fourth, research into the function of contractile characteristics is considered important, too, because it was reported that the maintenance contractile protein is more easily affected by non-weight bearing than is the maintenance of muscle mass. Lastly, young growing rats were used in this study, but examination of other subjects is necessary, too. For example, Thompson et al. evaluated the effect of non-weight bearing in terms of age differences, and reported that the effect is greater in aged than in young rats at the single fiber level. It can therefore be assumed that the reaction to weight bearing differs with age, and this aspect needs to be examined in detail.

Conclusion

The results of this study were regarded as normal in view of differences in the total time of weight bearing. The data also suggested that disuse muscle atrophy can be prevented quantitatively if weight bearing (one hour per day) is initiated from the day following the start of suspension, and that early start of weight bearing is more effective for controlling atrophy progression.

References


