

REPORT

## Effects of Combined Stretching and Clenbuterol on Disuse Atrophy in Rat Soleus Muscle

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**ABSTRACT. Background and Purpose:** Clinically, disuse muscle atrophy is often seen among patients who are severely debilitated and are on prolonged bed rest. Common physical therapy interventions are not successful in preventing disuse muscle atrophy early in the medical treatment of critically ill patients. In situations such as this, the use of a  $\beta_2$ -adrenergic agonist such as clenbuterol (Cb) may be of benefit in preventing atrophy. Also, recent studies have suggested that stretching is possible in preventing disuse muscle atrophy and the decline in muscle strength. The objective of this study was to evaluate the effects of Cb medication combined with stretching (ST) on rat soleus muscle (SOL) during the progression of disuse muscle atrophy. **Subjects:** Thirty-five male Wistar rats were used in this study. **Methods:** The rats were divided into five groups: control (CON), hindlimb-unweighting (HU) only, HU+ST, HU+Cb medication, and HU+ST+Cb groups. The right SOL in stretching groups was maintained a stretched position for one hour daily by passively dorsiflexing the ankle joint under non-anesthesia. The experimental period was 2 weeks. **Results:** In the ST group, peak twitch tension per cross-sectional area in soleus muscle was significantly larger than in the Cb group, while there was no significant difference between the CON and ST groups. The conversion of type I to type II fibers that was observed in the Cb group was not recognized in the combined ST and Cb group. **Discussion and Conclusion:** Distinct effect of combined stretching and Cb medication was not recognized statistically. The results indicate that Cb affects muscle morphological characteristics while stretching affects contractile properties. These data suggest that a combined ST and Cb intervention considered the type-specificity of muscle fiber may be need more consideration for preventing disuse muscle atrophy and the decline in muscle strength.

**Key words:** stretching, clenbuterol, disuse muscle atrophy, rat, atrophy prevention

(*J Jpn Phys Ther Assoc* 12: 13-19, 2009)

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**D**isuse muscle atrophy has a substantial effect on activities of daily living (ADL) such as standing and walking<sup>1,2</sup>). Clinically, disuse muscle atrophy is often seen among patients who are severely debilitated and have been on prolonged bed rest in an acute care setting. Early initiation of weight-bearing activities such as ambulation in the clinical course of a patient's critical illness has been advocated to prevent disuse atrophy of lower extremity muscles. However, due to underlying illness, early weight-bearing exercises are often contraindicated.

While hindlimb unweighting (HU) has been used as a

model for spaceflight, it is also used as a model for decreased muscle use. On the basis of the results from a study in rats, Brown *et al.*<sup>3</sup>) reported that one hour of weight-bearing per day, during two weeks of HU, is useful in suppressing the progression of disuse atrophy in soleus muscle (SOL). We have also been exploring effective means of preventing atrophy by using a clinically applicable program "intermittent weight-bearing (IWB)" involving one hour of weight-bearing per day in HU rats<sup>4,5</sup>). We have reported that four factors (frequency, duration, interval and timing of initiation of weight-bearing) affect muscle atrophy, and that IWB can suppress the progression of atrophy but does not completely prevent atrophy<sup>4,5</sup>).

Because of the difficulty in promoting early weight-bearing in critically ill patient, pharmacologic intervention to minimize muscle atrophy may be useful. Clenbuterol

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Received: November 28, 2007

Accepted: May 24, 2008

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(Cb) is a  $\beta_2$ -adrenergic agonist, with anabolic effects on protein reported to be useful in preventing skeletal muscle atrophy, and has been attracting attention as a countermeasure to skeletal muscle atrophy in micro-gravitational environments<sup>6,7</sup>). Although the specific cellular mechanism is unknown, Cb has been associated with an increase in protein synthesis, and increased cell size and muscular strength<sup>8-11</sup>). In an experiment using HU rats, Herrera *et al.*<sup>9</sup>) treated rats with Cb (1.5 mg/kg body weight) for 2 weeks daily during disuse atrophy progression. Consequently, muscle atrophy in SOL and EDL was suppressed by Cb, suggesting that treatment with Cb can significantly prevent muscle atrophy. Wineski *et al.*<sup>12</sup>) reported that Cb treatment (1.0 mg/kg) reduced HU-induced atrophy in rats. The results suggest that Cb exerts anabolic effects that are load-dependent and muscle-specific. Our previous study<sup>13</sup>) also demonstrated that Cb alone could suppress a decline in muscle weight with no effect on contractility, while IWB alone suppressed a decline in muscle contractile properties but had little effect on muscle morphological characteristics. The combination of IWB and Cb was useful in suppressing atrophy in type I fiber-predominant SOL<sup>13</sup>).

To prevent joint contracture and muscle shortening, passive muscle stretching is a common intervention performed by physical therapists. Gomes *et al.*<sup>14</sup>) reported that once weekly 40-minute stretching was useful in alleviating atrophy of SOL fiber immobilized in a shortened position in rats. Coutinho *et al.*<sup>15</sup>) also evaluated the effects of stretching once every 3 days (for 40 minutes each time) on rat SOL immobilized in a shortened position and reported that stretching did not prevent muscle contracture by alleviated muscle atrophy. In previous studies<sup>16,17</sup>), the authors reported the effects of stretching on suppressing atrophy during the progression of disuse muscle atrophy. These data showed that development of muscle atrophy in the SOL of HU rats was attenuated by stretching for 20 minutes (5 days/week) under anesthesia. To our knowledge, however, no study has been published that examines the potential use of a combination of stretching and Cb on the suppression of muscle atrophy. The objective of this study was to evaluate, from both morphological and functional perspectives, the effects of a combination of stretching and Cb medication on rat SOL during the progression of disuse muscle atrophy. In this study, stretching meant maintaining muscle-stretched position under non-anesthesia. A hypothesis underlying this study was that stretching would affect the contractile properties and Cb would affect morphological characteristics thus justifying the clinical use of stretching in combination with Cb. Additionally, the effects of combined stretching and Cb medication was compared to the effects of combined IWB and Cb medication in previous study<sup>13</sup>).

## Methods

### Materials

Thirty-five male Wistar rats were used in this study (age: 7 weeks, body weight:  $215 \pm 6$  g). The right soleus muscle (SOL) was selected as a representative slow muscle in the hindlimb. The rats were housed in individual cages under a 12-hour light-dark cycle, and maintained on standard rat feed and water *ad libitum*.

### Protocol

This experimental protocol was approved by the committee on animal experimentation of Kanazawa University (No. 050301, 060362). The rats were randomly divided into five groups: a control group (CON), an HU treatment only group (HU), an HU treatment + stretching group (ST), an HU treatment + Cb medication group (Cb), and an HU treatment + stretching + Cb medication group (ST+Cb). The three groups without Cb medication (CON, HU and ST) were injected with physiological saline under the same conditions used to inject the Cb medication in the other two groups. The experimental period was 2 weeks. Disuse atrophy was induced by an HU treatment device (a non-invasive device consisting of a simplified jacket) as described in our previous studies<sup>18</sup>). Bilateral hindlimbs were suspended so that they did not touch the floor. During suspension, the rats were checked daily for discoloration or any tissue damage and could move their forelimbs to aid in food and water consumption. The right SOL in the ST and ST+Cb groups was stretched maximally for one hour daily by passively dorsiflexing the ankle joint with non-elastic tape while maintaining the HU condition<sup>16,17</sup>). The rats were subcutaneously injected with Cb (1.0 mg/kg clenbuterol hydrochloride, Sigma Chemical Co., St. Louis, MO, USA) or saline (1.0 ml/kg) as part of a 2-day on/2-day off dosing regimen<sup>19</sup>) for 2 weeks (days 0, 1, 4, 5, 8, 9, 12 and 13). In the ST+Cb group, stretching was performed for one hour per day immediately after Cb injection.

### Muscle preparation / Measurement of contractile properties

On Day 14, body weight (BW) was measured, and the right SOL was excised under anesthesia (pentobarbital sodium, 50 mg/kg, ip). After measurement of contractile properties, the muscles were rapidly frozen and stored at  $-80$  °C until biochemical and histochemical analyses were performed. After measurement of the muscle length (ML) and circumference (MC) at rest, isometric contractile properties were measured *in vitro*. Using a slide caliper, muscle length was measured as the interval between the proximal and distal myotendinous junctions. To measure muscle circumference, a suture thread was removed from the muscle after ligation at the maximum muscle belly and circumference was assessed by measuring the length of the thread. Then the muscle was mounted onto a force-

recording device (LTS-500GA, Kyowa, Japan) and bathed in Ringer's solution (25 °C) which had been aerated with gas (95%O<sub>2</sub> / 5%CO<sub>2</sub>). The muscle was stretched to 110% of its resting length and stimulated with a supramaximal square wave (0.2 ms duration) delivered via two parallel platinum electrodes using an electric stimulator (SC6, Medelec, UK)<sup>13</sup>. Contractile responses were recorded and analyzed on an analog-to-digital converter coupled to a computer. The analysis parameters were peak isometric twitch tension (Pt), contraction time (CT), and one-half relaxation time (RT). The twitch contractile measurements of Pt, CT and RT are generally indicative of calcium handling by the muscle<sup>20,21</sup>. Contraction time is the amount of time it takes the muscle to develop Pt. One-half relaxation time is the time it takes after removal of the stimulus for Pt to return to one half of the tension generated during the twitch. Peak twitch tension generated per cross-sectional area (CSA) of muscle was calculated for comparison of tension generation between muscles of different sizes. The muscle CSA was estimated by dividing the muscle wet weight (MW) by ML using the technique described by Fitts *et al.*<sup>22</sup>. Following contractile measurements, muscle weight was measured after blotting the extra solution three times (5 sec. / time).

#### Histochemical analysis

Transverse sections (10 µm in thickness) at the muscle belly were cut using a cryostat microtome at -25°C and classified into muscle fiber type (I and II) with actomyosin ATPase staining (pH 10.6)<sup>4,5</sup>. The stained sections were examined with an image analysis system consisting of a light photomicroscope (BX-50; Olympus), personal computer (Power Macintosh G3) and image processing software (NIH Image 1.62)<sup>13</sup>. The muscle fiber type proportion and the CSA of more than 200 total muscle fibers in four fields from each muscle were measured.

#### Biochemical analysis

Muscle protein concentration was obtained using a

modification<sup>13</sup> of the technique described by Wineski *et al.*<sup>12</sup> and Caiozzo *et al.*<sup>23</sup>. All tissue preparations were performed on ice with buffer at 4 °C. Using a homogenizer, the muscle was homogenized in a solution containing 250 mM sucrose adjusted to pH 6.8. The crude homogenate was centrifuged at 1,000 g for 10 min. using a centrifugal separator. The pellet was resuspended in a solution containing 175 mM KCl (Solution A). This homogenate was centrifuged again as described above. The final pellet was suspended in 300 µl Solution A and used to determine myofibrillar protein (MP) concentration. MP concentration was determined using a bicinchoninic acid (BCA) protein assay and expressed as milligrams per muscle weight (gram).

#### Statistical analysis

The data are expressed as mean ± SD. The differences among the groups were statistically evaluated using one-way analysis of variance (ANOVA). When a significant difference was recognized (p<0.05), paired comparisons were performed using Scheffe's post hoc test.

## Results

#### Body weight

Body weight in the experimental groups (HU, ST, Cb and ST+Cb) was significantly less than in the CON group. Among the experimental groups, the Cb and ST+Cb groups had significantly greater body weights than the HU group (Table 1).

#### Muscle wet weight (MW)

As compared with the CON group, muscle weight of SOL was significantly decreased by 54.2% in the HU group, 47.4% in the ST group, 31.0% in the Cb group, and 25.1% in the ST+Cb group. Muscle weight in the Cb and ST+Cb groups was significantly greater than that in the HU group, but there was no significant difference between the ST and HU groups. The muscle-to-body weight ratio (MW/

**Table 1.** Morphometrical properties and myofibrillar protein concentration in SOL (Mean ± SD)

Groups(n)	CON(8)	HU(7)	ST(6)	Cb(7)	ST+Cb(7)
BW (g)	273.3 ± 11.0 <sup>†#</sup>	179.7 ± 7.2*	189.5 ± 12.2*	198.6 ± 8.0* <sup>†</sup>	205.1 ± 11.3* <sup>†</sup>
MW (mg)	126.9 ± 10.5 <sup>†#</sup>	58.1 ± 4.1*	66.8 ± 5.9*	87.6 ± 9.5* <sup>†#</sup>	95.0 ± 13.2* <sup>†#</sup>
MW/BW (mg/g body weight)	0.47 ± 0.04 <sup>†#</sup>	0.32 ± 0.02*	0.36 ± 0.04*	0.44 ± 0.04 <sup>†#</sup>	0.46 ± 0.04 <sup>†#</sup>
ML (mm)	24.1 ± 0.5 <sup>†#</sup>	19.3 ± 0.5* <sup>#</sup>	21.5 ± 0.6* <sup>†</sup>	19.8 ± 0.7* <sup>#</sup>	21.4 ± 1.0* <sup>†‡</sup>
MC (mm)	10.3 ± 0.8 <sup>†#</sup>	7.2 ± 0.9*	7.9 ± 0.4*	9.4 ± 1.0 <sup>†</sup>	9.0 ± 0.9 <sup>†</sup>
MP (mg/g muscle weight)	109 ± 10 <sup>†#</sup>	56 ± 11*	62 ± 10*	85 ± 12* <sup>†#</sup>	88 ± 9* <sup>†#</sup>

\* p<0.05 when compared to CON. <sup>†</sup> p<0.05 when compared to HU. <sup>#</sup> p<0.05 when compared to ST. <sup>‡</sup> p<0.05 when compared to Cb. CON: control group. HU: hindlimb unweighting (HU) only group. ST: HU + stretching group. Cb: HU + Cb medication group. ST+Cb: HU + stretching + Cb medication group. BW: body weight. MW: muscle wet weight. ML: muscle length. MC: muscle circumference. MP: myofibrillar protein. SOL: soleus muscle.

BW) of SOL in the Cb and ST+Cb groups was not significantly different from that in the CON group, indicating that Cb medication has the effect of preventing muscle atrophy (Table 1).

#### *Muscle length (ML) and circumference (MC)*

Muscle length in all experimental groups significantly decreased compared with that in the CON group. Among the experimental groups, muscle length in the ST and ST+Cb groups was significantly longer than that in the HU group, indicating that stretching has the effect of preventing decreases in muscle length. However, there was no significant difference between the HU and Cb groups. Muscle circumference in the Cb and ST+Cb groups was not significantly different from that in the CON group, but was differed from the HU group, indicating that Cb medication has the effect of preventing decreases in muscle circumference. There was no significant difference between the HU and ST groups, and muscle circumference in these groups was significantly smaller than in the CON group, showing that stretching did not affect muscle circumference (Table 1).

#### *Contractile properties*

One-half relaxation time did not differ among groups. Contraction time in the Cb and ST+Cb groups was significantly shorter than that in the CON group, but CT in the HU and ST groups was not significantly different from that in the CON group. Peak twitch tension in all experimental groups significantly decreased compared with that in the CON group. There was no significant difference

in peak twitch tension among the experimental groups (Table 2). In the ST group, Pt/cm<sup>2</sup> was significantly larger than in the Cb group, while there was no significant difference between Pt/cm<sup>2</sup> in the CON and ST groups, indicating that stretching has the effect of preventing the loss of strength. Pt/cm<sup>2</sup> in the ST group trended to be greater as compared to the HU group, but not significant (p=0.18), illustrating the possibility that stretching has the effect of minimizing the loss of strength that is commonly seen in HU (Table 2).

#### *Type proportion and cross-sectional area (CSA) of SOL*

The proportion of type I fibers in the Cb group was significantly decreased compared with that in other groups. Conversely, the proportion of type II fibers in the Cb group was significantly increased compared with that in other groups, showing a tendency toward fast muscle (Table 3). The mean CSA of type I fibers was significantly decreased in all experimental groups (Cb > ST+Cb > ST > HU) as compared to CON group. Similarly, the mean CSA of type II fibers was decreased in all experimental groups (ST ≈ ST+Cb > Cb > HU). Statistically significant differences were observed among all the experimental groups, except between the ST and ST+Cb groups for type II fibers (Table 3).

#### *Protein concentration*

Myofibrillar protein concentration in all experimental groups was significantly decreased compared with that in the CON group. Among the experimental groups, myofibrillar protein concentration in the Cb and ST+Cb

**Table 2.** Contractile properties of twitch tension in SOL (Mean ±SD)

Groups	CON	HU	ST	Cb	ST+Cb
CT (ms)	94.2 ± 8.6	77.5 ± 13.7	74.2 ± 14.6	69.2 ± 7.4*	73.3 ± 5.2*
RT (ms)	221.7 ± 19.1	207.5 ± 36.6	171.7 ± 27.0	179.2 ± 49.9	178.3 ± 30.1
Pt (N)	0.18 ± 0.01 <sup>#</sup>	0.06 ± 0.01*	0.09 ± 0.03*	0.06 ± 0.02*	0.08 ± 0.02*
Pt/cm <sup>2</sup> (N/cm <sup>2</sup> )	3.47 ± 0.43	2.10 ± 0.44*	2.95 ± 0.86	1.43 ± 0.52* <sup>#</sup>	1.88 ± 0.46*

\* p<0.05 when compared to CON. <sup>#</sup> p<0.05 when compared to ST. CT: contraction time. RT: one-half relaxation time. Pt: peak isometric twitch tension.

**Table 3.** Proportion and cross sectional area of SOL fiber (Mean ±SD)

Groups	CON	HU	ST	Cb	ST+Cb
Proportion (%)					
Type I fiber	80.1 ± 7.2 <sup>‡</sup>	75.2 ± 7.7	77.5 ± 2.6	65.9 ± 9.1*	73.8 ± 5.4
Type II fiber	19.9 ± 7.2 <sup>‡</sup>	24.8 ± 7.7	22.5 ± 2.6	34.1 ± 9.1*	26.2 ± 5.4
CSA (μm <sup>2</sup> )					
Type I fiber	2458 ± 773 <sup>#‡</sup>	1035 ± 322* <sup>#‡</sup>	1432 ± 498* <sup>‡</sup>	1990 ± 937* <sup>#</sup>	1822 ± 953* <sup>#‡</sup>
Type II fiber	2222 ± 491 <sup>#‡</sup>	758 ± 198* <sup>#‡</sup>	1361 ± 370* <sup>‡</sup>	1047 ± 520* <sup>#</sup>	1354 ± 638* <sup>‡</sup>

\* p<0.05 when compared to CON. <sup>#</sup> p<0.05 when compared to ST. <sup>‡</sup> p<0.05 when compared to Cb. CSA: cross sectional area.

groups was significantly greater than that in the HU and ST groups, indicating that Cb medication has the effect of increasing myofibrillar protein. There was no significant difference between the HU and ST groups (Table 1).

## Discussion

The objective of this study was to evaluate the effects of combined stretching and Cb medication on SOL during progression of disuse muscle atrophy in rats, additionally the effects were compared to the effects of a previous study (combined IWB and Cb medication). The wet weight and the mean CSA of type I fibers of SOL in the ST+Cb group were, respectively, 74.9% and 74.2% of the values for the CON group. In a previous study<sup>13)</sup>, these parameters in the IWB+Cb group were 92.0% and 92.3% respectively. Therefore the combined stretching and Cb medication was not as effective as the combined IWB and Cb medication. In both the present (stretching) and previous (IWB) studies, the duration of intervention was one hour per day. While IWB in previous study<sup>13)</sup> was performed by temporarily removing the HU device, stretching in this study was performed while maintaining the HU condition. This difference in HU between the present and previous studies may indicate that the distribution of body fluid and Cb during the intervention probably influences the effects of combined stretching and Cb medication. Hindlimb unweighting-related differences in plasma and tissue Cb concentrations<sup>19)</sup> might be attributed to differences in headward fluid shifts in the two studies. Changes in the volume distribution and losses in total body water may lead to change in the rate of Cb clearance<sup>19)</sup>. During HU, the ankle joint gradually assumes a position of plantar flexion<sup>24)</sup>. If the rat in this state bears body weight, the ankle joint will undergo passive dorsi-flexion, resulting in stretching of the SOL. Furthermore, bearing body weight will increase muscular work and thus suppress the progression of muscle atrophy<sup>25)</sup>. Therefore, we may speculate that stretching in the present study is less effective than IWB in suppressing muscle atrophy. Because this study was performed under non-anesthesia, it was thought that stretching induced both passive extension and isometric contraction of SOL. In the previous study<sup>16)</sup>, the authors compared the effects of stretching under anesthesia and non-anesthesia in preventing disuse muscle atrophy. As a result, the effects was recognized under anesthesia (passive extension), but there were few effects than under non-anesthesia (passive extension + isometric contraction). An additional experiment under anesthesia will be necessary to clarify the effect of passive extension alone in this study after this.

Contraction time and one-half relaxation time showed a tendency to decrease in the experimental groups. Generally, these two measures change in parallel<sup>20)</sup>. While contraction

time in the Cb and ST+Cb groups was significantly decreased compared to the CON group, one-half relaxation time did not statistically differ among each group. As a reason for these results, the possibility of the difference (time lag) in calcium dynamics by the sarcoplasmic reticulum (SR) during fiber type shifts was suspected. Many studies have reported Cb-induced shifts from slow-twitch (type I) toward fast-twitch (type II) fiber types in SOL<sup>26-29)</sup>. Contraction time and one-half relaxation time are indicative of calcium release and uptake by SR respectively<sup>21)</sup>. However, the experimental conditions in this study were complex, because the intervention (HU, stretching and Cb medication) were repeated. Therefore, the distinct reason was not clear in this study for 2 weeks.

In this study, Cb medication increased the proportion of type II fibers and reduced the contraction time. However, these parameters in the ST+Cb group did not statistically differ from the Cb group. Consequently, distinct effect of combined stretching and Cb medication was not recognized. No difference in the proportion of type II fibers in the ST+Cb and CON groups indicated that the conversion of the slow muscle into fast muscle through a change in muscle fiber composition was influenced by stretching in the combined stretching and Cb medication group. The CSA of type I fiber was significantly greater in the Cb group than in the ST+Cb group, indicating that (1) type I fiber responded to Cb predominately, and (2) the muscle length (Cb<ST+Cb) affected to the CSA, because the type I fiber was dominant in soleus muscle. Conversely, the CSA of type II fiber was significantly greater in the ST+Cb group than in the Cb group. It seems that type II fiber responded to stretching predominately, because the CSA of type II fiber did not differ significantly between the ST and ST+Cb groups. These results suggest that the response to Cb medication or stretching during HU varies depending on the type-specificity<sup>19)</sup> of muscle fibers. The twitch tension per unit CSA did not differ significantly between the ST and CON groups, indicating that stretching can prevent twitch tension reduction. The decrease in twitch tension in the Cb medication groups (Cb and ST+Cb groups) seems to result from the following two changes: (1) a smaller decrease in CSA due to Cb medication, and (2) shortening of the muscle by HU, thus reducing the tension per unit CSA. Therefore it is indicative that using stretching to improve contractility and using Cb to increase CSA could be clinically useful.

Clinically, the benefits of Cb have been shown in various studies. Maltin *et al.*<sup>8)</sup> mediated Cb to patients after meniscectomy of the knee joint and reported that Cb led to a more rapid rate of rehabilitation in the operated leg. They concluded that Cb had therapeutic potential in the treatment of muscle-wasting conditions. Also, the attempt to use Cb on patients with muscular dystrophy was tried in Japan. Oya *et al.*<sup>30)</sup> reported that Cb did not suppress the

progression of the disease but it was useful for retaining muscle mass and strength. However, these studies have been investigated the effects of Cb medication alone. No study has been published that examines the potential use of a combination of stretching and Cb on the suppression of muscle atrophy. Although medication is usually not considered when performing physical therapy, it has the potential to elevate the efficacy of stretching-based physical therapy. If a benefit is expected for the patient who cannot bear weight, and if the patient consents to it in advance, the use of combined stretching and Cb medication may provide a valid means of minimizing disuse muscle atrophy and the loss of muscle strength<sup>8,31)</sup>. However, considering that Cb medication alone can have an adverse effect<sup>26-29)</sup> of converting slow fibers into fast fibers, it is essential that Cb not be used alone but be combined with physical therapy in the form of stretching. Complete prevention of atrophy using the intervention (stretching alone) approximately one hour daily may be difficult. In this study, distinct effect of combined stretching and Cb medication was not recognized. However, the approach by using a method of intervention tailored to the type-specificity of muscle fiber and by adopting an optimal frequency and interval of intervention may be worth considering after this. Even if complete prevention is not possible, innovative combined-approach is expected to shorten the time required for recovery by effectively suppressing the progression of muscle atrophy and the decline in muscle strength. Therefore, additional studies in future may have implications for geriatric patients facing prolonged inactivity specially.

In summary, the effects of combined stretching and Cb medication were not as great as the results in our previous study<sup>13)</sup> of the effects of combined IWB and Cb medication. Distinct effect of combined stretching and Cb medication was not recognized statistically. However, these results may be interpreted as indicating that Cb affects muscle morphology while stretching affects function (contractility). Furthermore, the conversion of the muscle into fast muscle in the Cb group was not recognized in the combination group of stretching and Cb medication. These data suggest that innovative intervention combined stretching and Cb medication considered the type-specificity of muscle fiber may be need more consideration for preventing disuse muscle atrophy and the decline in muscle strength during the progression of atrophy in rat SOL.

### Acknowledgements

The authors thank Kazumi Adachi and Maiko Nakajima for skillful technical assistance.

The study was funded by Grant-in-Aid for Scientific Research (C) in Japan Society for the promotion of Science

(No. 17500351).

This research, in part, was presented as a Research Report Poster at the 15<sup>th</sup> International WCPT Congress, 2-6 June 2007, Vancouver, Canada.

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