Acupuncture Ameliorated Skeletal Muscle Atrophy Induced by Hindlimb Suspension in Mice

INTRODUCTION
Loss of skeletal muscle mass has a profound effect on a daily life, however, interventions such as exercise training are often hard, for the elderly and patients with serious diseases. Acupuncture is a branch of traditional East Asian medicine and used for recovery from skeletal muscle disorders. The effect of acupuncture on muscle atrophy, however, has not yet been sufficiently elucidated. Given all this, we hypothesized that acupuncture is a promising non-pharmacological strategy for preventing skeletal muscle atrophy.

METHODS

Animals and acupuncture conditions
Twenty-eight male mice (C57 BL/6, 8 weeks; Sankyo Lab Service, Tokyo, Japan) were randomly assigned into 4 groups; A) control, B) untreated hindlimb suspension (HS), C) HS + manual acupuncture (MA), D) HS + MA + electrical stimulation (EA) (n=7/group). Acupuncture was performed on the gastrocnemius muscle for 30 minutes every day for two weeks. The needle was inserted about 5 mm into the muscle. Electrical stimulation was simultaneously administered to the EA group using an Ohm Pulser LFP-4000 pulse generator (Edenki Inc., Kyoto, Japan). The interval and current of this electrical stimulation were 1 Hz and 6.5 mA, respectively. This study was approved by the Ethical Committee on Animal Experiments of Waseda University (10A58).

Statistical analysis
The statistical significance of the differences between means was assessed using one-way ANOVA followed by the Tukey-Kramer post-hoc test. A probability of less than 5% was considered significant (P < 0.05). Data are expressed as the mean ± SEM. Statistical significance is indicated as †p < 0.05, ‡p < 0.01, §p < 0.001 when compared to the control group, and as *p < 0.05, **p < 0.01, ***p < 0.001 when compared to the HS group.

RESULTS
After two weeks of HS, muscle mass and cross-sectional area (CSA) were significantly reduced in the soleus muscle (56% and 45% reductions, respectively) (Figure 1A, B). HS-induced reduction in soleus muscle mass was significantly improved in the MA and EA groups (7% and 10% increases, respectively; Figure 1A). Furthermore, HS-induced reduction in the CSA of the soleus muscle was significantly improved in the EA group (18% increase; Figure 1B). Consistent with previous studies, we found that unloading by HS significantly increased the expression levels of atrogin-1, MuRF1 mRNA. At the end of the two-week period of acupuncture treatment, significant increases in mRNA expression levels in the soleus were observed for both atrogin-1 (302%, Figure 2A) and MuRF1 (247%, Figure 2B). The HS-induced increase in atrogin-1 expression in the soleus muscle was significantly decreased in the MA and EA groups (Figure 2A). The expression level of Akt1 mRNA expression was not significantly increased by HS, the expression levels of the Akt1 mRNA group in the soleus muscle were significantly upregulated by MA compared to the control group (213%, Figure 2D). We also found that the expression of TRPV4 mRNA was higher in the MA group than in the control and HS groups (Figure 2F).

DISCUSSION
To the best of our knowledge, our data indicated for the first time that acupuncture partially prevents skeletal muscle atrophy caused by HS. We presume that acupuncture downregulated the genes involved in muscle-specific degradation, such as atrogin-1 and MuRF-1 (1, 2), and upregulated the genes involved in protein synthesis, such as Akt1 and TRPV4. Similarly, chronic resistance training and amino acid-containing diets are known to decrease muscle-proteolytic genes including atrogin-1 and MuRF-1.

Acupuncture can serve as an alternative non-pharmacological intervention that is feasible, versatile, and associated with little risk.

REFERENCE

Figure 1. Muscle weight/body weight and cross-sectional area. After acupuncture treatment, soleus muscle weights were significantly greater in the acupuncture groups than in the HS group. CSA was significantly greater in the EA group than in the HS group. Data is shown as the mean ± SEM. Statistical significance is indicated as †p < 0.05, ‡p < 0.01, §p < 0.001 when compared to the control group, and as *p < 0.05, **p < 0.01, ***p < 0.001 when compared to the HS group. (n=7/group).

Figure 2. mRNA expression levels as measured through quantitative RT-PCR. After acupuncture treatment, A) atrogin-1 and B) MuRF1 mRNAs were significantly lower in the acupuncture groups than in the HS group. D) Akt1 and F) TRPV4 mRNAs were significantly higher in the MA groups than in the HS group. Data is shown as the mean ± SEM. Statistical significance is indicated as †p < 0.05, ‡p < 0.01, §p < 0.001 when compared to the control group, *p < 0.05, **p < 0.01, ***p < 0.001 when compared to the HS group. (n=7).