**NEW MOUSE MODEL OF SKELETAL MUSCLE ATROPHY USING SPIRAL WIRE IMMOBILIZATION**

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**ABSTRACT:** Disuse-induced skeletal muscle atrophy is a serious concern; however, there is not an effective mouse model to elucidate the molecular mechanisms. We developed a noninvasive atrophy model in mice. **Methods:** After the ankle joints of mice were bandaged into a bilateral planter flexed position, either bilateral or unilateral hindlimbs were immobilized by wrapping in bonsai steel wire. Results: After 3, 5, or 10 days of immobilization of the hip, knee, and ankle, the weight of the soleus and plantaris muscles decreased significantly in both bilateral and unilateral immobilization. MAFbx/atrogin-1 and MuRF1 mRNA was found to have significantly increased in both muscles, consistent with disuse-induced atrophy. Notably, the procedure did not result in either edema or necrosis in the fixed hindlimbs. **Conclusions:** This method allows repeated, direct access to the immobilized muscle, making it a useful procedure for concurrent application and assessment of various therapeutic interventions.


Disuse-induced skeletal muscle atrophy is characterized by a decrease in muscle mass and an increase in the risk of debilitating diseases and orthopedic problems. Understanding the mechanisms of disuse-induced muscle atrophy is important for developing countermeasures to preserve physiological function. To elucidate the molecular mechanisms of muscle atrophy in vivo, various mouse models have been developed1–6; however, there is as yet no effective rodent model due to the small size. The previous models also suffered from the unwanted effects of specific devices, invasive surgical procedures, local edema, and the animals escaping immobilization. Here, we report a new mouse model using steel bonsai wire, which enables repeated direct access to the immobilized muscle and allows concurrent application and assessment of various therapeutic interventions.

**MATERIALS AND METHODS**

**Animals.** This study was approved by the ethics committees on animal experimentation at Waseda University (2011-A040) and Teikyo University (2013-13-026). C57BL/6 mice (8–9 weeks old, Sankyo Lab Service, Tokyo) were randomly assigned to the spiral wire immobilization (SWI) group or control group and were housed in independent plastic cages with access to food and water *ad libitum* in a 12-h light/dark cycle. The numbers of mice used for the experiments are shown in the figure legend.
SWI Procedures. The mice were anesthetized with Somnopentyl (Kyoritsu Seiyaku, Tokyo) and maintained in the supine position while their ankle joints were taped (1.5 × 1.5 cm, Kinesio Taping Association, Tokyo) in a bilateral plantar flexed position (Fig. 1A) with nonelastic bandage tape.
Then, the center of vinyl-coated steel wire (Garden soft wire free, diameter 2.5 mm, length 40 cm; Fujiwara Sangyo Co., Ltd. Hyogo, Japan) was applied around to the level of the L4–5 spine and coiled around the hip joints and both hindlimbs for bilateral SWI immobilization (Fig. 1B). The hindlimbs were fixed at a right angle to the trunk at the hip joint. The unilateral SWI procedure was the same as the bilateral SWI to coil 1 leg by using 30 cm length steel wire (Supporting Fig. S1A, which is available online).

**Muscle Sample Collection of the SWI Group.** The mice were euthanized after 3, 5, or 10 days of SWI. The weights of slow-twitch (soleus) and fast-twitch (plantaris) muscles were measured immediately after dissection.

**Body Weight and Food Intake of the SWI Group.** The body weight and food intake of mice were measured once daily around 11:00 AM.

**Statistical Analysis.** The results are presented as mean ± standard error. Muscle mass was assessed using 1-way analysis of variance (ANOVA), and body weight and food intake were analyzed using 2-way ANOVA followed by multiple comparative tests with Bonferroni correction. Statistical significance was set at *P* < 0.05.

**RESULTS**

The soleus muscle weight at 3, 5, or 10 days of bilateral SWI decreased significantly to 82% (*P* < 0.001), 72% (*P* < 0.001), and 56% (*P* < 0.001) compared with the nonimmobilized control group, respectively (Fig. 1C). The plantaris muscle weight at 3, 5, or 10 days of SWI also decreased significantly to 80% (*P* < 0.01), 84% (*P* < 0.01), and 78% (*P* < 0.001), respectively (Fig. 1D). The bilateral SWI group had a significant decrease in body weight compared with the control group (*P* < 0.01; Fig. 1E). The amount of food intake temporarily decreased after bilateral SWI (Fig. 1F).

The immobilized soleus muscle weight of 5 and 10 days of unilateral SWI decreased significantly to 81% (*P* < 0.001) and 73% (*P* < 0.001) compared with the nonimmobilized leg, respectively (Supporting Fig. S1B). The immobilized plantaris muscle weight of unilateral SWI of 5 days decreased significantly to 85% (*P* < 0.05; Supporting Fig. S1C). The unilateral SWI group had a significant decrease in body weight compared with the control group (*P* < 0.001, Supplementary Fig. S1D, available online). The amount of food intake temporarily decreased after unilateral SWI (Supplementary Fig. S1E).

To determine whether the muscle atrophy was mediated by the ubiquitin-proteasome pathway, mRNA of the E3 ubiquitin ligases MAFbx/atrogin-1 and MuRF1 was measured in the targeted muscles of the bilateral SWI group. The mRNA expression level of MAFbx/atrogin-1 had increased significantly in the soleus (608 ± 75%; *P* < 0.01) and plantaris muscles (582 ± 116%; *P* < 0.01; Supplementary Fig. S2I, K). Likewise, the expression level of MuRF1 had increased significantly in both the soleus (456 ± 60%; *P* < 0.01) and plantaris muscles (505 ± 79%; *P* < 0.01; Supplementary Fig. S2J, L). The SWI procedure did not cause edema, ulcer, or necrosis during immobilization.

**DISCUSSION**

SWI resulted in consistent muscle atrophy in previous disuse-induced muscle atrophy models. In previous studies, the soleus decreased by 11% and 22% by days 3 and 7 of cast-induced immobilization in mice. There was 12% reduction of the gastrocnemius after 5 days of immobilization in a study using rats. In addition, increased expression of MAFbx/atrogin-1 and MuRF1 was observed in the immobilized muscle. These results indicate that the SWI procedure induced significant upregulation of E3 ubiquitin ligases in both the slow-twitch soleus and fast-twitch plantaris muscles, leading to muscle atrophy.

The SWI procedure has several advantages over previous models. First, SWI does not require surgery or specially-designed devices. Mice subjected to surgery suffer from trauma incidental to the procedure. SWI does not cause local injury such as edema or necrosis. In addition, the commonly available bandages and steel wire were inexpensive. Second, SWI does not require continuous monitoring against damage to the constraints caused by the animals’ attempts to escape. Third, SWI can be placed on, or removed from, both hindlimbs within 3 min, allowing researchers to access the immobilized muscle directly. This feature makes it possible to engage in experimental interventions such as electrical stimulation or topical medication. Furthermore, unilateral immobilization allows 1 animal to provide both an experimental and control hindlimb.

This model proposed here can be used to replicate various aspects of human disuse-induced muscle atrophy as an aid to better understanding the underlying pathophysiology and to exploring potential treatments.

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STABILITY OF BICEPS BRACHII MMAX WITH ONE SESSION OF STRENGTH TRAINING

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ABSTRACT: Introduction: The maximal compound muscle action potential (Mmax) of biceps brachii is altered by 1 session of strength training. We examined whether the number of training sets in a session plays a role in this effect. Methods: Ten subjects completed 1 session of isometric strength training of the elbow flexors (2 sets, 75% maximal force with 1 arm; 12 sets with the other). Biceps Mmax was acquired in both arms immediately after training, and every 5 min for 30 min. Results: Mmax area was initially potentiated after 2 sets (7.2%) and 12 sets (13.6%) but returned to baseline within 5 min. Conclusions: Biceps Mmax is similarly affected by 2 and 12 sets of strength training. The overall effect is minimal compared with ~25% depression reported after similar training in a different arm posture. Thus, changes in Mmax appear more dependent on training posture than number of training sets.


The maximal compound muscle action potential (Mmax) is the sum of the single muscle fiber action potentials. Mmax is an important control parameter in neurophysiological experiments. It is used by researchers to normalize evoked responses of the corticospinal pathway, such as the motor evoked potential (MEP). Normalization enables researchers to conclude that changes in MEPs after exercise are likely due to mechanisms within the nervous system, rather than the muscle.1 Mmax does not always remain constant throughout an experiment.2 It can be influenced by factors such as contraction history and limb posture.3–6 We recently reported that biceps brachii Mmax area is significantly depressed (~25%) for at least 30 min after 12 sets of isometric strength training of the elbow flexors.4 Such a change may be methodologically problematic, because it influences the MEP/Mmax ratio and may inaccurately suggest altered corticospinal excitability. Normalizing MEPs to a changing Mmax is a valid procedure, but only if there is a uniform change across all muscle fibers. Within an experiment, it is difficult to determine whether these conditions are met. Thus, there is a need to understand further what factors influence Mmax and how they can be controlled, so as to not confound measures of corticospinal excitability.

The purpose of this study was to assess whether Mmax is influenced by the number of sets performed in 1 session of strength training. We acquired biceps brachii Mmax before and after 2 and 12 sets of isometric strength training of the elbow flexors. We implemented an arm posture (shoulder flexed, forearm supinated) that was different from our previous work (shoulder abducted, forearm neutral),4 which allowed us to also draw conclusions about the role of training posture. We hypothesized that biceps Mmax would not be affected by 2 sets of training but would be depressed after 12 sets, as in our previous study.4
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