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Original Article

A new model of skeletal muscle atrophy induced by immobilization using a hook-and-loop fastener in mice

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Abstract. [Purpose] To study muscle atrophy, the muscle atrophy model mice have been used frequently. In particular, cast immobilization is the most common method to induce muscle atrophy. However, it is time consuming and often causes adverse events including skin injury, edema, and necrosis. The present study, we developed a hook-and-loop fastener (Velcro) immobilization method as a new, simple, and less invasive approach to induce muscle atrophy. [Subjects and Methods] Mice were bandaged in the knee joint extension and ankle plantar extension position. Muscle atrophy was induced by either winding a cast or Velcro around the limb. [Results] According to weight and fiber size, Velcro immobilization induced equivalent muscle atrophy to cast immobilization. Velcro immobilization reduced significantly the time for the procedure and the frequency of adverse events. [Conclusion] Velcro immobilization can induce muscle atrophy comparable to cast immobilization, but in a shorter time and with less complications. Velcro immobilization may contribute to the study of disuse muscle atrophy in clinical practice of physical therapy using a mouse model.

Key words: Skeletal muscle atrophy, Cast immobilization, Velcro immobilization

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INTRODUCTION

Skeletal muscle atrophy is a major health problem in aging societies[1, 2]. Understanding the pathogenesis and effects of therapeutic interventions for muscle atrophy is becoming increasingly important in physical therapy. To study muscle atrophy, the muscle atrophy model mice have been used frequently.

Various methods are used to induce muscle atrophy, e.g., denervation, hindlimb suspension, cast immobilization, and immobilization using a splint, staple, plastic pipette, or spiral wire[3–9]. Among them, the cast immobilization method is the most commonly used because it is a practical method for clinical practice. However, it is time consuming, needs some skill, and may cause adverse events including skin injury, edema, and necrosis[10, 11]. As a result, cast immobilization requires experience, frequent observation, and replacement. For these reasons, a simple and less painful method for inducing muscle atrophy is required.

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Here, we developed a novel Velcro immobilization method using commercially available hook-and-loop fastener (Velcro® tape) to induce muscle atrophy (hereafter referred to as "Velcro immobilization"). We found that Velcro immobilization is faster and has less adverse events than cast immobilization and can induce muscle atrophy to the same extent. Thus, Velcro immobilization could substitute for cast immobilization and allow the immobilization-intervention process to be repeated easily. These advantages of Velcro immobilization may contribute to study muscle atrophy by reproducing various clinical conditions of daily physical therapy.

SUBJECTS AND METHODS

Fifteen 10-week-old male C57BL6 mice (Japan SLC, Hamamatsu, Japan) were housed in independent plastic cages in a 12-h light/dark cycle. This study was approved by the ethics committees on animal experimentation at Teikyo University of Science (14C037).

The mice were divided into 3 groups of 5: control group (Group Co), cast immobilization group (Group CI), and Velcro immobilization group (Group VI). Muscle atrophy was induced in Groups CI and VI (Fig. 1). The left hindlimb was maintained in a knee joint extension and ankle plantar extension position and taped with a non-elastic bandage tape (Multipore™ Sports White Athletic Tape; 3 M Japan, Tokyo, Japan) to prevent dermatitis. The left hindlimb was fixed by winding 12–15 mm-wide cast (Scotchcast™ Plus-J; 3 M Japan) (Group CI) or commercially available hook-and-loop fastener (Velcro® tape) (one-touch belt; 3 M Japan) (Group VI) around the limb. Both immobilization procedures were carried out without anesthetization. If necessary, light isoflurane (2%) inhalation was used. Detailed procedures of cast immobilization method are; Step 1: Cut the surgical tape to 7 × 150 mm and cast to 10–13 × 150 mm. Step 2: Hold mouse hindlimb in knee joint extension and ankle plantar flexion position. Step 3: Before cast immobilization, the left hindlimb is wrapped with surgical tape (Fig. 1A, upper row). Step 4: Wind the cast from the proximal portion. Avoid overtightening at the proximal part. Step 5: When winding is finished, check the looseness of the cast. Leave the forefoot portion exposed in order to monitor adverse events. Finally, shape the cast with water and wait 40 s for the cast to become dry and hard (Fig. 1A, middle row). Appearance of mouse when cast immobilization is completed (Fig. 1A, lower row). Detailed procedures of Velcro immobilization method are; Step 1: Cut the surgical tape to 7 × 80 mm and Velcro® tape to 13 × 150 mm. Step 2: Hold mouse hindlimb in knee joint extension and ankle plantar flexion position. Step 3: Wrap the surgical tape to the foot to prevent Velcro being pulled out (Fig. 1B, upper row). Step 4: Wind the Velcro along the hind limb from the distal portion of the foot. Step 5: When winding is finished, check the looseness of the Velcro. Leave the forefoot portion exposed in order to monitor adverse events (Fig. 1B, middle row). Finally, apply an instant glue with alpha cyanoacrylate (Aron Alpha®; Toagosei, Tokyo, Japan) to two parts of the Velcro to reinforce adhesion. Appearance of mouse when Velcro immobilization is completed (Fig. 1B, lower row). The mice were kept for 2 weeks in each group. They could move in the cage using their forelimbs and right hindlimb, so they were able to take food and water freely. The mice were monitored on alternate days. The cast or Velcro was replaced when it became loose or if any signs of adverse events (e.g., skin injury, edema, and necrosis) were observed in the fixed hindlimb. We also recorded the time required for the procedure and the occurrence of adverse events.

The mice were euthanized after 2 weeks of immobilization. Muscles were isolated, blood was extracted from the abdominal aorta and centrifuged immediately to obtain serum. The tibialis anterior (TA), gastrocnemius (Gc), and soleus (Sol) muscles were weighed immediately after dissection[12]. They were frozen in liquid nitrogen-cooled isopentane, and 8-μm cryosections were prepared using a cryostat (HM560; Microm, Walldorf, Germany)[13]. In order to analyze muscle fiber diameter, the cross-sectional area of more than 400 muscle fibers in Gc of each group was analyzed. The sections were immunostained with an anti-laminin α2 polyclonal antibody (L9393; Sigma, MO, USA), observed under an FSX100 fluorescence microscope (Olympus, Tokyo, Japan), and minimum diameter (μm) was measured by using ImageJ software[14].

Hydroperoxide content, an oxidative stress marker, was measured from serum at the end of immobilization by performing a reactive oxygen metabolites test[15] using the FREE Carpe Diem system (Diacron International, Grosseto, Italy) according to the manufacturer's instructions.

Statistical analyses were performed using the Mann-Whitney U-test. The significance level of the statistical methods was less than 5%.

RESULTS

First, we compared Velcro and cast immobilization for their ability to induce muscle atrophy. Representative pictures of the TA, Gc, and Sol muscles in each group are shown (Fig. 2A). The muscle wet-weight divided by body weight of the TA, Gc, and Sol muscles in Groups VI and CI showed a significant reduction compared to Group Co ($p<0.05$). No significant differences were found between Groups VI and CI (Table 1).

In histological analysis, the distribution of muscle fiber diameter in Group VI was similar to that of Group CI (Fig. 2B). A reduction of muscle fiber diameter was observed in Groups CI and VI in comparison with Group Co ($p<0.05$). The average muscle fiber diameter of Gc was 46.8 ± 2.1, 31.7 ± 1.6, and 34.6 ± 1.9 μm in Groups Co, CI, and VI, respectively.

To evaluate whether Velcro immobilization is practical in an experimental setting, we analyzed the time required for the procedure and frequency of rewinding due to adverse events. The time required was reduced significantly in Group VI

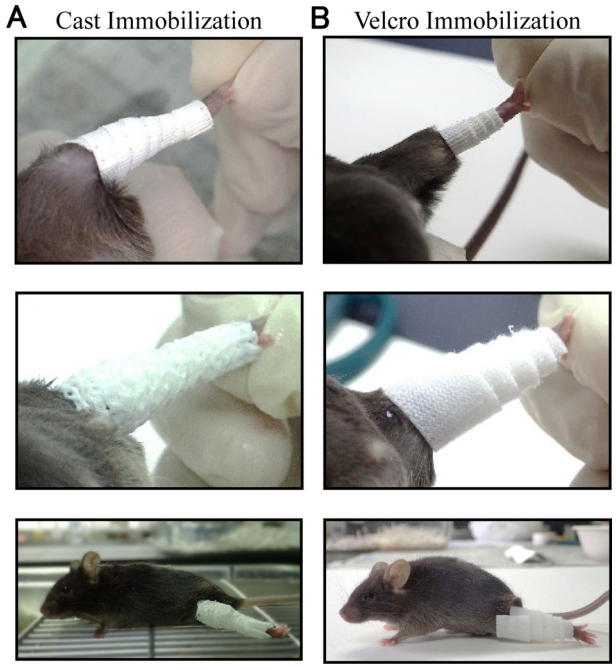

Fig. 1. Velcro immobilization in comparison with cast immobilization

(A) cast immobilization, (B) Velcro immobilization. Velcro® (hook-and-loop fastener) tape was wound around the limb as described in the text. The Velcro method immobilized the mouse lower limb similar to the casting method.

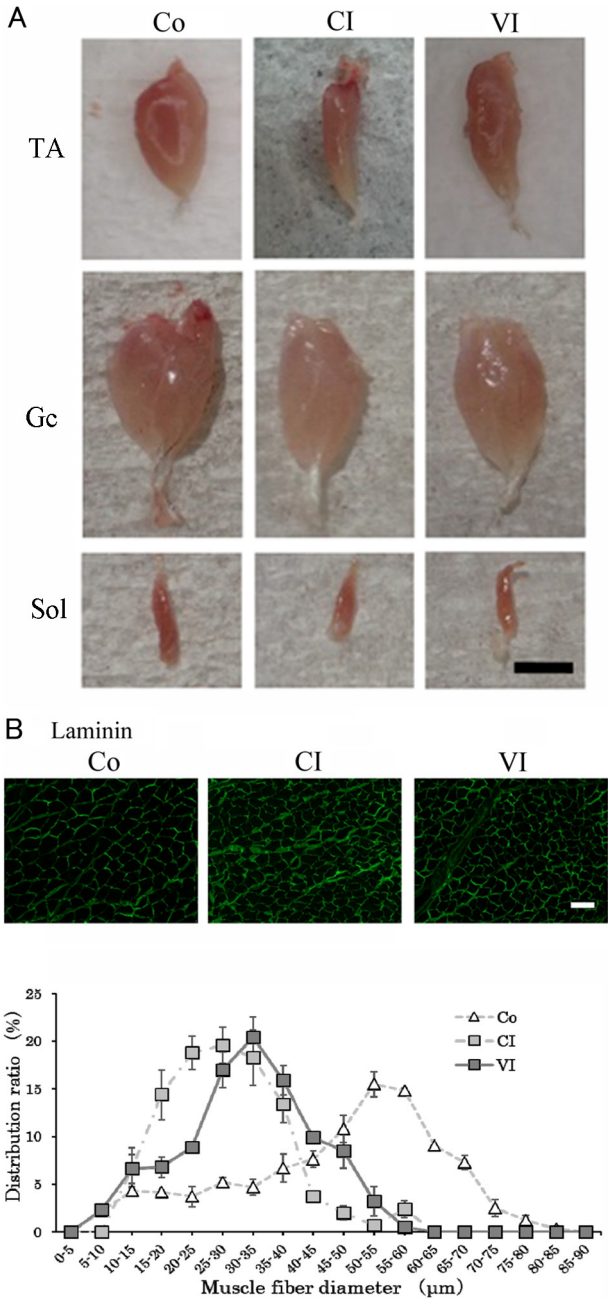

Fig. 2. Outcome of Velcro immobilization

(A) Representative pictures of the TA, Gc, and Sol muscles in each group. Scale bar=5 mm. (B) Upper: Laminin immunostaining of the Gc muscle in each group. Scale bar=100 μm. Lower: Distribution of muscle fiber diameter of the Gc muscle. Reduction of muscle fiber diameter was observed in Groups CI and VI. The distribution of muscle fiber diameter in Group VI was similar to that of Group CI.

compared to Group CI both for the first application and rewinding (both $p<0.05$) (Table 2). Notably, for rewinding, the time required for the Velcro method was markedly shortened by up to 5 min, which was approximately one-third of the time required for the cast method and not significantly different from the time required for the initial winding.

We further analyzed hydroperoxide content to examine whether the muscle was damaged by the procedure. There was no significant difference in hydroperoxide content between the groups ($p<0.05$) (Table 3).

Table 1. Muscle wet-weight divided by body weight

		Group Co (n= 5)	Group CI (n= 5)	Group VI (n= 5)
Body weight (BW; g)		26.6 ± 1.8	25.1 ± 1.1	27.2 ± 1.1
TA	Wet weight (WW; mg)	71.0 ± 15.0	51.2 ± 7.4*	51.3 ± 5.5*
	WW/BW	2.6 ± 0.6	1.2 ± 0.4*	1.2 ± 0.2*
GC	Wet weight (WW; mg)	150.3 ± 10.4	116.1 ± 10.2*	123.0 ± 11.4*
	WW/BW	5.1 ± 2.1	4.6 ± 0.3*	4.5 ± 0.4*
Sol	Wet weight (WW; mg)	8.6 ± 1.7	5.0 ± 1.4*	5.0 ± 1.6*
	WW/BW	0.3 ± 0.06	0.2 ± 0.05*	0.2 ± 0.06*

All values are given as mean ± SD. *vs. group Co ($p<0.05$).

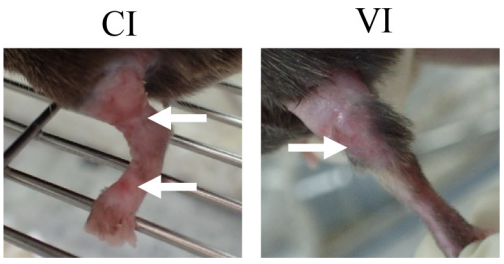

Fig. 3. Representative images of adverse events
Representative images of adverse events, e.g., reddening, edema, and skin injury, at the immobilized hindlimb (arrow) in Groups CI and VI. In Group VI, these adverse events occurred less often and were milder than in Group CI.

Table 2. Treatment time and frequency of rewinding

	Group CI (n= 5)	Group VI (n= 5)
Treatment time		
First time (min)	5'49"± 1'50"	3'17"± 43"*
Rewind (min)	13'07"± 7'25"	4'40"± 2'13"*
Frequency of rewinding		
Rewind (times)	1.83 ± 0.98	1.00 ± 0.57*

All values are given as mean ± SD. *vs. group CI ($p<0.05$).

Table 3. oxidative stress marker (hydroperoxide content)

	Group Co (n= 5)	Group CI (n= 5)	Group VI (n= 5)
Oxidative stress hydroperoxide (U.CARR)	93.7 ± 30.0	95.1 ± 30.0	114.7 ± 25.0

All values are given as mean ± SD.

Representative images of adverse events, e.g., reddening, edema, and skin injury, at the immobilized hindlimb (arrow) in Groups CI and VI are shown (Fig. 3). In Group VI, these adverse events occurred less often and were milder than in Group CI. The frequency of rewinding during the immobilization period in Group VI was significantly less than that of Group CI ($p<0.05$) (Table 2).

DISCUSSION

The Velcro immobilization method resulted in equivalent muscle atrophy in comparison with the conventional cast immobilization method. Velcro immobilization also reduced significantly the time for the procedure and the frequency of adverse events. Furthermore, no increase of an oxidative stress marker was observed in Velcro-immobilized muscle. These results indicate that Velcro immobilization induced muscle atrophy to the same extent as cast immobilization, but in a shorter time and with less complications.

Velcro immobilization has several major advantages over conventional methods. First, Velcro immobilization is a simple procedure and needs no special skills. Velcro tape is commercially available and inexpensive. It does not require specially designed equipment or surgery. In addition, Velcro immobilization causes less local damage, e.g., skin injury, edema, and necrosis. Mice subjected to cast immobilization often suffer from these adverse events. The greatest advantage of the Velcro method compared to the conventional methods is that it can be repeated easily. As shown, Velcro immobilization can be completed in up to 5 min. In contrast, conventional cast immobilization required a longer time, especially during rewinding,

because removing the cast is laborious and requires considerable time during rewinding. The Velcro method made it possible to rewind in one-third of the time required for the cast method, which allows the immobilization-intervention process to be repeated easily to reproduce various clinical conditions.

To date, several methods to induce muscle atrophy have been reported. The Velcro immobilization method proposed here is simple, inexpensive, safe, and easy-to-repeat. This could be the most suitable method to substitute for cast immobilization. Furthermore, Velcro immobilization easily makes it possible to repeat the immobilization-intervention process which reproducing daily clinical conditions. This new approach may contribute to the future development of research on muscle atrophy in clinical practice of physical therapy using a mouse model.

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REFERENCES

1) Jasuja R, LeBrasseur NK: Regenerating skeletal muscle in the face of aging and disease. Am J Phys Med Rehabil, 2014, 93: S88–S96. [Medline] [CrossRef]
2) Rudrappa SS, Wilkinson DJ, Greenhaff PL, et al.: Human skeletal muscle disuse atrophy: effects on muscle protein synthesis, breakdown, and insulin resistance-a qualitative review. Front Physiol, 2016, 7: 361. [Medline] [CrossRef]
3) Loermans H, Wirtz P: Inhibition of the expression of pathology in dystrophic mouse leg muscles by immobilization. Br J Exp Pathol, 1983, 64: 225–230. [Medline]
4) Mokhtarian A, Lefaucheur JP, Even PC, et al.: Hindlimb immobilization applied to 21-day-old mdx mice prevents the occurrence of muscle degeneration. J Appl Physiol 1985, 1999, 86: 924–931. [Medline]
5) Morey-Holton ER, Globus RK: Hindlimb unloading rodent model: technical aspects. J Appl Physiol 1985, 2002, 92: 1367–1377. [Medline] [CrossRef]
6) Frimel TN, Kapadia F, Gaidosh GS, et al.: A model of muscle atrophy using cast immobilization in mice. Muscle Nerve, 2005, 32: 672–674. [Medline] [CrossRef]
7) Caron AZ, Drouin G, Desrosiers J, et al.: A novel hindlimb immobilization procedure for studying skeletal muscle atrophy and recovery in mouse. J Appl Physiol 1985, 2009, 106: 2049–2059. [Medline] [CrossRef]
8) Khan MA, Sahani N, Neville KA, et al.: Nonsurgically induced disuse muscle atrophy and neuromuscular dysfunction upregulates alpha7 acetylcholine receptors. Can J Physiol Pharmacol, 2014, 92: 1–8. [Medline] [CrossRef]
9) Onda A, Kono H, Jiao Q, et al.: New mouse model of skeletal muscle atrophy using spiral wire immobilization. Muscle Nerve, 2016, 54: 788–791. [Medline] [CrossRef]
10) Ohmichi Y, Sato J, Ohmichi M, et al.: Two-week cast immobilization induced chronic widespread hyperalgesia in rats. Eur J Pain, 2012, 16: 338–348. [Medline] [CrossRef]
11) Guo TZ, Wei T, Li WW, et al.: Immobilization contributes to exaggerated neuropeptide signaling, inflammatory changes, and nociceptive sensitization after fracture in rats. J Pain, 2014, 15: 1033–1045. [Medline] [CrossRef]
12) Kawahara Y, Nikawa T, Hirasaka K, et al.: Preventive effect of isometric contraction exercise on disuse muscle atrophy using tail suspension mice. J Phys Ther Sci, 2008, 20: 39–44. [CrossRef]
13) Hagiwara H, Ohsawa Y, Asakura S, et al.: Bone marrow transplantation improves outcome in a mouse model of congenital muscular dystrophy. FEBS Lett, 2006, 580: 4463–4468. [Medline] [CrossRef]
14) Saito F, Kanagawa M, Ikeda M, et al.: Overexpression of LARGE suppresses muscle regeneration via down-regulation of insulin-like growth factor 1 and aggravates muscular dystrophy in mice. Hum Mol Genet, 2014, 23: 4543–4558. [Medline] [CrossRef]
15) Maruoka H, Fujii K, Inoue K, et al.: Long-term effect of ubiquinol on exercise capacity and the oxidative stress regulation system in SAMP1 mice. J Phys Ther Sci, 2014, 26: 367–371. [Medline] [CrossRef]