EFFECT OF TIMING OF ELECTROMYOSTIMULATION ON DENERVATED SKELETAL MUSCLE ATROPHY

EUN SIL KOH, MD, PhD,1,2 HEE CHAN KIM, PhD,2 and JAE-YOUNG LIM, MD, PhD3

1Department of Rehabilitation Medicine, National Medical Center, Seoul, Republic of Korea
2Department of Biomedical Engineering, Seoul National University College of Medicine, Seoul, Republic of Korea
3Mechanic & Molecular Myology Laboratory, Department of Rehabilitation Medicine, Seoul National University Bundang Hospital, 173-82, Gumi-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13620, Republic of Korea

ABSTRACT: Introduction: In this study we evaluated the effect of electromyostimulation (EMS) on myosin heavy chain (MHC) isoform expression in denervated rat muscles to determine the optimal timing for EMS application. Methods: EMS was initiated on post-injury day 1 for the group with denervation receiving immediate EMS (DIMEIS) and on post-injury day 15 for the group with denervation receiving delayed EMS (DDEMS) in rat denervated muscles. Muscle wet weight and muscle fiber cross-sectional area (FCSA) were measured. MHC isoforms were analyzed in both protein homogenates and single muscle fibers. Results: The expression levels of IIx and IIB isoforms of MHC were significantly lower and higher, respectively, in the gastrocnemius muscles of the DIMEIS group, but not the DDEMS group. The DIMEIS group also showed larger FCSA and a lower proportion of hybrid single fibers compared with the DDEMS group. Conclusions: These results indicate that immediate EMS is more effective than delayed EMS for aiding recovery of denervation-induced MHC changes.

Muscle Nerve 000:000–000, 2017

After complete denervation, skeletal muscles have no contractile activity and undergo rapid muscle fiber atrophy and loss of muscle mass, resulting in impaired function.1–3

The ability of muscle to adapt in response to the imposed functional demands is called muscle plasticity, which involves changes in the quantity or types of proteins expressed. Myosin is regarded as a cellular marker for muscle plasticity in response to new environmental requirements.1 It is an important structural and regulatory protein that constitutes the contractile apparatus of skeletal muscle, and is very sensitive to the degree of mechanical stress imposed on the muscle. The quantity and isoform type of myosin heavy chain (MHC) are altered in response to mechanical stress. In adult mammalian skeletal muscles, at least 4 different isoforms of MHC are expressed, I, IIa, IIx, and IIB, corresponding to fiber types I, IIa, IIx, and IIB, respectively. Mammalian skeletal muscle fibers can be categorized into slow- and fast-twitch types based on contractile properties, which, in part, vary depending on differences in MHC isoforms.4,5 The maximal contractile velocities of rodent muscle fibers are known to be slowest in type I followed by types IIa, IIx, and IIB.6–10 A fiber containing only a single MHC isoform is referred to as a pure fiber, whereas a fiber that expresses >1 MHC isoform is referred to as a hybrid fiber. The proportion of hybrid fibers to pure fibers is typically low in normal skeletal muscle. It is widely accepted that the proportion of hybrid fibers is higher in muscle in a transitional state compared with normal muscle.11–13 Denervation-induced muscle atrophy leads to adaptation of MHC expression, where the MHC content decreases14 and the relative proportions of MHC isoforms change.14–17 Conversion from slow- to fast-twitch fibers has been reported in non-human animals,18,19 as well as in humans.20–22 The relative proportion of hybrid fibers expressing multiple MHC isoforms increases markedly in denervated muscles.17,23

Various therapeutic modalities, such as electrical stimulation, stretching, and strengthening exercises, are widely used to prevent denervated muscle atrophy before reinnervation occurs.24–27 These interventions are designed to overcome non-physiologic use, overuse, or disuse based on early mobilization. In addition, such therapies may broaden the therapeutic window of time for functional restoration.28 One of the therapeutic approaches generally used for the treatment of denervated muscle is electromyostimulation (EMS). EMS is a method to evoke muscle contraction by direct muscle stimulation, leading to muscle contraction with the intention to minimize muscle atrophy and substitute for neural input during denervation.27,29,30 Although the benefits of EMS in patient and animal models remain controversial, EMS has been successfully translated into

Electromyostimulation Timing

Abbreviations: CMAP, compound muscle action potential; Dcon, group receiving no electromyostimulation after partial denervation; DDEMS, group with denervation receiving delayed electromyostimulation; DIMEIS, group with denervation receiving immediate electromyostimulation; EMS, electromyostimulation; EPT, extensor postural thrust; FCSA, fiber cross-sectional area; H&E, hematoxylin and eosin; MHC, myosin heavy chain; Ncon, group without any intervention; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis

Key words: application timing; denervation; electromyostimulation; muscle atrophy; myosin heavy chain; skeletal muscle

This work was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare (Grant No. HI11C0698), and by the Seoul National University Bundang Hospital Research Fund (Grant No. SNUBH-03-2010-000).
The rationale for application of EMS in denervated muscle is that the most important factor regulating the properties of muscle fibers is not the presence of neurotrophic factors, but rather muscle activity. Thus, EMS is considered a useful tool to delay muscle atrophy and preserve the normal properties of denervated muscle. EMS is also reported to be a potential inhibitor of axon terminal sprouting and has been shown to impair early functional recovery and accentuate skeletal muscle atrophy. The optimal timing of EMS application is also unclear. EMS initiated immediately after nerve injury is generally assumed to be most beneficial. As the interval between the onset of denervation and the start of stimulation increases, the success of recovery decreases. Andreose et al. reported that EMS applied in acutely denervated muscles reversed denervation, whereas EMS initiated after the acetylcholine receptors at the neuromuscular junction became destabilized did not demonstrate reversal. The lack of consensus on the use of EMS in denervated muscles is complicated by the fact there is wide variation in the onset time to stimulation, stimulus parameters, types of electrode, types of lesion, and treatment regimens that have been used in previous studies, which makes comparisons very difficult.

We have focused on the important but frequently neglected question concerning the effect of timing of EMS application on denervated skeletal muscles. Therefore, the purpose of this study was to establish the effect of EMS on muscle plasticity in partially denervated rat skeletal muscles, and to establish the optimal timing for initiation of EMS to obtain optimal recovery in this experimental system.

**METHODS**

**Animals.** Eighteen 8-week-old male Sprague-Dawley rats (350–400 g) were used for this study. The rats were housed under pathogen-free conditions at ~20°C and exposed to a reverse light–dark cycle (12:12h) each day. All procedures related to animal housing and surgical intervention were performed in accordance with the Canadian Council on Animal Care Guidelines, under institutionally reviewed animal protocols. The study was approved by the institutional animal care and use committee of Seoul National University Bundang Hospital.

**Experimental Protocol.** Before nerve injury, all rats were anesthetized with zolazepam and tiletamine (30 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. Under sterile conditions, the sciatic nerve trunk was exposed via a posterior thigh-splitting incision. The 18 rats were divided into 4 groups. Except for the normal control group (Ncon, n = 4), 3 groups of rats (n = 14) received a partial denervation injury. For partial denervation, the sciatic nerve trunk 10 mm distal to the sciatic notch was crushed for 1.5 min with a non-serrated hemostat. To apply constant pressure to the nerve trunk, the compression force was controlled using locking forceps set at the same level, and the inner margin of the nerve trunk was positioned 3 mm proximal to the end of the jaws, between the arms of the forceps. After the muscle and skin were closed, 10 mg/kg of gentamycin was injected into the gluteal muscle.

Ten of the 14 rats with partial denervation injury were assigned to the EMS groups and received EMS applied to the gastrocnemius muscle. The denervation with immediate EMS group (DIEMS, n = 5) and the denervation with delayed EMS group (DDEMS, n = 5) received EMS from day 1 to day 14, and from day 15 to day 28, respectively. The remaining rats with partial denervation injury were assigned to the denervation control group (Dcon, n = 4). All rats were euthanized and examined 4 weeks after the injury. The rats in the normal control group did not receive any intervention (Ncon, n = 4). The experimental protocol is summarized in Figure 1.

**EMS.** With the rat in a prone position, both legs were tied to a fixation frame, and the stimulation electrodes were applied to the motor points of the gastrocnemius (Fig. 2). Using a portable electrostimulator (Cefar Rehab 4 Pro, CefarCompex, Malmö, Sweden), EMS was applied at the motor point of the right gastrocnemius for 30 min/day 5 days/week (Monday–Friday) for 2 weeks. The stimulation parameters were as follows: 1-Hz frequency; 10-ms stimulation duration; 5-mA stimulus intensity; and biphasic triangular impulses. To minimize the difference between the groups in the level and amount of activity after denervation, the rats in the Ncon and Dcon groups were also placed in a prone position, and both legs were tied to the fixation frame.

![FIGURE 1. Overview of the experimental protocol. EMS, electromyostimulation.](image-url)
Thereafter, the soleus muscles were separated from the calcaneal insertion of the triceps surae distally in both legs. The gastrocnemius proximally to immediately above the calf muscles were resected from immediately below the origin of the bundles. The tibial nerve was stimulated at the popliteal fossa using a stimulator constructed in-house with a needle evoked potential electrode. The EPT was measured at least 5 times on each side, with a 1–2-min interval between measurements. The same test was performed on at least 5 times on each side, with a 1–2-min interval between measurements. The same test was performed on

Percentage motor deficit (%) = (NEPT – DEPT)/NEPT × 100

where NEPT is the value of the EPT on the normal side and DEPT is the value of the EPT on the denervated side.

Electrophysiological Examinations. At 4 weeks after the partial denervation injury, the tibial nerve compound muscle action potentials (CMAPs) were measured before harvesting of the muscle tissue. The tibial nerve was stimulated at the popliteal fossa using a stimulator constructed in-house with a needle evoked potential electrode.

CMAPs were recorded from active and reference electrodes overlying the gastrocnemius muscles (filtering frequency 10 Hz to 10 kHz, sweep speed 1 ms/division, sensitivity 1 mV/division) using a portable 2-channel electromyography/nerve conduction system (Medelec Synergy, Oxford Instrument Medical Systems, Oxford, UK). The ratio of the amplitude of the injured side to the intact side was calculated.

Muscle Wet Weight Measurement. Whole gastrocnemius muscles were resected from immediately below the origin of the gastrocnemius proximally to immediately above the calcaneal insertion of the triceps surae distally in both legs. Thereafter, the soleus muscles were separated from the triceps surae muscles by detaching the small longitudinal muscles lying underneath the gastrocnemius. The wet weights of the gastrocnemius and soleus muscles were measured, and the ratio of denervated muscle wet weight to that of the intact side was calculated for each specimen.

Morphometric Analysis of the Gastrocnemius Muscle. A block of muscle taken from the mid-belly of the muscle was fixed in 4% paraformaldehyde. The fixed muscle specimens were washed with water, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin, and cut into 7-μm sections. The sections were stained with hematoxylin and eosin (H&E), and the images were observed under a light microscope. Photographs were taken from 5 fields (100×) chosen randomly in the central region of 1 cross-section of each gastrocnemius muscle, with the aid of a digital camera (AxioCam HR, Carl Zeiss, Göttingen, Germany).

Images were digitalized using AxioVision imaging software (Carl Zeiss), and ImageJ 1.44a software (Wayne Rasband, National Institutes of Health, Bethesda, Maryland; http://rsb.info.nih.gov/ij) was used to measure the cross-sectional area of the muscle fibers (FCSA). Muscle sections were generated from 1 animal per group, and a minimum of 150 fibers were measured per animal.

Tissue Sampling and Extraction. Samples taken from the gastrocnemius and soleus muscles for protein analysis were frozen in liquid nitrogen and stored at −80°C. A 100-mg sample of muscle tissue was homogenized with 200 μl of lysis buffer containing ethylene-diamine tetraacetic acid, pepstatin A, leupeptin, and aprotonin. Sonication and centrifugation were repeated 3 times before the supernatant was separated from the pellet. The total protein content of the supernatant was measured using a 280-nm absorbance assay and a NanoDrop instrument (NanoDrop Technologies, Wilmington, Delaware), and the concentration was adjusted to 10 mg/ml with washing buffer. Subsequently, myofibrils were solubilized with 5 μl of sodium dodecylsulfate sample buffer. The samples were heated at 50°C for 20 min and stored at 4°C before sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

MHC Isoform Composition. MHC isoform composition was analyzed using 2 samples: protein homogenates and single muscle fibers (>20 per muscle). The MHC composition of muscle homogenates was determined using 6% SDS-PAGE. The acrylamide concentration was 4% (v/v) in the stacking gel and 6% in the separating gel, and the gel matrix included 30% glycerol, SDS-PAGE was run at a constant voltage of 90 V for 30 min and 140 V for 5.5 h. Protein bands were visualized with Coomassie Brilliant Blue. Densitometry was performed using analytic software (Bio-1D Light, Vilber Lourmat, Marne La Vallee, France) to measure the relative proportions of MHC isoforms.

To prepare single muscle fibers, bundles of fibers were chemically skinned in relaxing solution containing 50% (v/v) glycerol at 4°C to disrupt membrane-bound organelles, such as sarcosomes, t-tubules, sarcoplasmic reticulum, and mitochondria. After incubation for 24 h at 4°C, the bundles were stored at −20°C. Each single fiber was extracted under a stereomicroscope and dissolved with lysis buffer. Single-fiber MHC isoforms were detected by 6% SDS-PAGE in the same manner as described for the protein homogenates. Protein homogenate mixtures of the gastrocnemius and soleus muscles were used as an MHC standard. Gels loaded with single muscle fibers were subsequently stained with silver nitrite to identify the MHC isoform of...
each single fiber. The relative proportion of hybrid fibers in each muscle was calculated (Fig. 3).

Statistical Analysis. The mean values for each group were compared using the non-parametric Mann–Whitney U-test for 2 independent samples. Differences in the frequency of hybrid single muscle fibers among groups were tested by chi-square analysis. Analyses were performed using statistical software (SPSS version 18.0 for Windows, SPSS, Inc., Chicago, Illinois). \( P < 0.05 \) was considered statistically significant.

RESULTS

EPT Test. The percent motor deficits after 4 weeks of denervation were not significantly different among the Dcon, DDEMS, and DIEMS groups (Table 1).

CMAP Amplitude Ratio. The ratios of the CMAP amplitude of the injured side to the intact side are shown for each of the 4 groups in Table 1. Neither the DDEMS nor the DIEMS showed any significant difference in the CMAP amplitude ratio relative to Dcon.

Muscle Wet Weight Comparison. Dcon showed no significant decrease in the muscle weights of the gastrocnemius and soleus at 4 weeks post-injury. The muscle wet weights of the gastrocnemius and soleus in the DDEMS and DIEMS groups were not significantly different from those of Dcon after 4 weeks of denervation. There was no significant difference in the wet weights of the gastrocnemius and soleus muscles of DDEMS and DIEMS (Table 1).

Morphometric Analysis. Representative images of muscle fiber cross-sections of the 4 groups at 4 weeks after denervation injury are shown in Figure 4. The FCSAs of the gastrocnemius muscle fibers in each group are shown in Figure 5. Compared with Ncon, a significant decrease in type IIx MHC was observed in Dcon and DDEMS (\( P < 0.021 \)), but not in DIEMS, at 4 weeks after denervation (Fig. 6A). There was no significant difference in proportions of MHC isoforms between Dcon and DDEMS. However, DIEMS showed a significant decrease in type IIx (\( P = 0.014 \) ) and a significant increase in

<table>
<thead>
<tr>
<th>Group</th>
<th>Percent motor deficit</th>
<th>CMAP ratio</th>
<th>Muscle wet weight</th>
<th>D/N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gastrocnemius</td>
<td>Soleus</td>
<td></td>
</tr>
<tr>
<td>Ncon</td>
<td>1.76 ± 4.40</td>
<td>0.89 ± 0.01</td>
<td>2.86 ± 0.09</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Dcon</td>
<td>36.78 ± 15.61</td>
<td>0.17 ± 0.18</td>
<td>1.42 ± 0.06*</td>
<td>0.12 ± 0.02*</td>
</tr>
<tr>
<td>DDEMS</td>
<td>28.76 ± 17.45</td>
<td>0.17 ± 0.08</td>
<td>1.50 ± 0.13*</td>
<td>0.13 ± 0.01*</td>
</tr>
<tr>
<td>DIEMS</td>
<td>36.74 ± 7.37</td>
<td>0.18 ± 0.16</td>
<td>1.37 ± 0.14*</td>
<td>0.11 ± 0.02*</td>
</tr>
</tbody>
</table>

Percent motor deficit as determined by the extensor postural thrust (EPT) test. The CMAP amplitude ratio is the ratio of measurements for the intact side relative to the injured side. Muscle wet weights and the ratio of denervated muscle wet weight to that of the intact side are indicated for the gastrocnemius and the soleus. CMAP: compound muscle action potential; D/N, ratio of denervated muscle wet weight to that of the intact side; NA, not applicable. Ncon: group without any intervention; Dcon: group receiving no electromyostimulation (EMS) after partial denervation; DDEMS: group with denervation receiving delayed EMS; DIEMS: group with denervation receiving immediate EMS.

* \( P < 0.05 \) vs. Ncon.
type IIb ($P = 0.027$) compared with Dcon. The proportion of type IIb MHC in DIEMS was higher than that of the DDEMS, but this result was not statistically significant ($P = 0.076$).

The MHC protein of the Ncon soleus muscle homogenates consisted of $19.0 \pm 9.9\%$ of type IIa isoform and $81.0 \pm 9.9\%$ of type I isoform. At 4 weeks after denervation, the proportion of MHC isoforms did not change significantly in the soleus muscles. There were also no significant changes in MHC proportions in DDEMS and DIEMS compared with control (Fig. 6B).

Based on single muscle fiber analysis, the expression patterns of MHC isoforms were identified to be pure fiber (i.e., expressing only 1 MHC isoform) or hybrid fiber (i.e., expressing multiple types of MHC isoforms), such as type I/IIx, I/IIb, I/IIb/IIx, and IIb/IIx in the gastrocnemius or type I/IIa in the soleus. Figure 7 shows the changes in proportion of hybrid MHC fibers relative to pure fibers after 4 weeks of denervation. The proportions of hybrid single muscle fibers in the control gastrocnemius and soleus were $43.5 \pm 24.6\%$ and $11.2 \pm 9.9\%$, respectively. Four weeks after denervation, the proportion of hybrid fibers in the gastrocnemius was increased significantly ($P < 0.001$). In DIEMS, but not DDEMS, the proportion of hybrid fibers in the gastrocnemius decreased significantly when compared with Dcon ($P < 0.001$). At 4 weeks after denervation, the proportion of hybrid fibers did not change significantly in the soleus muscle. The delayed EMS, but not the immediate EMS, showed a significant increase in the proportion of hybrid fibers compared with the 3 other groups ($P < 0.05$; Fig. 7).
DISCUSSION

EMS is direct muscle stimulation that causes muscle contraction. Although EMS has been applied successfully for the treatment of denervated muscle, only limited information is available about its exact effects, particularly on MHC isoform expression and the optimal timing of application. Therefore, in this study we examined the effects of EMS on denervated muscles, focusing on identification of alterations in MHC isoform proportions, and investigating the effect of EMS application timing. When the effects of immediate and delayed EMS paradigms were compared, immediate application was found to have greater potential to reverse some of the changes induced by denervation. The analysis of MHC isoform proportions revealed that expression of MHC type IIx in the gastrocnemius was increased 4 weeks after denervation. With immediate EMS, however, this increase in the MHC type IIx proportion was restored to levels comparable with control. Delayed EMS did not replicate this effect. The expression ratios of each MHC type in the soleus muscle was not affected by either denervation or EMS. In addition, muscle fiber CSA increased in the rats treated with immediate EMS, but not in rats treated with delayed EMS, to levels similar to those of the control condition.

The changes induced by denervation of the gastrocnemius were consistent with the results of other fast-twitch muscles described in previous reports. EMS in tongue muscle induced a fast-to-slow shift in the MHC content in normal rabbits. Application of EMS to the tibialis anterior muscle also led to a fast-to-slow shift in MHC content in normal rats. There have been no reports on EMS-induced alterations in the MHC expression after partial denervation. In this study, EMS applied immediately after denervation led to changes in the proportions of MHC isoforms in the gastrocnemius in a manner opposite to the denervation-induced changes. This result suggests that immediate EMS may be a good candidate for treatment of denervation-induced muscle atrophy based on its effect of reversing denervation-induced changes. However, the effect of immediate EMS was not observed in the slow-twitch type I fiber soleus muscle.

MHC isoform analysis of single muscle fibers also suggested positive effects of immediate EMS. Denervation increased the proportion of hybrid fibers (i.e., muscle fibers co-expressing multiple MHC isoforms) in the gastrocnemius after 4 weeks. This increased proportion of hybrid fibers was not
found in the DIEMS group. The fraction of hybrid fibers in the gastrocnemius of the DIEMS group was similar to that of the control group; however, delayed EMS had no effect on the proportion of hybrid muscle fibers in the denervated gastrocnemius. In the soleus muscle, neither denervation nor immediate EMS appeared to change the proportion of hybrid fibers. However, a higher proportion of hybrid single fibers in the soleus muscle was observed in the DDEMS group.

Hybrid fibers have the potential to transform into a pure fiber type after reinnervation. It is now widely accepted that the fraction of MHC hybrids increases in muscles during molecular and functional transformations. Therefore, the decreased proportion of hybrid fibers in fast-twitch muscle in the DIEMS group implies that immediate EMS may rapidly stabilize denervation-induced changes in a manner that cannot be achieved using delayed EMS.

A noteworthy finding is that no change was observed after denervation and EMS in the soleus muscle. It has been well described in the literature that slow-twitch muscles exert an antigravity function, and are more responsive to immobilization or disuse compared with fast-twitch muscles. Because we studied partial denervation-induced changes, the soleus, which is a slow-twitch muscle, was less affected than the gastrocnemius. Further studies on the optimal treatment paradigm for the recovery of muscle atrophy should be designed, taking into account the cause of muscle atrophy (denervation or disuse) and muscle fiber type (fast or slow twitch). It is important to note that the stimulation electrodes were applied to the motor points of the gastrocnemius muscles. Because the soleus lies deep to the gastrocnemius, the stimulus may not have been delivered effectively to the soleus muscle. This electrode placement may be a reason for the absence of EMS-induced changes in the soleus.

A recent study revealed harmful effects of EMS based on 6 EMS sessions on a denervated tibialis anterior muscle applied every other day starting on day 3 after sciatic nerve crush injury in rats. It was reported that EMS was associated with a delay of functional recovery, muscle hypoexcitability, and severe muscle atrophy. Conversely, other investigators reported that chronic EMS partially favored the recovery of mechano- and metabosensitivity in denervated muscle after nerve repair by self-anastomosis. After muscle denervation, embryonic MHC, which is not expressed in adult healthy muscles, was expressed in some denervated fibers, as well as in small activated satellite cells, and maximal expression was observed 2–3 weeks after denervation. To reverse or inhibit these degenerative changes in denervated muscle, earlier application of EMS may be more effective. These contradictory results concerning the effects of EMS seem to be related to the different types of electrode (implanted vs. surface), onset time to stimulation, amount of stimulation, interval between stimulation sessions, and lesion type (transsection vs. crush injury).

Although immediate EMS reversed the changes in MHC isoform proportions to levels close to normal and decreased the proportion of hybrid single fibers in a denervated muscle, it should be noted that this effect may not directly lead to improvement in motor function. Immediate EMS in denervated muscle did not result in significant changes in the percent motor deficit or muscle wet weight. An extended follow-up period may be needed to evaluate whether immediate EMS can recover muscle mass or motor function, in addition to reversing denervation-induced changes in contractile protein expression. In our previous study, we reported that degenerative changes predominated for 4 weeks after injury, and most of the regeneration occurred actively from 4 to 8 weeks. Therefore, the 4-week follow-up period used in the present study protocol was insufficient to effectively determine the effects of EMS on the recovery of muscle mass and motor function. Skeletal muscle is a soft tissue that includes other components in addition to muscle fibers. The discrepancy between changes of whole muscle weight and individual muscle fiber may be due to a relatively small change of other tissues such as connective tissues and intermuscular fat rather than changes of number of total muscle fibers. It would be considered a limitation that we did not include the mechanical properties of muscle fibers in this study. In addition, to induce sufficient muscle contraction, in this study we used a 1-Hz frequency and 100-ms stimulation duration. The motor unit activation caused by this stimulation may not be as physiologically relevant as voluntary activation. Further studies with an EMS method that can induce more physiological muscle activity will be required.

EMS applied immediately after nerve injury increased the FCSA of denervated muscle fibers, reversed denervation-induced changes in MHC isoform expression, and reduced the proportion of hybrid single muscle fibers in a partial denervation model, whereas delayed EMS had no effect. These results indicate that immediate EMS is more effective than delayed EMS in accelerating the recovery of denervation-induced MHC changes.

REFERENCES
