**Original**

# EFFECT OF ELECTRICAL STIMULUS ON DENERVATED MUSCLE

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**Abstract**

1. As a model of inactive skeletal muscle, Wistar rats were performed sciatic nerve excision and the regressive changes of skeletal muscle were induced. In addition, the forced contraction was induced by applying electrical stimulus to the denervated skeletal muscle.

2. It was suggested that the forced contraction due to electrical stimulation could inhibit to some extent the decrease of wet muscle weight and atrophy of muscle fibers.

3. There was a possibility that the effects differed depending on the stimulus conditions in frequencies, and it should be clarified in the further study.

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—**Key words**—
Electrical Stimulation, Denervated Muscle, Muscle Atrophy

## Introduction

An electrical stimulation therapy is widely used in clinical practices including acceleration of assimilation of fracture, reduction of pain through transcutaneous electrical stimulation, and reduction of spasm. In the field of rehabilitation, it has recently been applied to the functional electrical stimulation (FES)[1)2)] and therapeutic electrical stimulation (TES)[3)]. The present study was conducted in order to investigate whether the electrical stimulus reduced the regressive change caused by denervation in a model of inactive skeletal muscle[4)], with establishing the wet weight and fiber structure of skeletal muscle as indicators.

## Method

Forty-eight male Wistar rats (initial body weights: 263.5 ± 7.9g, 7 weeks of age) were used in the present study. The animals were divided into 4 groups of a control group (Con Group), a group performed denervation (Den Group), a group performed denervation and applied electrical stimulus of 15Hz (St15 Group), and a group performed denervation and applied electrical stimulus of 60Hz (St60 Group). In Den, St15 and St60 Groups, the rats at 8 weeks of age were anesthetized with pentobarbital sodium (1.5m/kg (weight; intraperitoneal administration), and not less than 10mm of sciatic nerves in both hind limbs were cut so that no regeneration of nerves occurred in the posterior surface of femoral region. From the following day of denervation operation, muscle contraction was forced with direct electrical stimulus to triceps muscle of the calf using electrically conductive stainless needles (Seirin Co., Ltd., acupunctural needles 0.20–0.23 × 30mm in diameter) and an electrostimulator (Asahi Denshi Kogyo Co., Ltd., Stimulator AT-300) in St15 and St60 Groups.

As conditions of electrical stimulation, the frequencies were set at 15Hz or 60Hz, and the wave form was an asymmetric rectangle with duration of 200μsec. A stimulus of combination of 4-second contraction and 2-second relaxation was continuously given to the muscle for 15 minutes (1 cycle). The strength of stimulation was set within the range of 5 to 10V so that the animals did not feel discomfort. In addition, it was visually confirmed that ankle angles of 30 degrees dorsal flexion (−30 degrees) changed to 45 degrees or over of plantar flexion in the both hindlimbs when these were placed against gravity after applying electrical stimulus. Two cycles of electrical stimulation were given to the rats anesthetized with diethyl ether for contraction of muscle per day, for 5 days a week. Between each cycle, the animals were given a respite of at least 10 minutes or more.

A rearing room was kept under the specific conditions (room temperature: 23°C, humidity: 50%, 12: 12 light-dark cycle). The animals could freely access to water and diets during whole the study period. They were reared until 4 weeks after denervation under the above conditions, and the analysis to investigate the time course of the changes was performed 1,2, 3, and 4 weeks after denervation.

Analytical method

Soleus (Sol) was used as a sample muscle. The rats were painlessly killed using excessive dose of pentobarbital sodium, and Sol was extirpated from both hindlimbs and its wet weight was measured. Then the muscle belly of right Sol (the second one-third portion of Sol) was frozen using isopentane cooled by liquid nitrogen. The frozen section was sliced to be approximately 10–12μm in thickness using cryostat. These sections were stained with hematoxylin and eosin (H-E stain) or with nicotinamide adenine dinucleotide and tetrazolium reductase (NADH-TR) stain. Their forms were observed with the optical microscope and their photographs were taken (shown in Fig. 1). The left Sol was fixed in 10% formalin and was hung with a loaded weight of 5.25g within 24 hours after the fixation and muscle length between the tendon at each end was measured. In addition, the circumference of central region of muscle belly was measured using a nylon thread 0.148mm in diameter.

The ratio of wet muscle weight to body weight, the circumference of central region of Sol, the muscle length and cross-section area of the muscle fiber were measured and analyzed. The cross-section area was measured for not less than 100 muscle fibers per a muscle, by capturing their photomicrographs into a personal computer and analyzing them using software for image analysis (NIH image 1.62f). One-way analysis of variance was performed to compare the data among 4 groups. P value of less than 0.05 was regarded to be statistically significant. All data are presented as means ± SE. The present study was conducted after being approved by Animal Study Facility Ethics Committee in Hiroshima University, School of Medicine.

## Results

Fig. 2 showed the ratio of wet muscle weight to body weight in each group. The weight of skeletal muscle decreased after denervation, and there observed a significant difference among Con, Den and St Groups ($p<0.01$) 1 week after denervation, which were maintained for residual 3 weeks. However, there was no significant difference

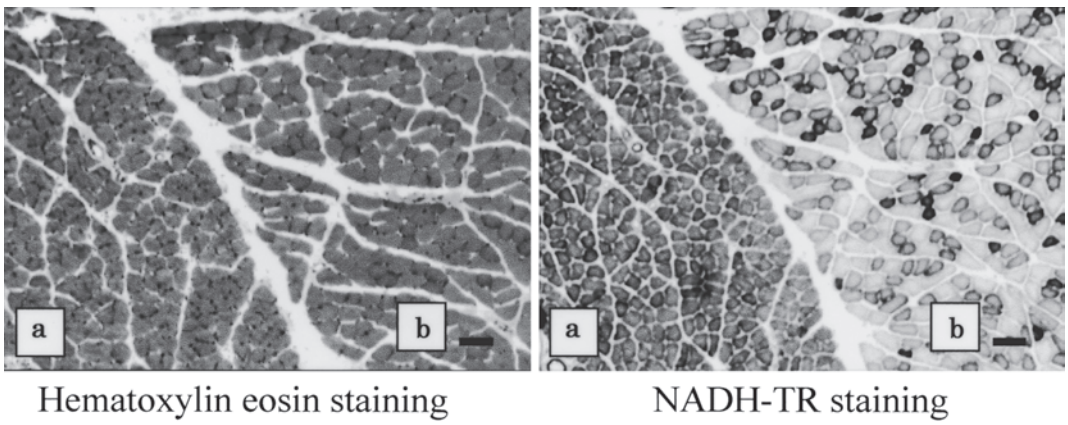


**Fig. 1** Cryostat cross-sections of rat soleus muscle stained for Hematoxylin eosin (Left) and nicotinamide adenine dinucleotide and tetrazolium reductase (Right). The left portion of pictures represents soleus muscle (a), and the right portion is tibialis anterior muscle (b). Scale bar = 100 micrometer.

between St15 and St60 Groups. The circumference of central region of Sol and the length were shown in Fig. 3 and 4. The circumference in Den and St Groups significantly differed from that in Con Group ($p<0.01$), which were maintained for residual 3 weeks. However, there was no significant difference between Den and St Groups. No significant difference in the muscle length was observed among these 4 groups.

Cross sectional area of denervated soleus muscle fiber decreased significantly ($p<0.05$–$0.01$) in all groups by 48–92% as compared with control muscles (Table 1). After 1 week, percentages of cross sectional area in the den-

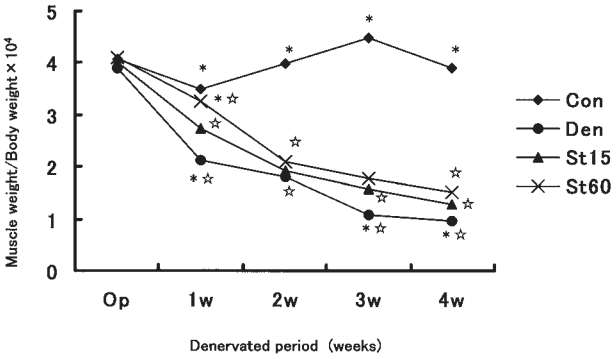


**Fig. 2** Muscle weight/body weight × 10,000 of different period in the soleus muscle of control (Con), denervation (Den), electrical stimulus plus frequency 15pps (St15) and electrical stimulus plus frequency 60pps (St60). ☆p<0.05 vs Con group, *p<0.05 vs St15.

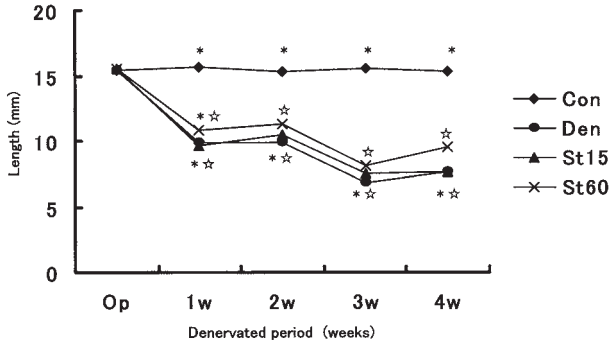


**Fig. 3** Muscle circumference of defferent period in the belly soleus muscle of control (Con), denervation (Den), electrical stimulus plus frequency 15pps (St15) and electrical stimulus plus frequency 60pps (St60). ☆p<0.05 vs Con group, *p<0.05 vs St15.

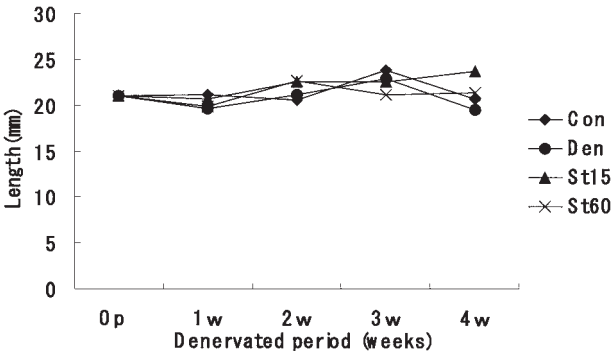


**Fig. 4** Muscle length of different period in the soleus muscle of control (Con), denervation (Den), electrical stimulus plus frequency 15pps (St15) and electrical stimulus plus frequency 60pps (St60). ☆p<0.05 vs Con group, *p<0.05 vs St15.

**Table 1** Cross sectional area of different period in the soleus muscle fiber

Mean ± SE（$\mu m^2$）【%】

| | 1w | 2w | 3w | 4w |
|---|---|---|---|---|
| Con | 2,435.2 ± 538.3<br>【100】※＊ | 2,474.7 ± 540.2<br>【100】※＊ | 3,011.1 ± 727.8<br>【100】※＊ | 2,994.9 ± 544.7<br>【100】※＊ |
| Den | 992.6 ± 267.7<br>【40.8】☆ | 265.5 ± 52.3<br>【10.7】☆＊ | 247.7 ± 53.1<br>【8.2】☆ | 218.7 ± 59.5<br>【7.3】☆ |
| St15 | 1,039.5 ± 305.1<br>【42.7】☆ | 470.2 ± 144.7<br>【19.0】☆※ | 296.8 ± 123.6<br>【9.9】☆ | 298.9 ± 47.5<br>【10.0】☆ |
| St60 | 1,252.1 ± 233.9<br>【51.4】☆※＊ | 761.7 ± 159.1<br>【30.8】☆※＊ | 589.5 ± 156.4<br>【19.6】☆※＊ | 310.1 ± 99.7<br>【10.4】☆# |

Con : control group, Den : denervation group, St 15 : denervation + electrical stimulation（pulse frequency 15 pps）, St 60 : denervation + electrical stimulation（pulse frequency 60 pps）group

☆ $p < 0.01$ vs control group

※ $p < 0.01$ vs denervation group（#$p < 0.05$ vs den. group）

＊ $p < 0.01$ vs stimulation 15 group

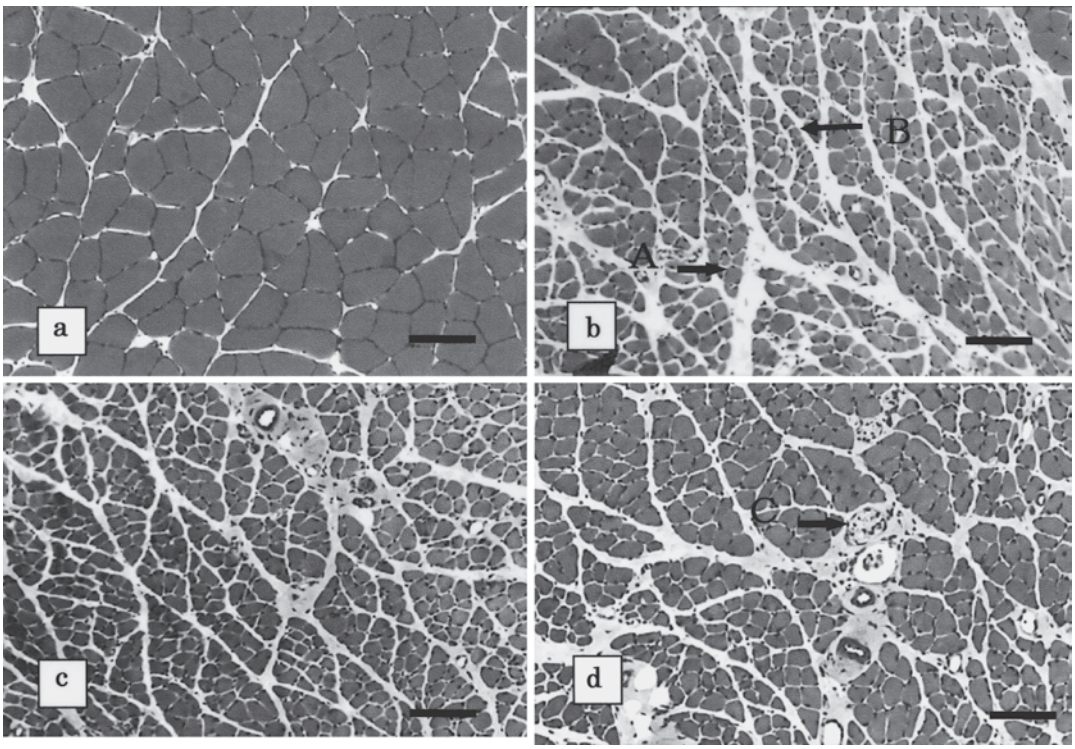


**Fig. 5** Photomicrographs of soleus muscle cross-section taken 3 weeks after sciatic nerve denervation. a; control, b; denervation, c; St15, d; St60. Hematoxylin and eosin staning. Scale bar = 100 micrometer.

ervated soleus muscles compared with Con Group were 40.8% in Den Group, 42.7% in St15 Group, and 51.4% in St60 Group. After 2 weeks, cross sectional area decreased to 10.7% in Den Group, 19.0% in St15 Group, and 30.8% in St60 Group. The significant differences in cross sectional area of soleus muscles between Con Group and other Group were recognized 1 week after the denervation. Moreover, after 2 weeks, the significant differences in cross sectional area of soleus muscles between Den Group and St Groups were recognized. Furthermore, after 3 weeks, a significant difference was recognized also between St60 Group and St15 Group. However, after 4 weeks, there was no significant difference between St60 Group and St15 Group.

Fig. 5 showed the photomicrographs of Sol cross-section (at right angle to the road of muscle fiber) taken 3 weeks after denervation in each group. In Con Group, the cross-section area of each muscle fiber was nearly equal, and each muscle fiber existed densely. In addition, there were a small amount of connective tissues around muscle fiber. On the other hand, the muscle fiber atrophied and connective tissues around them increased after denervation in Den and St Groups (A). Disparity of each muscle fiber size was also observed in Den and St Groups (B). It

was clear that the degree of muscle atrophy was reduced to some extent by forced contraction due to electrical stimulation in St Groups, but the amount of connective tissues increased as observed in Den Group. Some muscle fibers were damaged due to direct electrical stimulation, and were denatured in St Groups (C). Moreover, the occurrence of phlogocyte was observed in St Groups.

## Discussion

Kosman et al.[5] report that the stimulus that is strong enough to induce muscle contraction is more effective than the weak stimulus for inhibiting the regressive changes of the muscle, and supramaximal stimulus is the most effective if the pain is not taken into consideration. However, the electrical stimulation performed to inhibit the regressive changes due to denervation often required more electricity than that needed in normal condition, since it was difficult to induce contraction without stimulating the muscle directly. In addition, the electrical stimulation often induced muscle weakness, which might result in overwork weakness and intensifying the alteration of the muscle. Hatano et al.[6] show the inhibitory effect on muscle atrophy by giving the stimulus of approximately 30V as a transcutaneous electrical stimulation. In the present study, the large muscle contraction was obtained by giving only low intensity of stimulus of 5–10V since the stimulus was given directly to the muscle using a needle electrode. However, this method had several problems; the insertion of a needle worsened the damage of muscle fibers, and the electrification induced the heat injury of muscle fibers.

Not less than 80% of Sol in rats used as a sample muscle in the present study consists of Type 1 fiber, and is one of the skeletal muscles greatly attributable to the support of body weight. The degree of changes of the structure and the functional characteristics in an inactive state in Type 1 fiber is large compared to that in Type 2 fiber[4]. Since the turnover of protein is larger in Type 1 fiber than in Type 2 fiber, the atrophy of muscle occurs earlier in Type 1 fiber than in Type 2 fiber. In addition, since tonic fiber regularly performs stereotyped action, the contractility easily changes in an inactive state. Therefore, it was thought that Sol easily induced regressive changes due to inactivity and was one of the skeletal muscles whose degree of regressive changes was the largest.

The muscle contraction due to electrical stimulation becomes tonic contraction when the frequency of 30Hz or more is applied. In addition, the functional characteristics of muscle fiber can be changed by using various frequencies. When the stimulus at relatively low frequency (around 10Hz) is applied, the contraction speed decreases, oxidation enzyme activity increases, and skeletal muscle remarkably shows the characteristics of Type 1 fiber[7][8]. On the other hand, when the stimulus at high frequency (not less than 50Hz) is applied, skeletal muscle is reported to show the characteristics of Type 2 fiber[9]. The present study was conducted with employing 2 types of frequency of electrical stimulation to forcefully induce the contraction of skeletal muscle; 15Hz, at which the muscle was expected to show the characteristics of Type 1 fiber, and 60Hz, at which the muscle was expected to show those of Type 2 fiber. In addition, NADH-TR stain was performed in order to investigate the dominance of each type of fiber in the muscle after giving stimuli at 15 and 60Hz, but it was difficult to compare the dominance of these 2 types between the muscles given stimuli at 15 and 60Hz since only pachychromatic fibers which indicated the dominance of Type 1 fiber were observed in both the muscles given stimuli at 15 and 60Hz.

Relative weight of skeletal muscle gradually decreased due to denervation, and the significant difference of it was observed among Con, Den and St Groups 1 week after denervation. Our results were in agreement with those of other studies[4][10], and showed that the influence of denervation certainly occurred in Sol. Although the weight of skeletal muscle decreased after denervation, DNA amount is reported to increase due to the increase of nucleus[11]. In addition, there observed a significant difference in skeletal muscle weight between Den and St Groups 1 week after the start of electrical stimulation. It is widely known that the skeletal muscle enlarges as muscle contraction is maintained, but it is still not well known whether the forced contraction due to denervation can reduce the degree of muscle atrophy. As shown in photomicrographs of cross-section of skeletal muscle, it was clear that the degree of atrophy of muscle fibers themselves was reduced by forced contraction induced by electrical stimulation. Mokrusch et al.[12] suggest a possibility that the forced contraction induced by electrical stimulation at frequency of 25Hz recover the reduced cross-section area of the denervated skeletal muscle. These results indicated that there was a possibility that the forced contraction reduced to some extent the degree of atrophy even in the skeletal muscle without the information from motor nerves. However, the direct trigger which reduced muscle atrophy had been

still unknown, and the further study was required to be conducted.

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# 除神経筋に対する電気刺激の影響

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—キーワード—
電気刺激，除神経筋，筋萎縮

除神経筋が萎縮を示すことは周知のとおりである．また，それらの萎縮に対し，電気刺激療法を施行することも治療場面で数多く経験する．電気刺激療法は骨折の癒合促進や疼痛に対する経皮的電気刺激，痙縮の軽減など多方面の治療場面で利用されており，近年，リハビリテーションの分野においてもFES（functional electrical stimulation）やTES（therapeutic electrical stimulation）としての応用が知られている．本研究は，不活動モデルとしてWistar系ラットに坐骨神経切除術を施し，骨格筋の退行性変化を観察した．さらに除神経に伴う萎縮筋に対し，刺入電極による直接的電気刺激を加え，骨格筋の強制収縮を行った．電気刺激による収縮で筋湿重量の低下，筋線維の萎縮速度を抑制出来ることが示唆された．また，電気刺激における周波数の違いによっても，効果の違いが現れる可能性が考えられた．