（論文題目）

Strenuous eccentric contractions induce the peripheral nervous injury

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過度な伸張性収縮は末梢神経損傷を誘発する

（英　訳）

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Chapter 1. General introduction

1-1. Characteristics of muscle contraction

Exercises such as resistance training and endurance training are known as the beneficial tool that maintains and/or improves physical activity levels in the sport performance or the health promotion (1, 2).

Especially, the skeletal muscle assumes the important role during these physical activities. Muscles contract to perform appropriately movements such as walking, running, and lifting. Contractive properties of muscle are mainly divided into three types that are named as isometric (ISOs), concentric (CONs) and eccentric (ECs) contractions (3). These contractions have applied in several situations. For instance, ISOs is frequently used in the situation of re-habilitations because of without joint movement (Figure 1-1). CONs and ECs are also used in late phase of re-habilitations. In addition, these contractions are observed in exercise and training situations (4, 5). CONs and ECs are the behavior with joint movement (Figure 1-1). These produce the force as the muscle strength during change of the muscle length.

ECs is known that length of muscle is passively stretched during contractions, while CONs produces the force by shortening of the muscle length. Specifically, ECs has the low stress to respiratory system (2) despite the highly exersional force among three contraction types (6, 7). Therefore, ECs is widely used in several fields such as re-habilitation, conditioning and promotion.

Figure 1-1

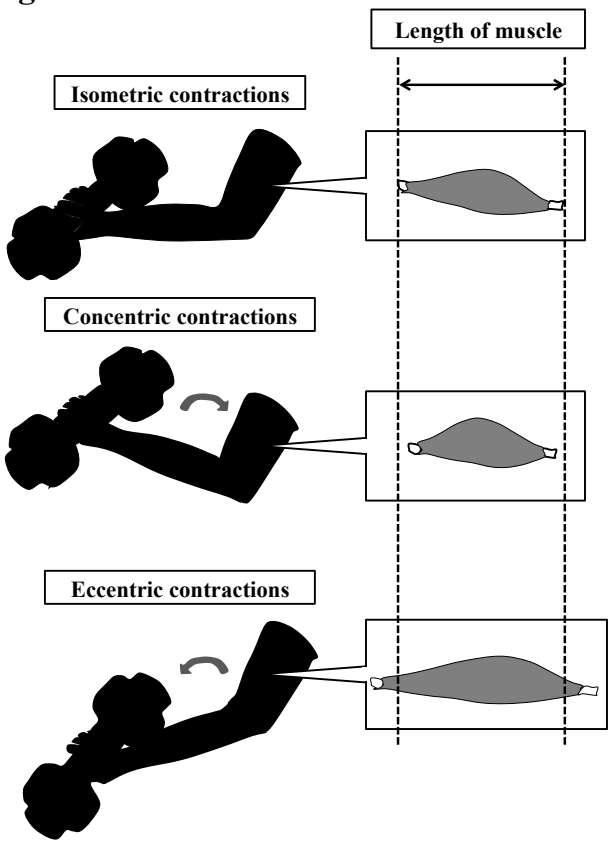


Figure 1-1. Types of the skeletal muscle contractions

The skeletal muscle length is short during the concentric contractions. In the eccentric contractions, the skeletal muscle length is prolonged. ISOs is not change length of the skeletal muscle. Arrows indicate the direction of joint movement.

1-2. Excessive eccentric contractions induce muscle damage

ECs contribute to improvement of muscle strength and/or muscular hypertrophy (4, 8), whereas it is reported that ECs causes skeletal muscle injuries (9, 10). In the previous report, experimental animals (rats) were passively applied ECs by divers joint angular velocity in their gastrocnemius muscles (4, 8, 10, 11). Positive effects such as increase of muscle strength and hypertrophy were induced by slow joint angular velocity (SLOW) ECs (4, 8). On the other hand, fast joint angular velocity (FAST) ECs induced negative effects such as functional and pathological damages of muscle (10-12). Hence, the joint angular velocity during ECs is one of a material factor whether ECs conducts positive or negative effects on the skeletal muscle.

The injuries by ECs broadly termed as exercise induced muscle damage, which has some physiological and structural symptoms (9, 12-15). Especially in the physiological symptom, muscle strength deficit, decrease of joint range of motion (ROM), delayed onset of muscle soreness (DOMS) and increased creatine kinase (CK) activity are typically observed. Disorders of muscle strength and ROM are observed immediately after the exercise, that are approximately 50% decreased in both animal and human experiments (12, 16, 17). DOMS is characterized that the

expression of muscle soreness is observed from 24 hours of the exercise (9, 12, 17). However, the mechanism has been unclear that time course of symptoms are differently indicated. In the pathological observation, changes such as muscle fiber disarrangement are observed (14). Additionally, the expression of muscle repair associated proteins (Pax7, MyoD and myogenin) is also observed as the molecular events in recovery process of exercise induced muscle damage.

1-3. Contribution of neuromuscular system in the muscle contractions

The nervous system plays an important role in the mechanism of skeletal muscle contractions. The nervous system is composed of two systems: central nervous system (CNS) and peripheral nervous system (PNS). Component of CNS is brain and spinal. On the other hand, PNS is originated from CNS (spinal) that PNS involves autonomic, sensory and motor neurons. Specifically, contractile activity of skeletal muscles is regulated by the motor neuron. To contract muscles, there are three important processes (18) (Figure 1-2). First and second process of PNS plays a bridging between the CNS and the muscle. Firstly, electrical signals are conducted the motor neuron as the action potential from CNS to the skeletal muscle (19) (Figure 1-2). Next, action potentials are passed the neuromuscular junction (NMJ) that is composed of presynaptic motor axon and postsynaptic skeletal muscle fiber (18, 20) (Figure 1-2). Action potentials are chemically transmitted to the skeletal muscle fiber. The chemical transmitter is termed the acetylcholine that is released from synapses (18). Finally, transmitted action potentials are propagated the skeletal muscle fiber by the excitation-contraction (E-C) coupling (18, 21) (Figure 1-2). Therefore, nervous systems and skeletal muscles are closely related in the process of the skeletal muscle contraction.

Figure 1-2

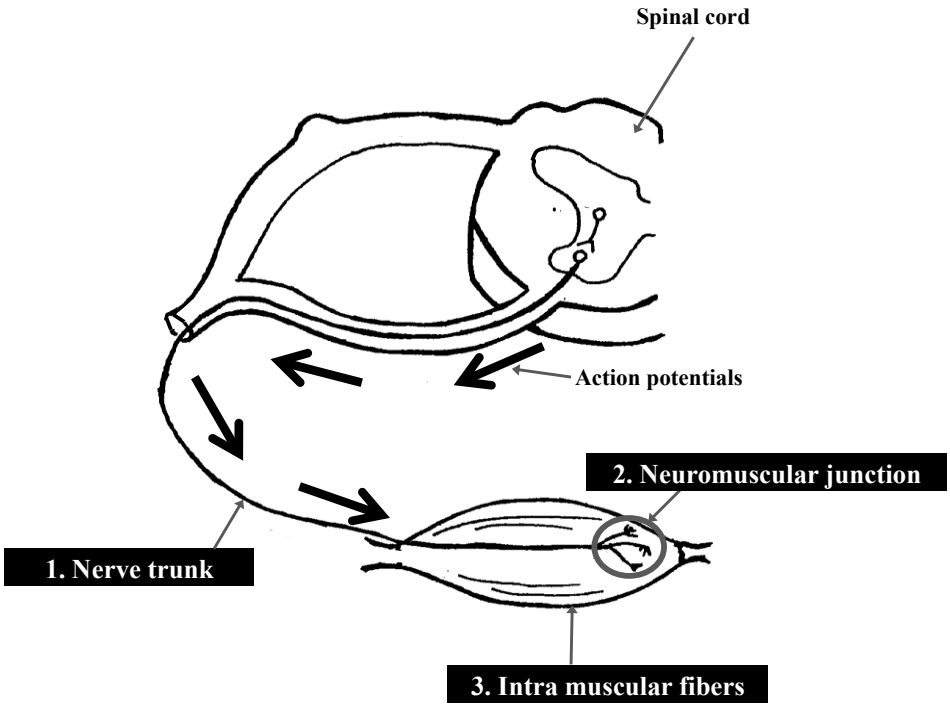


Figure 1-2. Mechanisms of muscle contractions

Action potentials from brain are firstly conduct the nerve trunk: 1. They are transmitted the

neuromuscular junction: 2, and propagate intra muscular fibers in the skeletal muscle: 3. Black arrows indicate the direction of action potentials.

1-4. Neuromuscular abnormality affect to nerve and muscle tissues

Nerve tissues are damaged by the several stresses such as acute and chronic injury or neurogenic diseases (22-25). It has been indicated that the nerve damage relates to functional and structural muscle disorders (25-28). Amyotrophic lateral sclerosis (ALS) is the typical muscle disorder due to motor neuron disease (25). In the experimental model of ALS mice, strength deficit and muscular atrophy was observed due to NMJ disarrangement, nerve degeneration and neurotransmitter inhibition in their hind limb muscle (25). Therefore, it has showed that despite the nerve is impaired, the skeletal muscle weakness and atrophy also induced.

Mechanical stresses (compression, elongation, contusion and/or rupture) in the nerve lead the functional and structural muscle impairments (28, 29). Colak et al. evaluated whether the physical movement during the exercise induces compressed nerve damage by overuse of upper limb muscles (28). In their study, athletes (ice hockey player) are subjected that they frequently use the upper limb muscles in the situation of racket swing. Their innervation nerves of upper limb muscles such as axillary, musculocutaneous and radial nerves are assessed by to measure propagating time of action potentials. In the result, each nerve conductivity are impaired (6-13%) relative to not exercised subjects. Hence, nerves are subject to damage from respective stresses.

1-5 Effect of strenuous eccentric contractions in innervation nerve of skeletal muscle

In a previous study, authors reported that the neuronal regeneration associated markers (p75 neurotrophin receptor, growth associated protein 43 and glial cell derived neurotrophic factor, among other things) are expressed in intramuscular nerves and spinal motor neurons after muscle such as contusion (30). Therefore, it is indicated a possibility that the nerve damage is induced from the muscle injury.

Unaccustomed ECs is a cause of the skeletal muscle injury as described above. There is the study whether ECs leads the nerve damage like functional and pathological impairments (16). In this study, sciatic nerve and gastrocnemius muscle of rat is subjected. The muscle is passively treated the FAST 20 ECs (5 contractions ×4 sets). Their sciatic nerves are functionally assessed by nerve conduction velocity (NCV) measurement after 3, 7 and 10 days. NCV is a assessment tool which values are decreased by neuronal disorders (28, 31, 32). NCV is significantly 21 % decreased on 7 days compared with not treated control group (Figure 1-3A). In addition, macrophage related protein (ED1) is markedly expressed that it is a marker for macrophages of the tissue damage. Also, loss of myelin protein zero (p0) is observed that p0 is the important component of the nerve (16).

Hence, these results indicate that unaccustomed ECs induce not only skeletal muscle injury,

but also its innervation nerve injury in both animal and human studies.

Figure 1-3

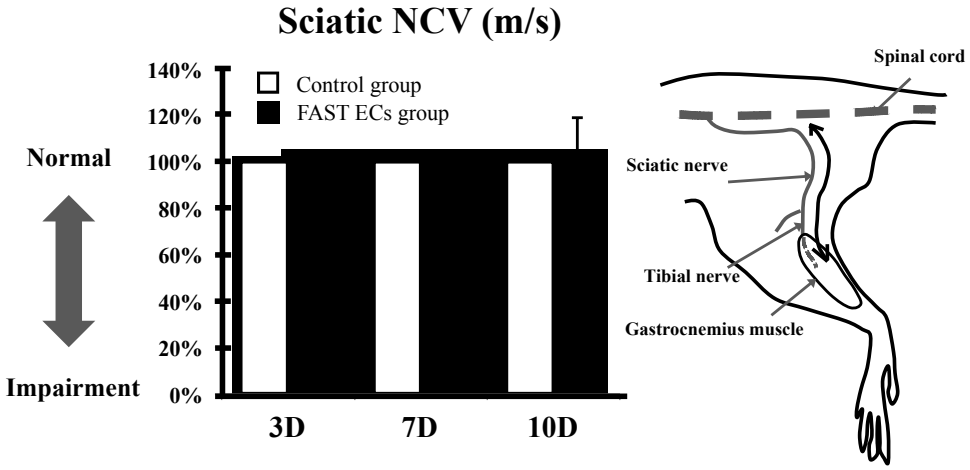


Figure 1-3. Results of nerve conductivity impairment from ECs induced innervation nerve injury (modified from Lee et al. 2014).

NCV was decreased after unaccustomed ECs in animal experiments. Black arrows indicate

the pathway of action potentials. ECs: eccentric contractions, NCV: nerve conduction velocity, 3D: 3 days, 7D: 7 days, 10D: 10 days.

1-6. Objectives

However, there are unclear points about physiological and/or pathological mechanisms of "ECs induced nerve injury". Based on these backgrounds, objectives of this study are composed of three theses as follows.

1. To measure musculocutaneous nerve conductivity after single bout of ECs in biceps brachii muscle.

Nerve impairment was induced by high angular velocity ECs in animal experimental model. However, it has not cleared about ECs induced nerve impairment in the human experiment. Therefore, the purpose of this chapter is to evaluate musleocutaneous nerve conductivity after unaccustomed ECs.

2. To evaluate whether repeated bouts of FAST ECs cause severe damage to the sciatic nerve in medial gastrocnemius muscle of rats.

In the previous report, FAST ECs had been applied only one time. On the other hand, the skeletal muscle injury become more severe by repetitive ECs (33). Hence, there is the possibility

which severe nerve injury also lead by repetitive ECs. To evaluate nerve damage, rats are exercised FAST ECs that their sciatic nerves are analyzed by NCV measurement and microscopic observation as functional and pathological assessment.

3. To investigate nerve latency and NCV of the sciatic nerve in athletes with a history of hamstring muscle strain injuries.

Hamstrings strain injuries (HSI) is a sports injury. It is thought that a main contributing factor is due to ECs. Based on previous and present experiments, a hypothesis is suggested following: The innervation nerve is impaired in athletes with a history of muscle strain injuries. To assess nerve impairment, athletes are measured their NCV using the pulsed magnetic stimulator.

Chapter 2. Increases in M-wave latency of fast eccentric contraction produce musculocutaneous nerve damage

2-1. Abstract

Purpose: ECs induce muscle damage that is indicated by prolonged loss of muscle function and delayed onset muscle soreness. It is possible that ECs affect motor nerve, which is attributed to the prolonged decreases in force generating ability. The present study investigated the hypothesis that musculocutaneous nerve M-wave latency would be increased after maximal elbow flexor ECs resulting in prolonged loss of muscle force.

Methods: Fifteen women performed 60 maximal ECs of the elbow flexors using their non-dominant arm. M-wave latency was assessed by the time taken from electrical stimulation applied the Erb's point to the onset of M-wave of the biceps brachii before, immediately after, and 1-4 days after the ECs. MVC torque, ROM and expression of DOMS using numerical rating scale (NRS) were also assessed at the same time points.

Results: Prolonged decreases in MVC torque (1 day: 54%, 4 days: 15%) and ROM (1 day: 32%, 2 days: 22%), and increased NRS (peak: 4.2 out of 10) were evident after exercise ($p<0.05$). The

M-wave latency increased ($p<0.01$) from 5.8 ± 1.0 ms before exercise to 6.5 ± 1.7 ms at 1 day and 7.2 ± 1.5 ms at 2 days after exercise for the exercised arm only. No significant changes in M-wave amplitude were evident after exercise.

Conclusion: The increased latency suggests a deficit in motor nerve function, and could be associated with efferent nerve damage induced by eccentric contractions.

2-2. Introduction

ECs induces muscle damage indicated by a prolonged loss of muscle strength and ROM, development of DOMS, and increases in muscle proteins in the blood such as creatine kinase activity (34, 35). Histological alterations are also found after ECs in contractile apparatus at z-line, A-band, transverse (t)-tubule, triads and terminal cisternae of sarcoplasmic reticulum (SR), and extracellular matrix (14, 36). It has been shown that E-C coupling failure is associated with the decreases in muscle strength after ECs (37).

Muscle fiber conduction velocity (MFCV) is indicative of action potential conduction along a muscle, which has been shown to be decreased after ECs (38). Previous study showed 27% decrease in muscle fiber conduction velocity of biceps brachii during maximal voluntary isometric contractions at 2 hours after 50 maximal ECs of the elbow flexors. The same authors reported that MFCV during maximal voluntary isometric contractions was decreased 12% at 2 hours after 60 (20 × 3 sets) maximal ECs of the elbow flexors (39). They stated that the decrease in MFCV was associated with loss of sarcolemmal excitability, and might indicate an impairment of motor nerve function.

To assess motor nerve function, NCV, M-wave latency and M-wave amplitude are often used (28). NCV is measured by the time between the electrical stimulation and the onset of M-wave (40), and has been used to examine nerve disorders such as neuropathy and neural muscular atrophy in previous studies (28, 41). Kaplan compared the latency of median and ulnar nerves between control and neuropathy groups, and showed that the motor terminal latency was longer (ulnar; 33%, medial; 41%) for the neuropathy than control group. It was reported that the latency of the median nerve was 38% longer for neuronal disease patients such as Crow-Fukase syndrome (42), and the femoral nerve latency prolonged by 15% for diabetes patients when compared with non-diseased population (43). These indicate that an increase in the latency is related to an impairment of motor nerve function.

Lee et al. recently reported that NCV decreased 21% at 7D after 20 fast velocity (180°/s) eccentric contractions of the plantar flexors in rats, and p0 that is an indicator of myelin sheath damage, increased in the sciatic nerve after the eccentric contractions (16). This suggests that ECs could damage nerve tissue; however, no previous studies have investigated the effect of eccentric exercise on efferent nerve in humans.

The biceps brachii is innervated by the musculocutaneous nerve (MsN), and MsN latency was measured as the conduction time from the onset of the stimulus to the onset of the muscle action

potential (44). The present study investigated MsN latency of biceps brachii in responses to eccentric exercise of the elbow flexors. It was hypothesised that MsN latency would be increased after eccentric exercise of the elbow flexors.

2-3. Methods

Participants

Fifteen young women (age: 24.6 ± 3.5 y, height: 160.3 ± 5.3 cm, body mass: 55.3 ± 5.5 kg) were recruited for this study. Their average ± standard deviation (SD) upper arm length measured by the distance between the acromion and lateral epicondyle was 28.4 ± 1.3 cm. They had not been participating in any regular resistance training prior to this study. The participants were requested to avoid any interventions such as massage and stretching during the experimental period. They were given detailed explanation of the study protocol before participation, and signed an informed consent form. The study was approved by the Ethics Committee of the Nippon Sports Science University (012-H01). The sample size was estimated by a power analysis (G power, Heinrich-Heine University of Dusseldorf) by setting the effect size as 0.25, α level of 0.05 and power of 0.8 for the possible latency changes after eccentric exercise, and it was shown that 15 participants were required.

Experimental protocols

All participants performed maximal eccentric exercise of the elbow flexors using their non-dominant arms, and the dominant arms were assigned to control (CNT) in which the latency

measurements were taken without eccentric exercise. The dependent variables included MVC torque, ROM of the elbow joint, muscle soreness of the elbow flexors assessed by a NRS, M-wave latency after electrical stimulation and maximal M-wave amplitude of biceps brachii. These measures except muscle soreness were taken immediately before, immediately after, and 1 to 4 days (1D - 4D) after eccentric exercise. Muscle soreness assessment was not included immediately after exercise, but was taken at all other time points. These measurements were performed in a room maintained at 26-28℃.

ECs protocol

Each participant was seated on the chair of an isokinetic dynamometer (Biodex Multi-Joint System 3, New York, USA), and the non-dominant arm was set at a shoulder joint angle of 45° flexion and the elbow joint was aligned with the rotation axis of the isokinetic dynamometer, while the lever arm of the isokinetic dynamometer was secured to the subject's wrist in a supinated position. The exercise consisted of 10 sets of 6 maximal voluntary eccentric contractions of the elbow flexors at a constant velocity of 90°/s for the ROM from 90° flexion to 0° (full extension) (12). The participants were verbally encouraged to maximally resist throughout the ROM for 1s, and after each contraction, the isokinetic dynamometer returned the arm to the 90° flexed position at a

constant velocity of 30°/s, creating a 3-s passive recovery between contractions. The rest period between sets was 50 s. Torque produced during eccentric contractions was saved in a computer connected to the isokinetic dynamometer, and peak torque and work were obtained later.

Maximal voluntary isometric contraction (MVC) torque

MVC torque was measured on the same apparatus and positioning as those described for the eccentric exercise. Subjects performed two 3-s MVC at 90° elbow joint angle with a 15-s rest between contractions (12). Higher peak torque of the two contractions was used for further analysis.

Elbow range of motion (ROM)

To examine the ROM of elbow joint, two elbow joint angles (extended and flexed joint angles) were measured using a plastic goniometer by the same investigator once for each. The extend joint angle was recorded when each participant attempted to fully extended the joint, with the elbow held at the side and the hand in supination, and the flexed joint angle was determined when the participant attempted to fully flex the joint with the hand in supinated position of the elbow joint

was fully extended (12). The ROM was determined as the difference between the two angles (extended angle – flexed angle).

Muscle soreness

Muscle soreness was assessed using a numerical rating scale (NRS), where 0 indicates "no pain", and 10 is "worst pain imaginable" (45). Each participant was asked to indicate the pain intensity on the scale when the investigator palpated the mid-belly of biceps brachii (9 cm from cubital fossa) using a thumb while each participant relaxed the arm at the side while standing (natural position). All muscle soreness assessments were made by the same examiner to ensure that the same pressure was applied on biceps brachii. After applying pressure three consecutive times, the examiner asked each participant to rate the soreness level.

M-wave latency and amplitude

Since M-wave latency has been reported to be affected by body temperature (40), the skin temperature of the upper extremity was checked using a thermal imager (TVS-200 NEC, Tokyo, Japan), and the room temperature was kept at 26-28°C throughout the measurements (28). The

musculocutaneous nerve was stimulated (pulse duration 10 ms) by a monopolar surface electrode connected to an electric stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan) combined with an isolator (ML408, AD-instruments, Japan), and the maximal stimulus current was 14-18 mA according to the instruction in the Manual of Nerve Conduction Studies. M-wave of biceps brachii muscle contraction was induced by the electrical stimulation at the Erb's point (supraclavicular fossa). M-wave latency was recorded with monopolar surface electrode placed at the mid-belly of the biceps brachii long head. Data acquisition and analysis were performed by a Power Lab Chart 7 (AD instruments, Australia). The placement of the electrode and a reference electrode were marked by a permanent marker to ensure that their locations did not change over time. M-wave latency was calculated from the start of the electrical stimulation to the starting time of the fast negative peak of M-wave (Figure 2-1). Starting point of M-wave was defined as above the mean ± 2 SD of the base line value (46). The maximal amplitude of M-wave was determined as the negative peak to the positive peak amplitude. Based on the value obtained from the control arm of all participants (n=15) across measures taken at six time points from before to four days after exercise, the coefficient of variation (CV) for the latency measurement was found to be 11%, and intra-class correlation coefficient was 0.89.

Figure 2-1

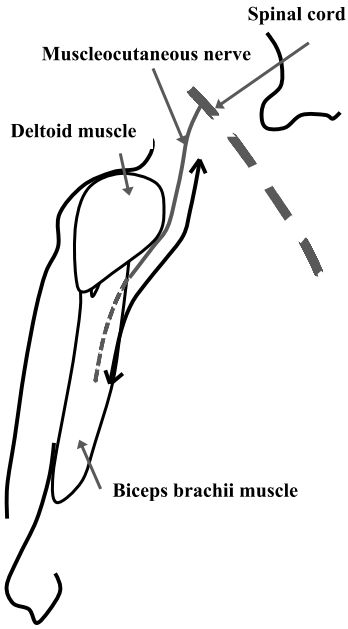


Figure 2-1. Measurement of muscleocutaneous nerve M-wave latency

Nerve stimulation was applied the space between sternocleidomastoid muscle and scalene.

Black arrows indicate the pathway of action potentials.

Statistical analyses

Changes in MVC, ROM and muscle soreness (NRS) over time were analysed by a one-way analysis of variance (ANOVA). Changes in M-wave latency and M-wave amplitude over time were compered between exercised and non-exercised (control) arms by a two-way repeated measures ANOVA. When a significant time effect or interaction effect was found, a Bonferroni's multiple comparison was followed as a post-hoc test. A significance level was set at $p < 0.05$. All values are expressed as means ± SD.

2-4. Results

MVC Torque

MVC torque decreased ($p<0.05$) by 54 ± 14% of the pre-exercise level at immediately after exercise, and remained lower than the baseline at 1D (32 ± 25%), 2D (26 ± 24%), 3D (18 ± 22%) and 4D days (15 ± 31%) after exercise (Figure 2-2A).

ROM

ROM decreased from the pre-exercise value at immediately (32 ± 27%) to 2D (22 ± 15%) after exercise, but returned to the baseline at 3D post-exercise (Figure 2-2B).

Muscle soreness

Muscle soreness developed at 1-3D after exercise, and peaked (4.2 ± 1.6 out of 10) at 2D post-exercise (Figure 2-2C).

M-wave Latency and amplitude

Pre-exercise M-wave latency was the same between arms (5.8 ± 1.0 ms), but a significant

interaction effect was found after exercise (Figure. 2-3A). Only for the exercised arm, M-wave latency increased at 1D (6.5 ± 1.7 ms) and 2D (7.2 ± 1.5 ms) after exercise ($p<0.05$). All participants showed a maximum increase in the latency at 1D or 2D after exercise from the baseline value by 43-88%. No significant changes in M-wave amplitude (baseline: 9.6 ± 4.7 mV) were observed over time for either arm (Figure. 2-3B).

Figure 2-2

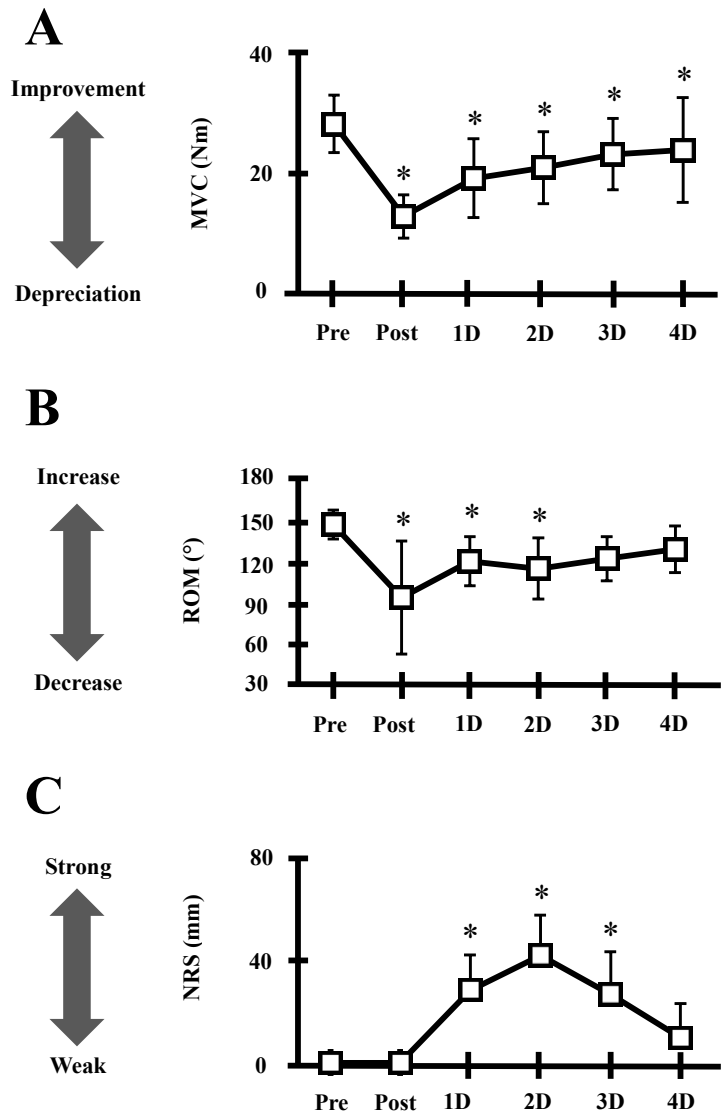

A
Improvement
Depreciation
MVC (Nm)
40
20
0
Pre
Post
1D
2D
3D
4D
B
Increase
Decrease
ROM (°)
180
150
120
90
60
30
C
Strong
Weak
NRS (mm)
80
40
0

Figure 2-2. Muscles functions after unaccustomed ECs

Decreased MVC (A) and ROM (B) were indicated immediately after ECs. Significant increase of muscle soreness (C) was shown on 2D. Pre: Before exercise, Post: After exercise, 1D: 1 day, 2D: 2 days, 3D: 3 days, 4D: 4 days.

Figure 2-3

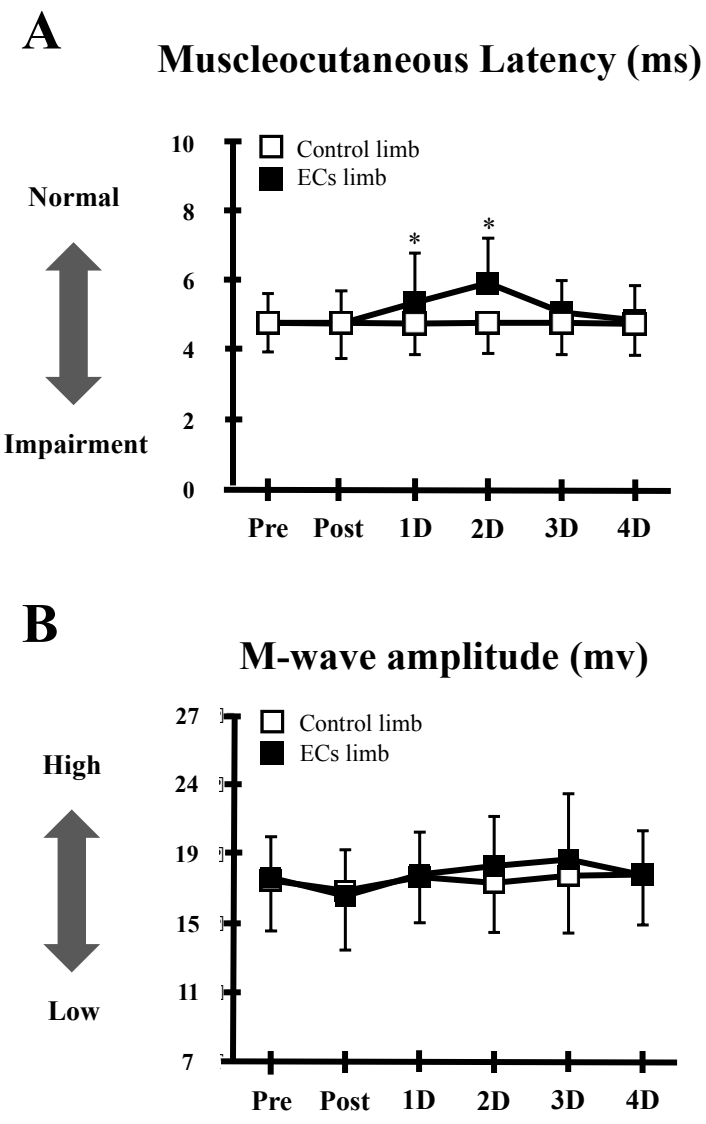

A
Muscleocutaneous Latency (ms)
Normal
Impairment
10
8
6
4
2
0
Control limb
ECs limb
*
*
Pre
Post
1D
2D
3D
4D
B
M-wave amplitude (mv)
High
Low
27
24
19
15
11
7
Control limb
ECs limb
Pre
Post
1D
2D
3D
4D

Figure 2-3. Results of latency and amplitude after unaccustomed ECs.

NCV was significantly increased on 1D and 2D after unaccustomed ECs: A. * $p<0.05$ vs pre M-wave amplitude was not changed between CNT limb and ECs limb: B. Pre: Before exercise, Post: Immediately after exercise, 1D: 1 day, 2D 2 days, 3D: 3 days, 4D: 4 days.

2-5. Discussions

The present study tested the hypothesis that M-wave latency of biceps brachii in response to electrical stimulation would be prolonged after eccentric exercise of the elbow flexors resulting in muscle damage. The changes in MVC torque, ROM and muscle soreness (Figure 2-2) indicate that muscle damage was induced by the exercise. M-wave latency significantly increased at 1D and 2D after exercise without changes in M-wave amplitude when compare with the pre-exercise value (Figure 2-3), which supported the hypothesis.

In the present study, female participants were recruited, because I wanted to have participants who had not performed resistance training, and it was easier for us to find such participants in females. Although the menstrual cycle of the participants was checked, I did not measure serum hormones to determine the exact cycle. The response to eccentric exercise might differ at the phases (e.g. follicular, ovulating and luteal), but the effect of menstrual cycle on eccentric exercise-induced muscle damage is still controversial (47-49). It is possible that NCV is affected by body temperature; however, the effect of menstrual cycle on NCV has not been examined previously. It is known that body temperature fluctuates in menstrual cycle about 0.5°C (Mouzon et al. 1984) which may affect NCV. In my experiment, the skin temperature of the participants were monitored using a thermal

imager, and the skin temperature was consistent around 36.0°C during the NCV measurement among subjects, and no significant difference was found between days for the same participants. Furthermore, no significant changes in NCV were observed for the non-exercise arm (Figure 2-3A). Thus, it seems unlikely that the menstrual cycle affected the changes in NCV found for the exercised arm.

The magnitude of the changes in MVC torque and ROM after eccentric exercise was similar to those reported in the previous study in which a similar eccentric exercise of the elbow flexors was performed by untrained participants (12). Regarding muscle soreness, the present study used NRS, instead of a visual analog scale that most of previous studies used, but the time course of the changes in muscle soreness and its magnitude appear comparable to those of the previous studies (12, 50, 51). Collectively, it seems reasonable to assume that moderate muscle damage was induced by the eccentric exercise.

The most important finding of the present study was that M-wave latency increased at 1D (12%) and 2D (24%) after exercise without changes in the M-wave amplitude (Figure 2-3). This was the first study to show the effect of eccentric exercise-induced muscle damage on the M-wave latency. However, Colak et al. reported a delay of M-wave latency in ice hockey players such that

M-wave latency of the dominant arm was 4.39 ms, which was significantly longer than that of sedentary control individuals (4.12 ms). They discussed that the MsN was compressed by surrounding muscles such as coracobrachialis and biceps brachii, which increased the latency. The baseline M-wave latency in the present study was 5.6 ± 1.5 ms, which was more than 1 ms longer than that of the previous study (28). Buschbacher et al. reported that average M-wave latency of healthy male and female adults was 5.1 ± 0.4 ms, which was comparable to that of the present study at baseline (52). M-wave latency and NCV are influenced by the length of limb and age, as well as environmental temperature and the electrode position (40, 44). Thus, the difference in the M-wave latency among studies may be explained by some differences in these factors. It is important to note that the M-wave latency measurements were performed in the same temperature, electrode sites and position between days in the present study, and no significant changes in the M-wave latency were found for the non-exercise arm (Figure 2-3A). Thus, it seems reasonable to assume that the significant increases in the M-wave latency were due to the eccentric exercise that induced some symptoms of muscle damage.

Tsur and Ring evaluated brachial plexus nerve latency in stroke patients whose shoulder muscles were paralysed, and showed that the axillary nerve M-wave latency of the paralysed

shoulder was 38% longer than that of the normal side, and the M-wave amplitude was decreased by 62% for the paralysed side than normal side. In contrast, the M-wave amplitude did not significantly change after eccentric exercise in the present study (Figure 2-3B). Nerve injuries are classified into three categories; neurapraxia, axonotmesis and neurotmesis (53, 54). It was stated that if nerve damage occurred only in myelin sheath (neurapraxia), M-wave latency increased without apparent change in M-wave amplitude, but if axonal damage occurred, amplitude of nerve impulse was decreased (53). Thus, it is possible that the eccentric exercise in the present study induced myelin sheath damage but not axonal damage. Lee et al. reported that p0, an indicator of myelin sheath damage, increased in the sciatic nerve after 20 fast velocity (180°/s) ECs of the plantar flexors in rats. Thus, it is possible that ECs induced myelin sheath damage in the present study.

It should be noted that the M-wave latency returned to the baseline at 3D after exercise (Figure 2-3A) when MVC was still decreased from the baseline (Figure 2-2A). The magnitude of the increase in the latency (1D post-exercise: 12%, 2D post-exercise: 24%) was smaller than the magnitude of decrease in MVC (32%, 26%, respectively). These suggest that the nerve repair process may be faster than skeletal muscle regeneration. It is known that nerve conduction is changed by extension and compression of the nerve, which is referred to as transient nerve

conduction block (29). Previous studies have shown that ECs induce structural changes in various tissues (vessel, extra-cellar matrix, nerve) surrounding muscle fibres (9). Since nerve axons are surrounded by connective tissue, it is possible that a temporary nerve conduction block is induced by ECs.

2-6. Conclusions

In conclusion, the present study found that latency of biceps brachii muscle increased 1 - 2D after eccentric exercise of the elbow flexors by 12-24%. It seems likely that motor nerve damage was associated with the increased M-wave latency, but it is not clear from the present study what kind of nerve damage was induced by eccentric contractions. Further studies are necessary to investigate the effect of eccentric contractions on efferent as well as afferent nerves including histological and physiological changes.

Chapter 3. Repeated bouts of fast eccentric contraction produce sciatic nerve damage in rats

3-1. Abstract

Introduction: I evaluated sciatic nerve impairment after ECs in rat medial gastrocnemius muscle (MG).

Methods: Wistar rats were randomly assigned to different joint angular velocity: 180°/s (FAST), 30°/s (SLOW), or CNT. FAST and SLOW groups were subjected to multiple (1-4) bouts of 20 (5 reps, 4 sets) ECs. NCV and isometric tetanic ankle torque was measured 24 h after each ECs bout. I also assessed nerve morphology.

Results: After 4 ECs bouts, NCVs and isometric torque in the FAST group were significantly lower than those in the CNT (NCV: 42%, torque: 66%; $p<0.05$). After 4 bouts, average nerve diameter was significantly smaller in the FAST group (CNT: 2.39 ± 0.20 μm vs. 2.69 ± 0.20 μm and SLOW: 2.93 ± 0.24 μm; $p<0.05$) than that in other two groups

Conclusion: Chronic ECs with high angular velocity induce serious nerve damage.

3-2. Introduction

ECs involve the forcible lengthening of activated muscles. In particular, unaccustomed ECs induce strength loss, muscle soreness, sarcolemma disruption, and/or activation of muscle degradation signals (11, 12, 14, 15). Histological changes have also been demonstrated in myofibrils, the extracellular matrix, and triads of the cytoplasmic membrane system (55, 56).

Recently, Lee et al. examined the effects of a single bout of ECs on the sciatic nerve in a rat model of ECs induced muscle damage (16). They used ECs with 2 different joint angular velocities (180°/s: fast and 30°/s: slow). Twenty (5 ×4 sets) acute ECs were applied to the MG of the rats. The isometric tetanic ankle torque significantly decreased on 1D to 7D after ECs in the FAST group. They also observed a significant decline (21%) in NCV, indicating a nerve disorder in the fast group only. In addition, loss of myelin structural protein, p0, and an increase in a macrophage-related protein: ED1 were detected by western blotting analysis. In the slow group, ECs did not induce any apparent damage in the sciatic nerve. They concluded that acute ECs with fast angular velocity induce transient damage not only in the muscle but also in the innervating nerve (16).

In general, overuse injuries are thought to result from the accumulation of microtraumas (28). For example, Colak et al. reported that nerve conductive function is impaired due to nerve

compression by a repetitive racket-swinging motion. According to their data, the brachial plexus nerves become entrapped and damaged in active high-level ice hockey players (28). Apparently, the brachial plexus nerves (axillary, musculocutaneous, and radial) become strangulated by surrounding muscles. Lee et al. reported that parameters indicating nerve damage (i.e. NCV, loss of p0 and increased ED 1) were observed on 7D post-ECs, and that all parameters had returned to normal on 10D. Although these results indicate that a single bout of ECs induces temporary sciatic nerve damage, the acute response is not always reflected by chronic adaptation (16). In this study, I tested whether repeated bouts of ECs cause severe nerve damage, as observed in overuse injuries. Because nerve injuries are frequently induced by repetitive stress (29, 57), I hypothesized that repeated bouts of ECs seriously impair nerve structure and function.

The aim of the present study was to evaluate whether repeated bouts of MG maximal ECs cause severe damage (e.g., axonal damage) to the sciatic nerve. To evaluate nerve damage, I measured the NCV of the sciatic nerve for functional assessment. Morphological examination of nerve fiber size and myelin thickness was also performed with an electron microscope.

3-3. Methods

Animal care

Fifty-four male Wistar rats (9 weeks old) were purchased from CLEA Japan (Tokyo, Japan). All rats were reared separately in ventilated cages and supplied with water and food. The room temperature was maintained at 22°C to 24°C, with a 12-h light/dark cycle. Rats were anesthetized with isoflurane for torque measurement and ECs training. All experimental procedures were approved by the ethical committee of Nippon Sport Science University (permit number 012-A03).

Experimental design

Wistar rats were divided into 3 groups: fast (180°/s) joint angular velocity ECs group (FAST, n = 24), slow (30°/s) joint angular velocity ECs group (SLOW, n = 24), and non-treated control (CNT, n = 6). To assess changes in NCV over time, 24 rats in the FAST and SLOW groups were divided into 4 multiple-EC-bout groups (1, 2, 3, and 4 bouts; 20 contractions per bout, n = 6 for each group). Animals in the 4-bout groups were tested with isometric tetanic ankle torque. Two additional animals were assigned to 4 bouts of ECs and used for morphological observation of the sciatic nerve. The experimental time schedule is described in Figure 3-1.

Figure 3-1

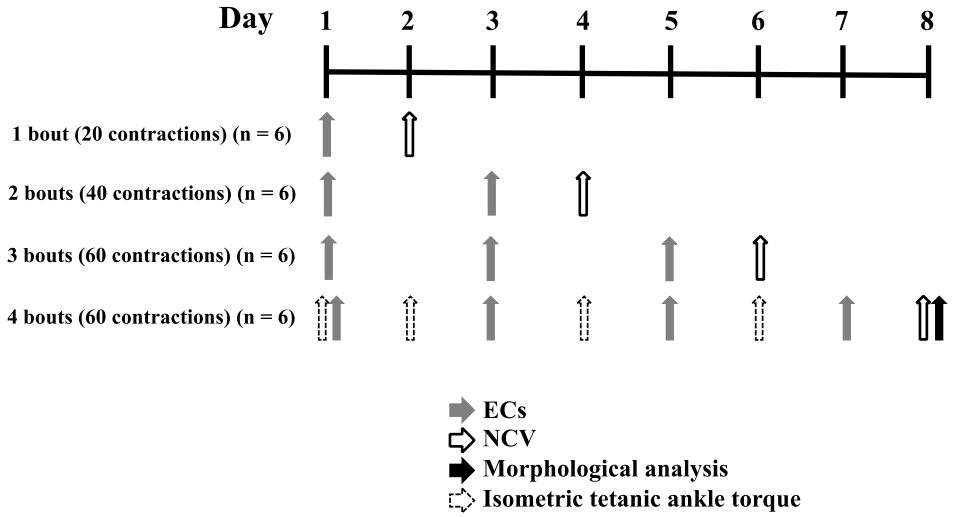


Figure 3-1. Experimental time schedule

ECs were divided into 1 to 4 bouts and NCV was measured 24 h after ECs. Isometric tetanic torque and nerve morphology were assessed in the 4-bout group. ECs: Eccentric contractions, NCV:

Nerve conduction velocity

ECs protocol

The method used to administer ECs was similar to that described previously (11, 16). Briefly, a maximal tetanic contraction was electrically induced in MG of anesthetized rats, as described in "isometric tetanic ankle torque assessment". During contraction, the right ankle joint was simultaneously dorsiflexed. The range of dorsiflexion was from 90° to 135° (range of motion: 45°) in both the FAST and SLOW groups. One bout of ECs consisted of 20 contractions (5 contractions, 4 sets) and the interval between each set was 5 min, as reported previously (58). Each ECs bout (20 contractions) was applied every 2D for repeated bouts groups. The total number of contractions was 40 for 2 bouts (1D and 3D), 60 for 3 bouts (1D, 3D and 5D), and 80 for 4 bouts (1D, 3D, 5D and 7D) (Figure 3-1).

Isometric tetanic ankle torque assessment

Maximal isometric tetanic ankle torque was measured in FAST and SLOW 4-bout groups the day after ECs were administered (Figure 3-1). The measurements were performed at 4 time points consisting of pre-ECs, 2D, 4D, and days 6 (6D). In the present study, I sequentially measured isometric tetanic torque in only those receiving 4 bouts. The detailed protocol for isometric torque

measurement shown below is essentially the same as that used in a previous study (16).

Isoflurane was used as an inhalant analgesic (aspiration rate: 450 mL/min, concentration: ~2.2%). The right ankle joint was positioned at 90°, and the extended right knee was fixed. A full activation of the MG was electrically induced (pulse duration: 0.4 ms, frequency: 100 Hz, intensity: ~35 V). Surface skin electrodes (7.5 mm × 7.5 mm; Vitrode V, Nihon Kohden, Japan) were connected to an electric stimulator isolator (Nihon Kohden Japan). To avoid redundant muscle responses (e.g., hypertrophy and injury), the maximum torque was averaged over 3 contractions.

Sciatic NCV examination

NCV assessment was conducted as recently described (16). NCV was analyzed using distance and time differences in proximal latency (PL) and distal latency (DL) in a manner similar to that described previously (16). PL and DL were detected as the M-wave response of the MG and generated with electrical stimulation at the proximal and distal nerve points. PL was designated as the time from the branching point of the sciatic nerve to the MG, whereas DL was defined as the time from the branching point of the tibial nerve to the MG. The sciatic nerve was electrically stimulated using a hook-type stainless-steel electrode (EKM 2-5050; Bioresearch Center, Tokyo,

Japan) connected to an electric stimulator and isolator (SEN-3301, SS-104 J; Nihon Kohden, Tokyo, Japan). The needle electrode was used for recording of induced M-wave (SS-104 J, Nihon Kohden). Data acquisition and analyses were performed using Power Lab Chart 7 (AD Instruments, Australia).

Nerve fiber observation with electron microscopy

Analysis of nerve fiber diameter was conducted after 4 bouts of ECs in the FAST, SLOW, and CNT groups. The sciatic nerve was observed using a scanning electron microscope (Quanta 3D FEG; FEI, Netherlands), as previously described (59). Rats were perfused through the left ventricle with saline and a fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M phosphate buffer [pH 7.4]). After chemical fixation, the sciatic nerve was dissected 2 mm from the origin of the spinal cord. After immersion fixation, nerve samples were placed in a solution containing 2% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M phosphate buffer at 4°C. Then, nerve samples were washed 3 times with distilled water and immersed in 1% thiocarbohydrazide solution for 1 h, soaked in 2% osmium tetroxide in distilled water, washed 3 times with distilled water, stained with a solution of 4% uranyl acetate dissolved in a 25% methanol solution overnight for contrast enhancement, and then washed with distilled water. The specimens were dehydrated using a graded

ethanol series (25%, 50%, 70%, 80%, 90%, and 2 rounds of 100%) for 10 min. Specimens were embedded in epoxy resin (Epon 812; TAAB, England) and polymerized for 72 h at 60°C. Treated nerve samples were collected from a completely flat portion of the specimen for use in scanning electron microscopy analyses as material contrast images. The blocks were cut into 1.5 mm × 1.5 mm squares and set on the sample holder for imaging. Images were captured using the ImageJ software package (NIH, Maryland, USA). Eight representative fields of view (x2500 and x5000) were randomly chosen within whole specimens. The number of nerves examined was: 418 in the CNT group, 379 in the SLOW group and 479 in the FAST group. Nerve fiber diameter for each nerve fiber was calculated as the mean value of the largest and smallest diameters (60). Myelin thickness for each nerve fiber was obtained by subtracting the axon diameter from the fiber diameter (61). Mean values for fiber diameter and myelin thickness in each group were averaged from all examined nerve fibers and myelin sheath.

Statistical analyses

All values are expressed as mean ± SD. One-way ANOVA was used to compare body mass, muscle wet weight, and results of morphological analysis of nerve fiber diameter (CNT vs. SLOW

vs. FAST). Two-way ANOVA was used to evaluate changes in isometric tetanic ankle torque. Torque was measured over time in the SLOW and FAST groups. When a significant time effect or interaction effect was found, Bonferroni's multiple comparison was used as a post-hoc test. NCV values were assessed by Dunnet analysis to make comparisons between each assigned contraction group and the CNT group (62, 63). The significance level was set at $\alpha = 0.05$. All values are expressed as means ± SD. Statistical analyses were conducted with SPSS version 22 (IBM Japan, Tokyo, Japan).

3-4. Results

Body weight and muscle wet weight

In the FAST, SLOW, and CNT groups, no significant differences were observed in body weight or in the weight of the soleus or plantaris muscles after 4 bouts of ECs. The weight of the MG was significantly lower in the FAST group than in the CNT group (Table 3-1). However, no significant differences were observed between the CNT and SLOW groups (Table 3-1).

Table 3-1. Changes in body weight and muscle mass after 4 bouts of ECs

	CNT (n = 6)	SLOW (n = 6)	FAST (n = 6)
Body weight (g)	294.6 ± 5.0	289.3 ± 8.3	291.1 ± 7.3
Soleus (mg)	112.4 ± 12.5	115.9 ± 12.4	115.3 ± 7.2
Plantaris (mg)	295.5 ± 13.5	299.6 ± 12.7	285.8 ± 31.1
Gastrocnemius (mg)	1,387.0 ± 62.4	1,430.3 ± 76.3	1,306.7 ± 85.1*

All values are expressed as mean ± SD. *$p<0.05$ vs CNT. CNT: Non-treated control group, SLOW: Slow angular velocity.

Isometric tetanic ankle torque

Isometric tetanic torque values (Table 3-2) and relative changes (Figure 3-2) are shown. Using raw values, significant torque deficits were observed on 2D and 4D in both groups, but further torque deficit at 6D was observed in only the FAST group. Using relative values, a significant decrease at 2D was observed both in the SLOW and FAST groups, but further significant decline was observed only in the FAST group.

Changes in sciatic NCV

Sciatic NCV was significantly lower in the FAST group after repeated bouts of ECs. However, no significant deficits were observed in the SLOW group (Figure 3-3).

Fiber diameter and myelin sheath thickness

Assessments of morphological changes were done in the FAST and SLOW 4-bout groups and the CNT group. Myelin sheath thickness of the sciatic nerve decreased only in the FAST group. Sciatic nerve fiber diameter significantly decreased only in the FAST group in comparison with the CNT group. No significant decreases were observed in the SLOW group (Figure 3-4A, 4B).

Table 3-2. Changes in isometric tetanic torque during the experimental period in the 4-bout groups (Isometric tetanic torque [mN·m])

		Pre	2D	4D	6D
Isometric tetraninc torque (mN·m)	**SLOW**	144.8±12.6	124.7±8.4†	134.9±13.0†	144±5.1
	FAST	150.5±8.4	127.7±15.4*	121.4±10.2*	99.5±10.0*

All values are expressed as mean ± SD. †$p<0.05$ vs pre in the SLOW group, * $p<0.05$ vs pre in the FAST group. Pre: Before exercise, 2D: 2 days, 4D: 4 days, 6D: 6 days, SLOW: Slow angular velocity, FAST: Fast angular velocity.

Figure 3-2

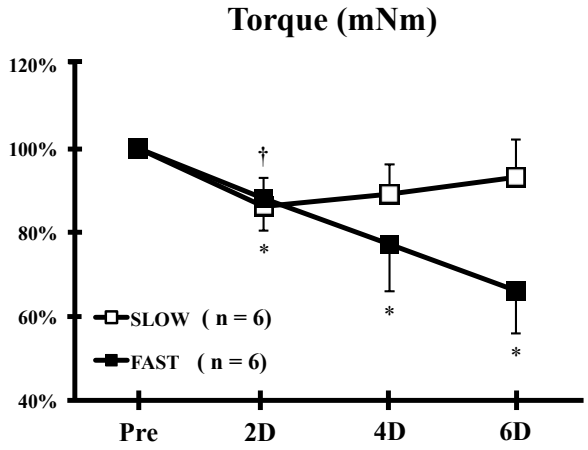


Figure 3-2. Isometric tetanic torque in the FAST and SLOW groups after 4 bouts of ECs

Deficit was observed in both groups on day 2 post-ECs. Torque continued to decline in the FAST group until 6D. (2D: 84%, 4D: 80%, 6D: 66% ; † $p<0.05$ vs pre in the SLOW group, * $p<0.05$ vs pre in the FAST group). SLOW: Slow angular velocity, FAST: Fast angular velocity, Pre: Before exercise, Post: After exercise, 1D: 1 day, 2D: 2 days, 4D: 4 days, 6D: 6 days.

Figure 3-3

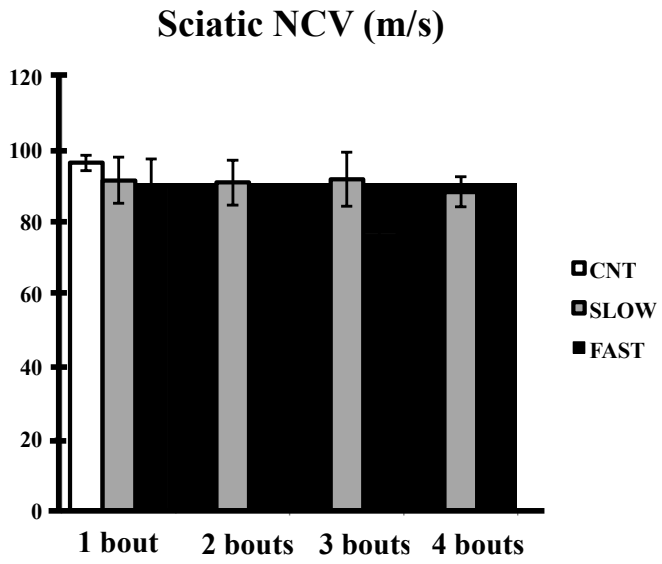


Figure 3-3. Changes in sciatic NCV

Sciatic NCV was significantly lower in the FAST group after repeated bouts of ECs (2 bouts: 78%, 3 bouts: 78%, 4 bouts: 42%; *$p<0.05$ vs CNT). No significant deficits were observed in the

SLOW group. NCV: Nerve conduction velocity, CNT: Control, SLOW: Slow angular velocity, FAST: Fast angular velocity.

Figure 3-4

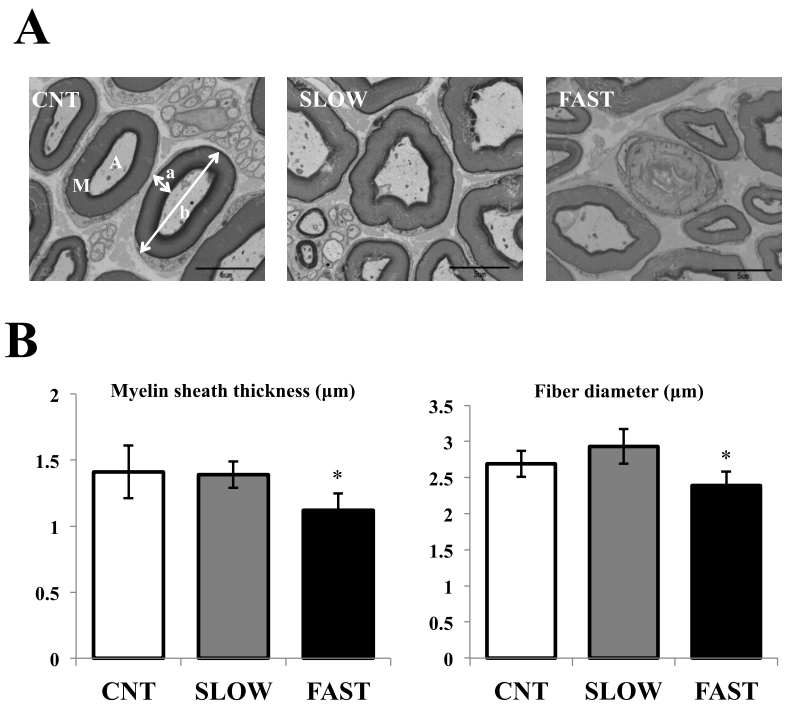


Figure 3-4. Morphological observation of sciatic nerve after 4 bouts of ECs

4A. Images show a transverse section (scale: 5 μm) obtained from each group. M: Myelin sheath, A: Axon, A: Myelin sheath thickness, B: Fiber diameter.

4B. Myelin thickness and fiber diameter decreased in the FAST group (*p<0.05). Myelin (CNT: 1.41 ± 0.20 μm, n=418, SLOW: 1.39 ± 0.10 μm, n=379, FAST: 1.12 ± 0.13 μm, n=479, left), Diameter (CNT: 2.69 ± 0.20 μm, n=418, SLOW: 2.93 ± 0.24 μm, n=379, FAST: 2.39 ± 0.20 μm, n=479, right). CNT: Control, SLOW: Slow angular velocity, FAST: Fast angular velocity.

3-5. Discussions

I investigated whether repeated bouts of ECs cause damage to the sciatic nerve in a rat model. I previously reported that ECs with fast angular velocity induce transient nerve impairment, and a significant NCV reduction caused by repeated ECs bouts was observed in the FAST ECs bouts groups (16). Additionally, a more than 50% reduction in torque deficit and NCV was observed in the FAST 4-bout ECs group. I further found that morphological nerve fiber changes (e.g., nerve diameter atrophy and myelin sheath thinning) were observed in the FAST 4-bout ECs group. On the other hand, signs of sciatic nerve injury were not observed in the SLOW group.

Muscle force is frequently used as an indicator of muscle damage in both humans and animals (10, 16, 39). A significant deficit in torque was observed in both ECs groups after repeated ECs. Acute ECs temporarily induce a force reduction, independent of angular velocity, and the torque gradually recovers over time (16). In this study, I observed that repeated bouts of ECs induced a continuous torque decline throughout the experimental period in the FAST group. These results suggest that consecutive bouts of ECs with a high angular velocity cause severe functional impairment in muscle. In the SLOW group, significant torque deficit was observed on 2D and 4D (Table 3-2). Using relative values, a significant decline was observed only at 2D (Figure 3-2). It is

well known regarding muscular adaptation in the repeated bout effect (RBE) that muscle functions (e.g. muscle strength and soreness) are less impaired after a second bout of ECs than after the initial ECs (12, 64). Diminishment of a significant torque deficit at 6D in SLOW group suggests that RBE occurs after slow angular velocity ECs. Nerve damage was induced by repeat bouts of ECs with high angular velocity. A significant NCV reduction was observed only in the FAST group (2 bouts: 78%, 3 bouts: 78%, 4 bouts: 42%). NCV is a primary indicator of chronic and traumatic nerve damage (28, 65-67). Peripheral nerve tissue is easily damaged by direct/indirect stresses through continuous compressions and entrapment, such as that seen in patients with sports injuries and disease (29, 42). When the damage occurs via stretching and/or compression of the peripheral nerve (e.g., myelin sheath or axon), the NCV is decreased due to impaired action potential traffic (53, 68, 69). Lee et al. (16) reported decreased NCV (21%) after a single bout of fast ECs. ECs-induced nerve damage was also observed in the present study (16). Additionally, the nerve was damaged more severely than the previous study (16). In particular, a 42% decrease in NCV was observed after 4 bouts of fast ECs, suggesting a cumulative effect occurs, as hypothesized. I speculated that the cause of the largest decrease in the 4-bout group is the timing of the fourth ECs bout. The previous study showed that NCV impairment appears 7D after a single bout of fast ECs. The day on which the fourth ECs bout

was performed (7D) coincided with the time point at which a significant decrease in NCV was observed after a single ECs (16). Therefore, I speculate that the accumulation of repeated temporary damage causes serious deterioration. Repeat bouts of slow ECs did not induce any impairment, and this result is in agreement with those of a previously reported single-bout experiment (16). In morphological analysis, damaged nerve fibers and myelin sheaths thinning were observed only in the 4-bout FAST ECs group. Neuronal degeneration is repeatedly induced by mechanical stress (compression, elongation, and denervation) or neuropathy (70, 71). Thinning of the myelin sheath and atrophy of fibers are typically observed after nerve transection (71). Additionally, I quantitatively analyzed nerve fiber morphology after ECs bouts. Only in the FAST group, myelin thickness (21%) and fiber diameter (12%) were significantly lower when compared with CNT values ($p<0.05$, Figure 2-4A, 4B). These results suggest that FAST ECs bouts strongly induce nerve thinning. Ikeda et al. reported a strong correlation between NCV and fiber diameter. The morphological changes measured in nerves correlated with the NCV reduction observed after ECs with a higher angular velocity. These results indicate that repeated bouts of ECs with faster angular velocity induce both functional and structural nerve disorders.

Although the mechanisms of ECs induced nerve damage are unclear, it is reported that nerve

damage is induced by several stresses such as transection, ischemia, immunologic changes, avulsion or metabolic disturbances (29, 72, 73). Although I have no data regarding the injury mechanism, I would like to suggest squeeze stress is applied to nerve fibers during ECs. A previous study reported that muscle-tendon behavior shifts after an initial bout of ECs; in that study, muscle fascicle length was shortened by 16%, suggesting that high indirect force was applied to connective tissues by ECs (74). Because nerve fibers are embedded in connective tissues, squeeze injuries may be applied during ECs with fast angular velocity. Additionally, the damage occurs in the neuromuscular junction. I and others have reported that the discontinuous area at muscle tendon units, such as the myotendinous junction and myofascial junction, is frequently damaged by ECs (10, 74). In the mouse model of muscular dystrophy (mdx mice), 15 maximal quadriceps ECs disrupted the motor end plate (75). Although this effect of ECs has only been reported in mdx mice, it is possible that neuromuscular junctions are disrupted after ECs with high angular velocity.

In this study, the MG wet weight of FAST group was significantly decreased in comparison to the CNT group (Table 3-1). Recent study showed that the MG wet weight after repeated bouts of 180°/sec ECs was significantly lower (6%) than that in the non-treated control group (33). The ECs protocol was same as the current study. In that paper, Ochi et al. also showed an increase in protein

breakdown by induction of forkhead box transcription factor O (FoxO) and myostatin contents in the MG after ECs. Although tissue swelling due to inflammatory responses also occurred, enhanced protein breakdown may lead to tissue atrophy. Since muscle atrophy is generally caused by denervation or immobilization muscle atrophy after ECs might be induced by nerve damage (76).

In humans, muscle strain injuries are induced by ECs (77). Strength loss and muscle atrophy are major symptoms in strain injury (78). In the current study, I found that repeated bouts of severe ECs induced myelin and axonal damage, concomitant with strength loss and muscle atrophy in an animal model. This line of evidence suggests that ECs inducing muscle strain might also damage nerve tissues. Since nerve damage induces muscle strength loss and atrophy, I speculate that motor nerve impairment might also occur in strain injuries. I'm now investigating sciatic nerve conduction velocity in athletes with hamstring strain injuries (HSI).

3-6. Conclusions

I conclude that nerve impairment induced by ECs is a velocity-dependent phenomenon. I observed nerve dysfunction and morphological abnormalities in the repeated FAST ECs group. However, no differences were observed in the SLOW group. Therefore, I conclude that repeated bouts of ECs with high angular velocity induce damage to the nerve in a cumulative fashion.

Chapter 4. Sciatic nerve conductivity is impaired by hamstring strain injuries

4-1. Abstract

Introduction: The aim of this study was to assess sciatic nerve conductivity in athletes with a history of HSI.

***Methods*:** Twenty-seven athletes with a history of HSI were included in the injured group. The control group consisted of 16 uninjured participants. I measured the proximal and distal latencies and calculated the sciatic NCV to evaluate neuronal conductivity. The results were expressed as median values and interquartile ranges.

Results: Both PL and DL of the injured limb in the injured group were significantly longer than those of the uninjured limb ($p<0.05$). The NCV of the injured limb in the injured group was significantly lower than that of the uninjured limb ($p<0.05$). There were no significant side-to-side differences in the CNT group.

Conclusion: Sciatic nerve conductivity impairments may exist in athletes with a history of HSI.

4-2. Introduction

Hamstring strain injuries are frequently observed in athletes who participate in strenuous sports such as rugby, American football, soccer, and track and field events (78, 79). Decreased strength and loss of flexibility are the typical symptoms (78, 80). In addition, HSI are characterised by high re-injury rates (81). It is generally known that the risk factors for HSI are mainly categorized according to non-modifiable factors (age, previous HSI, and/or ethnic origin) and modifiable factors (fatigue, strength imbalances, and/or early return to sports) (81-83). Colak et al. reported that the peripheral nerves of the upper extremities of ice hockey players are exposed to acute and chronic mechanical motions, such as the racket swing (28). In their study, conductive time of the brachial plexus was 6% prolonged in athletes compared with non-athlete controls. In addition, I found that nerve conductivities were impaired in animal and human models with ECs induced muscle damage (16, 17, 84). Degenerated nerves, thinner myelin, and decreased axon diameters were also observed after ECs (84). Further, Kami et al. reported that crush injury in the MG induced sciatic nerve injury (30). These reports indicate that muscle injuries induced by overuse and/or strenuous exercises are possibly accompanied by functional and structural nerve responses.

A previous commentary suggested that entrapment of the lumber spinal nerves may predispose

towards high recurrence rate of HSI (85). The lumbar nerves supply the several peripheral nerves that contract the lower limb muscles. Specifically, the hamstring and calf muscles are innervated by the sciatic nerve which branches at the L5 and S1 regions (85). Although these reports strongly suggest the existence of nerve impairments in muscle injuries, direct evidence of this conclusion is lacking.

The aim of this study was to investigate the nerve impairment of the sciatic nerve in athletes with a history of HSI. Here, I hypothesized that sciatic nerve conductivities are impaired by a history of muscle strain injuries.

4-3. Methods

Participants

Before participating, the subjects received a detailed description of the study assessment method and provided informed consent. This study was performed in the ethical committee of Nippon Sport Science University (013-H08). Forty-three collegiate students were enrolled; of them, 27 were athletes with a history of HSI defined as non-traumatic injuries of the hamstring (excluding muscle contusions) who were assigned to the injured (INJ) group; 16 uninjured participants were assigned to the CNT group. Especially, subjects of the INJ group were recruited that the medical staff advertised from their sports clubs respectively. The INJ group was asked about their history of HSI and time to returning to sports. The duration of return to sport was 2 weeks to 2 months. Subjects with acute and chronic strain injuries were recruited. In this study, an acute strain injury was defined as the state in which the athletes had not returned to full participation in training and were not available for selection. On the other hand, the definition of a chronic strain injury was the state in which the athletes had returned to full participation on training and game (79, 86). Twenty-three athletes had already returned to their sports while three subjects were not return to play. The INJ group had a hamstring injury only once. The subjects in the CNT group had not exercised

regularly in the past year but occasionally participated in recreational sports. In this study, an inclusion criteria of the CNT group was defined that subjects had not had a HSI in the past. All participants were asked to refrain from performing strenuous activities to eliminate the effects of acute activities. None of the subjects in either group had undergone surgery for HSI or other diseases.

Sciatic NCV examination

The hamstring is composed of the biceps short head, which is innervated by the common fibular nerve, and the biceps long head, semitendinosus, and semimembranosus muscles, which are innervated by the tibial nerve. Both nerves are branches of the sciatic nerve, which originates at the lumbosacral plexus. Therefore, I targeted the sciatic nerve for the nerve conduction assessment.

The nerve function was examined to determine nerve conduction impairments due to neuropathy and traumatic injury (32). The NCV was indicated as the conductive speed of the action potential per second in this study. It was calculated using the distance and time differences of two M-wave latencies, PL and DL. The PL is the conductive time from the initiation site of the sciatic nerve to the hamstring, while the DL is detected from the inferior division of the piriformis muscle

to the hamstrings. The action potential was induced by stimulating two points (lower lumbar and gluteal) as described previously (32). The proximal stimulation focused on the L5 root at the midpoint between the L5 spinal process and the posterior iliac crest; the distal stimulation was placed over the sciatic nerve at the gluteal fold, which is located at the midpoint between the ischial tuberosity and the greater trochanter of the femur (32) (Figure 4-1). Both latencies were detected as the compound muscle action potential detected from the hamstring contractions. The stimulation device used a magnetic stimulator (Magstim Company Ltd., Whitland, UK). A 70-mm-diameter figure eight-shaped coil was used for the stimulation. Intensities were set from 1.6 to 2.0 Tesla to the supramaximal stimulus. Subjects were standardised during the NCV testing. A recording electrode was placed at 50% of the hamstring length, consisting of the biceps femoris, semimembranosus, and semitendinosus muscles. A reference electrode was affixed to the tendon of the semitendinosus muscle. The room temperature was maintained at 26–28°C to exclude temperature changes in conductivity. The NCV value reliability was estimated as the CV of 2.1%-3.5% obtained from 4 healthy male volunteers.

Figure 4-1

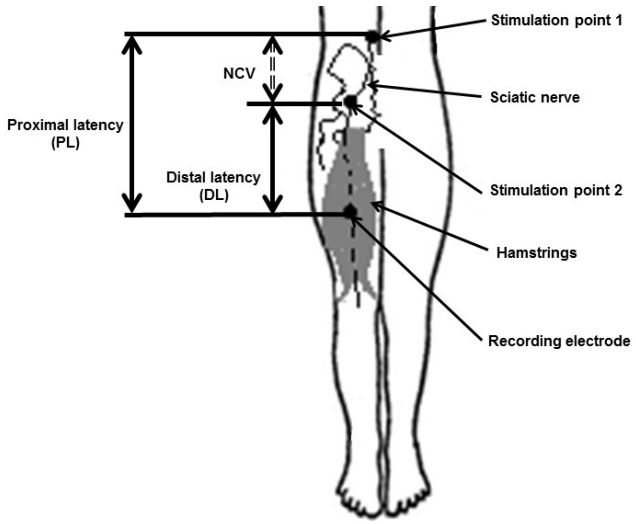


Figure 4-1. Measurement of NCV

NCV is calculated using the distance and time differences of two latencies as follows: NCV = distance/(PL – DL). PL indicates refers to the time of nerve root stimulation to the recording of the hamstring contraction. DL is achieved by a nerve stimulation passed through the bottom of the

piriformis to record the hamstring contraction. PL: Proximal latency, DL: Distal latency, NCV: Nerve conduction velocity.

Magnetic resonance Imaging (MRI)

In this study, INJ group consisted of chronic and acute cases. Therefore, magnetic resonance imaging (MRI; 0.3-Tesla; AIRIS II; Hitachi, Ltd., Tokyo, Japan) was used to observe the structural change of the injured site. The signal region was confirmed based on the short inversion time inversion recovery in the coronal and axial planes (86). The obtained images were divided into signal findings or no signal in accordance with the observation of an experienced orthopaedist.

Statistical analyses

Using the Shapiro-Wilk test, the data did not have a normal distribution, except for the physical characteristics. Therefore, nerve functions were expressed as median values and interquartile ranges. The statistical analysis used non-parametric tests. The Wilcoxon's signed-rank test was used to compare the intra-subject differences (CNT, right vs. left; INJ, uninjured vs. injured) in the NCV, PL, and DL. To compare the M-wave latencies and NCV between both groups, relative side-to-side differences were calculated. In the CNT group, NCV was obtained by dividing the lower value by the higher value in each participant. Relative side-to-side differences in the PL and DL were calculated by dividing the higher value by the lower value in each participant. To obtain the

relative values of the INJ group, the value of the injured limb was divided by that of the uninjured limb in each participant.

The Mann-Whitney U test was used to compare the inter-subject differences. The NCV and other variables were analysed using the non-parametric Spearman's product-moment correlation by using the SPSS ver. 22.0 for Mac (IBM Japan, Tokyo, Japan), and the significance level was set at $p<0.05$. Effect sizes (ES) and statistical power were calculated according to Cohen's d effect sizes. Physical characteristics are expressed as means and SD.

4-4. Results

Physical characteristics

The physical characteristics showed no significant differences between the CNT (age, 19.6 ± 1.4 years; height, 167.7 ± 7.0 cm; weight, 61.9 ± 7.3 kg) and INJ (age, 19.9 ± 1.1 years; height, 170.4 ± 9.2 cm; weight, 72.7 ± 16.0 kg) groups.

Changes in sciatic NCV

In the CNT group, no significant side-to-side differs were observed for the PL and DL (Table 4-1A). In the INJ group, significant side-to-side differences were found for PL and DL. (Table 4-1B). Further, relative side-to-side differences the PL in the INJ group was higher than that in the CNT group (CNT, 3%; INJ, 13%; ES = 0.78; 95 % CI = 0.13–1.41; $p<0.05$; Table 4-1C). However, there was no significant difference in the DL between the CNT and INJ groups.

In the CNT group, the NCV of both legs showed almost similar median values, such as 72.7 m/s (right) and 72.4 m/s (left). Conversely, the NCV of the injured limb [64.5 m/s (51.7 m/s–74.3 m/s)] was significantly lower than that of the uninjured limb [76.4 (71.6-84.2)] (ES = 0.87, 95% CI = 0.3–1.42; $p < 0.05$; post hoc power: 99%, Table 4-2, Figure 4-2). When comparing the INJ group

with the CNT group, the relative side-to-side NCV differences in the INJ group were significantly higher than that in the CNT group (CNT group, 98%; INJ group, 84%; ES = 2.04; 95% CI = 1.26–2.75; $p<0.05$; post hoc power: 98%; Table 4-2).

Table 4-1A. Proximal and distal latencies of the subjects in the CNT group

		PL	DL
CNT group	Right limb (m/s)	4.43 (4.05–4.92)	2.45 (1.44–3.10)
(n = 16)	Left limb (m/s)	4.48 (4.04–4.79)	2.25 (1.44–2.86)

All data are expressed as medians (interquartile ranges). CNT: Control, PL: Proximal latency, DL: Distal latency.

Table 4-1B. Proximal and distal latencies of the subjects in the INJ group

		PL	DL
INJ group	Uninjured limb (m/s)	5.55 (4.15–7.13)	3.60 (1.78–4.28)
(n = 27)	Injured limb (m/s)	5.85 (4.45–7.87)*	3.60 (2.10–4.48)*

All data are expressed as medians (interquartile ranges). INJ: Injured, PL: Proximal latency, DL: Distal latency, *$p<0.05$ vs. the uninjured limb.

Table 4-1C. Percent increase in the proximal and distal latencies of the subjects in the CNT and INJ groups

	PL	DL
CNT group (%)	3 (1–6)	5 (1–13)
INJ group (%)	13 (8.5–19.2)†	7 (1–14)

All data are expressed as medians (interquartile ranges). CNT, Control; INJ, Injured; PL, Proximal latency; DL, Distal latency; †$p<0.05$ vs. the CNT group.

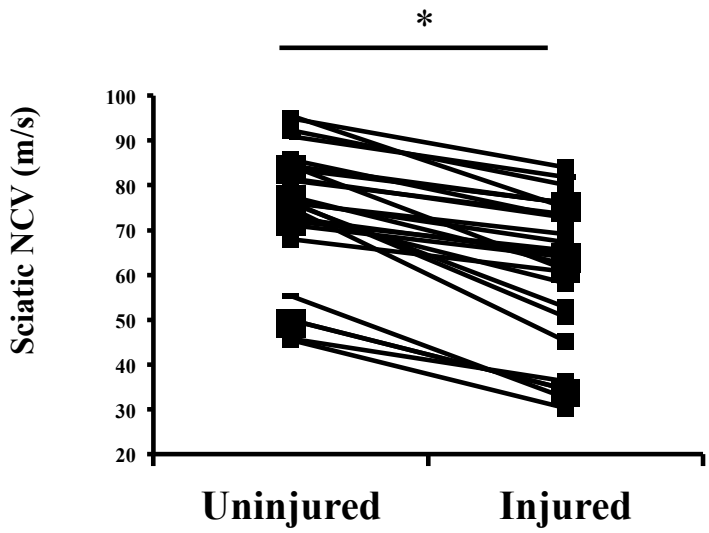
Table 4-2. Sciatic NCV among subjects in the CNT and INJ groups

CNT group (n = 16)	Right limb (m/s)	72.7 (64.8-79.1)
	Left limb (m/s)	74.0 (64.6-78.8)
	Left/right (%)	98
INJ group (n = 27)	Uninjured limb (m/s)	76.4 (71.6-84.2)
	Injured limb (m/s)	64.5 (51.7-74.3)*
	Injured/uninjured (%)	84†

All data are expressed as medians (interquartile ranges). CNT: Control, INJ: injured, NCV: Nerve conduction velocity, †$p<0.05$ vs. the CNT group, *$p<0.05$ vs. the uninjured limb.

Figure 4-2. NCV values between the uninjured and injured limbs in the INJ group

Figure 4-2



The NCV values significantly decrease in the injured limb (*$p<0.05$). Slower NCV values in the injured limb are observed in all examined injured subjects. NCV: Nerve conduction velocity.

Magnetic resonance Imaging (MRI)

High signal intensity on MRI was observed in 10 of the 27 subjects (37%) in the INJ group. There was no significant correlation in the NCV values between the two groups with or without high signal intensity (rho, -0.124; p = 0.539).

Time after injury

The time after injury was 1–70 months. There was no significant association between the nerve functions (rho, -0.267; p = 0.178). Therefore, such respective variables were not correlated, except for the PL and DL. The physical characteristics, MRI observations, sport backgrounds, and nerve functions of the patients in the INJ group are shown in Table 4-3.

Table 4-3. Physical characteristics, MRI findings, and nerve conductivity in the INJ group

No.	Sex	injury	(°)	Signal	Sport	(Uninjured/	(Uninjured	(Uninjured/
1	Male	5	75 / 80	Yes	American football	8.82 / 10.40	6.42 / 6.42	75.0 / 45.2
2	Male	6	85 / 85	Yes	American football	4.15 / 5.95	1.61 / 3.09	72.7 / 64.5
3	Male	2	85 / 85		American football	4.18 / 5.00	1.85 / 2.25	85.7 / 72.7
4	Male	6	90 / 90		American football	6.91 / 7.66	4.40 / 4.90	83.6 / 76.1
5	Male	24	90 / 90	Yes	Rugby	5.91 /6.79	3.60 / 3.90	77.6 / 62.3
6	Male	9	80 / 65		Rugby	7.35 / 8.07	4.60 / 4.95	76.4 / 67.3
7	Male	1	70 / 80		Rugby	3.90 / 4.20	1.69 / 1.70	95.0 / 84.0
8	Male	12	85 / 70	Yes	Rugby	4.75 / 4.20	1.65 / 1.95	84.4 / 61.2
9	Male	18	90 / 90		Rugby	4.15 / 4.20	1.70 /1.70	81.6 / 72.8
10	Male	2	90 / 90	Yes	Rugby	8.05 / 8.60	5.48 / 5.85	76.0 / 69.1
11	Male	1.5	90 / 90	Yes	Rugby	4.20 / 4.45	2.25 / 2.50	84.4 / 76.0
12	Male	70	80 / 80		Rugby	4.45 / 5.85	2.19 / 3.35	84.0/ 76.0
13	Male	6	90 / 90		Rugby	6.75 / 7.30	4.25 / 4.50	68.0 / 60.7
14	Male	8	80 / 90		Track and field	5.55 / 5.85	3.60 / 3.60	92.3 / 80.0
15	Male	18	90 / 80		Track and field	3.73 / 4.15	1.45 / 1.65	71.1 / 64.0
16	Male	12	90 / 90	Yes	Track and field	3.79 / 5.00	1.60 / 1.70	50.0 / 34.5
17	Female	18	90 / 90		Track and field	3.90 / 4.45	1.95 / 2.25	91.0 / 81.8
18	Female	18	90 / 105	Yes	Track and field	4.09 / 4.45	1.89 / 1.65	95.5 / 75.0
19	Male	16	90 / 90		Track and field	8.59 / 9.70	4.45 / 4.45	45.8 / 36.2
20	Female	14	90 / 80	Yes	Track and field	5.25 / 5.85	2.80 / 2.76	73.5 / 58.1
21	Male	36	90 / 90		Track and field	6.40 / 7.60	3.90 / 4.00	76.0 / 52.8
22	Female	16	80 / 80		Track and field	8.25 / 9.70	4.30 / 4.75	45.5 / 30.3
23	Male	60	80 / 80		Track and field	6.55 / 7.45	4.05 / 4.70	72.0 / 65.5
24	Male	18	90 / 70		Track and field	3.60 / 3.85	1.40 / 1.41	81.1 / 73.5
25	Male	3	90 / 90		Sepak takraw	6.85 / 8.30	4.10 / 4.15	76.4 / 50.6
26	Female	1	120 /120		Sepak takraw	7.65 / 9.40	3.85 / 3.90	50.0 / 34.5
27	Male	11	90 / 80	Yes	Soccer	10.50 / 10.15	4.45 / 4.45	55.4 / 32.5

MRI: Magnetic resonance imaging, INJ: Injured; PL: Proximal latency, DL: Distal latency, NCV: Nerve conduction velocity.

4-5. Discussions

This study explored whether the sciatic nerve conductivity is impaired in athletes with the history of hamstring stain injuries. To assess nerve functions, I measured sciatic NCV and latencies in CNT and INJ groups that results were compared with between both groups. For injured group, prolonged M-wave latencies and decreased NCV of the sciatic nerve were observed in the injured limb compared to uninjured side. There was no significant difference in the side-to-side comparison in the CNT group. In addition, there were significant inter-group differences in the relative side-to-side differences between the CNT and INJ groups, regardless of similar physical characteristics. Side-to-side NCV differences were observed in all of the athletes who experienced hamstring strain injuries in the INJ group.

PL, DL, and NCV measurements are used to assess nerve function in a variety of temporal nerve functional impairments and injuries (28, 32, 41). In this study, the PL was significantly longer in the INJ group (Figure 4-2). I previously found longer latency in the musculocutaneous nerve after ECs of the biceps brachii in humans (17). I also confirmed that ECs reduced maximal voluntary elbow flexor torque and muscle pain. These results indicated that muscle damage might be associated with increased PL and decreased NCV. When taken together, these findings show that

sciatic nerve function was affected by hamstring strain injuries. Although there were no significant relative side-to-side differences in the DL between the INJ and CNT groups, the data distributions showed that the DL in the INJ group was longer than that in the CNT group. I would like to discuss the reason why only the PL, but not the DL, showed a significant difference in the latter half of the discussion section.

Since M-wave latency includes axonal conductivity, neuromuscular transmission, and electrical conductivity in the muscle, I calculated NCV to focus on axonal conductivity. I found that NCV of the injured limb was significantly decreased compared to that of the uninjured side (ES = 0.87; Table 4-2). I further confirmed that side-to-side differences of the sciatic NCV in HSI athletes were significantly greater than the side-to-side differences in the CNT group (ES = 2.04; Table 4-2). I previously observed a significant 21 % decrease of sciatic NCV that is indicated between from origin of sciatic nerve to branching point to MG after ECs in rats (84). Although I cannot rule out whether functional impairments also exist in the NMJ and electrical conductivities of the muscle, I believe that nerve conductive impairments are associated with HSI.

My recent studies found the innervation nerve impairment after unaccustomed ECs. NCV deficit (a single EC bout: 21%; multiple EC bouts: 42%) and prolonged M-wave latency (a single

ECs bout: 24%) were induced in both animal and human studies (16, 17, 84). In this study, I found an 11% prolonged PL and 10–30 % reduction of sciatic NCV in athletes with history of HSI, suggesting that the reduction rate in strain injury was comparable with those in ECs-induced nerve impairment. Unaccustomed ECs during high-velocity sprinting and sudden accelerations are major risk factors for HIS (87). Therefore, the present results might support previous observations, showing that nerve damage was possibly induced by ECs.

However, it is unclear whether nerve conductivity impairment was caused by actual nerve tissue damages. In the previous study, macrophage invasion and loss of myelin protein were also caused by a single bout of ECs (16). In addition, myelin sheath and axonal thinning were observed on electron microscopy after repetitive ECs (84). In the present study, impaired nerve conductivity was sustained for months or years, although the subjects already returned to their play except for subjects had not returned to play. Therefore, I think that the persisting nerve conductivity impairment is due to the actual nerve tissue damage, such as myelin sheath and/or axonal thinning.

In this study, I measured the sciatic NCV between the origin of the sciatic nerve at the lumbosacral plexus and the piriformis area in which the NCV was calculated from the PL and DL. The NCV and PL were significantly impaired in the INJ group; however, the relative side-to-side

difference in the DL was not significant. Chang et al. reported that retrograde axonal atrophy was confirmed by measuring the NCV in patients with carpal tunnel syndrome (CTS) (88). They observed significant reductions in the wrist-palm motor conduction velocity (36.2%) as the typical symptom of CTS. In addition, the median forearm motor conduction velocity also significantly decreased compared with the normal control group (4.43%). In the previous study, the forearm motor conduction velocity, rather than the wrist-palm motor conduction velocity, was measured at a distal site. Therefore, if the initial nerve conductivity impairment occurs near the exercised muscle, the effect of the hamstring muscle injury is propagated to the remote region of the sciatic nerve. Kami et al. (30) reported that a contusion injury to the gastrocnemius muscle induces a sciatic nerve injury and that the damage occurs in a retrograde fashion toward the lumbar spinal nerve. Similarly, the dying-back phenomenon is also known as a retrograde nerve degeneration process that is observed in neuronal disorders, such as Charcot-Marie-Tooth and amyotrophic lateral sclerosis (89, 90). Hence, I think that sciatic nerve conductivity impairment initially occurs near the injured site of the hamstring and that the impairment might propagate toward the spinal cord.

Nerve conductive function is assessed using electrical stimulation (28, 29). However, electrical stimulation is invasive because the sciatic nerve is located deep beneath the skin. On the

other hand, magnetic stimulation is a beneficial tool that enables a direct percutaneous stimulation of the sciatic nerve (32). Therefore, I used a pulsed magnetic field to induce electrical activity in the sciatic nerve. Chang et al. measured sciatic NCV by magnetic field stimulation and reported that the normal sciatic NCV was 68.7 ± 10.1 m/s (32). In this study, the NCV value of the CNT group was 72.3 ± 12.3 (right) and 71.5 ± 12.3 (left), values that were similar to the value reported by Chang et al. They also found that the sciatic NCV was decreased by 19% in patients with piriformis syndrome. I also found a similar decrease of 14% in the injured limb. These lines of evidence suggest that magnetic field stimulation is an applicable substitute for electrical stimulation for measuring sciatic NCV.

This study has several limitations. First, it is unclear whether the significant differences in the NCV and PL are reliable because the sample size was small in the present study. However, I analysed the effect size of the NCV and latency as follows: PL: CNT group vs INJ group; ES = 0.78; 95 % CI = 0.13–1.41; NCV: CNT group vs INJ group ES = 2.04; 95% CI = 1.26–2.75. Although the sample size was small, the relatively large effect sizes indicate that the results obtained in this study are reliable. Second, I did not examine the injured site and damage degree of the HSI. There may be differences in the M-wave latencies and NCV characteristics if the damaged areas are different.

However, I found that all athletes who had HSI showed side-to-side differences in the sciatic NCV. Thus, I think that sciatic NCV would be affected by HSI regardless of injured sites and severity. Third, all participants in the INJ group were athletes, while those in the CNT group sometimes participated in recreational sports. Hence, the observed differences in the nerve function may be because of the different activity levels of the participants. Since I observed that the nerve function was almost similar among the uninjured limbs of all participants, I think that the participants in the CNT group serve as a suitable reference for the INJ group. Finally, a longitudinal examination is necessary to clarify the contribution of nerve impairments to HSI.

4-6. Conclusions

In conclusion, sciatic nerve conductivity is impaired in athletes with a history of HSI. I suggest that sciatic nerve disorders might be induced by strain injuries owing to the eccentric movements of the hamstrings. This is the first study to show that sciatic nerve conductivity impairment is observed in athletes with a history of HSI.

Chapter 5. General discussions

5-1. Summary

The purpose of this thesis is to investigate as follows:

1. To measure musculocutaneous nerve conductivity after single bout of ECs in biceps brachii muscle (Chapter 2).

2. To evaluate whether repeated bouts of FAST ECs cause severe damage to the sciatic nerve in MG of rats (Chapter 3).

3. To investigate nerve latency and NCV of the sciatic nerve in athletes with a history of HSI (Chapter 4).

In the chapter 2, the experiment was applied that whether ECs induced innervation nerve impairment is observed in human. Subjects were received single bout of 60 ECs with the muscle damage on biceps brachii muscle. Their MsN latency and amplitude were measured. After ECs, there is the observation that values of latency significantly increased (12 - 24 %) on 1 - 2 days. Therefore, it is established that unaccustomed ECs lead to MsN nerve impairment by this experiment.

In the chapter 3, functional and morphological sciatic nerve variations were assessed in rat's

MG after multiple bouts of ECs. Rats were applied different angular velocity ECs which is SLOW and FAST. Sciatic NCV was gradually decreased with increasing bouts of ECs (2 bouts: 78%, 3 bouts: 78%, 4 bouts: 42%) only the FAST group. Especially in the 4 bouts ECs of FAST group, degenerated sciatic nerves were observed that these fiber diameters were narrowed. On the other hand, functional and morphological disorders were not shown both CNT and SLOW groups. These results indicated the knowledge that severe ECs such as multiple bouts cause seriously innervation nerve impairment.

In the chapter 4, clinical application was applied whether sciatic nerve conductivity exists in athletes with a history of HSI. Previous chapters clarified that ECs lead to not only skeletal muscle injury, but also innervation nerve impairment. ECs contribute to characteristic movements such as cutting and leg swing during running. ECs is one of the factor of muscle strain injury that is induced by rapid ECs. Therefore, It is a possibility that innervation nerve impairment is observed in the case of muscle strain injury. To examine this possibility, 43 subjects were assessed their sciatic nerve that innervates hamstrings. Significant NCV deficit (14 %) and prolonged PL (10%) were observed in 27 subjects who have the history of HSI. On the other hand, there were not observed these phenomena in not injured 16 subjects. In addition, side-to-side difference of NCV values was observed in 27

injured athletes. Hence, it is clarified that innervation nerve impairment is observed in athletes with a history of HSI.

5-2. Future perspective

The possible mechanism of ECs induced peripheral nerve damage

It has been studied for long years about ECs induced muscle damage. In this study, innervation nerve impairment has been found after strenuous ECs. In human and animal studies, time course of innervation nerve impairment was later than decrease of MVC and ROM while similar time course of DOMS. And then, more severe NCV deficit and pathological abnormality were induced by chronic ECs. In the chapter 4, the result supported a hypothesis that nerve dysfunction is observed in athletes with history of HSI. These results indicated that impairment of nerve function might be related to clinical symptoms such as continuous strength loss, neuromuscular un-coordination and re-injury of HSI. However, process of pathological condition and the inducible factor of ECs induced nerve impairment are remained unclear.

I have already obtained few data about the mechanism of ECs induced nerve impairment, by use of animal experimental model. After FAST ECs, sciatic nerve and intramuscular nerve of MG were divided into 5mm segments to examine by histochemical analysis. Sciatic nerve tissue was analyzed by evans blue dying (EBD) that can detection the vascular permeability due to nerve damage (91, 92). Intramuscular nerve was stained by EBD and lectin. In the intramuscular nerve,

EBD infiltrations were observed on lectin-stained site only 1D and 3D (Figure 5-1, 2). In the sciatic nerve tissue, EBD was detected in distal portion of sciatic nerve on immediately post after FAST ECs. Interestingly, EBD stained sciatic nerve tissue was observed in proximal segments close to the spinal cord on 1D, 3D and 7 D after FAST ECs (Figure 5-3).

Figure 5-1

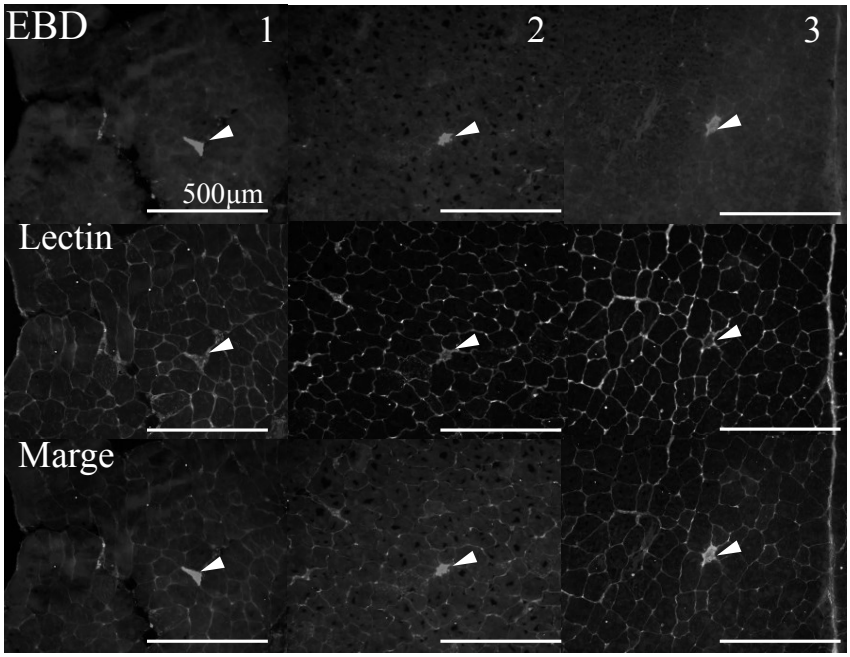


Figure 5-1. EBD and Lectin staining for intramuscular nerve on 1D after strenuous ECs

EBD infiltrations were observed on lectin-stained site. Top: EBD strained, Middle: Lectin stained, Bottom: Marged, White arrows: Positive stained nerve tissue.

Figure 5-2

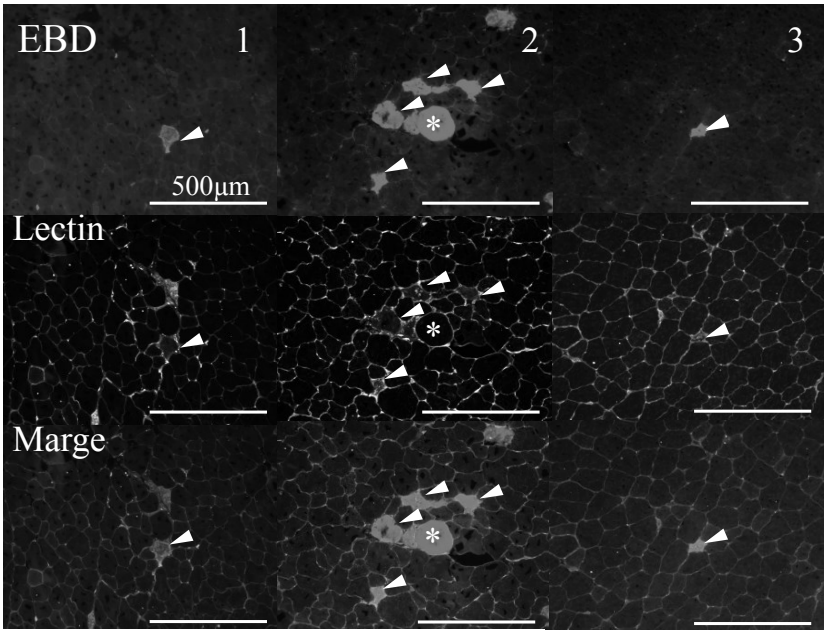


Figure 5-1. EBD and Lectin staining for intramuscular nerve on 3D after strenuous ECs

EBD infiltrations were observed on lectin-stained site. Top: EBD strained, Middle: Lectin stained, Bottom: Marged, *: Positive stained muscle fiber, White arrows: Positive stained nerve tissue.

Figure 5-3

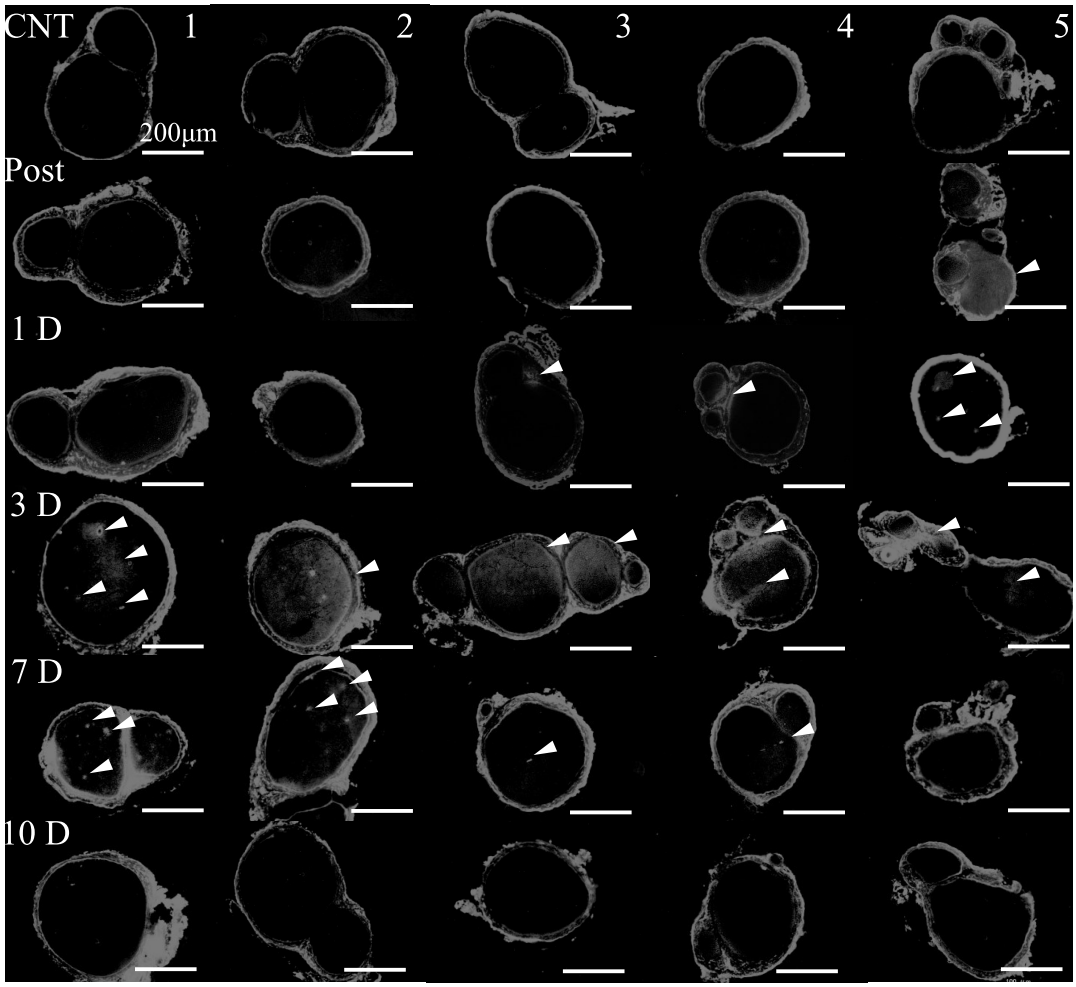


Figure 5-3. EBD for sciatic nerve tissue after strenuous ECs

EBD infiltrations were observed from immediately post to 7D after strenuous ECs. Top: White arrows; Positive stained nerve tissue, CNT: Non-treated control group, Post: immediately after, 1D:

1 day, 3D: 3 days, 7D: 7 days, 10D: 10 days.

Hence, ECs induced nerve impairment possibly occurred near connection of neuron and muscle, and retrogradely enlarged and/or transitioned from the damaged site.

In addition, time course of nerve conductivity impairment is similar to expression of DOMS. Especially, sensory nerve dysfunction might be an important factor to resolve the generating mechanism of DOMS. In fact, there are indications that neurotrophic factors such as nerve growth factor and/or brain derived neurotrophic factor were increased in similar time point after strenuous ECs (93-95).

Therefore, more studies are necessary to understand the mechanisms such as process of pathological condition and the inducible factor in ECs induced innervation nerve impairment.

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Abbreviations

1D: 1 day after ECs

2D: 2 days after ECs

3D: 3 days after ECs

4D: 4 days after ECs

5D: 5 days after ECs

6D: 6 days after ECs

7D: 7 days after ECs

10D: 10 days after ECs

ALS: Amyotrophic lateral sclerosis

ANOVA: Analysis of variance

CNS: Central nervous system

CNT: Non-treated control group or Not injured control group

CONs: Concentric contractions

CTS: Carpal tunnel syndrome

CV: Coefficient variation

DL: Distal latency

DOMS: Delayed onset of muscle soreness

EBD: Evans blue dye

ED1: Macrophage-related protein:

E-C coupling: Excitation-Contraction coupling

ECs: Eccentric contractions

EIMD: Exercise induced muscle damage

ES: Effect size

FAST: Fast joint angular velocity

FoxO: Forkhead box proteins O

HSI: Hamstrings strain injuries

INJ: Injured group

ISOs: Isometric contractions

NCV: Nerve conduction velocity

NMJ: Neuromuscular junction

p0: Protein 0

PL: Proximal latency

PNS: Peripheral nervous system

RBE: Repeated bout effect

ROM: Range of motion

SD: Standard deviation

SLOW: Slow joint angular velocity

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List of publications

1. **Kouzaki K, Nosaka K, Ochi E, Nakazato K.** Increases in M-wave latency of biceps brachii after elbow flexor eccentric contractions in women, European Journal of Applied Physiology, 2016, Mar 19, 116: 939-46

2. **Kouzaki K, Kobayashi M, Nakamura K, Ohta K, Nakazato K.** Repeated bouts of fast eccentric contraction produce sciatic nerve damage in rats, Muscle & Nerve, 2016, Mar 19, doi: 10.1002/mus.25110 [Epub ahead of print]

3. **Kouzaki K, Nakazato K, Mizuno M, Yonechi T, Higo Y, Kubo Y, Kono T, Hiranuma K.** Sciatic nerve conductivity is impaired by hamstring strain injuries, International Journal of Sports Medicine, 2017, Oct, 38(11): 803-808 doi: 10.1055/s-0043-115735. Epub 2017 Sep 11

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論 文 の 和 文 要 旨

氏 名 鴻崎香里奈

（博士論文の題目）

Strenuous eccentric contractions induce the peripheral nervous injury
過度な伸張性収縮は末梢神経損傷を誘発する

（博士論文の要旨）

第 1 章 過度な伸張性収縮は骨格筋の支配神経損傷を誘発する

骨格筋の収縮運動は等尺性、短縮性、伸張性収縮（ECs）の 3 様式に大別される.特に ECs は収縮条件によっては筋損傷が誘発され,近年ではラットに過度な ECs を課すと,筋の支配神経に異常が誘発されることが明らかとなった.しかし,ヒトで同様の現象が観察されるか否か,あるいは ECs の条件変化が神経へ及ぼす影響は未解明である.本研究では,3 つの課題を検証した.

第 2 章 過度な伸張性収縮は上腕二頭筋支配神経である筋皮神経潜時を遅延させる

本章では ECs 誘発性の神経損傷がヒトにおいて観察されるか否かを検証した.対象者は運動習慣のない 15 名の成人女性（年齢: 24.6 ± 3.5 歳,身長: 160 ±5.3 cm,体重: 55.3 ± 5.5 kg）とし,非利き手側の上腕二頭筋に ECs を実施した.ECs は先行研究に則り,90°/秒の関節角速度にて 1 セットあたり 6 回の ECs を 10 セット課した.5 日間の実験期間中,ECs は初日のみ,直後から翌日以降の 4 日間（1-4 日後）で筋および神経機能を評価した.筋機能は最大等尺性発揮筋力（MVC）,肘関節可

動域（ROM）,遅発性筋痛（DOMS）を,神経機能は上腕二頭筋を支配する筋皮神経の潜時測定によって評価した.潜時は複合活動電位が神経を伝導し筋収縮を誘発させるまでの時間を反映する.したがって神経機能が障害された場合,活動電位の伝導が阻害され潜時が遅延する.筋機能の結果では MVC と ROM が ECs 実施直後に顕著に低下し,DOMS は 2 日後で最も増悪した（$p<0.05$）.潜時は 1-2 日後において ECs 実施前より 12-24%有意に遅延した（$p<0.05$）.

本実験結果より,ヒト対象の実験においても ECs 誘発性の神経機能低下が示された.

第 3 章 ラット腓腹筋に対する繰り返しの伸張性収縮が坐骨神経へ及ぼす影響

過度な伸張性収縮を慢性的に負荷すると,重度な筋損傷が誘発される.本章ではラット腓腹筋に高角速度の ECs を繰り返し,慢性的な ECs が支配神経へ及ぼす影響を検証した.雄性 Wistar 系ラット（9 週齢）の内側腓腹筋に電気刺激を施し,収縮を誘発させながら足関節を背屈させ,ECs を課した.群分けは関節角速度の速い 180°/s 群（FAST）,遅い 30°/s 群（SLOW）,未処置群（CNT）とし, 1 日おきに 1-4 セット(1 日あたり 5 回×4 セット)を実施した.神経は腓腹筋支配神経を含む坐骨神経伝導速度（NCV）を評価した.NCV は複合活動電位が神経から筋へ伝導する際の速度であり,NCV の低下は支配神経における機能障害の発生を反映する.FAST 群では ECs 実施回数の増加に伴い段階的な NCV の低下が誘発された（2 セット: 78%, 3 セット: 78%, 4 セット: 42%, $p<0.05$）.特に 4 セット群では,足関節発揮トルクが CNT より 36%低下し,腓腹筋が萎縮した（$p<0.05$）.また電子顕微鏡下で坐骨神経線維を観察すると,明らかに変性した神経線維が観察され,ミエリン鞘は狭小化し線維径が減少した（$p<0.05$）.

本実験結果から,高角速度の伸張性収縮を繰り返し行うと,重篤な神経損傷が

誘発されることが示された.

第 4 章 ハムストリングスの肉離れは坐骨神経機能低下をもたらす

肉離れ損傷はスポーツ現場において頻発し,発揮筋力の低下や筋痛,重篤な例では損傷部の線維化や筋萎縮が観察される.さらに高い割合での再受傷が指摘されているが,原因は不明である. 肉離れはトップスピードでの疾走や切り返しなど, 繰り返しの ECs を主な損傷機転とするため,支配神経異常の関与が考えられる. 本章では, 肉離れが好発するハムストリングス肉離れ既往者の坐骨神経 NCV を評価した. 27 名の肉離れ既往者から成る INJ 群(年齢: 19.6 ± 1.4 歳, 身長: 167.7 ± 7.0 cm, 体重: 61.9 ± 7.3 kg)を対象に, 腰部からハムストリングスまでの坐骨神経 NCV を測定し,16 名の非損傷者から成る CNT 群(年齢: 19.9 ± 1.1 歳, 身長: 170.4 ± 9.2 cm, 体重: 72.7 ± 16.0 kg)と比較した. INJ 群では,CNT 群より NCV が有意に 14%低下していた($p<0.05$).さらに INJ 群の 27 名全症例において,損傷側の NCV は非損傷側より有意な低値を示した($p<0.05$).したがって, ECs を主な起点とした肉離れ損傷においても神経機能の低下が確認された.

第 5 章 今後の展望

本研究によって,過度な ECs を単回あるいは繰り返しおこなうと,支配神経の機能・構造異常を誘発することがヒトおよび動物実験によって明らかとなった.ECs が神経へ及ぼす影響の実態が解明されつつある一方で,発生機序や病態の進行過程は不明である.既に幾つかの検証によって,ECs 実施後の筋内神経における血管透過性の亢進(神経損傷を示唆する現象)や,坐骨神経線維の血管透過性の亢進が日数経過に伴い損傷部近傍から遠位(脊髄側)で発生することを確認した.今後は病態メカニズムや発生機序の解明に向けた細胞・分子レベルでの調査が重要課題である.

論 文 の 和 文 概 要

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（博士論文の題目）

Strenuous eccentric contractions induce the peripheral nervous injury

過度な伸張性収縮は末梢神経損傷を誘発する

（博士論文の概要）

伸張性収縮(ECs)は筋が伸張しながら大きな力発揮を行う運動であり,健康増進やトレーニングなどに用いられるが,実施条件によっては筋損傷を誘発する.最近,支配神経機能の低下や構造破綻が誘発されることが動物実験によって報告されたが詳細には検討されていない.本研究では以下の検証をおこなった.

第 2 章ではヒト上腕二頭筋に過度な ECs を実施し支配神経機能を評価した.運動習慣のない女性被験者 15 名の上腕二頭筋に,伸張性収縮を初日のみ 60 回実施し,支配神経である筋皮神経の機能を測定した.その結果 ECs 側で神経損傷時に生じる潜時の遅延が観察された.したがって動物実験と同様,ヒトにおいても ECs 誘発性の神経機能の低下が観察された.

第 3 章では,慢性的な繰り返しの ECs によって神経機能および構造異常が重症化するか否かを検証した.ラット内側腓腹筋に対し高角速度の ECs を 1 日おきにそれぞれ 1〜4 セット(1 セットあたり 20 回)行い,支配神経である坐骨神経伝導速度(NCV)を測定した. NCV は実施回数の増加に伴い著しく低下し,4 セット実施群では変性、狭小化した神経線維が観察された. したがって,ECs の繰り返しに伴い神経機能および構造異常が重症化することが示された.

第 4 章では,ハムストリングス肉離れ損傷既往者を対象として坐骨神経の機能低下が観察されるか否かを検証した. ハムストリングス肉離れは, 疾走や切り返しなど, 繰り返しの伸張性収縮を主な損傷機転とする. 27 名の肉離れ既往者(INJ 群)およ

び非損傷者 16 名(CNT 群)を対象に，坐骨神経伝導速度を測定した.その結果,INJ 群では CNT 群と比較して NCV が 14%低下していた.さらに INJ 群全症例において損傷側 NCV は非損傷側より有意な低値を示した.したがって，伸張性収縮を起点とした筋損傷と捉えられている肉離れ損傷においても神経機能の低下が確認された.

論 文 の 欧 文 概 要

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(Title) **Strenuous eccentric contractions induce the peripheral nervous injury**

(Abstract)

Eccentric muscle contractions (ECs) has two aspects that effect positive or negative. Especially, unaccustomed ECs induces loss of muscle strength, limit of joint range of motion, muscle soreness and structural disruption. Hence, it is the important to obtain the effect of ECs with avoiding muscle injury. Recent study has indicated that strenuous ECs induces innervation nerve impairment in rats. In this study, three experiments were applied to investigate detail of ECs induced nerve impairment.

In the chapter 2, the experiment was applied that whether ECs induced innervation nerve impairment is observed in human. After unaccustomed ECs, musculocutaneous nerve latency and amplitude were measured in 15 female subjects. There is the observation that values of latency significantly increased on 1 - 2 days after ECs. Therefore, it is established that unaccustomed ECs lead to musculocutaneous nerve impairment in human.

In the chapter 3, rats were respectively applied ECs which is slow (SLOW) and fast (FAST)

angular velocities. Sciatic nerve conduction velocity (NCV) was gradually decreased with increasing bouts of only the FAST group. Especially most severe ECs of FAST group, degenerated nerves and narrowed fiber diameters were observed. Therefore, ECs such as multiple bouts causes seriously innervation nerve impairment.

In the chapter 4, clinical application was applied whether sciatic nerve conductivity exists in athletes with a history of hamstrings strain injuries (HSI). ECs is one of the factor of muscle strain injury. Fourty-three subjects were measured their sciatic nerve that innervates hamstrings. Significant impairments of nerve conductivity were observed in 27 subjects with a history of HSI. However, there were not observed these phenomena in not injured 16 subjects. In addition, side-to-side difference of NCV values was observed in 27 injured athletes. Hence, it is clarified that innervation nerve impairment is observed in athletes with history of HSI.