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Research article

Effects of acute eccentric contractions on rat ankle joint stiffness

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Abstract

The sensation of joint stiffness is frequently observed after eccentric contractions (ECs) in human, but the joint stiffness of animals after ECs has not been examined previously. This study tested whether a bout of ECs affects rat ankle joint stiffness. We also evaluate muscle passive tension in the rat hindlimb to examine the relationships of ankle joint stiffness with muscle passive tension. Anesthetized male Wistar rats ($n = 23$) were firmly secured on a platform in the prone position. A bout of ECs was performed on the gastrocnemius muscle with a combination of electrically induced tetanic contractions via a skin electrode and simultaneous forced dorsiflexion of the ankle joint (velocity, 15°/s; from 0°to 45°). Passive resistive torque (PRT) of the ankle joint was measured to evaluate joint stiffness. Passive tension of the exposed gastrocnemius muscle was also measured when the maximum value of joint stiffness was obtained. The PRT on days 2, 3, and 4 was significantly higher than the pre-treatment value (days 2 and 4; $p < 0.001$, days 3; $p < 0.01$). The passive tension on day 4 was significantly higher than that of the sham-operated group. The muscle wet mass was identical in both groups, suggesting the absence of edema. We conclude PRT increases after ECs in rat ankle joint. We also show the possibility that it is associated with muscle passive tension, independent of edema formation.

Key words: Lengthening, flexibility, passive torque, passive tension, animal model.

Introduction

The mechanical stiffness of a joint is defined as the absolute torque that is required to maintain the joint specific angle or the ratio of the change in the joint torque to the change in joint angle (Kearney and Hunter, 1990). Joint stiffness depends on the following three properties that have different origins: (i) the elastic properties of non-contractile connective tissues (including joint capsule and skin), (ii) the elastic properties of the muscle-tendon complex, and (iii) the reflex activation of a muscle following a change in its length. In particular, the static passive stiffness of joints largely depends on the elastic properties of non-contractile and contractile tissues (Gajdosik et al., 1999).

It has been reported that acute eccentric contractions (ECs) induce muscle weakness, soreness, and the sensation of stiffness (Chleboun et al., 1998; Clarkson et al., 1992; Nosaka and Clarkson, 1995; Porter et al., 2002). Compared to other symptoms, the sensation of stiffness has not been well analyzed. The sensation of stiffness has been described as a reluctance to stretch the affected muscle and has been most commonly evaluated by measuring the post-exercise resting position of the joint (regarded as range of motion (ROM)) (Clarkson et al., 1992; Nosaka and Clarkson, 1995; Stauber et al., 1990). The elbow angle of a relaxed arm becomes more acute following EC exercises of the elbow flexors. Immediately after exercise, this angle begins to decrease and continues to decrease until day 3 in the previous human studies (Clarkson et al., 1992; Whitehead et al., 2003). The resting angle then increases gradually. In addition, Howell et al. (1993) and Chleboun et al. (1998) measured the joint stiffness (regarded as passive resistive torque (PRT)) in an intact human elbow. They showed that the PRT increased immediately after ECs and remained elevated for approximately 4 days. Regardless of the evaluation method used, joint stiffness is observed to increase after EC exercises.

Various theories have been proposed to explain this increase in joint stiffness. Clarkson et al. (1992) proposed that an influx or accumulation of calcium could activate specific enzymes and cause excessive contractures in the damaged fibers. Howell et al. (1993) stated that the restriction of motion and the apparent decrease in the resting length of the muscles was due to the occurrence of an edematous change in the perimuscular connective tissues. Stauber et al. (1990) concurred and proposed that the swollen tissues that pushed against the fascia could shorten the muscle passively. The focus of these hypotheses is to determine whether the elastic properties of the muscle-tendon complex are associated with the increase in joint stiffness. However, the direct association of joint stiffness and passive muscle tension after ECs has not been examined previously.

To directly compare joint stiffness and muscle passive tension, it is essential to employ experimental animals. With regard to the effects of ECs on muscle-tendon complex passive tension, Whitehead et al. (2001; 2003) performed ECs on exposed cat medial gastrocnemius muscles. Electrical stimulation was applied via the motor neurons, and the distal tendon of the medical gastrocnemius was extended to induce ECs. By using such experimental systems, they clearly demonstrated that passive tension of the medial gastrocnemius was significantly elevated immediately after the ECs. Since Whitehead et al. (2001) did not examine ankle joint stiffness in the experimental animal, the direct relationship between joint stiffness and muscle passive tension remained unclear.

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With regard to joint stiffness, Gillette and Fell (1996) measured ankle joint stiffness (static PRT) in rats. They revealed that 7-day hindlimb suspension significantly increased the PRT of the rat ankle joint. They also measured the ankle joint PRT after each individual muscle tendon (gastrocnemius, soleus, and plantarius muscles) was cut. The results revealed that the gastrocnemius and soleus muscles contributed to the increase in joint PRT in hindlimb-suspended animals. Direct comparison is important for evaluating the effects of ECs, as shown by Gillette and Fell; however, such a trial has not been pursued previously.

In this study, we measured the PRT of rat ankle joints and examined the relationships between the ankle joint PRT and the gastrocnemius muscle-tendon complex extensibility. We addressed the following two specific hypotheses: (i) the ankle joint PRT is increased by the ECs of the gastrocnemius, and (ii) the ankle joint PRT is related to the gastrocnemius passive tension. To assess these hypotheses, based on the reports of Gillette and Fell (1996) and Gajdosik et al. (1999), we developed equipment for measurement of the PRT of the rat ankle joint. The passive tension of gastrocnemius was also evaluated as reported by Whitehead et al. (2001). We also measured the muscle mass after ECs to investigate edema formation.

Methods

Study 1

Animals

The protocol used in this experiment was approved by the Ethics Committee of Nippon Sports Science University. Six male Wistar rats (9 weeks old, 267–317 g) were purchased from CLEA Japan (Tokyo, Japan). The animals were housed individually and maintained on a 12:12-hour light-dark cycle with the lights on from 7:00 PM until 7:00 AM. Water and food were provided ad libitum during the experiments.

Equipment setup for eccentric contraction and torque measurement

The ankle torque of each animal was measured on a dynamometer, the mechanical setup of which is shown in Figure 1a (same as Nakazato et al. 2007). The torque of a stepping motor (RKD514HA, Oriental Motor, Japan) was transmitted to a footplate. The footplate and its angular velocity were adjustable at 5^{o} intervals. The final deceleration and backlash movement of the footplate were damped using a magnetic powder brake (ZKB-0.3AN, Mitsubishi Electric, Japan). The footplate was positioned such that the anatomical axis of the ankle coincided with the axis of the dynamometer shaft. The plantar flexion force was measured by installing a strain-gauge force transducer (LTB-2KA, Kyowa Electronic Instruments, Japan). The angular position was measured with a potentiometer (LP06M3R1HA, Murata Manufacturing, Japan). Both the force and position signals were sampled at 4000 Hz by using a data acquisition system (PowerLab/16SP, ADInstruments, Australia). We confirmed that this system could evaluate linear relationships from 1.225 to 2450 mNm. The coefficient of variance (CV) of 5 measurements in same subject was 0.0251.

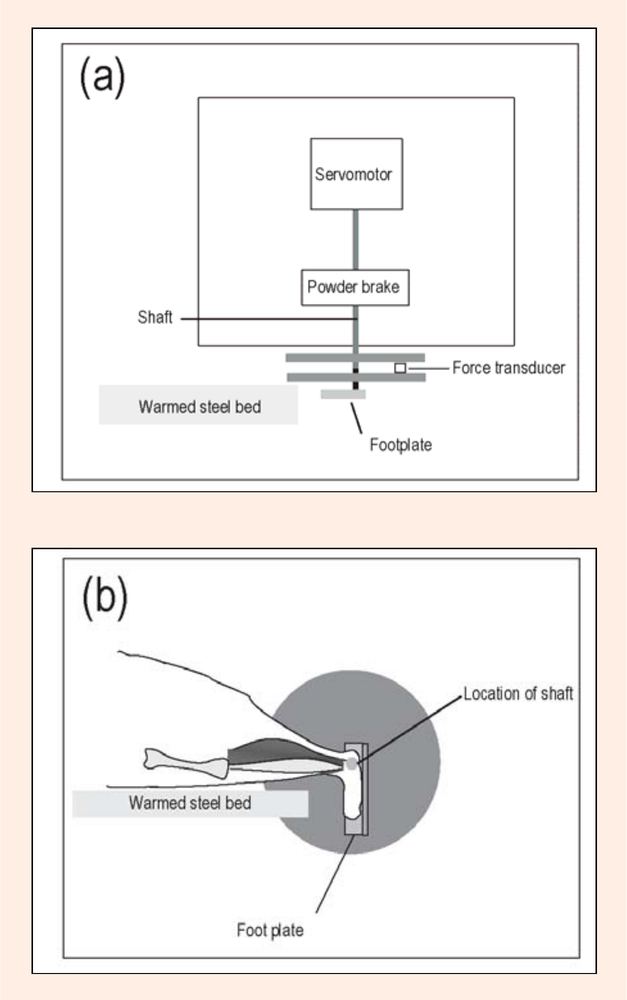

Figure 1. Diagram of the equipment. Top (a) and side (b) views are shown. See text for details (same as Nakazato et al. 2007).

Procedure for performing eccentric contractions on rat gastrocnemius muscles

Before we performed the ECs, all the animals were anaesthetized with sodium pentobarbital (1 mg/100 g body mass). The right lower leg of each animal was used for experimental intervention. As shown in Figure 1b (same as Nakazato et al. 2007), the anesthetized rats were firmly secured on the platform at ankle joint angle of 0°(defined as the angle at which the sole of foot and tibial bone are orthogonally positioned). The electrically stimulated contraction force was measured as follows. The gastrocnemius muscle was stimulated using pulses of 0.4-ms duration at supramaximal voltage (30 V). The stimulus voltage was adjusted to produce maximal isometric twitch force. The muscle was stimulated at 100 Hz to cause tetanic contraction. During tetanic contraction via a skin electrode, ECs were performed 10 times as a single bout with simultaneous forced dorsiflexion of the ankle joint (velocity, 15°/s; range of motion, from 0°to 45°).

Passive torque against ankle dorsi-flexion

The static PRT of the ankle joint was measured to evaluate joint stiffness as shown in our previous study (Ochi, E et al. 2007). The ankle joint of anesthetized rats was dorsi-flexed from 0° (defined as the angle at which the sole of the foot and tibial bone are orthogonally positioned) to either 30° or 45° at an angular velocity of 30°/s. The stress relaxation was allowed to proceed for >90 s until the passive torque reached an almost steady level. The values of torque measured 90 s after the stretch were used as static PRTs. A joint angle of 30 degrees was selected because this angle was the optimal angle observed in preliminary experiment. As for the reason for selecting PRT45 is to compare with previous study(Gillette and Fell, 1996). Six animals were measured at pretreatment, immediately after treatment, and days 1, 2, 3, 4, 5, 6, 8 and 10 after treatment.

Study 2

Animals

We did not only measure the joint stiffness in vivo but also the muscle passive tension in situ. Seventeen male Wistar rats (9 weeks old, 287–337 g) were purchased from CLEA Japan (Tokyo, Japan). The animals were housed as well as study 1. Seventeen rats were randomly assigned into two groups: the EC group (n = 9) and the sham-operated group (was not performed eccentric contractions, n = 8)

The passive tension of the exposed gastrocnemius muscle was measured to evaluate for the muscle-tendon extensibility. The knee joint and ankle of the anesthetized rats were fixed to a rigid metal frame. The exposed tissues were covered with phosphate-buffered saline that was retained in baths fashioned from skin flaps. The passive tension was recorded after the muscle tendon was fixed. The hindlimb was dissected to expose the gastrocnemius muscle. For this, it was necessary to free the gastrocnemius from the soleus muscle and cut and separate their tendons from the Achilles tendon. The distal tendon of the gastrocnemius muscle was connected to the lever arm of a servomotor at an arbitrary muscle-tendon unit length (Lf). The length was then increased in a series of 2-mm increments and stretched to 10 mm and holding the muscle at each length for 20 seconds. The passive tension was measured at each length by averaging the tension over 1 second at the end of the holding period. After the passive tension was measured, the gastrocnemius muscle was removed, trimmed free of connective tissue, and weighed.

Statistical analysis

Results are expressed as mean ± S.D. In the study of PRT, Dunnett's multiple comparison test was performed to compare the forces during pretreatment, immediately after treatment, and on days 1, 2, 3, 4, 5, 6, 8 and 10 after the treatment. The force during pre-treatment at day 0 was used as a control. In the study of the muscle passive tension, the Student's t test was used to compare the EC group and the control group. Significant differences were set at $P < 0.05$. Pearson's product–moment correlation coefficient was used to assess the relationship between parameters. Significant level was set at $P < 0.05$.The analysis program used was the statistical package for the social sciences SPSS software for Windows (SPSS Japan Inc., Japan).

Results

Study 1

Passive resistive torque of the ankle joint after eccentric contractions

Time course change of PRT at 30° after ECs is shown in Figure 2. The static PRT gradually increased and showed significantly higher values on days 2 ($p < 0.001$), 3 ($p < 0.01$), and 4 ($p < 0.001$) after the ECs. Time course change of PRT at 45° showed the same tendency (Figure2). Figure 3 shows the relative increase of ankle joint PRT30 after ECs. Relative PRT increased around 300%

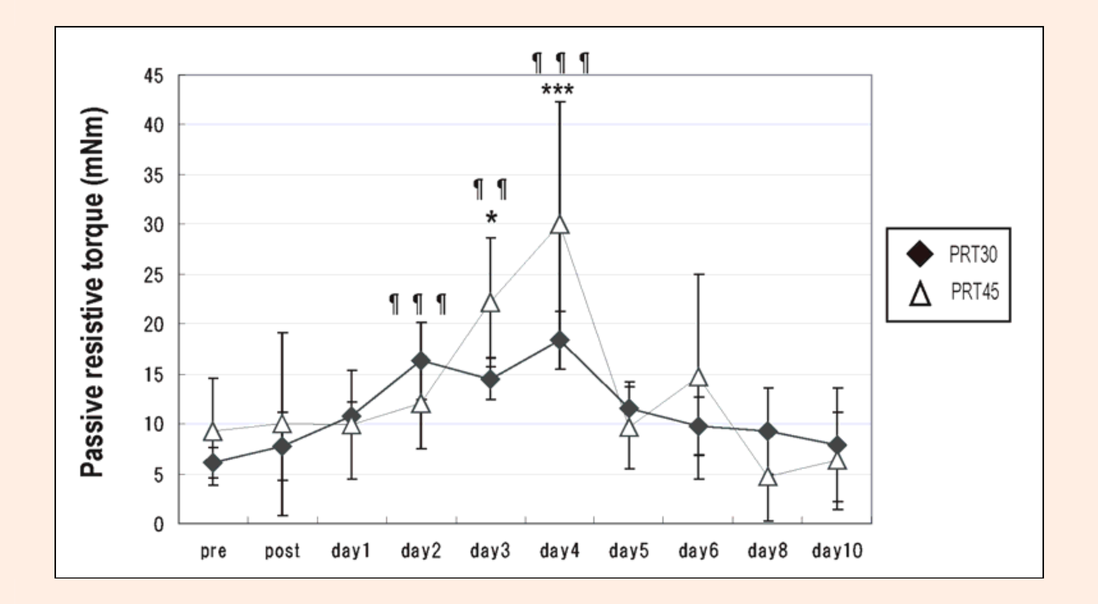

Figure 2. Changes in Passive resistive torque 30 and 45 degrees after eccentric contractions. Values are means and S.D. ¶¶¶ $p < 0.001$, ¶¶ $p < 0.01$, Significantly different from pre-treatment(PRT30). *** $p < 0.001$, * $p < 0.05$, Significantly different from pre-treatment (PRT45). pre: pretreatment, post: post-treatment.

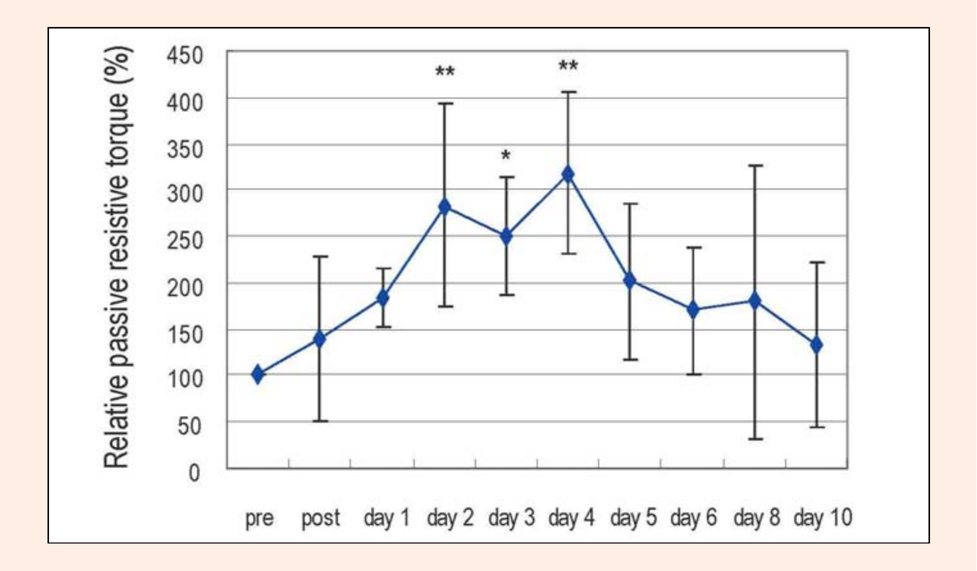

Figure 3. **Time course changes in relative passive resistive torque. Values are means and S.D.** ** $p < 0.01$, * $p < 0.05$, Significantly different from pre-treatment. pre: pretreatment, post: post-treatment

on day 2 after ECs. The higher tendency of PRT continued until day 4.

Correlation between PRT30 and PRT45

We calculated correlation between PRT30 and PRT45. We confirmed that positive correlations existed between PRT30 and PRT45 ($r = 0.839$; $p < 0.01$).

Study 2

Passive tension of the exposed gastrocnemius muscle

The passive tension of the exposed gastrocnemius muscle on day 4 after ECs was measured (Figure 4). The passive tensions of the EC group at 4, 6, 8, and 10 mm were significantly higher than those of the sham-operated group. On the other hand, muscle wet mass of the ECs group was similar to the sham-operated group (ECs group; 1.49 ± 0.07, Sham-operated group; 1.45 ± 0.13).

Correlation between passive resistive torque and passive tension

In the study 2, we calculated that correlation between PRT30 and passive tension of gastrocnemius at 10 mm elongation (shown in Figure 5). Significant positive correlation was observed between these two parameters ($r = 0.682$, $p < 0.01$).

Discussion

In this study, we confirmed that ECs raise joint PRT and are associated with reduction of muscle passive tension. The changes in joint stiffness and muscle passive tension after ECs have been examined independently and the direct relationships have not been examined previously (Nosaka and Clarkson, 1995; Whitehead et al., 2001). We experimentally showed that ECs increased both joint PRT and muscle passive tension and these two parameters were significantly correlated. Although the variation of each parameter existed, we think that it is due to the individual differences. Hereafter, we will discuss changes in PRT and muscle passive tension after ECs.

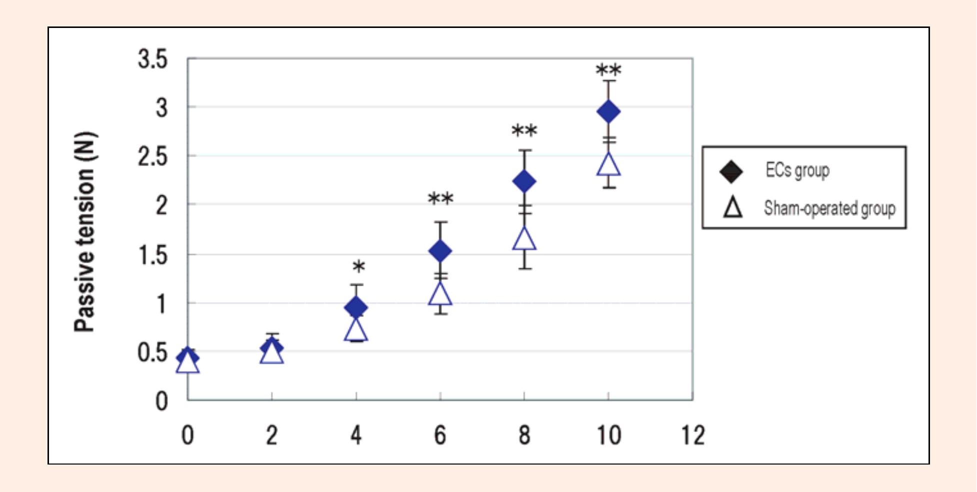

Figure 4. **Effect of eccentric contractions on passive tension of the gastrocnemius muscle. Values are means and S.D.** ** $p < 0.01$, * $p < 0.05$, Significant differences between groups.

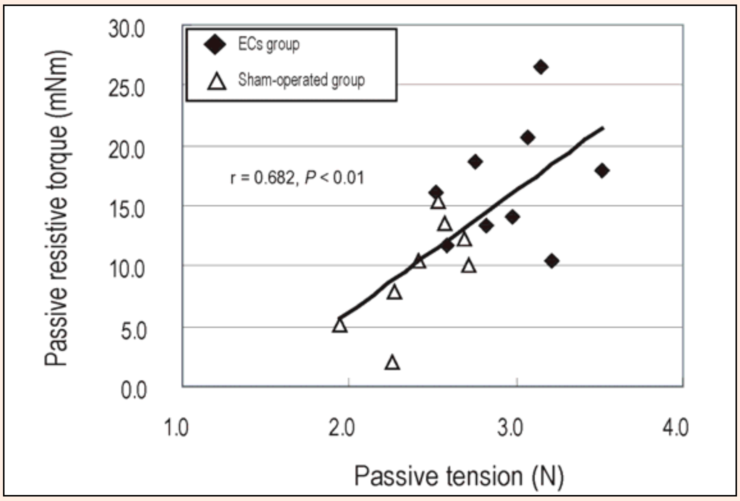

Figure 5. **Correlations between passive resistive torque of ankle joint and passive tension of gastrocnemius muscle.** Statistical analysis reveals that passive resistive torque has a positive correlation with muscle passive tension (r = 0.682; p < 0.01).

We measured PRT as rat ankle joint stiffness. PRTs of rat ankle joint have not been fully examined previously. Gillette and Fell (1996) showed that passive resistive tension of rat ankle joint at 45° was about 50 – 60 g, but they did not show absolute torque. Given that the lever arm of the rat ankle joint is 1.5 cm in our present data, then the PRT at 45° is 7.35 – 8.82 mNm. We measured PRT45 and the obtained value ranged from 4 to 15 mNm. Thus, we confirmed that our experimental setups gave relational value for PRT. We also confirmed that significant correlations existed between PRT30 and PRT45 ($r = 0.839$, $p < 0.01$, shown in Table.1). Above all, we conclude that our employed parameters of PRT30 and PRT45 accurately reflect stiffness of rat ankle joint.

We confirmed that a significant correlation exists between gastrocnemius muscle passive tensions and PRT on day 4 after ECs. This is the first study to show a direct correlation between them after ECs. Gillette and Fell (1996) showed that hindlimb uploading made rat ankle joint stiff, and this increased passive torque was due to musculotendinous units, especially gastrocnemius. Although another model was reported, Gillette's study and ours indicate that the viscoelastic properties of gastrocnemius are a major factor for determining ankle joint stiffness. On the other hand, thixotropic behaviors at a relaxed joint are attributed both to the joint structures and to short-range stiffness of muscles acting at the joint (Wiegner, 1987). Since thixotropic behavior is a normal joint characteristic, abnormal gastrocnemius extensibilities become apparent in the eccentric contracted joint.

Gastrocnemius muscle wet mass of the ECs group was similar to that of the sham-operated group. Chleboun et al. (1998) showed that elbow flexor volume increased when elbow joint stiffness increased after ECs of elbow flexor. Since elevated muscle wet mass and muscle volume after ECs suggest existence of edema, our results suggest that factors other than edema contribute to this phenomenon. Proske et al. (2005) proposed that the reason for the rise in passive tension is that after sarcomere disruption by the ECs, there is the likelihood of membrane damage, perhaps at the level of the t-tubules or sarcoplasmic reticulum. The consequent uncontrolled release of Ca^{2+} into the sarcoplasm activates the contractile filaments to develop an injury contracture (Proske and Allen, 2005). We cannot exclude the contribution of edema, but we consider that Ca^{2+} also plays an important role in joint and muscle stiffness after ECs.

Conclusion

In the present study, we determined that one bout of ECs increases both joint stiffness and gastrocnemius passive tensions. We also confirm that there were significant correlations between ankle joint PRT and gastrocnemius muscle-tendon complex extensibilities. We also show the possibility that joint stiffness is associated with muscle passive tension, independent of edema formation.

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Key points

- We confirmed that ECs raise joint PRT and are associated with reduction of muscle passive tension.
- The changes in joint stiffness and muscle passive tension after ECs have been examined independently and the direct relationships have not been examined previously.
- We experimentally showed that ECs increased both joint PRT and muscle passive tension and these two parameters were significantly correlated.

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