Measurement of excitation-contraction coupling time in lower extremities

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ABSTRACT. Objective: The aim of this study was to apply a novel method to measure excitation-contraction coupling time (ECCT) in normal soleus muscles. Methods: We performed simultaneous recordings of soleus compound muscle action potential (CMAP) and foot movement-related potential (MRP), and measured ankle plantar flexion torque in 36 healthy subjects. We calculated ECCT and examined the relations between CMAP, MRP, ECCT and ankle plantar flexion torque. Results: Statistical analyses established reference ranges (mean ± SE) for CMAP (13.4 ± 0.9 mV), MRP (5.3 ± 0.4 m/s²), ECCT (5.2 ± 0.1 ms), torque (85.9 ± 6.4 Nm) and torque/body weight (1.4 ± 0.1 Nm/kg). The torque showed a positive linear correlation with CMAP (p = 0.041) and a negative linear correlation with ECCT (p = 0.045). Conclusion: Soleus ECCT can be recorded easily, and is useful to assess the impairment of E-C coupling in muscles of the lower extremities.

Key words: excitation-contraction coupling, soleus muscle, plantar flexion, torque, accelerometer

Excitation-contraction (E-C) coupling in skeletal muscles involves a number of steps. After generation of a muscle action potential, sarcolemmal depolarization spreads transversely to the T tubules. The voltage sensor of the T tubules transmits the electric charge to the ryanodine receptor, resulting in channel opening. This opening leads to Ca²⁺ release from the sarcoplasmic reticulum into the myoplasm. Binding of the released Ca²⁺ to troponin triggers an interaction between actin and myosin filaments. Finally, these filaments slide and muscle fiber length shortens. In our previous studies, we developed a novel method (Imai’s method) to estimate the time from muscle depolarization to initiation of muscle contraction. In Imai’s method, masseteric compound muscle action potentials (CMAPs) and mandibular movement-related potentials (MRPs) are simultaneously recorded using an accelerometer, after trigeminal nerve stimulation with a needle electrode. The E-C coupling time (ECCT) is obtained from the difference in onset latencies between masseteric CMAP and mandibular MRP. By measuring the bite force using a specialized pressure-sensitive sheet, the correlation between ECCT and bite force can be analyzed. We also applied the principles of this method to intrinsic hand muscles, and recorded ECCTs and MRPs in the hand muscles to estimate the contribution of impairment of E-C coupling to muscle weakness in neuromuscular diseases.

Although decreased MRPs and prolonged ECCTs have been demonstrated in the masseter and intrinsic hand muscles in patients with various neuromuscular diseases, these procedures have not been applied to muscles in the lower extremities. The lower limb muscles are an important target for physical therapy. Applying the technique of ECCT measurement to unconscious patients would allow detection of the onset of E-C coupling impairment even in the absence of any voluntary movement, and facilitate decision on the timing of initiating physical therapy.

In the present study, the soleus muscle was chosen as the lower limb muscle, and the relations between CMAP, MRP, ECCT, and muscle strength (torque) were examined in healthy subjects.
Methods

1. Subjects

We studied 36 healthy subjects (22 males and 14 females) aged 22 to 82 years (median, 24 years; mean, 40.3 years) at Sapporo Medical University Hospital.

The inclusion criteria and exclusion criteria were as follows:

- **Inclusion criteria**
  - Person 18 years of age or older
  - Person who could maintain supine and prone positions, which are the measurement positions
  - Person who could understand the explanations of the assessor and undergo measurements
  - Person who gave informed consent to participate in this research

- **Exclusion criteria**
  - Person with neurological or orthopedic disorders that may affect measurement
  - Person who had significant contractures on the joints of the extremities and who had difficulty performing measurements
  - People who had inflammation, abrasions, incision, or other wounds on the skin of the body part on which electrical stimulation and electrodes were installed

The median (mean, range) height, weight, body mass index (BMI) and shoe size were 166.0 (166.1, 149.0-181.0) cm, 59.5 (61.8, 46.0-89.0) kg, 22.2 (22.3, 17.3-29.1) and 24.5 (25.0, 23.0-28.5) cm, respectively. A subject was instructed to lie supine on the bed for measurement of range of motion (ROM) and E-C coupling time (Fig. 1), and then lie prone on a custom-made apparatus for torque measurement (Fig. 2). These measurements were performed successively within an hour in our laboratory.

This study was approved by the ethics committee, Sapporo Medical University, Sapporo, Japan (reference number 23-86). All subjects gave informed consent for participation in this study.

2. Measurement of excitation-contraction coupling time

All stimulating and recording procedures were performed using an electromyograph (Nicolet Biomedical, Nicolet Viking Select). Before the electrophysiological assessment, the baseline ankle joint angle was measured using a goniometer (Tokyo University type, 300 mm in length). CMAPs were recorded from the soleus muscle using surface disc electrodes in belly-tendon arrangement, after tibial nerve stimulation at the popliteal fossa. The active elec-
trode (G1) was placed over the belly of soleus muscle at one-third of the distance from the popliteal crease to the heel, and reference electrode (G2) over the Achilles tendon at one-third of the distance from the heel to the popliteal crease. Simultaneously, MRPs were recorded using an accelerometer (NEC, SV1101) taped at the base of hallux (Fig. 1). The amplitude of CMAP was measured from the baseline to the negative peak using a cursor. The maximal acceleration of foot movement was obtained from the initial peak amplitude of MRP. For stimulation, a 0.2-ms rectangular pulse was delivered to the tibial nerve at the popliteal fossa with gradually increasing intensity to reach a supramaximal response. Once a supramaximal CMAP was obtained, resting CMAP and MRP were recorded successively.

The ECCT was defined as the difference in onset latencies between soleus CMAP and plantar MRP (Fig. 1). We confirmed the reproducibility of the test by repeating a set of several recordings (four times minimum), although our previous study already showed that the latencies of mandibular and thumb MRP were highly reproducible with minimal inter-trial variation. The MRP with the shortest latency was used as the representative data for measurements of ECCT and maximal acceleration.

3. Measurement of torque during isometric ankle plantar flexion

The isometric ankle plantar flexion task was performed using a custom-made ankle joint torque meter (Takei Scientific Instruments Co. Ltd.) (Fig. 2). The subject was instructed to lie prone on the bed. The foot was fixed to the foot plate by a strap, and the ankle joint angle was flexed at 0° for ankle dorsiflexion. The subject was asked to exert force only to execute ankle plantar flexion during isometric contraction, the subject was asked to exert force to execute only ankle plantar flexion. Each subject performed three trials with ≥ 2 min of rest between trials. If the three exerted forces differed by more than 5% between two successive trials, an additional trial was imposed. To record the maximum ankle plantar flexion torque, vigorous encouragement was given to the subject during isometric contraction. After three favorable recordings were completed, the three torques obtained were averaged, and the averaged value was then used as the representative torque during isometric ankle plantar flexion. We also calculated the ratio of torque per body weight (Nm/kg) using the averaged torque and body weight of each subject.

4. Effects of age

The CMAP, MRP, ECCT and ankle plantar flexion torque measured in this study may be affected by age. Therefore, we divided the subjects by the mean age of the group (40.3 years) as the cutoff point into a younger group (< 40 years) and an older group (> 40 years), and compared the variables between the two groups.

5. Statistical analysis

Before correlation analysis, Shapiro-Wilk test was conducted to examine if each variable was normally distributed. We used Pearson’s correlation coefficient when the variable is normally distributed, and Spearman’s rank correlation coefficient when the variable is not normally distributed. Correlation between variables was examined using linear regression analysis. The t-test was used to compare the differences between groups for each variable. The computer software package IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis, and a p-value of below 0.05 was considered as statistically significant.
Results

The median (mean, range) ROM of ankle joint was 17.5 (19.2, 5.0-50.0)° for dorsiflexion and 55.0 (51.4, 30-75)° for plantar flexion. In supine position for ECCT measurement (Fig. 1), the baseline angle of the ankle joint (mean ± SD) was 38.3 ± 7.2° for dorsiflexion. The median (mean, range) ROM of ankle joint was 17.5 (19.2, 5.0-50.0)° for dorsiflexion and 55.0 (51.4, 30-75)° for plantar flexion. In supine position for ECCT measurement (Fig. 1), the baseline angle of the ankle joint (mean ± SD) was 38.3 ± 7.2° (plantar flexion). Table 1 shows the average values of electrical and mechanical parameters in 36 normal subjects. We confirmed that all these variables were normally distributed using Shapiro-Wilk test before conducting correlation analysis. The values (mean, SE) of were normally distributed using Shapiro-Wilk test before conducting correlation analysis. The values (mean, SE) of CMAP, MRP, ECCT, ankle plantar flexion torque and torque/body weight were 13.4 ± 0.9 mV, 5.3 ± 0.4 m/s², 5.2 ± 0.1 ms, 85.9 ± 6.4 Nm and 1.4 ± 0.1 Nm/kg, respectively.

Table 2 showed Pearson’s correlation coefficients for the relations between CMAP, MRP, ECCT, ankle plantar flexion torque and torque/body weight. A significant correlation was observed between CMAP and MRP (p = 0.031), CMAP and torque (p = 0.041), and ECCT and torque (p = 0.045). The plantar flexion ROM correlated significantly with mechanical parameters such as plantar flexion torque (p = 0.015) and torque/body weight (p = 0.004), but did not correlate with electrophysiological parameters of CMAP, MRP and ECCT. In addition, the shoe size and the measured angle of the ankle joint, which were expected to affect MRP, did not correlate with MRP (r = 0.052; p = 0.764, r = 0.158; p = 0.359).

CMAP and MRP increased while ECCT decreased as ankle plantar flexion torque increased (Fig. 3). The torque of plantar flexion showed a positive linear correlation with CMAP amplitude (R² = 0.116, p = 0.041; regression equation: [mV] = 0.047 × plantar flexion torque [Nm] + 9.368) and a negative linear correlation with ECCT (R² = 0.113, p = 0.045; regression equation: [mV] = -0.008 × plantar flexion torque [Nm] + 5.824). The relation between torque and MRP did not reach statistical significance (R² = 0.0142, p = 0.178; regression equation: [mV] = 0.0142 × plantar flexion torque [Nm] + 4.042).

The variables for each group are presented as mean ± SE. Since each variable had a normal distribution, Student’s t-test was performed and the results are shown in Table 2. The CMAP was significantly lower in the older group (10.5 ± 1.1 mV) than in the younger group (15.0 ± 1.1 mV) (t = 5.926; df = 33.905; p = 0.001). Similarly, MRP was significantly lower in the older group (4.3 ± 0.4 m/s²) than in the younger group (5.8 ± 0.6 m/s²) (t = 5.751; df = 34; p = 0.001). On the other hand, ECCT was not significantly different between the younger (5.3 ± 0.2 ms) and the older groups (5.0 ± 0.3 ms) (t = 0.751; df = 34; p = 0.458). The ankle plantar flexion torque was significantly lower in the older group (53.0 ± 5.0 Nm) than in the younger group (104.5 ± 7.1 Nm) (t = 5.926; df = 33.905; p <0.0005). These results show that CMAP amplitude, MRP, and ankle plantar flexion torque are affected by age, whereas ECCT is not affected.

Discussion

The present results showed a significant correlation between soleus CMAP and MRP amplitude, and between

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<th>Table 1. Average values of electrical and mechanical parameters in normal subjects (n=36)</th>
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<td>CMAP amplitude</td>
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Data are expressed as mean ± standard error. Torque denotes ankle plantar flexion torque.
CMAP, compound muscle action potential; MRP, movement-related potential; ECCT, excitation-contraction coupling time

| Table 2. Pearson’s correlation coefficients of electrical and mechanical parameters |
|-----------------|----------------|----------------|----------------|----------------|
|                 | MRP             | ECCT           | Torque         | Torque/body weight |
| CMAP amplitude  | 0.361*          | 0.003          | 0.343*         | 0.477**         | 0.135 |
| MRP             | -0.137          | 0.230          | 0.270          | 0.119          |
| ECCT            | -0.336*         | -0.185         | 0.198          |
| Torque          | 0.926**         | 0.402*         |
| Torque/body weight |                | 0.470**       |

* p < 0.05, ** p < 0.01, significant correlation between 2 parameters:
Torque and ROM indicate ankle plantar flexion torque and range of motion of ankle plantar flexion, respectively.
CMAP, compound muscle action potential; MRP, movement-related potential; ECCT, excitation-contraction coupling time
soleus CMAP and torque of ankle plantar flexion. In addition, soleus MRP was higher as torque increased, although the relationship did not reach statistical significance for linear correlation. These results indicate that soleus CMAP and muscle strength of ankle plantar flexion could be estimated using soleus MRP. Furthermore, a significant correlation was found between soleus ECCT and muscle strength, similar to the significant correlation between masseteric ECCT and bite force\(^7\). A change in bite force should represent a change in strength of the masseter muscle, because a significant correlation between the magnitude of bite force and masseter morphology has been reported\(^{11,12}\). On the other hand, the torque of ankle plantar flexion may include activities of muscles other than the soleus muscle, such as posterior tibial muscle and toe flexor muscles\(^{13}\). Therefore, it should be noted that our results may not indicate a direct correlation between soleus muscle strength and soleus CMAP, MRP or ECCT.

The present method can be applied to assess the impairment of E-C coupling associated with neuromuscular disorders even in unconscious patients. For example, intensive care unit-acquired weakness (ICUAW) may be a major target for clinical application of this method. Studies have indicated that ICUAW may be caused by inactivation of muscle membrane derived from sodium channel dysfunction and selective loss of myosin in the skeletal muscle fibers\(^{14,15}\). In addition, an experimental study revealed impaired calcium ion release from the sarcoplasmic reticulum, which may in turn cause impairment of E-C coupling in a rat model of ICUAW\(^{16}\). Currently, there is no useful technique for assessment of the onset of ICUAW in unconscious patients. The present technique would allow detection of the development of ICUAW even in the absence of any voluntary movement, and facilitate clinical decision of the prescription of physical therapy as soon as ICUAW occurs.

Previous publications have reported the effects of aging on joint ROM as reflected by plastic changes of the muscle-tendon complex and age-related deterioration in flexibility\(^{17,18}\). However, our results show no significant correlation between torque of plantar flexion and CMAP (A), MRP (B) or ECCT (C). Linear regression analyses show a significant positive correlation between torque and CMAP (p = 0.041) and a negative correlation between torque and ECCT (p = 0.045), but no significant correlation between torque and MRP (p = 0.178). CAMP, compound muscle action potential; MRP, movement-related potential; ECCT, excitation-contraction coupling time.
relation between ankle ROM and electrophysiological parameters including CMAP, MRP and ECCT. These results indicate an additional advantage of our technique in allowing robust measurement without being affected by ankle ROM. On the other hand, aging may affect the distribution of skeletal muscle fiber type and sarcoplasmic function. Age-related increase of slow twitch fibers may induce slowing of muscle contraction and decrease of phasic muscle power. Also, functional decline of sarcoplasmic reticulum may induce decrease of calcium ion release in the muscle fiber, which may result in impairment of E-C coupling. Further studies in healthy subjects aged over 60 are required to elucidate the possible relationship between aging and the electrophysiological parameters measured using our technique, and to establish the precise age-matched normal ranges of these parameters.

Conclusions

In this study, the normal values of CMAP, MRP, ECCT, and muscle strength of the triceps surae muscles were established, and the relationships of these parameters were demonstrated in healthy subjects. The present method is useful for assessing E-C coupling impairment in the lower limb muscles.

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Conflict of Interest: None of the authors has any conflict of interest to disclose.

References