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Research paper

A new method to evaluate staircase phenomenon in skeletal muscle using piezoelectric sensor

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A B S T R A C T

Objective: The staircase phenomenon, which refers to the increases in the force of contraction with repetitive stimulation of the muscle, has been studied for many years, but the method is difficult and not widely used. Our objective was to evaluate the staircase phenomenon in skeletal muscle using a piezoelectric sensor.
Methods: Thirty-five subjects without neuromuscular diseases (normal controls), 11 patients with Becker muscular dystrophy (BMD), and 19 patients with myotonic dystrophy type 1 (MyD) were studied. Compound muscle action potential (CMAP) and movement-related potential (MRP) waveforms were recorded using piezoelectric sensors during repetitive stimulation of the median nerve, and the amplitudes and durations were measured. Excitation-contraction (E-C) coupling time (ECCT) was calculated from the difference between onset latencies of CMAP and MRP.
Results: In normal controls, MRP amplitude ratio (relative to baseline) increased significantly with increase in stimulation duration and with increase in stimulation frequency. In BMD and MyD, however, MRP amplitude ratio did not change significantly with increase in stimulation duration. Especially, in MyD, there was no change in MRP amplitude ratio with increase in frequency.
Conclusion: Staircase phenomenon abnormalities can be evaluated easily using piezoelectric sensors, indicating their potential utility for evaluating E-C coupling impairment in myopathies.
Significance: Piezoelectric sensors may be a useful tool to evaluate staircase phenomenon in skeletal muscle.

1. Introduction

The staircase phenomenon is a contraction response discovered in cardiac muscle of frog. The phenomenon was named for the fact that when cardiac muscle is stimulated at a certain frequency, the force of contraction increases and the graph capturing this phenomenon resembles a staircase (Niedergerke, 1956; Puglisi et al., 2013). Similar effect is also observed in skeletal muscles (Ritchie and Wilkie, 1955).

Although the staircase phenomenon has been researched mainly *in vitro*, *in vivo* studies in humans have been reported since the 1950 s (Desmedt. 1958). Subsequent studies have shown that abnormalities of the staircase phenomenon suggest impairment of excitation–contraction (E-C) coupling, and that the staircase phenomenon is impaired in myasthenia gravis (MG) (Slomić et al., 1968) and myopathies (Krarup and Horowitz, 1979). However, the method of evaluating the staircase phenomenon by measuring the contraction force has not been widely used because of the complexity of the equipment.

Our group has studied E-C coupling impairments by measuring excitation–contraction coupling time (ECCT), which is calculated from the difference in onset latency between compound muscle action potential (CMAP) and movement-related potential (MRP) measured using an accelerometer, and we have shown that ECCT is prolonged in various pathological conditions (Tsuda et al., 2010; Imai et al., 2012; Yamamoto et al., 2016; Asada and Imai, 2020; Hirose et al., 2022). However, the accelerometers that we have been using are industrial products used to measure vibrations in machinery, and it is difficult to apply them to clinical settings. In contrast, piezoelectric sensors are widely accepted for medical purposes and are easy to use. In the field of clinical neurophysiology, these sensors are commonly used in diagnostic tests for sleep apnea (Berry et al., 2012). The piezoelectric sensor detects mechanical deformation by generating an electrical signal in response to applied force. When the sensor is placed on the surface of a muscle, it measures the strain caused by muscle contraction, which is proportional to the force exerted. We succeeded in obtaining MRPs using a polyvinylidene fluoride (PVDF) sensor, which is one of the piezoelectric sensors (Xu et al., 2023). This sensor allows quantification of the force applied to the

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sensor and evaluation of the staircase phenomenon.

The purpose of this study was to prove that the staircase phenomenon can be evaluated using a piezoelectric sensor during repetitive nerve stimulation, and to examine whether the staircase phenomenon is impaired in patients with muscular dystrophy. Changes in ECCT were also evaluated simultaneously.

2. Materials and methods

2.1. Subjects

We studied 11 patients with Becker muscular dystrophy (BMD) aged 34 to 75 years (mean, 58.8 years), 19 patients with myotonic dystrophy type 1 (MyD) aged 28 to 67 years (mean, 48.8 years) and 35 healthy individuals (normal controls; 18 males and 17 females) aged 25 to 65 years (mean, 45.6 years) between May 2023 and February 2024 at the National Hospital Organization Hakone Hospital.

Healthy volunteers without neuromuscular diseases were enrolled in the normal control group. BMD and MyD patients were diagnosed based on the clinical picture and genetic testing. All patients manifested muscle weakness, with BMD predominantly affecting the proximal muscle and MyD affecting the distal muscles. We excluded patients with joint contractures that would interfere with the examination, those who were bedridden, and those on ventilators.

All subjects gave informed consent to participate in the examinations. The study was reviewed and approved by the ethics committees of National Hospital Organization Hakone Hospital (approval number: 22–2).

2.2. Protocol

The subject was instructed to lie supine on the bed, with the forearms in external rotation and the shoulder joint in 0-30° abduction. All stimulating and recording procedures were performed using an electromyograph (Neuropack 2300; Nihon Kohden Co., Tokyo, Japan). A piezoelectric sensor, PIEZO FILM SENSOR (TP-028, Kureha Trading Co., Ltd. Tokyo, Japan) for MRP study was attached to surface electrode R1 for nerve conduction study, which was placed on the abductor pollicis brevis muscle (Fig. 1). Surface electrode R2 was placed on the tendon of the first metacarpophalangeal joint. Surface electrodes, which were rectangular (25 × 45 mm) and made of silver/silver chloride (Ag/AgCl), were used. The potentials from the surface electrodes R1 and R2 were

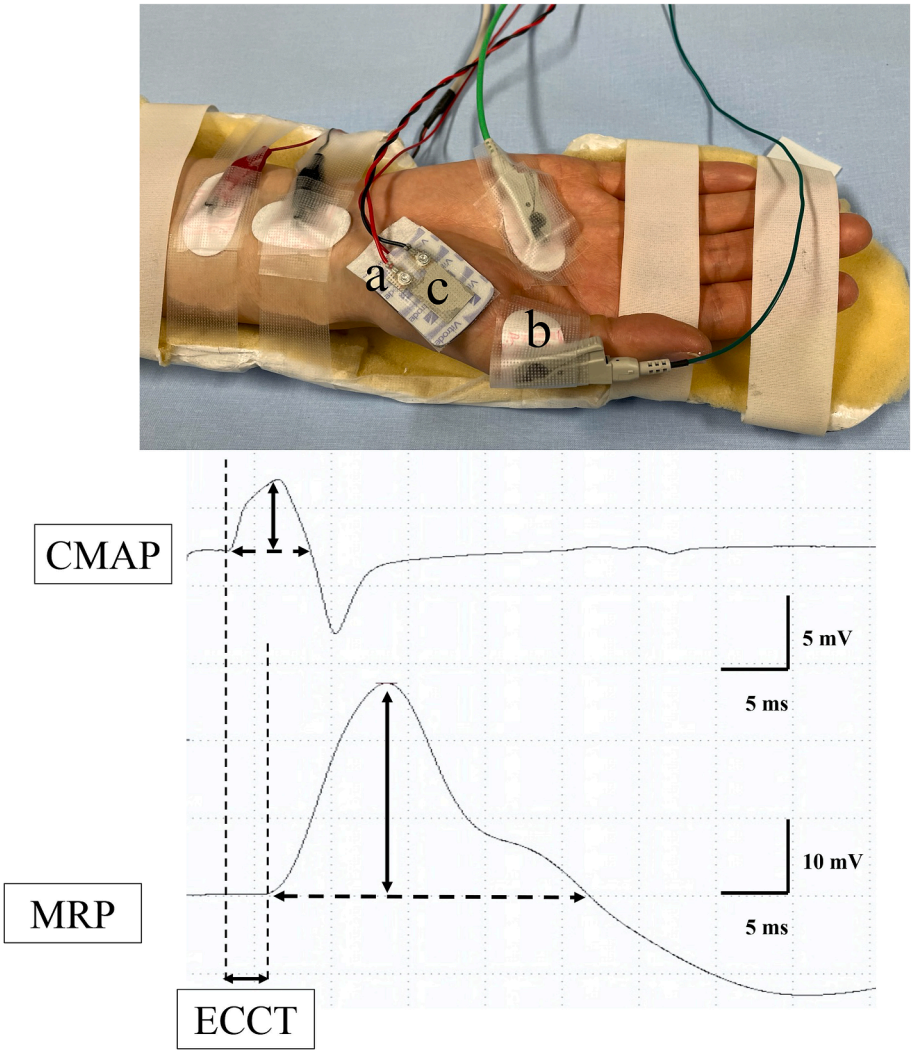


Fig. 1. Placement of stimulation and recording electrodes and piezoelectric sensor, and typical recorded waveforms. Upper panel: Surface electrode R1 is placed on the abductor pollicis brevis muscle (a), and surface electrode R2 on the tendon of the first metacarpophalangeal joint (b). A piezoelectric sensor is attached to surface electrode R1 (c). Lower panel: Vertical solid double-ended arrows indicate the CMAP and MRP amplitudes. Horizontal dashed double-ended arrows show the CMAP and MRP durations. Vertical dashed lines denote the onset latencies of CMAP and MRP, and the difference in the two onset latencies is the ECCT. CMAP, compound muscle action potential; MRP, movement-related potential; ECCT, excitation–contraction coupling time.

input to channel 1 and that from the piezoelectric sensor was input to channel 2 of the electromyograph, and these potentials were recorded on the screen as CMAP and MRP, respectively. Four fingers other than the thumb were fixed to a fixture made of thermoplastic and Velcro, so that MRP of the thumb could be recorded. The bandpass of amplifier was 10 – 5000 Hz for CMAP and 20 – 2000 Hz for MRP recordings.

The stimulation protocol was as follows: the median nerve at the wrist was repetitively stimulated electrically for 120 s followed by 120 s of rest. CMAP and MRP were recorded simultaneously before stimulation (baseline) and at 5 s, 15 s, 30 s, 60 s, 90 s and 120 s after nerve stimulation was started, then during rest at 30 s, 60 s, 90 s and 120 s after stimulation ended (150, 180, 210, and 240 s from start of stimulation). Recording was conducted at electrical stimulation 20 % higher than the maximum, which sufficiently ensured that the stimulation was supramaximal. Stimulation was conducted at frequencies of 1, 2 and 3 Hz in the controls. In BMD and MyD, the stimulation frequencies were 2 and 3 Hz, considering patient tolerance to the test and time constraint.

We recorded CMAP and MRP from the abductor pollicis brevis muscle. Typical CMAP and MRP waveforms are shown in Fig. 1. On the CMAP and MRP waveforms, amplitude was measured from the baseline to the negative peak, and duration from the onset to the return of the signal to the baseline.

The onset latency was recorded with a recording sensitivity of 2 mV/division for CMAP and 5 mV/division for MRP. Basically, the automatically measured latencies were used, but in cases where the generated latencies clearly differed from the actual measurements, the latencies were determined by the investigator (BH), and reproducibility was checked by the raters (RY and ET). ECCT was calculated from the difference between the onset latencies of CMAP and MRP.

2.3. Evaluation of the staircase phenomenon

Fig. 2 illustrates the MRP amplitude ratio relative to baseline during the 2-Hz stimulation protocol for a representative normal control subject, BMD patient, and MyD patient. In this study, normal staircase phenomenon was defined as the increase in MRP amplitude ratio relative to baseline with increase in duration of continuous stimulation and with increase in stimulation frequency, as shown by the normal control in Fig. 2.

We compared the MRP amplitude ratios between normal control and BMD groups as well as between normal control and MyD groups at the 60-second and 120-second time points during the 120-second stimulation protocol. Abnormality of the staircase phenomenon was evaluated based on the lower limit of the 95 % range of the MRP amplitude ratios in normal controls as the cutoff.

2.4. Evaluation of excitation–contraction coupling time

Excitation-contraction (E-C) coupling is a series of processes from the onset of action potentials in the muscle membrane to the propagation of excitation to the T-tubules and the release of Ca^{2+} from the sarcoplasmic reticulum via dihydropyridine receptors and ryanodine receptors, resulting in muscle contraction. The time between the generation of CMAP derived from the electrode and that of MRP derived from the piezoelectric sensor can be taken as the time required for the E-C coupling. This latency difference was defined as ECCT in this study. Changes in ECCT during recordings in the stimulation protocol were evaluated.

2.5. Statistical analysis

The JMP statistical program (SAS Institute Inc., Cary, NC, USA) was used for data analysis. Shapiro-Wilk test was used to examine the normality of continuous variables. Steel-Dwass multiple comparison test was used to test the difference between groups. Friedman test was used to assess significant differences in the trend of each parameter during repetitive stimulation. Repeated measures analysis was performed using a mixed model with the restricted maximum likelihood (REML) method to test the differences in the trend of MRP amplitude ratio and ECCT among frequencies, with fixed effects of time and frequency. Mann-Whitney *U* test was used to compare MRP amplitude ratios between normal control and BMD, as well as between normal control and MyD, at 60 s and 120 s of stimulation. A P value < 0.05 was considered statistically significant. ROC (Receiver Operating Characteristic) analysis was performed to evaluate the diagnostic performance of MRP amplitude ratios at different frequencies and times. The area under the curve (AUC) was calculated to determine the overall accuracy of the test. Cutoff values were established based on the maximum Youden index, which optimally balanced sensitivity and specificity. Sensitivity and specificity at each cutoff point were also computed to assess the test's ability to correctly identify patients with BMD and MyD compared to normal controls.

3. Results

3.1. Baseline parameters in controls and muscular dystrophies

Demographics and electrophysiological findings at baseline (before

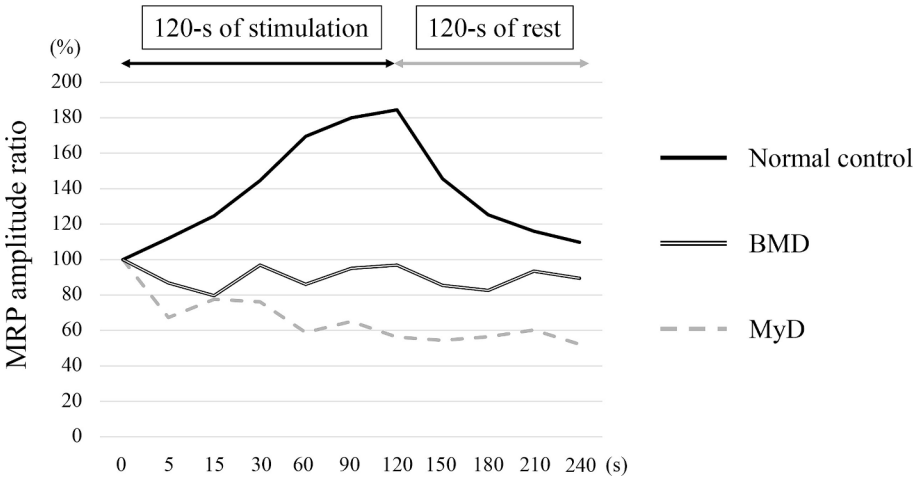


Fig. 2. Representative data of one subject each in normal control, BMD and MyD groups showing changes in MRP amplitude ratio (value at each time point divided by baseline value) during the stimulation protocol at 2 Hz. The black line represents normal control, the double line represents BMD, and the gray dashed line represents MyD.

starting nerve stimulation) of normal controls, BMD and MyD are shown in Table 1. The BMD patients was significantly older than the normal controls. BMD and MyD showed lower baseline CMAP amplitude, MRP amplitude, and higher ECCT compared to normal controls. Baseline CMAP and MRP durations did not differ among three groups, except CMAP duration between control and MyD. No significant differences in baseline electrophysiological findings were found between BMD and MyD.

3.2. Changes in parameters during stimulation protocol in controls

Mean ratios (value at each time point divided by baseline value) of CMAP and MRP amplitude and duration, and mean ECCT during the stimulation protocol (stimulation for 120 s followed by resting for 120 s) in normal controls are shown in Fig. 3. The CMAP amplitude ratio fluctuated only slightly, and was maintained within the 1–2 % range at stimulation frequencies of 1 Hz, 2 Hz, and 3 Hz. The MRP amplitude ratio significantly increased with increase in duration of nerve stimulation ($p < 0.001$ for all frequencies). The mean ratios at 120 s of stimulation were 122 %, 138 % and 150 % at 1 Hz, 2 Hz and 3 Hz, respectively, showing higher amplitude with increase in frequency. After the end of stimulation, the amplitude ratios decreased at all frequencies. MRP amplitude ratio varied significantly with time and with frequency ($p < 0.001$). An interaction between time and frequency was also observed ($p = 0.0022$). ECCT was shortened significantly at 120 s of stimulation at 1, 2, and 3 Hz ($p < 0.001$ for all frequencies), but ECCT did not change significantly with increase in stimulation frequency ($p = 0.9150$).

3.3. Changes in parameters during stimulation protocol in BMD

Changes in CMAP and MRP parameters in BMD patients are shown in Fig. 4. CMAP amplitude did not change significantly at 2 Hz or 3 Hz. At 120 s of stimulation, mean MRP amplitude ratio increased to 111 % at 2 Hz and to 137 % at 3 Hz. At 2-Hz, the mean MRP amplitude ratio was below 100 % for up to 90 s of stimulation, and there was no significant increase during 120 s of stimulation ($p = 0.4775$). At 3 Hz, the mean MRP amplitude ratio apparently increased with time, but the increase at 120 s of stimulation was not significant ($p = 0.0637$) due to high inter-patient variability. However, there was a significant difference in MRP amplitude ratio between 2 Hz and 3 Hz ($p = 0.0342$). ECCT decreased significantly during 120 s of stimulation at 2 Hz and 3 Hz ($p = 0.0245$ and $p < 0.001$, respectively), but no significant difference was observed between 2 Hz and 3 Hz ($p = 0.2474$).

Table 1
Comparison of demographics and electrophysiological findings at baseline (before start of nerve stimulation) in three groups.

		Muscular dystrophy	
	Normal control (n = 35)	BMD (n = 11)	MyD (n = 19)
Age	45.6 ± 13.0*	58.8 ± 12.6*	48.8 ± 10.1
Male / Female	18 / 17	11 / 0	10 / 9
CMAP amplitude (mV)	6.5 ± 1.9*†	3.2 ± 1.6*	3.6 ± 1.2†
CMAP duration (ms)	5.6 ± 0.8†	5.4 ± 0.9	6.5 ± 1.7†
MRP amplitude (mV)	20.0 ± 6.9*†	8.0 ± 5.2*	9.5 ± 7.4†
MRP duration (ms)	25.6 ± 4.9	28.1 ± 9.8	27.2 ± 8.7
ECCT (ms)	2.8 ± 0.5*†	4.1 ± 1.0*	3.9 ± 1.0†

Data are expressed as number of subjects or mean ± standard deviation. Values to the second decimal place were rounded off. Asterisk (normal control vs. BMD), dagger (normal control vs. MyD) denote $p < 0.05$. BMD, Becker muscular dystrophy; MyD, myotonic dystrophy; CMAP, compound muscle action potential; MRP, movement related potential; ECCT, excitation–contraction coupling time.

3.4. Changes in parameters during stimulation protocol in MyD

Changes in CMAP and MRP parameters in MyD patients are shown in Fig. 5. Mean CMAP amplitude ratio decreased to 94.3 % at 2 Hz and 92.6 % at 3 Hz at 15 s after nerve stimulation was started, remained unchanged thereafter until 120 s of stimulation, and then recovered gradually after the end of stimulation to almost 100 %. Mean MRP amplitude ratio increased to 111.2 % at 2 Hz and 109.4 % at 3 Hz at 120 s of stimulation. Changes in mean MRP amplitude ratio during 120-second repetitive stimulation were almost the same at 2 Hz and 3 Hz, and the increases at 120 s of stimulation were not significant at both frequencies ($p = 0.3886$, $p = 0.1834$, respectively). There was no significant difference between 2 Hz and 3 Hz ($p = 0.9076$). ECCT decreased significantly at 120 s of stimulation compared to baseline at both 2 Hz and 3 Hz ($p < 0.001$ and $p = 0.0034$, respectively), but no significant difference was observed between 2 Hz and 3 Hz ($p = 0.9492$).

3.5. Comparison of BMD and MyD with normal controls

We compared BMD and MyD with normal control with respect to the MRP amplitude ratios at 60 s and 120 s of 2-Hz and 3-Hz stimulations. For 2-Hz stimulation, the MRP amplitude ratio showed a significant difference between normal control and BMD at 60 s ($p = 0.0081$) and 120 s ($p = 0.0436$), as well as between normal control and MyD at 60 s ($p = 0.001$) and 120 s ($p = 0.0015$). For 3-Hz stimulation, the MRP amplitude ratio was not significantly different between normal control and BMD at 60 s ($p = 0.3153$) or 120 s ($p = 0.3971$), whereas significant differences were observed between normal control and MyD at 60 s ($p < 0.001$) and 120 s ($p = 0.0016$).

The time courses of MRP amplitude ratio in individual BMD and MyD patients at 2-Hz and 3-Hz stimulations, along with the mean values ± 2SD of normal controls, are shown in Supplementary Figure S1.

The percentage of patients with MRP amplitude ratio below −2SD was as follows. For 2-Hz stimulation at 60 s, 4 of 11 BMD patients and 1 of 19 MyD patients (total 5 of 30 total; 17 %) were below the cutoff. At 120 s, 3 of 11 BMD patients and 0 of 19 MyD patients (total 3 of 30 total; 10 %) were below the cutoff. For 3-Hz stimulation at 60 s, 3 of 11 BMD patients and 5 of 19 MyD patients (8 of 30 total; 27 %) were below the cutoff. At 120 s, 2 of 11 BMD patients and 3 of 19 MyD patients (5 of 30 total; 17 %) were below the cutoff.

ROC analysis evaluated cutoff values of MRP amplitude ratios at different frequencies and durations to distinguish normal controls from patients with BMD and MyD (Fig. 6). At a frequency of 2 Hz for 60 s, the cutoff MRP amplitude ratio was 108.8 %, with an area under the curve (AUC) of 0.79 (95 % CI: 0.657–0.923), sensitivity of 60 %, and specificity of 94.3 %. At a frequency of 2 Hz for 120 s, the cutoff MRP amplitude ratio was 114.9 %, also yielding an AUC of 0.79 (95 % CI: 0.657–0.923), sensitivity of 66.7 %, and specificity of 88.6 %. When the frequency was 3 Hz for 60 s, the cutoff MRP amplitude ratio was 103.6 %, with an AUC of 0.76 (95 % CI: 0.621–0.899), sensitivity of 56.7 %, and specificity of 97.2 %. Finally, when the frequency was 3 Hz for 120 s, the cutoff MRP amplitude ratio was 109.4 %, resulting in an AUC of 0.72 (95 % CI: 0.574–0.866), sensitivity of 53 %, and specificity of 94.3 %.

4. Discussion

The staircase phenomenon is defined as a condition in which repetitive stimulation of a muscle or dominant nerve results in a gradual increase in muscle force while the action potential remains constant. This phenomenon is proposed to be caused by an increase in the Ca^{2+} concentration in muscle fibers (Smith et al., 2014) or an increase in Ca^{2+} sensitivity due to phosphorylation of myosin regulatory light chains (Zhi et al., 2005), thereby promoting the E-C coupling. Abnormalities of the staircase phenomenon have been used to evaluate E-C coupling impairment in myopathies (Slomić et al.,1968). The staircase

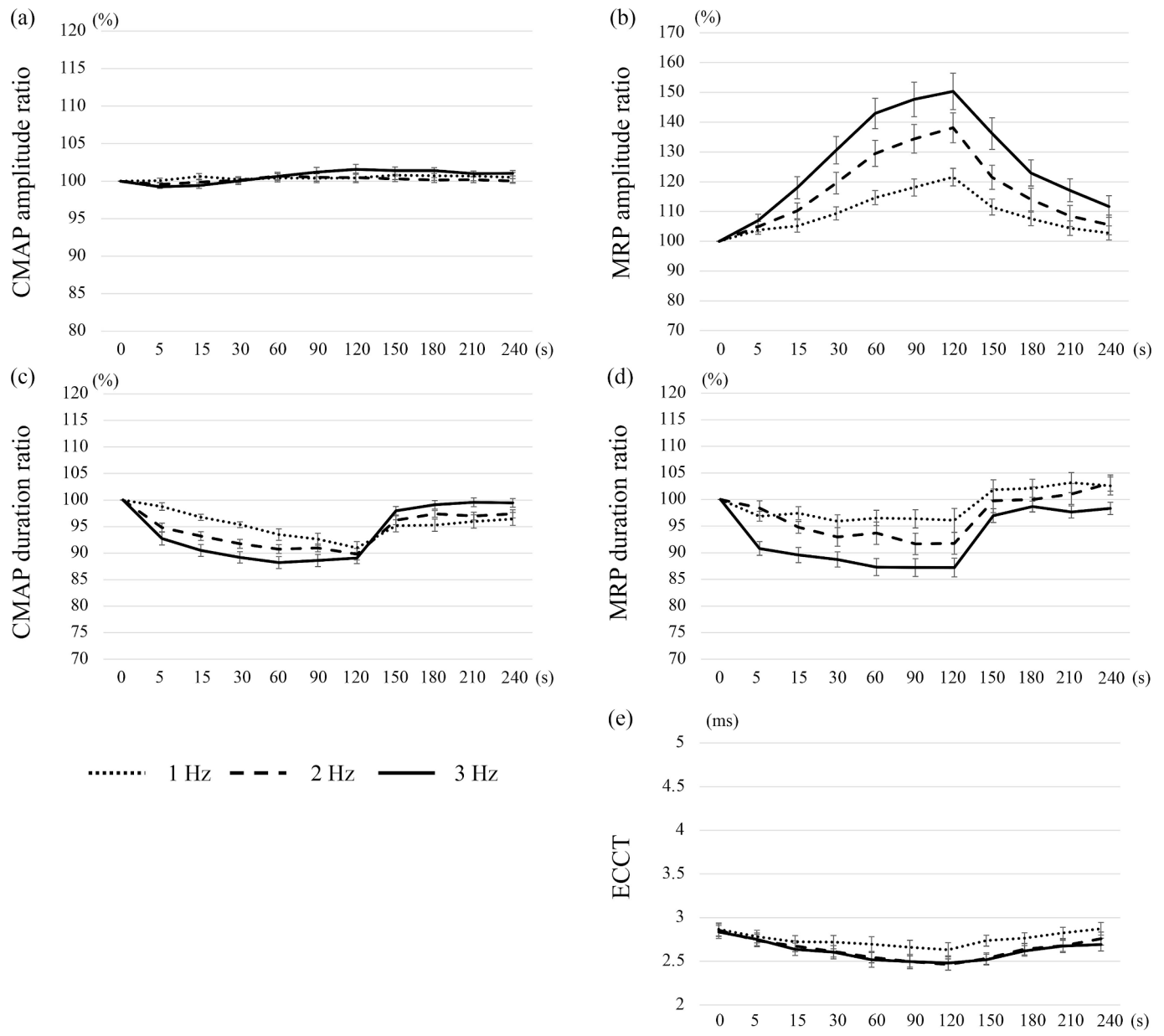


Fig. 3. Changes in CAMP and MRP parameters during the stimulation protocol in normal controls. (a) CMAP amplitude ratio (value at each time point divided by baseline value), (b) MRP amplitude ratio, (c) CMAP duration ratio, (d) MRP duration ratio, (e) ECCT. Data are expressed as mean ± standard error (vertical bar). Solid lines indicate data of 3-Hz stimulation, dashed lines indicate data of 2-Hz stimulation, and dotted lines indicate data of 1-Hz stimulation.

phenomenon has been reported to increase reaching a plateau with repetitive nerve stimulation for 15–18 min or longer (Deschamps et al., 2005), but prolonged electrical stimulation for 10 min or longer is considered too painful and intolerable for the patient. In the present study, the nerve stimulation time was set at 120 s because Slomić et al. (1968) performed repetitive stimulation up to 100 s, and we observed that the staircase phenomenon reached a plateau at approximately 2 min.

The previously reported method of measuring muscle contraction force associated with nerve stimulation was complicated (Slomić et al.,1968; Krarup and Horowitz, 1979; Deschamps et al., 2005). The method has not been widely used because it requires a specific device or amplifier to output waveforms separately from the electromyograph. Piezoelectric sensors can be connected directly to an electromyograph to evaluate potentials related to muscle contraction. In particular, the PVDF sensor we use is a thin film that can be attached more easily. The PVDF film used in piezoelectric sensors is a ferroelectric material in which the monomer units are line-aligned with the polarity of dipoles aligned in the β crystal (Fukuda, 2000; Kalimuldina et al., 2020). Therefore, it remains piezoelectric unless the crystal melts. The piezoelectric sensor converts the force received into electric potential. Consequently, it does not measure the muscle force per se. Nevertheless, our research shows that the piezoelectric sensor can be used to evaluate the staircase phenomenon.

In the controls, MRP amplitude increased as stimulation frequency increased from 1 Hz to 2 Hz to 3 Hz. This result is consistent with previous reports (Slomić et al.,1968; Krarup and Horowitz, 1979). Statistically, this change was significantly related to time and frequency. Furthermore, an interaction between time and frequency was detected. In the BMD and MyD, on the other hand, MRP amplitude ratio was not significantly related to stimulation time or frequency, suggesting that the stair phenomenon did not occur properly.

In BMD, decrease in MRP was observed up to 90 s in the 2-Hz stimulation, whereas MRP increased during this period in the 3-Hz

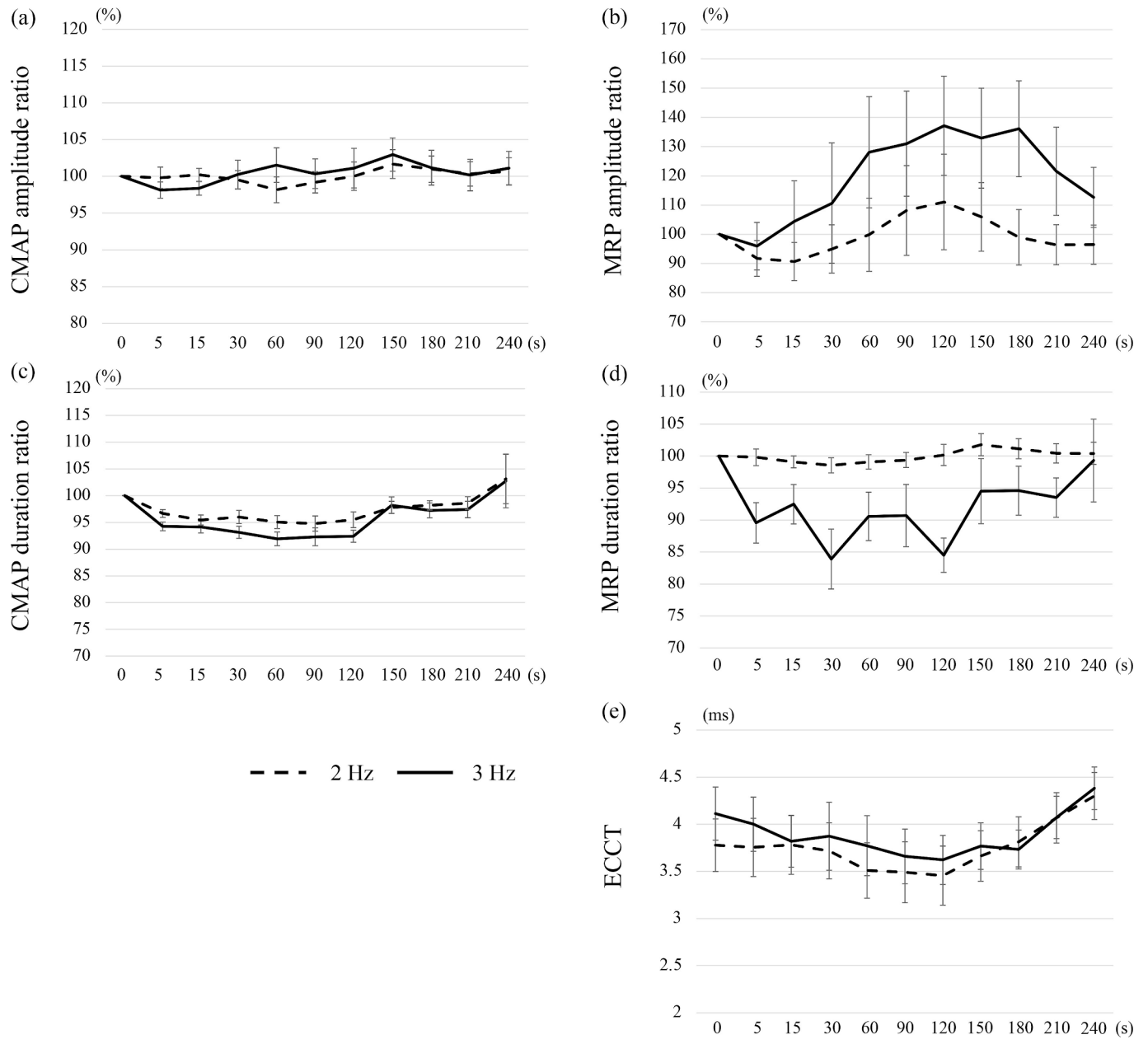


Fig. 4. Changes in CAMP and MRP parameters during the stimulation protocol in Becker muscular dystrophy. (a) CMAP amplitude ratio (value at each time point divided by baseline value), (b) MRP amplitude ratio, (c) CMAP duration ratio, (d) MRP duration ratio, (e) ECCT. Data are expressed as mean ± standard error (vertical bar). Solid lines indicate data of 3-Hz stimuli, dashed lines indicate data of 2 Hz-stimuli.

stimulation; a significant difference was observed between 2 Hz and 3 Hz. In MyD, however, MRP ratio did not increase, and no difference was observed between 2 Hz and 3 Hz. Both diseases exhibited impaired staircase phenomenon compared to normal controls. The different responses of BMD and MyD to the 2-Hz and 3-Hz stimulations may be attributed to the fact that BMD predominantly affects proximal muscles while MyD affects distal muscles. Other potential reasons include differences in ion channel abnormalities (Tang, et al. 2012.; Acket et al. 2016) and disruptions in Ca^{2+} homeostasis (Dubinin and Belosludtsev. 2023), which may lead to varying responses to repetitive stimulation frequencies among diseases. In addition to impairment in the staircase phenomenon, MyD also showed a decrease in CMAP amplitude with repetitive stimulation. A reduction in CMAP amplitude with repetitive stimulation has been reported in MyD patients (Bombelli et al. 2016), and this decrease may have contributed to the differences between BMD and MyD. When −2 SD was applied as a cutoff value, up to 27 % of patients fell below this threshold, thus failing to detect abnormalities in BMD and MyD. This finding may be due to the large individual differences and standard deviations in the normal control group. Therefore, we did not observe any cases in which the MRP amplitude ratio decreased to a mean of −2 SD (Supplementary Figure S2) and considered it inappropriate to use −2 SD as the cutoff value. In order to pursue a more appropriate cutoff value, we performed ROC analysis. ROC analysis showed that the sensitivity was low (50–60 %), but the specificity was high (97.2 %) when measurements were made at 3 Hz over 60 s. Therefore, this method applying piezoelectric sensors may be a useful diagnostic tool due to its high specificity.

Previous reports have shown that the staircase phenomenon is impaired in patients with various types of myopathies. Krarup and Horowitz (1979) reported impaired staircase phenomenon in various myopathic populations, including mitochondrial myopathy, nemaline myopathy, and myositis. Sunohara et al. (1982) also reported impaired

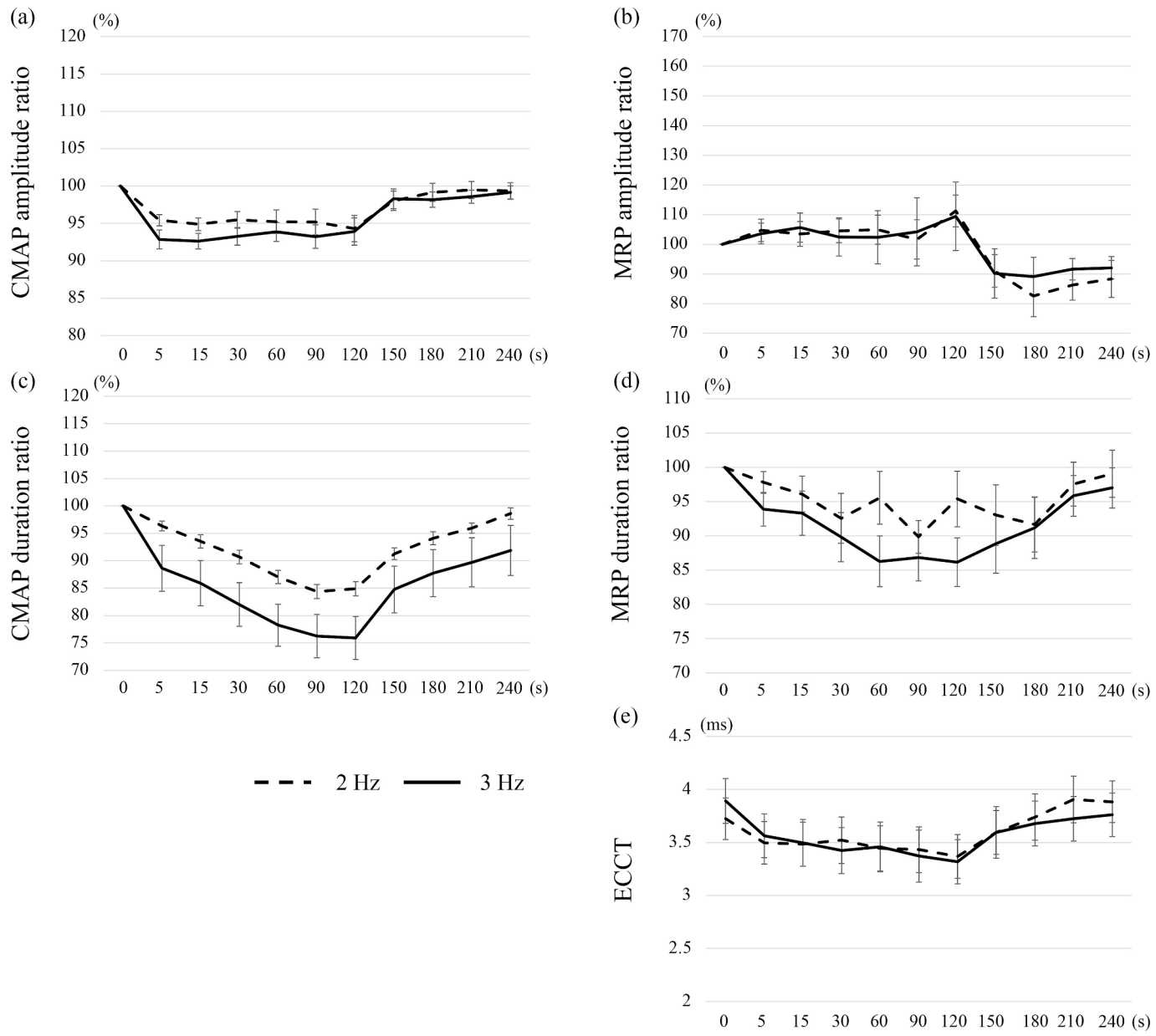


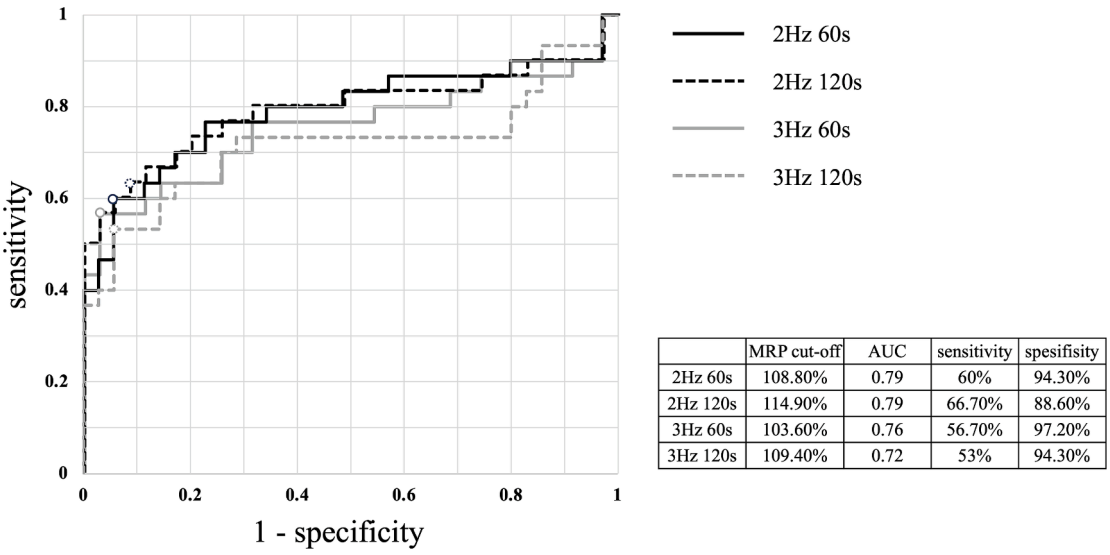
Fig. 5. Changes in CAMP and MRP parameters during the stimulation protocol in myotonic dystrophy. (a) CMAP amplitude ratio (value at each time point divided by baseline value), (b) MRP amplitude ratio, (c) CMAP duration ratio, (d) MRP duration ratio, (e) ECCT. Data are expressed as mean ± standard error (vertical bar). Solid lines indicate data of 3-Hz stimuli; dashed lines indicate data of 2-Hz stimuli.

staircase phenomenon in mitochondrial myopathy and limb-girdle muscular dystrophy. McArdle's disease was also reported to lack staircase phenomenon (Brandt et al., 1977).

ECCT was shortened by nerve stimulation in normal controls and patients with BMD and MyD, but no significant differences were observed between stimulation frequencies of 2 Hz and 3 Hz in both BMD and MyD. Since the increase in frequency was not associated with an increase in MRP amplitude in BMD and MyD, shortening of ECCT is considered a change that occurs independent of the staircase phenomenon. We speculate that ECCT shortening is caused by the reduction of MRP duration and steepening of the MRP waveform, as well as the faster onset latency of MRP potential resulting from the increased synchrony of contraction of muscle fibers by nerve stimulation. Our group has reported that ECCT is a useful marker that sensitively detects E-C coupling impairment in MG (Tsuda et al., 2010). Using ECCT as a marker of E-C coupling impairment, we have demonstrated that the ice-pack test induces a prolonged effect of ameliorating impaired E-C coupling in MG (Yamamoto et al., 2017), and that tacrolimus improves E-C coupling in ryanodine receptor antibody-positive MG (Imai et al., 2012). However, in the present study, the ECCT changes during nerve stimulation are distinct from the staircase phenomenon. We therefore conclude that ECCT change is not a useful indicator of E-C coupling impairment in the present protocol.

The present study demonstrates that the staircase phenomenon is impaired in BMD and MyD, and evaluating the staircase phenomenon using piezoelectric sensor allows detection of abnormalities with high specificity. In particular, this method may be useful for detecting early changes in ICU-acquired weakness, especially in critical illness myopathy. We have previously reported early E-C coupling impairment in ICU-acquired weakness using an accelerometer (Hirose et al., 2022). Our present findings may be used to develop a simpler detecting tool for assessing ICU-acquired weakness.

This study had several limitations. First, only distal muscles were examined in this study. We were able to show impairment of the



	MRP cut-off	AUC	sensitivity	spesifisity
2Hz 60s	108.80%	0.79	60%	94.30%
2Hz 120s	114.90%	0.79	66.70%	88.60%
3Hz 60s	103.60%	0.76	56.70%	97.20%
3Hz 120s	109.40%	0.72	53%	94.30%

Fig. 6. ROC curve analysis for the diagnostic performance of our proposed method. The receiver operating characteristic (ROC) curve was plotted to evaluate the diagnostic accuracy of our proposed method for distinguishing normal controls from patients with BMD and MyD. 2 Hz at 60 s indicate a solid black line, 2 Hz at 120 s indicate dashed black line, 3 Hz at 60 s indicate a solid gray line, and 3 Hz at 120 s indicate a dashed gray line. The cut-off points are marked with open circle on each curve. The MRP amplitude ratio at the cut-off point, along with the AUC, sensitivity, and specificity values, are presented in the table within the figure.

staircase phenomenon in muscle diseases by including patients with muscular dystrophies in whom the distal muscles were affected. However, this method would be more widely applicable to study muscle diseases if the proximal muscles can be examined. Evaluation of proximal muscles using the protocol of this study would require nerve stimulation with a stronger current, which would likely cause greater patient distress. Therefore, it is necessary to design a simpler examination method targeting the proximal muscles. Second, we did not investigate whether the degree of impairment of the staircase phenomenon correlates with clinical severity. This investigation was not possible in this study because of the slow clinical course of the disease and the large individual differences among patients.

In conclusion, use of piezoelectric sensor allows easy detection of staircase phenomenon abnormality, which has been difficult to evaluate in clinical practice. The present study demonstrates that E-C coupling is impaired in BMD and MyD, in a pattern different from the method using ECCT reported previously. Evaluation of the staircase phenomenon using piezoelectric sensor may be a more sensitive marker to evaluate E-C coupling impairment in myopathies.

Author contributions.

BH and TI were involved in conception and design of the work. BH, ET, RY, AT and TI were involved in acquisition of data. KI contributed to the development of the methodology described in this paper. TI was involved in analysis and interpretation of data. SH conducted literature searches and edited the article. BH and TI drafted the article, and all other co-authors revised it critically for important intellectual contents.

Declaration of generative AI in scientific writing.

AI tools were not used in the preparation of this paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cnp.2024.12.002.

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