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Motor unit firing patterns in older adults with low skeletal muscle mass

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HIGHLIGHTS

- Older adults were divided to two group based on their skeletal muscle mass.
- Individuals with low physical functions, i.e., sarcopenia, were excluded.
- Normally, motor units fire with hierarchy depending on recruitment thresholds.
- In low skeletal muscle mass group, the hierarchical firing pattern was absence.

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ABSTRACT

Muscular dysfunctions involving a decline in muscle strength are often induced by loss of muscle mass in older adults. Understanding neural activation in older adults in addition to muscular characteristics may be important to prevent such age-related dysfunctions. This study aimed to investigate the difference in motor unit firing patterns between community-dwelling older individuals with normal and low skeletal muscle mass. Sixty-six older adults (62–90 years) performed muscle strength and function tests. On conducting high-density surface electromyography of the vastus lateralis, individual motor unit firing properties were assessed. Individual motor units were divided into three different recruitment threshold groups and their firing rates were compared. The skeletal muscle quantity and quality were assessed using bioimpedance methods and ultrasound images. They were divided into two groups according to sarcopenia criteria: a normal group ($n = 39$) and presarcopenia group with low skeletal muscle mass but normal physical functions ($n = 21$). Skeletal muscle mass and muscle thickness were greater and echo intensity was lower in the normal group than presarcopenia group. Motor units in normal older adults fired at different rates with a hierarchy depending on their recruitment threshold, observed as a normal phenomenon. However, motor units in the presarcopenia group fired without showing the hierarchical pattern. The results suggest that older adults with low skeletal muscle mass exhibited an abnormal neural input pattern, in addition to declines in muscle quantity and quality.

1. Introduction

The increasing number of older adults has become a major social issue worldwide, and age-related muscle wasting is a serious medical and economic problem, leading to increased morbidity and mortality (Kitamura et al., 2021; Xu et al., 2022). To prevent muscle wasting and maintain physical functions in older individuals, muscle weakness and low skeletal muscle mass are often focused on (Chen et al., 2014, 2020; Cruz-Jentoft et al., 2010, 2019). Indeed, low skeletal muscle mass and low physical functions or strength were used for the diagnosis of

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sarcopenia in the European Working Group of Sarcopenia in Older People (EWGSOP) in 2010 (Cruz-Jentoft et al., 2010) and 2019 (Cruz-Jentoft et al., 2019). Additionally, EWGSOP also defined presarcopenic individuals as exhibiting only low skeletal muscle mass. Previous studies reported that low skeletal muscle mass is a risk factor for many diseases (Szulc et al., 2005; Veronese et al., 2021). A five-year cohort study in Japan reported that the prevalence of presarcopenia was 21.8% in men and 30.4% in women (average age 70.2 years), and also stated that presarcopenia was significantly correlated with osteoporosis (Kobayashi et al., 2019). Furthermore, a recent study showed a higher risk of mortality in those with not only sarcopenia but also older individuals with presarcopenia than healthy older individuals (Lera et al., 2021). Thus, it is necessary to understand the physiological characteristics of older individuals at the onset of muscular dysfunction such as presarcopenia for the prevention of sarcopenia.

Muscle strength and skeletal muscle mass, which are criteria for sarcopenia, are often concerns, but it is also important to focus on the central nervous system that controls muscle force output. Manini et al. (2013) pointed out that the reduced muscle strength of older individuals cannot by solely explained by the decline in muscle mass, suggesting that the decline in neural factors also contributes to muscle weakness. Weak older adults exhibit less neural activities evaluated by motor evoked potentials elicited by transcranial magnetic stimulation (Clark et al., 2021). While impairments of skeletal muscle quality and quantity have been reported with aging (Fukumoto et al., 2012, 2018), neural factors that drive muscle force exertion also degenerate in older adults (Clark et al., 2015; Kirk et al., 2021b; Klass et al., 2008). This neuromuscular dysfunction progresses with aging (Clark & Manini, 2012; Manini & Clark, 2012; Manini et al., 2013), being one of the most important topics regarding older adult locomotion.

The pathway from the central nervous system to muscle is evaluated by motor unit firing properties, and each motor unit has different firing properties depending on its recruitment threshold. Normally, motor units with a low recruitment threshold discharge at a high frequency, whereas motor units with a high recruitment threshold discharge at a low frequency during submaximal contractions (Erim et al., 1996). This hierarchical firing pattern is well-known as the 'onion skin phenomenon'. However, older adults exhibit different motor unit firing patterns compared with young healthy adults, indicating that the hierarchical pattern is often indistinct in older adults (Watanabe et al., 2016). A previous report (Watanabe et al., 2016) did not consider the difference in skeletal muscle mass of older individuals; thus, the difference between older individuals with normal and low skeletal muscle mass remains unclear. Since previous studies suggested that the dissociation between muscle strength and muscle mass was mainly ecplained by neural factors, Motor unit firing properties can be influenced by increases (Watanabe et al., 2020a) or decreases (Hirono et al., 2022b) in skeletal muscle mass, suggesting that a low skeletal muscle mass can alter motor unit firing properties. Therefore, it is considered that these characteristics of motor unit firing may differ between older individuals with normal and low skeletal muscle mass.

The purpose of the present study was to investigate the motor unit firing pattern between older adults with normal and low skeletal muscle mass, that is healthy versus presarcopenia, respectively. It was hypothesized that motor unit firing rates would differ depending on the recruitment threshold in normal older adults, but that the pattern would not be observed in the presence of presarcopenia. Insight into neural factors in individuals with low skeletal muscle mass would be beneficial information to advance understanding of muscular dysfunction.

2. Materials and methods

2.1. Participants

Sixty-six community-dwelling older adults participated in the present study. They were recruited at a health promotion class held at the university and a hospital. They were all independent and living in the community. The mean ± standard deviation of their age, height, and body mass were 75.2 ± 5.5 yrs (range: 62–90 yrs), 154.9 ± 8.0 cm, and 54.8 ± 8.7 kg, respectively. The experimental procedures were explained to the participants before they provided informed written consent to participate in the present study. This study was approved by the Research Ethics Committee of Chukyo University (2021–101) and conducted in accordance with the Declaration of Helsinki.

2.2. Body composition

Skeletal muscle mass and body fat of the whole body underwent bioimpedance measurements (InBody 430, InBodyJapan Inc., Tokyo, Japan). Participants stood on separate metallic electrodes under each foot and held metallic grip electrodes. Appendicular skeletal muscle mass was estimated by measuring the impedance of each segment. The appendicular skeletal muscle mass was converted into the skeletal muscle mass index (SMI) by dividing the muscle mass by height squared.

2.3. Physical function tests

The participants performed usual gait speed, grip strength, five-time chair stand, and timed up and go tests (Hirono et al., 2023). They were instructed to walk at their usual pace along a straight path, the speed was calculated, and the mean value of two trials was used. Maximal grip strength was measured twice with both hands using a hand dynamometer (Grip-D, TKK5401, Takei Scientific Instruments Co., Ltd., Niigata, Japan). The maximum value including both hands was used. As the five-time chair stand test, they repeated standing up until full knee extension and sitting back down on a chair as quickly as possible, and the time needed to complete five consecutive repetitions was measured. The timed up and go test was the time taken by participants to stand up from a chair, walk a distance of 3 m, turn around, walk back to the chair, and sit down as fast as possible.

2.4. Knee extension strength

The participants performed maximal voluntary isometric contraction (MVC) of knee extensors twice. They were seated with their hips and knees fixed at 90°. A force transducer (Takei Scientific Instruments Co., Ltd., Niigata, Japan; LU-100KSE; Kyowa Electronic Instruments, Aichi, Japan) was fixed on their right distal shank. After several knee extensions for familiarization and warm-up, MVC of knee extension was measured twice. The peak force during the 5-s MVC was recorded, and the greatest value between the two was used for further analysis. Knee extension torque was calculated by multiplying MVC force and arm length, which was defined as the distance between the knee joint axis and force transducer. Knee extension strength was also assessed by knee extension torque per body weight.

2.5. Ultrasound images

The assessments of ultrasound images were performed before the assessments of knee extension strength and motor unit firing behavior to avoid muscular hydration following muscle contractions. The participants were seated in a relaxed state. They were resting naturally with 90-deg knee flexion during measuring ultrasound images. A B-mode ultrasound device (MicrUS EXT-1H, TELEMED, Vilnius, Lithuania) with a multifrequency linear array probe (L12–5L40S-3) was used to measure the muscle thickness and echo intensity. The frequency was 7.5 MHz, and dynamic range was 66 dB. Power was −7 dB, gain was 72%, and depth was 70 mm. The settings were constant for all participants. The measurement site was the middle point between the greater trochanter and superior and lateral edges of the patella. A coupling gel was applied to compensate for depression of the tissues. Longitudinal images were obtained twice with careful adjustment of the probe angle to catch the brightest bone echo. Subcutaneous thickness and muscle thickness of the vastus lateralis was measured, and the mean value between two images was used for further analysis. Transverse images were also obtained twice with the same care. The two images were transferred to software (Image J version 1.53k; National Institutes of Health, Bethesda, MD, USA), and the region of interest of the vastus lateralis was manually set as large as possible, excluding the surrounding fascia. The echo intensity was calculated by the mean grayscale value of the region of interest, which was computed using the standard histogram function. The grayscale values ranged from 0 (black) to 255 (white). A higher value indicates a greater amount of fat and fibrous tissue within muscle (Pillen et al., 2009). The mean value between the two images was used for further analysis.

2.6. High-density surface electromyography

High-density surface electromyography (EMG) signals were recorded from the vastus lateralis during submaximal knee extension contraction using an EMG acquisition device (Sessantaquatro, OT Bioelettronica, Torino, Italy). A semi-disposable adhesive grid of 64 electrodes (GR08MM1305, OT Bioelettronica, Torino, Italy) was used. After the participants' hair was shaved and skin was cleaned with alcohol and water, a grid of electrodes with a bi-adhesive sheet (KITAD064, OT Bioelettronica) and conductive paste (Elefix Z-181BE, Nihonkohden, Tokyo, Japan) was attached on the skin over the vastus lateralis at the midpoint between the greater trochanter and superior and lateral edges of the patella. A reference electrode was attached on the tibial tuberosity using a wet electrode strap (WS2, OT Bioelettronica, Torino, Italy). Monopolar signals were filtered with a bandpass filter from 10 to 500 Hz, amplified by a factor of 150, sampled at 2000 Hz, and converted to digital form by a 16-bit analog-to-digital converter (Sessantaquatro, OT Bioelettronica). The force signal from the transducer was synchronized with the EMG signals on this converter.

The participants performed submaximal ramp-up contraction, consisting of a 17-s increasing phase to 50% of MVC and 10-s sustained phase at 50% of MVC. The exerted and target levels were shown on a monitor in real-time as visual feedback.

Recorded EMG signals were transferred to analysis software (MATLAB R2019a, MathWorks GK, MA, USA) and individual motor units were identified by the Convolution Kernel Compensation (CKC) technique using DEMUSE software (ver. 6; The University of Maribor, Slovenia) (Farina et al., 2010; Holobar et al., 2009). All decomposition results were manually inspected by a single experienced investigator (TH). The sustained phase was only incorporated to ensure reliable motor unit identification. Any physiologically irregular motor unit firing (< 4 or > 50 pps) and individual motor units with firing rates showing a coefficient of variation of over 30% were discarded (Adam & De Luca, 2005; Hirono et al., 2022a). Detected motor units were divided into three motor unit groups based on their recruitment thresholds (Fig. 1). The recruitment threshold of individual motor units was defined at the force level when the initial firing was identified. Motor units recruited at less than 15% of MVC were defined as L-MU, those recruited at 15–30% of MVC were defined as M-MU, and those recruited at 30–45% of MVC were defined as H-MU. The individual MU firing rate was calculated as the median value during 45 to 50% of MVC. These data on the motor unit firing rate from individuals were pooled in each group.

2.7. Definition of grouping

According to the study definition of Yamada et al. (2017) based on AWGS (Chen et al., 2014) and EWGSOP (Cruz-Jentoft et al., 2010), participants were divided into two groups (Fig. 2). First, participants with a gait speed below the cutoff value (< 0.8 m/s) or grip strength below the cutoff values (men: < 26 kg, women: < 18 kg) were excluded. Based on the cutoff value of SMI (men: 7.0 kg/m^2, women: 5.7 kg/m^2), participants were divided into a normal skeletal muscle mass group or low skeletal muscle mass group, with the latter group being defined as showing presarcopenia.

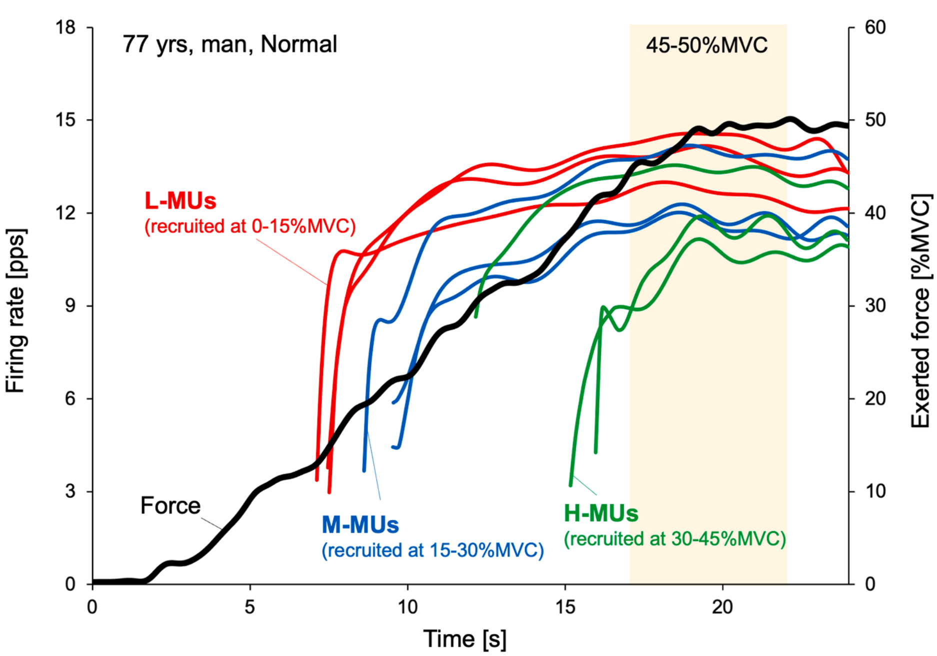

Fig. 1. Example data from an older adult with normal skeletal muscle mass (77 years old, man). The black line shows exerted force, which is increasing with a ramp-up rate of approximately 3% of maximum voluntary contraction (MVC) force level per second. The colored lines are described as individual motor unit firing rates. Red lines indicate the motor unit group with a relatively low recruitment threshold, defined as motor units recruited at 0–15% of MVC (L-MU). Blue lines indicate the motor unit group with a middle recruitment threshold, defined as motor units recruited at 15–30% of MVC (M-MU). Green lines indicate the motor unit group with a relatively high recruitment threshold, defined as motor units recruited at 30–45% of MVC (H-MU). The firing rate of each motor unit was calculated as the median during 45–50% of MVC.

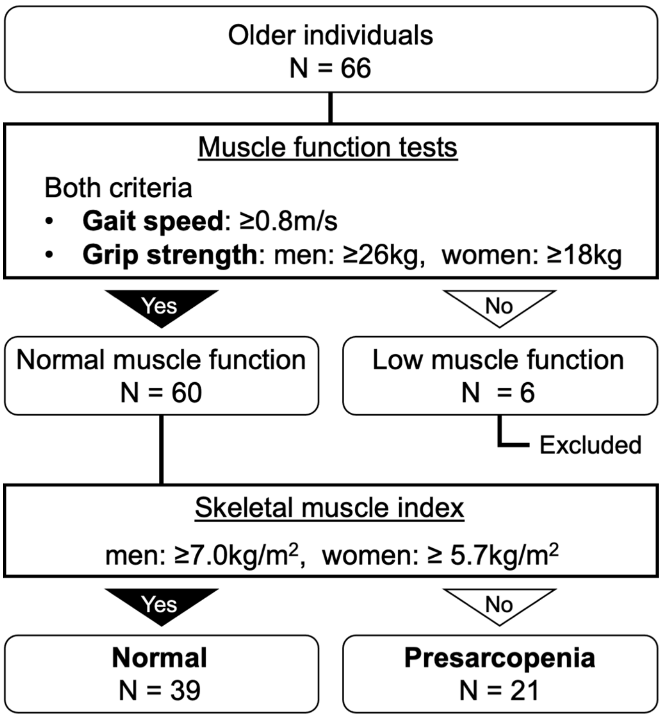

Fig. 2. Algorithm to determine whether normal or presarcopenic individual.

2.8. Statistical analysis

All statistical analyses were performed with SPSS (Statistical Package for the Social Sciences ver. 28.0, IBM Japan Inc., Tokyo, Japan). To compare the variables, other than the motor unit firing rate, between normal and presarcopenia groups, analysis of covariance (ANCOVA) with sex and age as covariates was performed because physical functions and muscular characteristics are influenced by sex and age. To compare motor unit firing rates among motor unit groups in each group, Kruskal Wallis tests were performed because the normality of the distribution was negated by Shapiro Wilk tests. When the tests identified significances, post-hoc tests were performed using Mann-Whitney U tests with Bonferroni correction. The significance level was set at 0.050.

3. Results

According to the diagnosis, 6 individuals were excluded from the present study who had physical dysfunctions such as sarcopenia (4 sarcopenia and 2 individuals with only low physical functions). Finally, the number of older adults belonging to the normal group was 39, whereas the number of presarcopenic older adults was 21 (Fig. 2).

Table 1
Comparison of characteristics in normal and presarcopenic older adults.

	Normal older adults $N = 39$	Presarcopenia $N = 21$	P-value
Age, years	74.5 ± 5.0	75.5 ± 6.3	0.536
Men, number (%)	11 (28%)	5 (24%)	–
Height, cm	156.8 ± 7.8	154.1 ± 7.0	0.144
Body mass, kg	58.6 ± 8.0	50.0 ± 6.5	< 0.001
SMI, kg/m^2	6.7 ± 0.7	5.6 ± 0.7	< 0.001
Subcutaneous thickness, mm	5.6 ± 4.8	4.9 ± 2.3	0.447
Knee extension strength, Nm	99.5 ± 35.3	82.3 ± 24.1	0.021
Knee extension strength / BW, Nm/kg	1.7 ± 0.5	1.6 ± 0.3	0.834
Gait speed, m/s	1.5 ± 0.2	1.5 ± 0.2	0.457
Grip strength, kg	29.1 ± 7.6	25.8 ± 6.1	0.038
Five-time chair stand test, s	7.1 ± 1.7	7.4 ± 1.8	0.554
Timed up and go, s	6.3 ± 1.1	6.4 ± 0.8	0.964

Values are mean ± standard deviation. P-values were calculated using analysis of covariances with sex and age as covariates, excluding the comparison of age. BW: body weight. SMI: skeletal muscle index. N: number of participants.

Table 1 shows the results of ANCOVAs. ANCOVAs for body mass, SMI, knee extension strength, and grip strength revealed significant differences between the two groups. ANCOVAs for muscle thickness and echo intensity are shown in Fig. 3A and B, indicating that muscle thickness was significantly lower and echo intensity was significantly higher in the presarcopenia group than in the normal group ($p = 0.002$ and $p = 0.023$, respectively). The other ANCOVAs identified no significant differences.

In the normal group, numbers of detected L-MU, M-MU, and H-MU were 109, 191, and 139, respectively. In the presarcopenia group, numbers of detected L-MU, M-MU, and H-MU were 56, 89, and 60, respectively. Fig. 4A shows the results of the motor unit firing rate. The Kruskal Wallis test for the normal group revealed significance ($p < 0.001$), and post-hoc tests also revealed significant differences between L-MU and M-MU ($p = 0.014$), between L-MU and H-MU ($p < 0.001$), and between M-MU and H-MU ($p < 0.001$). On the other hand, the Kruskal Wallis test for the presarcopenia group revealed a significance ($p = 0.025$), and post-hoc tests also identified a significant difference between L-MU and H-MU ($p = 0.030$), but not between L-MU and M-MU ($p = 0.115$), or between M-MU and H-MU ($p = 1.0$).

4. Discussion

The present study aimed to investigate the motor unit firing pattern in normal older adults and older adults with presarcopenia who have a low skeletal muscle mass but maintain physical functions. Novel findings were as follows: the firing rate of motor units with a low recruitment threshold in normal older adults was greater than that of motor units with a high recruitment threshold, with a hierarchy depending on their recruitment threshold (Fig. 4A left panel), which is a normal motor unit firing pattern well-known as the 'onion skin phenomenon', as indicated by normal individual data in Fig. 4B. On the other hand, such a firing pattern hierarchy was absent in those with presarcopenia (Fig. 4A right panel and Fig. 4C). To our best knowledge, this is the first study to investigate motor unit firing patterns in older adults with presarcopenia.

Based on the size principle (Henneman et al., 1965), motor units with a small motoneuron cell size are initially recruited, followed by motor units with a large motoneuron cell size. Motor units with a large motoneuron cell size, defined as motor units recruited with a relatively high-intensity contraction, show higher-amplitude and shorter-duration twitch responses; therefore, they can contribute to high force exertion, while they fatigue faster than motor units with low recruitment thresholds (Erim et al., 1996). During gradual ramp-up contraction, motor units with low recruitment thresholds fire at higher rates, and motor units with high-recruitment thresholds fire at lower rates, based on the minimum-metabolic-energy cost to compensate for fatigue (Erim et al., 1996). A previous study (Watanabe et al., 2016) reported that the hierarchical firing pattern was partly absent, but it did not consider the difference in skeletal muscle mass of individual older adults. In the present study, where participants were divided into two groups with or without sufficient skeletal muscle mass, this physiologically normal firing pattern was observed in normal older adults but not in those with presarcopenia (Fig. 3).

As older individuals' muscle undergoes atrophy, the neuromuscular system, such as neuromuscular junctions, also degenerate with aging (Hepple & Rice, 2016; Jones et al., 2022), and the number of motor units decreases (McNeil & Rice, 2018). If these alterations in the neuromuscular system occur in the presence of presarcopenia, this is one of the possibilities to explain the characteristic motor unit firing patterns observed (Fig. 4); motor units with a high recruitment threshold may have fired at a high rate to compensate for the low force exerted by motor units with a low recruitment threshold at 45–50% of MVC. In a previous study investigating motor unit firing patterns of the tibialis

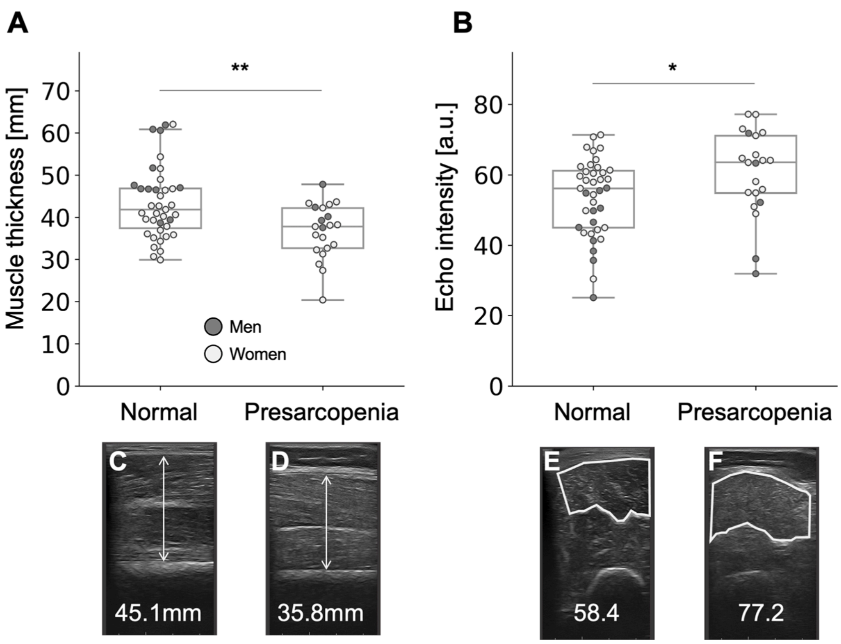

Fig. 3. Muscle thickness (A) and echo intensity (B) in normal and presarcopenia groups. Analysis of covariances with sex as a covariate were performed. There were significant differences between normal and presarcopenia groups in both muscle thickness and echo intensity. C and E represent muscle thickness and echo intensity data from one participant (74 years, woman) in the normal group, respectively. D and F represent muscle thickness and echo intensity data from one participant (71 years, woman) in the presarcopenia group, respectively. * denotes a significant difference at $p < 0.05$, and ** at $p < 0.01$.

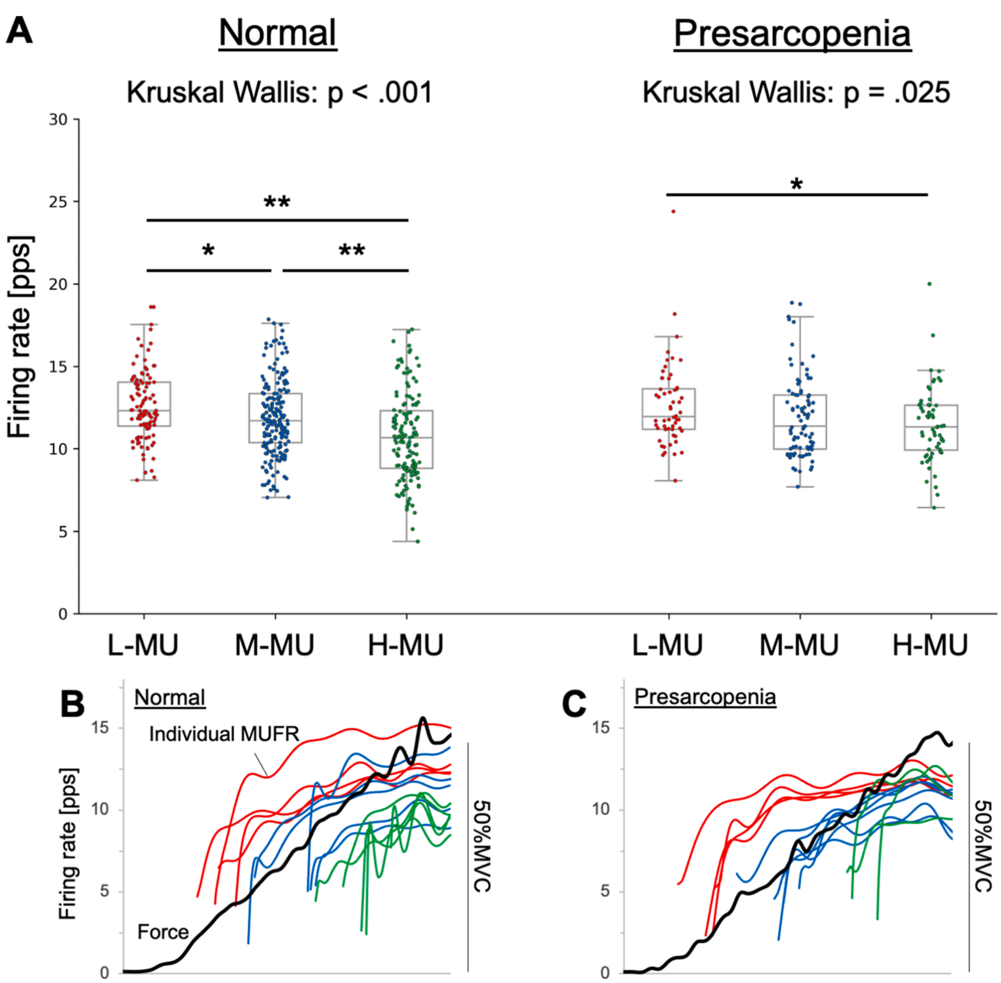

Fig. 4. Motor unit firing rate in normal and presarcopenia groups. A shows results of the firing rate of merged motor units in each group. L-MU: motor units recruited at 0–15% of MVC. M-MU: motor units recruited at 15–30% of MVC. H-MU: motor units recruited at 30–45% of MVC. B and C show representative data from normal and presarcopenia groups, respectively. Motor unit firing rates in B show a hierarchical pattern, whereas those in C do not show such a hierarchical pattern. * denotes a significant difference at $p < 0.05$, and ** at $p < 0.01$.

anterior in healthy young adults (Oya et al., 2009), later-recruited motor units fired at a higher rate during almost maximal contraction than earlier-recruited motor units, which is the reverse of the 'onion skin phenomenon'. In a simulation study, during high-intensity muscle contraction, force was exerted mainly by rate coding rather than by an increase in recruited motor units (Moritz et al., 2005). The present results may also suggest that high recruitment threshold motor units in presarcopenia fire with a strategy to increase the rate even during middle-intensity contraction, such as 50% of MVC. Additionally, age-related electrophysiological alterations, such as elongation of the afterhyperpolarization (AHP) duration, were reported (Piotrkiewicz et al., 2007) and this may also explain the differences in motor unit firing patterns between normal and presarcopenic older populations. Since the duration of AHP is related to the motor unit firing rate (Christie & Kamen, 2010), older individuals' motor unit firing rate is lower than that of young individuals (Girts et al., 2020; Kirk et al., 2021a; Orssatto et al., 2021; Watanabe et al., 2016). On the other hand, motor units with a high recruitment threshold are associated with short AHP durations (Zwaagstra & Kernell, 1980) and have the potential to increase the firing rate at a high frequency (Oya et al., 2009), suggesting that motor units with a high recruitment threshold might be little affected by a elongation of AHP duration. We consider that the greater elongation of afterhyperpolarization in the presence of presarcopenia might inhibit the increasing firing rate of motor units with a low recruitment threshold. Also, motor units with a high recruitment threshold, which have the potential to increase their firing rate because of faster contraction, would actually increase their firing rate to compensate, resulting in a non-hierarchical firing pattern in presarcopenia. Further studies are warranted to examine the detailed electrophysiological characteristics of motor units in older populations with presarcopenia.

Echo intensity of presarcopenic muscle was altered in addition to low skeletal muscle mass (Table 1). Echo intensity can be affected by subcutaneous thickness because of the attenuation of ultrasound wave (Young et al., 2015). However, we found no difference in subcutaneous thickness between two groups (Table 1), the influence was believed minimum in the present results. Echo intensity is a reflection of the amount of fat and fibrous tissue within muscle (Pillen et al., 2009), and is related to many physical functions and physical activity (Cadore et al., 2012; Fukumoto et al., 2012; Yoshiko et al., 2019). The results support the findings of a previous report (Fu et al., 2023). Although the grip strength was significantly weaker in presarcopenic than in normal individuals, it still exceeded the sarcopenia diagnostic criteria and other physical function test results were not different between the groups (Table 1). While presarcopenic individuals could maintain physical functions related to daily activities, skeletal muscle showed declines in both quantity and quality. These alterations can influence neural system changes in motor unit firing, but the cause-effect relationship remains unclear based on the present study.

We noted reductions in muscle quantity and quality (Fig. 3) and an abnormal motor unit firing pattern (Fig. 4) in presarcopenic compared with normal individuals, but all exhibited plasticity to improve mediated by interventions, such as resistance training or ingestion of nutrients. Resistance training can improve muscle quantity (Orssatto et al., 2020), muscle quality evaluated by echo intensity (Radaelli et al., 2013, 2019), and the motor unit firing pattern (Watanabe et al., 2018, 2020a). Ingestion of nutrients can also improve muscle quantity (Kirwan et al., 2022; Mori et al., 2022) and the motor unit firing pattern (Watanabe et al., 2020b). Therefore, it is possible to prevent or improve the declines observed in presarcopenia. Further studies with a focus on neural factors are necessary to investigate the effectiveness of interventions to prevent and improve the declines with aging and the preventive effects against sarcopenia in the future.

We have some limitations. We did not control the participants' physical activity, sleep and diets before taking part in the present study. Since these can be potential confounding variables, the uncontrolled conditions were limitations in the present study. In the present participants, there were a few sarcopenia individuals. If 4 sarcopenia individuals were included in a group with low skeletal muscle mass, the significances were little different from the original results (supplementary data). More number of sarcopenia individuals were should investigated neuromuscular properties in future studies.

In conclusion, presarcopenic older adults, who exhibit low skeletal muscle mass without physical dysfunctions, have a characteristic motor unit firing pattern depending on the recruitment threshold. Neuromuscular functions in older individuals with low skeletal muscle mass are not often investigated; however, the present results indicate that some aspects of neuromuscular alterations were beginning in those individuals. These findings suggest the possibility of a strategy to compensate for a decrease in muscle mass and maintain physical functioning; however, they are also a warning that the abnormal neural system might cause further, diverse dysfunctions.

CRediT authorship contribution statement

Tetsuya Hirono: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ryosuke Takeda:** Writing – review & editing, Validation, Formal analysis, Data curation. **Taichi Nishikawa:** Writing – review & editing, Validation, Data curation. **Masamichi Okudaira:** Writing – review & editing, Formal analysis, Data curation. **Shun Kunugi:** Writing – review & editing, Formal analysis, Data curation. **Akito Yoshiko:** Writing – review & editing, Formal analysis, Data curation. **Saeko Ueda:** Writing – review & editing, Data curation. **Akane Yoshimura:** Writing – review & editing, Data curation. **Kohei Watanabe:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.archger.2023.105151.

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