

Association between physical fitness tests and neuromuscular properties

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Running title

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Data availability

Data that support the findings of the present study are presented in the text, figures

39 and table, and are available from the corresponding author upon reasonable request.
40

41 **ABSTRACT**

42 Purpose: While various fitness tests have been developed to assess physical
43 performances, it is unclear how these tests are affected by differences, such as, in
44 morphological and neural factors. This study was aimed to investigate associations
45 between individual differences in physical fitness tests and neuromuscular properties.

46 Methods: One hundred and thirty-three young adults participated in various general
47 physical fitness tests and neuromuscular measurements. The appendicular skeletal
48 muscle mass (ASM) was estimated by bioelectrical impedance analysis. Echo
49 intensity (EI) was evaluated from the vastus lateralis. During submaximal knee
50 extension force, high-density surface electromyography of the vastus lateralis was
51 recorded and individual motor unit firings were detected. Y-intercept (i-MU) and slope
52 (s-MU) from the regression line between the recruitment threshold and motor unit firing
53 rate were calculated.

54 Results: Stepwise multiple regression analyses revealed that knee extension strength
55 could be explained (adjusted $R^2=.712$) by ASM ($\beta=.723$), i-MU (.317), EI (-.177), and
56 s-MU (.210). Five-sec stepping could be explained by ASM (adjusted $R^2=.212$).
57 Grip strength, side-stepping, and standing broad jump could be explained by ASM and
58 echo intensity (adjusted $R^2=.686$, .354, and .627, respectively). Squat jump could be
59 explained by EI (adjusted $R^2=.640$). Counter-movement jump could be explained by

60 EI and s-MU (adjusted $R^2=.631$). On the other hand, i-MU and s-MU could be
61 explained by five-sec stepping and counter-movement jump, respectively, but the
62 coefficients of determination were low (adjusted $R^2=.100$ and $.045$).
63 Conclusion: Generally-developed physical fitness tests were mainly explained by
64 morphological factors, but were weakly affected by neural factors involved in
65 performance.

66

67 **KEYWORDS**

68 Motor unit, decomposition, high-density surface electromyography, ultrasound, echo
69 intensity

70

INTRODUCTION

Physical fitness tests, such as hand grip strength, stepping test, vertical jump, and balance test, have been developed to assess physical functions or performances. Results of individual physical fitness tests reflect different physical functions, such as strength, agility, balance, exact force control, and coordination. Therefore, we can interpret these results as individual characteristics of physical performances. For example, the results are used to understand the development levels of children's physical functions (Lesinski et al. 2020; Tambalis et al. 2011), degree of locomotor dysfunction in older adults or patients (Burton et al. 2013; Yamada et al. 2017; Kimura et al. 2012), and the effects of exercise interventions (Lopez et al. 2018). However, how physiological backgrounds affect interpretation of the results of physical fitness tests has not been fully clarified. It is well-known that physical functions or performances are mainly determined by morphological and neural factors, i.e., muscle volume/quality and central nervous control (Balshaw et al. 2017; Moritani and deVries 1979). The ability to quantify the components of individual physical fitness tests from these factors would provide useful information to interpret the results of such tests.

Morphological factors are evaluated by ultrasound images (Paris and Mourtzakis

2021; Van Hooren et al. 2020) or magnetic resonance imaging (MRI) (Naruse et al. 2022; De Smet 1993). The quantity of morphology is estimated by the muscle thickness, muscle cross-sectional area, or appendicular skeletal muscle mass (Naruse et al. 2022; Paris and Mourtzakis 2021), while the quality of morphology is estimated by the muscle echo intensity calculated from B-mode ultrasound images or water and fat values using MRI (Fukumoto et al. 2015; Fukumoto et al. 2022). Morphological indexes are commonly employed to investigate muscular function (Hill et al. 2021), while the central nervous system, especially cerebral cortex control, also contributes to muscle contraction and physical performances (Perez et al. 2004). Both morphological and neural factors should be considered when implementing physical performance assessments or improvements.

Evaluation of the central nervous system that contributes to muscle contraction requires expensive and specific devices. To evaluate motor control, electromyography (EMG) is widely used to assess neural drive from the central nervous system to a peripheral, malleable organ, that is muscle. Especially, high-density surface EMG (HDsEMG) is used to detect motor unit (MU) behavior, providing detailed neurophysiological information (Holobar et al. 2009; Farina et al. 2010). HDsEMG can quantify and qualify neural drive to muscle directly (Parra et al. 2021;

109 Sterczala et al. 2020). The MU firing rate is negatively associated with the
110 recruitment threshold of MUs in healthy and young individuals (Parra et al. 2021).
111 The regression line between the MU firing rate and recruitment threshold is calculated
112 and the y-intercept and slope mean that normalized quantity of firing rate with
113 consideration of recruitment thresholds of each MU and relationship among each MU
114 with different recruitment threshold, respectively (Parra et al. 2021; Sterczala et al.
115 2020).

116

117 These neurophysiological properties could be related to physical fitness performances
118 as well as morphological properties. The first purpose of the present study was to
119 examine the contribution of morphological and neural factors, which are both
120 determinants of the muscle contraction strength, to each physical fitness test. The
121 second purpose was to examine whether physical fitness tests could estimate the
122 neural drive quantity and quality, which are evaluated by specific devices and
123 algorithms. The hypotheses of the purposes were that physical fitness tests could be
124 explained by both morphological and neural properties. In addition, subanalyses
125 comparing sex differences was conducted to investigate whether the abovementioned
126 associations were observed in each sex.

127

128

129 **METHODS**

130

131 **Participants**

132

133 One hundred and thirty-three healthy young adults participated in the present study.

134 Their ages ranged from 18 – 29 years old. Eighty-one males (height: 172.0 ± 5.2 cm,

135 body mass: 65.4 ± 9.5 kg) and 52 females (height: 159.4 ± 5.0 cm, body mass: $52.6 \pm$

136 7.0 kg) participated. They had no neuromuscular disorders or pain preventing

137 physical fitness tests or neuromuscular measurements involving their legs. The

138 purpose and procedures were explained to the participants before they provided

139 informed written consent to participate in the present study. This study was

140 conducted in accordance with the Declaration of Helsinki and approved by the

141 institutional review board (2021-101).

142

143 **Physical fitness tests**

144

145 The participants performed knee extension strength measurements and 10 physical

146 fitness tests that are typically used in Japan (Oshita and Yano 2010; Tamura et al.

2020; Kimura et al. 2012; Kawakami et al. 2020; Fujita et al. 1995). The grip strength is used as an index of muscular strength, whereas the whole-body reaction time, five-sec stepping, and side-stepping are employed as tests of agility. Single-leg standing with the eyes closed on a rigid or foam surface is used as an index of balance ability. The five-time chair stand test, standing broad jump, squat vertical jump, and counter-movement vertical jump are used as indexes of muscle power (Guralnik et al. 1994; Caspersen et al. 1985).

Knee extension strength

Participants were seated in a custom-made dynamometer (Takei Scientific Instruments Co., Ltd.) with a force transducer (LU-100KSE; Kyowa Electronic Instruments). Their hips and knees were flexed at 90 deg. After several knee extensions for familiarization and warm-up, they performed maximum voluntary isometric knee extension contraction twice. The peak force during the contractions was recorded, and the greater value of the two was determined as the maximum voluntary contraction (MVC) force. Knee extension strength was determined by the torque calculated by multiplying the MVC force and arm length. The moment arm length was measured as the distance between the knee joint axis and force transducer.

166 Grip strength

167 Maximum grip strength is widely used (Kim et al. 2022; Katsimpris et al. 2021) as an
168 estimation of whole-body muscle strength because the measurement is straight-
169 forward. Maximal grip strength was measured using a hand dynamometer (Grip-D,
170 TKK5401, Takei Scientific Instruments Co., Ltd.). The participants stood upright with
171 their upper limbs by their sides and were instructed to perform maximal voluntary grip
172 strength tests. Grip strengths of left and right hands were recorded, and the mean
173 values of both hands were used for analyses.

174

175 Five-time chair stand test

176 This test is general to check older adults' physical function (Whitney et al. 2005), such
177 as diagnosis of sarcopenia (Chen et al. 2020). The participants were seated first,
178 and they repeated standing up until full knee extension and sitting back down on a
179 chair as quickly as possible. The time to complete five consecutive repetitions was
180 recorded (Guralnik et al. 1994). The test was repeated twice, and the fastest time
181 was used for further analysis.

182

183 Reaction time

184 Whole-body reaction time was measured using a pressure-sensing mat (T.K.K. 5408;

185 Takei Scientific Instruments Co.) (Kawakami et al. 2020). The participants were
186 instructed to stand on the mat first, and to jump as quickly as possible immediately
187 after a light placed in front of them came on. The time between the light coming on
188 and disappearance of the foot pressure on the mat was recorded, and five trials were
189 conducted. The mean time was used for further analysis (Kawakami et al. 2020).

190

191 Five-sec stepping

192 The participants were instructed to keep stepping as quickly as possible on the spot
193 for 5 sec. The stepping was recoded using digital slow video with a frame rate of 120
194 Hz (iPad Pro, Apple Inc.). The number of steps was counted manually by watching
195 a slow video until 5 sec after the start of stepping.

196

197 Side-stepping

198 Three lines of tape were placed parallel on the floor at intervals of 100 cm. First, the
199 participants stood, straddling the center line. They stepped with one foot to touch or
200 cross the outside line, and then returned to the original position, again straddling the
201 center line. They then stepped with the other foot to touch or cross the other outside
202 line, and then returned to the original position. These side-step cycles were repeated
203 as quickly as possible for 20 sec (Tamura et al. 2020). The number of steps touching

204 or crossing the lines was counted as the side-stepping score.

205

206 Single-leg standing with the eyes closed on a rigid or foam surface

207 The participants stood on a rigid floor or foam pad (AIREX® Balance-pad). Their

208 arms were kept on their chest and they maintained single-leg standing for as long as

209 possible until their opposite foot touched the floor or pad, or until the standing foot

210 moved. The upper time limit was 180 sec. The time was recorded and the average

211 value (including both legs) was used for further analysis.

212

213 Standing broad jump

214 The participants stood with their feet behind the start line. They were required to

215 jump forward as far as possible with their knees bent and arms swinging freely. The

216 distance between the start line and point where their heel landed was measured.

217 They performed this three times, and the greatest distance was used for further

218 analysis.

219

220 Vertical jump

221 The participants performed two different vertical jumps: a squat jump, and counter-

222 movement jump. The squat jump was performed with both knees bent at an angle of

223 90 deg between the thigh and shank, and the maximum vertical height they could jump
224 to was measured using a jump tester (T.K.K.5406, Takei Scientific Instruments Co.,
225 Ltd.). Counter-movement jump was performed from an upright, standing position.
226 They were permitted to use the recoil on bending the knees, and performed a vertical
227 jump to reach as high as possible, where the height was measured using the same
228 instrument as for the squat jump. During each vertical jump, their hands were fixed
229 around their hips to avoid arm-swinging during jumping. Each vertical jump was
230 repeated three times, and the highest value was used for further analysis.

231

232 **Measurements of neuromuscular characteristics**

233

234 Appendicular skeletal muscle mass (ASM) and body fat mass were estimated using a
235 bioelectrical impedance analysis device (Inbody 430, Inbody Japan Inc., Tokyo, Japan).
236 The device assesses the resistance and reactance of arms, the trunk, and legs with a
237 segmental multifrequency approach (5, 50, and 250 kHz).

238

239 The participants were seated on a chair without their feet touching the ground. They
240 were asked to relax with their lower legs resting naturally vertical with 90-deg knee
241 flexion. A B-mode ultrasound device (MicrUS EXT-1H, TELEMED) with a

242 multifrequency linear array probe (L12-5L40S-3) was used to measure the muscle
243 morphological characteristics. The frequency was 7.5 MHz, and dynamic range was
244 66 dB. Power was -7 dB, gain was 72%, and the depth was 70 mm. The settings
245 were constant for all participants. The measurement site was the middle point
246 between the greater trochanter and superior and lateral edge of the patella. An
247 adequate coupling gel was applied to compensate for depression of the tissues.
248 Transverse images were obtained while adjusting the probe angle when the bone echo
249 was the brightest. The images were converted by software (Image J version 1.53k;
250 National Institutes of Health, Bethesda, MD, USA). The region of interest of the
251 vastus lateralis was manually set as large as possible, excluding the surrounding
252 fascia. The echo intensity was calculated by the mean grayscale value of the region
253 of interest, which was computed using the standard histogram function. The
254 grayscale values ranged from 0 (black) to 255 (white). A higher value indicates a
255 greater amount of fat and fibrous tissue within the muscle (Pillen et al. 2009). The
256 mean value between two images was used for further analysis as an index of muscle
257 morphological quality.

258

259 HDsEMG signals were recorded from the vastus lateralis during submaximal knee
260 extension contraction using the same instrument as for measuring maximum knee

extension strength. A semi-disposable adhesive grid of 64 electrodes (13 rows and 5 columns with one missing electrode in the upper left corner) with a 1-mm diameter and 8-mm inter-electrode distance (GR08MM1305, OT Bioelettronica, Torino, Italy) was used to record HDsEMG signals. After the participants' hair was shaved and skin was cleaned with alcohol, the grid of electrodes was attached to the skin over the vastus lateralis with a bi-adhesive sheet (KITAD064, OT Bioelettronica) after applying conductive paste (Elefix Z-181BE, NIHONKOH DEN). The center of the grid was placed at the midpoint of the line between the greater trochanter and superior and lateral edges of the patella. The long side of the grid was set along the line. A reference electrode was attached on the tibial tuberosity using a wet electrode strap (WS2, OT Bioelettronica, Torino, Italy). Monopolar surface EMG signals were filtered with a bandpass filter from 10 to 500 Hz, amplified by a factor of 150, sampled at 2,000 Hz, and converted to digital form by a 16-bit analog-to-digital converter (Sessantaquattro, OT Bioelettronica, Torino, Italy). The signal from the force transducer was synchronized with HDsEMG signals on this analog-to-digital converter.

The participants performed three ramp-up contractions, which consisted of a 15, 17, and 14-sec increasing phase to 30, 50, and 70% of the MVC force level and 15, 10, and 5-sec sustained phase at each force level, respectively. The exerted and target

280 forces were shown on a monitor in real-time as visual feedback. During these trials,
281 HDsEMG signals were recorded.

282

283 Recorded monopolar EMG signals were transferred to analysis software (MATLAB
284 R2019a, MathWorks GK), and individual MUs were identified by the Convolution
285 Kernel Compensation (CKC) technique using Decomposition of Motor Unit Surface
286 EMG (DEMUSE) software (ver. 5.0.1; The University of Maribor, Slovenia) (Farina et
287 al. 2010; Holobar et al. 2009). All decomposed results were manually inspected.

288 The sustained phase was only incorporated to ensure reliable MU identification. The
289 70% of MVC ramp-up contraction data were used for subsequent analysis. The 30
290 and 50% of MVC ramp-up contraction data were combined with the data of 70% of
291 MVC ramp-up because those data could more reliably ensure the detection of MUs
292 (Francic and Holobar 2021). The recruitment threshold of individual MUs was
293 defined at the force level where the initial firing was identified. The individual MU
294 firing rate was calculated as the mean value between 60 and 70% of MVC exerted,
295 because the peak firing rate could be ascertained with this duration. Any
296 physiologically irregular MU discharge (less than 4 and over 50 Hz) and individual MUs
297 with firing rates showing a coefficient of variation of over 30% were discarded (Adam
298 and De Luca 2005; Welsh et al. 2007; Kirk et al. 2021; Kamen and Knight 2004;

Fuglevand et al. 1993; Hirono et al. 2022b). Scatter plots of the firing rates and recruitment thresholds of individual MUs were analyzed in each participant. The linear regression was calculated and the y-intercept and slope of the regression line were computed. The y-intercept indicates the amount of neural excitability (Hirono et al. 2022c; Herda 2022), and the slope indicates the maturity of the 'onion skin' phenomenon, which is a potential characteristic of MU firing. If examining MU firing in healthy individuals, the slope can be a negative value (Parra et al. 2021). However, if examining MU firing in older adults or patients with neuropathologies, the slope can be near zero because they have lost the phenomenon (Watanabe et al. 2016; Nishikawa et al. 2022; Nishikawa et al. 2021). Even in young healthy males and females, MUs with high recruitment threshold fires at high frequency during high-intensity contraction (Inglis and Gabriel 2021; Oya et al. 2009), which causes the slope to be near zero or positive regression line. We defined the y-intercept (i-MU) and slope (s-MU) as the quantity and quality of neural drive to muscle, respectively (Figure 1).

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social

Sciences (SPSS) version 28 (IBM Japan, Inc.). Descriptive data are presented as the mean $\pm$ standard deviation. To investigate which of the morphological or neural factors could explain each physical fitness test, stepwise multiple linear regression analyses were performed with each physical fitness test value as a dependent variable, and ASM, body fat mass, echo intensity, s-MU, i-MU, sex, and body mass weight as independent variables. On the other hand, to investigate which of the physical fitness test values could explain the neural factors of s-MU and i-MU, stepwise multiple linear regression analyses were performed with s-MU or i-MU as a dependent variable, and physical fitness test values as independent variables. The level of significance was set at p-value < 0.05.

As an additional analyses to investigate sex differences, all variables were compared between sex by t-test, and same stepwise multiple linear regression analyses were also performed for each sex.

RESULTS

The number of detected MUs was 833. Twenty-seven participants (2 males and 25

337 females) from whom the number of detected MUs was one or zero were excluded
338 because the regression line between the MU firing rate and recruitment threshold
339 could not be calculated. Another male participant whose thigh fat and muscle were
340 too large to clearly capture the bone in ultrasound images was also excluded. After
341 excluding these twenty-eight participants (3 males and 25 females), one hundred and
342 five participants' data (78 males and 27 females) were analyzed in the present study.
343 Their demographics, the number of detected MUs, physical fitness tests, muscle
344 thickness, echo intensity, s-MU, and i-MU are shown in Table 1.

345

346 The results of the stepwise multiple regression analyses with physical fitness tests as
347 dependent variables are shown in Table 2. The significant models were explained by
348 individual sex, ASM, echo intensity or neural properties (adjusted $R^2 = 0.204 - 0.712$).
349 The regression analyses with the five-time chair stand test, reaction time, and single-
350 leg standing on both rigid and foam surfaces as dependent variables did not select
351 any significant independent variables.

352

353 The results of the stepwise multiple regression analyses with s-MU or i-MU as a
354 dependent variable are shown in Table 3. Both s-MU and i-MU were significantly
355 explained by the physical fitness test, but the coefficients of determination were low

(adjusted $R^2 = 0.100$ and 0.045 , respectively). The associations between s-MU and counter-movement jump and between i-MU and five-sec stepping are described in Figure 2.

For additional analyses to investigate same statistics in each sex, the results were described in appendix tables. Almost all variables were different between sex by t-tests ($p < 0.05$, Appendix Table 1). Especially, s-MU was significant lower in male (-0.13 ± 0.09 pps/%MVC) than that in female (-0.06 ± 0.12 pps/%MVC, $p = 0.011$), but i-MU did not differ ($p = 0.372$). The results of the stepwise multiple regression analyses in males showed that ASM and echo intensity were significantly associated with physical fitness tests, but neural properties were not selected in many cases. However, in females, i-MU was selected as a significant independent variable in addition to ASM and echo intensity to explain many physical fitness tests (Appendix Table 2). The results of the stepwise multiple regression analyses with s-MU or i-MU as a dependent variable in each sex are shown in Appendix Table 3. In males, s-MU nor i-MU were not explained by any physical fitness tests, but in females s-MU was significantly explained by five-time chair stand test (adjusted $R^2 = 0.158$) and i-MU was significantly explained by counter-movement jump (adjusted $R^2 = 0.211$).

DISCUSSION

We investigated the association between individual differences in physical fitness tests and neuromuscular properties that were evaluated based on bioelectrical impedance analysis, ultrasound images and HDsEMG in more than one hundred healthy young adults. Our results revealed that the knee extension strength, grip strength, five-sec stepping, side-stepping, standing broad jump, squat jump, and counter-movement jump could be significantly explained by both morphological and neural factors, while the five-time chair stand test, reaction time, and single-leg standing were not significantly explained by them. We also revealed that neural factors could not be sufficiently explained by individual parameters of physical fitness tests. This is the first reported study to demonstrate the influence of morphological and neural factors of human performance on physical fitness tests by multiple regression analyses.

Multiple regression analyses of physical fitness tests as dependent variables

Knee extension strength could be effectively explained by ASM, muscle quality, and neural quality and quantity (Table 2). The standard coefficient of i-MU when using model 4 including ASM, echo intensity, i-MU, and s-MU as dependent variables was

higher than that when using model 3, which excluded s-MU from model 4. This result suggests that high i-MU with a less negative s-MU could contribute to greater knee extension strength (in Figure 1, ID C has a less negative s-MU than ID A and B.). Generally, a more negative s-MU (the regression line inclines to the right) is normal and healthy but the present multiple regression analysis revealed that if i-Mus are the same, a less negative s-MU (the line becomes progressively flatter) could be effective for muscle strength. A high i-MU accompanying a less negative s-MU suggested that the whole MU firing rate was high and activated with marked excitability, which contributed to greater muscular exertion. Thus, high-level excitability of the whole neural drive can contribute to greater muscle strength. Skeletal muscle mass and echo intensity were also primary contributors to muscle strength in the present study. Both factors have been reported to be associated with muscle strength in older adults (Taniguchi et al. 2017; Fukumoto et al. 2012), and our results revealed that skeletal muscle mass and echo intensity significantly contributed to muscle strength even when neural factors were additionally considered.

i-MU and s-MU were selected with moderate coefficients ($\beta = 0.317, 0.210$) for knee extension strength, but i-MU was not selected for any physical fitness tests. S-MU was selected with low coefficients for counter-movement jump ($\beta = -0.127$). Motor

413 unit firing properties can be influenced by motor unit neuron afterhyperpolarization
414 duration in the muscle (Duchateau and Enoka 2011), for example, elongation of the
415 duration is related to a low firing rate (Christie and Kamen 2010). Therefore, motor
416 unit firing properties would be hypothesized to relate to physical fitness tests, but it
417 was not supported by our findings. MU firing properties were assessed during
418 isometric contractions because of a methodological issue in the present study. Motor
419 unit behaviors during dynamic contraction might be important to relate to physical
420 fitness. These neural factor assessments using HDsEMG are good tools to evaluate
421 neural excitability in the presence of longitudinal changes (Del Vecchio et al. 2019;
422 Hirono et al. 2022c), and they indicate the characteristics in aged individuals or
423 patients (Nishikawa et al. 2022; Noto et al. 2021; Watanabe et al. 2016), but the
424 present results suggest that they are not good indicators for physical fitness tests
425 involving dynamic muscle contractions, because physical performances may require
426 specific neural modulation with specific physical movements.

427

428 Echo intensity, which was used as an index of muscle morphological quality, was
429 selected for not only muscle strength (knee extension and grip strength) but also side-
430 stepping, standing broad jump, and both vertical jumps. A previous study identified
431 an association between echo intensity and rapid force development (Gerstner et al.

2017), which is considered an important factor to perform physical tests well (Maffiuletti et al. 2016; Waugh et al. 2013). Therefore, individuals with high-quality muscle could achieve high-level performance in agility and jump tasks.

The five-time chair stand test requires high-speed and repeated knee extension and flexion; therefore, it is used to examine the physical function in older adults (Uusi-Rasi et al. 2017; Zhu et al. 2019), but the chair stand ability may not be impaired in healthy young adults. Although knee extensors are key muscles when performing repeated chair sit and standing, this test would not be associated with neuromuscular properties in knee extensors in healthy young adults. Reaction time and single-leg standing with the eyes closed were not influenced by muscle morphological or neural properties, suggesting that they might be affected by afferent or reflex pathways, such as somatosensory stimulation or the connection between visual sensor and motor control (Lupinacci et al. 1993; Horak 2006; Peterka 2002), not voluntary muscle contraction potential. Actually, postural control has been reported to be associated with low-intensity contraction ability, such as 5 or 20% of MVC, not moderate-intensity levels (Hirono et al. 2020; Hirono et al. 2022a). Neural factors, which were evaluated during 70% of MVC ramp-up tasks, were not associated with postural tasks.

Multiple regression analyses of neural properties as dependent variables

Our secondary purpose was to confirm which physical fitness tests could easily estimate the neural quantity or quality, which could be investigated by specific devices and algorithms. Even if significant dependent variables were selected to estimate s-MU or i-MU, the accuracies of the estimation were poor since the coefficients of determination were low (adjusted $R^2=0.045$, 0.100 , Table 3, Figure 2). Neural adaptation is commonly focused on in healthy subjects because children often adopt specific neuromuscular coordination patterns and their neuromotor systems are developing (Lloyd et al. 2014; Gogtay et al. 2004), or older adults show impaired muscle strength involving mainly neural factors (Manini et al. 2013) and impaired neuromuscular control (Oomen and van Dieen 2017). It is difficult to estimate the invisible neural activities by physical fitness tests without expensive and specific instruments and technologies. We only examined the values of physical fitness tests in the present study, but it is possible that more effective estimates could be made by investigating the processes during the tests, such as calculating the movement speed and joint velocities using wearable accelerators or gyro sensors. In the future, it will be of interest to conduct such detailed analyses.

Subanalyses investigating sex differences

As shown in the appendix tables, there were some differences in associations between neuromuscular properties and all physical fitness tests. In males, almost physical tests were explained by ASM and echo intensity, not by neural factors. Whereas in females, not only muscle strength but also some dynamic physical performances were explained by neural properties. It has been previously reported that there were sex differences in motor unit behaviors (Harwood et al. 2014; Lulic-Kuryllo and Inglis 2022). It was difficult to detect a large number of motor units from female subjects in the present study (25 females from 52 were excluded due to the number of detected motor units); therefore, sample size of females was smaller than that of males. The present study was a pilot study to suggest sex differences in neuromuscular properties. Future studies will be developed to examine the reason that physical fitness performances were explained by neural properties in female.

Limitation

The present study had some limitations. We only measured one site of thigh muscles in both ultrasound measurements and HDsEMG recording. Each physical fitness

test necessitates various joint torque and motor functions, not only the vastus lateralis or quadriceps femoris. It is impossible to measure the morphological and neural properties of all muscles, and so the present study focused on only one site, which is measurable.

Conclusion

In conclusion, various physical fitness tests, which are widely used as indicators of physical performances or functions, were explained by morphological and neural quantities or qualities. Morphological properties were mainly selected with higher coefficients than neural properties. On the other hand, various physical fitness tests failed to estimate the neural properties well, which were determined by MU firing properties assessed using HDsEMG. Regarding sex differences, many physical tests were explained by ASM and echo intensity, not by neural factors in males. Whereas in females, both muscle strength and some physical performances were explained by neural properties.

506 **Conflict of interest**

507 The authors have no conflicts of interest relevant to this article.

508

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754 **FIGURE CAPTIONS**

755 Figure 1

756 Three participants' data of motor unit firing properties. Upper figures show the
757 individual motor unit firing rate during the 70% of maximum voluntary contraction
758 (MVC) ramp-up task. Motor units with low-intensity recruitment threshold (for
759 example #MU01 and #MU02 in participant A data) fired with a high frequency during
760 60 – 70% of MVC force exertion. Whereas, motor units with high-intensity
761 recruitment threshold (for example #MU15 and #MU18 in participant A data) fired with
762 a low frequency rate during 60 – 70% of MVC force exertion. The regression line
763 between the individual motor unit firing rate and recruitment threshold was calculated
764 (lower figures). Generally, the regression line shows a negative association
765 according to the size principle. The y-intercept (i-MU) and slope (s-MU) indicate the
766 motor unit firing properties in individual subjects. Comparing participants A and B,
767 the individual motor unit firing rate of B was high during 60 - 70% of MVC (upper
768 figures), indicating a greater i-MU value (lower figures). Comparing participants A
769 and C, the individual motor unit firing rate overlapped in C, indicating a less negative
770 s-MU value (the regression line becomes progressively flatter). i-MU indicates the
771 quantity as neural excitability, and s-MU indicates the quality as individual motor unit
772 variability.

773 Figure 2

774 The associations between the slope and counter-movement jump (left), and between
775 i-MU and five-sec stepping (right). Stepwise regression analyses revealed that s-MU
776 was only explained by counter-movement jump (standard coefficient = -0.330), and
777 that i-MU was only explained by five-sec stepping (standard coefficient = 0.232). i-
778 MU and s-MU were determined as y-intercept and slope, respectively, which were
779 calculated from the regression line between the individual motor unit firing rate and
780 recruitment threshold.

1 Table 1. Demographics, physical fitness tests, and neuromuscular measurements

N=105 (78 males and 27 females)	Mean $\pm$ standard deviation			Range	
Age, years	20.6	$\pm$	2.0	18.2	- 29.0
Height, cm	168.6	$\pm$	7.5	141.3	- 187.5
Body mass, kg	61.1	$\pm$	9.9	39.9	- 102.6
Neuromuscular properties					
Appendicular skeletal muscle mass, kg	21.7	$\pm$	4.3	11.9	- 32.4
Body fat mass, kg	9.4	$\pm$	4.3	2.7	- 31.5
Echo intensity, a.u.	43.5	$\pm$	8.1	23.7	- 64.0
s-MU, pps/%MVC	-0.11	$\pm$	0.11	-0.85	- 0.18
i-MU, pps	19.8	$\pm$	4.1	7.1	- 31.8
Number of detected motor units	7.9		6.8	2	- 39
Physical fitness tests					
Knee extension strength, Nm	191.2	$\pm$	59.5	54.9	- 424.2
Grip strength, kg	41.5	$\pm$	8.9	22.5	- 65.4
Five-time chair stand test, sec	5.33	$\pm$	0.80	3.71	- 8.10
Reaction time, sec	0.353	$\pm$	0.043	0.272	- 0.493
Five-sec stepping, steps	56.2	$\pm$	8.8	25	- 73
Side-stepping, score	54.5	$\pm$	6.9	39	- 69
Single-leg standing on rigid surface, sec	72.8	$\pm$	57.4	6.3	- 180.0
Single-leg standing on foam surface, sec	6.7	$\pm$	5.4	1.5	- 37.5
Standing broad jump, cm	215.6	$\pm$	27.7	142.0	- 282.5
Squat jump, cm	45.2	$\pm$	7.8	24	- 61
Counter-movement jump, cm	48.9	$\pm$	8.2	28	- 66

2 s-MU and i-MU were determined as y-intercept and slope, respectively, which were
3 calculated from the regression line between the individual motor unit firing rate and
4 recruitment threshold.

5

Table 2. Stepwise multiple regression analyses for physical fitness tests

Dependent variable	Independent variable	R ² (adjusted R ²)	Standardized β Coefficient	Unstandardized B Coefficient	(95%CI)	p
Knee extension strength	Model 1	.668 (.665)				
	ASM		0.818	11.282	(9.729, 12.835)	< .001
	Model 2	.690 (.684)				
	ASM		0.793	10.945	(9.415, 12.475)	< .001
	i-MU		0.149	2.159	(0.553, 3.765)	.009
	Model 3	.708 (.699)				
	ASM		0.713	9.841	(8.108, 11.574)	< .001
	i-MU		0.153	2.211	(0.644, 3.778)	.006
	Echo intensity		-0.156	-1.135	(-2.042, -0.229)	.015
	Model 4	.723 (.712)				
	ASM		0.723	9.978	(8.277, 11.678)	< .001
	i-MU		0.317	4.594	(2.055, 7.133)	< .001
Grip Strength	Echo intensity		-0.177	-1.290	(-2.187, -0.394)	.005
	s-MU		0.210	118.526	(17.880, 219.173)	.021
	Model 1	.649 (.646)				
	ASM		0.806	1.665	(1.426, 1.904)	< .001
	Model 2	.692 (.686)				
	ASM		0.684	1.412	(1.150, 1.674)	< .001
	Echo intensity		-0.239	-0.262	(-0.400, -0.123)	< .001

Five-sec stepping	Model 1	.212 (.204)				
	ASM		0.460	0.943	(0.587, 1.299)	< .001
Side-stepping	Model 1	.290 (.284)				
	ASM		0.539	0.858	(0.596, 1.120)	< .001
	Model 2	.367 (.354)				
	ASM		0.375	0.597	(0.307, 0.886)	< .001
	Echo intensity		-0.321	-0.271	(-0.424, -0.118)	< .001
Standing broad jump	Model 1	.536 (.532)				
	Sex		-0.732	-46.147	(-54.535, -37.760)	< .001
	Model 2	.606 (.598)				
	Sex		-0.540	-34.018	(-43.641, -24.394)	< .001
	Echo intensity		-0.326	-1.109	(-1.627, -0.590)	< .001
	Model 3	.638 (.627)				
	Sex		-0.299	-18.834	(-32.462, -5.206)	.007
	Echo intensity		-0.311	-1.056	(-1.557, -0.556)	< .001
Squat jump	Model 1	.537 (.533)				
	Sex		-0.733	-12.959	(-15.310, -10.607)	< .001
	Model 2	.640 (.633)				
	Sex		-0.498	-8.808	(-11.386, -6.229)	< .001
	Echo intensity		-0.398	-0.379	(-0.518, -0.240)	< .001
Counter-movement jump	Model 1	.546 (.542)				

Sex		-0.739	-13.732	(-16.177, -11.288)	< .001
Model 2	.627 (.620)				
Sex		-0.532	-9.882	(-12.640, -7.123)	< .001
Echo intensity		-0.352	-0.352	(-0.501, -0.203)	< .001
Model 3	.642 (.631)				
Sex		-0.496	-9.217	(-12.010, -6.425)	< .001
Echo intensity		-0.351	-0.351	(-0.497, -0.205)	< .001
s-MU		-0.127	-9.832	(-19.393, -0.270)	.044

Sex (dependent variable) was calculated as 0 for males and 1 for females. R^2 means the coefficient of determination. 95%CI means the 95% confidence interval. s-MU and i-MU were determined as y-intercept and slope, respectively, which were calculated from the regression line between the individual motor unit firing rate and recruitment threshold. ASM, Appendicular skeletal muscle mass

Table 3. Stepwise multiple regression analyses of s-MU or i-MU

Dependent variable	Independent variable	R ² (adjusted R ²)	Standardized β Coefficient	Unstandardized β Coefficient	(95%CI)	p
s-MU	Model 1	.109 (.100)				
	Counter-movement jump		-0.330	-0.004	(-0.007, -0.002)	< .001
i-MU	Model 1	.054 (.045)				
	Five-sec stepping		0.232	0.108	(0.019, 0.196)	.017

R² means the coefficient of determination. 95%CI means the 95% confidence interval. s-MU and i-MU were determined as y-intercept and slope, respectively, which were calculated from the regression line between the individual motor unit firing rate and recruitment threshold.

App. Table 1. Comparison of demographics, physical fitness tests, and neuromuscular measurements between males and females

	Males N=78			Females N=27			P-value
Age, years	20.7	±	2.2	20.3	±	0.9	.231
Height, cm	171.8	±	5.1	159.3	±	5.4	<.001
Body mass, kg	64.7	±	8.3	50.8	±	6.0	<.001
Neuromuscular properties							
Appendicular skeletal muscle mass, kg	23.8	±	2.7	15.8	±	2.0	<.001
Body fat mass, kg	8.7	±	4.5	11.2	±	2.6	.008
Echo intensity, a.u.	40.7	±	6.9	51.7	±	5.5	<.001
s-MU, pps/%MVC	-0.13	±	0.09	-0.06	±	0.12	.011
i-MU, pps	20.0	±	3.8	19.2	±	4.8	.372
Number of detected motor units							
Physical fitness tests							
Knee extension strength, Nm	214.6	±	48.5	123.6	±	28.5	<.001
Grip strength, kg	45.3	±	6.4	30.4	±	5.0	<.001
Five-time chair stand test, sec	5.4	±	0.8	5.2	±	0.8	<.001
Reaction time, sec	0.350	±	0.042	0.361	±	0.045	.240
Five-sec stepping, steps	58.4	±	7.6	50.0	±	9.2	.259
Side-stepping, score	56.5	±	6.3	48.7	±	4.8	<.001
Single-leg standing on rigid surface, sec	74.6	±	59.4	67.5	±	51.9	<.001
Single-leg standing on foam surface, sec	6.9	±	6.0	6.0	±	2.7	<.001
Standing broad jump, cm	227.5	±	18.5	181.3	±	20.3	<.001
Squat jump, cm	48.6	±	5.1	35.6	±	6.0	<.001
Counter-movement jump, cm	52.4	±	5.5	38.7	±	5.5	<.001

s-MU and i-MU were determined as y-intercept and slope, respectively, which were calculated from the regression line between the individual motor unit firing rate and recruitment threshold.

App. Table 2. Stepwise multiple regression analyses for physical fitness tests in each sex

Dependent variable	sex	Independent variable	R ² (adjusted R ²)	Standardized β Coefficient	Unstandardized Coefficient (95%CI)	B	p
Knee extension strength	Male	Model 1	.405 (.397)				
		ASM		0.636	11.430 (8.264, 14.595)		< .001
		Model 2	.437 (.422)				
		ASM		0.628	11.280 (8.177, 14.382)		< .001
		Echo intensity		-0.180	-1.260 (-2.467, -0.053)		.041
		Model 1	.359 (.334)				
	Female	ASM		0.600	8.622 (3.881, 13.362)		< .001
		Model 2	.519 (.478)				
		ASM		0.564	8.108 (3.887, 13.328)		< .001
		i-MU		0.400	2.371 (0.633, 4.109)		.010
		Model 3	.595 (.542)				
		ASM		0.507	7.292 (3.248, 11.336)		< .001
		i-MU		0.448	2.653 (0.997, 4.309)		.003
		Echo intensity		-0.286	-1.473 (-2.934, -0.012)		.048
Grip Strength	Male	Model 1	.292 (.282)				
		ASM		0.540	1.275 (0.821, 1.729)		< .001
		Model 2	.337 (.320)				
		ASM		0.530	1.252 (0.809, 1.695)		< .001

			Echo intensity	-0.214	-0.197	(-0.369, -0.025)	.026
Female			Model 1	.241 (.210)			
			Echo intensity	-0.491	-0.442	(-0.766, -0.119)	.009
			Model 2	.554 (.517)			
			Echo intensity	-0.575	-0.518	(-0.774, -0.262)	< .001
			i-MU	0.566	0.586	(0.292, 0.880)	< .001
			Model 3	.656 (.611)			
			Echo intensity	-0.509	-0.459	(-0.694, -0.223)	< .001
			i-MU	0.527	0.546	(0.279, 0.813)	< .001
			ASM	0.326	0.819	(0.167, 1.471)	.016
Five-time stand test	chair	Female	Model 1	.211 (.179)			
			i-MU	-0.459	-0.077	(-0.138, -0.016)	.016
Five-sec stepping		Female	Model 1	.155 (.121)			
			ASM	0.394	1.828	(0.071, 3.585)	.042
Side-stepping		Male	Model 1	.100 (.088)			
			Echo intensity	-0.317	-0.288	(-0.486, -0.091)	.005
			Model 2	.153 (.130)			
			Echo	-0.306	-0.279	(-0.472, -0.086)	.005

			intensity					
			ASM		0.230	0.539	(0.043, -1.036)	.034
Standing jump	broad	Male	Model 1	.140 (.129)				
			Echo		-0.374	-0.996	(-1.559, -0.432)	< .001
			intensity					
	Female	Model 1	.351 (.325)					
		ASM		0.592	6.056	(2.662, 9.450)	.001	
		Model 2	.480 (.437)					
		ASM		0.560	5.726	(2.607, 8.845)	< .001	
		i-MU		0.361	1.520	(0.236, 2.805)	.022	
		Model 3	.647 (.602)					
		ASM		0.476	4.869	(2.185, 7.552)	.001	
i-MU			0.431	1.817	(0.718, 2.915)	.002		
	Echo		-0.422	-1.549	(-2.518, -0.580)	.003		
		intensity						
Squat jump	Male	Model 1	.230 (.220)					
			Echo		-0.480	-0.350	(-0.497, -0.204)	< .001
			intensity					
	Female	Model 1	.228 (.197)					
		Echo		-0.478	-0.514	(-0.904, -0.125)	.012	
			intensity					
Model 2		.411 (.361)						
	Echo		-0.542	-0.584	(-0.936, -0.231)	.002		

		intensity					
		i-MU		0.4332	0.534	(0.130, 0.939)	.012
		Model 3	.530 (.469)				
		Echo		-0.470	-0.507	(-0.835, -0.178)	.004
		intensity					
		i-MU		0.390	0.482	(0.110, 0.854)	.013
		ASM		0.354	1.065	(0.155, 1.974)	.024
Counter-movement jump	Male	Model 1	.194 (.183)				
		Echo		-0.440	-0.352	(-0.515, -0.188)	< .001
		intensity					
	Female	Model 1	.300 (.272)				
		ASM		0.548	1.509	(0.560, 2.458)	.003
		Model 2	.497 (.455)				
		ASM		0.508	1.399	(0.573, 2.225)	.002
		i-MU		0.446	0.505	(0.165, 0.846)	.005
		Model 3	.614 (.564)				
		ASM		0.438	1.206	(0.450, 1.962)	.003
		i-MU		0.504	0.572	(0.263, 0.882)	< .001
		Echo		-0.353	-0.348	(-0.622, -0.075)	.015
		intensity					

Sex (dependent variable) was calculated as 0 for males and 1 for females. R^2 means the coefficient of determination. 95%CI means the 95% confidence interval. s-MU and i-MU were determined as y-intercept and slope, respectively, which were calculated from the regression line between the individual motor unit firing rate and recruitment threshold. ASM,

Appendicular skeletal muscle mass

App. Table 3. Stepwise multiple regression analyses of s-MU or i-MU for each sex

Dependent variable	Sex	Independent variable	R ² (adjusted R ²)	Standardized β Coefficient	Unstandardized β Coefficient	(95%CI)	p
s-MU	Female	Model 1	.190 (.158)				
		Five-time chair stand test		0.436	0.066	(0.010, 0.122)	.023
i-MU	Female	Model 1	.241 (.211)				
		Counter-movement jump		0.491	0.433	(0.117, 0.749)	.009

R² means the coefficient of determination. 95%CI means the 95% confidence interval. s-MU and i-MU were determined as y-intercept and slope, respectively, which were calculated from the regression line between the individual motor unit firing rate and recruitment threshold.

Figure 1

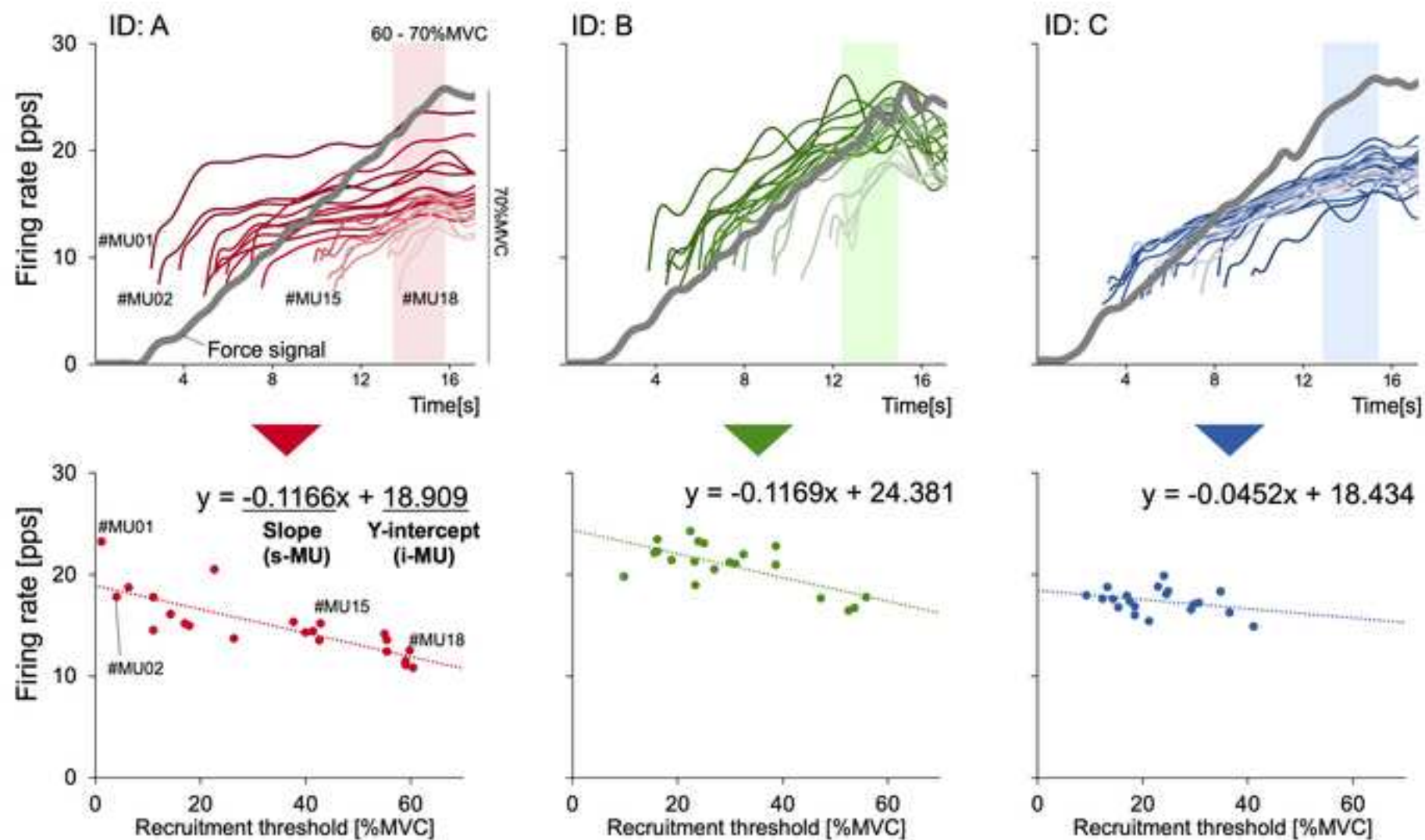
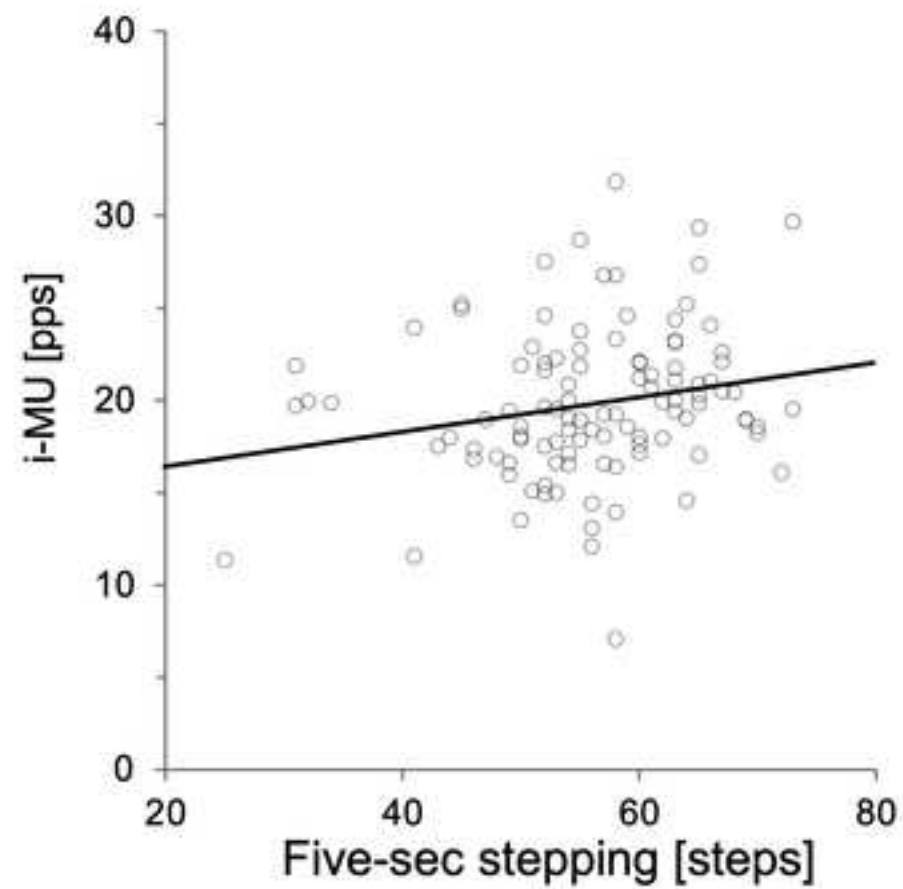
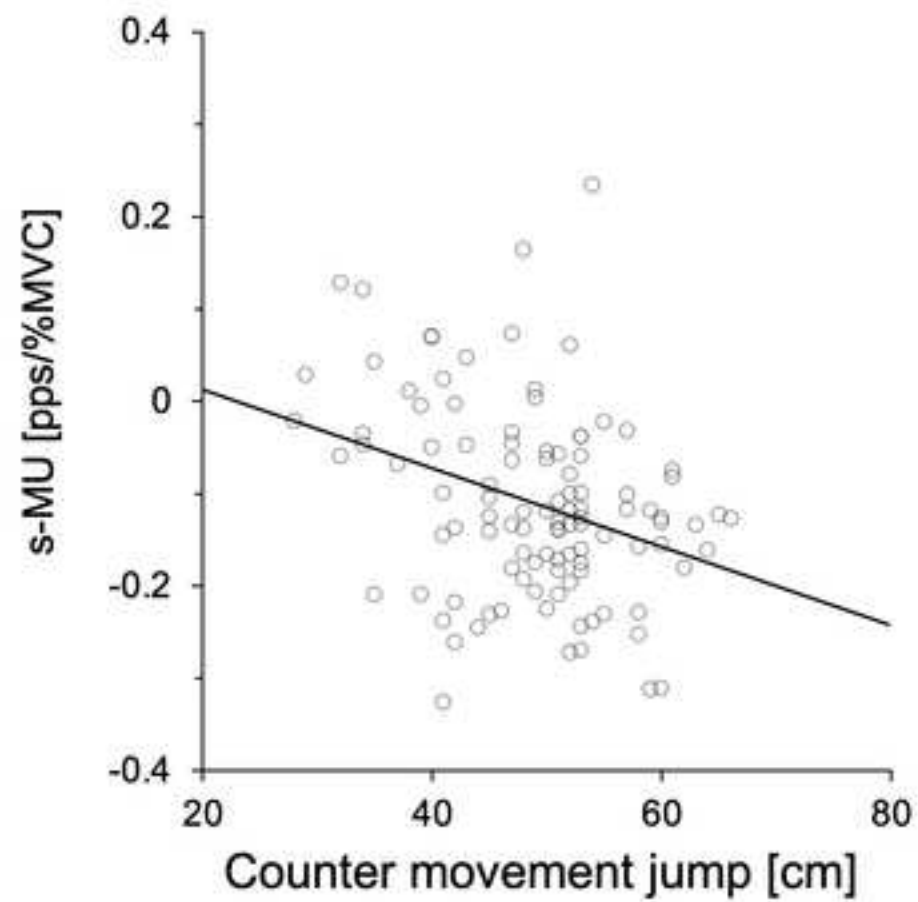


Figure 2



Author Contribution Statements

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by TH and KW. The first draft of the manuscript was written by TH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.