

Strength training increases conduction velocity of high-threshold motor units

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27 **ABSTRACT**

28 **Purpose:** Motor unit conduction velocity (MUCV) represents the propagation velocity of action
29 potentials along the muscle fibres innervated by individual motor neurons and indirectly reflects the
30 electrophysiological properties of the sarcolemma. In this study, we investigated the effect of a 4-
31 week strength training intervention on the peripheral properties (MUCV and motor unit action
32 potential amplitude, RMS_{MU}) of populations of longitudinally tracked motor units (MUs).

33 **Methods:** The adjustments exhibited by 12 individuals who participated in the training (INT) were
34 compared with 12 controls (CON). Strength training involved ballistic (4x10) and sustained (3x10)
35 isometric ankle dorsi flexions. Measurement sessions involved the recordings of maximal voluntary
36 isometric force (MViF) and submaximal isometric ramp contractions, while high-density surface
37 EMG (HDsEMG) was recorded from the tibialis anterior. HDsEMG signals were decomposed into
38 individual MU discharge timings and MUs were tracked across the intervention.

39 **Results:** MViF (+14.1%, $P=0.003$) and average MUCV (+3.00%, $P=0.028$) increased in the INT
40 group, while normalized MUs recruitment threshold (RT) decreased (-14.9%, $P=0.001$). The slope
41 (rate of change) of the regression between MUCV and MUs RT increased only in the INT group
42 (+32.6%, $P=0.028$), indicating a progressive greater increase in MUCV for higher-threshold MUs.
43 The intercept (initial value) of MUCV did not change following the intervention ($P=0.568$). The
44 association between RMS_{MU} and MUs RT was not altered by the training.

45 **Conclusion:** The increase in the rate of change in MUCV as a function of MU recruitment threshold,
46 but not the initial value of MUCV, suggests that short-term strength training elicits specific
47 adaptations in the electrophysiological properties of the muscle fibre membrane in high-threshold
48 motor units.

49 **Keywords:** Resistance training; motor unit; peripheral properties; conduction velocity; amplitude;
50 EMG decomposition

51

52

53 INTRODUCTION

54 Strength training is one of the most common modalities of exercise since it is known to improve
55 musculoskeletal health and enhance athletic performance (1). It is well established that physical
56 activity involving repeated bouts of strong voluntary contractions, increases the maximal force-
57 generating capacity of skeletal muscles. There is evidence that the early increase in voluntary muscle
58 force that occurs after very few training sessions (< 2-4 weeks) is determined predominantly by neural
59 factors (2–4), before significant hypertrophy and muscle architectural adjustments take place
60 (typically > 30-35 days) (5–8). Recently, we showed that the increase in muscle force following 4
61 weeks of strength training is likely mediated by an increase in the net excitatory input to the motor
62 neuron pool or to adaptations in the intrinsic motor neuron properties (9). Although muscle contractile
63 properties typically change in longer training times, the electrophysiological muscle fibre membrane
64 properties may show faster changes.

65 An electromyography (EMG) derived parameter that reflects the fibre membrane properties is muscle
66 fibre conduction velocity (MFCV) which represents the average velocity of propagation of motor unit
67 action potentials (MUAPs) along the sarcolemma. MFCV is a basic physiological parameter that can
68 be estimated either from the interference EMG as the weighted mean of the conduction velocities of
69 the several concurrently active motor units (10) or for single motor units (MUCV) as the average
70 propagation velocity of action potentials along the muscle fibres innervated by individual motor
71 neurons (11–14), by decomposing the surface EMG signal and extracting action potentials for isolated
72 motor units (15). At the single muscle fibre level, MFCV is related to the diameter of the fibres (16–
73 18) and this association can be mathematically derived because of a biophysical association between
74 diameter and conduction velocity (19). Moreover, MFCV linearly increases with force because of the
75 progressive recruitment of higher-threshold motor units innervating fibres with larger diameters.
76 Indeed, Del Vecchio et al., (20, 21) have recently reported a strong association between MFCV,
77 estimated during increasing-force contractions and MUCV ($R^2 = 0.71$), which in turn significantly
78 correlated with MU recruitment threshold ($R^2 = 0.70$). Therefore, MFCV is considered an indicator

79 of the progressive recruitment of motor units (i.e. a “size-principle” parameter) and has been generally
80 adopted to indirectly infer neural control strategies in a wide range of contractions (20, 21).
81 Additionally, MFCV provides an indirect window into the electrophysiological properties of the
82 muscle fibre membrane since it is influenced by the polarization state, i.e. electrical excitability, of
83 the sarcolemma (22, 23). Indeed, the velocity of propagation of MUAPs is influenced by intracellular
84 and extracellular ionic concentrations (mainly Na^+ - K^+), and hence by Na^+ - K^+ -ATPase pump activity,
85 changes of the membrane potential, resistance and capacitance, as well as changes of intramuscular
86 pH and temperature (23). Moreover, the propagation velocity of MUAPs is also influenced by motor
87 unit discharge rate (24).

88 The adaptations in the neural and peripheral properties of motor units following short-term strength
89 training in longitudinally tracked motor units have yet to be clarified (25). In particular, it is currently
90 unknown whether short-term strength training influences the electrophysiological properties of the
91 muscle fibre membrane of individual motor units. The only available evidence has been obtained by
92 cross-sectional studies that have estimated MFCV from chronic strength and power trained athletes
93 (18, 20, 26), or interventional studies that have investigated changes in MUCV following high-
94 intensity interval training (HIIT) and/or endurance training (27) or strength training (28). In particular,
95 Del Vecchio and colleagues (20) recently observed significantly higher MFCV in a cohort of strength-
96 trained individuals compared to untrained, which was also accompanied by an association to the rate
97 of force development. Moreover, Martinez-Valdez et al., (27) reported increased MUCV from low-
98 to high-threshold motor units after two weeks of HIIT, whereas increased MUCV occurred only in
99 low-threshold motor units after endurance training. Similarly, Vila-Chã et al., (28) observed an
100 increase in MUCV, assessed in contractions at 30 % of the maximum voluntary contraction, following
101 6 weeks of either endurance or strength training. These studies collectively suggest that the
102 propagation velocity of action potentials along muscle fibres might be altered by a training
103 intervention, although further investigations are warranted. Indeed, the time-course of conduction
104 velocity in single motor units after strength training is currently unknown.

105 Technological advancements in the recording and decomposition of high-density surface EMG
106 (HDsEMG) signals allow the behaviour of large samples of motor units to be evaluated in-vivo and
107 for a wide range of voluntary forces (15, 29). The non-invasive estimation of MUCV allows the
108 electrophysiological properties of the muscle fibre membrane to be characterised for different
109 populations of motor units (e.g. low-threshold and high-threshold motor units) (12, 13). Moreover,
110 such methodology provides a reliable tracking of the same motor units across different experimental
111 sessions (9, 30). This implies that potential training-associated changes in the neural and peripheral
112 properties of motor units can be directly investigated at the individual subject level and for the
113 recruitment range of a muscle.

114 In this study, we concurrently evaluated the changes in MUCV and MU action potential amplitude
115 as well as adjustments in recruitment threshold and discharge rate of motor units from the tibialis
116 anterior muscle tracked over time, following a four week strength training intervention. In order to
117 indirectly relate motor neurone and muscle fibre properties, the association between MUCV and
118 recruitment threshold of the corresponding motor unit was compared before and after the training
119 intervention at the individual-subject level. Although MU recruitment threshold is a measure of force
120 and hence not strictly a motor neuron property, here we consider it as an indirect measure of the
121 activation threshold of a motor neuron.

122 In particular, based on the aforementioned evidences that highlight the potential adaptability of
123 MUCV following a training intervention, it was hypothesized that the exposure to a short term
124 strengthening intervention involving the combination of ballistic and submaximal sustained isometric
125 contractions, would be sufficient to induce changes in MUCV and that the adjustments would differ
126 between low- and high-threshold motor units.

127

128 **METHODS**

129 **Participants**

130 The participants enrolled in this study were the same as in our previous publication, which
131 investigated strength training-induced changes in motor neuron output (9). In this study, we focused
132 on the conduction velocity of single motor units. Specifically, 28 healthy, recreationally active and
133 non-smoking young men took part. The exclusion criteria were the presence of any neuromuscular
134 disorder and previous history of lower limb pathology or surgery. Volunteers were physically active
135 of light to moderate intensity at a recreational level (e.g. running, soccer, basketball) no more than
136 twice a week. Participation in regular or competitive lower body strength or power training in the last
137 6 months was a further exclusion criterion. Participants were randomly allocated to either an
138 intervention group (INT, n=14) or to a control group (CON, n=14), which were very homogeneous
139 at the baseline with respect to their anthropometrical features, physical activity habits based on their
140 score on the International Physical Activity Questionnaire (IPAQ) and maximal voluntary isometric
141 force (MViF) (see Table 1). Three participants withdrew following recruitment for personal reasons
142 (i.e. time demands). Additionally, one participant from the INT group was excluded a posteriori from
143 the analysis because of poor EMG signal quality for the estimation of conduction velocity (coefficient
144 of correlation (CC) between channels < 0.70 , see below). Thus, a total of 24 participants, 12
145 volunteers in the INT group and 12 volunteers in the CON group completed the study and were
146 considered in the current analysis (see **Table 1**).

147 The study protocol and procedures were approved by the University of Rome “Foro Italico” Ethical
148 Committee (approval no. 44 680) and conformed to the requirements of the *Declaration of Helsinki*.
149 After being informed of the purpose and experimental procedures of the study a written informed
150 consent was signed by all participants prior to the start of the study.

151

152 **Study overview**

153 Experimental protocols, procedures and strength training regimen have been described previously in
154 details (9) and are therefore only briefly summarized here.

155 The experimental protocol consisted of fifteen laboratory sessions over a 7-week period. Sessions
156 one and two consisted of familiarization and baseline assessment session, respectively. Sessions three
157 to fourteen consisted of the 4-week strength training intervention for the INT group and session fifteen
158 involved the post intervention assessment.

159 The first session involved explanation of the study, and familiarization with the experimental setup
160 and testing protocol. In particular, the familiarization session involved maximal voluntary as well as
161 submaximal isometric ankle dorsi-flexion of the dominant foot (selected based on a self-report).
162 Additionally, a standard health questionnaire was used to evaluate their eligibility to the study and
163 they were screened for their physical activity habits (IPAQ, short form). Following recruitment, and
164 three to five days after the familiarization session, the participants underwent the main baseline
165 assessment session which involved the concomitant recordings of muscle force during maximal and
166 submaximal isometric voluntary contractions and HDsEMG recordings from the tibialis anterior
167 muscle.

168 The training intervention, which involved 3 sessions a week for 4 weeks (12 sessions in total) was
169 based on unilateral isometric strength training of the ankle dorsiflexors. The control subjects were
170 instructed to continue to exercise as usual and not to change their physical activity daily habits. During
171 the last session, which was performed 48-72 hours after the final training session, of all the baseline
172 measurements performed in session two, were repeated.

173 All participants were asked to abstain from strenuous physical exercise 48 hours prior to the main
174 measurement sessions and additionally to avoid caffeine consumption 24 hours prior to these
175 sessions. In order to minimize diurnal variability in muscle contractility, the two measurement
176 sessions were held at a consistent time of the day for each participant.

177

178 **Experimental procedure**

179 *Baseline and post-test assessments (Force and HDsEMG measurements)*

180 Following placement of the HDsEMG electrodes (see below), the participants performed a
181 standardized warm-up involving 8 isometric contractions of ankle dorsiflexion at different intensities
182 of self-perceived maximal voluntary force (4x50%, 3x70%, 1x90%, with 15-30 s rest in-between).
183 To determine the maximal voluntary isometric force (MViF) of the dorsiflexors, the participants
184 performed 3-4 maximal voluntary contractions (MVCs) separated by 30 s of rest. The participants
185 were instructed to “push as hard as possible” and to achieve the MViF within 3-5 s. During the
186 contractions, the participants were motivated with verbal encouragement by an investigator. A
187 horizontal cursor displayed on a monitor indicated the peak force achieved in the preceding MVC.
188 The highest force recorded out of the 3-4 trials was set as a reference to determine the relative intensity
189 of the submaximal contractions in each of the two measurement sessions.

190 Five minutes after the MVCs, the participants completed 6 trapezoidal contractions (2 x each of the
191 target forces set at 35%, 50%, 70% of MViF) that were characterized by a linear increase in force to
192 the target value, 10 s of steady state at the achieved target force, and a linear force decrease back to
193 the baseline value. The rate of force development was kept constant in all trapezoidal contractions
194 and was equal to 5% MViF·s⁻¹ for both the ramp-up and ramp-down phases. In this task, the
195 participants were instructed to match as precisely as possible a visual force template corresponding
196 to the three target forces, which was displayed on a computer monitor placed at 1 m distance from
197 participants’ eyes.

198 Trapezoidal contractions were separated by 3-to-5 minutes of recovery and were performed in a
199 randomized order, which was in turn kept constant for each participant both at baseline and post
200 intervention assessment, in order to minimize the potential effects of fatigue, i.e., reduction in force
201 capacity, on motor unit behaviour after the training intervention (27).

202

203 *Training Protocol*

204 The intervention involved 12 training sessions lasting approximately ~30 min each, separated by 48-
205 72 hours, over a period of four weeks. Each session were supervised by an investigator (A.C and/or

206 A.D.V) and involved a warm-up, maximal voluntary contractions and a combination of ballistic and
207 sustained isometric contractions.

208 The standardized warm-up consisted of five submaximal isometric contractions of ankle dorsiflexion
209 (2x50%, 2x70%, 1x90% of perceived MVIF) of the dominant foot and was followed by three MVCs
210 to determine the reference values for submaximal contractions. Approximately 5 minutes after the
211 MVCs, the participants performed a total of 40 ballistic contractions and 30 sustained ramp
212 contractions. In the ballistic contractions (4 sets x 10 repetitions), the participants were instructed to
213 contract “as fast and as hard as possible” up to a horizontal target placed at 75% of their MVIF,
214 without any pre-tension and/or countermovement, and immediately relax thereafter. A resting time
215 of 5 s and 1 min were allowed between the repetitions and each set, respectively. In the sustained
216 isometric ramp contractions (3 sets x 10 repetitions), the participants were instructed to reach a target
217 force of 75% MVIF in 2 s ($37.5 \text{ MVIF} \cdot \text{s}^{-1}$) and hold a steady state phase at the target force for 3 s. A
218 resting interval of 2 s and 2 min were given between the sustained repetitions and each set,
219 respectively.

220

221 **Data acquisition**

222 *Force recording*

223 The familiarization, main trials and the training sessions were carried out on the same apparatus,
224 which consisted of a rigid custom-made ankle ergometer (OT Bioelettronica, Turin, Italy) fixed to a
225 massage table. Individual variability in lower limb length was accounted for by regulating the position
226 of the ergometer on the table with two adjustable straps. The testing and training configurations were
227 defined during the initial session and were then subsequently replicated.

228 The participants were seated on the massage table in a comfortable position with their back against
229 the seat back ($\sim 120^\circ$ hip flexion), their knee extended to $\sim 180^\circ$ and their ankle positioned in $\sim 100^\circ$
230 ($90^\circ =$ perpendicular to the tibia) of plantar flexion. In order to minimize extraneous movements, their

231 dominant leg was tightly secured to the table and to the ergometer with Velcro straps (~3 cm) placed
232 at the knee (above the patella), ankle (foot dorsum) and foot (over the distal third of metatarsals).
233 Muscle force produced during isometric ankle dorsiflexion was recorded with a calibrated load cell
234 (CCT TRANSDUCER s.a.s, Turin, Italy) that was positioned in series with an adjustable footplate to
235 which the foot was fastened. The analogue force signal from the load cell was amplified (x 200) and
236 sampled at 2048 Hz with an external analogue to digital (A/D) converter (EMG-Quattrocento, OT
237 Bioelettronica, Turin, Italy), and in turn synchronized with the EMG data. A personal computer was
238 used to record force and HDsEMG data with the software OT BioLab (Version 2.0.6352.0, OT
239 Bioelettronica, Turin, Italy). Force templates and feedback were provided with a customized
240 LabVIEW program (LabVIEW 8.0, national Instruments, Austin, USA) from a second computer, and
241 displayed on a monitor (see above).

242

243 *HDsEMG recording*

244 Myoelectrical activity during the isometric contractions of ankle dorsiflexion was recorded from the
245 tibialis anterior muscle using two bi-dimensional adhesive grids of 64 equally spaced electrodes each
246 (5 columns x 13 rows; gold-coated; 1 mm diameter; 8 mm interelectrode distance (IED); OT
247 Bioelettronica, OT Bioelettronica, Turin, Italy). The electrode positioning and orientation has been
248 described previously (9) and was performed according to the anatomical description for the location
249 of an easily identifiable innervation zone (IZ) in the distal portion of tibialis anterior muscle (31, 32).
250 Briefly, in order to determine the placement of the high-density grids, the muscle belly was identified
251 through palpation by an experienced investigator and its profile was delineated with a surgical marker.
252 To optimize the orientation of the grids, a 16-electrode dry array was used to identify the IZ located
253 in the distal portion of the tibialis anterior and to estimate muscle fibre direction (13, 21). The IZ was
254 located by identifying the point of inversion in the propagation direction of action potentials
255 proximally (toward proximal tendon of tibialis anterior) and distally (toward the distal tendon of

256 tibialis anterior) along the electrode column (13, 21). The estimation of the anatomical direction of
257 muscle fibres corresponded to alignment that led to the identification of action potentials propagating
258 clearly along the array, without substantial changes in waveform shapes. Once the IZ and the
259 estimated fibre direction were determined, the skin surface was shaved, lightly abraded and cleansed
260 with 70% ethanol. Disposable bi-adhesive foam layers (SpesMedica, Battipaglia, Italy) were used to
261 attach the grids to the surface of the muscle. The first adhesive grid of electrodes was positioned with
262 the first column of electrode aligned in the direction of the muscle fibres and with the first four rows
263 on the IZ. In order to cover most of the muscle belly, the second high-density grid was attached
264 proximally to the first. The skin-to-electrode contact was optimized by filling each adhesive layer
265 hole, corresponding to one electrode, with conductive paste (SpesMedica, Battipaglia, Italy).
266 The reference electrode was placed in proximity of the styloid process of the ulna on the wrist on the
267 tested side. The reference electrodes for the two preamplifiers were positioned on the tuberosity of
268 the tibia and on the medial malleolus of the tested limb.

269 The HDsEMG signals were recorded in monopolar configuration, amplified (x 150) and band pass
270 filtered (10-500 Hz) at source, and converted to digital data by a 16-bit analogue-to-digital converter
271 (EMG-Quattrocento, 400-channel amplifier, OT Bioelettronica, Turin, Italy) before being stored on
272 a computer hard-disk for off-line analysis (Matlab R2016a, The Mathworks Inc., Natick,
273 Massachusetts, USA). The HDsEMG signals were sampled at 2048 Hz.

274 In order to allow similar electrode positioning between the baseline and final measurement session,
275 the exact profiles of the two grids were marked on the participants' skin at the baseline session using
276 a surgical pen. Participants were instructed to re-mark carefully the grid profiles daily. Additionally,
277 the electrodes position with respect to anatomical landmarks was also traced on transparent sheets (9,
278 27).

279

280 **Force and HDsEMG analysis**

281 *Muscle force*

282 After the conversion to digital data, the force signal was transformed into newtons (N) and low-pass
283 filtered (4th order, zero-lag, Butterworth) with a cut-off frequency of 15 Hz. The offset was removed
284 by correcting for the effect of gravity and for each participant, only the trapezoidal contraction trial
285 at each force target (35%, 50%, 70% MViF) showing the best tracking of force with respect to the
286 given template and with no pre-tension or countermovement (≤ 0.5 N from the baseline of force in
287 the 150 ms prior to force onset^{x1}), was included in the analysis (9).

288

289 *Motor unit analysis*

290 In the present study, we focused solely on the decomposition and analysis of HDsEMG signals
291 recorded from the grid located on the distal portion of tibialis anterior muscle. Indeed, proper
292 electrode placement (i.e. identification of IZ, estimation of muscle fibres orientation) was the sine-
293 qua-non to observe the propagation of MUAPs from the IZ to the tendon region and hence allow
294 MUCV to be reliably calculated (13, 21). For clarity, in our previous publication (9) only the
295 HDsEMG signals recorded from the grid located on the proximal portion of tibialis anterior muscle
296 were decomposed and analysed given the divergent aims of each work.

297 In an offline analysis, monopolar HDsEMG signals were band-pass filtered between 20 and 500 Hz
298 (2nd order, Butterworth). The HDsEMG signals were decomposed into individual MUAPs, with an
299 extensively validated convolutive blind source separation method (15, 29). This decomposition
300 algorithm is highly reliable and sensitive to detect changes in motor unit behaviour after different
301 training interventions (9, 30). Additionally, it can accurately identify discharge timings even at high
302 (70%) force levels (29). Once the motor unit discharge times were identified, they were converted to
303 binary spike trains and manually inspected by experienced investigators. Only those motor units with
304 a pulse-to-noise ratio (PNR) higher than 30dB and/or by a time interval < 2 s between the spikes were
305 retained and further analysed (29).

306 For each identified motor unit, the recruitment threshold (RT) and mean discharge rate (DR) were
307 calculated. Motor unit RT was defined as the percentage of force (%MViF) produced by the ankle
308 dorsiflexors at which the first motor unit action potential was discharged. Mean motor unit DR was
309 calculated as the average of the first 20 MUAPs, in the ramp-up phase (e.g. at the recruitment) of the
310 trapezoidal contraction. This number of firings minimizes the effect of interspike interval (ISI)
311 variations on the assessment of average MU discharge rate in the recruitment phase of the trapezoidal
312 contraction and on the estimation of MU conduction velocity (13, 21, 27).

313

314 *Motor unit conduction velocity and amplitude estimation*

315 Motor unit action potential waveforms were extracted via spike-triggered averaging. The
316 multichannel MUAP waveforms were extracted by averaging HDsEMG signals using the discharge
317 times identified by decomposition as triggers (13). The first 20 discharge timings for each motor unit
318 were used for the spike-triggered averaging, which was performed in 15 ms (MUAPs duration)
319 intervals. Double differential derivations were then computed from averaged monopolar MUAPs
320 along the electrode columns and used for MUCV and motor unit amplitude (e.g. root mean square,
321 RMS_{MU}) estimation. Double differential EMG channels were visually inspected (customized Matlab
322 script) and a minimum of 4 up to a maximum of 8 double differential channels belonging to the same
323 electrode column were selected for MUCV and RMS_{MU} calculation. To date, manual selection of
324 EMG channels is considered the most accurate method for MUCV and RMS_{MU} estimation (13, 21).
325 The criteria for channel selection were the clearest propagation of action potentials along the electrode
326 columns with minimal change in MU shape, and the highest correlation coefficient (CC) between the
327 channels ($CC \geq 0.70$) (33). Since the number of EMG channels influences the accuracy of MUCV
328 estimation, we selected the greatest number of channels showing a $CC \geq 0.70$. Once the channels
329 were selected, a multi-channel maximum likelihood algorithm was adopted to calculate MUCV. This
330 algorithm has shown to estimate MUCV with a considerably low standard deviation ($< 0.1 \text{ m}\cdot\text{s}^{-1}$)
331 (12). On the same selected channels, RMS_{MU} was calculated by applying the same procedures adopted

332 for global EMG variable estimates. Moreover, the same number and location (column of electrodes)
333 of the selected channels adopted at the baseline assessment was maintained for MUCV and RMS_{MU}
334 estimation at the post-intervention measurement.

335

336 *Motor unit tracking*

337 A validated motor unit tracking approach was adopted to investigate training-related changes in motor
338 unit neural (RT, DR) and peripheral properties (MUCV, RMS_{MU}) on the same motor units identified
339 before and after the intervention (30). This procedure can accurately and reliably identify the same
340 motor units longitudinally across multiple experimental sessions in different days/weeks and has
341 already been adopted in at least two different training studies (9, 27), which have confirmed the
342 possibility to track 30 to 40% of all motor units identified by HDsEMG decomposition across
343 different sessions. The tracking method is based on the two-dimensional cross-correlation between
344 MUAP waveforms, which are in turn extracted with the spike-triggered averaging, following
345 HDsEMG decomposition (see above). A minimum CC value between MUAP waveforms of 0.70 was
346 accepted (9).

347

348 **Statistical analysis**

349 The Shapiro-Wilk test was adopted to evaluate the distribution of the data for all the variables
350 considered. In the case of non-normal distribution, the correspondent non-parametric tests were
351 applied. The sphericity assumption was assessed with the Mauchly's test, and if this condition was
352 not satisfied, the Greenhouse-Geisser correction was applied. Baseline between-group differences in
353 anthropometrical features (age, height, body mass), physical activity habits (IPAQ score) and baseline
354 muscle force levels (MViF) were investigated with one-way ANOVAs. Similarly, between-group
355 differences with regard to baseline neural and peripheral properties of motor units (RT, DR, MUCV,
356 RMS_{MU}) were assessed with the same test. Differences in the total number of motor units identified

357 by HDsEMG decomposition between groups (INT vs. CON) and conditions (PRE vs. POST) were
358 assessed with one-way ANOVA and paired-t-test, respectively.

359 The effects of strength training on the outcome variables, i.e. MVIF, motor unit RT (in absolute and
360 normalized terms) and DR, were investigated with two-way repeated-measures analysis of variance
361 ANOVA (time: PRE vs. POST; group: INT vs. CON). When significant time x group interactions
362 were found, results were determined following adjustment with Bonferroni correction. For each
363 participant, motor unit variables (RT, DR, MUCV, RMS_{MU}) were averaged among contractions
364 (35%, 50%, 70% of MVIF) whereas, for each group, individual values were averaged. Subject-
365 specific changes in MUCV and RMS_{MU} of the tracked motor units were studied as a function of their
366 RT. Firstly, the association between MUCV/RMS_{MU} and motor unit RT for each participant in each
367 condition (PRE vs. POST) was assessed with Pearson product-moment correlation coefficient.
368 Secondly, the slopes and intercepts of the regression lines between MUCV/RMS_{MU} and motor unit
369 RT from all participants, at all force targets (35%, 50%, 70% of MVIF) and in both test conditions
370 (PRE vs. POST) were compared with two-way repeated measure ANOVAs. The effect size of
371 changes for all the variables analysed after the training intervention was calculated as partial eta
372 square (η^2) from the ANOVAs (34). Statistical analyses were performed with the software SPSS,
373 Version 23.0 (SPSS Inc, Chicago, IL, USA). The significance level was set at $\alpha < 0.05$ for all tests.
374 Results are presented as mean \pm standard deviation (SD).

375

376 **RESULTS**

377 **Baseline assessment**

378 At the baseline, there were no significant differences between the two groups with regard to age,
379 anthropometric features, physical activity habits and MVIF of ankle dorsiflexors (see **Table 1**).
380 Similarly, no between-group differences were detected for any of the electrophysiological variables,

381 i.e., absolute and normalized RT, mean MU DR, average MUCV and RMS_{MU} , at the baseline (see
382 **Table 2**).

383

384 **Motor unit decomposition and tracking**

385 A total of 948 motor units from the tibialis anterior muscle were included in the analysis. This number
386 is the sum of all motor units detected for both groups and conditions. The total number of identified
387 motor units was not statistically different between groups (INT: 475; CON: 473; $P = 0.961$) and
388 conditions (PRE: 493; POST: 455; $P = 0.062$). A total of 210 motor units could be tracked between
389 the baseline and post-intervention session (INT: 94; CON: 116; $P = 0.245$), corresponding to 22.2%
390 of the total number of motor units identified. The average number of tracked motor unit per participant
391 was 8 ± 2 and 10 ± 5 for INT and CON group, respectively. The cross-correlation between the action
392 potential waveforms of the tracked motor units pre- and post-intervention was 0.81 ± 0.01 and 0.88
393 ± 0.03 in INT and CON group, respectively. (See Table 3, Supplemental Digital Content 1, Overview
394 of the total number of identified and tracked motor units by group, pre-to-post intervention).

395

396 **Neuromotor adaptations**

397 Maximal voluntary isometric ankle dorsiflexion force increased significantly after four weeks of
398 strength training from 284.3 ± 64.0 to 324.4 ± 61.5 N (+14.1%; $P = 0.003$, $\eta^2 = 0.576$; Figure 1 A).

399 Conversely, no change was observed for the CON group (PRE: 299.2 ± 40.6 N; POST: 304.3 ± 35.4
400 N; $P = 0.422$).

401 Similarly, recruitment threshold of the pool of tracked motor units, in both absolute and normalized
402 values, changed following the intervention. Normalized motor unit RT (% MViF), averaged across
403 contractions and subjects, decreased significantly from 32.2 ± 18.1 to 27.4 ± 15.7 % MViF (-14.9%;
404 $P = 0.001$; $\eta^2 = 0.665$; Figure 1 B) following training. The absolute motor unit RT decreased from

405 93.9 ± 51.9 to 85.0 ± 46.4 N (-9.4%) following training, although this was not significantly ($P =$
406 0.238). In the CON group no differences were observed for RT in both absolute (PRE: 95.6 ± 53.3
407 N; POST: 95.9 ± 53.8 N; $P = 0.952$) and normalized values (PRE: 31.5 ± 17.5 % MVIF; POST: 31.3
408 ± 17.7 % MVIF; $P = 0.886$).

409 Because of its influence on the conduction velocity of action potentials, according to the velocity
410 recovery function (24), the motor unit discharge rate was also investigated. The mean discharge rate
411 of the tracked motor units at recruitment (average of the first 20 spikes) did not change significantly
412 as a consequence of the intervention (Figure 1 C). The mean MU discharge rate values for the INT
413 group were 15.5 ± 3.1 pps and 16.1 ± 2.5 pps at the baseline and the post-test assessment, respectively
414 ($P = 0.125$). For the CON group, the mean discharge rate was 14.8 ± 2.8 pps and 14.8 ± 2.7 pps at
415 the baseline and post-test, respectively ($P = 0.955$).

416

417 **Motor unit properties**

418 *Motor unit conduction velocity (MUCV)*

419 The observed average MUCV values of the tracked motor units ($n = 210$) were within the
420 physiological range (2-6.5 m·s⁻¹) in all cases and in agreement with previous studies conducted on
421 healthy populations (11, 13, 14, 21, 31). Specifically, the MUCV range was 2.98-6.07 m·s⁻¹, with an
422 average of 4.35 ± 0.63 m·s⁻¹.

423 Motor unit propagation velocity of the longitudinally tracked motor units recorded from tibialis
424 anterior, represented by an average of values among contractions and participants, changed
425 significantly following 4 weeks of strength training (Interaction: time x group; $P = 0.004$, $\eta^2 =$
426 0.327). MUCV significantly increased following the intervention from 4.52 ± 0.39 to 4.66 ± 0.44 m·s⁻¹
427 (+3.00%; $P = 0.028$; $\eta^2 = 0.367$), on average. Moreover, when mean MUCV was computed
428 separately for the tracked lower-threshold (RT between 0-30% MVIF) and higher-threshold (RT
429 between 50-70% MVIF) motor units, significant changes were observed solely for the higher-

430 threshold motor units (n=48; low-threshold, PRE: 4.14 ± 0.53 ; POST: $4.19 \pm 0.53 \text{ m}\cdot\text{s}^{-1}$; $P = 0.066$;
431 n=20; high-threshold, PRE: 5.14 ± 0.45 ; POST: $5.28 \pm 0.55 \text{ m}\cdot\text{s}^{-1}$; $P = 0.037$; $\eta^2 = 0.210$).
432 Conversely, no significant changes were observed for the CON group for either low-threshold MUs
433 (n=64; PRE: 3.96 ± 0.51 ; POST: $3.93 \pm 0.46 \text{ m}\cdot\text{s}^{-1}$, $P = 0.227$) and high-threshold MUs (n=23; PRE:
434 4.76 ± 0.49 ; POST: $4.70 \pm 0.38 \text{ m}\cdot\text{s}^{-1}$; $P = 0.056$).

435 We observed a linear correlation between motor unit RT and MUCV in all conditions (PRE vs. POST)
436 and groups (INT vs. CON). This indicates a faster action potential propagation velocity in higher-
437 threshold compared to lower-threshold motor units, and is in agreement with previous studies (11,
438 13, 14, 21). Individual R^2 values ranged from 0.31 to 0.99 with a mean value of 0.71 ± 0.16 ($P < 0.05$
439 in all cases). (See Table 4, Supplemental Digital Content 2, Participant-specific values for MUCV
440 linear regression analysis pre-to-post intervention).

441 Motor unit propagation velocity changes to strength training were further investigated by linear
442 regression. As depicted in Figure 2 A-B, the rate of change of MUCV as a function of their RT
443 adapted differently in the two groups, when considering the same motor units before and after the
444 intervention (interaction: time x group; $P = 0.035$; $\eta^2 = 0.186$). In the INT group, the rate of change
445 of MUCV, i.e. the regression slope, changed significantly after the intervention and increased on
446 average from 0.019 ± 0.007 to $0.025 \pm 0.011 \text{ m}\cdot\text{s}^{-1}\cdot\% \text{MViF}$ (+32.6%; $P = 0.028$; $\eta^2 = 0.367$; Figure
447 3 B). Conversely, the y-intercept of MUCV (PRE: 3.93 ± 0.50 ; POST: $3.97 \pm 0.56 \text{ m}\cdot\text{s}^{-1}$; $P = 0.314$)
448 was not significantly influenced by the training intervention (interaction: time x group; $P = 0.568$;
449 $\eta^2 = 0.015$; Figure 3 A). These findings indicate a predominant increase in MUCV for high threshold
450 motor units and suggest specific electrophysiological changes in the motor units recruited at higher
451 muscle forces.

452 On the contrary, the rate of change in MUCV (slope, PRE: 0.018 ± 0.008 ; POST: $0.017 \pm 0.007 \text{ m}\cdot\text{s}^{-1}$
453 $\cdot\% \text{MViF}$; $P = 0.696$) and the initial value (intercept, PRE: 3.71 ± 0.54 ; POST: $3.71 \pm 0.47 \text{ m}\cdot\text{s}^{-1}$; P
454 $= 0.999$) of the linear regressions remained similar in the CON group (Figure 3 A-B).

455 Because of the association between force and MUCV (21), we also assessed the association between
456 the changes in MViF (Δ MViF) and the changes in MUCV (Δ MUCV) at the individual level. The
457 correlation was not statistically significant ($r = 0.045$, $P = 0.889$).

458

459 *Motor unit amplitude (RMS_{MU})*

460 The action potential amplitude of the pool of tracked motor units, did not change following the
461 training intervention (Interaction: time x group; $P = 0.478$, $\eta^2 = 0.023$). When averaged among
462 contractions and participants, RMS_{MU} ranged from 59.51 ± 29.62 (PRE) to 56.17 ± 27.66 (POST) μ V
463 and from 55.51 ± 37.51 to 47.60 ± 22.27 μ V in the INT group and CON group, respectively.

464 The regression analysis indicated less consistent associations between RMS_{MU} and motor unit
465 recruitment thresholds. Indeed, only 17 out of 24 individuals showed significant correlations in all
466 testing conditions. Considering both conditions (PRE vs. POST) and groups (INT Vs. CON),
467 individual R^2 ranged from 0.04 to 0.98 with a mean value of 0.67 ± 0.24 . (See Table, Supplemental
468 Digital Content 3, Participant-specific values for RMS_{MU} linear regression analysis pre-to-post
469 intervention). The absence of changes in RMS_{MU} at the group level as a consequence of the strength
470 training intervention was confirmed by individual linear regressions. Indeed, as reported in Figure 3
471 C-D, the intervention did not modify the y-intercepts (Interaction: time x group; $P = 0.531$, $\eta^2 =$
472 0.018) nor the slopes (Interaction: time x group; $P = 0.100$, $\eta^2 = 0.118$) of the regression lines.
473 Indeed, the y-intercept of RMS_{MU} ranged from 13.82 ± 42.77 at the baseline to 18.10 ± 23.52 μ V at
474 post-test in INT group and from -5.24 ± 28.29 to 7.88 ± 15.26 μ V in the CON group, respectively.
475 The rate of change of RMS_{MU} relative to RT ranged from 1.24 ± 1.04 to 1.35 ± 0.68 μ V·%MViF and
476 from 1.87 ± 1.79 to 1.19 ± 0.68 μ V·%MViF in the INT and CON group, respectively. Therefore, the
477 association between RMS_{MU} and motor unit RT was not modified by training, likely because of the
478 considerable inter-subject variability (See Table 5, Supplemental Digital Content 3, Participant-
479 specific values for RMS_{MU} linear regression analysis pre-to-post intervention). These values are in

480 accordance with previous literature (21). Because of the association between force and EMG
481 amplitude (21), we also assessed the association between the changes in MVIF (Δ MVIF) and the
482 changes in RMS_{MU} (Δ RMS_{MU}) at the individual level. The correlation was not statistically significant
483 ($r = 0.400$, $P = 0.197$).

484

485

486 **DISCUSSION**

487 This study showed differential adjustments in conduction velocity of longitudinally tracked low- and
488 high-threshold motor units recorded from the tibialis anterior muscle after four weeks of strength
489 training. In particular, MUCV and RMS_{MU} changes were studied in relation to the corresponding
490 motor unit recruitment threshold at the individual subject level, in the recruitment range of the tibialis
491 anterior muscle. We showed that MUCV of motor units recruited at higher muscle forces significantly
492 increased following the training intervention, while no changes in MUCV of lower threshold motor
493 units were observed. On the other hand, these specific adjustments were not accompanied by changes
494 of motor unit action potential amplitude. These results provide the first in-vivo evidence of specific
495 strength-training induced adaptations in peripheral properties of high threshold motor units.

496

497 **Neuromotor adaptations**

498 As expected, four weeks of isometric strength training induced significant changes in maximal
499 voluntary force of the ankle dorsiflexors, which increased by 14%, on average. Our results are in
500 agreement with previous investigations on neuromuscular responses to isometric strength training
501 that reported significant increases in muscle force following short-term interventions (3, 35).
502 Considering the short duration of the training, these early gains in maximal force are unlikely
503 associated with any increase in muscle thickness, fascicle angle or length (5), cross-sectional area or
504 twitch torque, which are known to occur only after 4-to-5 weeks of regular strength training (2, 3, 5).

505 Nevertheless, these variables have not been examined in the current study. Similarly, changes in
506 antagonist muscle coactivation, which might have contributed to the muscle force gains by a training-
507 induced increase in reciprocal inhibition, was not quantified (36). Considering that the force generated
508 by a muscle in a voluntary contraction depends on the modulation of the number of recruited motor
509 units and their discharge rate, as well as by the mechanical properties of the muscle units, we
510 monitored adjustments in motor unit behaviour following the training intervention. We recently
511 demonstrated that an increase in the net excitatory input to the motor neuron pool for the same relative
512 force, could partly account for the observed gains in motor output (9). In the current study, we
513 observed that the early gains in force-generating capacity were accompanied by a substantial and
514 consistent decrease of normalized motor unit recruitment thresholds during the submaximal
515 trapezoidal contractions, in all subjects. Specifically, this means that the prescribed force trajectory
516 during the ramp-up phase of trapezoidal contractions was achieved by recruiting motor units earlier,
517 i.e. at lower force intensities relative to the maximum. The fact that only non-significant changes
518 were observed for the absolute motor unit recruitment thresholds excludes, although indirectly, the
519 possible implication of a training-induced decrease and/or impairment in motor unit twitch forces.
520 One possible explanation for the decrease in normalized recruitment threshold could be related to
521 changes in musculotendinous stiffness. Indeed, a previous study (37) reported that a training program
522 characterized by high-force contractions decreases musculotendinous stiffness, which in turn was
523 correlated with a decrease of neuromechanical delay (NMD). Since it has recently been demonstrated
524 by Del Vecchio et al., (38) that the NMD, which refers to the latency between the neural command
525 to a muscle unit and the force generated during voluntary tasks, is broadly modulated by the central
526 nervous system by varying the activation of motor units (e.g. their recruitment thresholds), the
527 training-induced decrease in muscular stiffness might therefore account for the observed decrease in
528 MU recruitment threshold. Nevertheless, this hypothesis needs to be verified. Additionally, according
529 to previous evidence, an increase in conduction velocity would also affect the force rise time in single
530 motor units (11, 20, 39). Therefore, the increase in rate of force development of single motor units

531 could potentially result in a better summation of motor unit twitches and thus total force output for a
532 given synaptic input. Accordingly, the number of active motor units to generate a force directory may
533 be lower. However, also in this case, this hypothesis needs to be verified.

534 As reported in our recent investigation (9), in addition to the decrease in normalized motor unit
535 recruitment thresholds, the strength-training protocol induced an increase of motor unit discharge
536 rate, which was exhibited by most identified motor units and hence independently of their recruitment
537 threshold, during the steady state of the contractions. Conversely, in the current analysis the peripheral
538 properties of lower and higher threshold motor units i.e., MUCV, showed distinct adjustments after
539 the short-term training intervention, as discussed below.

540

541 **Motor unit conduction velocity**

542 Considering both groups and conditions, the observed average MUCV values of the tracked motor
543 units ($n = 210$) were in agreement with previous reports where MUCV was quantified for the tibialis
544 anterior during electrical stimulation (11) and voluntary contractions (13, 14, 21). A strong positive
545 association between MUCV and recruitment threshold during submaximal isometric ramp
546 contractions was observed in all subjects and in both testing conditions (mean $R^2 = 0.71 \pm 0.16$). This
547 strong correlation is in agreement with previous studies (14, 40) and with recent reports (13, 21)
548 where the association between motor neuron properties (e.g. MU RT) and muscle unit properties (e.g.
549 MUCV) has been systematically investigated for large populations of motor units. For example, in
550 agreement with our results, in two previous studies by Del Vecchio, mean R^2 values of 0.64 ± 0.14
551 (21) and 0.70 ± 0.09 (13) were reported between MUCV and RT of motor units from tibialis anterior.
552 The observed strong association between voluntary recruitment of motor units and muscle unit
553 properties confirmed that CV is higher for motor units with larger diameters and hence higher
554 recruitment thresholds, compared to lower-threshold, smaller-diameter motor units.

555

556 **Motor unit conduction velocity and training**

557 Previously, the effects of physical training on the velocity of propagation of action potentials along
558 the sarcolemma of muscle fibres innervated by individual motor neurons (i.e. MUCV) have been
559 indirectly investigated. The majority of previous studies focused on training-associated changes in
560 muscle fibre conduction velocity (MFCV), an EMG-derived parameter that reflects an average value
561 of the conduction velocities of the active motor units, in different types of contraction. In these
562 studies, MFCV was estimated in resting conditions (18), during maximal voluntary contractions (26)
563 or in ballistic contractions (20), and primarily compared cross-sectionally between chronically
564 trained-individuals and control cohorts. For instance, Sadoyama et al. (26) observed significantly
565 higher MFCV in the vastus lateralis muscle of sprinters ($4.84 \pm 0.24 \text{ m}\cdot\text{s}^{-1}$) compared to endurance
566 runners ($4.31 \pm 0.10 \text{ m}\cdot\text{s}^{-1}$). Additionally, a strong and positive correlation was found between MFCV
567 and the relative area of fast-twitch fibres. Similarly, Methenitis et al. (18) confirmed the close
568 association between MFCV and muscle fibre % cross-sectional area (CSA). In particular, the authors
569 found that the % CSA of type II and IIX fibres explained a large part of the correlation between MFCV
570 and rate of force development and power performance in sedentary, endurance and strength/power-
571 trained individuals. However, the methodology adopted in this previous study, i.e. MFCV derived
572 from electrical stimulation of individual motor units, only allowed analysis of the compound motor
573 unit activity. In a recent study, Del Vecchio et al. (20) observed that the higher rate of torque
574 development in the very early phase (~electromechanical delay and 0-50 ms) of ballistic isometric
575 contractions in chronically strength-trained individuals was associated with significantly higher
576 MFCV compared to controls. Accordingly, strength-trained individuals seem to be able to achieve
577 higher force levels by recruiting motor units with greater MFCV (i.e., larger motor neurons
578 innervating larger diameter and fast-twitch muscle fibres) in a shorter amount of time compared to
579 untrained individuals. In the only study where longitudinal changes in MFCV were assessed, Cadore
580 et al. (41) reported a significant increase in maximal MFCV after either 6 weeks of concentric (22.2
581 $\pm 65.1\%$) or eccentric ($27.3 \pm 73.8\%$) strength training.

582 Overall, the current evidence suggests that physical training might elicit changes in MFCV, which
583 might be associated with changes in muscle fibre size, as well as with changes in the excitability and
584 conduction properties of the sarcolemma. Moreover, MFCV adaptations seem to be training-specific.
585 In fact, different exercise training protocols (e.g. strength vs endurance training) might induce a
586 predominant recruitment of different populations of motor units (higher vs lower-threshold MUs) and
587 hence affect their properties specifically.

588 Nevertheless, because of methodological limitations related with the estimation of single MUCV and
589 with the impossibility until very recently to identify and track representative populations of motor
590 units across experimental sessions (9, 30), specific training-associated adjustments in MUCV of
591 lower- and higher- threshold motor units remained unclear. By assessing the association between
592 MUCV and recruitment threshold of the same motor units before and after a training intervention and
593 hence by relating motor neurone and muscle fibre properties for a large sample of motor units, here
594 we have been able to evaluate chronic MUCV adaptations to strength training at the individual subject
595 level.

596 There are no previous studies that assessed the adaptations in motor unit peripheral properties (i.e.
597 MUCV) following strength training when considering the same motor units tracked before and after
598 the intervention. In this study, the MUCV of the tracked motor units estimated during submaximal
599 isometric contractions significantly increased after four weeks of strength training, when averaged
600 among contractions and subjects. More interestingly, the linear regressions between individual
601 MUCV values and normalized RT of the same motor units pointed out distinct adjustments in motor
602 unit peripheral properties between lower- and higher-threshold motor units. Indeed, when comparing
603 the regression lines at the baseline and post-intervention, a significant increase in MUCV rate of
604 change (slope) relative to MU RT was observed. Conversely, the initial value of MUCV (intercept)
605 did not change, indicating that the CV of motor units recruited at the lower- force levels and hence
606 their peripheral properties were not influenced by training.

607 Similarly, Martinez-Valdez et al. (27) reported distinct MUCV adjustments following two weeks of
608 either high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT). In
609 particular, an overall increase in MUCV of lower threshold motor units during submaximal voluntary
610 contractions was observed following both training interventions, while MUCV of higher-threshold
611 motor units increased significantly only after the HIIT intervention. It was concluded that these
612 differential changes could be due to differences in load intensity and exercise volume between the
613 two training protocols, which might have induced a predominant recruitment of different populations
614 of motor units for the two interventions. However, the two training protocols adopted were designed
615 to achieve similar adaptations in aerobic metabolism and endurance performance and therefore the
616 results cannot be directly compared to the findings of the current study, where the training protocol
617 was designed to enhance muscle strength. In line with this interpretation (27), the nature of our
618 training might have induced a greater activation and hence larger adaptation in higher threshold motor
619 units, whose recruitment is necessary and essential to achieve increased peak muscle forces (25).
620 However, we have recently shown an increase in motor unit discharge rate at the plateau of a
621 trapezoidal contraction in most motor units after strength training, independently on their recruitment
622 threshold (9). Therefore, taken together the results of these studies, our findings suggest that, although
623 central adaptations in motor unit behaviour (e.g. motor unit discharge rate) occurred in the whole
624 population of identified motor units, short-term strength training elicited specific adjustments in
625 peripheral properties (e.g. MUCV) of low- and high- threshold motor units. In particular, these
626 differential changes might reflect a potential greater adaptability in the electrophysiological properties
627 of muscle membrane of higher-threshold motor units.

628

629 In the only study that focused on long-term changes in motor unit properties after a training
630 intervention, Vila-Chã and colleagues (28) reported a significant increase in MUCV of lower
631 threshold motor units, i.e. recruited at 30% MV_iF, during submaximal contractions following either
632 6 weeks of endurance or strength training. Considering the longer duration of the training intervention

633 (6 vs 4 weeks), changes in the contractile apparatus (e.g. increase in muscle fibre size) cannot be
634 completely ruled out and this may have influence the MUCV results. Furthermore, because of
635 technical constraints related with the invasive assessment of MUCV, conduction velocity was
636 computed only on motor units recruited at low torque levels (e.g. lower-threshold motor units) and
637 hence potential adjustments in higher-threshold motor units could not be documented in this previous
638 study. Again, a direct comparison with the current results is difficult.

639 The specific changes in conduction velocity observed in higher threshold motor units following the
640 short-term strength training might be due to specific adaptations in the voltage-sensitive ionic
641 channels (e.g. Na^+ and K^+) and/or modifications of the transport activity and capacity of $\text{Na}^+ - \text{K}^+$
642 pump (e.g. $\text{Na}^+ - \text{K}^+ - \text{ATPase}$). In fact, both factors play a significant role in the transmission of motor
643 unit action potentials along the muscle fibres by influencing membrane excitability (22, 23). On one
644 side, ionic channels are responsible for the propagation of action potentials from the sarcolemma to
645 the terminal cisternae of the sarcoplasmic reticulum (SR) triggering the release of Ca^{2+} from the SR
646 to the muscle fibrils, on the other, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ contributes to the recovery and maintenance of
647 the resting membrane potential and modulates muscle contractile function (42). For instance, previous
648 studies have pointed out that motor unit action potential velocity is impaired by an increased
649 concentration of extracellular K^+ (e.g. hyperkalaemia) (43). On the other hand, $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ plays
650 a key role in reducing extracellular K^+ concentration and in particular, a study (44) reported that the
651 stimulation of the $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ with adrenaline, increases motor unit action potential propagation
652 velocity in muscle fibres with high extracellular levels of K^+ . Moreover, studies conducted on both
653 animals and humans revealed that type I and type II muscle fibres seem to exhibit a different number,
654 density and isoforms of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (42, 45). In particular, type II muscle fibres, which are
655 generally innervated by larger α -motor neurons and hence generally found in higher-threshold motor
656 units, have a greater amount of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ compared to type I fibres, innervated by smaller α -
657 motor neurons and hence usually found in lower-threshold motor units (42). Furthermore, the $\beta 2$ -
658 subunit isoform of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$, characterized by a greater rate of $\text{Na}^+ - \text{K}^+$ ion transfer and lower

659 inactivity period, seems to be the predominant isoform in type II muscle fibres (45). Accordingly, the
660 different electrophysiological features manifested in type II fibres seem to justify the faster spread of
661 action potentials along the sarcolemma of higher-threshold motor units compared to lower-threshold
662 motor units. In this regard, an activity-dependent up-regulation of Na⁺-K⁺-ATPase activity has been
663 observed after different training interventions over the last 20 years (46, 47). Conversely, inactivity
664 and immobilization lead to a down-regulation of the content of Na⁺-K⁺-ATPase in skeletal muscle.
665 For instance, Green et al. (47) reported an increase in Na⁺-K⁺-ATPase concentration (+16%),
666 measured with the ³[H]-ouabain technique, after 12 weeks of high-resistance training. However,
667 although no significant changes were observed in the first 4 weeks of the intervention, a direct
668 comparison between the present results and the results of Green et al. (47) is difficult due to
669 methodological differences and to the different duration (e.g. 12 vs 4 weeks) and type (dynamic vs
670 isometric) of strength training applied. Nevertheless, to date, the effects of training on the distribution
671 of subunit isoforms of Na⁺-K⁺-ATPase in skeletal muscle remains unclear. Therefore, it is plausible
672 to assume that the short-term strength training intervention proposed might have induced a greater
673 stimulation of the Na⁺-K⁺ pump synthesis in higher-threshold motor units than in lower threshold
674 motor units.

675 In agreement with this interpretation of selective adjustments in motor unit behaviour, Piitulainen et
676 al. (48) documented specific changes in MUCV for higher-threshold motor units (e.g. only at 50-70%
677 MVC) following a single session of maximal eccentric exercise. The authors concluded that the high-
678 intensity of the contractions to which participants' muscles were subjected, might have stimulated
679 predominantly fast-twitch fibres compared to slow-twitch fibres. Similarly, it is possible that the
680 training intervention adopted in the current study might have elicited a predominant
681 recruitment/activation of larger-diameter, fast-twitch, higher-threshold motor units, whose
682 progressive activation and recruitment determines the increase in muscle strength. Another factor that
683 might have induced the selective increase in MUCV of higher-threshold motor units is a specific
684 change in the diameter of muscle fibres belonging to these motor units (i.e. hypertrophy). However,

685 although changes in contractile properties cannot be completely ruled out because they were not
686 directly quantified in the current study, it is very unlikely that the short-term protocol (12 training
687 sessions over 4 weeks) induced any changes in muscle fibre size or architecture, considering that
688 significant morphological changes generally occur after longer training interventions (> 30-35 days)
689 (7). In fact, changes in MUCV do not necessarily imply changes in the contractile properties (49).
690 Indeed, although not directly investigated in the current study, there seem to exist a close association
691 between muscle fibre electrophysiological and contractile properties. For instance, the speed of
692 release of calcium from the sarcoplasmic reticulum increases with increasing depolarization (50),
693 which is related to the propagation speed of MUAPs, and in turn determined by fibre diameter.
694 Indeed, the time-to-peak of MU twitch forces decreases as the MUCV increases (11). Moreover, it
695 was reported that MU twitch force increases when two discharges occur close to each other (51),
696 paralleling an increase in MFCV. These mechanisms, suggest that adaptations in the muscle fibre
697 electrophysiological properties may affect contractile properties regardless of either an increase or
698 decrease in muscle fibre diameter (39).

699 In support of this explanation, we observed that motor unit action potential amplitude (e.g. RMS_{MU}),
700 an EMG-derived parameter that is suggested to reflect changes in muscle fibre size and morphology
701 (hypertrophy) (52), of the tracked motor units did not change significantly after the intervention at
702 the group level. Furthermore, the lack of changes in motor unit action potential amplitude was
703 confirmed at the single individual level by examining the regression lines between RMS_{MU} and the
704 normalized recruitment threshold of the same motor units. In this regard, although positive and
705 significant correlations were observed for the majority of participants (18 out of 24), EMG amplitude
706 estimates exhibited a high level of inter-individual variability particularly with regard to the rate of
707 change in RMS_{MU} as a function of recruitment threshold and in the initial value of RMS_{MU} regression
708 values. These results are aligned with previous reports (13), which showed that motor unit action
709 potential amplitudes are only moderately correlated with recruitment thresholds, with high variability
710 across subjects (21, 33). This observation is related to the fact that motor unit action potential

711 amplitude does not always relate to muscle force. In fact, HDsEMG decomposition algorithms tend
712 to identify predominantly the largest motor units, which might not always show the greatest action
713 potential amplitude (15). Deeper motor units having a higher recruitment threshold and therefore
714 larger size might show smaller motor unit action potential amplitude (21). Moreover, the lack of
715 changes in motor unit discharge rate at the recruitment of trapezoidal contractions, excludes the
716 potential influence of this variable on the increase of MUCV.

717

718 **Conclusion**

719 This study revealed that four weeks of isometric strength training elicited specific adaptations in the
720 electrophysiological properties of muscle fibre membrane of higher-threshold motor units. Although
721 the specific neurophysiological mechanisms underlying these early and selective adjustments in
722 higher-threshold motor units need to be further elucidated in future investigations, we provided the
723 first in vivo evidence of the effects of strength training on motor unit conduction velocity, likely due
724 to intrinsic changes in the muscle membrane properties. Our findings support the importance of the
725 implementation of isometric strength training in rehabilitation programs (e.g. neuromuscular
726 disorders) or as a recovery tool for exercise programs normally inducing a mechanical damaging of
727 the sarcolemma (e.g. eccentric contractions) and hence might have important implications for
728 exercise prescription.

729

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733 The authors declare that they have no competing interests. All authors affirm that the results of the
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737

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870 **FIGURE CAPTIONS**

871 **Figure 1. A.** Bar plots representing the average values for maximal voluntary isometric force (N) for
872 the INT and CON group, before (PRE, grey bars) and after (POST, white bars) the strength training
873 intervention. **B.** Bar plots representing the average values for MU normalized recruitment threshold
874 (% MVIF) for the INT and CON group, before (PRE, grey bars) and after (POST, white bars) the
875 strength training intervention. **C.** Bar plots representing the average values for motor unit discharge
876 rate (pps) at the recruitment for the INT and CON group, before (PRE, grey bars) and after (POST,
877 white bars) the strength training intervention. In all the graphs (A, B, C), individual average values
878 are also reported and each subject is indicated with a filled circle of a different colour. * $P < 0.05$; **
879 $P < 0.001$.

880

881 **Figure 2.** Motor unit conduction velocity ($\text{m}\cdot\text{s}^{-1}$) regression lines plotted as a function of normalized
882 recruitment threshold (% MVIF) of the identified pool of longitudinally tracked motor units recorded
883 from the TA muscle before (PRE, orange) and after (POST, blue) the strength training intervention
884 for the INT (**A**) and CON (**B**) group. PRE intervention regression lines are represented with an orange
885 dashed line, whereas POST intervention regression lines are represented with a blue dashed line. Each
886 filled dot in the graphs represents a single motor unit ($n = 210$). A total of 94 and 116 motor units
887 were tracked across the two main measurement sessions for INT and CON group, respectively. The
888 coefficient of determination (R^2) of the linear regressions are reported as mean (\pm SD) across
889 participants and are shown in the upper left corner of each graph.

890

891 **Figure 3.** Bar plots representing the average values for the y-intercept (initial value) of MUCV (**A**)
892 and the rate of change (slope) of MUCV relative to MU recruitment threshold (**B**), derived from the
893 regressions between MUCV and normalized recruitment threshold (% MVIF), before (PRE, grey
894 bars) and after (POST, white bars) the strength training intervention. Bar plots representing the

895 average values for the y-intercept (initial value) of RMS_{MU} (**C**) and rate of change (slope) of RMS_{MU}
896 relative to MU recruitment threshold (**D**), derived from the regressions between RMS_{MU} and
897 normalized recruitment thresholds (% MVIF), before (PRE, grey bars) and after (POST, white bars)
898 the strength training intervention. In all the graphs (A, B, C, D), individual average values are also
899 reported and each subject is indicated with a filled circle of a different colour. * $P < 0.05$

900 **SUPPLEMENTAL DIGITAL CONTENT**

901 Supplemental digital content 1, Table 3, Overview of the total number of identified and tracked
902 motor units by group, pre-to-post intervention.docx

903 Supplemental digital content 2, Table 4, Participant-specific values for MUCV linear regression
904 analysis pre-to-post intervention.docx

905 Supplemental digital content 3, Table 5, Participant-specific values for RMS_{MU} linear regression
906 analysis pre-to-post intervention.docx