# Strength training increases conduction velocity of high-threshold motor units

Casolo, Andrea; Farina, Dario; Falla, Deborah; Bazzucchi, Ilenia; Felici, Francesco; Del Vecchio, Alessandro

*DOI:*
10.1249/MSS.0000000000002196

# Strength training increases conduction velocity of high-threshold motor units

Andrea Casolo [1,2], Dario Farina [2], Deborah Falla [3], Ilenia Bazzucchi [1], Francesco Felici [1], Alessandro Del Vecchio [1,2]

**Affiliations:**

[1] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy

[2] Department of Bioengineering, Imperial College London, SW7 2AZ, London, UK

[3] Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom

**Corresponding author:**

Dario Farina. Department of Bioengineering, Imperial College London, SW7 2AZ, London, UK. Tel: Tel: +44 (0)20 759 41387, Email: d.farina@imperial.ac.uk

**Abbreviated title:**

Motor unit adaptations to strength training

## ABSTRACT

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

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

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

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



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

## INTRODUCTION

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

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

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

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

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

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

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

## METHODS

### Participants

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

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

**Study overview**

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

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

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

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

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

## Experimental procedure

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

Following placement of the HDsEMG electrodes (see below), the participants performed a standardized warm-up involving 8 isometric contractions of ankle dorsiflexion at different intensities of self-perceived maximal voluntary force (4x50%, 3x70%, 1x90%, with 15-30 s rest in-between).

To determine the maximal voluntary isometric force (MViF) of the dorsiflexors, the participants performed 3-4 maximal voluntary contractions (MVCs) separated by 30 s of rest. The participants were instructed to "push as hard as possible" and to achieve the MViF within 3-5 s. During the contractions, the participants were motivated with verbal encouragement by an investigator. A horizontal cursor displayed on a monitor indicated the peak force achieved in the preceding MVC. The highest force recorded out of the 3-4 trials was set as a reference to determine the relative intensity of the submaximal contractions in each of the two measurement sessions.

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

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

*Training Protocol*

The intervention involved 12 training sessions lasting approximately ~30 min each, separated by 48-72 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 $MViF \cdot s^{-1}$) 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 (~120° hip flexion), their knee extended to ~180° and their ankle positioned in ~100°
230 (90° = perpendicular to the tibia) of plantar flexion. In order to minimize extraneous movements, their

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

*HDsEMG recording*

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

tibialis anterior) along the electrode column (13, 21). The estimation of the anatomical direction of muscle fibres corresponded to alignment that led to the identification of action potentials propagating clearly along the array, without substantial changes in waveform shapes. Once the IZ and the estimated fibre direction were determined, the skin surface was shaved, lightly abraded and cleansed with 70% ethanol. Disposable bi-adhesive foam layers (SpesMedica, Battipaglia, Italy) were used to attach the grids to the surface of the muscle. The first adhesive grid of electrodes was positioned with the first column of electrode aligned in the direction of the muscle fibres and with the first four rows on the IZ. In order to cover most of the muscle belly, the second high-density grid was attached proximally to the first. The skin-to-electrode contact was optimized by filling each adhesive layer hole, corresponding to one electrode, with conductive paste (SpesMedica, Battipaglia, Italy).

The reference electrode was placed in proximity of the styloid process of the ulna on the wrist on the tested side. The reference electrodes for the two preamplifiers were positioned on the tuberosity of the tibia and on the medial malleolus of the tested limb.

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

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

**Force and HDsEMG analysis**

*Muscle force*

After the conversion to digital data, the force signal was transformed into newtons (N) and low-pass filtered ($4^{th}$ order, zero-lag, Butterworth) with a cut-off frequency of 15 Hz. The offset was removed by correcting for the effect of gravity and for each participant, only the trapezoidal contraction trial at each force target (35%, 50%, 70% MViF) showing the best tracking of force with respect to the given template and with no pre-tension or countermovement (≤ 0.5 N from the baseline of force in the 150 ms prior to force onsetx1), was included in the analysis (9).

*Motor unit analysis*

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

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

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

*Motor unit conduction velocity and amplitude estimation*

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

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

*Motor unit tracking*

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

**Statistical analysis**

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

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

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

## RESULTS

### Baseline assessment

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

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

## Motor unit decomposition and tracking

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

## Neuromotor adaptations

Maximal voluntary isometric ankle dorsiflexion force increased significantly after four weeks of strength training from 284.3 ± 64.0 to 324.4 ± 61.5 N (+14.1%; $P = 0.003$, $\eta p^2 = 0.576$; Figure 1 A). Conversely, no change was observed for the CON group (PRE: 299.2 ± 40.6 N; POST: 304.3 ± 35.4 N; $P = 0.422$).

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

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

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

## Motor unit properties

### *Motor unit conduction velocity (MUCV)*

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

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

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

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

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

On the contrary, the rate of change in MUCV (slope, PRE: 0.018 ± 0.008; POST: 0.017 ± 0.007 $m \cdot s^{-1} \cdot$%MViF; $P = 0.696$) and the initial value (intercept, PRE: 3.71 ± 0.54; POST: 3.71 ± 0.47 $m \cdot s^{-1}$; $P = 0.999$) 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, $\eta p^2$ = 0.023). When averaged among
462 contractions and participants, $RMS_{MU}$ ranged from 59.51 ± 29.62 (PRE) to 56.17 ± 27.66 (POST) μV
463 and from 55.51 ± 37.51 to 47.60 ± 22.27 μV 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, $\eta p^2$ =
472 0.018) nor the slopes (Interaction: time x group; *P* = 0.100, $\eta p^2$ = 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 μV at
474 post-test in INT group and from -5.24 ± 28.29 to 7.88 ± 15.26 μV 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 μV·%MViF and
476 from 1.87 ± 1.79 to 1.19 ± 0.68 μV·%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

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

## DISCUSSION

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

### Neuromotor adaptations

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

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

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

**Motor unit conduction velocity**

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

## Motor unit conduction velocity and training

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

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

In the only study that focused on long-term changes in motor unit properties after a training intervention, Vila-Chã and colleagues (28) reported a significant increase in MUCV of lower threshold motor units, i.e. recruited at 30% MViF, during submaximal contractions following either 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 $Na^+$ - $K^+$
642 pump (e.g. $Na^+$-$K^+$-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, $Na^+$-$K^+$-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, $Na^+$-$K^+$-ATPase plays
650 a key role in reducing extracellular $K^+$ concentration and in particular, a study (44) reported that the
651 stimulation of the $Na^+$-$K^+$-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 $Na^+$-$K^+$-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 $Na^+$-$K^+$-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 β2-
658 subunit isoform of $Na^+$-$K^+$-ATPase, characterized by a greater rate of $Na^+$ - $K^+$ ion transfer and lower

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

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

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

## Conclusion

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

## Acknowledgements

The authors would like to extend their sincere gratitude to the participants for their commitment to the study. This work was funded by the University of Rome Foro Italico, Rome, Italy.

The authors declare that they have no competing interests. All authors affirm that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data

735 manipulation. The results of the study do not constitute endorsement by the American College of
736 Sports Medicine.
737

738 **References**


739 1. Folland JP, Williams AG. Morphological and neurological contributions to increased
740 strength. *Sport Med*. 2007;37(2):145–68.

741 2. Van Cutsem M, Duchateau J, Hainaut K. Changes in single motor unit behaviour contribute
742 to the increase in contraction speed after dynamic training in humans. *J Physiol*.
743 1998;513(1):295–305.

744 3. Nuzzo JL, Barry BK, Jones MD, Gandevia SC, Taylor JL. Effects of Four Weeks of Strength
745 Training on the Corticomotoneuronal Pathway. *Med Sci Sports Exerc*. 2017;49(11):2286–96.

746 4. Weier AT, Pearce AJ, Kidgell DJ. Strength training reduces intracortical inhibition. *Acta*
747 *Physiol*. 2012;206(2):109–19.

748 5. Blazevich AJ, Gill ND, Deans N, Zhou S. Lack of human muscle architectural adaptation
749 after short-term strength training. *Muscle Nerve*. 2007;35(1):78–86.

750 6. Aagaard P, Andersen JL, Dyhre-Poulsen P, et al. A mechanism for increased contractile
751 strength of human pennate muscle in response to strength training: changes in muscle
752 architecture. *J Physiol*. 2001;534(2):613–23.

753 7. Seynnes OR, de Boer M, Narici M V. Early skeletal muscle hypertrophy and architectural
754 changes in response to high-intensity resistance training. *J Appl Physiol*. 2007;102(1):368–
755 73.

756 8. Balshaw TG, Massey GJ, Maden-Wilkinson TM, Tillin NA, Folland JP. Training-specific
757 functional, neural, and hypertrophic adaptations to explosive- vs. sustained-contraction
758 strength training. *J Appl Physiol*. 2016;120(11):1364–73.

759 9. Del Vecchio A, Casolo A, Negro F, et al. The increase in muscle force after 4 weeks of
760 strength training is mediated by adaptations in motor unit recruitment and rate coding. *J*

761 *Physiol*. 2019;597(7):1873–87.

762 10. Farina D, Merletti R. Estimation of average muscle fiber conduction velocity from two-
763 dimensional surface EMG recordings. *J Neurosci Methods*. 2004;134(2):199–208.

764 11. Andreassen S, Arendt-Nielsen L. Muscle fibre conduction velocity in motor units of the
765 human anterior tibial muscle: a new size principle parameter. *J Physiol*. 1987;391(1):561–71.

766 12. Farina D, Muhammad W, Fortunato E, Meste O, Merletti R, Rix H. Estimation of single
767 motor unit conduction velocity from surface electromyogram signals detected with linear
768 electrode arrays. *Med Biol Eng Comput*. 2001;39(2):225–36.

769 13. Del Vecchio A, Negro F, Felici F, Farina D. Distribution of muscle fibre conduction velocity
770 for representative samples of motor units in the full recruitment range of the tibialis anterior
771 muscle. *Acta Physiol*. 2018;222(2):e12930.

772 14. Masuda T, De Luca CJ. Recruitment threshold and muscle fiber conduction velocity of single
773 motor units. *J Electromyogr Kinesiol*. 1991;1(2):116–23.

774 15. Holobar A, Zazula D. Multichannel blind source separation using convolution Kernel
775 compensation. *IEEE Trans Signal Process*. 2007;55(9):4487–96.

776 16. Hakansson CH. Conduction Velocity and Amplitude of the Action Potential as Related to
777 Circumference in the Isolated Fibre of Frog Muscle. *Acta Physiol Scand*. 1956;37(1):14–34.

778 17. Blijham PJ, ter Laak HJ, Schelhaas HJ, van Engelen BGM, Stegeman DF, Zwarts MJ.
779 Relation between muscle fiber conduction velocity and fiber size in neuromuscular disorders.
780 *J Appl Physiol*. 2006;100(6):1837–41.

781 18. Methenitis S, Karandreas N, Spengos K, Zaras N, Stasinaki AN, Terzis G. Muscle Fiber
782 Conduction Velocity, Muscle Fiber Composition, and Power Performance. *Med Sci Sports*
783 *Exerc*. 2016;48(9):1761–71.

19. Plonsey R, Barr C. R. *Bioelectricity: A Quantitative Approach*. New York, NY: Springer Science and Business Media; 2007.

20. Del Vecchio A, Negro F, Falla D, Bazzucchi I, Farina D, Felici F. Higher muscle fiber conduction velocity and early rate of torque development in chronically strength-trained individuals. *J Appl Physiol*. 2018;125(4):1218–26.

21. Del Vecchio A, Negro F, Felici F, Farina D. Associations between motor unit action potential parameters and surface EMG features. *J Appl Physiol*. 2017;123(4):835–43.

22. Christiansen D. Molecular stressors underlying exercise training-induced improvements in K + regulation during exercise and Na + ,K + -ATPase adaptation in human skeletal muscle. *Acta Physiol*. 2019;225(3):e13196.

23. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*. 2008;88(1):287–332.

24. Farina D, Falla D. Effect of muscle-fiber velocity recovery function on motor unit action potential properties in voluntary contractions. *Muscle Nerve*. 2008;37(5):650–8.

25. Duchateau J, Semmler JG, Enoka RM, et al. Training adaptations in the behavior of human motor units. *J Appl Physiol*. 2006;101(6):1766–75.

26. Sadoyama T, Masuda T, Miyata H, Katsuta S. Fiber conduction velocity and fibre composition in human vastus medialis. *Eur J Appl Physiol*. 1988;57:767–71.

27. Martinez-Valdes E, Farina D, Negro F, Del Vecchio A, Falla D. Early Motor Unit Conduction Velocity Changes to High-Intensity Interval Training versus Continuous Training. *Med Sci Sports Exerc*. 2018;50(11):2339–50.

28. Vila-Chã C, Falla D, Farina D. Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training. *J Appl Physiol*.

2010;109(5):1455–66.

29. Holobar A, Minetto MA, Farina D. Accurate identification of motor unit discharge patterns from high-density surface EMG and validation with a novel signal-based performance metric [Internet]. *J Neural Eng*. 2014;11(1) doi:10.1088/1741-2560/11/1/016008.

30. Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, Farina D. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. *J Physiol*. 2017;595(5):1479–96.

31. Farina D, Arendt-nielsen L, Merletti R, Graven-nielsen T. Assessment of single motor unit conduction velocity during sustained contractions of the tibialis anterior muscle with advanced spike triggered averaging. 2002;115:1–12.

32. Farina D, Arendt-Nielsen L, Merletti R, Graven-Nielsen T. Effect of Experimental Muscle Pain on Motor Unit Firing Rate and Conduction Velocity. *J Neurophysiol*. 2004;91(3):1250–9.

33. Del Vecchio A, Bazzucchi I, Felici F. Variability of estimates of muscle fiber conduction velocity and surface EMG amplitude across subjects and processing intervals. *J Electromyogr Kinesiol*. 2018;40(February):102–9.

34. Cohen J. *Statistical power analysis for the behavioral sciences*. Lawerence Erlbaum Associates, Hillsdale, NJ; 1988. 302 p.

35. Tillin NA, Pain MTG, Folland JP. Short-term training for explosive strength causes neural and mechanical adaptations. *Exp Physiol*. 2012;97(5):630–41.

36. Bazzucchi I, Riccio ME, Felici F. Tennis players show a lower coactivation of the elbow antagonist muscles during isokinetic exercises. *J Electromyogr Kinesiol*. 2008;18(5):752–9.

37. Grosset J-F, Piscione J, Lambertz D, Pérot C. Paired changes in electromechanical delay and

musculo-tendinous stiffness after endurance or plyometric training. *Eur J Appl Physiol*. 2009;105(1):131–9.

38. Del Vecchio A, Úbeda A, Sartori M, Azorín JM, Felici F, Farina D. Central nervous system modulates the neuromechanical delay in a broad range for the control of muscle force. *J Appl Physiol*. 2018;125(5):1404–10.

39. Farina D, Arendt-Nielsen L, Graven-Nielsen T. Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. *J Appl Physiol*. 2005;99(1):197–203.

40. Hogrel J-Y. Use of surface EMG for studying motor unit recruitment during isometric linear force ramp. *J Electromyogr Kinesiol*. 2003;13(5):417–23.

41. Cadore EL, González-Izal M, Pallarés JG, et al. Muscle conduction velocity, strength, neural activity, and morphological changes after eccentric and concentric training. *Scand J Med Sci Sport*. 2014;24(5):e343–52.

42. Clausen T. Na + -K + Pump Regulation and Skeletal Muscle Contractility. *Physiol Rev*. 2003;83(4):1269–324.

43. Fortune E, Lowery MM. Effect of extracellular potassium accumulation on muscle fiber conduction velocity: A simulation study. *Ann Biomed Eng*. 2009;37(10):2105–17.

44. Kössler F, Lange F, Caffier G, Küchler G. External potassium and action potential propagation in rat fast and slow twitch muscles. *Gen Physiol Biophys*. 1991;10(5):485–98.

45. Zhang L, Morris KJ, Ng Y-C. Fiber type-specific immunostaining of the Na+,K+-ATPase subunit isoforms in skeletal muscle: Age-associated differential changes. *Biochim Biophys Acta - Mol Basis Dis*. 2006;1762(9):783–93.

46. Klitgaard H, Clausen T. Increased total concentration of Na-K pumps in vastus lateralis

muscle of old trained human subjects. *J Appl Physiol*. 1989;67(6):2491–4.

47. Green HJ, Dahly A, Shoemaker K, Goreham C, Bombardier E, Ball-Burnett M. Serial effects of high-resistance and prolonged endurance training on Na+-K+pump concentration and enzymatic activities in human vastus lateralis. *Acta Physiol Scand*. 1999;165(2):177–84.

48. Piitulainen H, Holobar A, Avela J. Changes in motor unit characteristics after eccentric elbow flexor exercise. *Scand J Med Sci Sports*. 2012;22(3):418–29.

49. Farina D, Arendt-Nielsen L, Graven-Nielsen T. Spike-triggered average torque and muscle fiber conduction velocity of low-threshold motor units following submaximal endurance contractions. *J Appl Physiol*. 2005;98(4):1495–502.

50. Struk A, Lehmann-Horn F, Melzer W. Voltage-Dependent Calcium Release in Human Malignant Hyperthermia Muscle Fibers. *Biophys J*. 1998;75(5):2402–10.

51. Thomas CK, Johansson RS, Bigland-Ritchie B. Pattern of Pulses That Maximize Force Output From Single Human Thenar Motor Units. *J Neurophysiol*. 1999;82(6):3188–95.

52. Pope ZK, Hester GM, DeFreitas JM. Action Potential Amplitude as a Non-invasive Indicator of Motor Unit Specific Hypertrophy. *Med Sci Sport Exerc*. 2016;48:114.

## FIGURE CAPTIONS

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

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

**Figure 3.** Bar plots representing the average values for the y-intercept (initial value) of MUCV (**A**) and the rate of change (slope) of MUCV relative to MU recruitment threshold (**B**), derived from the regressions between MUCV and normalized recruitment threshold (% MViF), before (PRE, grey 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. * $P < 0.05$

## SUPPLEMENTAL DIGITAL CONTENT

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

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

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