Strength Training Increases Conduction Velocity of High-Threshold Motor Units

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ABSTRACT

CASOLO, A., D. FARINA, D. FALLA, I. BAZZUCCHI, F. FELICI, and A. DEL VECCHIO. Strength Training Increases Conduction Velocity of High-Threshold Motor Units. Med. Sci. Sports Exerc., Vol. 52, No. 4, pp. 955–967, 2020. Purpose: Motor unit conduction velocity (MUCV) represents the propagation velocity of action potentials along the muscle fibers innervated by individual motor neurons and indirectly reflects the electrophysiological properties of the sarcolemma. In this study, we investigated the effect of a 4-wk strength training intervention on the peripheral properties (MUCV and motor unit action potential amplitude, RMSMU) of populations of longitudinally tracked motor units (MU). Methods: The adjustments exhibited by 12 individuals who participated in the training (INT) were compared with 12 controls (CON). Strength training involved ballistic (4 × 10) and sustained (3 × 10) isometric ankle dorsiflexions. Measurement sessions involved the recordings of maximal voluntary isometric force and submaximal isometric ramp contractions, whereas high-density surface EMG was recorded from the tibialis anterior. High-density surface EMG signals were decomposed into individual MU discharge timings, and MU was tracked across the intervention. Results: Maximal voluntary isometric force (+14.1%, P = 0.003) and average MUCV (+3.0%, P = 0.028) increased in the INT group, whereas normalized MU recruitment threshold (RT) decreased (−14.9%, P = 0.001). The slope (rate of change) of the regression between MUCV and MU RT increased only in the INT group (+32.6%, P = 0.028), indicating a progressive greater increase in MUCV for higher-threshold MU. The intercept (initial value) of MUCV did not change after the intervention (P = 0.568). The association between RMSMU and MU RT was not altered by the training. Conclusion: The increase in the rate of change in MUCV as a function of MU RT, but not the initial value of MUCV, suggests that short-term strength training elicits specific adaptations in the electrophysiological properties of the muscle fiber membrane in high-threshold MU. Key Words: RESISTANCE TRAINING, MOTOR UNIT, PERIPHERAL PROPERTIES, CONDUCTION VELOCITY, AMPLITUDE, EMG DECOMPOSITION

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trength training is one of the most common modalities of exercise because 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 wk) is determined predominantly by neural factors (2–4) before significant hypertrophy and muscle architectural adjustments take place.

(typically >30–35 d) (5–8). Recently, we showed that the increase in muscle force after 4 wk 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 fiber membrane properties may show faster changes. An EMG-derived parameter that reflects the fiber membrane properties is muscle fiber conduction velocity (MFCV), which represents the average velocity of propagation of motor unit action potentials (MUAP) 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 (MU) (10) or for single MU as the average propagation velocity of action potentials along the muscle fibers innervated by individual motor neurons (11–14), by decomposing the surface EMG signal and extracting action potentials for isolated MU (15). At the single muscle fiber level, MFCV is related to the diameter of the fibers (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 MU innervating fibers with larger diameters. Indeed, Del Vecchio et al. (20,21) have recently reported a strong association between MFCV, estimated during increasing-force contractions and motor unit conduction velocity (MUCV) \((R^2 = 0.71)\), which in turn significantly correlated with MU recruitment threshold (RT) \((R^2 = 0.70)\). Therefore, MFCV is considered an indicator of the progressive recruitment of MU (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). In addition, MFCV provides an indirect window into the electrophysiological properties of the muscle fiber membrane because it is influenced by the polarization state, i.e., electrical excitability, of the sarcolemma (22,23). Indeed, the velocity of propagation of MUAP 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; and changes of intramuscular pH and temperature (23). Moreover, the propagation velocity of MUAP is also influenced by MU discharge rate (DR) (24).

The adaptations in the neural and peripheral properties of MU after short-term strength training in longitudinally tracked MU have yet to be clarified (25). In particular, it is currently unknown whether short-term strength training influences the electrophysiological properties of the muscle fiber membrane of individual MU. 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 after 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 with 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 MU after 2 wk of HIIT, whereas increased MUCV occurred only in low-threshold MU after endurance training. Similarly, Vila-Chã et al. (28) observed an increase in MUCV, assessed in contractions at 30% of the maximum voluntary contraction, after 6 wk of either endurance or strength training. These studies collectively suggest that the propagation velocity of action potentials along muscle fibers might be altered by a training intervention, although further investigations are warranted. Indeed, the time course of conduction velocity in single MU after strength training is currently unknown.

Technological advancements in the recording and decomposition of high-density surface EMG (HDsEMG) signals allow the behavior of large samples of MU to be evaluated in vivo and for a wide range of voluntary forces (15,29). The noninvasive estimation of MUCV allows the electrophysiological properties of the muscle fiber membrane to be characterized for different populations of MU (e.g., low-threshold and high-threshold MU) (12,13). Moreover, such methodology provides a reliable tracking of the same MU across different experimental sessions (9,30). This implies that potential training-associated changes in the neural and peripheral properties of MU 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 RT and DR of MU from the tibialis anterior muscle tracked over time, after a 4-wk strength training intervention. To indirectly relate motor neuron and muscle fiber properties, the association between MUCV and RT of the corresponding MU was compared before and after the training intervention at the individual subject level. Although MU RT 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 evidence that highlight the potential adaptability of MUCV after 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 MU.

**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 MU. Specifically, 28 healthy, recreationally active and nonsmoking 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 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

| Variables                      | INT       | CON       | \(P\)  |
|-------------------------------|-----------|-----------|-------|
| Age (yr)                      | 24.2 ± 3.2| 25.8 ± 3.4| 0.229 |
| Height (m)                    | 1.78 ± 0.1| 1.79 ± 0.1| 0.868 |
| Body mass (kg)                | 74.8 ± 8.9| 73.3 ± 8  | 0.668 |
| IPAQ score (MET min kg\(^{-1}\)) | 2422 ± 1297| 2304 ± 1153| 0.690 |
| MViF (N)                      | 284.3 ± 64.0| 299.2 ± 40.6| 0.503 |

Data are presented as mean ± SD. Between-group comparisons were performed with one-way ANOVA.
participants withdrew after recruitment for personal reasons (e.g., time demands). In addition, 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 before 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 15 laboratory sessions over a 7-wk period. Sessions 1 and 2 consisted of familiarization and baseline assessment session, respectively. Sessions 3 to 14 consisted of the 4-wk strength training intervention for the INT group, and session 15 involved the postintervention 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 dorsiflexion of the dominant foot (selected based on a self-report). In addition, 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). After recruitment, and 3 to 5 d 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 three sessions a week for 4 wk (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 h after the final training session, all the baseline measurements performed in session 2 were repeated.

All participants were asked to abstain from strenuous physical exercise 48 h before the main measurement sessions and additionally to avoid caffeine consumption 24 h before these sessions. 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 posttest assessments.** After placement of the HDsEMG electrodes (see below), the participants performed a standardized warm-up involving eight isometric contractions of ankle dorsiflexion at different intensities of self-perceived maximal voluntary force (4% \(\times\) 50%, 3% \(\times\) 70%, 1% \(\times\) 90%, with 15–30 s of rest in between).

To determine the MViF of the dorsiflexors, the participants performed three to four maximal voluntary contractions (MVC) 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 three to four 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 MVC, the participants completed six trapezoidal contractions (two times each of the target forces set at 35%, 50%, and 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 per second 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 a 1-m distance from participants’ eyes.

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

**Training protocol.** The intervention involved 12 training sessions lasting approximately ~30 min each, separated by 48–72 h, over a period of 4 wk. Each session was supervised by an investigator (A.C. and/or A.D.V.) and involved warm-up, MVC, and a combination of ballistic and sustained isometric contractions.

The standardized warm-up consisted of five submaximal isometric contractions of ankle dorsiflexion (2% \(\times\) 50%, 2% \(\times\) 70%, and 1% \(\times\) 90% of perceived MViF) of the dominant foot and was followed by three MVC to determine the reference values for submaximal contractions. Approximately 5 min after the MVC, the participants performed a total of 40 ballistic contractions and 30 sustained ramp contractions. In the ballistic contractions (4 sets \(\times\) 10 repetitions), the participants were instructed to contract “as fast and as hard as possible” up to a horizontal target placed at 75% of their MViF, without any pretension and/or countermovement, and immediately relax thereafter. Resting periods of 5 s and 1 min were allowed between the repetitions and each set, respectively. In the sustained isometric ramp contractions (3 sets \(\times\) 10 repetitions), the participants were instructed to reach a target force of 75% MViF in 2 s (37.5 MViF·s\(^{-1}\)) and hold a steady-state phase at the target force for 3 s. Resting intervals of 2 s
and 2 min were given between the sustained repetitions and each set, respectively.

Data Acquisition

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

The participants were seated on the massage table in a comfortable position with their back against the seat back (~120° hip flexion), their knee extended to ~180°, and their ankle positioned in ~100° (90° = perpendicular to the tibia) of plantarflexion. 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 analog force signal from the load cell was amplified (×200) and sampled at 2048 Hz with an external analog-to-digital converter (EMG-Quattrocento, OT Bioeletronica) 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 Bioeletronica). Force templates and feedback were provided with a customized LabVIEW program (LabVIEW 8.0; National Instruments, Austin, TX) 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 bidimensional adhesive grids of 64 equally spaced electrodes each (5 columns × 13 rows; gold-coated; 1-mm diameter; 8-mm interelectrode distance (IED); OT Bioeletronica). 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, 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 fiber 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 fibers 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 fiber direction were determined, the skin surface was shaved, lightly abraded, and cleansed with 70% ethanol. Disposable biadhesive foam layers (SpesMedica, Battipaglia, Italy) were used to attach the grids to the surface of the muscle. The first adhesives grid of electrodes was positioned with the first column of electrode aligned in the direction of the muscle fibers and with the first four rows on the IZ. 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).

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 (×150) and band-pass filtered (10–500 Hz) at source, and converted to digital data by a 16-bit analog-to-digital converter (EMG-Quattrocento, 400-channel amplifier, OT Bioeletronica) before being stored on a computer hard-disk for offline analysis (Matlab R2016a; The Mathworks Inc., Natick, MA). The HDsEMG signals were sampled at 2048 Hz.

To allow similar electrode positioning between the baseline and the 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 remark carefully the grid profiles daily. In addition, the electrode position with respect to anatomical landmarks was also traced on transparent sheets.

**Force and HDsEMG Analyses**

**Force Analysis.** After the conversion to digital data, the force signal was transformed into newtons (N) and low-pass filtered (fourth-order, zero-lag, Butterworth) with a cutoff 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%, and 70% MViF) showing the best tracking of force with respect to the given template and with no pretension or countermovement (<0.5 N from the baseline of force in the 150 ms before force onset) was included in the analysis (9).

**MU 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 fibers orientation) was the *sine qua non* to observe the propagation of MUAP 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 analyzed given the divergent aims or each work.

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

For each identified MU, the RT and the mean DR were calculated. MU RT was defined as the percentage of force (%MViF) produced by the ankle dorsiflexors at which the first MU action potential was discharged. Mean MU DR was calculated as the average of the first 20 MUAP, in the ramp-up phase (i.e., 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 DR in the recruitment phase of the trapezoidal contraction and on the estimation of MU conduction velocity (13,21,27).

**MUCV and amplitude estimation.** MU 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 MU were used for the spike-triggered averaging, which was performed in 15-ms (MUAP duration) intervals. Double differential derivations were then computed from averaged monopolar MUAP along the electrode columns and used for MUCV and MU amplitude (e.g., MU root mean square $[\text{RMS}_{\text{MU}}]$) estimation. Double differential EMG channels were visually inspected (customized Matlab script), and a minimum of four up to a maximum of eight double differential channels belonging to the same electrode column were selected for MUCV and RMS$_{\text{MU}}$ calculation. To date, the manual selection of EMG channels is considered the most accurate method for MUCV and RMS$_{\text{MU}}$ 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). Because 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 multichannel maximum likelihood algorithm was adopted to calculate MUCV. This algorithm has shown to estimate MUCV with a considerably low standard deviation ($<0.1 \text{ m} \cdot \text{s}^{-1}$) (12). On the same selected channels, RMS$_{\text{MU}}$ 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$_{\text{MU}}$ estimation at the postintervention measurement.

**MU tracking.** A validated MU tracking approach was adopted to investigate training-related changes in MU neural (RT, DR) and peripheral properties (MUCV, RMS$_{\text{MU}}$) on the same MU identified before and after the intervention (30). This procedure can accurately and reliably identify the same MU 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 MU 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, after 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 nonnormal distribution, the correspondent nonparametric 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, and body mass), physical activity habits (IPAQ score), and baseline muscle force levels (MViF) were investigated with one-way ANOVA. Similarly, between-group differences with regard to baseline neural and peripheral properties of MU (RT, DR, MUCV, and RMS$_{\text{MU}}$) were assessed with the same test. Differences in the total number of MU 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, MU RT (in absolute and normalized terms), and DR, were investigated with a two-way repeated-measures ANOVA (time, PRE vs POST; group, INT vs CON). When significant time–group interactions were found, results were determined after adjustment with Bonferroni correction. For each participant, MU variables (RT, DR, MUCV, and RMS$_{\text{MU}}$) were averaged among contractions (35%, 50%, and 70% of MViF), whereas for each group, individual values were averaged. Subject-specific changes in MUCV and RMS$_{\text{MU}}$ of the tracked MU were studied as a function of their RT. First, the association between MUCV/RMS$_{\text{MU}}$ and MU RT for each participant in each condition (PRE vs POST) was assessed with Pearson product–moment correlation coefficient. Second, the slopes and intercepts of the regression lines between MUCV/RMS$_{\text{MU}}$ and MU RT from all participants, at all force targets (35%, 50%, and 70% of MViF), and in both test conditions (PRE vs POST) were compared with two-way repeated-measures ANOVA. The effect size of changes for all the variables analyzed after the training intervention was calculated as partial
et al. (34). The significance level was set at \( \alpha < 0.05 \) for all tests. Results are presented as mean ± SD.

**RESULTS**

**Baseline Assessment**

At the baseline, there were no significant differences between the two groups with regard to age, anthropometrical 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 RMSMU at the baseline (see Table 2).

**MU Decomposition and Tracking**

A total of 948 MU from the tibialis anterior muscle were included in the analysis. This number is the sum of all MU detected for both groups and conditions. The total number of identified MU 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 MU could be tracked between the baseline and the postintervention session (INT, 94; CON, 116; \( P = 0.245 \)), corresponding to 22.2% of the total number of MU identified. The average number of tracked MU per participant was 8 ± 2 and 10 ± 5 for INT and CON groups, respectively. The cross correlations between the action potential waveforms of the tracked MU pre- and postintervention were 0.81 ± 0.01 and 0.88 ± 0.03 in INT and CON groups, 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, http://links.lww.com/MSS/B819).

**Neuromotor Adaptations**

Maximal voluntary isometric ankle dorsiflexion force increased significantly after 4 wk of strength training from 284.3 ± 64.0 to 324.4 ± 61.5 N (+14.1%; \( P = 0.003, \eta^2_p = 0.576; \) Fig. 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, RT of the pool of tracked MU, in both absolute and normalized values, changed after the intervention. Normalized MU RT (%MVIF), averaged across contractions and subjects, decreased significantly from 32.2 ± 18.1 to 27.4% ± 15.7% MVIF (−14.9%; \( P = 0.001, \eta^2_p = 0.665; \) Fig. 1 B) after training. The absolute MU RT decreased from 93.9 ± 51.9 to 85.0 ± 46.4 N (−9.4%) after training, although this was not significant (\( P = 0.238 \)). 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 MU DR was also investigated. The mean DR of the tracked MU at recruitment (average of the first 20 spikes) did not change significantly as a consequence of the intervention (Fig. 1 C). The mean MU DR values for the INT group were 15.5 ± 3.1 and 14.8 ± 2.8 pps at the baseline and posttest, respectively (\( P = 0.422 \)). For the CON group, the mean DR values were 14.9 ± 3.1 and 14.8 ± 2.5 pps at the baseline and posttest, respectively (\( P = 0.955 \)).

**MU Peripheral Properties**

**MUCV.** The observed average MUCV values of the tracked MU (\( n = 210 \)) were within the physiological range (2–6.5 m·s\(^{-1}\)) in all cases and in agreement with previous studies conducted on

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**TABLE 2. Baseline characteristics of the pool of tracked MU identified from tibialis anterior muscle by group (INT, \( n = 94; \) CON, \( n = 116 \)).**

| Variables | INT | CON | P |
|-----------|-----|-----|---|
| Absolute MU RT (N) | 93.9 ± 51.9 | 95.6 ± 53.3 | 0.870 |
| Normalized MU RT (%MVIF) | 32.2 ± 18.1 | 31.5 ± 17.5 | 0.777 |
| MU DR (pps) | 15.5 ± 3.1 | 14.8 ± 2.8 | 0.503 |
| MUCV (m·s\(^{-1}\)) | 4.52 ± 0.39 | 4.28 ± 0.39 | 0.203 |
| RMSMU (\( \mu \text{V} \)) | 59.51 ± 29.62 | 55.51 ± 37.51 | 0.775 |

Data are presented as mean ± SD. Between-group comparisons were performed with one-way ANOVA.

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**FIGURE 1**—A. Bar plots representing the average values for MVIF (N) for the INT and CON groups, before (PRE, gray bars) and after (POST, white bars) the strength training intervention. B. Bar plots representing the average values for MU normalized RT (%MVIF) for the INT and CON groups, before (PRE, gray bars) and after (POST, white bars) the strength training intervention. C. Bar plots representing the average values for MU DR (pps) at the recruitment for the INT and CON groups, before (PRE, gray 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 color. *\( P < 0.05 \); **\( P < 0.001 \).
The MU propagation velocity of the longitudinally tracked MU recorded from tibialis anterior, represented by an average of values among contractions and participants, changed significantly after 4 wk of strength training (time–group interaction; \( P = 0.004, \eta_p^2 = 0.327 \)). MUCV significantly increased after the intervention from 4.52 ± 0.39 to 4.66 ± 0.44 m·s\(^{-1}\) (+3.0%; \( P = 0.028, \eta_p^2 = 0.367 \)), on average. Moreover, when mean MUCV was computed separately for the tracked lower-threshold (RT between 0% and 30% MViF) and higher-threshold (RT between 50% and 70% MViF) MU, significant changes were observed solely for the higher-threshold MU \((n = 48); \) low threshold: \( PRE, 4.14 ± 0.53; \) POST, \( 4.19 ± 0.53 \) m·s\(^{-1}\); \( P = 0.066, n = 20 \); high threshold: \( PRE, 5.14 ± 0.45; \) POST, \( 5.28 ± 0.55 \) m·s\(^{-1}\); \( P = 0.037, \eta_p^2 = 0.210 \)). Conversely, no significant changes were observed for the CON group for either low-threshold MU (\( n = 64 \); \( PRE, 3.96 ± 0.51; \) POST, \( 3.93 ± 0.46 \) m·s\(^{-1}\); \( P = 0.227 \)) and high-threshold MU (\( n = 23 \); \( PRE, 4.76 ± 0.49; \) POST, \( 4.70 ± 0.38 \) m·s\(^{-1}\); \( P = 0.056 \)).

We observed a linear correlation between MU 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 with lower-threshold MU 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, http://links.lww.com/MSS/B820).

MU propagation velocity changes to strength training were further investigated by linear regression. As depicted in Figure 2A and B, the rate of change of MUCV as a function of their RT adapted differently in the two groups, when considering the same MU before and after the intervention (time–group interaction; \( P = 0.035, \eta_p^2 = 0.186 \)). 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·s\(^{-1}\)·%MViF (+32.6%; \( P = 0.028, \eta_p^2 = 0.367 \); Fig. 3B). Conversely, the \( y \)-intercept of MUCV (PRE, \( 3.93 ± 0.50 \); POST, \( 3.97 ± 0.56 \) m·s\(^{-1}\); \( P = 0.314 \)) was not significantly influenced by the training intervention (time–group interaction; \( P = 0.568, \eta_p^2 = 0.015 \); Fig. 3A). These findings indicate a predominant increase in MUCV for high-threshold MU and suggest specific electrophysiological changes in the MU 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·s\(^{-1}\)·%MViF; \( P = 0.696 \)) and the initial value (intercept; PRE, \( 3.71 ± 0.54 \); POST, \( 3.71 ± 0.47 \) m·s\(^{-1}\); \( P = 0.999 \)) of the linear regressions remained similar in the CON group (Fig. 3A and B).

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

**MU amplitude.** The action potential amplitude of the pool of tracked MU did not change after the training intervention (time–group interaction; \( P = 0.478, \eta_p^2 = 0.023 \)). When averaged among contractions and participants, RMSMU ranged from 59.51 ± 29.62 (PRE) to 56.17 ± 27.66 (POST) \( \mu \)V and from 55.51 ± 37.51 to 47.60 ± 22.27 \( \mu \)V in the INT and CON groups, respectively.

The regression analysis indicated less consistent associations between RMSMU and MU RT. Indeed, only 17 out of 24 individuals showed significant correlations in all testing conditions. Considering both conditions (PRE vs POST) and groups (INT vs CON), individual \( R^2 \) ranged from 0.04 to 0.98 with a mean value of \( 0.67 ± 0.24 \) (see Table 5, Supplemental Digital Content 3, Participant-specific values for RMSMU linear regression analysis pre-to-post intervention, http://links.lww.com/MSS/B821). The absence of changes in RMSMU at the group level as a consequence of the strength training intervention was confirmed by individual linear regressions. Indeed, as reported in Figure 3C and D, the intervention did not modify the

FIGURE 2—MUCV (m·s\(^{-1}\)) regression lines plotted as a function of normalized RT (%MViF) of the identified pool of longitudinally tracked MU recorded from the TA muscle before (PRE, orange) and after (POST, blue) the strength training intervention for the INT (A) and CON (B) group. Preintervention regression lines are represented with an orange dashed line, whereas postintervention regression lines are represented with a blue dashed line. Each filled dot in the graphs represents a single MU (\( n = 210 \)). A total of 94 and 116 MU were tracked across the two main measurement sessions for INT and CON groups, respectively. The coefficient of determination (\( R^2 \)) values of the linear regressions are reported as mean ± SD across participants and are shown in the upper left corner of each graph.
y-intercepts (time–group interaction; $P = 0.531$, $\eta^2_p = 0.018$) or the slopes (time–group interaction; $P = 0.100$, $\eta^2_p = 0.118$) of the regression lines. Indeed, the y-intercept of RMSMU ranged from $13.82 \pm 42.77$ at the baseline to $18.10 \pm 23.52$ μV at posttest in the INT group and from $-5.24 \pm 28.29$ to $7.88 \pm 15.26$ μV in the CON group, respectively. The rate of change of RMSMU relative to RT ranged from $1.24 \pm 1.04$ to $1.35 \pm 0.68$ μV·%MViF and from $1.87 \pm 1.79$ to $1.19 \pm 0.68$ μV·%MViF in the INT and CON groups, respectively. Therefore, the association between RMSMU and MU RT was not modified by training, likely because of the considerable intersubject variability (see Table 5, Supplemental Digital Content 3, Participant-specific values for RMSMU linear regression analysis pre-to-post intervention, http://links.lww.com/MSS/B821). 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 ($\Delta$ MViF) and the changes in RMSMU ($\Delta$ RMSMU) 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 MU recorded from the tibialis anterior muscle after 4 wk of strength training. In particular, MUCV and RMSMU changes were studied in relation to the corresponding MU RT at the individual subject level, in the recruitment range of the tibialis anterior muscle. We showed that the MUCV of MU recruited at higher muscle forces significantly increased after the training intervention, whereas no changes in MUCV of lower-threshold MU were observed. On the other hand, these specific adjustments were not accompanied by changes of MU action potential amplitude. These results provide the first in vivo evidence of specific strength training–induced adaptations in peripheral properties of high-threshold MU.

**Neuromotor adaptations.** As expected, 4 wk 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 after 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 (CSA), or twitch torque, which are known to occur only after 4 to 5 wk of regular strength training (2,3,5). Nevertheless, these variables have not been examined in the current study. Similarly, changes in antagonist muscle coactivation, which might have contributed to the muscle force gains by a training-induced increase...
in reciprocal inhibition, were not quantified (36). Considering that the force generated by a muscle in a voluntary contraction depends on the modulation of the number of recruited MU and their DR, as well as by the mechanical properties of the muscle units, we monitored adjustments in MU behavior after the training intervention. We recently demonstrated that an increase in the net excitatory input to the motor neuron pool for the same relative force could partly account for the observed gains in motor output (9). In the current study, we observed that the early gains in force-generating capacity were accompanied by a substantial and consistent decrease of normalized MU RT during the submaximal trapezoidal contractions in all subjects. Specifically, this means that the prescribed force trajectory during the ramp-up phase of trapezoidal contractions was achieved by recruiting MU earlier, i.e., at lower-force intensities relative to the maximum. The fact that only nonsignificant changes were observed for the absolute MU RT excludes, although indirectly, the possible implication of a training-induced decrease and/or impairment in MU twitch forces. One possible explanation for the decrease in normalized RT could be related to changes in musculotendinous stiffness. Indeed, a previous study (37) reported that a training program characterized by high-force contractions decreases musculotendinous stiffness, which in turn was correlated with a decrease of neuromechanical delay. Because it has recently been demonstrated by Del Vecchio et al. (38) that the neuromechanical delay, which refers to the latency between the neural command to a muscle unit and the force generated during voluntary tasks, is broadly modulated by the central nervous system by varying the activation of MU (e.g., their RT), the training-induced decrease in muscular stiffness might therefore account for the observed decrease in MU RT. Nevertheless, this hypothesis needs to be verified. In addition, according to previous evidence, an increase in conduction velocity would also affect the force rise time in single MU (11, 20, 39). Therefore, the increase in rate of force development of single MU could potentially result in a better summation of MU twitches and thus total force output for a given synaptic input. Accordingly, the number of active MU 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 MU RT, the strength training protocol induced an increase of MU DR, which was exhibited by most identified MU and hence independently of their RT, during the steady state of the contractions. Conversely, in the current analysis, the peripheral properties of lower- and higher-threshold MU showed distinct adjustments after the short-term training intervention, as discussed below.

**MUCV.** Considering both groups and conditions, the observed average MUCV values of the tracked MU \((n = 210)\) 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 RT during submaximal isometric ramp contractions was observed in all subjects and in both testing conditions \((\text{mean } R^2 = 0.71 \pm 0.16)\). 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 MU. For example, in agreement with our results, in two previous studies by Del Vecchio et al. (21), mean \(R^2\) values of 0.64 ± 0.14 and 0.70 ± 0.09 (13) were reported between MUCV and RT of MU from tibialis anterior. The observed strong association between voluntary recruitment of MU and muscle unit properties confirmed that CV is higher for MU with larger diameters and hence higher RT, compared with lower-threshold, smaller-diameter MU.

**MUCV and training.** Previously, the effects of physical training on the velocity of propagation of action potentials along the sarcolemma of muscle fibers innervated by individual motor neurons (i.e., MUCV) have been indirectly investigated. The majority of previous studies focused on training-associated changes in MFCV, an EMG-derived parameter that reflects an average value of the conduction velocities of the active MU, in different types of contraction. In these studies, MFCV was estimated in resting conditions (18), during MVC (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 \((4.84 \pm 0.24 \text{ m·s}^{-1})\) compared with endurance runners \((4.31 \pm 0.10 \text{ m·s}^{-1})\). In addition, a strong and positive correlation was found between MFCV and the relative area of fast-twitch fibers. Similarly, Methenitis et al. (18) confirmed the close association between MFCV and muscle fiber \% CSA. In particular, the authors found that the \% CSA of type II and IIx fibers 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 MU, only allowed analysis of the compound MU 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 with controls. Accordingly, strength-trained individuals seem to be able to achieve higher force levels by recruiting MU with greater MFCV (i.e., larger motor neurons innervating larger-diameter and fast-twitch muscle fibers) in a shorter amount of time compared with 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 wk of concentric \((22.2\% \pm 65.1\%)\) or eccentric \((27.3\% \pm 73.8\%)\) strength training.

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

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

There are no previous studies that assessed the adaptations in MU peripheral properties after strength training when considering the same MU tracked before and after the intervention. In this study, the MUCV of the tracked MU estimated during submaximal isometric contractions significantly increased after 4 wk of strength training, when averaged among contractions and subjects. More interestingly, the linear regressions between individual MUCV values and normalized RT of the same MU pointed out distinct adjustments in MU peripheral properties between lower- and higher-threshold MU. Indeed, when comparing the regression lines at the baseline and postintervention, a significant increase in MUCV rate of change (slope) relative to MU RT was observed. Conversely, the initial value of MUCV (intercept) did not change, indicating that the CV of MU recruited at the lower-force levels and hence their peripheral properties were not influenced by training.

Similarly, Martinez-Valdez et al. (27) reported distinct MUCV adjustments after 2 wk of either HIIT or moderate-intensity continuous training. In particular, an overall increase in MUCV of lower-threshold MU during submaximal contractions was observed after both training interventions, whereas MUCV of higher-threshold MU 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 MU 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 with 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 MU, whose recruitment is necessary and essential to achieve increased peak muscle forces (25). However, we have recently shown an increase in MU DR at the plateau of a trapezoidal contraction in most MU after strength training, independently on their RT (9). Therefore, taken together the results of these studies, our findings suggest that although central adaptations in MU behavior (e.g., MU DR) occurred in the whole population of identified MU, short-term strength training elicited specific adjustments in peripheral properties (e.g., MUCV) of low- and high-threshold MU. In particular, these differential changes might reflect a potential greater adaptability in the electrophysiological properties of muscle membrane of higher-threshold MU.

In the only study that focused on long-term changes in MU properties after a training intervention, Vila-Chá and colleagues (28) reported a significant increase in MUCV of lower-threshold MU, i.e., recruited at 30% MViF, during submaximal contractions after either 6 wk of endurance or strength training. Considering the longer duration of the training intervention (6 vs 4 wk), changes in the contractile apparatus (e.g., increase in muscle fiber size) cannot be completely ruled out, and this may have influenced the MUCV results. Furthermore, because of technical constraints related with the invasive assessment of MUCV, conduction velocity was computed only on MU recruited at low torque levels (e.g., lower-threshold MU), and hence potential adjustments in higher-threshold MU could not be documented in this previous study. Again, a direct comparison with the current results is difficult.

The specific changes in conduction velocity observed in higher-threshold MU after the short-term strength training might be due to specific adaptations in the voltage-sensitive ionic channels (e.g., Na+ and K+) and/or modifications of the transport activity and capacity of Na+–K+ pump (e.g., Na+–K+–ATPase). In fact, both factors play a significant role in the transmission of MU action potentials along the muscle fibers by influencing membrane excitability (22,23). On one side, ionic channels are responsible for the propagation of action potentials from the sarcolemma to the terminal cisternae of the sarcoplasmic reticulum (SR) triggering the release of Ca2+ from the SR to the muscle fibrils; on the other side, Na+–K+–ATPase contributes to the recovery and maintenance of the resting membrane potential and modulates muscle contractile function (42). For instance, previous studies have pointed out that MU action potential velocity is impaired by an increased concentration of extracellular K+ (e.g., hyperkalemia) (43). On the other hand, Na+–K+–ATPase plays a key role in reducing extracellular K+ concentration, and in particular, a study (44) reported that the stimulation of the Na+–K+–ATPase with adrenaline increases MU action potential propagation velocity in muscle fibers with high extracellular levels of K+. Moreover, studies conducted on both animals and humans revealed that type I and type II muscle fibers seem to exhibit a different number, density, and isoforms of Na+–K+–ATPase (42,45). In particular, type II muscle fibers, which are generally innervated by larger α-motor neurons and hence generally found in higher-threshold MU, have a greater amount of Na+–K+–ATPase compared with type I fibers, innervated by smaller α-motor neurons and hence usually found in lower-threshold MU (42). Furthermore, the β2-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 fibers (45). Accordingly, the different electrophysiological features manifested in type II fibers seem to justify
the faster spread of action potentials along the sarcolemma of higher-threshold MU compared with lower-threshold MU. In this regard, an activity-dependent upregulation of Na\(^+\)-K\(^+\)-ATPase activity has been observed after training interventions over the last 20 yr (46,47). Conversely, inactivity and immobilization led to a downregulation 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 wk of high-resistance training. However, although no significant changes were observed in the first 4 wk of the intervention, a direct comparison between the present results and the results of Green et al. (47) is difficult because of methodological differences and different duration (e.g., 12 vs 4 wk) 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 MU than in lower-threshold MU.

In agreement with this interpretation of selective adjustments in MU behavior, Pitulainen et al. (48) documented specific changes in MUCV for higher-threshold MU (e.g., only at 50%–70% MVC) after 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 fibers compared with slow-twitch fibers. 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 MU, 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 MU is a specific change in the diameter of muscle fibers belonging to this MU (i.e., hypertrophy). However, although changes in contractile properties cannot be completely ruled out because they were not directly quantified in the current study, it is very unlikely that the short-term protocol (12 training sessions over 4 wk) induced any changes in muscle fiber size or architecture, considering that significant morphological changes generally occur after longer training interventions (>30–35 d) (7). In fact, changes in MUCV do not necessarily imply changes in the contractile properties (49). Indeed, although not directly investigated in the current study, a close association between muscle fiber electrophysiological and contractile properties seems to exist. For instance, the speed of release of calcium from the SR increases with increasing depolarization (50), which is related to the propagation speed of MUAP, and in turn determined by fiber diameter. Indeed, the time to peak of MU twitch forces decreases as the MUCV increases (11). Moreover, it was reported that MU twitch force increases when two discharges occur close to each other (51), paralleling an increase in MFCV. These mechanisms suggest that adaptations in the muscle fiber electrophysiological properties may affect contractile properties regardless of either an increase or decrease in muscle fiber diameter (39).

In support of this explanation, we observed that MU action potential amplitude (RMS\(_{MU}\)), an EMG-derived parameter that is suggested to reflect changes in muscle fiber size and morphology (hypertrophy) (52), of the tracked MU did not change significantly after the intervention at the group level. Furthermore, the lack of changes in MU action potential amplitude was confirmed at the single individual level by examining the regression lines between the RMS\(_{MU}\) and the normalized RT of the same MU. In this regard, although positive and significant correlations were observed for the majority of participants (18 out of 24), EMG amplitude estimates exhibited a high level of interindividual variability particularly with regard to the rate of change in RMS\(_{MU}\) as a function of RT and in the initial value of RMS\(_{MU}\) regression values. These results are aligned with previous reports (13), which showed that MU action potential amplitudes are only moderately correlated with RT, with high variability across subjects (21,33). This observation is related to the fact that MU action potential amplitude does not always relate to muscle force. In fact, HDsEMG decomposition algorithms tend to identify predominantly the largest MU, which might not always show the greatest action potential amplitude (15). Deeper MU having a higher RT and therefore larger size might show smaller MU action potential amplitude (21). Moreover, the lack of changes in MU DR at the recruitment of trapezoidal contractions excludes the potential influence of this variable on the increase of MUCV.

**CONCLUSION**

This study revealed that 4 wk of isometric strength training elicited specific adaptations in the electrophysiological properties of muscle fiber membrane of higher-threshold MU. Although the specific neurophysiological mechanisms underlying these early and selective adjustments in higher-threshold MU need to be further elucidated in future investigations, we provided the first in vivo evidence of the effects of strength training on MUCV, 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.

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REFERENCES

1. Folland JP, Williams AG. The adaptations to strength training: morphological and neurochemical contributions to increased strength. Sports Med. 2007;37(2):145–68.

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

3. Nuzzo JL, Barry BK, Jones MD, Gandevia SC, Taylor JL. Effects of four weeks of strength training on the corticomotorneuronal pathway. Med Sci Sports Exerc. 2017;49(11):2286–96.

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

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

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

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

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

9. Del Vecchio A, Casolo A, Negro F, et al. The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. J Physiol. 2019;597(7):1873–87.

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

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

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

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

14. Mascuda T, De Luca CJ. Recruitment threshold and muscle fibre conduction velocity of single motor units. J Electromyogr Kinesiol. 1999;1:116–23.

15. Holobar A, Azzud A. Multichannel blind source separation using convolution kernel compensation. IEEE Trans Signal Processing. 2007;55(9):4487–96.

16. Hakansson CH. Conduction velocity and amplitude of the action potential as related to circumference in the isolated fibre of frog muscle. Acta Physiol Scand. 1956;37(1):14–34.

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

18. Methenis S, Karandreas N, Spengos K, Zaras N, Stasinaki AN, Terzis G. Muscle fiber conduction velocity, muscle fiber composition, and power performance. Med Sci Sports Exerc. 2016;48(9):1761–71.

19. Plonsey R, Barr CR. 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. Sadowska-T, Masuda T, Miyata H, Katsuta S. Fibre conduction velocity and fibre composition in human vastus lateralis. Eur J Appl Physiol Occup 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-Chà 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. J Neural Eng. 2014;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. J Neurosci Methods. 2002;115:1–12.

32. Farina D, Arendt-Nielsen L, Merletti R, Graven-nielsen T. Effect of muscular strain 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 fibre conduction velocity and surface EMG amplitude across subjects and processing intervals. J Electromyogr Kinesiol. 2018;40:102–9.

34. Cohen J. Statistical Power Analysis for the Behavioral Sciences. Hillsdale (NJ): Lawrence Erlbaum Associates; 1988. 302 p.

35. Tillin NA, Pain MT, Folland JP. Short-term training for explosive strength causes neural and mechanical adaptations. Exp Physiol. 2012;97(3):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, Péro 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, Übeda A, Sartori M, Azorin 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*. 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 H, 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. Pitulainen 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.