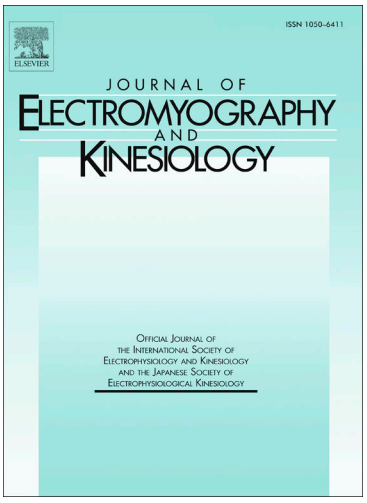
Journal Pre-proofs



Reproducibility of muscle fibre conduction velocity during linearly increasing force contractions

Andrea Casolo, Stefano Nuccio, Ilenia Bazzucchi, Francesco Felici, Alessandro Del Vecchio

PII: S1050-6411(20)30081-X
DOI: https://doi.org/10.1016/j.jelekin.2020.102439
Reference: JJEK 102439

To appear in: *Journal of Electromyography and Kinesiology*

Received Date: 14 January 2020
Revised Date: 14 May 2020
Accepted Date: 6 June 2020

Please cite this article as: A. Casolo, S. Nuccio, I. Bazzucchi, F. Felici, A. Del Vecchio, Reproducibility of muscle fibre conduction velocity during linearly increasing force contractions, *Journal of Electromyography and Kinesiology* (2020), doi: https://doi.org/10.1016/j.jelekin.2020.102439

Reproducibility of muscle fibre conduction velocity during linearly increasing force contractions

Andrea Casolo [1,2], Stefano Nuccio [1,2], 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

***Corresponding author**

a.del-vecchio@imperial.ac.uk, Department of Bioengineering, Imperial College London SW7 2AZ, UK

Keywords

Global EMG estimates, propagation velocity, linearly increasing force contractions, coefficient of variation, intraclass correlation coefficient.

6.1 ABSTRACT

Muscle fibre conduction velocity (MFCV) is a basic physiological parameter biophysically related to the diameter of muscle fibres and properties of the sarcolemma. The aim of this study was to assess the intersession reproducibility of the relation between voluntary force and estimates of average muscle fibre conduction velocity (MFCV) from multichannel high-density surface electromyographic recordings (HDsEMG). Ten healthy men performed six linearly increasing isometric ankle dorsiflexions on two separate experimental sessions, 4 weeks apart. Each session involved the recordings of voluntary force during maximal isometric (MViF) and submaximal ramp contractions at 35-50-70 % of MViF. Concurrently, the HDsEMG activity was detected from the tibialis anterior muscle and MFCV estimates were derived in 250-ms epochs. Absolute and relative reproducibility of MFCV initial value (intercept) and rate of change (regression slope) as a function of force were assessed by within-subject coefficient of correlation (CV_w) and with intraclass correlation coefficient (ICC). MFCV was positively correlated with voluntary force (R^2 =0.75±0.12) in all individuals and test conditions (P<0.001). Average CV_w for MFCV intercept and slope were of 2.6±2.0% and 11.9±3.2% and ICC values of 0.96 and 0.94, respectively.

Overall, MFCV regression coefficients showed a high degree of intersession reproducibility in both absolute and relative terms. These results may have important practical implications in the tracking of training-induced neuromuscular changes and/or in the monitoring of the progress of neuromuscular disorders when a full sEMG signal decomposition is problematic or not possible.

INTRODUCTION

During voluntary contractions, the central nervous system (CNS) controls the motor output by the orderly recruitment of motor units and the concurrent modulation of their discharge rate (Henneman, 1957). As a consequence of the discharge of action potentials from the motor neurons, which travel along the axon and arrive at the neuromuscular junction, a local depolarization of muscle fibre membrane generates muscle fibre action potentials in the innervated muscle fibres (Heckman and Enoka, 2004).

The average propagation velocity of action potentials along the sarcolemma of muscle fibres, namely muscle fibre conduction velocity (MFCV), can be measured in vivo from a surface interferential electromyographic (EMG) signal in a wide range of voluntary contractions (Del Vecchio et al., 2018b; Farina et al., 2007; Pozzo et al., 2004; Sbriccoli et al., 2009) and it represents the weighted mean of conduction velocities of the active motor units (Farina and Merletti, 2004a). MFCV is usually estimated from EMG recordings with linear or two-dimensional grids of electrodes parallel to the anatomical direction of muscle fibres (Farina and Merletti, 2004a).

Because of the biophysical association of conduction velocity and muscle fibre diameter, described in simulation models (Nandedkar et al., 1985) and experimental observations (Blijham et al., 2006; Hakansson, 1956; Methenitis et al., 2016), MFCV increases linearly during voluntary force contractions due to the progressive and orderly recruitment of larger-diameter, higher-threshold motor units (Andreassen and Arendt-Nielsen, 1987; Arendt-Nielsen and Zwarts, 1989; Del Vecchio et al., 2017). Indeed, it has been recently shown that the increase in MFCV with respect to force is associated to the recruitment of high threshold motor units with greater conduction velocities (Del Vecchio et al., 2017). In particular, there is a strong association (R^2 = 0.71) between MFCV, estimated during increasing-force contractions and motor unit conduction velocity (MUCV), which in turn is significantly correlated (R^2 = 0.70) with motor unit recruitment threshold forces (Del Vecchio et al., 2018; Del Vecchio et al., 2017). Therefore, global MFCV can be used as an indirect biomarker of motor unit recruitment (Andreassen and Arendt-Nielsen, 1987; Del Vecchio et al.,

2018c; Del Vecchio et al., 2017) to be used in situations when a full EMG decomposition is not possible (Del Vecchio et al., 2018b).

Because of the many potential applications of MFCV estimates for the non-invasive monitoring of changes in muscle properties and neural control strategies during normal muscle function, a systematic assessment of the intersession reproducibility of MFCV estimates is a critical issue (Farina et al., 2004d). Indeed, although from a methodological perspective the estimation of MFCV is rather simple, many factors other than the physiological phenomena under study might affect the quality of its estimate and hence its reproducibility (Beretta-Piccoli et al., 2019; Farina et al., 2004d). For instance, the quality of the MFCV estimates is affected by the processing and detection system's features (e.g. electrode positioning, IED, electrode number, processing time-interval, estimation method) (Farina et al., 2002b; Farina and Merletti, 2004b).

Moreover, MFCV estimation is also influenced by muscle fibre membrane properties (e.g. Na^+, K^+ ionic concentrations, Na^+-K^+-ATPase pump activity) (Allen et al., 2008; Christiansen, 2019), muscle acidosis and temperature (Brody et al., 1991; Farina et al., 2005), motor unit discharge rate (Farina and Falla, 2008), local muscle fatigue (Merletti et al., 1990) and by the subjects' training status (Casolo et al., 2020; Martinez-Valdes et al., 2018; Vila-Chã et al., 2010).

A recent study by Beretta-Piccoli et al. (Beretta-Piccoli et al., 2019) has systematically reviewed the literature on test-retest (n=2), intrasession (n=3), and intersession reliability (n=12) of MFCV estimates derived from the sEMG signal recorded from different muscles, during both voluntary and electrically-induced contractions. Moderate to high intersession reliability of MFCV estimates were reported in 7 out of 12 studies included ($0.7<ICC<1$). However, a common methodological approach to all previous studies is that MFCV reproducibility was mainly assessed during sustained, steady-state isometric contractions. Despite the employment of steady state contractions at different force levels could still provide reliable information about motor unit recruitment strategies, the adoption of isometric linearly increasing force contractions, which emulate the gradual development phase of

muscular force on a continuum, could provide a more direct opportunity to use MFCV as an indirect parameter of the progressive motor unit recruitment. Indeed, there are currently no data on the reproducibility of MFCV estimates during the development of muscular force. Because we have previously shown that the rate of change of MFCV is associated to the progressive recruitment of motor units in a relatively large cohort of subjects (Del Vecchio et al., 2018c, 2018a; Del Vecchio et al., 2017) here we used those developed methods to assess the intersession reproducibility of MFCV during linearly increasing force contractions.

The aim of this study was to assess the absolute (coefficient of variation, CV_w) and relative (intraclass correlation coefficient, ICC) intersession reproducibility of MFCV estimates during linearly increasing isometric ankle dorsi-flexions, over 4 weeks.

METHODS

Participants

Ten healthy and recreationally active young men were recruited to participate in this study (mean ± SD, age: 25.2 ± 3.0 yr, body mass: 73.4 ± 8.9 kg, height: 1.79 ± 0.07 m). The exclusion criteria were the presence of any neuromuscular disorder, current or previous history of lower limb surgery and the involvement in any regular physical exercise training program. All participants were right-leg dominant (self-reported) and were engaged in light-to-moderate aerobic physical activity ($\leq 2 \cdot week^{-1}$), which was assessed with the IPAQ (International Physical Activity Questionnaires) prior their enrolment (IPAQ: 2407 ± 1240 MET $min \cdot wk^{-1}$). All procedures and protocols were approved by the local Ethical Committee of the University of Rome "Foro Italico" (approval no. 44 680) and were in accordance with the Declaration of Helsinki. A written informed consent was obtained from all participants prior to inclusion.

Study overview

Participants attended the laboratory on three distinct occasions over a 7-week period. After a familiarization session (Session 1), two duplicate main measurement sessions were conducted 4

weeks apart. Each measurement session involved the concurrent recording of ankle dorsiflexors forces and tibialis anterior myoelectrical activity with high-density surface EMG (HDsEMG), whilst participants performed maximal and submaximal trapezoidal ankle dorsi flexions. Session 2 was carried out 3-to-5 days after session 1, while session 3 four weeks after session 2. During the familiarization session participants completed the same contraction of the main protocol but without the recording of data. Between the two main measurement sessions participants were told to maintain their habitual physical activity and diet unchanged.

Experimental procedures and protocols

The three laboratory sessions were conducted at a consistent time of the day for each participant in order to reduce the potential effect of diurnal variability of muscle contractility and hence force production (Racinais et al., 2005). The room temperature was controlled and kept constant at 19-21° C. The same customized ankle ergometer (see *Force recording*), whose configuration was defined according to individual lower limb length during session 1, was adopted in all the following experimental sessions (**Figure 1, A**). All participants were instructed to avoid caffeine consumption and demanding physical exercise in the 24 and 48 hours prior each main measurement session, respectively. Following a brief standardized warm-up based on a set of 8 isometric ankle dorsi flexions (4 x 50%, 3 x 70% and 1 x 90% of self-perceived maximum, ≤ 30s rest in-between), participants performed 3-to-4 maximal voluntary isometric contractions (MVC) and a total of 6 submaximal isometric trapezoidal contractions (2 x 35%, 2 x 50%, 2 x 70% of MVC). In MVCs, participants were instructed to "push as hard as possible" over a period of 3-to-5 s, while being verbally encouraged to exceed the previously obtained force level. These trials were separated by 30 s of rest. The highest value recorded among the MVCs, i.e. the maximal voluntary isometric force (MViF), was used as a reference for the definition of the submaximal contractions (%MViF). Approximately 5 minutes after the MVCs, participants completed six trapezoidal contractions characterized by a linear increase in force from baseline to a target force level, 10s of plateau at the target force, and a linear decrease in force to the baseline values, in a randomized order. The speed of the contraction was set at 5% MViF·s^{-1} and was kept constant in all the trials. The trials were

separated by 3-to-5 minutes of rest. Participants received visual feedback of their ankle dorsiflexion force and were instructed to match as precisely as possible a trapezoidal template showed on a monitor (~1 m from participants' eyes). The same randomized order was kept constant for each participant in the two main measurement sessions.

Data acquisition

Force recording

In each experimental session, participants were seated comfortably on a massage table to which a custom adjustable ankle ergometer (OT Bioelettronica, Turin, Italy) was secured (**Figure 1, A**). The trunk was reclined to 30° (180° = supine position), the knees extended at 180° and the dominant ankle at 100° of plantar flexion (90° = neutral position). The knee, the ankle and the foot of the dominant leg were firmly strapped to the massage table and to an adjustable foot plate, respectively. In particular, Velcro straps (~3 cm width) were arranged above the patella, on the distal third of metatarsals and on the foot dorsum. The foot plate was connected in series with a calibrated force transducer (CCT Transducer s.a.s, Turin, Italy), located perpendicular to the sole of the foot. The contralateral leg rested on the massage table. The analogue signal recorded by the force transducer was amplified (x 200), sampled at 2048 Hz and converted to digital data by a 16-bit external analogue-to-digital converter (EMG-Quattrocento, OT Bioelettronica, Turin, Italy). Force and HDsEMG signals were acquired with the software OTbiolab (OT Bioelettronica, Turin, Italy) and synchronized at source.

HDsEMG signal recording

Surface EMG signals were recorded in monopolar derivation from the tibialis anterior muscle using two high-density adhesive grids of 13 x 5 equally spaced electrodes (gold-coated, 1 mm diameter, 8 mm inter-electrode distance (IED); OT Bioelettronica, Turin, Italy). The two grids, positioned as described in previous studies (Casolo et al., 2020; Del Vecchio et al., 2019), were connected to a multichannel amplifier (EMG-Quattrocento, OT Bioelettronica, Turin, Italy). The EMG signals

detected were amplified (x 150), sampled at 2048 Hz, band-pass filtered (3dB bandwidth, 10-500 Hz) and converted to digital data by the same multichannel amplifier (**Figure 1, B**).

To ensure an optimal placement of the high-density grids, a linear 16-electrode dry array (silver bar, 5 mm electrode length, 1 mm electrode thick, 5 mm IED; OT Bioelettronica, Turin, Italy) was moved over the skin, according to the anatomical description (Farina et al., 2004c, 2002a) for the location of an easy identifiable innervation zone (IZ) in the distal region of tibialis anterior muscle. Briefly, the accessible and superficial IZ located in the distal portion of TA was identified by visual inspection of multichannel EMG recordings, as the point of inversion in propagation direction of action potentials (APs) along the electrode columns (Del Vecchio et al., 2018; Del Vecchio et al., 2017). Once the IZ was located, the anatomical direction of muscle fibres was visually estimated by choosing the angle of inclination of the array that led to the most similar propagation of motor unit APs from the IZ to the distal tendon region without significant change of APs waveforms (**Figure 1, C**). The IZ, the distal tendon region as well as the estimated anatomical direction of muscle fibres (mostly parallel to the lateral margin of the tibia in the distal region of tibialis anterior) were marked with a surgical pen. After this procedure, the portion of the skin delineated was prepared (shaving, light skin abrasion and cleansing with 70% ethanol). One adhesive grid was fixed over the skin of the distal portion of tibialis anterior with the first column of electrodes aligned with the estimated direction of muscle fibres and particularly, the fourth row of the grid in correspondence of the IZ. The second adhesive grid was placed proximally in order to cover most of the muscle belly. The reference electrodes (one for each grid) were positioned on the medial malleolus and on the tuberosity of the tibia of the tested leg, respectively. The main ground electrode was placed at the wrist of the tested side.

Signal analysis

Force processing

In offline analysis, the analogue force signal was converted to newtons (N) and low-pass filtered (4^{Th} order, zero-lag, Butterworth, cut-off frequency 15 Hz). The offset of force was corrected for the

effect of gravity. For each participant, only the best contraction trial at each force level (35-50-70% MViF) was analysed. In particular, the criterion adopted for the selection of the contraction to include in the analysis was the lowest deviation of the force trajectory from the given force template. Contractions that showed any pre-activation or countermovement (≤ 0.5 N from baseline force in 150 ms prior to force onset) were excluded (Casolo et al., 2020; Del Vecchio et al., 2019).

HDsEMG signal processing

For clarity, the dataset adopted for this analysis is the same as in our previous publications (Casolo et al., 2020; Del Vecchio et al., 2019). Considering the divergent aim of each work, in this study we only focused on the analysis of EMG signals recorded from the grid located on the distal portion of the TA muscle, which represents the optimal location to observe the propagation of action potentials along the muscle fibre membrane to the distal tendon region of TA muscle.

After being low-pass filtered offline (20-500 Hz, Butterworth, 2nd order), double-differential derivations were computed from the monopolar recordings in the longitudinal direction of the bi-dimensional grids (columns of the grid). In this study, only HDsEMG signals recorded from the distal grid, aligned with the estimated direction of muscle fibres in the distal portion of tibialis anterior were adopted for MFCV estimation (Del Vecchio et al., 2018a; Del Vecchio et al., 2019). Double-differential signals were visually inspected and the channels that showed the clearest propagation of motor unit action potentials (MUAPs), with minimal change in MUAP waveforms, were manually selected for MFCV estimation (min 4, max 7 channels from the same electrode column) (Casolo et al., 2020; Del Vecchio et al., 2018c, 2018a). Because the number of channels also influences the accuracy of MFCV estimation, the highest number of channels showing a CC ≥ 0.5 between consecutive EMG signals was selected for each participant (Del Vecchio et al., 2018a). Once the EMG channels were selected, a multi-channel maximum likelihood algorithm was used to estimate MFCV during the ramp-up phase of the isometric trapezoidal contraction in consecutive 250-ms intervals without overlapping. The validity and reliability of the adopted algorithm to estimate MFCV with an associated standard deviation (SD) < 0.1 m·s^{-1} (Farina et al., 2004d, 2001; Farina and Merletti, 2004a) has been previously assessed in both static and dynamic contractions (Farina et al.,

2004d, 2004c) and it has been adopted in different muscles (Del Vecchio et al., 2018b, 2018c). Maximal muscle fibre conduction velocity ($MFCV_{MAX}$) was estimated during MVC in the 250 ms time window in correspondence of MViF, in order to allow the normalization of MFCV estimates for $MFCV_{MAX}$. After the estimation of $MFCV_{MAX}$, MFCV was estimated during the submaximal contractions and precisely from the EMG onset to the steady state phase of the linearly increasing isometric ramp contractions (i.e., recruitment phase) (Farina et al., 2004d; Farina and Merletti, 2004b). The time-course of MFCV as a function of voluntary force was investigated at the individual level by linear regressions in both absolute (MFCV) and normalized (% $MFCV_{MAX}$) terms. In particular, the time course of MFCV as a function of force was investigated at each of the three force targets (35-50-70 % MViF). This means that for each subject, three regression lines were derived. Accordingly, the regression lines of MFCV over force were estimated using the MFCV estimates obtained from each single time window. Thereafter, the three regression lines where pooled together originating a unique regression line for each participant ranging from 0 to 70 % of MViF, which corresponds to the recruitment range of TA muscle. The MFCV initial value, corresponding to the intercept with the y-axis, and rate of change of the regression line (slope) were extracted by the regressions for each participant and compared four weeks apart (Merletti et al., 1990) (see Statistical Analysis for details). The minimum MFCV ($MFCV_{MIN}$), maximum MFCV ($MFCV_{MAX70}$) and mean MFCV ($MFCV_{MEAN}$) corresponded respectively to the minimum, maximum and average MFCV values estimated in the ramp-up phase of the linearly increasing force contractions. Only MFCV values within the physiological range were considered in the analysis (2-6.5 $m \cdot s^{-1}$) (Andreassen and Arendt-Nielsen, 1987; Del Vecchio et al., 2018c).

To assess the reproducibility of MFCV estimates, the same number of channels and location (column of electrodes) was precisely replicated for each participant in both the test sessions. Furthermore, the profiles of the 2-dimensional high-density grids were marked on participants' skin during session 1 and were carefully re-marked on a daily basis with a surgical pen. The grids' position with respect to anatomical references was also traced on transparent sheets to ensure similar electrode positioning for the test session 2 (Del Vecchio et al., 2019; Martinez-Valdes et al., 2017).

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of distribution of the extracted variables. For the variables that did not show a normal distribution, the correspondent non-parametric tests were adopted or if not possible (e.g. intraclass coefficient of correlation), they were log-transformed to meet the assumption of normality prior applying the correspondent statistical test.

Multiple paired sample *t*-tests were adopted to compare differences in anthropometric characteristics, MViF and surface EMG-derived parameters (e.g. $MFCV_{MAX}$, $MFCV_{MIN}$, $MFCV_{MAX70}$, $MFCV_{MEAN}$) between session 1 and session 2. Pearson product-moment correlation coefficient (R) was used to assess the relation between MFCV and force for each participant in both test sessions. Linear regressions (1st order polynomial) were adopted to assess the relation between MFCV and voluntary force for each participant as adopted in previous studies (Casolo et al., 2020; Del Vecchio et al., 2017; Del Vecchio et al., 2018a, 2018c; Farina et al., 2004c). The coefficient of determination (R^2) was adopted as an index of prediction power. The MFCV initial value (intercept) and rate of change (regression slope) related to force of the regression lines were extracted and statistically compared with paired sample *t*-tests to assess changes between test session 1 and 2, both in absolute and normalized (% $MFCV_{MAX}$) values. Moreover, the estimates of MFCV initial values and rate of change related to force were also correlated between the two test sessions.

To determine the level of reproducibility of MViF and surface EMG-derived estimates (e.g. MFCV intercept and slope of the regression lines) between the two test sessions, the within-participant coefficient of variation (CV_w, [SD/mean] X 100) was calculated as a measure of variability of an individual's value (i.e. absolute reproducibility). CV_w values were interpreted as "acceptable" if CV_w < 12%, "intermediate" if 12%< CV_w < 20% or "unacceptable" if CV_w > 20% (Balshaw et al., 2017). The intraclass coefficient of correlation (ICC) (two-way random effect model, absolute agreement) was adopted to assess the percentage of global variance that attributable to subject-to-subject variability (i.e., relative reproducibility). The ICC was calculated for MViF and surface EMG-

derived estimates (e.g. MFCV intercept and slope of the regression lines) between the two test sessions. ICC values were interpreted as "very high" if 0.9<ICC<1.0, "high" if 0.7<ICC<0.9, "moderate" if 0.5<ICC<0.7, "low" if 0.3<ICC<0.5, "negligible" if 0.0<ICC<0.3 (Hinkle et al., 2002).

The software SPSS, Version 23.0 (SPSS Inc, Chicago, IL, USA) was adopted to conduct all statistical tests and the statistical significance was set at $\alpha < 0.05$. Data are reported as means ± SD unless stated elsewhere.

Figure 1 goes here

RESULTS

Anthropometrical characteristics

Participant's anthropometrical characteristics did not differ between the two test sessions separated by 4 weeks ($P > 0.05$ in all cases).

Reproducibility of MViF and $MFCV_{MAX}$

MViF did not change between the two test sessions (session 1: 304.7 ± 42.6 N; session 2: 309.8 ± 36.1 N, $P = 0.500$). Average CV_w for MViF was 3.6 ± 3.9 % [95% CI: 1.2 to 6.0 %] between the two assessments. An ICC of 0.91 [95% CI: 0.66 to 0.98] displayed a "very high" reproducibility of MViF of ankle dorsiflexors over the four weeks ($P = 0.001$). Similarly, the maximum MFCV at the MViF ($MFCV_{MAX}$) did not differ between the two test sessions (session 1: 4.06 ± 0.59 $m \cdot s^{-1}$; session 2: 4.05 ± 0.57 $m \cdot s^{-1}$, $P = 0.874$). Average CV_w for $MFCV_{MAX}$ was 3.1± 2.4 % [95% CI: 1.6 to 4.6 %] between the two assessments. An ICC of 0.97 [95% CI: 0.87 to 0.99], corresponding to a "very high" reproducibility of $MFCV_{MAX}$ was observed over the four weeks ($P < 0.001$).

MFCV estimates and force

MFCV was estimated from an average of 5.6 ± 0.8 EMG channels per participant, which showed the clearest propagation of MUAPs along a single column of electrodes and the highest cross-correlation (CC) between consecutive action potentials (CC, session 1: 0.63 ± 0.10; session 2: 0.62 ± 0.13, P = 0.650). In particular, for all the participants the single column of electrodes selected for MFCV estimation was the one closer to the lateral margin of the tibia bone, overlying an easily identifiable IZ (Del Vecchio et al., 2018a; Farina et al., 2004d).

The individual ranges of conduction velocities for both test sessions is reported in **Table 1**. No significant changes were observed for average $MFCV_{MIN}$ (P = 0.847), $MFCV_{MAX70}$ (P = 0.435) and $MFCV_{MEAN}$ (P = 0.742) values estimated during linearly increasing force contractions up to 70% of MViF between test session 1 and 2. Average CV_w for $MFCV_{MIN}$ was 3.3 ± 2.0 % [95% CI: 1.8 to 4.71 %], for $MFCV_{MAX70}$ was 4.2 ± 0.4 % [95% CI: 0.8 to 4.3 %] and for $MFCV_{MEAN}$ was 2.1 ± 2.1 % [95% CI: 0.6 to 3.6 %]. An ICC of 0.90 [95% CI: 0.60 to 0.98], of 0.91 [95% CI: 0.68 to 0.98] and of 0.94 [95% CI: 0.76 to 0.98], corresponding to a "very high" reproducibility, was observed for $MFCV_{MIN}$, $MFCV_{MAX70}$ and $MFCV_{MEAN}$, respectively (P < 0.001 in all cases).

For all the participants, MFCV was positively correlated with force (P < 0.001 in all cases). The participant-specific coefficient of determination (R^2), intercept and slope of the regressions between MFCV and force are reported in **Table 2**. In particular, the average R^2 was equal to 0.75 ± 0.12 and 0.76 ± 0.13 during session 1 and 2, respectively. **Figure 2 A-B-C** shows an example of the regression lines between MFCV and voluntary force in the recruitment range of tibialis anterior muscle for three representative individuals derived from the two test sessions. In particular, as observable for S1 and S2 (**Figure 2 A-B**), MFCV linearly increased as a function of voluntary force in both test conditions (PRE vs POST). A similar trend was observed in the majority of participants (7 out of 10). Conversely, in S3 (**Figure 2 C**), despite a linear increase in MFCV was observed in the initial phase of the contraction (at lower voluntary forces), MFCV reached a plateau at higher forces. This trend was observed only in 3 out of 10 participants (S3, S5, S6) and could be attributable to different factors

(see Discussion). In these specific cases, the MFCV plateau (break out point of the linear relation with force) was observed on average at 48.2 ± 7.0 % and at 47.9 ± 7.5 % MViF, in measurement session 1 and 2, respectively. Nevertheless, an overlap of the regression lines between the time-course of MFCV against force during both test sessions is clearly observable in all the three representative individuals (S1, S2, S3).

We extracted from the regression line the MFCV initial value (intercept) and MFCV rate of change as a function of force production (slope). These variables were compared before and after the four weeks. The MFCV initial values, over all the conditions and participants, were not statistically different between the two test sessions (Session 1: 3.43 ± 0.39 $m \cdot s^{-1}$; Session 2: 3.39 ± 0.43 $m \cdot s^{-1}$; P = 0.432). Similarly, the estimates of the MFCV rate of change as a function of force, did not differ between the two test sessions (Session 1: 0.012 ± 0.004 $m \cdot s^{-1} \cdot$% MViF; Session 2: 0.012 ± 0.005 $m \cdot s^{-1} \cdot$% MViF; P = 0.646). **Figure 3 A** shows the scatter plot of the MFCV initial values for session 1 and session 2, for each participant. Similarly, a scatter plot of MFCV rate of change between the two test sessions is reported in **Figure 3 B**. In both cases, a strong and significant correlation was observed between the estimates of the two test sessions (MFCV Intercept: R^2 = 0.87; MFCV Slope: R^2 = 0.80; $P < 0.001$ in all cases).

Figure 2 goes here

Reproducibility of MFCV and force

After having observed that MFCV initial value and rate of change did not differ significantly between the two test sessions, we aimed at assessing the within-participant absolute variability (CV_w) and between-participant relative variability (ICC) of the MFCV estimates.

The within-participant CV_w for absolute MFCV initial value and rate of change between the two test sessions are shown, for each participant, in **Table 2**. In particular, an average CV_w of 2.6 ± 2.0 % (acceptable) was observed for MFCV initial value and an average CV_w of 11.9 ± 3.2 % (acceptable) was reported for MFCV rate of change as a function of force. On average, when considering the same

participants tested in different sessions four weeks apart, the variability of MFCV slopes was larger than that of MFCV intercepts. Similar results were obtained for normalized MFCV (% $MFCV_{MAX}$) and specifically a CV_w of 2.5 ± 2.1 % (acceptable) and 12.9 ± 5.3 % (acceptable) were observed for normalized MFCV intercept and rate of change, respectively.

The intraclass correlation coefficients of absolute MFCV initial value and rate of change were 0.96 [95% CI: 0.86 to 0.99] and 0.94 [95% CI: 0.76 to 0.98], respectively, showing a "very high" between participants repeatability. Similarly, the ICC of normalized MFCV initial value and rate of change were 0.96 [95% CI: 0.85 to 0.99] and 0.93 [95% CI: 0.70 to 0.98], respectively.

Figure 3 goes here

DISCUSSION

In this study we investigated the absolute and relative reproducibility of MFCV estimates related to voluntary force. The time-course of MFCV during linearly increasing isometric voluntary contractions was compared 4 weeks apart with regression analysis. The regression coefficients extracted from the association between MFCV and voluntary force were compared between the two test sessions in both absolute and normalized values. MFCV was linearly associated with voluntary force at least up to 60-70 % of MViF in the majority of participants and test conditions. MFCV intercept and slope estimates showed an "acceptable" CV_w (< 12%), and a "very high" ICC (> 0.9), in both absolute and normalized terms over the 4 weeks. These findings suggest that in linearly increasing voluntary contractions, MFCV estimates have an overall high level of absolute and relative reproducibility and could be adopted to indirectly infer changes in MFCV as they might occur with training or neuromuscular pathologies in a fully non-invasive way.

Reproducibility of MFCV vs force relation

The issue of reproducibility of MFCV estimates has been addressed by several previous studies, where a wide spectrum of experimental conditions (e.g. different contraction type and muscle

analysed, detection, processing and estimation method adopted) and test-retest periods were adopted (for review, Beretta-Piccoli *et al.*, 2019). In general, the reproducibility of MFCV estimation from non-invasive sEMG is known to be affected by at least three factors: 1) intrinsic properties of the acquisition system (e.g. type of electrodes, IED, estimation method adopted), 2) electrode repositioning in test-retest sessions and 3) intrinsic variability in the subject's performance and modality of muscle control (Farina et al., 2004d; Farina and Merletti, 2004a). In addition to these factors, MFCV estimation, and hence its reproducibility, can be also influenced by other physiological phenomena such as the electrophysiological properties of the sarcolemma, muscle acidosis and temperature, local muscle fatigue and motor unit discharge rate (Beretta-Piccoli et al., 2019; Farina et al., 2004d).

The majority of previous investigations assessed the intersession reproducibility of MFCV estimates during sustained or fatiguing contractions (Beretta-Piccoli et al., 2017; Falla et al., 2002; Farina and Merletti, 2004b; Linssen et al., 1993; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi et al., 2001, 1999) or during dynamic contractions (Macdonald et al., 2008) in lower and upper limb muscles, whereas only one previous investigation (Martinez-Valdes et al., 2017) assessed the intersession reproducibility of motor unit conduction velocity (i.e. average propagation velocity of action potentials along the muscle fibres innervated by individual motor neurons) during linearly increasing isometric knee extensions (e.g. ramp contractions). From our perspective, this contraction paradigm allows a more direct opportunity to study the time-course of MFCV during the gradual development phase of volitional force compared to a steady state or sustained contraction, thus providing a more direct window to investigate the progressive motor unit recruitment strategies.

In line with the interpretation of Beretta-Piccoli *et al.*, (2019), it has to be noted that a higher reproducibility of MFCV estimates ($0.7<ICC<1.0$) can be generally observed in the more recent studies, where the adoption of bi-dimensional arrays, reduced IED and multichannel algorithms significantly improved the quality of MFCV estimation. In general, the parameters showing the most reproducible and reliable estimates (i.e. highest ICC and lowest coefficient of variation) are the

average MFCV and initial MFCV (intercept), compared to the rate of change of MFCV (slope) related to voluntary force, whose lower degree of reproducibility depends on its higher sensitivity to electrode repositioning in test-retest sessions (Farina et al., 2004d; Farina and Merletti, 2004b). Indeed, Farina *et al.*, (2004) showed that by increasing the number of recording electrodes (from 2 to 7) and the interelectrode distance (IED) (from 2 to 10 mm), higher ICC values for MFCV estimates can be obtained probably as a result of the reduced experimental noise due to a lower sensitivity to electrode repositioning. The majority of previous investigations that addressed the issue of reproducibility of MFCV slope estimates during isometric contractions, adopted a four-electrodes acquisition system, whose associated larger sensitivity to electrode repositioning, could explain the rather consistent low-to-moderate ICC observed between separate test sessions (Falla et al., 2002; Ollivier et al., 2005; Rainoldi et al., 2001, 1999). Conversely, in the only study that reported high levels of reproducibility of MFCV slope estimates during fatiguing isometric contractions, bi-dimensional arrays with a 10 mm IED were adopted (Beretta-Piccoli et al., 2017).

To the best of our knowledge, a systematic reproducibility analysis of MFCV estimates has never been conducted during linearly increasing voluntary isometric ankle dorsiflexions. Indeed, because of the linear relation between MFCV and the diameter of muscle fibres (Blijham et al., 2006; Hakansson, 1956; Methenitis et al., 2016) and of the well-demonstrated association with motor unit recruitment threshold (Del Vecchio et al., 2018c; Del Vecchio et al., 2017), the estimation of MFCV during linearly increasing isometric force contractions could provide an unique indirect and non-invasive perspective on the progressive recruitment of motor units. Furthermore, a main advantage of MFCV estimation from the interference sEMG, is that it does not require full sEMG decomposition, which in turn may be limited to controlled laboratory conditions, such as slow-and low-force isometric contractions (Farina and Holobar, 2016). Conversely, MFCV can be estimated in a relatively simple way and assessed over a broader range of experimental conditions (Bazzucchi et al., 2015; Farina et al., 2007, 2004a; Pozzo et al., 2004).

Here, we adopted a multichannel maximum likelihood algorithm (Farina and Merletti, 2004b) to estimate MFCV from bi-dimensional HDsEMG recordings from the surface of TA muscle, whose anatomical characteristics (i.e. long fibres arranged parallel to the skin and easy-accessible IZ in the distal region) are optimal for MFCV estimation. In general, we observed a strong correlation between test-retest estimates of MFCV initial values (intercept, $R^2 = 0.87$) and MFCV rate of change (slope, $R^2 = 0.80$) related to voluntary force (**Figure 3 A-B**). Moreover, we observed an overall acceptable ($CV_w < 12\%$) absolute reliability for both MFCV initial value (intercept) and MFCV rate of change (slope) related to voluntary force and a "very high" degree of relative reproducibility (ICC > 0.8) for both parameters. In particular, in agreement with previous investigations, we observed a lower CV_w for MFCV initial value compared to MFCV rate of change related to force (CV_w: 2.6 ± 2.0 % vs. 11.9 ± 3.2 %). In accordance with these results, a higher ICC was observed for MFCV initial value compared to MFCV rate of change related to force (ICC: 0.96 vs. 0.94). These findings suggest a higher degree of both absolute and relative reproducibility for MFCV intercept estimates compared to MFCV rate of change, although both parameters showed an overall "very high" degree of intersession reproducibility.

Accordingly, MFCV initial value (i.e. indicator of behaviour and properties of the lower-threshold motor units) and rate of change related to force (i.e. indicator of progressive motor unit recruitment) are robust reproducible neurophysiological variables suitable for application in longitudinal studies (i.e. up to four weeks) to track changes in muscle properties and neural control strategies following training interventions or to monitor the progress of specific neuromuscular disorders. Interestingly, and in line with our findings, a recent study demonstrated the possibility to track reliably and accurately the same motor neurons over a period of one-month, highlighting the potential and feasible non-invasive monitoring of changes in neural control strategies over time (Del Vecchio and Farina, 2019).

It should be noted that in 3 out of 10 participants involved in the study, the time-course of MFCV exhibited a plateau at higher forces (on average, at 48% MViF). However, as shown in **Figure 2 C**

for one representative subject, this trend was consistent and reproducible between the two separate experimental sessions. This non-linear trend of MFCV against voluntary force at higher forces has been previously observed in the literature and could be the result of manifestation of local muscle fatigue (Merletti et al., 1990), the achievement of full motor unit recruitment (Sbriccoli et al., 2003), amplitude cancellation and/or the effect of volume conductor (Farina et al., 2004b; Holobar and Farina, 2014; Keenan et al., 2006), and the plateau of the limit of the size of the higher threshold motor units muscle fibre diameters (Del Vecchio et al., 2018c; Hakansson, 1956). In this regard, it is important to note that more studies are needed to confirm the associations between MFCV and motor unit recruitment and that MFCV only represents an indirect parameter of the recruitment order.

The protocol adopted here consisted of submaximal isometric trapezoidal contractions and it was not specifically designed to induce muscle fatigue, as opposed to fatiguing (to exhaustion) or sustained contraction tasks. Indeed, considering the primary aim of the study, MFCV was only estimated in the ascending phase of the contractions, ranging between a minimum of 7 s (at 35% MViF) and a maximum of 14 s (at 70% MViF). In such a short time frame, it very unlikely that a significant increase in muscle acidosis (Brody et al., 1991) as a result of local muscle fatigue or changes muscle temperature (Farina et al., 2005; Rutkove, 2001) occurred. However, considering the influence of the rate of force generation on motor unit recruitment and hence on MFCV (Sbriccoli et al., 2003), which in our case was relatively slow (~5 % MViF·s^{-1}), the fact that local muscle fatigue may have arisen in some subjects (i.e. S3, S5, S6) inducing a MFCV plateau or level off cannot be completely ruled out. Moreover, as reported by Sbriccoli et al., (2003), MFCV exhibited an earlier plateau (followed by a decay) during ramp contractions at slower rates of force generation (5% MViF·s^{-1}) compared to faster contractions (20 % MViF·s^{-1}), which was attributable to an earlier recruitment of high-threshold motor units and hence an earlier achievement of the full motor unit recruitment in biceps brachialis muscle. In agreement with this study, the MFCV plateau observed at higher forces, would imply that when the target force (% MViF) was reached, the faster late recruited MUs (i.e. higher threshold MUs) were already fatigued and additional force gains were mainly or likely accomplished by increasing the discharge rate of the active motor units. Nevertheless, for all participants very

similar and consistent MFCV estimates were observed over 4 weeks, suggesting that eventual manifestations of fatigue and individual fatigability status were both reproducible between the two experimental sessions at the individual subject level.

Another factor that may have influenced the non-linear relation between MFCV and force observed in some subjects at higher forces, could be the phenomenon of amplitude cancellation (Farina et al., 2004b). Indeed, as a result of the cancellation of positive and negative phases of MU action potential waveforms, the sEMG is known to underestimate the neural drive to the muscle. This implies that although the sEMG amplitude increases monotonically with the neural drive (output from the spinal cord) to the muscle, it may not reflect the effective underlying MU activity, which contributes to the voluntary force generation. Although many factors are known to potentially influence the relation between sEMG and force (e.g. volume conductor, location and anatomy of active muscle fibres) the specific mechanisms underpinning the manifestation of amplitude cancellation to a larger extent in some subjects are still poorly understood (Farina et al., 2004b).

Participants' training status could also affect MFCV estimates (Casolo et al., 2020; Del Vecchio et al., 2018b). However, the volunteers enrolled in this study were not involved in any regular physical training program and were asked to simply maintain their physical activity and nutritional habits over the 4 weeks. Accordingly, also the effect of changes in participants' training status on MFCV estimate and hence on its reproducibility over the 4 weeks could be neglected.

CONCLUSION

In the present study, we showed a high degree of absolute and relative intersession reproducibility of MFCV estimates during linearly increasing force contractions over four weeks. These results suggest that the relation between MFCV and voluntary force could be adopted to indirectly infer neural strategies of muscle control over time. Therefore, our findings may have important practical implications in the tracking of training-associated neuromuscular changes when a full sEMG signal decomposition is problematic or not possible.

References

Allen, D.G., Lamb, G.D., Westerblad, H., 2008. Skeletal muscle fatigue: cellular mechanisms. Physiol. Rev. 88, 287–332. https://doi.org/10.1152/physrev.00015.2007.

Andreassen, S., Arendt-Nielsen, L., 1987. Muscle fibre conduction velocity in motor units of the human anterior tibial muscle: a new size principle parameter. J. Physiol. 391, 561–71. https://doi.org/10.1113/jphysiol.1987.sp016756

Arendt-Nielsen, L., Zwarts, M., 1989. Measurement of muscle fiber conduction velocity in humans: techniques and applications. J.Clin.Neurophysiol. https://doi.org/10.1097/00004691-198904000-00004

Balshaw, T.G., Fry, A., Maden-Wilkinson, T.M., Kong, P.W., Folland, J.P., 2017. Reliability of quadriceps surface electromyography measurements is improved by two vs. single site recordings. Eur. J. Appl. Physiol. 117, 1085–1094. https://doi.org/10.1007/s00421-017-3595-z

Bazzucchi, I., De Vito, G., Felici, F., Dewhurst, S., Sgadari, A., Sacchetti, M., 2015. Effect of exercise training on neuromuscular function of elbow flexors and knee extensors of type 2 diabetic patients. J. Electromyogr. Kinesiol. 25, 815–823. https://doi.org/10.1016/j.jelekin.2015.06.008

Beretta-Piccoli, M., Cescon, C., Barbero, M., D'Antona, G., 2019. Reliability of surface electromyography in estimating muscle fiber conduction velocity: A systematic review. J. Electromyogr. Kinesiol. 48, 53–68. https://doi.org/10.1016/j.jelekin.2019.06.005

Beretta-Piccoli, M., D'Antona, G., Zampella, C., Barbero, M., Clijsen, R., Cescon, C., 2017. Test-retest reliability of muscle fiber conduction velocity and fractal dimension of surface EMG during isometric contractions. Physiol. Meas. 38, 616–630. https://doi.org/10.1088/1361-6579/aa614c

Blijham, P.J., ter Laak, H.J., Schelhaas, H.J., van Engelen, B.G.M., Stegeman, D.F., Zwarts, M.J., 2006. Relation between muscle fiber conduction velocity and fiber size in neuromuscular

disorders. J. Appl. Physiol. 100, 1837–1841. https://doi.org/10.1152/japplphysiol.01009.2005

Brody, L.R., Pollock, M.T., Roy, S.H., De Luca, C.J., Celli, B., 1991. pH-induced effects on median frequency and conduction velocity of the myoelectric signal. J. Appl. Physiol. 71, 1878–1885. https://doi.org/10.1152/jappl.1991.71.5.1878

Casolo, A., Farina, D., Falla, D., Bazzucchi, I., Felici, F., Del Vecchio, A., 2020. Strength Training Increases Conduction Velocity of High-Threshold Motor Units. Med. Sci. Sport. Exerc. 52, 955–967. https://doi.org/10.1249/MSS.0000000000002196

Christiansen, D., 2019. Molecular stressors underlying exercise training-induced improvements in K + regulation during exercise and Na + ,K + -ATPase adaptation in human skeletal muscle. Acta Physiol. 225, e13196. https://doi.org/10.1111/apha.13196

Del Vecchio, Alessandro, Bazzucchi, I., Felici, F., 2018a. Variability of estimates of muscle fiber conduction velocity and surface EMG amplitude across subjects and processing intervals. J. Electromyogr. Kinesiol. 40, 102–109. https://doi.org/10.1016/j.jelekin.2018.04.010

Del Vecchio, A., Casolo, A., Negro, F., Scorcelletti, M., Bazzucchi, I., Enoka, R., Felici, F., Farina, D., 2019. The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. J. Physiol. 597, 1873–1887. https://doi.org/10.1113/JP277250

Del Vecchio, A., Farina, D., 2019. Interfacing the neural output of the spinal cord: robust and reliable longitudinal identification of motor neurons in humans. J. Neural Eng. 17, 016003. https://doi.org/10.1088/1741-2552/ab4d05

Del Vecchio, Alessandro, Negro, F., Falla, D., Bazzucchi, I., Farina, D., Felici, F., 2018b. Higher muscle fiber conduction velocity and early rate of torque development in chronically strength-trained individuals. J. Appl. Physiol. 125, 1218–1226. https://doi.org/10.1152/japplphysiol.00025.2018

Del Vecchio, A., Negro, F., Felici, F., Farina, D., 2018. Distribution of muscle fibre conduction

velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. Acta Physiol. (Oxf). 222, e12930. https://doi.org/10.1111/apha.12930

Del Vecchio, Alessandro, Negro, F., Felici, F., Farina, D., 2018c. Distribution of muscle fibre conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. Acta Physiol. 222, e12930. https://doi.org/10.1111/apha.12930

Del Vecchio, A., Negro, F., Felici, F., Farina, D., 2017. Associations between motor unit action potential parameters and surface EMG features. J. Appl. Physiol. 123, 835–843. https://doi.org/10.1152/japplphysiol.00482.2017

Falla, D., Dall'Alba, P., Rainoldi, A., Merletti, R., Jull, G., 2002. Repeatability of surface EMG variables in the sternocleidomastoid and anterior scalene muscles. Eur. J. Appl. Physiol. 87, 542–549. https://doi.org/10.1007/s00421-002-0661-x

Farina, D., Arendt-Nielsen, L., Graven-Nielsen, T., 2005. Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. J. Appl. Physiol. 99, 197–203. https://doi.org/10.1152/japplphysiol.00059.2005

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

Farina, D., Cescon, C., Merletti, R., 2002b. Influence of anatomical, physical, and detection-system parameters on surface EMG. Biol. Cybern. 86, 445–456. https://doi.org/10.1007/s00422-002-0309-2

Farina, D., Falla, D., 2008. Effect of muscle-fiber velocity recovery function on motor unit action potential properties in voluntary contractions. Muscle Nerve 37, 650–658. https://doi.org/10.1002/mus.20948

Farina, D., Ferguson, R.A., Macaluso, A., De Vito, G., 2007. Correlation of average muscle fiber conduction velocity measured during cycling exercise with myosin heavy chain composition,

lactate threshold, and VO2max. J. Electromyogr. Kinesiol. 17, 393–400. https://doi.org/10.1016/j.jelekin.2006.03.003

Farina, D., Holobar, A., 2016. Characterization of Human Motor Units From Surface EMG Decomposition. Proc. IEEE 104, 353–373. https://doi.org/10.1109/JPROC.2015.2498665

Farina, D., Macaluso, A., Ferguson, R.A., De Vito, G., 2004a. Effect of power, pedal rate, and force on average muscle fiber conduction velocity during cycling. J. Appl. Physiol. 97, 2035–2041. https://doi.org/10.1152/japplphysiol.00606.2004

Farina, D., Merletti, R., 2004a. Estimation of average muscle fiber conduction velocity from two-dimensional surface EMG recordings. J. Neurosci. Methods 134, 199–208. https://doi.org/10.1016/j.jneumeth.2003.12.002

Farina, D., Merletti, R., 2004b. Methods for estimating muscle fibre conduction velocity from surface electromyographic signals. Med. Biol. Eng. Comput. 42, 432–445. https://doi.org/10.1007/BF02350984

Farina, D., Merletti, R., Enoka, R.M., 2004b. The extraction of neural strategies from the surface EMG. J. Appl. Physiol. 96, 1486–1495. https://doi.org/10.1152/japplphysiol.01070.2003

Farina, D., Muhammad, W., Fortunato, E., Meste, O., Merletti, R., Rix, H., 2001. Estimation of single motor unit conduction velocity from surface electromyogram signals detected with linear electrode arrays. Med. Biol. Eng. Comput. 39, 225–36. https://doi.org/10.1007/BF02344807

Farina, D., Pozzo, M., Merlo, E., Bottin, A., Merletti, R., 2004c. Assessment of Average Muscle Fiber Conduction Velocity From Surface EMG Signals During Fatiguing Dynamic Contractions. IEEE Trans. Biomed. Eng. 51, 1383–1393. https://doi.org/10.1109/TBME.2004.827556

Farina, D., Zagari, D., Gazzoni, M., Merletti, R., 2004d. Reproducibility of muscle-fiber conduction velocity estimates using multichannel surface EMG techniques. Muscle Nerve 29,

282–291. https://doi.org/10.1002/mus.10547

Hakansson, C.H., 1956. Conduction Velocity and Amplitude of the Action Potential as Related to Circumference in the Isolated Fibre of Frog Muscle. Acta Physiol. Scand. 37, 14–34. https://doi.org/10.1111/j.1748-1716.1956.tb01338.x

Heckman, C.J., Enoka, R.M., 2004. Physiology of the motor neuron and the motor unit, in: Handbook of Clinical Neurophysiology. Elsevier B.V., pp. 119–147. https://doi.org/10.1016/S1567-4231(04)04006-7

Henneman, E., 1957. Relation between Size of Neurons and their susceptibility to discharge. Science (80-.). 126, 1345–1347.

Hinkle, D.E., Wiersma, W., Jurs, S.G., 2002. Applied Statistics for the Behavioral Sciences, Fifth Edit. ed, Houghton Mifflin. Houghton Mifflin, Boston.

Holobar, A., Farina, D., 2014. Blind source identification from the multichannel surface electromyogram. Physiol. Meas. 35, R143–R165. https://doi.org/10.1088/0967-3334/35/7/R143

Keenan, K.G., Farina, D., Merletti, R., Enoka, R.M., 2006. Amplitude cancellation reduces the size of motor unit potentials averaged from the surface EMG. J. Appl. Physiol. 100, 1928–1937. https://doi.org/10.1152/japplphysiol.01282.2005

Linssen, W.H.J.P., Stegeman, D.F., Joosten, E.M.G., Notermans, S.L.H., van't Hof, M.A., Binkhorst, R.A., 1993. Variability and interrelationships of surface EMG parameters during local muscle fatigue. Muscle Nerve 16, 849–856. https://doi.org/10.1002/mus.880160808

Macdonald, J.H., Farina, D., Marcora, S.M., 2008. Response of Electromyographic Variables during Incremental and Fatiguing Cycling. Med. Sci. Sport. Exerc. 40, 335–344. https://doi.org/10.1249/mss.0b013e31815b491e

Martinez-Valdes, E., Farina, D., Negro, F., Del Vecchio, A., Falla, D., 2018. Early Motor Unit Conduction Velocity Changes To Hiit Versus Continous Training, Medicine & Science in

Sports & Exercise. https://doi.org/10.1249/MSS.0000000000001705

Martinez-Valdes, E., Negro, F., Laine, C.M., Falla, D., Mayer, F., Farina, D., 2017. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. J. Physiol. 595, 1479–1496. https://doi.org/10.1113/JP273662

McIntosh, K.C.D., Gabriel, D.A., 2012. Reliability of a simple method for determining muscle fiber conduction velocity. Muscle Nerve 45, 257–265. https://doi.org/10.1002/mus.22268

Merletti, R., Knaflitz, M., De Luca, C.J., 1990. Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions. J. Appl. Physiol. 69, 1810–1820. https://doi.org/10.1152/jappl.1990.69.5.1810

Methenitis, S., Karandreas, N., Spengos, K., Zaras, N., Stasinaki, A.N., Terzis, G., 2016. Muscle Fiber Conduction Velocity, Muscle Fiber Composition, and Power Performance. Med. Sci. Sports Exerc. 48, 1761–1771. https://doi.org/10.1249/MSS.0000000000000954

Nandedkar, S.D., Stalberg, E. V, Sanders, D.B., 1985. Simulation Techniques in Electromyography. IEEE Trans. Biomed. Eng. BME-32, 775–785. https://doi.org/10.1109/TBME.1985.325493

Ollivier, K., Portero, P., Maïsetti, O., Hogrel, J.-Y., 2005. Repeatability of surface EMG parameters at various isometric contraction levels and during fatigue using bipolar and Laplacian electrode configurations. J. Electromyogr. Kinesiol. 15, 466–473. https://doi.org/10.1016/j.jelekin.2005.01.004

Pozzo, M., Merlo, E., Farina, D., Antonutto, G., Merletti, R., Di Prampero, P.E., 2004. Muscle-fiber conduction velocity estimated from surface EMG signals during explosive dynamic contractions. Muscle and Nerve 29, 823–833. https://doi.org/10.1002/mus.20049

Racinais, S., Blonc, S., Jonville, S., Hue, O., 2005. Time of day influences the environmental effects on muscle force and contractility. Med. Sci. Sports Exerc. 37, 256–261. https://doi.org/10.1249/01.MSS.0000149885.82163.9F

Rainoldi, A., Bullock-Saxton, J.., Cavarretta, F., Hogan, N., 2001. Repeatability of maximal voluntary force and of surface EMG variables during voluntary isometric contraction of quadriceps muscles in healthy subjects. J. Electromyogr. Kinesiol. 11, 425–438. https://doi.org/10.1016/S1050-6411(01)00022-0

Rainoldi, A., Galardi, G., Maderna, L., Comi, G., Lo Conte, L., Merletti, R., 1999. Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle. J. Electromyogr. Kinesiol. 9, 105–119. https://doi.org/10.1016/S1050-6411(98)00042-X

Rutkove, S.B., 2001. Effects of temperature on neuromuscular electrophysiology. Muscle Nerve 24, 867–882. https://doi.org/10.1002/mus.1084

Sbriccoli, P., Bazzucchi, I., Rosponi, A., Bernardi, M., De Vito, G., Felici, F., 2003. Amplitude and spectral characteristics of biceps Brachii sEMG depend upon speed of isometric force generation. J. Electromyogr. Kinesiol. 13, 139–147. https://doi.org/10.1016/S1050-6411(02)00098-6

Sbriccoli, P., Sacchetti, M., Felici, F., Gizzi, L., Lenti, M., Scotto, A., De Vito, G., 2009. Non-invasive assessment of muscle fiber conduction velocity during an incremental maximal cycling test. J. Electromyogr. Kinesiol. 19, 380–386. https://doi.org/10.1016/j.jelekin.2009.03.008

Vila-Chã, C., Falla, D., Farina, D., 2010. Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training. J. Appl. Physiol. 109, 1455–1466. https://doi.org/10.1152/japplphysiol.01213.2009

Table 1. Participant-specific minimum ($MFCV_{MIN}$), maximal ($MFCV_{MAX70}$) and average ($MFCV_{MEAN}$) values of MFCV ($m \cdot s^{-1}$) estimated from the HDsEMG signals. MFCV estimates were derived from linearly increasing ankle dorsi flexions up to 70% of MViF at a rate of 5% $MViF \cdot s^{-1}$. Data are presented for both test sessions as mean ± SD.

	SESSION 1			**SESSION 2**		
Participants	$MFCV_{MIN}$	$MFCV_{MAX70}$	$MFCV_{MEAN}$	$MFCV_{MIN}$	$MFCV_{MAX70}$	$MFCV_{MEAN}$
S1	3.45	4.79	4.08	3.61	4.89	4.17
S2	2.95	3.93	3.42	3.01	3.98	3.49
S3	2.89	3.85	3.51	2.99	3.88	3.50
S4	3.36	3.94	3.62	3.22	3.98	3.63
S5	3.44	4.20	3.80	3.51	4.09	3.82
S6	3.48	4.38	4.08	3.53	4.24	3.99
S7	3.10	4.45	3.59	3.19	4.39	3.63
S8	3.56	4.31	4.02	3.86	4.59	4.28
S9	3.76	5.00	4.44	3.45	4.44	4.12
S10	3.06	3.74	3.47	2.80	3.53	3.22
Average	3.31	4.26	3.80	3.32	4.20	3.78
SD	0.29	0.41	0.34	0.33	0.39	0.35

Table 2. Participant-specific coefficient of determination (R^2), intercept ($m \cdot s^{-1}$) and slope ($m \cdot s^{-1} \cdot \%$ MViF) of the regressions between MFCV and voluntary force. Within-participant coefficient of variation (CV_w) for MFCV initial value (intercept) and MFCV rate of change (slope) of the regressions during voluntary ankle dorsi flexions is also reported. * $P < 0.001$

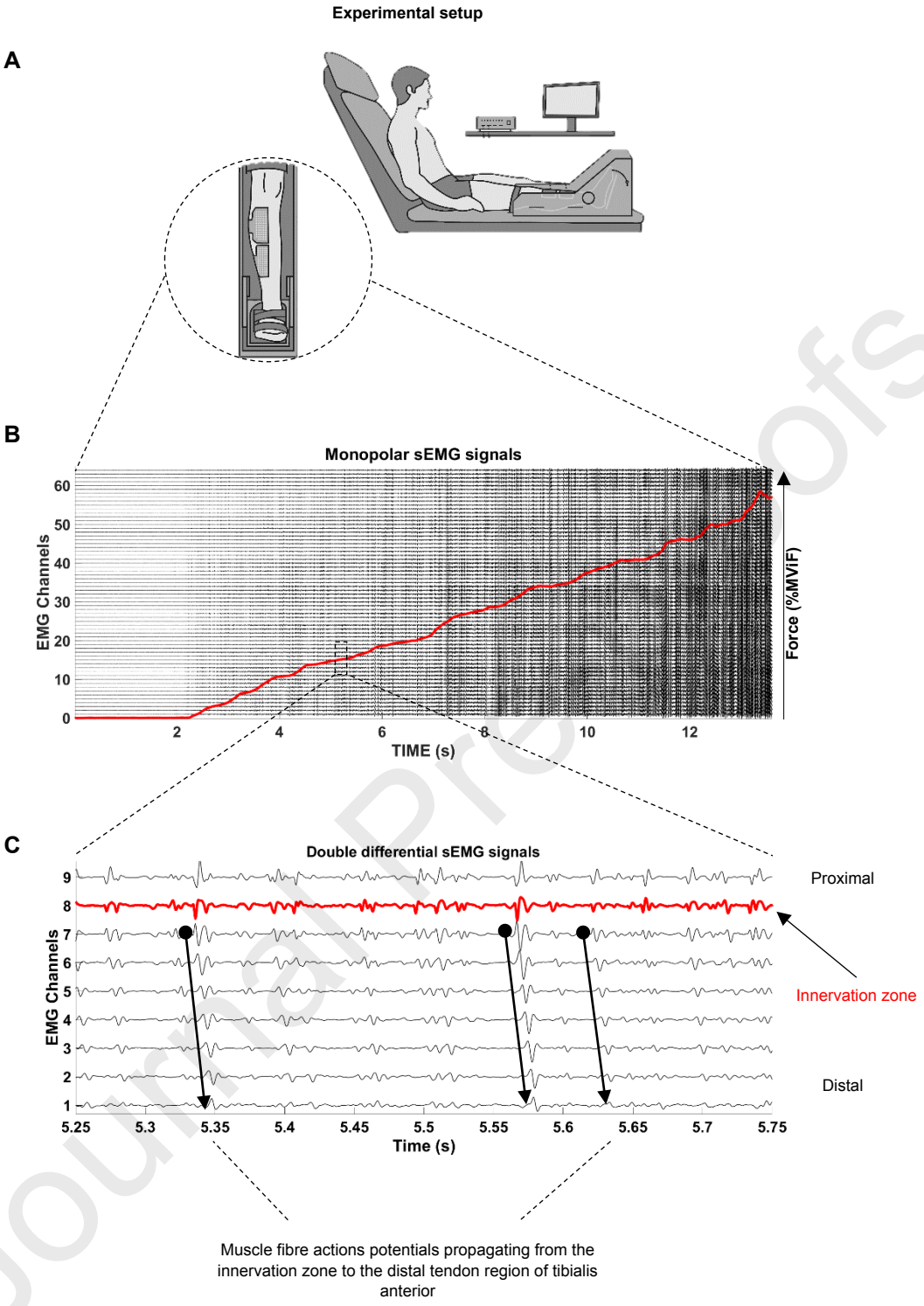
	MFCV - FORCE							
	SESSION 1			**SESSION 2**			**CV_W**	
Participants	R^2	Intercept	Slope	R^2	Intercept	Slope	Intercept	Slope
S1	0.93*	3.49	0.019	0.87*	3.62	0.018	2.53	4.68
S2	0.81*	3.13	0.011	0.87*	3.09	0.014	0.88	14.34
S3	0.66*	3.24	0.010	0.63*	3.27	0.009	0.76	13.07
S4	0.90*	3.16	0.010	0.95*	3.04	0.013	2.75	14.51
S5	0.51*	3.60	0.008	0.55*	3.64	0.006	0.74	11.57
S6	0.69*	3.79	0.012	0.64*	3.74	0.010	0.92	16.10
S7	0.80*	2.82	0.019	0.81*	2.74	0.023	1.96	12.06
S8	0.77*	3.78	0.008	0.77*	4.00	0.010	3.97	12.42
S9	0.73*	4.10	0.012	0.63*	3.86	0.010	4.26	11.64
S10	0.73*	3.23	0.009	0.84*	2.92	0.010	7.00	8.88

FIGURE CAPTIONS

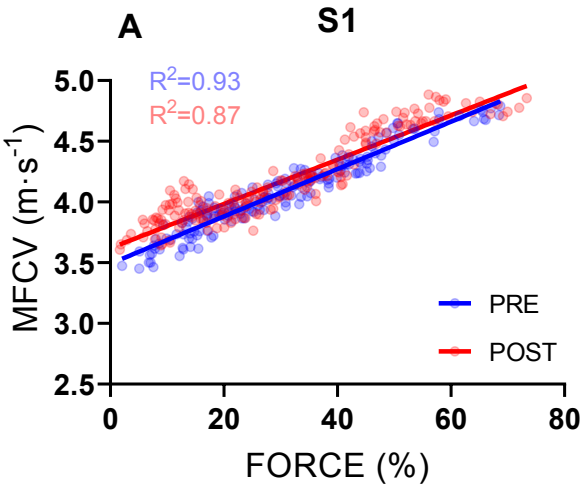
Figure 1. Experimental setup overview (**A**) and muscle fibre conduction velocity estimation (**B, C**). Participants were seated on a massage table with their dominant foot firmly strapped to a custom-made ankle ergometer. Two high-density grids of electrodes (64 electrodes each) were placed over the tibialis anterior muscle (**A**). Example of sixty four sEMG signals recorded in monopolar configuration during a linearly increasing (ramp) contraction at approximately 70% MViF. An increase in tibialis anterior myoelectrical activity can be clearly observed as the voluntary force generated is progressively increased (red line) (**B**). Nine double differential sEMG signals from one column of the distal high-density grid are shown in a 250 ms EMG processing window. The EMG channel overlying the identified innervation zone is indicated in red. Muscle fibre action potentials propagating from the innervation zone to the distal tendon region of tibialis anterior can be clearly observed (black arrow) (**C**).

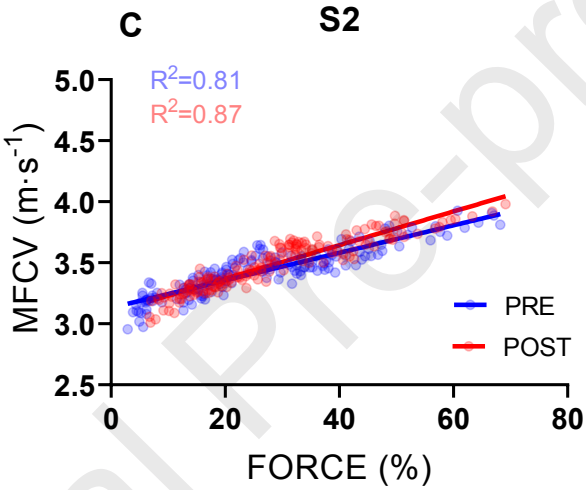
Figure 2. Regression lines for muscle fibre conduction velocity (MFCV, $m \cdot s^{-1}$) as a function of voluntary force (% MViF) from three representative individuals (**A-B-C**). The regression lines from test session 1 are indicated with a blue line, whereas regression lines from test session two are indicated with a red line. The coefficient of determination (R^2) for both test session 1 (in blue) and test session 2 (in red), are reported in the upper left corner of each graph.

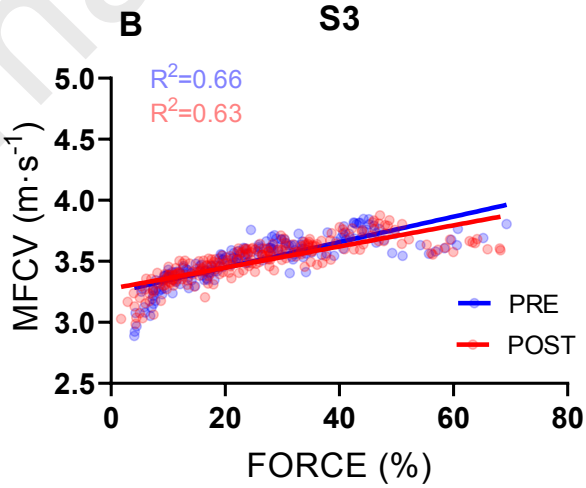
Figure 3. Scatter plots of the MFCV initial value and MFCV rate of change related to voluntary force for the same individuals between the two test sessions (A-B). Each individual is represented by an orange filled dot (n = 10). The MFCV initial value and MFCV rate of change related to voluntary force are shown for test session 1 (on the ordinate) and for test session 2 (on the abscissa). The coefficient of determination (R2) for each regression is reported in the upper left corner of each graph.

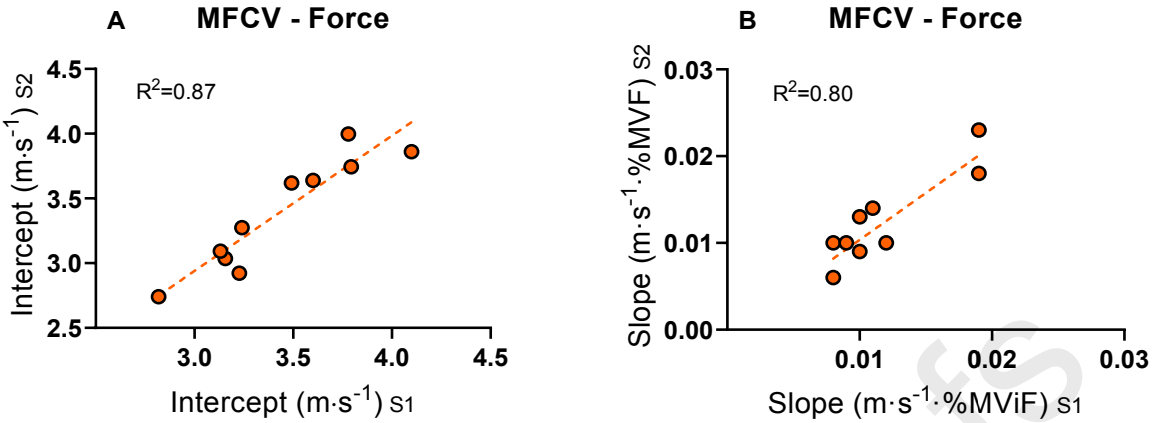


Muscle fibre actions potentials propagating from the innervation zone to the distal tendon region of tibialis anterior









Andrea Casolo is currently working as a Research Associate at the Department of Bioengineering, Imperial College London (United Kingdom). He received a BSc in Sports and Physical Education from the Catholic University of Milan (Italy) in 2014, a MSc in Health and Physical Activity from the University of Rome "Foro Italico" (Italy) in 2016 and a PhD (Doctor Europaeus) in Human Movement and Sport Sciences from the same institution in 2020. During his doctoral studies he spent eighteen months as a visiting researcher at the Imperial College London, where he gained experience with non-invasive techniques to record motor unit activity in vivo, in both healthy and pathological populations (HDsEMG). His main research interests are the study of motor control and of the neuromuscular adaptations to different training stimuli.

Stefano Nuccio received a MSc degree in Health and Physical Activity (2016) and a PhD (Doctor Europaeus) in Human Movement and Sport Science (2020) from the University of Rome "Foro Italico". During his PhD he spent six months as visiting researcher at the Department of Bioengineering of the Imperial College London, (United Kingdom). He is currently a Post-Doctoral Research Fellow at the University of Rome "Foro Italico". His main research interests are the neuromechanical adaptions of the knee joint to anterior cruciate ligament reconstruction in athletes.

Ilenia Bazzucchi received her PhD in Sport Science and Health and she is currently an Assistant Professor at the University of Rome “Foro Italico”, Italy. She has been part of the Organizing Committee of the XX ISEK Congress of and she is member of the Editorial Board of the Journal of Electromyography and Kinesiology. Her research work mainly concerns the study of neuromuscular control by means of surface electromyography, non-invasive assessment of muscle damage, neuromuscular effects of exercise and nutritional interventions in healthy individuals, athletes and patients.

Francesco Felici, MD and Specialist in Sport Medicine, is Full Professor of Human Physiology and Exercise Physiology at the University of Rome “Foro Italico” Italy. He has chaired the XX Congress of ISEK and is a member of the Italian Physiological Society of the Council of the International Society of Electrophysiology and Kinesiology, of the European College of Sport Sciences. He is a member of the Editorial Board of the Journal of Electromyography. He has authored more than 100 papers and scientific communications and his research activity focuses mostly in the human movement area – from energetic to control - in collaboration with national and international institutions. The main topics are: neuromuscular control, non-linear analysis of surface electromyograms, neuromuscular effects of exercise and sport, exercise physiology in healthy and pathological subjects.

Alessandro Del Vecchio received a PhD (Doctor Europaeus) in Human Movement Sciences from the University of Rome “Foro Italico”. During his PhD he spent one year at the Department of Neurorehabilitation Engineering, University of Medicine in Göttingen (Germany). Currently he is working as a Research Associate at the Department of Bioengineering, Imperial College in London. His main interests are the study of the neuromuscular function in health, training, and pathology.