TITLE PAGE

Title:

Distribution of muscle fiber conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle

Authors:

Alessandro Del Vecchio[1-3], Francesco Negro[2], Francesco Felici[1] and Dario Farina[3*]

Affiliations:

[1]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", 00135 Rome, Italy;

[2]Department of Clinical and Experimental Sciences, University of Brescia, 25123 Brescia, Italy;

[3]Department of Bioengineering, Imperial College London, London, UK

Short title

Motor unit conduction velocity distribution

***Corresponding author:**

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

Key words

Conduction velocity; Motor unit; Muscle fiber diameter; Recruitment; Size principle

Abstract

Aim: Motor units are recruited in an orderly manner according to the size of motor neurons. Moreover, because larger motor neurons innervate fibers with larger diameters than smaller motor neurons, motor units should be recruited orderly according to their conduction velocity (MUCV). Because of technical limitations, these relations have been previously tested either indirectly or in small motor unit samples that revealed weak associations between motor unit recruitment threshold (RT) and MUCV. Here we analyze the relation between MUCV and RT for large samples of motor units.

Methods: Ten healthy volunteers completed a series of isometric ankle dorsiflexions at forces up to 70% of the maximum. Multi-channel surface electromyographic signals recorded from the tibialis anterior muscle were decomposed into single motor unit action potentials, from which the corresponding motor unit RT, MUCV, and action potential amplitude were estimated. Established relations between muscle fiber diameter and CV were used to estimate the fiber size.

Results: Within individual subjects, the distributions of MUCV and fiber diameters were unimodal and did not show distinct populations. MUCV was strongly correlated with RT (mean (SD) R^2 = 0.7 (0.09), $p<0.001$; 406 motor units), which supported the hypothesis that fiber diameter is associated to RT.

Conclusion: The results provide further evidence for the relations between motor neuron and muscle fiber properties for large samples of motor units. The proposed methodology for motor unit analysis has also the potential to open new perspectives in the study of chronic and acute neuromuscular adaptations to ageing, training, and pathology.

INTRODUCTION

Motor neurons are recruited in an orderly manner according to the size of their soma[1]. Motor neurons with large cell bodies have a great number of dendrites, large axon diameter, and innervate large muscle units that produce great maximal tensions [2–4]. There is a quadratic relation between axon diameter and motor axon conduction velocity [2,5], so that larger axons have greater conduction velocity (CV) [2,5]. At the muscular level, the CV of action potentials (muscle fiber conduction velocity, MFCV) is linearly related to the diameter of muscle fibers (MFD) [6,7]. Moreover, MUCV is associated to MU recruitment threshold (RT) [8] and therefore muscle fibers innervated by larger motor axons have larger diameter than those innervated by smaller axons.

Previous studies analyzed the association between MUCV and RT for small numbers of motor units or during stimulation of single motor axons [8–10]. In these previous studies, motor units may have not been sampled in a representative way [8,9]. Moreover, because of the small MU samples, significant relations between MUCV and RT have been demonstrated only when pooling subjects together [8]. A systematic analysis of large populations of MUs and their distribution of MUCV is missing. Moreover, estimates of the properties of large samples of MUs would clarify whether MU properties in humans are clustered in discrete classes, as shown in animal and in-vitro research studies [3,11], or are continuously distributed, as recently discussed [4,12–15].

The access to CV measures for large populations of MUs would also provide estimates of average MFD because of the association between MFD and MFCV [6,16]. Currently, the study of muscle properties is often performed with muscle biopsies, which provide results dissociated from the neural control of the sampled muscle fibers. Furthermore, the same muscle fiber can co-express several myosin isoform compositions [4,17,18], thus inferring muscular and neural adaptations from muscle biopsies has limitations. For example, the decrease in muscle size with ageing has been correlated to specific fast-fiber atrophy (type II muscle fibers) [19,20]. Moreover, elderly individuals exhibit lower MU discharge rates [21]. However, the cause-effect relation in these age-related adaptations is unknown [19,20,22,23], mainly because of the lack of methods that allow direct access to motor neuron behavior and muscle unit properties concurrently, in large samples of MUs in vivo.

Here we report the measure of RT and MUCV (and indirectly MFD) in the full recruitment range of the tibialis anterior muscle and for a large sample of MUs, in a fully non-invasive way. The technique for this measurement is the combination of methods previously developed for MU identification. Recently, it became possible to study large samples of MUs in vivo in humans during voluntary contractions [24–26]. Moreover, the estimates of MUCV from multi-channel EMG signals have been advanced to reach estimation errors as low as 2-3% [27]. The aim of the study was to analyze the relation between MUCV and RT with these techniques to provide novel data on the associations between motor neuron and muscle fiber properties.

RESULTS

High-density EMG decomposition

Only reliable MU discharge patterns showing a regular discharge after recruitment were selected for the analysis. Across all subjects and contraction forces, the number of decomposed MUs was 406 (41 ± 17 per subject), with an average accuracy of 0.93 ± 0.021 (SIL). The average discharge rate was 15.4 ± 2.4 pulses per second (pps).

Conduction velocity

MUCV ranged from 2.78 to 6.21 m/s (4.32 ± 0.71 m/s) and was significantly correlated with RT in all subjects (mean R^2 = 0.70 ± 0.09, $p<0.001$, Fig 2). The CV of high threshold MUs was significantly greater compared to that of low threshold MUs. The average lower and upper limit of MUCV were respectively 3.89 ± 0.50 m/s (for MUs with threshold in the range 0-30% MVC) and 5.12 ± 0.45 m/s (range of thresholds 50-70 % MVC) ($p<0.001$). Fig 1. C-D shows a low-threshold and a high-threshold MU action potential. The action potential of the high threshold MU propagates with a greater velocity with respect to the low threshold unit.

Single MUCV estimates were converted in MFD by equation 1 (*see Methods*). The average estimated diameter was 67.46 µm, ranging from 36.60 to 105.32 µm. The group of lower-threshold units (0-30% MVC) had fibers with mean diameter of 58.97 ± 10.01 µm and that of higher threshold units (50-70% MVC) had mean diameter of 83.31 ± 9.10 µm. Fig.4 A shows the association between MFD and RT when pooling data from all subjects ($p<0.001$) and Fig. 4B reports the frequency of occurrence of muscle unit diameter

estimated values in the form of a histogram. It is relevant to note that, while this histogram does not show distinct clusters, there are limitations in discussing its specific shape with respect to fiber diameters and composition. The distribution estimated by this histogram is a pooled distribution from many subjects. Moreover, the values of fiber diameters are associated to an estimation error due to both the error in conduction velocity estimates and in the equation used to associate conduction velocity to diameter.

MUAP properties

MU action potential amplitudes ranged from 15.33 to 501.41 (μV) with an average of 120.22 ± 80.01 (μV). RMS_{MU} did not show a consistent relation with neither force nor RT of individual MUs, although RMS_{EMG} was associated to force in all subjects (mean RMS_{EMG} $R^2 = 0.88 \pm 0.040$, Table 1). Therefore, the surface action potential amplitudes and recruitment order (RMS_{MU}) were not the only determinant of the EMG amplitude-force relation. Only six subjects showed correlation between RMS_{MU} and RT, with weak strength ($R^2 = 0.29 \pm 0.23$, Table 1). Fig.3-A representatively shows that RMS_{EMG} was positively correlated with voluntary force in Subject 4, however when 77 MU action potentials were extracted from the surface EMG signal of this subject, the individual RMS_{MU} were poorly correlated with the respective RT (Fig. 3-B). Table 1 shows R^2 values for each subject between global and single MU amplitude estimates.

DISCUSSION

CV and amplitude of MU action potentials as well as of the interference EMG were studied in relation to MU RT or joint force for the full recruitment range of the tibialis anterior muscle. In all subjects, there was a strong relation between RT and MUCV (and therefore MFD [6,7,16]). The results indicate that 1) MUCV is strongly associated to RT; 2) MUCV, MFD (for individual MUs) and RT are continuously distributed in the tibialis anterior muscle without clustering in distinct groups; and 3) the size of surface MUAPs only moderately influences the relation between EMG amplitude and force.

MUCV in the full recruitment range

We have reported representative distributions of MUCV for individual subjects, rather than cumulative results over several subjects pooled together, as in previous studies. The observed average MUCV values were within the physiological range [8,28–30]. Similar values of MUCV were obtained following stimulation of

single motor axons in the tibialis anterior muscle [9]. In this previous study, MUCV was measured in a small sample of MUs (~11 MUs per subject, 3 subjects in total) and the range found was 2.6 to 5.3 m/s, with an average of 3.7 ± 0.7 m/s [9]. The lower limit of MUCV agrees with the results of the present study but there is a difference of ~1 m/s for the upper limit of MUCV [9]. This discrepancy is due to several differences among the studies. First, the number of subjects and identified MUs was substantially smaller in previous studies than in the present study [9]. Second, during the stimulation of motor axons previously employed, it is not certain that the detected MUs were uniformly sampled from the full motor pool. Third, the discharge of MUs influences MUCV with respect to values at rest [31,32].

There are only two other studies that have assessed MUCV during voluntary contractions for a range of RT [8,10] and they report different relations between RT and MUCV. Masuda and De Luca observed a linear relation between MUCV and RT in the tibialis anterior muscle during isometric ramp contractions [8]. However, the samples of MUs and subjects were very small (average of 6 MUs per subject, 3 subjects in total), and the strength of the associations was weak [8]. Indeed, the relations could be assessed only when grouping all subjects together and not at the individual subject level [8]. In the biceps brachii muscle, Hogrel reported an exponential relation between MUCV and RT in 3 subjects [10]. In addition to the limited subject and/or MU samples in these previous studies, it is important to note that MUCV estimates were less accurate than those obtained in the present study due to different algorithms applied [27,28,33].

The current estimates of MUCV are obtained with the most accurate MFCV estimation method available and indeed, when converted to MFD, the values for diameters are in precise accordance with those reported in muscle biopsies studies [11,34] (Fig. 4). However, muscle biopsies studies have also usually shown clusters of values for muscle fiber areas[11], while we could not detect clearly distinct classes of fibers when estimating their conduction velocity (Fig. 2) or their diameters (Fig. 4-A,B). The explanation for this disagreement is likely that biopsies cannot assess the whole spectrum of muscle fibers due to the classification based on enzyme staining [17,18] and cannot relate MFD to RT. Conversely, our results indicate that when MFD were estimated for the full recruitment range of the corresponding MUs, their distribution was unimodal, despite fiber typing. This continuous distribution of muscle fiber diameters is presumably a determinant factor for both the metabolic activity of the muscle and for force control.

It has to be noted that the estimates of MUCV, and therefore of MFD, represent averages over the values for the muscle fibers in the muscle unit. The distribution of MFCV for the muscle fibers comprising a muscle unit depends on the distribution of fiber diameters in the muscle unit, which is unknown. However, cross-innervation studies [35] and neurophysiological investigations [2,9,36] of motor unit properties support the assumption that muscle fibers in a MU have very similar diameters and properties. Therefore, the estimated MUCV well represent the conduction velocity of all the fibers of the muscle unit.

Significance of a continuous distribution of MUCV and muscle fiber diameters

Larger motor neurons begin to branch more proximally than smaller ones. Therefore, it has been hypothesized that larger motor neurons have a higher innervation number that produces higher maximal tensions [37]. Accordingly, it was later shown that there is a direct relation between size and function: smaller MUs are recruited first, due to the higher input resistance of the motor neurons, and are composed of smaller muscle units that produce lower maximal tensions [1,3,36].

This wide spectrum of relations led to the classification of MUs based on their physiological properties or on the myosin heavy chain isoforms composition [3,38]. However, there are no direct causal relations between MU behavior (i.e., RT) and muscle fiber characteristics (i.e., MFD). From human studies during voluntary contractions, MU mechanical properties do not cluster into distinct groups but are distributed continuously within a MU pool [4,12–14]. Similarly, muscle fibers have been shown to exhibit large co-expression of MHC isoforms [4,14,17,18,39]. Accordingly, in the present study, we show that MUCV, and indirectly MFD, are not clustered into distinct groups but increase linearly with force, thus indicating a continuum of motor unit properties and sizes. A continuous distribution of properties is presumably needed for a smooth generation of force in the full recruitment range and for energy control.

In most human muscles there is an inverse relation between MFD and enzyme oxidative capacity [34,40] due to the fact that a smaller diameter improves the surface-to-volume ratio for the perfusion of oxygen and exchange of metabolites [40]. However, from a force control perspective, the interpretation is challenging due to the limitations of in-vitro studies that cannot relate single muscle fiber characteristics to motor neuron properties. The 100-fold difference in MU tetanic forces is primarily related to the MU innervation number [41]. Indeed, when the specific force (force/cross sectional area) of the MU is taken into account, there are

no significant differences between muscle units (i.e., slow vs fast fatigable) [41]. However, the speed of release of calcium in the sarcoplasmic reticulum is related to the propagation speed of the action potential [42], which is determined by fiber diameter [43,44]. Indeed, CV is related to the time-to-peak of MU twitch forces [9,42]. Slow muscle fibers during slow movements shorten at a velocity that gives peak mechanical power and efficiency whilst the optimal shortening velocity for fast muscle fibers is when powering maximal movements [45]. Because of the slower force generation, small MUs tend to tetanize at significantly lower discharge rates [40]. The distribution of diameters according to recruitment may be an advantage for performing accurate, low-force motor tasks, since force variability at low forces is reduced when slower force twitches are elicited.

EMG-force relation

At the single muscle fiber level, the amplitude of the action potential increases as a function of fiber circumference [7]. However, when recorded from surface electrodes, the amplitude of action potentials is also influenced by the volume conductor. For this reason, the size of simulated surface MU action potentials has been shown to be poorly associated to RT [46,47]. Nonetheless, researchers have used surface action potential amplitudes to test the validity of the size principle [48,49] or to assess pathological adjustments in the neural drive to the muscle [50]. In the present study, although the surface EMG amplitude increased with force, the amplitudes of MU action potentials did not show a strong association with RT, with a large variability among subjects (Fig. 3. C-D). Therefore, the amplitude of surface action potentials provides only a crude information on recruitment strategies and their adaptations to pathology and exercise [49,50]. Moreover, the current results indicate that the orderly recruitment of MUs is not the main factor determining the EMG-force relation. The monotonous increase in EMG amplitude is not an evidence of orderly recruitment since it would be observed with any recruitment strategy because of rate coding.

In conclusion, we applied a non-invasive approach that allows the investigation of motor neuron behaviors concurrently with the properties of the innervated muscle units. We showed that MUCV and MFD are continuously distributed and strongly positively associated to RT in the tibialis anterior muscle. The proposed approach will open new perspectives in physiologic investigations that relate in-vivo motor neuron properties with the respective muscle unit characteristics (CV, MFD) in large representative samples of

MUs (covering the full muscle recruitment range). Since the approach is applied to motor unit action potentials, however, it is not possible to differentiate differences in fiber properties within individual muscle units. Finally, the results also indicate that the size of surface MUAPs influences the EMG amplitude to muscle force relation only moderately.

MATERIALS AND METHODS

Participants

Ten healthy, moderately active men (age 28.5 ± 1.8 yr; body mass 77.8 ± 6.7 kg; height: 180.2 ± 7.2 cm) volunteered and completed the experiment that was approved by the Ethical Committee of the Universitätmedizin Göttingen (n. 1/10/12). An informed written consent was signed by all the volunteers before participating in the experiments. None of the volunteers reported any previous history of neuromuscular disorders or lower limb pathology or surgery.

Experimental protocol

The volunteers performed three isometric maximal voluntary contractions (MVC). The greatest force was recorded and used as reference for the isometric submaximal contractions (Ramp contractions). Ramp contractions consisted of a trapezoidal paradigm with increasing and decreasing rate of 5% MVC/s^{-1} and sustained for 10 s at 15, 35, 50, and 70% MVC. The volunteers completed eight ramp contractions, two for each force level. The order of the ramps was randomized and a recovery time of 5 min between attempts was allowed.

Force and electromyogram recordings

Participants were seated in a Biodex System 3 chair in an upright position (Biodex Medical Systems Inc., Shirley, NY, USA), with the dominant leg extended and the ankle flexed at ~30° with respect to the neutral position. The ankle and the foot were tightly fastened by means of Velcro straps in a force transducer. High-density surface electromyography (HDsEMG) signals were recorded from the tibialis anterior muscle by using one grid of 64 electrodes (5 columns, 13 rows; gold-coated; 1-mm diameter; 8-mm interelectrode distance; OT Bioelettronica, Torino, Italy; Fig 1B). Before placing the high-density grid, an array of 16 electrodes was used to identify the distal innervation zone and a surgical pen marked its location. For proper

electrode placement, the array was moved over the muscle and oriented in order to observe the propagation of motor unit action potentials (MUAPs) from the innervation zone to the tendon region [28,51]. The signal quality was assessed by visual inspection by an experienced operator. After this procedure, the skin was prepared by shaving, light abrasion and cleansing with 70% ethanol and the electrode grid was placed over the muscle with conductive paste for establishing the skin-electrode contact (SpesMedica, Battipaglia, Italy). The EMG grid was located in the distal portion of the tibialis anterior in order to detect the MUAPs arising from the most distal innervation zone and propagating to the distal tendon (Fig 1. C) [28]. The columns of the grid were aligned longitudinally following the fiber direction. HDsEMG signals were recorded in monopolar derivation (3dB bandwidth 10-500 Hz; EMG-USB2+, multichannel amplifier, OT Bioelettronica, Torino, Italy) and converted to digital data on 12 bits and 2048 samples/s. The joint torque was recorded concurrently with the EMG with the same acquisition system.

High density EMG decomposition and conduction velocity estimation

HDsEMG signals were digitally band-pass filtered between 20-500 Hz (Butterworth). The signals were decomposed into series of MU discharges with a convolutive blind source separation method [24]. This algorithm has been previously validated and guarantees high accuracy in the identification of MU discharge times, even at high contraction levels [24,52]. The decomposition accuracy was estimated with the silhouette measure (SIL), with an acceptance threshold of 0.90 [24]. From EMG decomposition, the average discharge rate and the RT (force value in percentages of MVC corresponding to the first MU discharge) were computed for each MU. The decomposition algorithm directly identifies the discharge times of each motor unit but not the waveform of the corresponding multi-channel action potentials. The multichannel MUAPs were therefore estimated by averaging the surface EMG using the discharge times identified by decomposition as triggers, as shown for a representative example in Fig 1.C-D. This spike-triggered averaging was performed using only the first 50 discharge timings for each MU. The averaged interval (duration of the MUAPs) was 15 ms [28]. From the averaged monopolar MUAPs, double differential derivations were computed by differentiating in the longitudinal direction and were used for the estimation of MUCV [28], and MUAP amplitude (Root mean square, RMS_{MU}). For the estimation of these MUAP properties, we visually selected a minimum of 4 and a maximum of 6 double differential EMG derivations.

An example of two MUs with action potentials propagating along the electrode grid is shown in Fig. 1. In this example, the action potentials of individual motor units are similar in shape along the electrode grid and are delayed because of propagation along the muscle fibers. The small variations in shape of the action potentials along the grid are due to misalignment of the grid with respect to fiber direction and end-of-fiber components, as previously discussed [27]. The selection criteria for channels were the clearest propagation of the MUAP along the columns of the grid and a coefficient of correlation between channels >0.8 [28]. From the selected channels, MUCV estimation was performed using the multichannel maximum-likelihood algorithm that allows a calculation of CV in single MUs with an estimate standard deviation <0.1 ms^{-1} [27]. Single MUCV values were then used to estimate the average diameter of the fibers of the muscle units using the equation MFD (µm) = (MUCV m/s -0.95) / 0.05 (*equation 1*), as previously described [6,16]. MUCV and MFD were also computed separately for the groups of lower- and higher-threshold MUs, which were arbitrarily defined as the MUs with range of RT 0-30% MVC and 50-70% MVC, respectively.

Finally, we analyzed the interference surface EMG signal by computing the root mean square value (RMS_{EMG}) from the full duration of the recordings.

Statistics

Single MU properties (MUCV, RMS_{MU}) were studied as a function of RT. The global interference HDsEMG signal amplitude (RMS_{EMG}) was correlated with force (as % MVC). A Pearson product-moment correlation coefficient was computed to assess the association between MUCV or RMS_{MU}, and RT. The frequency distribution of fiber diameters was composed of 12 bins with a ~6µm width. Statistical analyses were performed using SPSS version 21 (SPSS Inc, Chicago, USA) and statistical significance was accepted for *P* values less than 0.05. Results are reported as mean and standard deviation (SD).

Acknowledgements

Alessandro Del Vecchio has received funding from the University of Rome “Foro Italico”. Francesco Negro has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 702491 (NeuralCon).

Conflict of interest

No conflict of interest.

References

1. Henneman E: Relation between size of neurons and their susceptibility to discharge. *Science*, 126: 1345–7, 1957.

2. Henneman E, Somjen G, Carpenter DO: Functional Significance of Cell Size in Spinal Motoneurons. *J Neurophysiol*, 28: 560–80, 1965.

3. Burke RE, Levine DN, Tsairis P, Zajac FE: Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J Physiol*, 234: 723–48, 1973.

4. Heckman CJ, Enoka RM: Motor Unit. *Compr Physiol*, 2: 2629–82, 2012.

5. Hursh JB: Conduction velocity and diameter of nerve fibers. *Am J Physiol*, 127: 131–9, 1939.

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

7. Håkansson CH: 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, 1956.

8. Masuda T, De Luca CJ: Recruitment threshold and muscle fiber conduction velocity of single motor units. *J Electromyogr Kinesiol*, 1: 116–23, 1991.

9. 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*, 391: 561–71, 1987.

10. Hogrel JY: Use of surface EMG for studying motor unit recruitment during isometric linear force ramp. *J Electromyogr Kinesiol*, 13: 417–23, 2003.

11. Helliwell TR, Coakley J, Smith PE, Edwards RH: The morphology and morphometry of the normal human tibialis anterior muscle. *Neuropathol Appl Neurobiol*, 13: 297–307, 1987.

12. 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*, 513: 295–305, 1998.

13. Bigland-Ritchie B, Fuglevand AJ, Thomas CK: Contractile properties of human motor units: Is man a cat? *Neuroscientist*, 4: 240–9, 1998.

14. Enoka RM, Duchateau J: Inappropriate interpretation of surface EMG signals and muscle fiber characteristics impedes progress on understanding the control of neuromuscular function. *J Appl Physiol*, 34: 1519, 2015.

15. Farina D, Merletti R, Enoka RM: The extraction of neural strategies from the surface EMG. *J Appl Physiol*, 96: 1486–95, 2004.

16. Nandedkar SD, Stålberg E: Simulation of single muscle fibre action potentials. *Med Biol Eng Comput*, 21: 158–65, 1983.

17. Caiozzo VJ, Baker MJ, Huang K, Chou H, Wu YZ, Baldwin KM: Single-fiber myosin heavy chain polymorphism: how many patterns and what proportions? *Am J Physiol Regul Integr Comp Physiol*, 285: R570-80, 2003.

18. Bottinelli R: Functional heterogeneity of mammalian single muscle fibres: Do myosin isoforms tell the whole story? *Pflugers Arch Eur J Physiol*, 443: 6–17, 2001.

19. Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M: Sarcopenia, dynapenia, and the impact of advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol*, 3: 1–18, 2012.

20. Evans WJ: Human Aging, Muscle Mass, and Fiber Type Composition. *Journals Gerontol Ser A Biol Sci Med Sci*, : 11–6, 1995.

21. Klass M, Baudry S, Duchateau J: Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. *J Appl Physiol*, 104: 739–46, 2008.

22. Bazzucchi I, De Vito G, Felici F, Dewhurst S, Sgadari A, Sacchetti M: Effect of exercise training on neuromuscular function of elbow flexors and knee extensors of type 2 diabetic patients. *J Electromyogr Kinesiol*, 25: 815–23, 2015.

23. Castelein B, Cools A, Bostyn E, Delemarre J, Lemahieu T, Cagnie B: Analysis of scapular muscle EMG activity in patients with idiopathic neck pain: A systematic review. *J Electromyogr Kinesiol*, 25: 371–86, 2015.

24. Negro F, Muceli S, Castronovo AM, Holobar A, Farina D: Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *J Neural Eng*, 13: 26027, 2016.

25. Farina D, Negro F, Muceli S, Enoka RM: Principles of motor unit physiology evolve with advances in technology. *Physiology*, 31: 83–94, 2016.

26. Negro F, Holobar A, Farina D: Fluctuations in isometric muscle force can be described by one linear projection of low-frequency components of motor unit discharge rates. *J Physiol*, 587: 5925–38, 2009.

27. 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*, 39: 225–36, 2001.

28. 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*, 115: 1–12, 2002.

29. Houtman CJ, Stegeman DF, Van Dijk JP, Zwarts MJ: Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. *J Appl Physiol*, 95: 1045–54, 2003.

30. Buchthal F, Guld C, Rosenfalck P: Innervation Zone and Propagation Velocity in Human Muscle. *Acta Physiol Scand*, 35: 174–90, 1955.

31. McGill KC, Lateva ZC: History dependence of human muscle-fiber conduction velocity during voluntary isometric contractions. *J Appl Physiol*, 111: 630–41, 2011.

32. Stålberg E: Propagation velocity in human muscle fibers in situ. *Acta Physiol Scand*, 287: 1–112, 1966.

33. Farina D, Zagari D, Gazzoni M, Merletti R: Reproducibility of muscle-fiber conduction velocity

estimates using multichannel surface EMG techniques. *Muscle and Nerve*, 29: 282–91, 2004.

34. Jaworowski Å, Porter MM, Holmbäck a. M, Downham D, Lexell J: Enzyme activities in the tibialis anterior muscle of young moderately active men and women: Relationship with body composition, muscle cross-sectional area and fibre type composition. *Acta Physiol Scand*, 176: 215–25, 2002.

35. Dubowitz V: Cross-innervated mammalian skeletal muscle: histochemical, physiological and biochemical observations. *J Physiol*, 193: 481–96, 1967.

36. Henneman E, Olson C: Relations between structure and function in the design of skeletal muscles. *J Neurophysiol*, 28: 581–98, 1965.

37. Eccles JC, Sherrington CS: Numbers and Contraction-Values of Individual Motor-Units Examined in some Muscles of the Limb. *Proc R Soc B Biol Sci*, 106: 326–57, 1930.

38. Bottinelli R, Canepari M, Pellegrino MA, Reggiani C: Force-velocity properties of human skeletal muscle fibres : myosin heavy chain isoform and temperature dependence. *J Physiol*, 495: 573–86, 1996.

39. Andersen JL, Aagaard P: Effects of strength training on muscle fiber types and size; consequences for athletes training for high-intensity sport. *Scand J Med Sci Sport*, 20: 32–8, 2010.

40. Burke RE: Motor units: anatomy, physiology, and functional organization. *Handb Physiol - Nerv Syst*, 543: 345–422, 2011.

41. Tötösy de Zepetnek JE, Zung H V, Erdebil S, Gordon T: Innervation ratio is an important determinant of force in normal and reinnervated rat tibialis anterior muscles. *J Neurophysiol*, 67: 1385–403, 1992.

42. 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*, 99: 197–203, 2005.

43. Plonsey R, Barr RC: Bioelectricity: A quantitative Approach. 1988.

44. Plonsey R, Barr RC: Bioelectricity: A quantitative Approach. 2007.

45. Rome LC, Funke RP, Alexander RM, Lutz G, Aldridge H, Scott F, Freadman M: Why animals have different muscle fibre types. *Nature*, 335: 824–7, 1988.

46. Keenan KG, Farina D, Merletti R, Enoka RM: Amplitude cancellation reduces the size of motor unit potentials averaged from the surface EMG. *J Appl Physiol*, 100: 1928–37, 2006.

47. Farina D, Cescon C, Negro F, Enoka RM: Amplitude Cancellation of Motor-Unit Action Potentials in the Surface Electromyogram Can Be Estimated With Spike-Triggered Averaging. *J Appl Physiol*, 100: 431–400, 2008.

48. Hu X, Rymer WZ, Suresh NL: Motor unit pool organization examined via spike-triggered averaging of the surface electromyogram. *J Neurophysiol*, 110: 1205–20, 2013.

49. Pope ZK, Hester GM, Benik FM, DeFreitas JM: Action potential amplitude as a noninvasive indicator of motor unit-specific hypertrophy. *J Neurophysiol*, 115: 2608–14, 2016.

50. Hu X, Suresh AK, Rymer WZ, Suresh NL: Assessing altered motor unit recruitment patterns in paretic muscles of stroke survivors using surface electromyography. *J Neural Eng*, 12: 66001, 2015.

51. Rainoldi A, Melchiorri G, Caruso I: A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J Neurosci Methods*, 134: 37–43, 2004.

52. Holobar A, Minetto M a, 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*, 11: 16008, 2014.

Figure captions

Fig. 1. A. Example of eight double differential surface EMG signals detected during an isometric contraction at 50% maximal voluntary force of the tibialis anterior muscle (interelectrode distance 8 mm). Clear propagation of several motor unit action potentials (MUAPs) along one column of the matrix can be observed. **B.** Grid of 64 electrodes used for the experiment. **C.** and **D.** Two MUAPs (low and high threshold), extracted by spike triggered averaging after EMG decomposition, showing propagation of single MUAPs along the grid. The innervation zone of the lower threshold motor unit is located at the top of the grid. The MUAPs propagate from the innervation zone (proximal) to the distal tendon region. * RT = Recruitment threshold (% of maximal voluntary force), CV = Conduction velocity.

Fig. 2. A. Motor unit conduction velocities (MUCV) from all the subjects versus the respective recruitment thresholds (% of maximal voluntary force). R^2 for the linear regression is reported as mean (SD) across subjects. Data are reported for all motor units (n= 406), with different symbols for each subject. Subject-specific values are reported in Table 1.

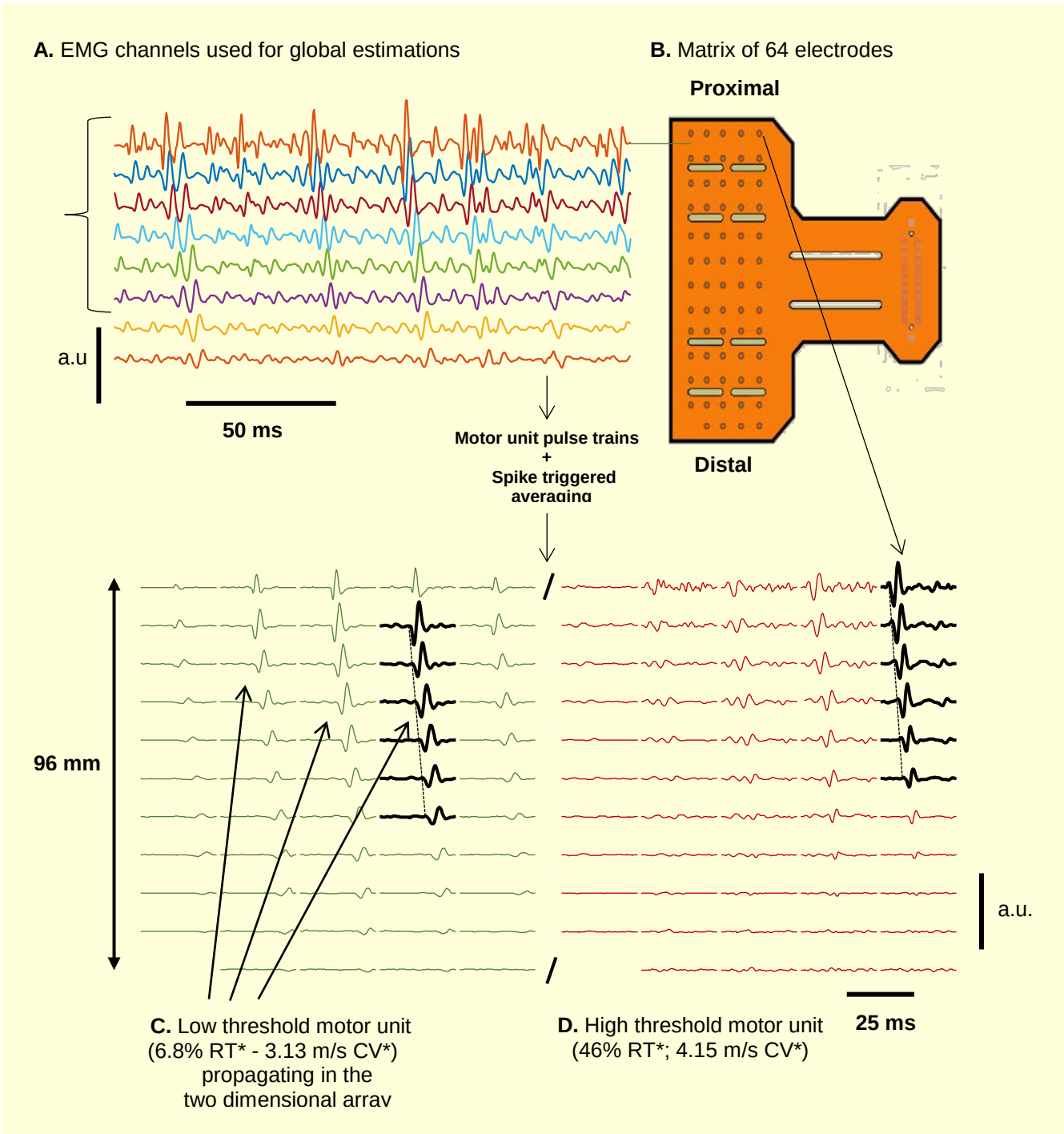
Fig. 3. A. Root mean square of the EMG (RMS_{EMG}) for each subject as a function of force in percentages of maximal voluntary force. **B.** Motor unit amplitudes (RMS_{MU}) versus the respective recruitment threshold (% of maximal voluntary force). Data from all the motor units (n= 406). The linear regression is reported as mean (SD) across subjects. Subject-specific values are reported in Table 1.

Fig. 4. A-B. Motor unit conduction velocities converted in muscle fiber diameters (MFD). Data from all the subjects (number motor units = 406) are shown. **A.** Relation between MFD in motor units (µm) and recruitment threshold (% of maximal voluntary force). Correlation coefficient (R^2) and regression line for the full sample are shown. * = $p < 0.001$ **B.** Histogram of MFD (µm) grouped in bins of ~6 µm along the *abscissa* and the number of motor units on the *ordinate*.

Table 1. Subject specific R^2 values

SUBJECT	RMS_{EMG}	RMS_{MU}	MUCV
S1	0.91‡	0.11	0.73‡
S2	0.94‡	0.18*	0.76‡
S3	0.83‡	0.45‡	0.50‡
S4	0.85‡	0.06	0.76‡
S5	0.90‡	0.66*	0.68‡
S6	0.89‡	0.007	0.60‡
S7	0.89‡	0.08	0.77‡
S8	0.91‡	0.40‡	0.85‡
S9	0.89‡	0.60‡	0.66‡
S10	0.82‡	0.41‡	0.75‡

Global EMG amplitude was correlated with force. Motor unit variables were correlated with recruitment threshold. ‡ = $p<0.001$; * = $p<0.05$


A. EMG channels used for global estimations
B. Matrix of 64 electrodes
Proximal
Distal
a.u
50 ms
Motor unit pulse trains
+
Spike triggered
averaging
96 mm
a.u.
25 ms
C. Low threshold motor unit
(6.8% RT* - 3.13 m/s CV*)
propagating in the
two dimensional arrav
D. High threshold motor unit
(46% RT*; 4.15 m/s CV*)

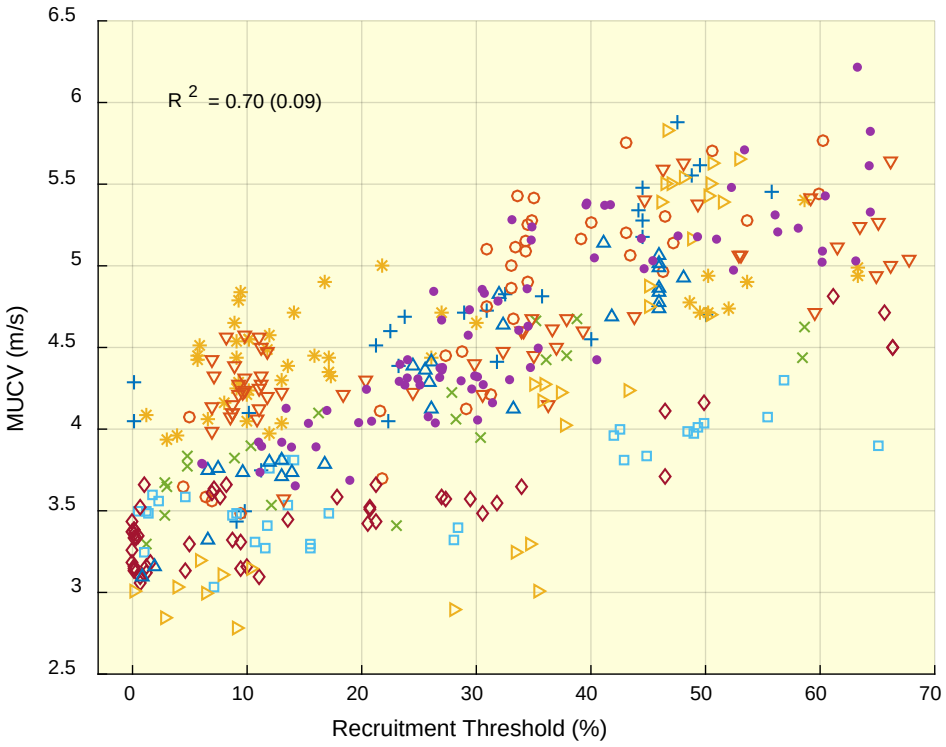

R 2 = 0.70 (0.09)
MUCV (m/s)
Recruitment Threshold (%)

Fig. 2.

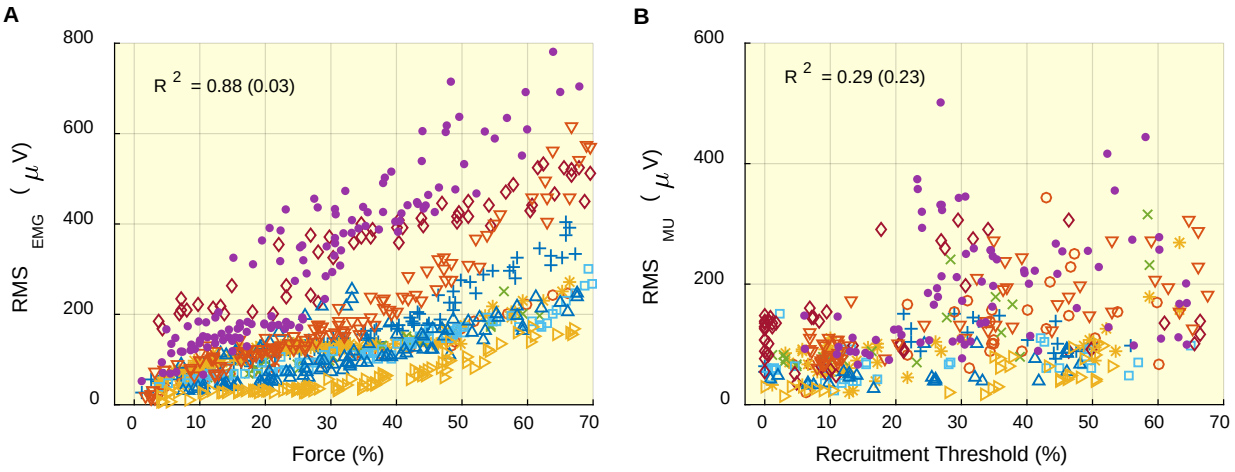

A
R 2 = 0.88 (0.03)
RMS EMG (μV)
Force (%)
B
R 2 = 0.29 (0.23)
RMS MU (μV)
Recruitment Threshold (%)

Fig. 3.

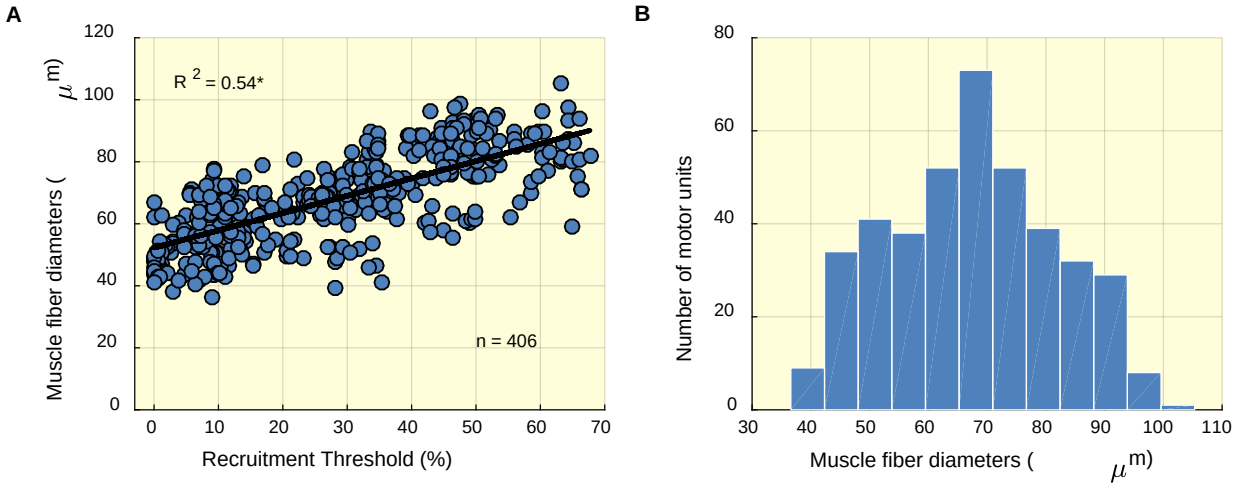

A
B
R 2 = 0.54*
n = 406
Muscle fiber diameters (μm)
Recruitment Threshold (%)
Number of motor units
Muscle fiber diameters (μm)

Fig. 4.