Surface EMG amplitude does not identify differences in neural drive to synergistic muscles

Martinez-Valdes, Eduardo; Negro, Francesco; Falla, Deborah; De Nunzio, Alessandro Marco; Farina, Dario

DOI:
10.1152/japplphysiol.01115.2017

Surface EMG amplitude does not identify differences in neural drive to synergistic muscles

Eduardo Martinez-Valdes[1, 2, 3], Francesco Negro[4], Deborah Falla[1], Alessandro De Nunzio [1], Dario Farina[5]

1- Centre of Precision Rehabilitation for Spinal Pain (CPR Spine), School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, UK
2- Department of Sports Medicine and Sports Orthopaedics, University of Potsdam, Potsdam, Germany
3- Centro de Investigación en Fisiología del Ejercicio (CIFE), Universidad Mayor, Santiago, Chile
4- Department of Clinical and Experimental Sciences, Universita' degli Studi di Brescia, Brescia, Italy
5- Department of Bioengineering, Imperial College London, Royal School of Mines, London, UK

Running Head:

Motor unit size and EMG of synergistic muscles

Corresponding author:

Dario Farina

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

Key words

Surface electromyography; Motor unit; amplitude; motor unit action potential; high-density surface EMG: synergistic muscles

ABSTRACT

Surface electromyographic (EMG) signal amplitude is typically used to compare the neural drive to muscles. We experimentally investigated this association by studying the motor unit (MU) behavior and action potentials in the vastus medialis (VM) and vastus lateralis (VL) muscles. Eighteen participants performed isometric knee extensions at four target torques [10, 30, 50 and 70% of the maximum torque (MVC)] while high-density EMG signals were recorded from the VM and VL. The absolute EMG amplitude was greater for VM than VL ($p<0.001$) while the EMG amplitude normalized with respect to MVC was greater for VL than VM ($p<0.04$). Because differences in EMG amplitude can be due to both differences in the neural drive and in the size of the MU action potentials, we indirectly inferred the neural drives received by the two muscles by estimating the synaptic inputs received by the corresponding motor neuron pools. For this purpose, we analyzed the increase in discharge rate from recruitment to target torque for motor units matched by recruitment threshold in the two muscles. This analysis indicated that the two muscles received similar levels of neural drive. Nonetheless, the size of the MU action potentials was greater for VM than VL ($p<0.001$) and this difference explained most of the differences in EMG amplitude between the two muscles (~63% of explained variance). These results indicate that EMG amplitude, even following normalization, does not reflect the neural drive to synergistic muscles. Moreover, absolute EMG amplitude is mainly explained by the size of MU action potentials.

New and Noteworthy

EMG amplitude is widely used to indirectly compare the strength of neural drive received by synergistic muscles. However, there are no studies validating this approach with motor unit data. Here, we compared between-muscles differences in surface EMG amplitude and motor unit behavior. The results clarify the limitations of surface EMG to interpret differences in neural drive between muscles.

INTRODUCTION

Surface electromyography (EMG) amplitude depends on the level of muscle activation (number of muscle fiber action potentials) and it is typically used to infer the strength of neural drive (number of motor neuron action potentials) received by muscles (6). Changes in the relative activations of synergistic muscles are believed to be associated to the development of musculoskeletal disorders (19). For example, researchers argue that pathologies such as patellofemoral joint pain and Achilles tendinopathy might occur due to misbalanced activation of the vasti and calf muscles, respectively (17, 19). For patellofemoral joint pain, it is assumed that a greater activation of the vastus lateralis (VL) compared to the vastus medialis (VM) muscle induces a lateral shift of the patella, leading to misalignment of the patellofemoral joint (17, 19). Although these explanations seem plausible, there is still no consensus in the literature (7, 31), mainly because of limitations of surface EMG amplitude in measuring the neural drive (6). While normalization of EMG amplitude with respect to its value during a maximal voluntary contraction (MVC) may increase reliability when comparing between subjects (4), normalization may cancel out changes in muscle activation following, e.g., training interventions. It has been recently shown that high-density EMG (HDEMG) systems provide more reliable estimates of signal amplitude without the need for normalization (14, 34). This is possible due to the large number of observation sites (tens of electrodes) over the muscle belly that compensate for the variability of EMG with electrode location. However, the use of several electrodes does not solve the problem of comparing between muscles and subjects.

In addition to the neural drive to the muscle, EMG amplitude estimates are influenced by several other factors, such as muscle architecture, geometry, EMG crosstalk, and subcutaneous tissue thickness (11). Although normalization could help to improve between-muscle amplitude estimates, it is still not known if such measures really reflect differences in neural drive to the muscles. The direct way to access the neural drive to muscles is by motor unit recordings. Recent research has shown the possibility to identify large populations of motor units non-invasively, with HDEMG (25, 27). However, even sampling relatively large numbers of motor units, it is not possible to directly compare the strength of the neural drive to different muscles since the decomposition cannot identify the entire pool of active motor units. Rather, the number of decomposed motor units varies among muscles, with a weak relation with the actual number of active units. For this reason, in this study we propose a way to indirectly infer differences in neural drive between muscles by estimating the synaptic

inputs received by their motor neuron pools. Assuming similar intrinsic properties of the motor neurons between the muscles, we analyzed the increase in discharge rate from recruitment to target torque for motor units matched by recruitment threshold in the two muscles. Differences in the increase of discharge rate for motor units with the same recruitment thresholds would indicate differences in synaptic input received by the corresponding motor neurons and therefore differences in the generated neural drive to the muscles. In addition, we estimated the amplitude of the individual motor unit action potentials to examine the associations between interference EMG amplitude and either motor unit action potential size or neural drive. Therefore, the aim of the study was to assess the strengths of neural drives received by VM and VL muscles and investigate their relations with EMG amplitude. We hypothesized that differences in EMG amplitude between VM and VL muscles would be largely determined by the size of the motor unit action potentials (MUAPs) rather than differences in neural drive to the two muscles, and that normalization would not completely compensate for this influence.

MATERIALS AND METHODS

Participants

Eighteen healthy and physically active men (mean (SD) age: 29 (3) years, height: 178 (6) cm, mass: 79 (9) kg) were recruited. None of the participants reported any history of neuromuscular disorders or previous lower limb surgery. Subjects were asked to avoid any strenuous activity 24 h prior to the measurements. The ethics committee of the Universität Potsdam approved the study (approval number 26/2015), in accordance with the declaration of Helsinki (2004). All participants gave written, informed consent.

Experimental protocol

The participants performed submaximal and maximal knee extension contractions on an isokinetic dynamometer (CON-TREX MJ, PHYSIOMED, Regensdorf, Switzerland). The isometric knee extensions were exerted with the knee flexed to 90°. After placement of the surface EMG electrodes (see Data acquisition), subjects performed three maximal voluntary contractions (MVC) of knee extension each over a period of 5 s. Each of these trials was separated by 2 min of rest. The highest MVC value served as a reference to define the submaximal torque levels. After 5 min of rest, and following familiarization trials at low torque levels (10 and 30% MVC), subjects performed submaximal isometric knee extension contractions at 10, 30, 50 and 70% MVC in random order. Contractions at 10 and 30% MVC were maintained for 20 s, while the contractions at 50 and 70% MVC were sustained for 15

and 10 s respectively. In each trial, the participants received visual feedback of the torque applied by the leg to the dynamometer, which was displayed as a trapezoid (5 s ramps with hold-phase durations as specified above). Each contraction level was performed twice with a rest of 2 min following each contraction.

Data Acquisition

The surface EMG signals of VM and VL were recorded in monopolar derivation with a two-dimensional adhesive grid (SPES Medica, Salerno, Italy) of 13 × 5 equally spaced electrodes (1 mm diameter, inter-electrode distance of 8 mm). EMG signals were initially recorded during a brief voluntary contraction during which a linear non-adhesive dry electrode array of 8 silver-bar electrodes (1-mm diameter, 5-mm length, 5 mm interelectrode distance; SA 8/5, OT Bioelettronica, Torino, Italy) was moved over the skin to detect the location of the innervation zone and tendon regions (23). After the skin was shaved and cleansed with abrasion and water, the electrode cavities of the grids were filled with conductive paste (SPES Medica, Salerno, Italy). Grids were positioned between the proximal and distal tendons of the VM and VL muscles with the electrode columns (comprising 13 electrodes) oriented along the muscle fibers. Therefore, the VM grid was positioned ~50º with respect to a line between the anterior superior iliac spine and the medial side of the patella while the VL grid was positioned ~30º with respect to a line between the anterior superior iliac spine and the lateral side of the patella ((1, 22, 24, 25) (Figure 1). Reference electrodes were positioned over the malleoli and patella of the dominant leg.

EMG and torque signals were sampled at 2048 Hz and converted to digital data by a 12-bit analogue to digital converter (EMG-USB 2, 256-channel EMG amplifier, OT Bioelettronica, Torino, Italy, 3dB, bandwidth 10-500 Hz). EMG signals were amplified by a factor of 2000, 1000, 500, 500 and 500 for the 10, 30, 50, 70 and 100% MVC contractions, respectively. Data were analysed offline using Matlab (The Mathworks Inc., Natick, Massachusetts, USA). The 64-monopolar EMG channels were re-referenced offline to form 59 bipolar channels as the differences between adjacent electrodes in the direction of the muscle fibers.

Signal analysis

Motor unit analysis. The EMG signals recorded during the submaximal isometric contractions (from 10 to 70% MVC) were decomposed offline with a method that has undergone extensive validation (28). The accuracy of the decomposition was tested with the silhouette measure, which was set to ≥0.90 (28). The signals were decomposed throughout the whole duration of the submaximal contractions and the discharge times of the identified motor units were converted into binary spike trains. The mean discharge rate and discharge

rate variability (coefficient of variation of the inter-spike-interval, CoVisi), were calculated during the stable plateau torque region. Discharge rate at recruitment was calculated using the first six discharges of the motor units (9). The motor unit recruitment threshold was defined as the knee extension torque (%MVC) at the time when the motor unit began discharging action potentials. Discharges that were separated from the next by <33.3 ms or >200 ms (30 and 5 Hz, respectively) were discarded from the mean discharge rate and CoVisi calculation since such discharges are usually considered decomposition errors (24). Motor unit conduction velocity (MUCV) was measured from a minimum of three to a maximum of nine double-differential channels (manual selection) (25). Channels that had the clearest propagation of MUAPs, with the highest amplitude in the columns of the grid and a coefficient of correlation between channels ≥0.9, were selected for further analysis. Finally, the amplitude of the MUAPs was calculated as the MUAP RMS averaged over all channels of the grid (MURMS). VM and VL motor units were matched by their recruitment threshold with a tolerance of ±0.5% MVC. The matched motor units were then grouped in four classes, according to their recruitment thresholds ([0-10] % MVC, [10-30] % MVC, [30-50] % MVC, [50-70] % MVC).

The discharge rate of motor units with the same recruitment thresholds (i.e., with a difference in threshold <0.5% MVC) in the two muscles was used as a measure to compare the synaptic inputs received by the pools of motor neurons. This measure corresponds to the increase in discharge rate from recruitment to the target torque relative to the increase in torque from the recruitment threshold [target torque (10, 30, 50 and 70% MVC) – recruitment threshold torque]. A difference in the relative rate of increase in discharge rate between motor units in the two muscles indicates differences in synaptic input received by the motor neuron pools of the two muscles. It was then assumed that the neural drive to the muscles depended on the synaptic input.

Interference EMG. The root mean square values (RMS) obtained from submaximal and maximal contractions, were averaged over all channels of the electrode grid (22). During the submaximal isometric contractions, the RMS was computed from the HDEMG signals in intervals of 1 s. These values were extracted from the stable-torque region of the contractions (e.g., hold-phase of 15 seconds at 50% MVC). RMSs of the maximal (MVC) contractions were analyzed in a time window of 250 ms centered at the peak EMG activity (22). The average conduction velocity (referred in the following as muscle fiber conduction velocity) was calculated from the interference EMG in double differential derivations obtained along the fiber direction (columns of the grid). In order to maximize the accuracy of muscle fiber

conduction velocity estimates, three contiguous columns with four to six channels with the highest cross-correlation in propagation were selected (10). Muscle fiber conduction velocity estimation was obtained with a multichannel maximum-likelihood algorithm that was previously shown to provide accurate estimates (standard deviation <0.1 ms) (13).

Amplitude normalization. Both absolute RMS and MURMS were normalized to the RMS value obtained during the MVC in order to analyze the effects of normalization on submaximal RMS amplitude of the interference EMG (absolute RMS) as well as on MURMS between muscles.

Statistical Analysis

The Shapiro-Wilk test was used to check the normality of all variables. Sphericity was checked by the Mauchley's test and if violated, the Greenhouse-Geisser correction was applied to the degrees of freedom. Statistical significance was set at $p < 0.05$. Results are expressed as mean and standard deviation (SD).

EMG (absolute RMS, normalized RMS and muscle fiber conduction velocity) and motor unit variables (MURMS, discharge rate, CoVisi, motor unit conduction velocity and normalized MURMS) were compared between muscles at each torque level with a two-way repeated measures analysis of variance (ANOVA) with factors muscle (VM and VL) and torque (10, 30, 50 and 70% MVC). When the repeated measures ANOVA was significant, pairwise comparisons were performed with a Student-Newman-Keuls (SNK) post-hoc test. Linear regression was used to characterize the association for each motor unit between the differences in discharge rate at the target torque (mean discharge rate at 10, 30, 50 and 70% MVC) and at recruitment (calculated from the first 6 motor unit discharges) and between the target torque (10, 30, 50 and 70% MVC) and motor unit recruitment threshold. The slopes of these linear regressions were compared between the two muscles by analysis of covariance (ANCOVA) (35). The same analysis was applied to VM and VL MURMS vs. recruitment threshold.

Finally, a multiple linear regression (stepwise) analysis was performed on EMG/motor unit parameters to identify the variables that predicted the differences between VM and VL absolute RMS. Therefore, the percent (%) difference in absolute RMS between VM and VL was used as the predictor variable and the % differences in MU behavior/properties were regarded as independent variables. Each torque level was analyzed independently (e.g. absolute RMS % difference between VM and VL at 30% MVC was compared with motor unit variables obtained at the same torque level). The partial eta-squared (ηp^2) for ANOVA

was used to examine the effect size of the differences between EMG and motor unit parameters between muscles. A ηp^2 less than 0.06 was classified as "small", 0.07-0.14 as "moderate", and greater than 0.14 as "large" (5).

RESULTS

Interference EMG

Absolute RMS (Figure 2a) was significantly greater for VM than VL at 30, 50 and 70% MVC (interaction: muscle-torque, $p<0.0001$, $\eta p^2=0.79$). However, muscle fiber conduction velocity (Figure 2b) was similar for the two muscles (interaction: muscle-torque, $p=0.96$, $\eta p^2=0.019$).

Decomposed motor unit populations

The average number of motor units accurately identified (with a SIL≥0.90) per subject at each torque level was 8 (0.7) and 7 (1.2) in VM and VL, respectively.

According to their recruitment threshold, 340 motor units were matched between VM and VL. Per subject, an average of 6.2 (3.0), 5.0 (2.5), 5.7 (2.8) and 3.3 (2.0) motor units were matched between VM and VL at 10, 30, 50 and 70% MVC, respectively. The average recruitment threshold of the matched motor units at 10, 30, 50 and 70% MVC was 7.5, 23.3, 38.2 and 56.2% MVC, respectively. Figure 3 shows the histograms of the number of matched motor units according to their recruitment thresholds.

Discharge rate and discharge rate variability

The mean motor unit discharge rate (at target torque) of VM was greater than for VL motor units as revealed by a significant effect of muscle ($p=0.009$, $\eta p^2=0.38$) (Figure 4). However, the regression lines of delta discharge rate [mean discharge rate at target torque – discharge rate at recruitment] vs. delta torque [target torque – recruitment threshold] were not different between muscles (slope of the regression lines, $p>0.35$, intercepts, $p>0.08$) at all target torques (10, 30, 50 and 70% MVC) (Figure 5). Finally, there was no difference in discharge rate variability between muscles as CoVisi (Figure 6) remained similar at all torque levels (interaction: muscle-torque, $p=0.4$, $\eta p^2=0.07$).

Size and conduction velocity of MUAPs

MURMS (Figure 7a) was significantly greater for VM than VL at 30, 50 and 70% MVC (interaction: muscle-torque, $p<0.0001$, $\eta p^2=0.57$). Moreover, MURMS increased at a greater rate with recruitment threshold for VM than for VL ($p<0.0001$, Figure 7b). Motor unit conduction velocity (Figure 8) was significantly higher at 70% MVC for VM than VL (interaction: muscle-torque, $p=0.023$, $\eta p^2=0.46$).

Multiple linear regression

Motor unit variables that significantly differed between muscles were entered into the multiple linear regression analysis to explain the differences in absolute EMG amplitude between muscles. Therefore, the difference (%) in VM-VL MURMS, discharge rate, and motor unit conduction velocity were regarded as independent variables. Table 1 reports the results of the multiple regression. At 10% MVC, only MURMS was entered in the model, explaining 71% of the variance for the difference (%) in VM-VL absolute RMS. At 30%, both MURMS and discharge rate entered in the model, however MURMS explained most of the variance (53% MURMS vs. 13.2% for discharge rate). Similar results were obtained at 50% MVC where MURMS explained 72% of the difference between VM-VL absolute RMS, with discharge rate only explaining 7.7% of the variance. Finally, at 70% MVC, only MURMS was entered in the model, explaining 57% of the %difference in VM-VL absolute RMS.

Normalized amplitude

Normalized RMS (Figure 9) showed systematically higher values for VL across all torque levels (effect: muscle, $p=0.039$, $\eta p^2=0.23$). Conversely, normalized MURMS did not show any difference between muscles at any torque level (effect: muscle, $p=0.46$, $\eta p^2=0.04$, interaction: torque-muscle, $p=0.12$, $\eta p^2=0.11$).

DISCUSSION

This study shows that differences in EMG amplitude between synergistic muscles are mostly explained by differences in MUAP size (MURMS), with little influence of other motor unit properties. Moreover, EMG normalization does not provide clear explanation of differences in muscle activation between the vasti. The observed differences in EMG amplitude between muscles (in absolute values or normalized) contrasted with the similar neural drive estimated for VM and VL. Taken together, the results suggest that EMG amplitude (in absolute values

or normalized) should not be used to infer differences in neural drive between synergistic muscles.

Neural drive to VM and VL muscles

Due to current limitations in EMG decomposition, it is not possible to identify the full populations of active motor units. For this reason, the neural drives cannot be directly compared between muscles. We compensated for this limitation by an indirect assessment of the strength of the neural drive. Matching synergistic muscles motor units by recruitment threshold allows a direct comparison of motor unit discharge rates across muscles. Because the discharge rate depends on the torque relative to the recruitment threshold, we focused on the rate of change in discharge rate (mean discharge rate at target torque – discharge rate at recruitment) with respect to the difference between exerted torque (10, 30, 50 or 70% MVC) and recruitment threshold across the decomposed motor unit populations. This analysis provides an estimate of the synaptic input received by the motor neuron pools of VM and VL, since discharge rates indicate the nonlinear transformation of synaptic inputs into motor neuron outputs (20). This approach indicated a similar change in motor unit discharge rate with torque (figure 5) despite a difference in absolute discharge rates that can be due to the random sampling of motor units in the two muscles (Figure 4). This suggests that the net excitatory synaptic input to the pool of motor neurons of the vasti was similar. Assuming that the intrinsic properties of the motor neuron pools in the two muscles were similar, this observation was interpreted as reflecting similar drives from the motor neurons to the muscle units. This conclusion is in agreement with a previous study that showed that VM and VL share most of their synaptic input (21).

We also did not observe differences in discharge rate variability (CoVisi) between the two muscles (Figure 6), in agreement with previous results (34). The present results show that, despite a difference in mean absolute discharge rates between motor units of the VM and VL, the two muscles did receive similar strengths of neural drives. Differences in VM and VL surface EMG amplitude therefore do not reflect differences in the neural drive between the vasti, as also confirmed by the multiple regression analysis.

EMG amplitude and muscle fiber conduction velocity

Surface EMG amplitude is commonly used to infer the magnitude of the neural drive to muscles. However, EMG amplitude depends on both motor unit behavior (recruitment, discharge rate and discharge rate variability) and muscle fiber properties (MUAP size and

conduction velocity) (11, 12). In this study, despite similar neural drives to the VM and VL, the EMG amplitude for VM was significantly greater than for VL for torques in the range 30%-70% MVC (Figure 7). These results are consistent with other reports on absolute EMG amplitude for these two muscles (15, 22, 34). EMG amplitude is influenced by muscle's geometry, architecture, crosstalk and subcutaneous tissue thickness (11, 29). Since the observed differences in EMG amplitude between muscles did not correspond to differences in neural drive, they are mainly explained by these anatomical factors, as confirmed by the differences in MUAP sizes. Although previous research has reported similar subcutaneous tissue thickness for the distal VM and VL (3), it has also been shown that the distal VM has a larger cross sectional area and greater fascicle angle compared to the distal VL (2). Indeed, recent research has shown that differences in muscle architecture can influence EMG amplitude, even when the muscle is activated at a similar intensity (32).

Muscle fiber conduction velocity estimated from the interference EMG was similar between the vasti, in agreement with previous studies (3). However, motor unit conduction velocity differed between muscles. Muscle fiber conduction velocity is associated to fiber diameter (16) but also depends on the level of muscle acidosis (30), temperature (8), muscle fatigability (23), subcutaneous tissue thickness (33), exercise training (25, 33), discharge rate (26). Because of these factors of influence, the relation between average and motor unit muscle fiber conduction velocity is not exactly linear.

EMG amplitude and MUAP size

As for absolute EMG amplitude, the size of MUAPs was significantly greater for VM in the range of torques above or equal to 30% MVC. Moreover, MURMS increased at a faster rate with recruitment threshold for VM than VL (Figure 7). This is consistent with a recent report comparing VM and VL MUAP peak-to-peak amplitude (24). As for EMG amplitude, MURMS is also influenced by muscle's geometry, architecture and subcutaneous tissue thickness (11, 29); therefore it is not surprising to find similar results for absolute RMS and MURMS. Accordingly, results from the multiple linear regression (Table 1) showed that most of the variance of the difference between absolute RMS of VM and VL was explained by MURMS. This result directly indicates that that the neural drive has a relatively small influence on EMG amplitude with respect to the MUAP waveforms.

Amplitude normalization

Since a vast number of studies apply normalization of the surface EMG prior to comparing levels of muscle activations (4, 17), we analyzed the effect of normalization of both EMG amplitude and MUAP size with respect to MVC. Even though normalization decreased the VM/VL activation ratio and cancelled out the differences in MUAP size between muscles, normalized EMG amplitude was greater for VL compared to VM that is contrary to the result without normalization. This result does not correspond to the estimated similar neural drive to the two muscles (figure 5) and explains the divergent results across studies on normalized activations of the VM and VL in healthy subjects (31) and patients with musculoskeletal disorders (e.g. patellofemoral pain syndrome) (18). Taken together, our findings suggest that neither absolute nor normalized EMG amplitude (even when recorded from HDEMG electrodes) are appropriate for inferring differences in neural drive between muscles.

Conclusion

The difference in surface EMG amplitude between VM and VL muscles was mostly explained by differences in MUAP size, with little effect of motor unit properties associated to the neural drive to muscles. EMG amplitude is therefore mainly determined by peripheral properties rather than by the neural activation. Normalization of the EMG compensates for the differences in MUAP sizes but is still a poor determinant of neural activation.

REFERENCES

1. **Barbero M, Merletti R, and Rainoldi A**. *Atlas of muscle innervation zones : understanding surface electromyography and its applications*. Milan ; New York: Springer, 2012, p. 131-132.
2. **Blazevich AJ, Gill ND, and Zhou S**. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat* 209: 289-310, 2006.
3. **Boccia G, Dardanello D, Beretta-Piccoli M, Cescon C, Coratella G, Rinaldo N, Barbero M, Lanza M, Schena F, and Rainoldi A**. Muscle fiber conduction velocity and fractal dimension of EMG during fatiguing contraction of young and elderly active men. *Physiol Meas* 37: 162-174, 2016.
4. **Burden A**. How should we normalize electromyograms obtained from healthy participants? What we have learned from over 25 years of research. *J Electromyogr Kinesiol* 20: 1023-1035, 2010.
5. **Cohen J**. *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J.: L. Erlbaum Associates, 1988, p. xxi, 567 p.
6. **Dideriksen JL, Enoka RM, and Farina D**. Neuromuscular adjustments that constrain submaximal EMG amplitude at task failure of sustained isometric contractions. *J Appl Physiol (1985)* 111: 485-494, 2011.

7. **Fagan V, and Delahunt E**. Patellofemoral pain syndrome: a review on the associated neuromuscular deficits and current treatment options. *Br J Sports Med* 42: 789-795, 2008.
8. **Farina D, Arendt-Nielsen L, and Graven-Nielsen T**. Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. *J Appl Physiol (1985)* 99: 197-203, 2005.
9. **Farina D, Holobar A, Gazzoni M, Zazula D, Merletti R, and Enoka RM**. Adjustments differ among low-threshold motor units during intermittent, isometric contractions. *J Neurophysiol* 101: 350-359, 2009.
10. **Farina D, and Merletti R**. Estimation of average muscle fiber conduction velocity from two-dimensional surface EMG recordings. *J Neurosci Methods* 134: 199-208, 2004.
11. **Farina D, Merletti R, and Enoka RM**. The extraction of neural strategies from the surface EMG. *J Appl Physiol (1985)* 96: 1486-1495, 2004.
12. **Farina D, Merletti R, and Enoka RM**. The Extraction of Neural Strategies from the Surface Emg: An Update. *J Appl Physiol (1985)* jap 00162 02014, 2014.
13. **Farina D, Muhammad W, Fortunato E, Meste O, Merletti R, and Rix H**. Estimation of single motor unit conduction velocity from surface electromyogram signals detected with linear electrode arrays. *Med Biol Eng Comput* 39: 225-236, 2001.
14. **Gallina A, Pollock CL, Vieira TM, Ivanova TD, and Garland SJ**. Between-day reliability of triceps surae responses to standing perturbations in people post-stroke and healthy controls: A high-density surface EMG investigation. *Gait Posture* 44: 103-109, 2016.
15. **Hedayatpour N, Arendt-Nielsen L, and Farina D**. Non-uniform electromyographic activity during fatigue and recovery of the vastus medialis and lateralis muscles. *J Electromyogr Kinesiol* 18: 390-396, 2008.
16. **Houtman CJ, Stegeman DF, Van Dijk JP, and Zwarts MJ**. Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. *J Appl Physiol (1985)* 95: 1045-1054, 2003.
17. **Hug F, Goupille C, Baum D, Raiteri BJ, Hodges PW, and Tucker K**. Nature of the coupling between neural drive and force-generating capacity in the human quadriceps muscle. *Proc Biol Sci* 282: 2015.
18. **Hug F, Hodges PW, and Tucker K**. Muscle Force Cannot Be Directly Inferred From Muscle Activation: Illustrated by the Proposed Imbalance of Force Between the Vastus Medialis and Vastus Lateralis in People With Patellofemoral Pain. *J Orthop Sports Phys Ther* 45: 360-365, 2015.
19. **Hug F, and Tucker K**. Muscle Coordination and the Development of Musculoskeletal Disorders. *Exerc Sport Sci Rev* 45: 201-208, 2017.
20. **Johnson MD, Thompson CK, Tysseling VM, Powers RK, and Heckman CJ**. The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *J Neurophysiol* 118: 520-531, 2017.
21. **Laine CM, Martinez-Valdes E, Falla D, Mayer F, and Farina D**. Motor Neuron Pools of Synergistic Thigh Muscles Share Most of Their Synaptic Input. *J Neurosci* 35: 12207-12216, 2015.
22. **Martinez-Valdes E, Falla D, Negro F, Mayer F, and Farina D**. Differential Motor Unit Changes after Endurance or High-Intensity Interval Training. *Med Sci Sports Exerc* 49: 1126-1136, 2017.
23. **Martinez-Valdes E, Guzman-Venegas RA, Silvestre RA, Macdonald JH, Falla D, Araneda OF, and Haichelis D**. Electromyographic adjustments during continuous and intermittent incremental fatiguing cycling. *Scand J Med Sci Sports* 26: 1273-1282, 2016.

24. **Martinez-Valdes E, Laine CM, Falla D, Mayer F, and Farina D**. High-density surface electromyography provides reliable estimates of motor unit behavior. *Clin Neurophysiol* 127: 2534-2541, 2016.
25. **Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, and Farina D**. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. *J Physiol* 595: 1479-1496, 2017.
26. **McGill KC, and Lateva ZC**. History dependence of human muscle-fiber conduction velocity during voluntary isometric contractions. *J Appl Physiol (1985)* 111: 630-641, 2011.
27. **Muceli S, Poppendieck W, Negro F, Yoshida K, Hoffmann KP, Butler JE, Gandevia SC, and Farina D**. Accurate and representative decoding of the neural drive to muscles in humans with multi-channel intramuscular thin-film electrodes. *J Physiol* 593: 3789-3804, 2015.
28. **Negro F, Muceli S, Castronovo AM, Holobar A, and Farina D**. Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *J Neural Eng* 13: 026027, 2016.
29. **Rainoldi A, Nazzaro M, Merletti R, Farina D, Caruso I, and Gaudenti S**. Geometrical factors in surface EMG of the vastus medialis and lateralis muscles. *J Electromyogr Kinesiol* 10: 327-336, 2000.
30. **Schmitz JP, van Dijk JP, Hilbers PA, Nicolay K, Jeneson JA, and Stegeman DF**. Unchanged muscle fiber conduction velocity relates to mild acidosis during exhaustive bicycling. *Eur J Appl Physiol* 112: 1593-1602, 2012.
31. **Smith TO, Bowyer D, Dixon J, Stephenson R, Chester R, and Donell ST**. Can vastus medialis oblique be preferentially activated? A systematic review of electromyographic studies. *Physiother Theory Pract* 25: 69-98, 2009.
32. **Vieira TM, Bisi MC, Stagni R, and Botter A**. Changes in tibialis anterior architecture affect the amplitude of surface electromyograms. *J Neuroeng Rehabil* 14: 81, 2017.
33. **Vila-Cha C, Falla D, Correia MV, and Farina D**. Adjustments in motor unit properties during fatiguing contractions after training. *Med Sci Sports Exerc* 44: 616-624, 2012.
34. **Vila-Cha C, Falla D, and Farina D**. Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training. *J Appl Physiol (1985)* 109: 1455-1466, 2010.
35. **Zar JH**. *Biostatistical analysis*. Upper Saddle River, N.J.: Prentice-Hall/Pearson, 2010, p. 944.

Acknowledgements

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).

Figure captions

Figure 1. Placement of the HDEMG electrodes. Vastus medialis (VM) electrode grid was placed ~50º with respect to a line between the anterior superior iliac spine and the medial side of the patella (dashed lines, left) while the VL grid was positioned ~30º with respect to a line between the anterior superior iliac spine and the lateral side of the patella (dashed lines, right).

Figure 2. Interference EMG parameters [mean (SD)] for vastus medialis (VM, white dots) and vastus lateralis (VL, black dots) at 10, 30, 50 and 70% of the maximum voluntary contraction torque (MVC). A) Absolute root mean square (ABS RMS). B) Muscle fiber conduction velocity. Presented values were averaged for each subject and presented at each submaximal target torque. * $P<0.001$.

Figure 3. Two subsets of motor units identified from the vastus medialis and lateralis muscles were matched for recruitment threshold. The histograms of the motor unit recruitment thresholds in these subsets are shown for the vastus medialis (left) and vastus lateralis (right) motor units.

Figure 4. Motor unit (MU) average discharge rate (target torque discharge rate) calculated from recruitment-threshold matched MUs from vastus medialis (VM, white dots) and vastus lateralis (VL, black dots) at 10, 30, 50 and 70% of the maximum voluntary contraction torque (MVC). MU discharge rate values [mean (SD)] were averaged for each subject and presented

535 at each submaximal target torque (10, 30, 50 and 70% MVC), # main effect of muscle
536 P=0.009.
537
538 Figure 5. Linear regression analysis of the difference between VM and VL mean discharge
539 rate at target torque and discharge rate at recruitment (Y-axis) and the difference between
540 target torque (10, 30, 50 and 70% MVC) and MU recruitment threshold (X-axis) at 10%
541 (upper left), 30% (upper right), 50% (lower left) and 70% (lower right) of the MVC torque.
542 Linear regression equations are shown in the figure. All regression lines had positive slopes
543 ($P<0.03$) and their R^2 values were 0.1 and 0.15 (10% MVC), 0.16 and 0.08 (30% MVC), 0.05
544 and 0.05 (50% MVC), and 0.17 and 0.14 (70% MVC) for VM and VL respectively. None of
545 the regression lines (slopes and intercepts) differed significantly between muscles ($p>0.09$).
546 DR, discharge rate.
547
548 Figure 6. Motor unit (MU) coefficient of variation of the inter-spike interval (CoVisi)
549 calculated from recruitment-threshold matched MUs from vastus medialis (VM, white dots)
550 and vastus lateralis (VL, black dots) at 10, 30, 50 and 70% of the maximum voluntary
551 contraction torque (MVC). Presented values were averaged for each subject and presented at
552 each submaximal target torque.
553
554 Figure 7. Motor unit (MU) root mean square amplitude (MURMS) [mean (SD)] extracted
555 from recruitment-threshold matched MUs from vastus medialis (VM, white dots) and vastus
556 lateralis (VL, black dots) at 10, 30, 50 and 70% of the maximum voluntary contraction torque
557 (MVC). A) MURMS values [mean (SD)] were averaged for each subject and presented at
558 each submaximal target torque (10, 30, 50 and 70% MVC), * $P<0.01$. B) VM and VL
559 MURMS vs. recruitment threshold regression lines. Both lines increased significantly with
560 torque ($P<0.0001$) and displayed significantly different slopes ($P<0.0001$); R^2 values are
561 shown in the figure.
562
563 Figure 8. Motor unit (MU) conduction velocity [mean (SD)] extracted from recruitment-
564 threshold matched MUs from vastus medialis (VM, white dots) and vastus lateralis (VL,
565 black dots) at 10, 30, 50 and 70% of the maximum voluntary contraction torque (MVC).
566 Presented values were averaged for each subject and presented at each submaximal target
567 torque. * $P<0.01$.
568

Figure 9. Normalized EMG and motor unit (MU) amplitude [mean (SD)] for vastus medialis (VM, white dots) and vastus lateralis (VL, black dots) at 10, 30, 50 and 70% of the maximum voluntary contraction torque (MVC). A) Normalized root mean square EMG (EMG RMS NORM), B) Normalized MU root mean square (MURMS NORM). # Main effect of muscle P=0.039.

Figure 1

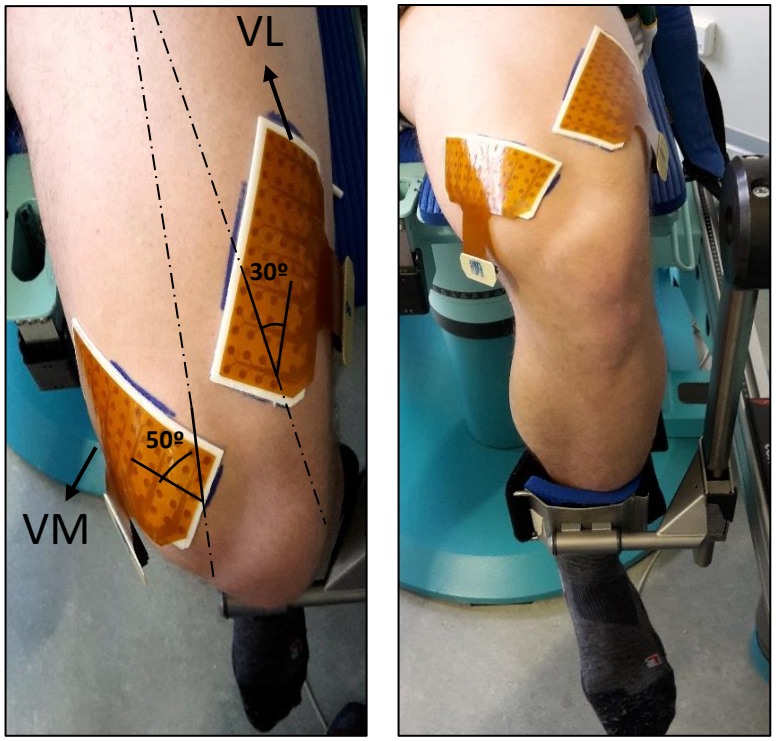
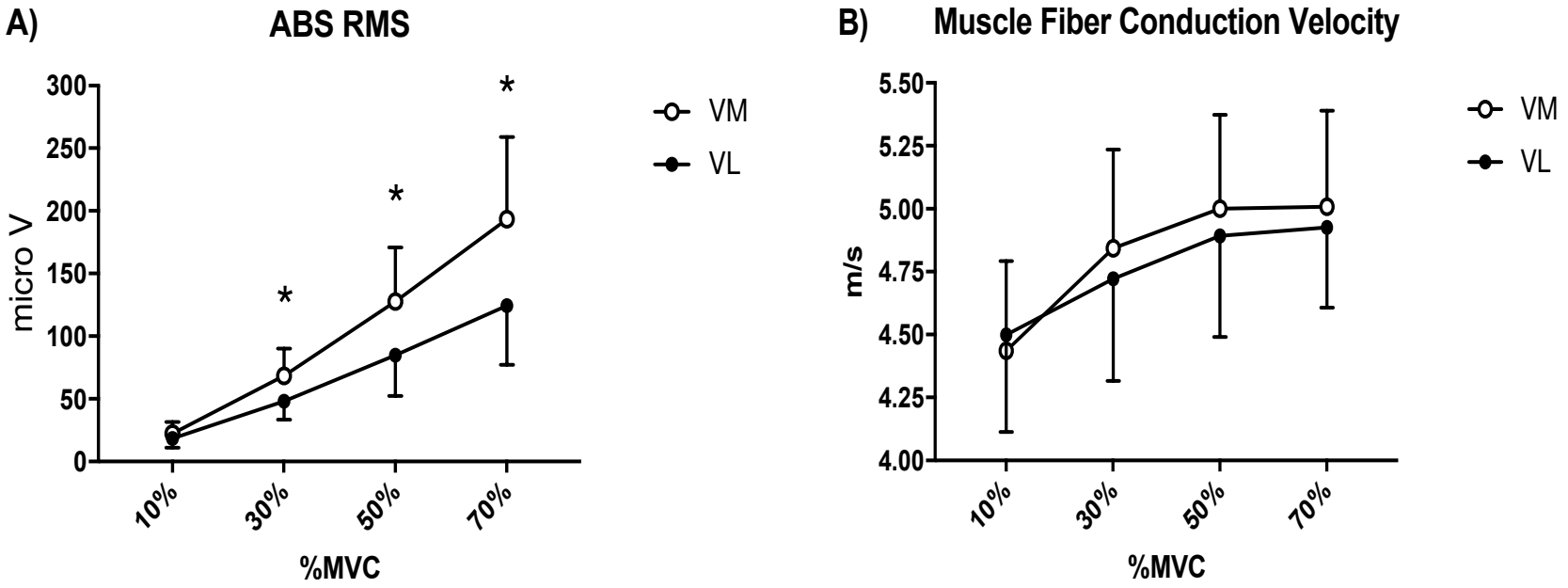
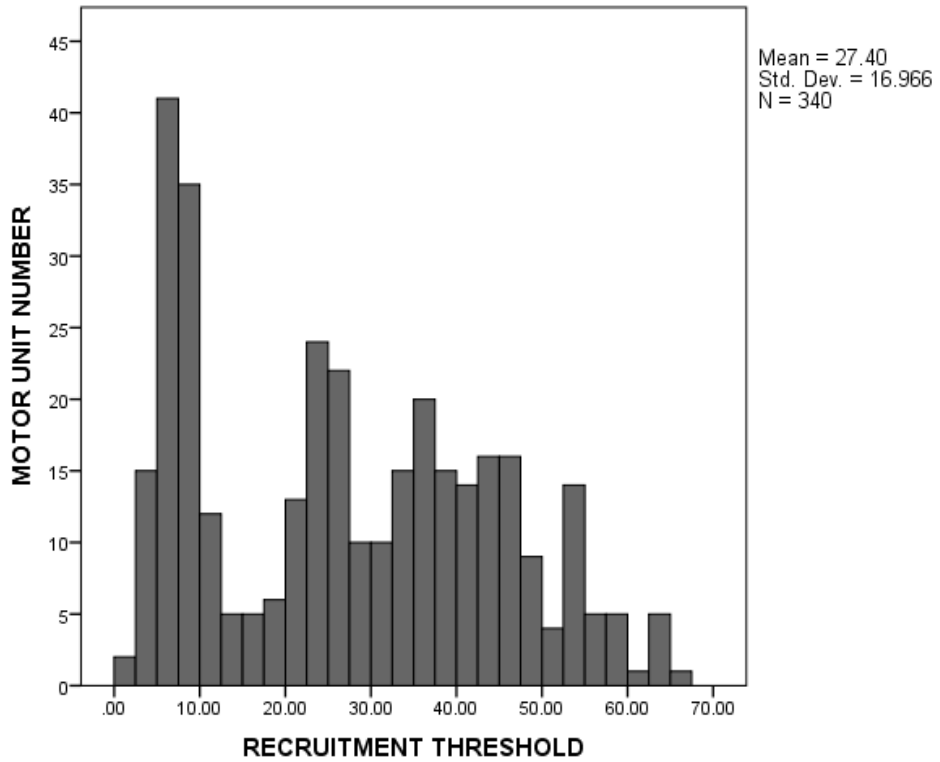


Figure 2



Vastus Medialis



Vastus Lateralis

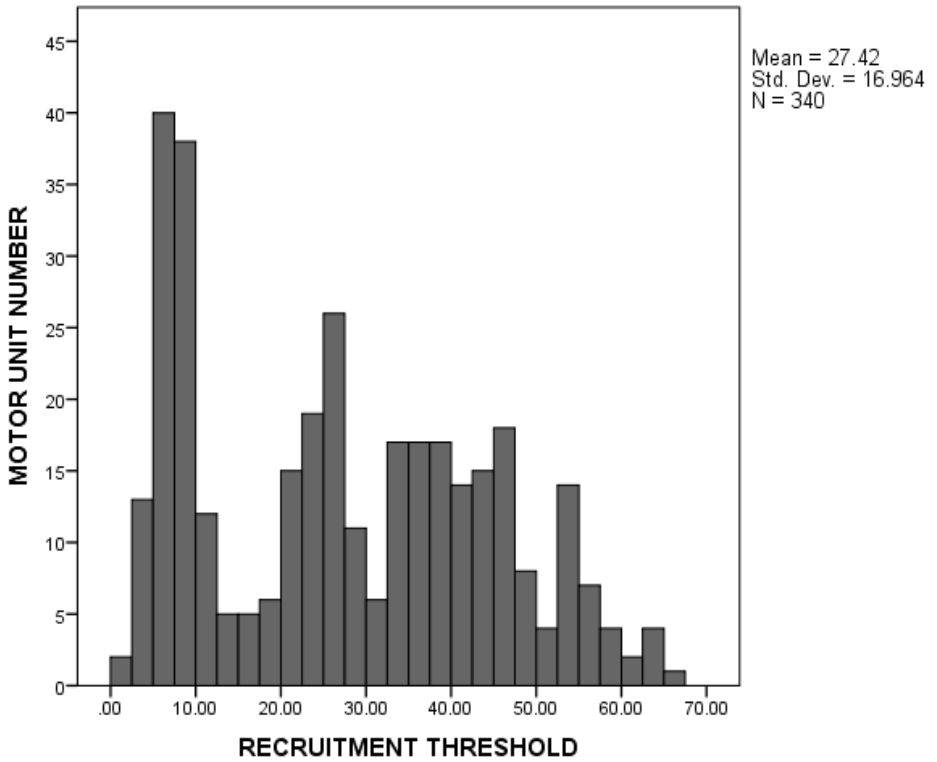


Figure 4

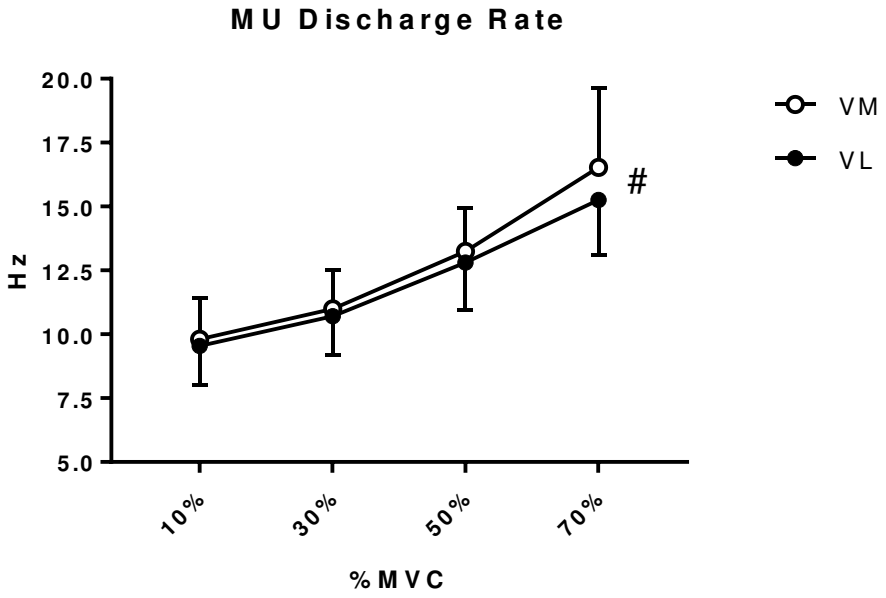


Figure 5

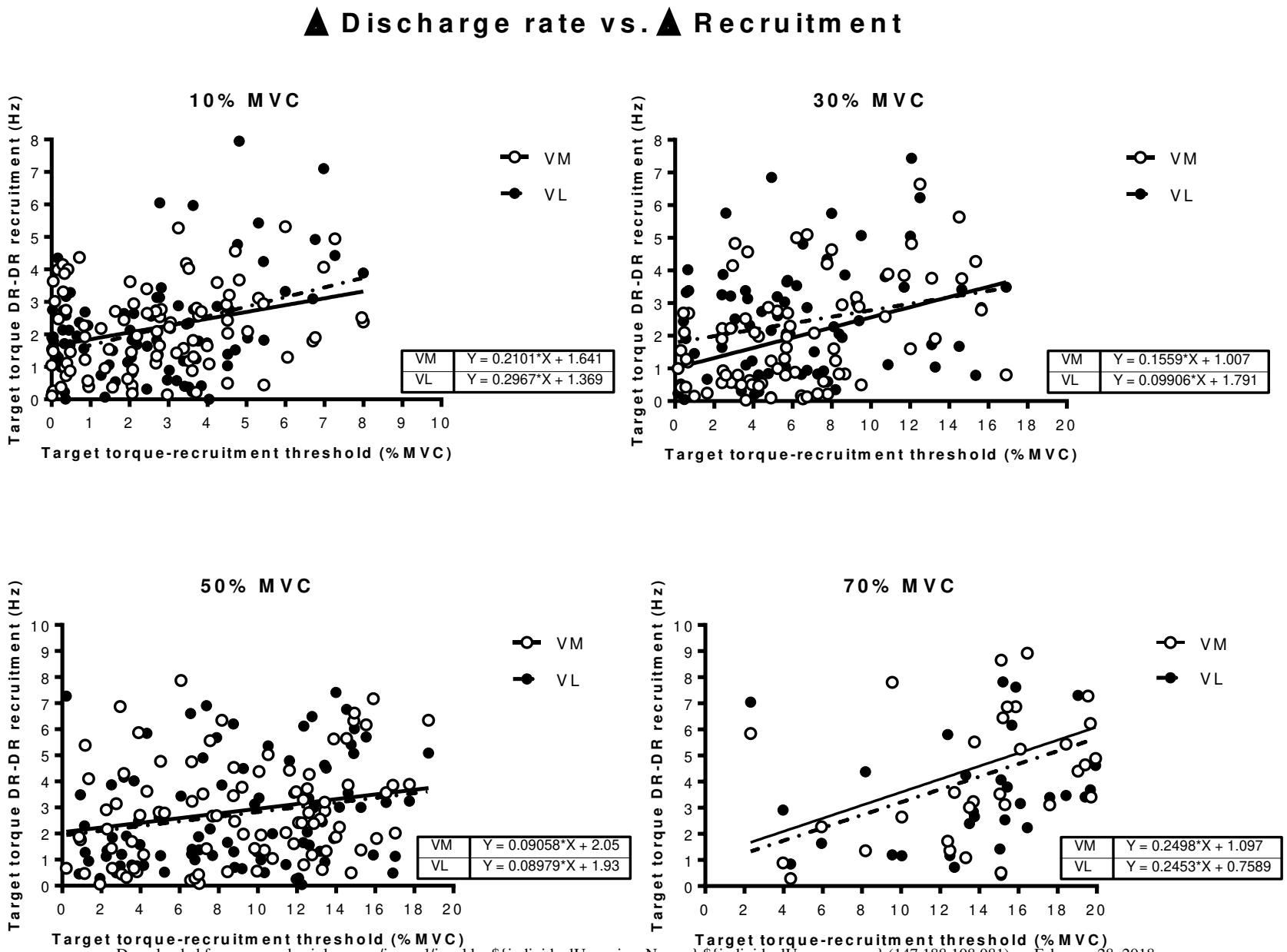


Figure 6

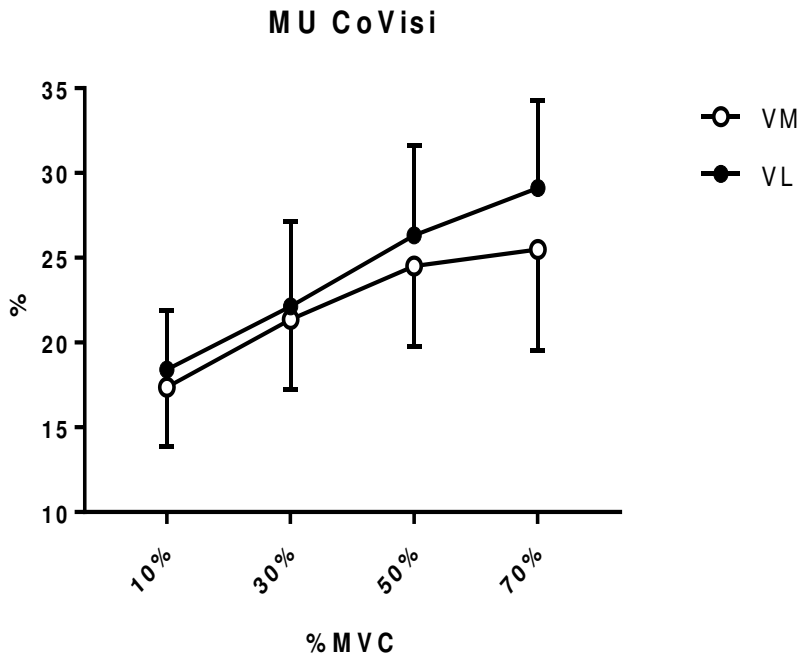


Figure 7

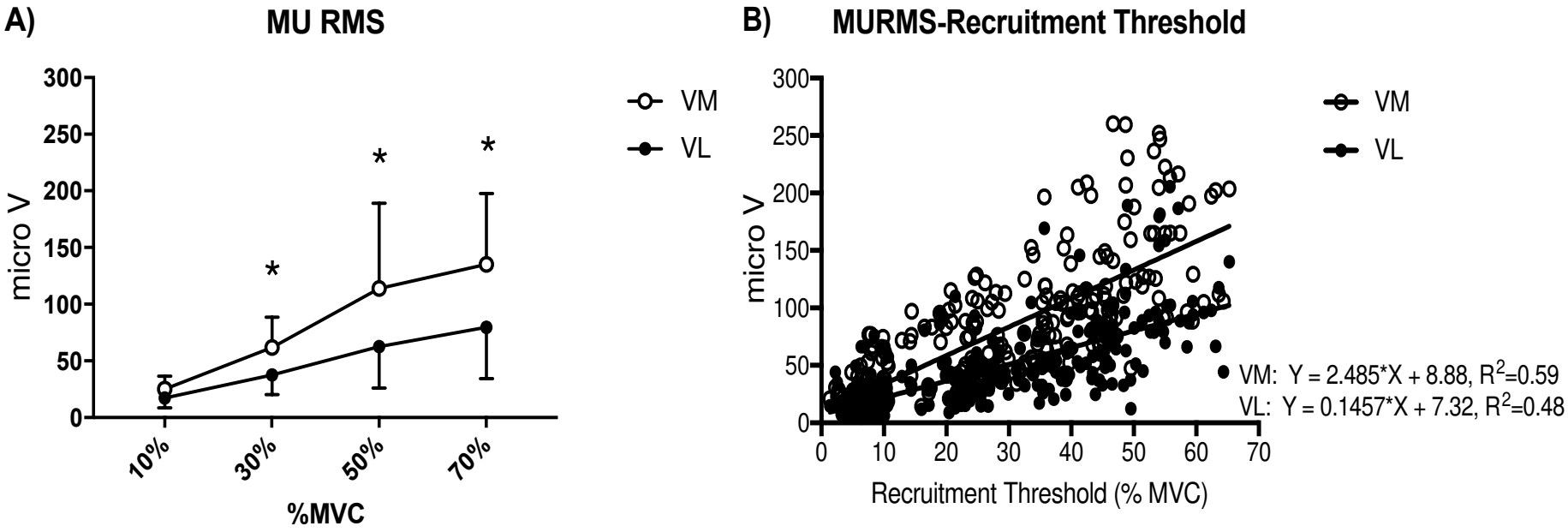


Figure 8

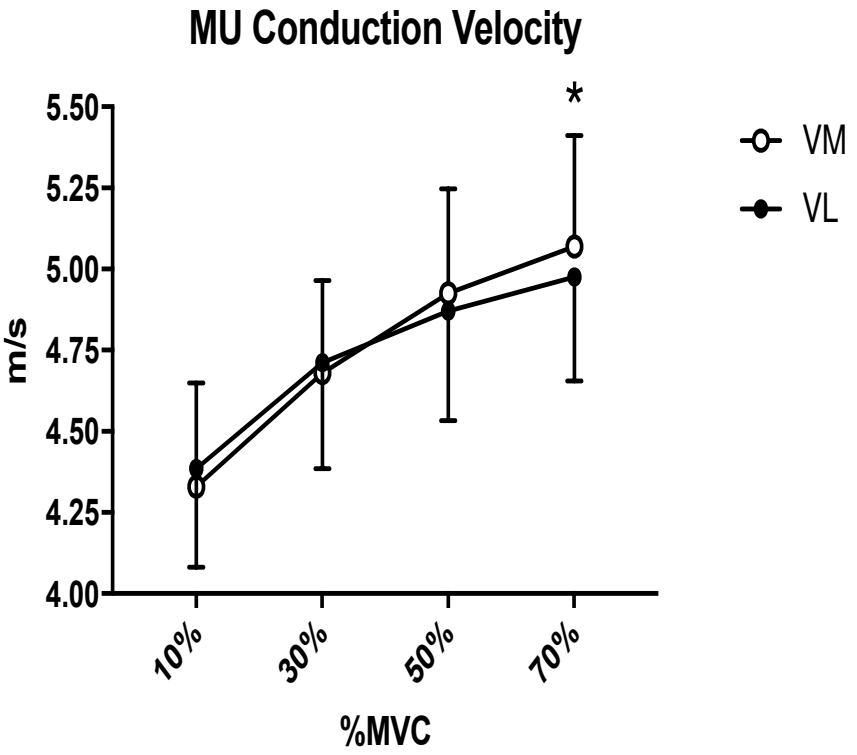
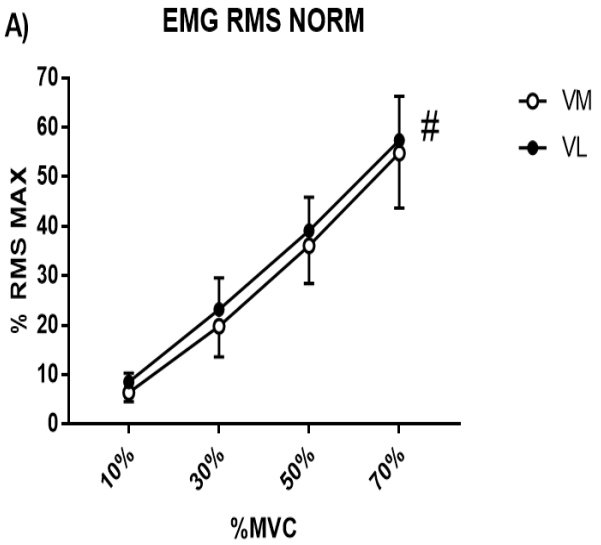


Figure 9



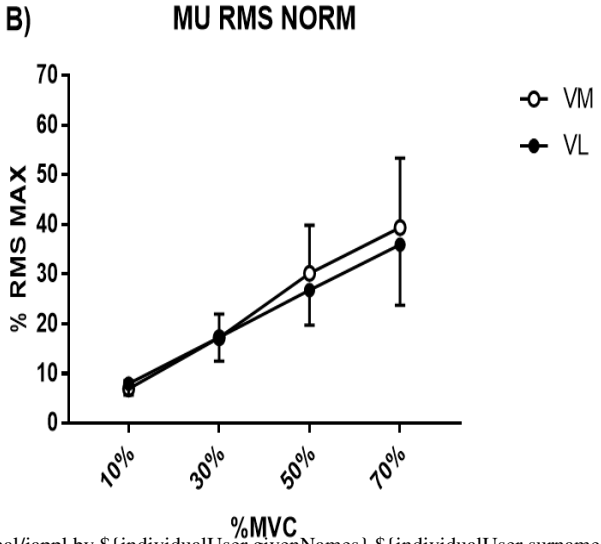


Table 1. Percent difference [%, mean (SD)] and bivariate correlation coefficients (r) between predictor variable (% change in VM-VL EMG RMS) and independent variables: %change in VM-VL motor unit (MU) RMS, %change in VM-VL in MU discharge rate (DR) and %change in VM-VL MU conduction velocity (CV)

Torque Level (%MVC)	%Difference in EMG RMS	% Difference in MU RMS	% Difference in MU DR	% Difference in MU CV
10%	14.8 (25.3)	25.2 (34.1), r= 0.84**	2.3 (7.8), r=-0.48	-1.4(4.9), r=-0.27
30%	27.2 (19.4)	36.5 (25.4), r=0.73**	2.3 (7.8), r=0.14	-0.7 (2.5), r=0.12
50%	32.8 (12.5)	42.3 (19.6), r=0.85**	4.1 (9.5), r=0.02	1.3 (3.1), r=-0.2
70%	34.9 (15.8)	42.2 (19.1), r=0.76**	6.2 (13.3),r=0.26	1.8 (3.9), r=0.07

** Significant correlation ($p<0.0001$)