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Variability of estimates of muscle fiber conduction velocity and surface EMG amplitude across subjects and processing intervals

Alessandro Del Vecchio[a,b,*], Ilenia Bazzucchi[b], Francesco Felici[b]

[a] Neuromuscular Research Centre & Technology, Department of Bioengineering, Imperial College London, SW7 2AZ London, UK
[b] Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", 00135 Rome, Italy

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ABSTRACT

The force developed by the human neuromuscular system can change very rapidly (15–50 ms). When processing EMG signals for inferring neural control strategies, it is therefore necessary to extract estimates from short time intervals. In this study, we investigate the relation between joint torque and estimates of average muscle fibre conduction velocity (MFCV) and amplitude (RMS) from surface EMG signals, when varying the duration of the processing interval. Moreover, we assessed the inter-subject variability in RMS and MFCV estimates. Ten healthy subjects performed isometric linearly increasing ankle dorsiflexion contractions up to 70% MVC at a rate of 5% $MVCs^{-1}$. High-density EMG signals were recorded from the tibialis anterior muscle and MFCV and RMS were estimated in eight time-intervals ranging from 15 to 2000 ms. MFCV and RMS were significantly correlated with force in all subjects and when using all time-intervals (MFCV = 0.77 ± 0.07, RMS = 0.79 ± 0.06 (R^2), Pearson-P < 0.01). The variability around the regression line for both MFCV and RMS estimates significantly increased when using intervals < 100 ms (P < 0.001). However, the slope of the regression between EMG variables and force did not change with the duration of the interval (P < 0.001). Moreover, MFCV showed a substantially smaller variability across subjects in its relation to force than RMS [average coefficient of variation of regression slopes across all time intervals, 24.48 ± 1.51 (%), whilst for the RMS it was 56.65 ± 0.69 (%)]. These results indicate that estimates of MFCV and RMS as a function of joint torque are unbiased with respect to processing interval duration. Moreover, they reveal that estimates of MFCV are more consistent across subjects than EMG amplitude.

1. Introduction

During voluntary contractions the central nervous system contract muscles by recruiting and modulating the discharge rate of pools of motor units. The behaviour and properties of the recruited motor units dictate the force level at which a muscle contracts and the characteristics of the generated surface electromyogram (EMG). Therefore, the interference EMG signal can be analysed to infer the neural strategies of muscle activation. Features of the EMG used for this purpose are the amplitude (e.g., root mean square, RMS), spectral moments, and conduction velocity of the propagating action potentials (muscle fiber conduction velocity, MFCV) (Farina et al., 2004b). Some of these features are associated to the force produced by the contracting muscle since they are influenced by motor unit recruitment (Del Vecchio et al., 2017a; Lippold, 1952).

The correlation between EMG amplitude and voluntary force is due to the progressive recruitment of motor units and increase in their discharge rates with increasing force (Del Vecchio et al., 2017b; Dideriksen et al., 2011; Farina et al., 2004a; Lippold, 1952). MFCV is a physiological parameter that is biophysically related to the diameter of the muscle fibers (Del Vecchio et al., 2017a; Håkansson, 1956) and during voluntary contractions represents the weighted mean of the conduction velocities of the action potentials in the processing time interval (Del Vecchio et al., 2017b; Farina et al., 2001). MFCV increases with force because of the orderly recruitment of motor units according to the size principle (Andreassen and Arendt-Nielsen, 1987; Del Vecchio et al., 2018; Henneman, 1957). Moreover, the increase of MFCV with force is related to the progressive recruitment of motor units with greater conduction velocities (Del Vecchio et al., 2017b). Therefore, it has been suggested that MFCV may be used as an indicator of the progressive recruitment of motor units (Andreassen and Arendt-Nielsen, 1987; Del Vecchio et al., 2017b; Masuda and De Luca, 1991).

EMG amplitude and, to a less extent, MFCV have been used to indirectly infer neural control strategies in a wide range of contractions (e.g., jumping, explosive tasks, gait analysis) (Aagaard et al., 2002a; Farina et al., 2004a; Pozzo et al., 2004; Sbriccoli et al., 2003; Tillin

* Corresponding author at: Neuromuscular Research Centre & Technology, Department of Bioengineering, Imperial College London, SW7 2AZ London, UK.
E-mail address: a.del-vecchio@imperial.ac.uk (A. Del Vecchio).

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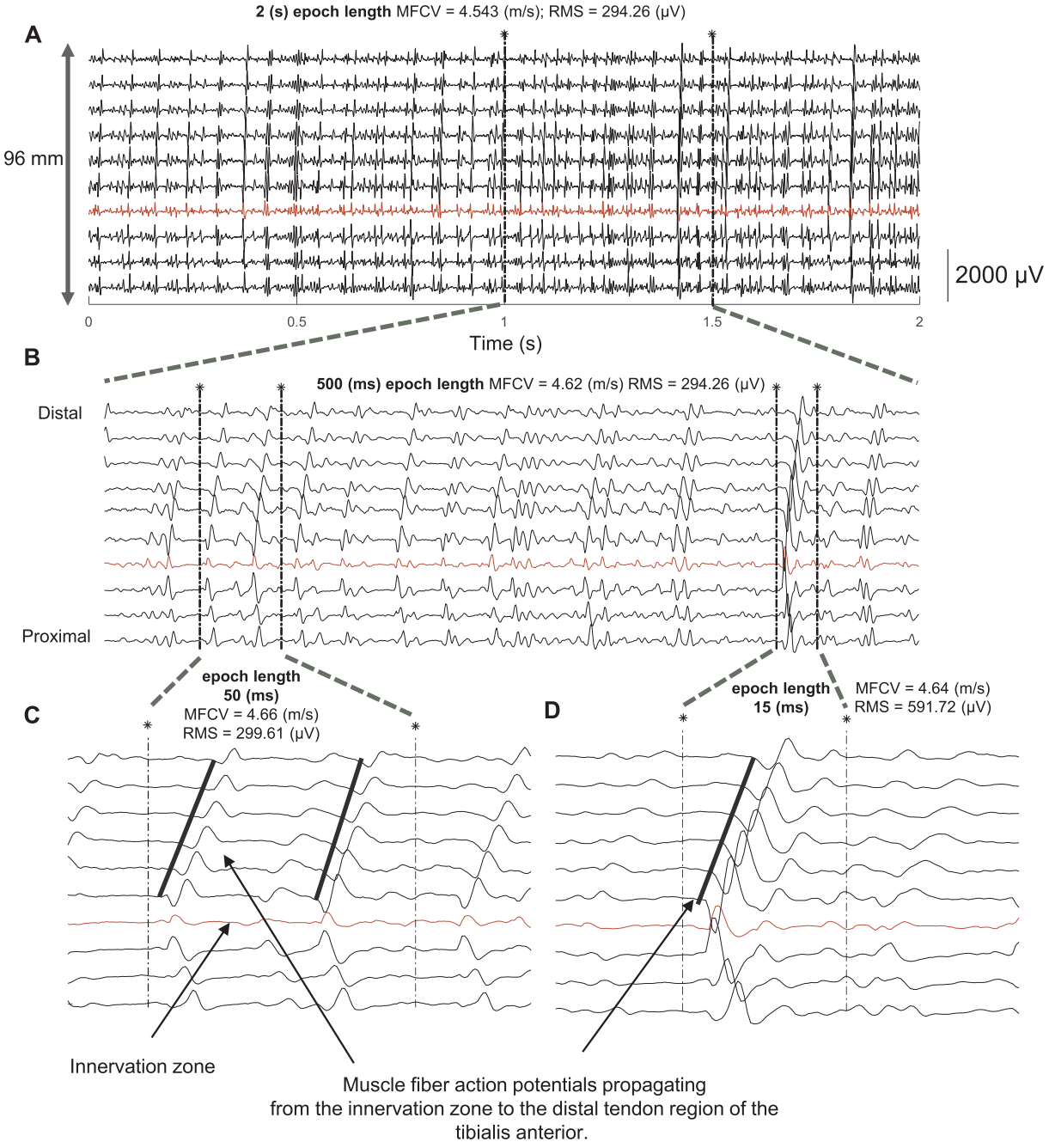

Fig. 1. A. Ten double differential EMG signals during a ramp contraction at approximately 35% MVC. The EMG channel depicted in red represents the innervation zone (IZ). **B, C, D.** Consecutive shortening of the two second EMG time window (**A**) in 500 (B), 50 (C) and 15 (D) ms time intervals. In the two smallest time frames the IZ can be clearly observed with clear propagation of muscle fiber action potentials propagating to the distal tendon region of the tibialis anterior muscle. The EMG signals corresponding to the tilted black line in **C** and **D** were chosen for both muscle fiber conduction velocity (MFCV) and EMG amplitude (root mean square, RMS) estimation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2010). Some of these contractions occur in very short time intervals. For example, during isometric ballistic contractions motor units are fully recruited at ~60 ms from EMG onset (Desmedt and Godaux, 1977). In studies that involve fast and forceful contractions, the EMG amplitude is extracted in time windows as short as ~50 ms (Aagaard et al., 2002b; de Ruiter et al., 2007; Tillin et al., 2010). However, there are no experimental data that evaluate the effect of EMG time interval on the amplitude and/or conduction velocity when correlated to voluntary force contractions. Previous evidence from a simulation (Farina and Merletti, 2000) and an experimental study (Buckthorpe et al., 2012) suggests that the coefficient of variations may significantly increase for EMG segments smaller than 250 ms. However, an experimental investigation of the associations between EMG variables and EMG processing intervals with voluntary force is missing. Moreover, the EMG amplitude shows a large variability between subjects due to different anatomies, thus normalization procedures have been introduced (Farina et al., 2014). These characteristics are critical especially when studying neuromuscular changes over time. Therefore, we studied the variability of MFCV and amplitude across subject and processing time intervals in relation to voluntary force.

The first aim of this study was to investigate the effect of the processing time interval on surface EMG variables when related to voluntary force. We also aimed at quantifying the inter-subject variability of MFCV and EMG amplitude during voluntary force contractions up to

70% maximal force in the tibialis anterior muscle.

2. Methods

Ten healthy men (26.4 ± 1.6 yr; body mass 78.6 ± 6.4 kg; height: 182.2 ± 5.2 cm) were recruited for this study. All volunteers signed an informant consent form that was approved by the Ethical Committee of the Universitätmedizin Göttingen (n. 1/10/12). None of the volunteers reported any history of neuromuscular disorders or lower limb surgery.

2.1. Experimental protocol

The volunteers performed three maximal voluntary isometric contractions (MVC). The greatest force value was recorded and stored on a custom MATLAB program (MathWorks, Inc, USA). The same program displayed the force biofeedback on a monitor. The force feedback consisted of six trapezoidal contractions that linearly increased at a rate of 5% $MVCs^{-1}$ and were sustained for 10 s at either 35, 50 and 70% MVC (two contractions for each force level) and decreased at a rate of 5% $MVCs^{-1}$. The trapezoidal contractions were performed in a random order with a recovery time of 5 min between contractions.

2.2. Force and EMG recordings

The volunteers were seated in a chair of a Biodex System 3 in upright position (Biodex Medical System Inc., Shirley, NY, USA), with the dominant leg (self-reported) extended and the ankle flexed at 30° with respect to neutral position. The ankle joint and the foot were securely fastened with Velcro straps in order to allow a secure connection between the force transducer and the foot. Two reference electrodes separated by ~1 cm were placed on the malleolus of the dominant leg. One dry array of 16 electrodes was placed on the tibialis anterior muscle in order to observe clear motor unit action potential propagation from the distal innervation zone to the tendon. The EMG signal was digitally visualized by a multichannel amplifier USB2+ (OT Bioelettronica, Turin, Italy). An experienced investigator identified the innervation zone and a surgical pen was used to mark its location on the skin (Farina et al., 2002). Afterwards, the skin was shaved and cleansed with 70% ethanol. One grid of high-density surface electrodes (5 columns, 13 rows, gold-coated 1-mm diameter, 8-mm interelectrode distance) (OT Bioelettronica, Torino, Italy) was placed on the skin between the innervation zone and distal tendon, with the fourth row of the electrodes placed on the innervation zone (Del Vecchio et al., 2017a). The grid was fixed on the skin with a bi-adhesive foam with conductive paste between each electrode and the skin. High density EMG 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 force and EMG signal were synchronized at source by the same acquisition system.

2.3. Muscle fiber conduction velocity estimates

After digitally band-pass filtering the signals between 20 and 500 Hz (3rd Butterworth), double differential derivations were computed from the monopolar recordings by differentiating in the longitudinal direction of the bi-dimensional array. From these derivations, 5–7 EMG channels were manually selected by an experienced investigator as corresponding to the clearest motor unit action potential propagation (Fig. 1). MFCV was estimated from the selected channels with a multichannel maximum-likelihood algorithm, previously proposed (Farina et al., 2004c, 2001; Farina and Merletti, 2004). The estimates of MFCV were computed only during the ramp-up phase of the ramp contractions. MFCV was calculated from consecutive time intervals without overlapping. The coefficient of correlation was computed in the selected signals. The duration of each time interval was varied between the values 2000, 1000, 500, 200, 100, 50, 25, and 15 ms. The EMG amplitude was obtained from the same EMG channels used for the estimates of MFCV. The root mean square (RMS) for each of the double-differential signal was first calculated for each double differential EMG signal and then averaged across all the selected channels. Fig. 1C, shows the propagating channels that were selected for the estimates of MFCV and RMS, highlighted with a black line. The association between MFCV and RMS with force was quantified by linear regression (Fig. 5). The mean squared error of the estimates was used as an index of estimation accuracy while the bias in the estimates was assessed from the slope and intercept of the regression line.

2.3.1. Statistical analysis

The Shapiro-Wilk test confirmed normal distribution of all the extracted variables reported in the study. A Pearson product-moment correlation coefficient assessed the significance level for the estimates of MFCV, RMS when plotted as a function of voluntary force in percentages of MVC. The sample standard deviation for each regression line was assessed by computing the normalized root mean square deviation (NRMSD) between the estimates of amplitude and conduction velocity with their respective rate of change as a function of force. Successively, the RMSD was normalized by the range (the maximum RMSD value minus the minimum). The NRMSD was computed for each of the regressions and then averaged across all subjects. The lower NRMSD values indicate less residual variance. The normalization procedure was necessary because of different absolute values for MFCV and EMG amplitudes. The coefficient of variations (standard deviation/mean, in percentages) for MFCV and RMS were obtained for the regression slopes and intercepts between all the subjects and processing time intervals. Finally, a repeated measure ANOVA with Bonferroni correction for multiple comparisons was used to assess differences in the EMG regression values coefficients and NRMSD values. The statistical analysis was completed with MATLAB and the significance P level was set at 0.05. The data are reported as mean ± standard deviation. The reported P value for the individual regressions corresponds to the maximum value obtained across subjects (closer to 0.05).

3. Results

The range of the conduction velocities and EMG amplitudes are reported in Table 1. Fig. 1 shows an example of the effect of interval duration on 10 double differential EMG signals propagating in the bi-dimensional array. The processing of these intervals for both EMG conduction velocity and EMG amplitude during the full duration of a representative contraction for one subject are shown in Fig. 2.

Table 1
Minimum and maximal values of muscle fiber conduction velocity (MFCV, m/s) and amplitude (RMS, μV) of the surface EMG signal. MFCV and RMS estimates are obtained during linearly increasing force contractions up to 70% of maximal voluntary force.

Subject	$MFCV_{MIN}$	$MFCV_{MAX}$	RMS_{MIN}	RMS_{MAX}
1	3.182	3.961	18.79	365.94
2	3.214	3.698	19.70	427.54
3	2.769	3.564	69.25	492.29
4	2.779	3.310	34.23	572.32
5	3.751	4.157	67.63	628.04
6	3.324	3.942	21.62	344.21
7	3.484	4.119	34.10	1067.61
8	3.369	4.033	43.98	1051.31
9	2.857	3.637	30.73	1416.91
10	3.370	3.947	38.05	898.62
Mean	3.210	3.837	37.81	726.5
SD	0.323	0.273	18.09	362.4

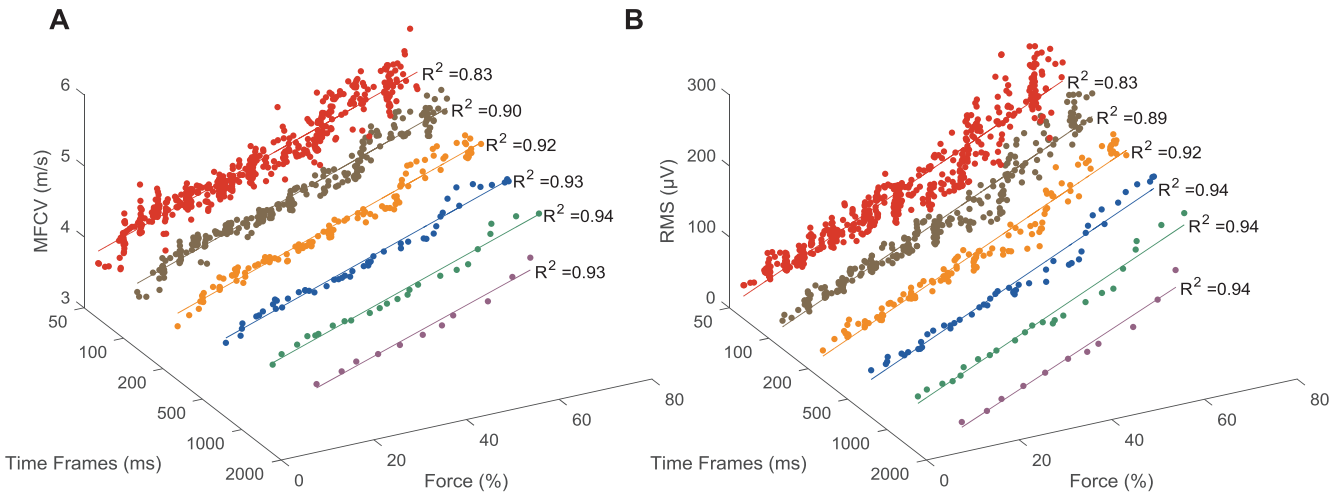

Fig. 2. A. Three-dimensional scatter plot with regression lines for muscle fiber conduction velocity (MFCV, m/s) (**A**) and EMG amplitude (RMS, μV) (**B**) when plotted as a function of force for one representative subject. The three-dimensional scatter plot shows the combined effect of processing time interval and force level during a linearly increasing ramp contraction up to 70% maximal voluntary force on MFCV and RMS.

3.1. Correlations between MFCV, RMS and force

MFCV and RMS were significantly correlated with force for all subjects when using all time interval durations (average R^2 across all subjects and epoch lengths MFCV = 0.77 ± 0.03p < 0.01, RMS = 0.79 ± 0.03Pearson P < 0.01). The average coefficient of correlation in propagation between the selected EMG signals across subject and epoch lengths was 0.56 ± 0.09.

The coefficient of determinations for each epoch length are reported in Fig. 3A. Force prediction power decreased non-linearly with smaller EMG epoch lengths and was maximum in the time frames ranging from 2000 and 200 ms. Below this value, there was a significant decrease for both MFCV and RMS estimates (ANOVA, p < 0.05). Correspondingly, the variability of MFCV and RMS increased with the shortening of processing interval (Fig. 3B). The variability in MFCV and RMS was evaluated with the standard deviation of the normalized estimates with respect to the regression line (normalized root mean square deviation, RMSD) (Fig. 3B). The normalized RMS increased significantly for epoch lengths ≥50 ms for both MFCV and EMG amplitude (Fig. 3B).

However, despite an increase in variability, the regression coefficients for the estimates of MFCV and EMG amplitude were not different for any processing interval (Fig. 4A and B). However, the RMS intercepts showed a larger variability compared to MFCV values (Fig. 4D).

3.2. Inter-subject variability of MFCV and RMS

When the coefficient of variations for the rate of change values was assessed for all the subjects, MFCV showed a one-fold smaller inter-subject variation when compared to the estimates of EMG amplitude (Fig. 5). Fig. 5 shows the MFCV and EMG amplitude inter-subject coefficient of variations of the regression coefficients for each individual epoch length. The average (across time epochs) coefficient of variation for the rate of change of MFCV when plotted as a function of force was 24.48 ± 1.51 (%), whilst for the RMS was 56.65 ± 0.69 (%). The normalized rate of change values for each individual subject are reported in Fig. 6. In this figure, MFCV and RMS values were first normalized for the maximal value during the ramp contractions and then the initial values of the regression coefficient (intercept) were subtracted for all the regression lines. It can be noted that the RMS has a larger inter-subject variability when compared to estimates of MFCV (Fig. 6).

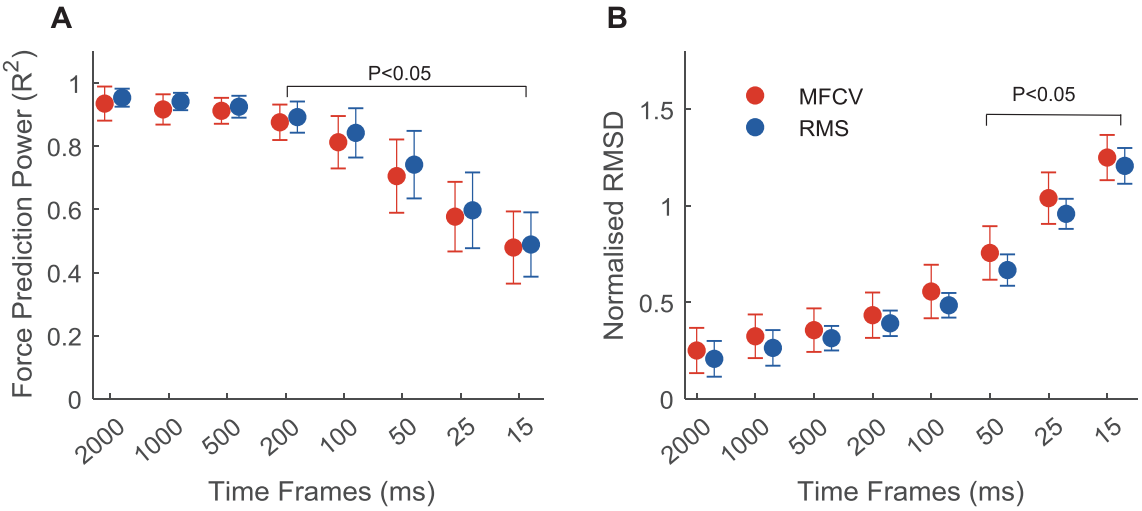

Fig. 3. A. Force prediction power (R^2, averaged across subjects) when plotted as a function of the processing time intervals (ms) for both muscle fiber conduction velocity (MFCV, m/s) and EMG amplitude (RMS, μV). **B.** Normalized root mean square deviations (RMSD, averaged across subjects) as a function of the time frames (ms) used in this study. Significance (P values) and sample standard deviation are given.

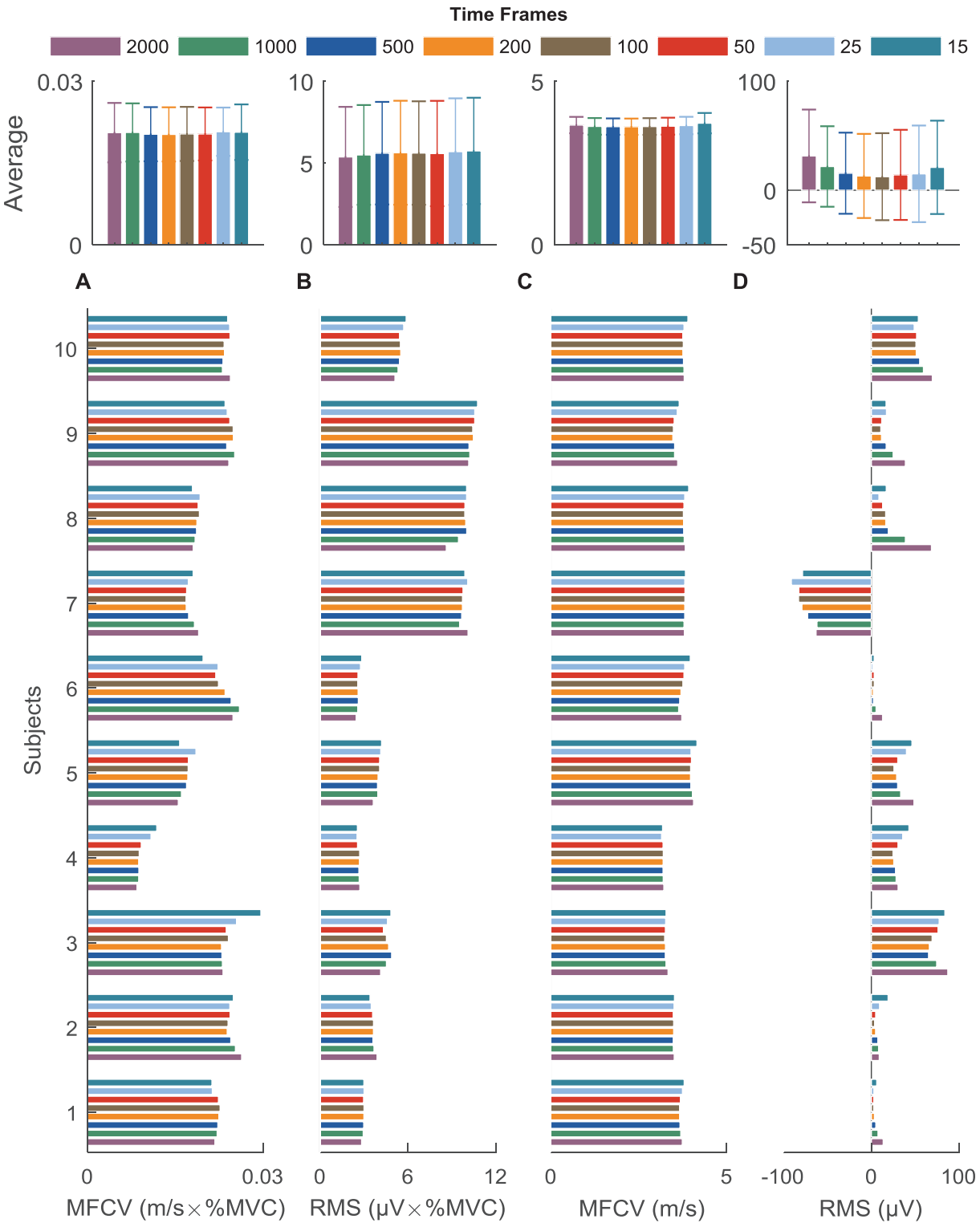

Fig. 4. Subject specific regression coefficient values for muscle fiber conduction velocity (MFCV m/s) and EMG amplitude (RMS, μV). Each color represents a specific processing time interval. The plots in the top side of the figure represent the average across subject for each time interval. **A and B** Regression slopes for MFCV (m/s) and RMS (μV). **C and D** Regression intercepts for MFCV (m/s) and RMS (μV). * = $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

In the present study we clarified in an experimental way the effect of processing time interval on the correlations between surface EMG estimates and voluntary force. Although both MFCV and RMS were linearly related with force in all the time intervals, the processing time interval significantly influenced the correlation strength between MFCV, RMS and force. Specifically, epoch lengths smaller than 100 ms may critically influence the estimates of MFCV and RMS during voluntary force contractions. On the other hand, the rate of change in MFCV and EMG amplitude were not affected by processing time interval. Conversely the RMS intercepts were affected by the processing

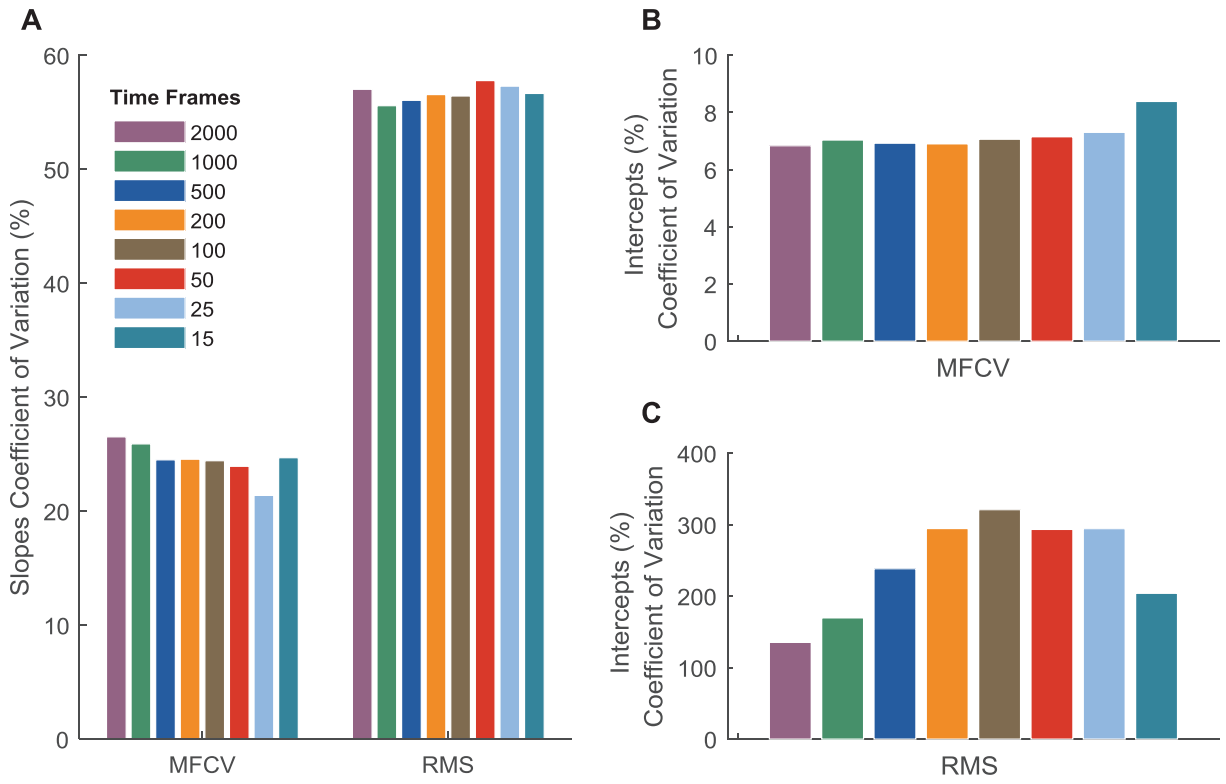

Fig. 5. Inter-subject coefficient of variations (in percentages) for the regression slopes for muscle fiber conduction velocity (MFCV, m/s) and EMG amplitude (RMS, μV) when plotted as a function of maximal voluntary force (%MVC). It can be noted that the RMS variation is approximately one-fold larger than MFCV. **B and C** Inter-subject coefficient of variations (in percentages) for the regression intercepts of MFCV and RMS estimates when plotted as a function of voluntary force. It can be noted that MFCV are more consisted through the different processing time intervals and largely smaller when compared to the RMS.

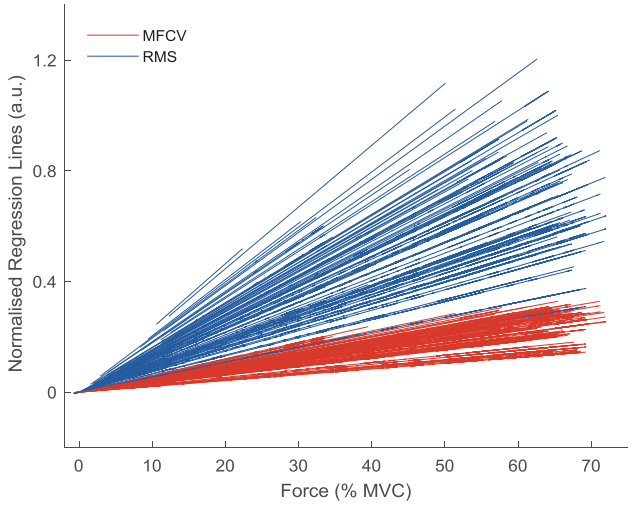

Fig. 6. Normalised regression lines for muscle fiber conduction velocity (MFCV, red) and EMG amplitude (RMS, blue) as a function of voluntary force in percentages of MVC. The intercept of the regression lines was subtracted from the regression slope coefficients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

time interval. Moreover, MFCV showed a one-fold lower variability when compared to those obtained from EMG amplitude estimates. These characteristics highlights the possibility of using MFCV as a neuromuscular parameter in small time frames.

4.1. Surface EMG variables and force

The conduction velocity and amplitude of the action potentials are highly related to voluntary force and this has been shown in many studies (Andreassen and Arendt-Nielsen, 1987; Farina et al., 2004a; Lippold, 1952; Masuda and De Luca, 1991). The conduction velocity of action potentials along muscle fibers is associated to fiber diameter and predicts the progressive increase in motor unit conduction velocity during voluntary force contractions (Blijham et al., 2006; Del Vecchio et al., 2017b; Håkansson, 1956). This association is explained by the fact that larger, higher threshold motor units innervate fibres with larger diameter compared to lower threshold units. This property can be used to indirectly infer the progressive recruitment of motor units during voluntary force contractions. For example, an increase in the rate of change in MFCV when plotted as a function of voluntary force can be associated to an increase in the recruitment of larger, higher threshold motor units. These characteristics require an understanding of the limitations and potentialities of MFCV for assessing the neural control of muscles in different epoch lengths.

For example, it has been recently shown that MFCV estimates obtained in 500 ms time frames predict the progressive increase in motor unit recruitment and motor unit conduction velocity (Del Vecchio et al., 2017b). Specifically, the rate of change in MFCV estimates as a function of force was highly correlated to the rate of change in single motor unit conduction velocity when plotted against motor unit recruitment (Del Vecchio et al., 2017b). Interestingly, in the present study the rate of change of MFCV even for the smallest time window (15) was similar when compared to those obtained in time windows > 500 ms, Fig. 4. This characteristic implies that MFCV obtained in time frames as small as 15 ms can be used to predict the changes in single motor unit conduction velocity (Figs. 3 and 4).

The results obtained in this study also show that the multichannel maximum likelihood algorithm allows an accurate estimates of MFCV even when the coefficient of correlation in propagation between EMG signals is considerably lower to previous proposed algorithms (Merletti et al., 2001). Merletti and colleagues reported that MFCV estimates

obtained with the cross-correlogram method provided reliable estimates only when the coefficient in correlation was more than 0.8. Conversely, we show that MFCV values corresponding to a coefficient of correlations higher ≥ 0.5 provide reliable estimates of force. The effect of processing time interval on conduction velocity estimates with different algorithm is unknown. However, the methodology used in this study has been shown to provide estimates with errors as small as (0.1 m/s, standard deviation) (Farina et al., 2002). Future studies may evaluate the effect of EMG epoch length on estimates of MFCV with different approaches (Farina and Negro, 2007).

The EMG amplitude estimates in the present study exhibited a high inter-individual variability, as shown previously in a vast literature (Basmajian and De Luca, 1985; Farina et al., 2004b). The rate of change in RMS as a function of force was for some subjects as low as $\sim 2\,\mu V$ and for others as high as $\sim 12\,\mu V \cdot \%MVC$. Similarly, the initial RMS regression values for one subject were even negative (Fig. 4). Moreover, the regression intercepts for the EMG amplitudes were also affected by the processing time interval (Fig. 4). The increase in the RMS that is noted in the present study is not related to the recruitment order of motor units, but it is the combination of the increase recruitment and rate coding (Del Vecchio et al., 2017b). Indeed, it has been recently shown that the motor unit action potential amplitudes when plotted as a function of their recruitment thresholds are highly variables between subjects (Del Vecchio et al., 2017b). For this reason, the amplitude of an EMG signals requires normalization, however this procedure is problematic particularly when assessing neuromuscular changes over time. On the other hand, MFCV seems to be a more robust neuromuscular parameter and normalization procedures may not be needed.

To conclude, in the present study we showed that the processing time-interval affects the conduction velocity and amplitude of the surface EMG signal. The variability for both MFCV and RMS significantly increased for epoch lengths smaller than 100 ms. However, the MFCV regression coefficients were not affected by the processing time interval and showed a substantial lower inter-subject variability when compared to amplitude estimates. These characteristics imply that MFCV may be used in time windows as small as 15 ms to infer neural strategies of muscle control and also highlight the robustness of MFCV as a neuromuscular parameter.

Conflict of interest

All authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jelekin.2018.04.010.

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Alessandro Del Vecchio completed an MSc in Exercise Physiology from Loughborough University. Afterwards he received a PhD (Doctor Europaeus) in Human Movement Sciences from the University of Rome "Foro Italico" with a thesis ranked top 2% in the research field. 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.

Ilenia Bazzucchi received her PhD in Sport Science and Health and she is currently an Assistant University of Rome "Foro Italico", Italy. She has recently been part of the Organizing Committee of the XX Congress of ISEK, International Society of Electrophysiology 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 an Associate 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 an Associate Editor (Section Sport and Exercise) 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.