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EVALUATION OF NEUROMUSCULAR FATIGUE THROUGH INNOVATIVE SURFACE EMG PARAMETERS IN HUMANS

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ABSTRACT

The surface electromyogram (EMG) undergoes several changes during an isometric fatiguing muscle contraction. Amplitude and spectral parameters, as well as muscle fiber conduction velocity and fractal dimension of the surface EMG signal, may be used as indirect fatigability indices to monitor these changes. The aims of this thesis were to determine whether surface EMG is a reliable tool for estimating conduction velocity, through a systematic review of the literature; to determine the relationship between muscle force and the selected fatigability indices; and to study the behavior of the fatigability indices in patients with facioscapulohumeral muscular dystrophy (FSHD) during a fatiguing task, with respect to healthy controls. It was hypothesized that the fractal dimension was not related to the intensity of muscle contraction, and that FSHD patients would have shown significant differences in the considered fatigability indices. In the first study (p. 32) high reliability was reported in eight studies and was, in general, associated with using the initial or mean conduction velocity value, using several electrodes (3 to 8), ensuring appropriate electrode positioning, and evaluating muscles with fibers that run parallel to the skin. It was next demonstrated in the second study (p. 52), that the values of fractal dimension and mean frequency of the power spectrum increased with force unless a plateau was reached at 30% maximal voluntary contraction. Finally, the third study (p. 61) showed that FSHD patients presented significantly less steeper slopes of mean frequency of the power spectrum, conduction velocity and fractal dimension, compared to the controls. The results of this thesis demonstrated firstly, that sEMG is suitable for use when investigating conduction velocity, which is sensible to peripheral aspects affecting performance fatigability; secondly, the use of fractal dimension, as index of central factors affecting performance fatigability, may be considered above a certain level of force, regardless of muscle contraction intensity; and lastly, that impaired neuromuscular function caused patients with FSHD to exert a smaller force, yield a longer endurance time and experience lower levels of performance fatigability compared to healthy participants. In conclusion, the use of the fractal dimension of the sEMG signal to infer central aspects of performance fatigability should be considered in particular, in those muscles where motor unit decomposition techniques are limited by anatomical constraints.

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ABBREVIATIONS

ApEn	Approximate Entropy
ANOVA	Analysis of variance
ARV	Average rectified value
AS	Anterior scalene
ATM	Anterior temporal muscle
BB	Biceps brachii
B&A	Bland and Altman
c-c	cross-correlation
CD	Correlation dimension
CIS-fatigue	Checklist Individual Strength, fatigue subscale
CNS	Central nervous system
CoV	Coefficient of variation
CV	Conduction velocity
DD	Double differential
DFA	Detrended fluctuation analysis
DFT-based AA	Discrete Fourier transform alignment algorithm
EMG	Electromyography / electromyogram (mV)
ES	Electrical stimulation
FFT	Fast Fourier transform
FD	Fractal dimension
FSHD	Facioscapulohumeral muscular dystrophy
GRAAS	Guidelines for reporting reliability and agreement studies
H^+	Hydrogen ion
HD-sEMG	High density surface EMG
IAP	Intracellular action potential
IED	Interelectrode distance
ICC	Intraclass correlation coefficient
iMNF	Instantaneous mean frequency
IQR	Inter-quantile range
IZ	Innervation zone
iEMG	Intramuscular electromyography
K^+	Potassium ion

LofA	Level of agreement
maxlike	Maximum likelihood
MDC	Minimal detectable change
MDF	Median frequency of the power spectrum
MeSH	Medical subject heading
MNF	Mean frequency of the power spectrum
MSE	Mean squared error
MSEn	Multiscale entropy
MU	Motor unit
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
NA	Not available
ND	Not defined
(n)SE	(normalized) standard error of the mean
(n)SEM	(normalized) standard error of measurement
n slope	Normalized slope
PPO	Peak power output
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RMS	Root mean squared value
RSE	Roughness scaling extraction
SampEn	Sample Entropy
SCM	Sternocleidomastoid
SD	Single differential
SEM	Standard error of measurement
sEMG	Surface electromyography
SENIAM	Surface EMG for a Non-Invasive Assessment of Muscles
STARD	Standards for Reporting of Diagnostic Accuracy
T1	Trial 1
TA	Tibialis anterior
VL	Vastus lateralis
VMO	Vastus medialis obliquus

PUBLICATIONS AND COMMUNICATIONS

The following publications and communications are a direct consequence of this work:

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

Beretta-Piccoli M, Boccia G, Ponti T, Clijsen R, Barbero M, Cescon C. Relationship between Isometric Muscle Force and Fractal Dimension of Surface Electromyogram. *Biomed Res Int*. 2018 Mar 15;2018:5373846. doi: 10.1155/2018/5373846.

Beretta-Piccoli M, Calanni L, Negro M, Barbero M, Berardinelli A, Siciliano G, Tupler R, Soldini E, Cescon C, D'Antona G. Increased resistance towards fatigability in patients with facioscapulohumeral muscular dystrophy. Submitted to the *European Journal of Applied Physiology*.

COMMUNICATIONS

Beretta-Piccoli M, Boccia G, Ponti T, Clijsen R, Barbero M, Cescon C. (2018).
Relationship between isometric muscle force and fractal dimension of surface electromyogram.
XXII Conference of the International Society of Electrophysiology and Kinesiology, Dublin.

Beretta-Piccoli M, Cescon C, Barbero M, D'Antona G. (2018).
Evaluation of motor fatigability in a facioscapulohumeral muscular dystrophy patient.
Swiss Congress for Health Professions, Zurich.

Beretta-Piccoli M, Calanni L, Negro M, Barbero M, Berardinelli A, Tupler R, Soldini E, Cescon C, D'Antona G. (2020).
Characterization of performance fatigability in patients with facioscapulohumeral muscular dystrophy using surface EMG.
XXIII Conference of the International Society of Electrophysiology and Kinesiology, Virtual Conference.

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BACKGROUND

The conceptual framework of muscle fatigue

It has been recognized since antiquity that intensively exercised muscles show a progressive decline in performance, a phenomenon physiologists term "neuromuscular fatigue" (Allen et al., 2008b). The study of neuromuscular fatigue and the factors that limit, or regulate performance during athletic events, ergonomic tasks and daily activities, intrigued scientists for centuries, but a clear explanation of the etiology of this condition remains elusive (Marino et al., 2011). For instance, between the XVIII and the XIX century, the prevailing theories concerning the causes of muscular fatigue pertained a lack of oxygen [Antoine Lavoisier (1743-1794)] and the presence of poisons [Edward Pflüger (1829-1910) and Nathan Zuntz (1847-1920)]. Later, Angelo Mosso (1889) showed that "fatigue had a central (the will) as well as a peripheral (muscular) component", represented by a reduction in muscular force. According to Mosso, the two phenomena may be distinguished. Although, he acknowledged the inherent difficulties in measuring purely central fatigue, by reminding readers that "it is not will, not the nerves, but it is the muscle that finds itself worn out after the intense work of the brain".

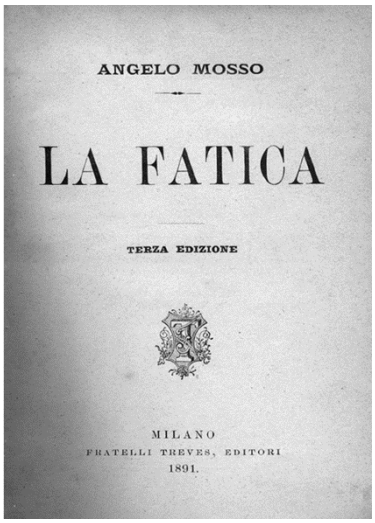

Figure 1 Frontispiece of the third edition of Angelo Mosso's "*La fatica*" (1891).

Mosso's original description of fatigue (Figure 1) was based on the concept of repetitive contractions that provoked neuromuscular adjustments in healthy subjects that were reversible by rest. Consequently, the Mosso dichotomy, in which the phenomenon of force reduction is regarded as distinct from the sensations that arise from prolonged muscular activity, was seen as the fundamental

basis of contemporary research on the physiology of fatigue (Enoka and Stuart, 1992). However, despite the large number of studies that have adopted the central-peripheral dichotomy (e.g. Bigland-Ritchie et al., 1978; Gandevia et al., 1995; Kent-Braun, 1999; Schillings et al., 2003), two major limitations with this approach have precluded the development of a consensus understanding on what causes fatigue (Kluger et al., 2013). In fact, recent studies suggested that it is not possible to identify the etiology of fatigue by attempting to separate the decline in muscle force from sensations about fatigue, particularly during long-lasting contractions (Taylor and Gandevia, 2008). For instance, adjustments in the activation signal discharged by motoneurons during a voluntary contraction begin before a detectable reduction in muscle force, and are attributable to changes occurring within the muscle (Carpentier et al., 2001; Farina et al., 2009). Moreover, most of the physiological processes involved in performing a voluntary action, such as the generation of the motor command or the cross-bridge cycle, can be challenged under appropriate experimental conditions and thereby contribute to the development of fatigue: a phenomenon that has become known as the task dependency of fatigue (Enoka and Stuart, 1992). Moreover, it is known that protocol specifications affect the findings and the underlying mechanisms of fatigue (Enoka, 1995). Different types of protocols are applied in healthy subjects to assess various aspects of fatigue, where submaximal protocols most likely challenge the central nervous system (CNS), while high-intensity exercises challenge the peripheral neuromuscular system (Taylor and Gandevia, 2008; Vøllestad, 1997).

Recently, Enoka and Duchateau (2016), suggested to refine the definition of fatigue and to adopt a unified taxonomy to guide its assessment and management. They proposed the conceptualization of fatigue as a disabling symptom or percept, characterized by feelings of tiredness and weakness, in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability. Fatigue is defined in terms of fatigability, to allow the normalization of the level of fatigue reported by an individual relative to the demands of the task that produces it (Eldadah, 2010). Thus, when a person reports the level of fatigue during ongoing activity, the value at a specific time point will depend on the rates of change in its two attributes: performance and perceived fatigability.

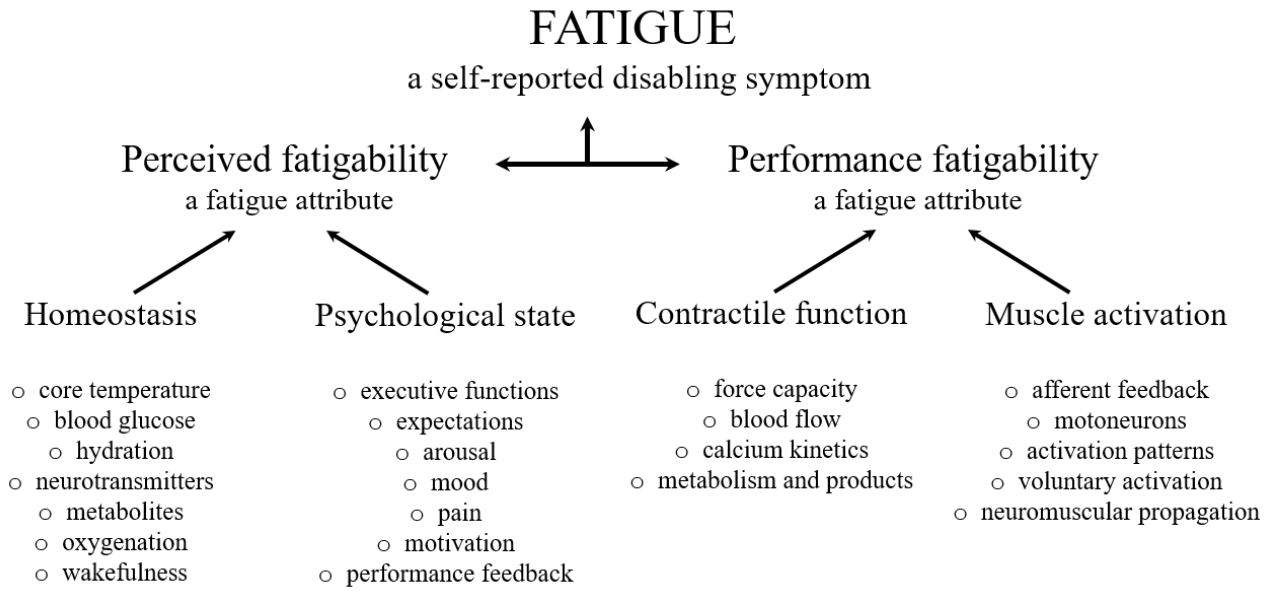

Figure 2 Major factors contributing to the two domains of fatigue: perceived and performance fatigability (adapted from Kluger et al. 2013).

Characterization of perceived fatigability

Perceived fatigability refers to the sensations that regulate the integrity of the performer; these sensations can be modulated by disruptions to homeostasis (e.g. core temperature, hydration status, substrate availability) and modifications in psychological state (e.g. arousal, motivation, mood) that contribute to the perception of effort required for the task (Figure 2). In contrast to performance fatigability, perceived fatigability can be assessed when a person is either at rest (Fieo et al., 2013; Glynn et al., 2015), or performing a physical task (Schnelle et al., 2012; Simonsick et al., 2014). High levels of perceived fatigability at rest may be attributed to deviation of one or more of the modulating factors (e.g. core temperature, hydration, motivation or pain) from normal baseline values. Conversely, perceived fatigability during ongoing activity is derived from rates of change in the modulating factors, which are used to regulate the pace of the performance and thereby control the development of fatigue. Duchateau and Enoka (2016) suggested that, although it seems that the influence of psychological factors on fatigue is mediated via perceived fatigability, it remains to be determined whether or not psychological factors can have a direct effect on fatigue without involving perceived fatigability. Moreover, perceived fatigability may be assessed by self-report scales under different constructs, such as physical or cognitive, or state versus trait.

Characterization of performance fatigability

Performance fatigability (commonly termed muscle fatigue) is an acute activity-induced reduction of force or power of a muscle, and in the laboratory is typically quantified as the decline in an objective measure of performance, such as the production of maximal voluntary force, the time to failure of a submaximal task, or the involuntary twitch response to electrical stimulation (Enoka and Duchateau, 2016). Performance and perceived fatigability are interdependent and they interact to modulate and determine the symptoms of fatigue.

Although the taxonomy illustrated in Figure 2 lists many of the factors that can influence each attribute of fatigue (performance fatigability and perceived fatigability), the scheme acknowledges that most voluntary actions performed by humans involve significant interactions between the two domains. For example, several of the modulating factors that contribute to perceived fatigability, including the levels of blood glucose (Nybo, 2003), core temperature (Nybo, 2008), arousal (Klass et al., 2012), and mood (Steens et al., 2012), can all modulate the capacity of the individual to generate the required amount of voluntary activation, which is a factor that influences performance fatigability. Similarly, afferent feedback generated during high-intensity exercise can influence the adjustments required to maintain homeostasis and thereby contribute to perceived fatigability (Kennedy et al., 2015; Sidhu et al., 2013). The key feature of this scheme is that the level of fatigue experienced by an individual emerges from the many adjustments that occur in the modulating factors within and between each fatigability attribute. With this construct, fatigue is defined as a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability. As indicated in Figure 2, the level of fatigue experienced by an individual can be modulated by challenges to homeostasis, disturbances in the psychological state, reductions in contractile function, and limitations in the capacity to provide an adequate activation signal to the involved muscles (Enoka and Duchateau, 2016).

According to the definition proposed by Kluger et al. (2013), fatigue may be considered as a single entity which does not need to be modified by an accompanying adjective, such as central fatigue, mental fatigue, muscle fatigue, peripheral fatigue, physical fatigue, or supraspinal fatigue. Therefore, since the new definition considers fatigue as a symptom, its assessment requires the individual to interpret relevant psychological and physiological factors by providing responses to standardized questions (Avlund, 2013; Bennett et al., 2014; Halson, 2014; Schmidt et al., 2012; Yang and Wu, 2005). Consequently, conventional measures of fatigue, such as the time to complete a defined task, the reduction in maximal voluntary contraction (MVC) force or the decline in power production, may

be regarded as indices of performance fatigability, but do not provide a measure of the symptom (Enoka and Duchateau, 2016).

Neuromuscular mechanisms

As illustrated in Figure 2, many factors contribute to performance fatigability, which may be differentiated in central and peripheral. Central factors results from a combination, though not well understood yet, of intrinsic motoneuronal properties and decrease in voluntary activation of the muscle, which causes a decrease in the number of recruited motor units (MUs) and their discharge rate. Gandevia (2001) suggested that a reduction in the neural drive command from supraspinal sites that controls a muscle, results in a decline in the tension development (Fuglevand, 1996). Moreover, the discharge rate of MUs decreases to match the change in the mechanical state of the muscle during the fatiguing task (Bigland-Ritchie et al., 1983a), a mechanism called the "muscle wisdom" (Barry and Enoka, 2007). The changes in the discharge rate have been suggested to be protective mechanisms to prevent muscle failure whenever the task was continued at the same intensity (Bigland-Ritchie and Woods, 1984).

In contrast, peripheral contributors, include alterations that occur locally from excitation to muscle contraction, such as in neuromuscular action potential propagation, and decreases in the contractile strength of the muscle fibers, thus affecting perturbation of calcium ions release from the sarcoplasmic reticulum, accumulation of inorganic phosphate, and/or transient large increase in adenosine diphosphate concentration (Boyas and Guevel, 2011). The peripheral factors concur in an hampered execution of the descending central commands (Allen et al., 2008b). Some of these mechanism are affected by blood flow, which is blocked during high contraction intensity (Crenshaw et al., 1997, Sjogaard et al., 1988), causing the muscle to operate in ischemic conditions, with progressive accumulation of metabolites (Dideriksen et al., 2011; Dideriksen et al., 2010a).

The quantification of performance fatigability

At present there is no gold-standard to assess performance fatigability nor in healthy subjects, neither in chronic conditions; nonetheless, three categories of outcome measures used in literature may be

identified: (1) strength-based outcomes, (2) neurophysiological outcomes and (3) indirect outcomes, as suggested in the systematic review of (Severijns et al., 2017).

In isometric conditions, strength decline is calculated as the ratio between the initial and the final strength during sustained or repetitive contractions, or the slope of the strength decline (e.g. Borji et al., 2013; Homma et al., 2015; Mehta and Agnew, 2012; Rantanen et al., 2000). Other authors reported a statistical comparison of the maximal strength, assessed before and after a specific task (e.g. Delextrat et al., 2018; Severijns et al., 2016). Moreover, during isokinetic protocols, the ratio between the work done during the first contractions versus the last contractions (or the slope of torque decline) may be used (e.g. Clarkson et al., 1982; Hameau et al., 2017).

Neurophysiological outcomes may help the researcher in exploring underlying mechanisms of fatigability. For instance, the twitch interpolation technique has been validated and is extensively employed in neuromuscular research to determine the changes in central and peripheral activation of the muscles under investigation, and used as an indication of loss of central drive ("central fatigue") and "peripheral muscle fatigue" (Allen et al., 1995; Gandevia et al., 2013; Kent-Braun and Le Blanc, 1996; Lepers et al., 2002). Nonetheless, recently the accuracy of the twitch interpolation technique has been questioned (Taylor, 2009), suggesting that it mainly reflects the amount of muscle activation in a qualitative way (Herzog, 2009).

Indirect measures comprise different types of outcomes, such as the time until a maximal voluntary force declined to 50% of the initial MVC (e.g. Peters and Fuglevand, 1999) or the number of repetitions performed until inability to maintain a target force (e.g. Dodd et al., 2011; Grisdale et al., 1990). In addition, particularly in people with impaired ambulation, performance fatigability may be assessed as the deceleration index after a walking task (e.g. Phan-Ba et al., 2012), or as the distance walked (e.g. Leone et al., 2016; McDonald et al., 2013; Mercuri et al., 2016). Finally, indexes of fatigability may be extracted from gait kinematics (e.g. Boudarham et al., 2013; Engelhard et al., 2016; Sehle et al., 2014) and electromyography (Vøllestad, 1997).

Electromyography

Changes in the electromyogram were first used to investigate fatigue in the 1950s (Cifrek et al., 2009), and are now one of the most widely used indirect indices of performance fatigability in humans (Vøllestad, 1997). There are two types of electromyography (EMG): intramuscular EMG (iEMG)

and surface EMG (sEMG); with sEMG being the most commonly used, due to the fact of being non-invasive, with signals recorded from the skin surface (Merletti and Farina, 2016).

Intramuscular EMG

Intramuscular EMG has been used for decades to investigate muscular pathophysiology, with concentric needles inserted into muscles. One of the major advantages of this technique consists in the fact that the effect of the volume conductor that separates the muscle fibers from the detecting electrodes is reduced (Merletti and Farina, 2009). Consequently, in 1929 Adrian and Bronk showed for the first time, that the action potentials from individual motor units (MUAPs) in a voluntary muscle contraction, can be easily identified and recorded by means of a concentric needle electrodes, at least at moderate force levels.

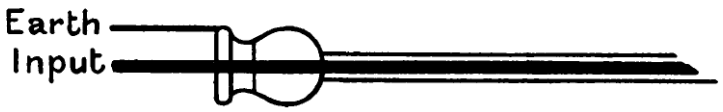

Figure 3 Representation of a concentric needle electrode used in the study of Adrian and Bronk (1929).

Nowadays, due to the high sensitivity of iEMG in detecting very small amplitude potentials (such as fibrillation potentials) and the accuracy to assess subtle changes in an individual MUAP, this technique is considered as a major component of a standard electrodiagnostic examination to assess clinical neuromuscular problems (Daube and Rubin, 2009; Rubin, 2012). In fact, iEMG provides complimentary information to nerve conduction studies, to help localize a disorder and characterize the underlying pathologic changes that occurs in MUs within muscles. Currently, for most clinical recordings, concentric or monopolar electrodes are used (Dumitru et al., 1997). In addition, to record the activity of only a single or few muscle fibers, single-fiber needle electrodes, with a 25-µm diameter, may be used (Ekstedt et al., 1969; Stålberg and Ekstedt, 1971). Single-fiber EMG was developed to study the microphysiology of the MU, such as the conduction velocity (CV) of individual muscle fibers (Stålberg, 1966), the distribution of muscle fibers within individual MUs, and neuromuscular jitter (Sanders and Stålberg, 1996; Stålberg and Trontelj, 1994), which is considered as the most sensitive measure of neuromuscular transmission and has become a widely accepted technique in assessing myasthenia gravis and Lambert-Eaton myasthenia (Giannoccaro et al., 2020; Wolfe et al., 2019).

Eventually, intramuscular bipolar fine-wire electrodes for the detection of iEMG signals were proposed by Basmajian and Stecko (1962). This type of electrodes consist of a pair of non-oxidizing wires with insulation, placed in the cannula of a needle and bent at the tip; the needle is inserted into the muscle and then removed and discarded, while the wires are left in the muscle (Basmajian, 1978). The advantage with regard to concentric needle electrodes is that the wires can be hardly felt when the needle is withdrawn, thereby allowing intense contractions without discomfort or pain for the subject. However, upon removal of the needle, their location cannot be changed, while a needle electrode may be relocated inside the muscle to search for a suitable position. Wire electrodes are usually favored in studies where the iEMG signals are collected over long periods of time or during movement because they are more stable than needle electrodes (Merletti and Farina, 2009).

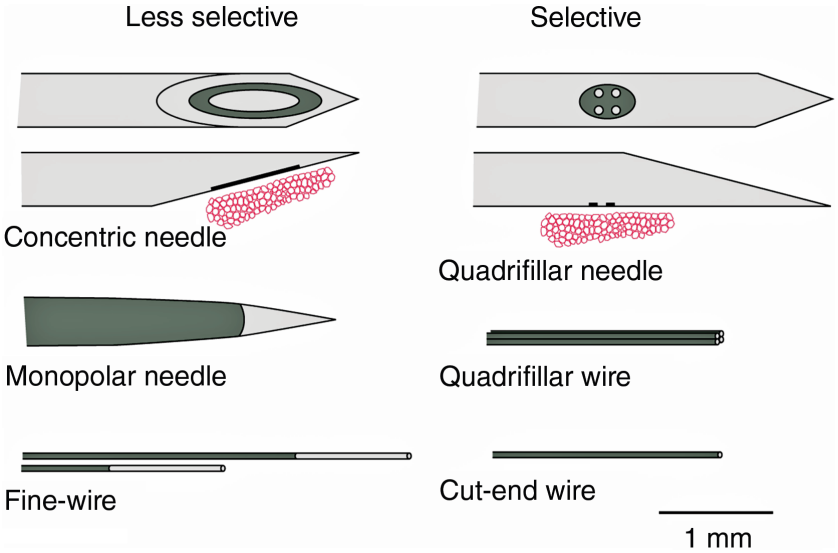

Figure 4 Examples of needle and fine-wire electrodes for recording iEMG signals. Selective electrodes are close to fewer fibers and detect fewer and simpler MUAP waveforms. Less selective electrodes are close to more fibers and detect more and more complex shaped MUAP waveforms (from Marateb and McGill, 2016).

Besides pathophysiological studies, iEMG may be used also to investigate changes in MU behavior (e.g. recruitment and firing patterns) induced by a fatiguing task, which gives information about CNS motor control and its disturbances (Bigland-Ritchie et al., 1983a; Moritani et al., 1986; Trontelj and Stålberg, 1995). Moreover, through full decomposition of multi-unit intramuscular signals it is possible to obtain complete discharge patterns of multiple, simultaneously active MUs (McGill et al., 2005), to investigate adaptations to fatigue, such as MU synchronization, a phenomenon described as a higher occurrence of nearly simultaneous discharge of different MUAPs than expected by chance (Datta and Stephens, 1990; Nordstrom et al., 1992).

Surface EMG

The signal from the sEMG is the instantaneous algebraic summation of the electrical contributions made by the recruited MUs, in response to the activation provided by innervating motor neurons (Farina et al., 2004a, 2014). In contrast to iEMG, the information extracted from the sEMG is considered a global measure of MU activity of the selected muscle. Moreover, amplitude and power spectrum of the sEMG signal are dependent on the timing of the MUAPs and the membrane properties of the muscle fibers, suggesting that the sEMG is reflective of both central and peripheral properties of the neuromuscular system (Farina et al., 2004a).

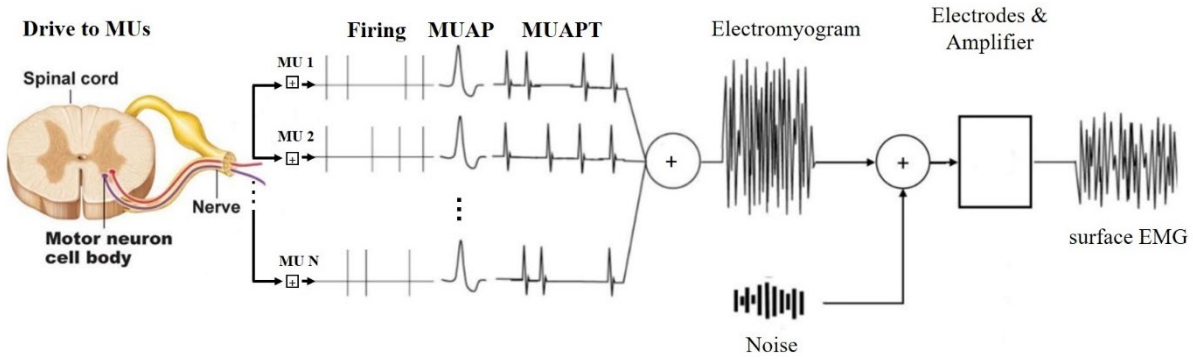

Figure 5 Generation of surface electromyogram (sEMG) from motor unit action potentials (MUAP). The recorded sEMG differs from the physiological EMG due to noise and filtering introduced by the detection (adapted from Merletti and Muceli, 2019). MU, motor unit; MUAPT, motor unit action potential train.

sEMG has been used in a number of different applications, such as in estimating muscle force, exploiting the almost linear relationship between signal amplitude and force (Bigland-Ritchie et al., 1981; Inman et al., 1952; Lippold, 1952); to investigate muscle activity during gait analysis (Sutherland, 1966); or to evaluate fatigability of skeletal muscles (Merletti et al., 1990). More recently, the development of multi-channel electrode arrays (Merletti et al., 2003) expanded the number of applications of sEMG to other fields, such as neurorehabilitation (e.g. Liu et al., 2020), obstetrics (e.g. Cescon et al., 2014; Zacesta et al., 2018), occupational medicine (e.g. Rathleff et al., 2016), ergonomics (e.g. Januario et al., 2016) and assessment of interventions (reviewed in Drost et al. 2006; Frigo and Crenna, 2009). Finally, the combination of techniques of spatial filtering (De Luca et al., 2006), spatial sampling (Gazzoni et al., 2004) and source separation (Holobar and Zazula, 2007) has provided a robust solution to fully decompose the sEMG signal into the discharge times of single MUs, with an accuracy comparable to iEMG decomposition (Del Vecchio et al., 2020). This technique was applied for example to investigate neural strategies for the generation of movement in

newborns, where iEMG is not applicable (Farina, 2020) or in wearable neural interfaces for prosthetic control (Aszmann et al., 2015, Farina et al., 2017).

One of the pioneer studies that used sEMG techniques to track changes in the EMG signal during a fatiguing task was conducted by Piper (1912), who observed a progressive "slowing" of the signal during isometric voluntary sustained contractions. This phenomenon consisted in a shift of the spectral components of the signal towards lower frequencies (Piper rhythm). Besides such a frequency shift, Cobb and Forbes (1923) found a consistent increase in the amplitude of the sEMG signal.

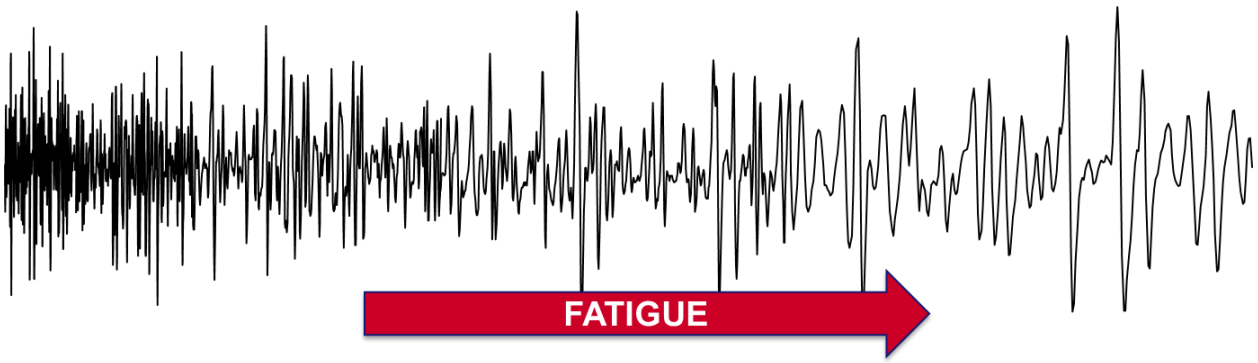

Figure 6 Exaggerated artificial representation of the changes affecting the surface electromyogram during an isometric fatiguing task.

The traditional approach to acquire sEMG signals, which is still very often used in physiological and clinical studies, is based on a pair of electrodes placed on the skin (bipolar detection) in the region above the muscle. The signal detected however, is strongly dependent on the location, the interelectrode distance (IED), the size of the electrode pair, and the position along the muscle fiber, which can result in very different amplitude and spectral characteristics (Barbero et al., 2012; Farina et al., 2002b; Farina and Merletti, 2001; Merletti and Muceli, 2019; Mesin et al., 2009b; Nishihara et al., 2010). The main advantage of bipolar sEMG is its high suitability for assessing global muscle activation in dynamic actions, such as sports, but inferences regarding MU behavior (such as recruitment properties and rate coding) are limited. A relatively more recent approach consists in the use of multiple electrodes aligned in one- or two-dimensional arrays. When the grid of electrodes is dense, this sEMG representation is referred to as high-density sEMG (HD-sEMG, Figure 7).

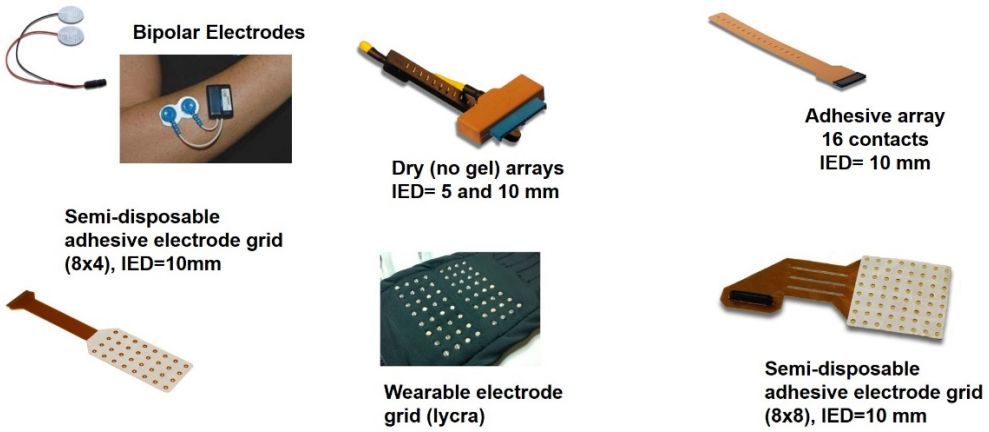

Figure 7 Examples of surface EMG electrodes. IED, interelectrode distance (from http://www.robertomerletti.it).

At least three detection modalities (also called electrode montages, or spatial filters) that can be applied when using linear electrode arrays: monopolar, single differential (SD) and double differential (DD; Figure 8) (Merletti and Farina, 2016). The monopolar provides the voltage of each electrode of the array with respect to a reference; the SD provides the output of the set of differential amplifiers and is obtained by taking the difference between adjacent channels; the DD provides the difference between adjacent SD channels. Each of these modalities gives three different signals with different properties. Monopolar montage senses all of the information in the signal but is the most prone to common disturbances affecting all channels, such as the end-of-fiber effect. The SD montage reduces the common components, and facilitates the identification of the innervation zone.

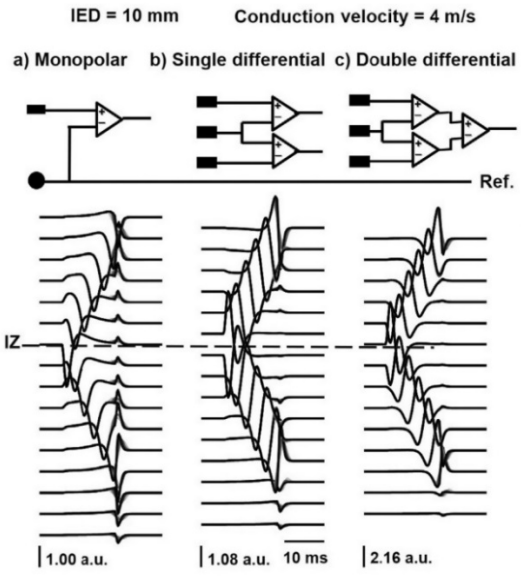

Figure 8 Examples of simulated monopolar (a), single differential (b) and double differential (c) motor units action potentials as detected with a linear array. The a.u. units are arbitrary units that allow amplitude comparisons (from Merletti and Muceli, 2019). IZ, innervation zone.

The DD montage further attenuates non-propagating signals and is therefore preferred in estimating muscle fiber CV (Cescon et al., 2008; Farina et al., 2001b), whose measurement is influenced by the presence of non-propagating signals. Moreover, the three detection modalities have different volumes of detection and therefore detect a different number of MUs: the monopolar montage can detect "far" sources while the SD and DD modalities are more selective and detect "close" sources (Merletti and Muceli, 2019).

The multi-channel approach grants access to a set of physiologically relevant variables on the global muscle level or on the level of single MUs, providing new methods for the study of performance fatigability. For instance, multi-channel sEMG allows (1) a more precise estimation of muscle fiber CV, (2) the assessment of regional changes in the sEMG signal due to fatigue and (3) the analysis of single MUs, with the chance to obtain information about MU control and fiber membrane changes (Gazzoni et al., 2017; Merletti et al., 2003).

Bipolar electrodes

The relation between the sEMG signal and changes occurring in a muscle during a fatiguing task is very complex and affected by many factors. Therefore, in order to reduce the influence of some of these factors, the first type of contraction that was studied using sEMG techniques, was isometric or static contractions (Lloyd, 1971). However, although the recording of the signals is easier with respect to dynamic contractions, even in isometric constant force contractions, many factors affect the sEMG features (Table 1), ranging from anatomical to the detection system, to the estimation algorithm used (Farina et al., 2002b; Farina et al., 2002d; Farina and Merletti, 2000; 2001; Farina et al., 2001a), complicating the interpretation of the acquired signals (several factors were described in details in Study 1). In contrast to isometric contractions, the interpretation of the sEMG signal changes during fatiguing dynamic contractions, is complicated by a number of other factors, such as the changes in joint angle, that causes a shift in the underlying muscle fibers with respect to the recording electrodes, and the non-stationary nature of the signal, which causes the fact that classical spectral parameters may not be appropriate for extracting information (Farina, 2006; Merletti and Farina, 2016).

Table 1 Factors that influence the surface EMG signal (modified from Negro, 2020).

BIOPHYSICAL	Anatomical	Spatial distribution of motor unit fibers Fibers' length Spread of the innervation zone and tendon regions among motor units Presence of more than one pinnation angle	PERIPHERAL	Fiber membrane properties	Average muscle fiber conduction velocity Distribution of motor unit conduction velocities Distribution of muscle fibers conduction velocities within the motor units Shape of the intracellular action potential
	Detection system	Skin-electrode contact (impedance, noise) Spatial filter for signal detection Interelectrode distance Electrode size and shape Electrodes location	CENTRAL	MU control properties	Number of the recruited motor units Distribution of motor unit discharge rates Statistics and coefficient of variation for discharge rate Motor unit synchronization
	Geometrical	Inclination of detection system relative to muscle fibers orientation Muscle fiber shortening Muscle shift relative to the detection system			
	Physical	Conductivities of the tissues			

For instance, the two main factors that impacts on the sEMG signal in isometric conditions, are the decrease in muscle fiber CV and the variations of shape and increase of the spatial support and time duration of the transmembrane action potential, also called intracellular action potential (IAP) (Andreassen and Arendt-Nielsen, 1987; Arendt-Nielsen et al., 1989; Dimitrov et al., 2008, Dimitrova and Dimitrov, 2003). The decrease in muscle fiber CV impacts on the sEMG power spectrum causing a compression towards lower frequencies (Brody et al., 1991; Kupa et al., 1995).

A fundamental characteristic of sEMG signals that are recorded during isometric constant force contractions is that the signal can be assumed to be stationary, thus allowing frequency-based techniques, such as Fourier transform or discrete fast Fourier transforms to be used to determine changes in the sEMG signal due to performance fatigability. However, even in such a controlled condition, non-stationarities may manifest, often related to the appearance of fatigue or changes in temperature (Bonato et al., 2001).

In the last 40 years a large number of parameters extracted from the sEMG signal to indirectly assess performance fatigability was developed. It is not the intention of this thesis to describe them all, but rather to review and further discuss the classical (amplitude and spectral parameters), non-linear parameters and the estimation of muscle fiber CV. Some recent works in literature give an exhaustive

overview and critical analysis of methods for EMG fatigue evaluation using bipolar electrodes (Cifrek et al., 2009; Gonzalez-Izal et al., 2012; Merletti and Farina, 2016; Rampichini et al., 2020; Rogers and MacIsaac, 2013) or with a multi-channel approach (Gazzoni et al., 2017).

1. Amplitude-based parameters

The averaged rectified value (ARV) and the root mean squared value (RMS, which is the square root of the area under the power spectrum) are the main parameters used to investigate the amplitude of the sEMG signal:

$$ARV = \frac{1}{n}\sum_{n} \left| x_n \right|$$

$$RMS = \sqrt{\frac{1}{n}\sum_{n} x_n^2}$$

where x_n are the values of the sEMG signal, and n is the number of samples.

Initially, the amplitude of the signal was related to central factors only (recruitment and discharge rates of the active MUs (Moritani et al., 1986; Solomonow et al., 1990)). In fact, during maximal isometric contractions amplitude falls progressively, in parallel with the decrease in force (Bigland-Ritchie et al., 1983a; Bigland-Ritchie et al., 1983b; Bigland-Ritchie and Lippold, 1979), whereas during submaximal contractions rises gradually, as consequence of additional muscle fibers recruitment, in an attempt to maintain the same contraction intensity (Bigland-Ritchie and Woods, 1984; Viitasalo and Komi, 1977). In addition the behavior of amplitude in dynamic contractions is similar, as it was shown to decrease during exercises at maximal intensity (Komi and Tesch, 1979) and increase during submaximal dynamic exercises (Tesch et al., 1990). Later, Dimitrov et al. (2006), Dimitrova and Dimitrov (2002 and 2003) found that changes in amplitude during submaximal contractions are mostly due to peripheral factors (the shape and conduction velocity of the IAPs). Moreover, Arabadzhiev et al. (2010) remarked that the use of RMS as estimate of neural drive in fatiguing submaximal contractions could be a misleading approach, since changes in amplitude result mainly from lengthening of the IAP profile. In order to reduce the effects of peripheral factors the authors suggested to normalize RMS, with respect to the initial value (i.e., intercept with the y-axis).

Other normalization methods were recently reviewed and discussed in Besomi et al. (2020). Finally, changes in the shape of IAP may modify also the EMG-force relation, which may become non-linear, as has been observed experimentally during contractions that are sustained at a constant force (Carpentier et al., 2001; De Luca, 1984; Fuglevand et al., 1993b; Merletti and Lo Conte, 1997). Moreover, Dideriksen et al. (2010b) showed through a computational model, that amplitude and force were different depending of the fatiguing protocol. The relation between amplitude and force during short isometric contractions was investigated in study 2.

2. *Spectral parameters*

Two characteristic frequencies have been used to quantify the changes in the spectral content, based on the Fourier transform: the mean (or centroid, MNF) and the median frequency of the power spectrum (MDF). The MDF is the 50th percentile of the power spectrum, i.e. the value spitting it in two parts of equal energy (Gonzalez-Izal et al., 2012):

$$\int_{f1}^{fmedian} PS(f) \cdot df = \int_{fmedian}^{f2} PS(f) \cdot df$$

where PS(f) is the power spectrum calculated using the Fourier transform, and f1 and f2 determine the lowest and highest frequency of the bandwidth, respectively, typically ranging from 20 to 400 Hz. MNF is however calculated as follows:

$$MNF = \frac{\int_{f1}^{f2} f \cdot PS(f) \cdot df}{\int_{f1}^{f2} PS(f) \cdot df}$$

where PS(f) is the sEMG power spectrum calculated using Fourier transform, and f1 and f2 determine the bandwidth of the surface electromyography (f1 = lowest frequency and f2 = highest frequency of the bandwidth).

MDF and MNF are related to changes in muscle fiber CV and subsequent changes in the IAP duration (Bigland-Ritchie et al., 1981). It was shown during static contractions that MNF shifts towards lower frequencies during increasing fatigue (Lindström et al., 1977; Merletti et al., 1990; Merletti and Lo Conte, 1997; Viitasalo and Komi, 1977), due to the diminished CV as a consequence of local

metabolic changes in the working muscle, mainly H^+ and K^+ distribution across the sarcolemma (Dimitrova and Dimitrov, 2003; Masuda et al., 1983). However, the modifications of the MUAP shape, MU firing rate and synchronization may also contribute to MNF changes (Bigland-Ritchie and Woods, 1984; Brody et al., 1991; Gabriel and Kamen, 2009). MDF is less sensible to noise (Hof, 1991) and more sensitive to simulated variations in the sEMG spectrum (Bonato et al., 2001) than MNF, in particular during dynamic contractions. The probability of discerning the relative contribution of physiological, anatomical and source of detection affecting spectral descriptors, as regards amplitude descriptors, requires careful reflection.

The behavior of the spectral variables during dynamic contractions was shown to be variable: Tesch et al. (Tesch et al., 1990) found decrements of MNF, whereas others observed no change during fatiguing walking exercises (Ament et al., 1996; Arendt-Nielsen and Sinkjær, 1991), for various reasons, collectively termed dynamic factors. Merletti and Farina (Merletti and Farina, 2016) indicated that those factors include recruitment and de-recruitment of active MUs near to the electrodes, the time-varying spatial filter which changes as the muscle change its shape (Mesin et al., 2006), and the movement of the innervation zone relative to the surface electrodes. Moreover, also the skin and intramuscular temperature may have an effect on spectral variable during dynamic tasks (Coletta et al., 2018; Petrofsky and Lind, 1980), though it does not act as a primary factor (Masuda et al., 1999). Collectively , these findings suggest that, when certain methodological measures are taken, traditional spectral descriptors may be well adapted for studying fatigue under both isometric and dynamic conditions.

Besides parameters derived from the Fourier-transform, which apparently were considered as not sufficiently sensitive for studying performance fatigability during dynamic contractions, the use of time-frequency techniques was proposed, such as the instantaneous mean frequency (iMNF, Bonato et al. 2001) and wavelet spectral parameters (González-Izal et al., 2010). However, even though the first studies suggested that iMNF was affected by the same physiological factors as the classical spectral parameters, Farina et al. (2014) showed that during simulated ramp contractions, no association between the estimates of iMNF and recruitment and de-recruitment of MUs was found, suggesting the iMNF was insensitive to changes in MU population during a fatiguing task.

3. Non-linear parameters

Over the last quarter of century, great interest has been given in literature, particularly in the fields of physics, mathematics and chaos theory, to non-linear dynamics. Unlike linear systems, which are simple, proportionate and can be viewed as the sum of their parts (Goldberger, 1996, 2006), non-linear systems are characterized by a lack of proportionality, with small adjustments having dramatic, unpredictable consequences, thus restricting their ability to predict their long-term behavior (Peng et al., 2009). Non-linear systems are regarded as complex, chaotic and unpredictable; characteristics which are of great interest to scientists.

Previous works suggested that also the sEMG waveform could be better modeled as an output of a non-linear dynamic system, rather than as a stochastic output of a linear white-noise driven system (Abarbanel et al., 1989; Nieminen and Takala, 1996). As a non-linear signal, sEMG displays chaotic behavior, i.e., its time series (1) evolves over the time, (2) depends on the initial state, and (3) is fractal in the terms of dimensionality (Nieminen and Takala, 1996). Non-linear analysis offers a powerful approach for the investigation of physiological time series because it provides a measure of the signal complexity, and may be able to detect additional EMG changes during a fatiguing task. Moreover, it has been found that non-linear parameters, such as a entropy, percent of determinism based on recurrence quantification analysis, and dimensionality based on fractal analysis are highly sensitive for hidden rhythms on sEMG in subjects under fatigue and condition of increased MU synchronization (Del Santo et al., 2007; Farina et al., 2002c; Filligoi and Felici, 1999; Gitter and Czerniecki, 1995).

3.1 Entropy

Entropy, as expressed in the second law of thermodynamics, is a measure of disorder or randomness which, in an isolated system, tends to a maximum (Schneider and Kay, 1994; Seely and Macklem, 2004). As far as dynamic systems are concerned, entropy can be defined as the rate of information output (Eckmann and Ruelle, 1985; Richman and Moorman, 2000; Seely and Macklem, 2004) and can be used to measure the apparent randomness and regularity of a system , i.e. the complexity (Pincus, 1991; Seely and Macklem, 2004). A number of parameters were developed to estimate the entropy of the sEMG signal, e.g. Pincus (1991) developed the Approximate Entropy (ApEn), as a model-independent quantification of the regularity of sequences and time-series data, motivated by applications to relatively short, noisy data sets. Richman and Moorman (2000) developed a new

parameter, called Sample Entropy (SampEn), which was shown to be more consistent and performant than ApEn, although the former parameter has been used less widely than the latter. Thereafter, Costa et al. (2002) introduced the multiscale entropy (MSEn) method, which was intended to better detect the presence of complexity in the time series. Cashaback et al. (2013) applied the MSEn to the sEMG signal to evaluate short-term complexity at different contraction intensities, although the complexity level at MVC was only slightly different compared to 70% MVC, probably due to the fact that the complexity of the signal was mostly influenced by the firing rate rather than MU recruitment.

Entropy methods have been applied to sEMG signal to detect fatigability changes. For instance, Hernandez and Camic (2019) found that SampEn values decreased differently during maximal concentric, eccentric and isometric knee extensions. Similar results were found by Cashaback et al. (2013) during submaximal and maximal isometric elbow flexions; and by Navaneethakrishna et al. (2015) during dynamic biceps brachii (BB) curl exercise until task failure. The authors of these studies hypothesized that the reduction of complexity was related to central (MUs synchronization) and/or peripheral factors (decrease in muscle fiber CV).

3.2 Fractal analysis

The fractal's theory refers to the discovery of Benoit Mandelbrot (1982): 'an object or a signal which can be split into parts, each of which is a reduced-size copy of the whole, might be defined as fractal and this property is called self-similarity'. Mandelbrot coined the term "fractal" few years earlier, from the Latin *fractus*, the past participle of the verb *frangere*, "to break," (1977). The definition of fractals was later generalized by Goldberger (1996) to structures consisting of sub-units (and sub-sub-units, etc.) resembling the overall object's structure.

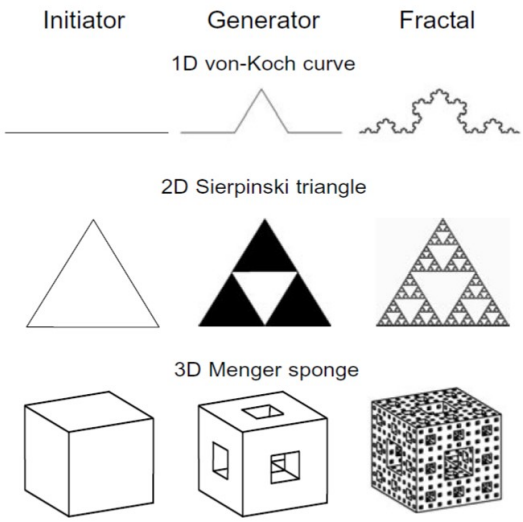

Figure 9 Examples of ideal geometric (exact) fractals. The von-Koch curve, Sierpinski triangle and Menger sponge are all generated by repetitively applying a single rule of generation (the generator), to a simple object (the initiator), in an infinite number of iterative steps (from Eke et al. 2002).

Fractals have subsequently come to be defined by a set of four characteristic properties: self-similarity, scaling, the fractal dimension (FD) and statistical properties (Di Ieva, 2016; Eke et al., 2002):

1. The self-similarity fractals exhibit, may be either geometrical or statistical. Geometrically self-similar objects are those with smaller, exact replicas of the entire object (Eke et al., 2002; Mandelbrot, 1982). Statistically self-similar objects are "kind of like" the whole; the pieces' statistical properties are proportionate to the statistical properties of the whole (Bassingthwaighte and Raymond, 1994).
2. Owing to the self-similarity, features in one resolution are correlated with features in other resolutions. Scaling refers to how the measured values depend on the resolution used to make the measurement (Di Ieva, 2016); thus, the length measured at finer resolutions would be longer, because it includes finer features. The scaling relationship is defined by self-similarity and can contribute to power-law scaling. Thus, the length measured at finer resolutions will be longer as it contains finer features (Eke et al., 2002).
3. The FD offers a quantitative measurement of self-similarity and scaling, explaining how many new pieces, similar to the entire object, are revealed when the resolution is finer (Di Ieva, 2016).
4. The statistical properties of fractals include the fact that there might not be a mean or variance in fractal processes. For the mean, as more data are analyzed, rather than converging to a single value, the mean tends to increase to an ever-larger value or decreases to an ever-smaller

value (Liebovitch, 1998). For the variance, self-similarity means that small irregularities are replicated on a larger scale as larger irregularities, and as more data are examined, those larger irregularities increase the variance, which then becomes infinite (Di Ieva, 2016).

Many complex anatomical structures display fractal-like geometry, and unlike the geometric fractals developed by mathematicians, which may be defined as exact fractals, these structures are statistical fractals (Eke et al., 2002). Anatomical structures are fractal, because their small form tends to be identical to their large-scale form (Glenny et al., 1991; Goldberger and West, 1987). Examples of fractal-like anatomic structures in the body have been reviewed in Di Ieva (2016) and comprise arterial and venous trees, the His-Purkinje conduction system, and the dendrites in the nervous system. Other fractal-like physiological processes include blood pressure, ion channel kinetics, gait (Chakraborty et al., 2015) and muscle force output. Moreover, fractal geometries have been applied to medical signal (1D, 2D or 3D) analysis applications like pattern recognition, texture analysis and segmentation. During the past two decades, results from numerous published articles have shown the ability of texture analysis algorithms to extract diagnostically meaningful information from medical images that were obtained with various imaging modalities, such as mammography, ultrasound, computed tomography, positron emission tomography and magnetic resonance imaging (reviewed in Lopes and Betrouni 2009). Indeed, some signals have a fractal character; this is particularly the case for repeated sequences, like palindromes (local repetitions) and homologies between two different nucleotide sequences (motifs along the genome) composing a self-similar (fractal) pattern in mitochondrial DNA (Oiwa and Glazier, 2004).

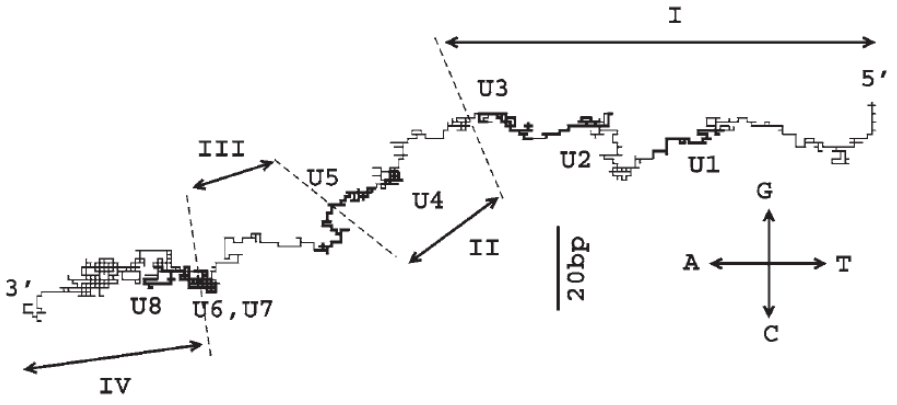

Figure 10 Two-dimensional DNA walk for mitochondrial 16S rRNA in Mus musculus. DNA walk exhibits fractal nature, and is a vectorial representation of DNA sequences transformed into a planer trajectory (from Oiwa and Glazier, 2004).

The sEMG signal itself, which originates from a strong non-linear combination of similar templates (i.e. action potentials of different MUs) that undergo spectral and magnitude compression, has self-similarity properties, and therefore fractal analysis seems appropriate (Anmuth et al., 1994). As outlined above, the description of a fractal structure occurs through the determination of the FD, which is a measure of self-similarity and geometrical complexity of the signal. FD gives a quantitative indication of the chaotic behavior of a signal, and is also related to the degree of interference of the signal, which is inversely related to the 'smoothness' of the signal (Mesin et al., 2009a).

At least eight different methods for estimating FD of sEMG waveforms have been applied in literature, including the box-counting method (Barnsley and Hurd, 1989), the Hurst exponent (Hurst, 1951), a method based on power spectral density (Kaplan, 1999; Raghav and Mishra, 2008; Spasic, 2007), the methods proposed by Higuchi (1988), Sevcik (2010), Petrosian (1995) and Katz (1988) and two variants of the latter (Castiglioni, 2010). These methods were compared and reviewed by Coelho and Lima (2014), who evidenced that the normalized version of the Katz's estimation method, followed by the Hurst exponent, significantly outperform the others in terms of generating more discriminatory features. However, the Katz's method provides FD estimates that may depend on the length of the time series (Castiglioni, 2010) and in another study, the Higuchi algorithm was preferred over the Katz's, since it provided a more accurate and consistent estimation of FD for physiological signals (Esteller et al., 2001). Thus, consensus has not yet been reached, and an accurate selection of FD algorithm is required for specific applications.

The Katz's method has been further revised by Anmuth et al. (1994) to be applied to sEMG signal during isometric contractions. Given a signal lasting 3 seconds, FD was estimated for the middle 1 s as:

$$FD = \frac{\log N}{\left[\log N + \log\left(\frac{d}{L}\right)\right]}$$

where N is the number of samples in the signal, d is the planar extent of the waveform (computed as the distance between the first point of the sequence and the point of the series that provides the farthest distance), and L the total length of the signal (sum of distances between successive points) (Rampichini et al., 2020).

In addition, Wang et al. (2019) introduced a new algorithm named 'roughness scaling extraction' (RSE) to evaluate FD based on a single morphological image. It was found that RSE algorithm was much more accurate than the traditional algorithms.

Eventually, another popular estimator of the FD is the box-counting method on the EMG signal interference pattern (Gitter and Czerniecki, 1995). The exact calculation of FD through the box-counting method is given in detail in the General methods. FD values close to 1 reflect smoothed signals whereas values approaching 2 are typical of signals with high space-filling propensity (Beretta-Piccoli et al., 2015). The box-counting algorithm has been used to evaluate sEMG signals during isometric contractions in healthy subjects (Beretta-Piccoli et al., 2017; Mesin et al., 2009a; Troiano et al., 2008), elderly (Boccia et al., 2016) and persons with multiple sclerosis (Beretta-Piccoli et al., 2020).

FD was initially used to characterize levels of muscle activation during isometric and isokinetic contractions (Anmuth et al., 1994; Gupta et al., 1997; Talebinejad et al., 2009) and patterns of MU recruitment (Gitter and Czerniecki, 1995; Xu and Xiao, 1997). Arjunan and Kumar (2010) investigated the complexity of muscle activation using the FD during wrist and finger flexions. Later, FD was proposed as an index to monitor changes in sEMG signal during a fatiguing task (Beretta-Piccoli et al., 2015; Boccia et al., 2016; Mesin et al., 2009a). Indeed, we clearly showed a significant negative normalized slope of FD, during fatiguing isometric contractions in different muscles (vastus lateralis (VL), vastus medialis (VM) and BB) and at different intensities (Beretta-Piccoli et al., 2015, 2017 and 2020; Boccia et al., 2016; Meduri et al., 2016), suggesting a reduction in signal complexity (Figure 11).

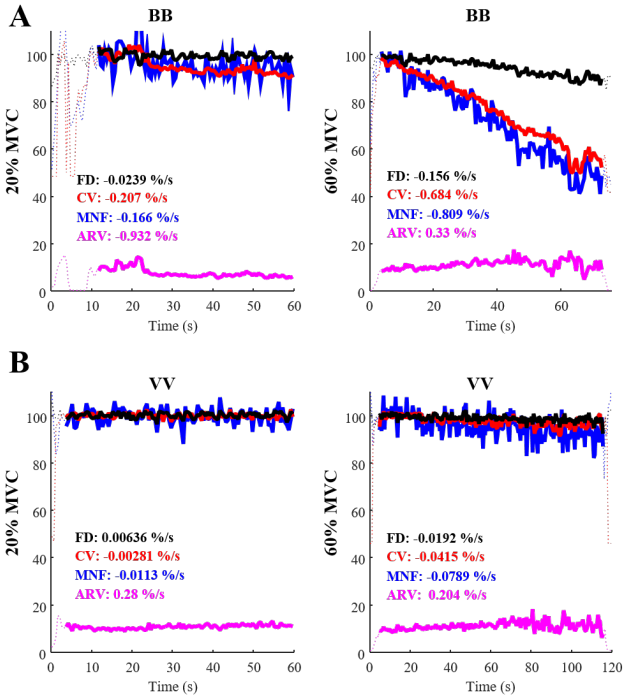

Figure 11 Time course of fractal dimension (FD), muscle fiber conduction velocity (CV), mean frequency of the power spectrum (MNF) and average rectified value (ARV) for a representative person with multiple sclerosis (from Beretta-Piccoli et al., 2020). BB, biceps brachii; VV, vastus lateralis and medialis.

Moreover, a decrease in FD was also associated to ageing and disease (Arjunan and Kumar, 2013; Boccia et al., 2016; Goldberger et al., 2002). These findings suggest a possible benefit of the fractal analysis of the sEMG signal as a complementary tool for the evaluation of fatigability during a performance test. However, although the use of non-linear analysis of the sEMG signal is desirable, as more sensitive than spectral analysis for the assessment of performance fatigability (Farina et al., 2002b), it is difficult to relate these parameters to physiological changes in muscle properties resulting from fatigue (Merletti and Farina, 2016). Mesin et al. (2009a) compared FD to other linear and non-linear muscle fatigue indices' computed from both synthetic and experimental sEMG signals: they found that FD was the parameter least affected by CV changes, weakly affected by fat layer thickness and mostly related to the level of MU synchronization, which suggested its possible use as index of central components of fatigue. Szu-Yu et al. (2015) using the Katz's calculation of FD, did not report any changes during isotonic repeated submaximal contractions (pedaling). Lastly, Mesin et al. (2016) investigated the effect on FD of both the percentages of MU synchronization (from 0–20%) and different firing rates (5–40 Hz), respectively. The authors demonstrated the presence of an inverse relationship between FD and MU synchronization and a positive relationship with the MU firing rate. Such results have brought new light to the understanding of FD changes induced by fatigue, rendering FD no longer regarded as an exclusive index of MU synchronization only. FD of the sEMG signal was investigated in studies 2 and 3.

Other authors have used different fractal parameters, such as detrended fluctuation analysis (DFA) and multifractality. DFA, developed by Peng et al. (1994), relates to the color of noise and detects long-range correlations in time-series, thus providing an indication of temporal fractal scaling. Further details of the DFA calculation are given by Stanley et al. (1999). An extended version of the DFA method was applied to identify the components of the multifractal dynamics, since complex systems may generate not only monofractal time series, but also multifractals. Wang et al. (2007) and Talebinejad et al. (2010) applied the multifractal DFA to investigate performance fatigability during static, as well as cyclic and random contractions, respectively.

Finally, among others non-linear methods, the evaluation of the correlation dimension (CD) (Grassberger et al., 1991) has been used to classify the sEMG dynamics, both at rest and during light and fatiguing muscle contractions. CD is a measure of the amount of correlation contained in a signal connected to the FD (Rampichini et al., 2020). During a fatiguing task a reduction in the dimensionality of the system, as assessed by CD was demonstrated: this has been ascribed to MU

synchronization and reduction in the propagation velocity of the IAP and firing rate, which may reduce the neuromuscular system adaptability (Nieminen and Takala, 1996).

However, a precise connection between the physiologic adaptation to fatigue in muscle activity and the changes in CD of sEMG signals is still lacking.

To sum up, although complex biosignals such as sEMG have recently been shown to represent non-linear, non-stationary, and non-equilibrium processes in nature, the methods used to analyze these data often assume linearity, stationary, and equilibrium-like conditions. In particular, studies have shown that sEMG signals may contain hidden information that cannot be retrieved using traditional analytical methods (Coelho and Lima, 2014; Rampichini et al., 2020). Such hidden information may provide important and critical information to be of clinical value as well as to relate to neural and muscle properties and activity function (Di Ieva, 2016).

Multi-channel electrodes

With respect to the classical bipolar approach, the use of methods based on more than two channels arranged serially (linear arrays) allows the detection of sEMG signals along the longitudinal or transverse axis of a muscle (Merletti et al., 2003; Wood et al., 2001). Additionally, bi-dimensional electrode arrays (grids of electrodes) may be used to determine the distribution of EMG amplitude and spectral descriptors across the entire skin area covering the target muscle (Falla and Farina, 2007; Gallina et al., 2013a; Vieira et al., 2015). Even if we recognize the important contribution that EMG amplitude maps can make to the study of fatigue, they will only be mentioned, because they go beyond the topic addressed in this thesis. In addition, multi-channel sEMG allows, from a global muscle level point of view, a more precise and reliable estimation of muscle fiber CV (reviewed in Beretta-Piccoli et al., 2019) and the assessment of regional changes in the sEMG signal due to fatigue (reviewed in Gazzoni et al., 2017).

1. *Muscle fiber conduction velocity*

Muscle fiber CV is not only a mathematical descriptor, but also a significant physiological variable directly related to fiber membrane properties, fiber diameter and fiber contractile properties (Andreassen and Arendt-Nielsen, 1987). Muscle fiber CV is associated to the size principle

(Henneman et al., 1965) has been positively correlated with the percentage of myosin heavy chain type I fibers (Farina et al., 2007) and can be used to assess MU recruitment during static and dynamic exercise (Merletti et al., 2010; Nicolò et al., 2015; Piitulainen et al., 2013; Pozzo et al., 2006). Muscle fiber CV has also been proposed as a non-invasive tool to infer MU recruitment and de-recruitment during incremental cycling exercises (Lenti et al., 2010; Sbriccoli et al., 2009). Moreover, since during dynamic contractions the number of active MUs changes significantly, the analysis of CV is preferred over spectral analysis to extract information on MU recruitment (Farina, 2006).

The CV of the action potentials in human muscles has been measured successfully by the use of needle electrodes in the late 50s: Buchthal et al. (1955) used 3 to 6 coaxial needle electrodes, while Stålberg (1966) used a multi-contact needle electrode. Later, Nishizono et al. (1979) conducted the first estimation of muscle fiber CV using up to 8 sEMG electrodes placed on the BB (Figure 12)

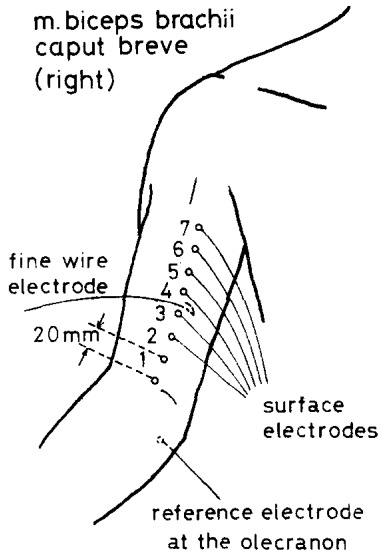

Figure 12 Surface and bipolar fine-wire intramuscular EMG electrodes placement (from Nishizono et al., 1979).

In their study Nishizono et al. (1979) suggested that "if muscle CV could be measured accurately using sEMG, it could be applied effectively, for instance, to the detection of muscle disorders and to the estimation of muscle fiber composition". This important physiological parameter will extensively be discussed in study 1.

As was described earlier, during isometric constant force contractions, changes in the EMG signal due to fatigue are mainly caused by three physiological factors: (1) a decay in muscle fibers CV (Buchthal et al., 1955; Stålberg, 1966), mainly related to a decrease of the intracellular pH (Bouissou et al., 1989; Brody et al., 1991; Komi and Tesch, 1979); (2) an increase of the level of MUs

synchronization by the CNS (Merletti et al., 1990) and (3) a reduction of the recruitment threshold of MUs (Adam and De Luca, 2003). In particular, changes in CV during fatiguing contractions, have profound impact on the shape of the MUAP waveform and therefore, on the amplitude and spectral variables extracted from the sEMG signal.

Therefore, the estimation of CV slope (i.e. rate of change), might be useful to characterize the peripheral components of muscle fatigue (during an isometric task) (Merletti and Farina, 2016) and this variable may be considered as one of the most robust EMG fatigue indices (Figure 11; Dedering et al., 2000; Kollmitzer et al., 1999; Linssen et al., 1993; Rainoldi et al., 2001).

Muscle fiber CV is generally estimated from sEMG signals collected with multi-channel electrodes positioned parallel to the muscle fibers. However, most fibers within in-depth pinnate muscles, do not lay in planes parallel to the skin, and as a result, electrodes and muscle fibers are not located in parallel planes. In such muscles, CV estimates are biased towards values far over the physiological range (Merletti and Farina, 2016). Moreover, a pair of electrodes placed above an unspecific muscle region may provide very misleading information. An electrode array covering the entire muscle surface of an in-depth pinnate muscle, is therefore essential to identify regional activations and to detect CV. Indeed, recent publications suggested that in pinnate muscles such as the medial gastrocnemius, and the tibialis anterior, it is possible to reliably assess physiological estimates of CV, using HD-EMG, from their distal region (Gallina et al., 2013b; Houtman et al., 2003), where fibers run parallel to the skin surface.

2. Muscle regional changes in the sEMG signal due to fatigue

Recent studies conducted with a multi-channel approach along the longitudinal axis of the muscle fibers have highlighted that changes in sEMG signals collected locally from a single region of a muscle, may not reveal the neural changes occurring somewhere else within the same muscle (Mesin et al., 2011; Vieira et al., 2011). For instance, even in small muscles such as those in the forearm (Gallina and Botter, 2013), the amplitude parameters extracted from the sEMG detected from different locations, were observed to change during fatiguing contractions (Falla and Farina, 2007; Gallina et al., 2011; Watanabe et al., 2013; Zijdewind et al., 1995). Farina et al. (2008) demonstrated that during a positional endurance task, amplitude and spectral changes were observed to manifest locally within the trapezius muscle. Collectively, it appears that fatigue is more likely to affect muscles locally, rather than globally.

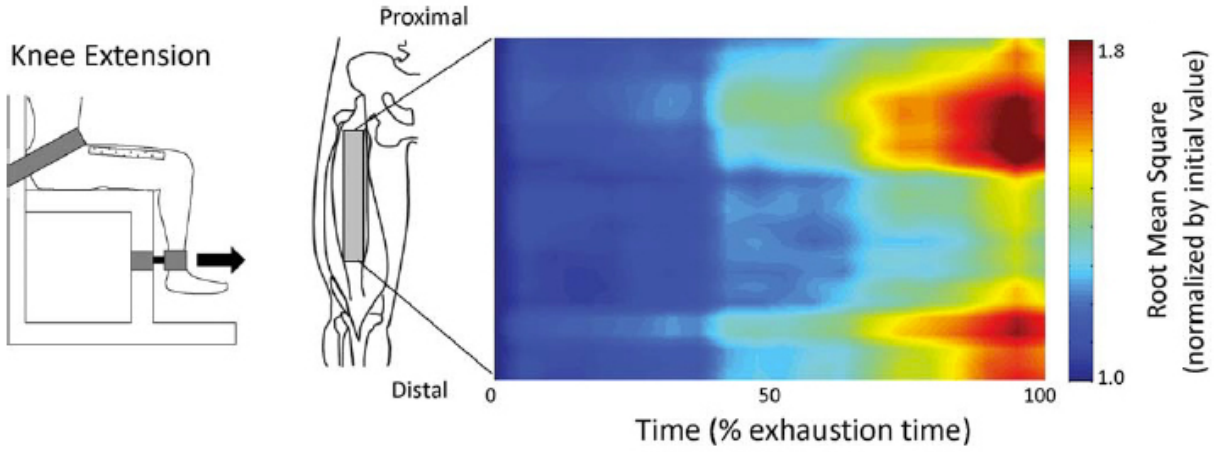

Figure 13 Temporal changes of normalized RMS. During knee extension, increases in normalized RMS values with time were mainly observed in the proximal and distal regions of rectus femoris muscle (from Watanabe et al., 2013).

However, regional variations in muscle activity (Figure 13) should be differentiated from anatomically-induced changes in sEMG signals, occurring when surface electrodes are positioned near or across the innervation zone, as well as near the tendon regions (Barbero et al., 2012; Farina et al., 2002b).

Several hypothesis upon the physiological mechanisms affecting regional changes in muscle activity during prolonged contractions, such as MU rotation or MU substitution (Westgaard and de Luca, 1999) were described in literature. Rotation indicates different MUs that are periodically and alternatively de- and re-recruited during a sustained contraction; whereas, substitution refers to the recruitment of fresh MUs, to replace active, tonically discharging MUs of lower recruitment threshold (Gazzoni et al., 2017).

AIMS AND HYPOTHESES

The overall aim of this thesis is to investigate changes in linear and non-linear sEMG parameters during a fatiguing task, in healthy subjects and patients with neuromuscular disorders. The specific aims of the three studies are as follows:

1. to verify whether muscle fiber conduction velocity may be reliably estimated during voluntary and electrically elicited contractions using sEMG, and to identify the experimental conditions that allow highly reliable CV estimation.
2. to determine the relationship between muscle force and FD of the sEMG signal during isometric contractions.
3. to investigate whether performance fatigability differs in patients with facioscapulohumeral muscular dystrophy (FSHD) during a fatiguing task, with respect to healthy controls.

The hypotheses to be tested within the two experimental studies (2 and 3) are:

1. that FD of the sEMG signal is not related to the intensity of muscle contraction (similarly to what reported in previous publications, but in different muscles)
2. that patients with FSHD will produce less force and probably show greater resistance towards fatigability. Since FSHD causes strong alteration of the membrane and contractile properties, the selected sEMG parameters will probably show different behaviors both for the estimates and the slopes.

GENERAL METHODS

Ethical concerns

Full ethical approval of the experimental designs and procedures were obtained from the local ethics committee of the Swiss Italian health and sociality department, Bellinzona, Switzerland (study 2) and from the ethics committee of the University of Pisa (study 3) prior the commencement of each data collection. All subjects gave their written informed consent to participate in the studies, which were conducted according to the Declaration of Helsinki (1964). It was made clear to participants that they could withdraw their consent at any time, without having to give a reason for their withdrawal.

Participants

Healthy participants were recruited among the students and staff at the University of Applied Sciences and Arts of Southern Switzerland (SUPSI) and at the University of Pavia. Patients with facioscapulohumeral muscle dystrophy (FSHD), who were enrolled in the Italian Nation Registry for FSHD (INRF), were recruited at the Criams-Sport Medicine Centre Voghera, University of Pavia.

Experimental procedures

During all experimental sessions, participants were seated in a height-adjustable chair with their dominant arm positioned on an isometric ergometer (MUC1, OTBioelettronica, Turin, Italy), equipped with a load cell (Model TF022, CCT Transducers, Turin, Italy). In order to isolate the action of their BB, the wrist was fastened to the ergometer, with the elbow at 120°, as shown in Figure 14.

Figure 14 sEMG signals were recorded from the BB during isometric contractions of the dominant arm.

Initially, two isometric MVCs were performed, separated by 2 min rest. During each contraction of the trial, the force trace was displayed to participants on a computer monitor as visual feedback. Participants were instructed to increase the force up to their maximum, and to hold it for 2-3 s, and were given strong verbal encouragement.

sEMG and force measurements

Myoelectric signals were detected from the dominant BB, in a monopolar configuration using a bidimensional array of 64 electrodes (3 mm diameter, 8x8 grid, 10 mm interelectrode distance; model ELSCH064NM3; OT Bioelettronica) (Figure 14). This muscle was chosen in order to obtain high-quality sEMG signals according to the qualitative criteria described in Beretta-Piccoli et al. (2014). Moreover, the anatomy of the BB (long fibers that run parallel to the skin) is mostly favorable to estimate muscle fiber CV as well as, to extract parameters from the sEMG interference signal. On the contrary, recent studies (reviewed in Del Vecchio et al., 2020) suggested that this muscle may not be suitable for sEMG decomposition techniques.

The electrode grid was applied on the muscle belly according to Barbero et al. (2012), with its distal edge close to the cubital fossa and the midline of the array aligned with the midline of BB along a line from the cubital fossa to the acromion (see Figure 14). A ground electrode was placed on the contralateral wrist. The EMG signals were amplified (EMG-USB2+; OT Bioelettronica), band-pass filtered (10–750 Hz), sampled at 2048 Hz using a 16-bit A/D converter, with 5 V dynamic range, and stored on a computer.

The isometric ergometer was used to measure elbow torque with a torque meter operating linearly in the range 0–1000 Nm. The torque signal was amplified (MISO II; OT Bioelettronica) and stored on a computer with the sEMG data. The torque signal was displayed on a screen, providing real-time biofeedback.

Signal processing

The number of channels used for CV estimation was selected based on visual inspection, of single differential signals, which comprised five steps (Beretta-Piccoli et al., 2017):

1) Identification of movement artifacts or missing channels: signals with large amplitude changes due to movement were removed from the analysis.

2) Identification of power line interference: signals which presented large sinusoidal components at 50 Hz or their harmonics, were removed from the analysis.
3) Manual identification of the main innervation zone (if present under the array) and of the distal tendons (through observation of propagating waves and inversion of MUAP shapes.).
4) Selection of the array column where the maximal amplitude and largest MUAPs were visible.
5) Selection of the channels between the innervation zone and the distal tendons, where the MUAPs appear similar in shape and shifted in time.

The number of channels selected for CV estimation usually ranged between 4 and 7 (according to Farina et al., 2004c). CV was estimated using a multichannel algorithm (Farina and Merletti, 2003) on single differential signals, based on the matching between signals filtered in the temporal and in the spatial domains, using non-overlapping signal epochs of 1-s, on the selected channels. Each of the selected signal epochs was used for the estimation of average rectified value (ARV), mean frequency of the power spectrum (MNF) and FD: these variables were averaged among all the selected channels. ARV and MNF were computed off-line with numerical algorithms (Merletti et al., 1990) using the following calculation formula (Gonzalez-Izal et al., 2012):

$$ARV = \frac{1}{n}\sum_{n} \left| x_n \right|$$

where x_n are the values of the sEMG signal, and n is the number of samples.

$$MNF = \frac{\int_{f1}^{f2} f \cdot PS(f) \cdot df}{\int_{f1}^{f2} PS(f) \cdot df}$$

where PS(f) is the sEMG power spectrum calculated using Fourier transform, and f1 and f2 determine the bandwidth of the surface electromyography (f1 = lowest frequency and f2 = highest frequency of the bandwidth).

FD was estimated using the box-counting method, as previously reported (Beretta-Piccoli et al., 2017; Gitter and Czerniecki, 1995). Briefly, a grid of square boxes is used to cover the signal, and the number of boxes that the sEMG waveform passes through is counted (Figure 15). When the box size decreases, the number of the boxes that are counted will increase exponentially. The range of box size is restricted in order to avoid saturation for high and low value of size (Gitter and Czerniecki, 1995).

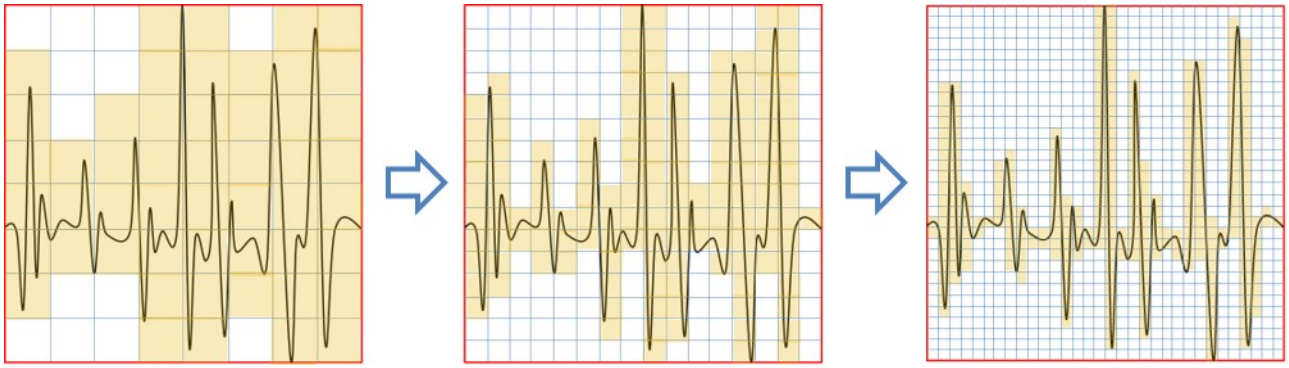

Figure 15 Graphical representation of the box-counting algorithm used to determine the fractal dimension of the EMG waveform. The total number of boxes entered by the waveform (shaded region) are counted as the size of the overlying grid is successively reduced in size.

The box size was fixed to 13 steps equally spaced in logarithmic scale, with the smallest box equal to 1/128th of a second and the largest box equal to 1/8th of a second. The vertical side of the boxes was normalized to the range of the signal during epochs of 1 second and divided in the same number of boxes. However, by plotting the logarithm of the number of boxes required to cover the signal vs. the logarithm of the inverse of the box area, the exponential relationship becomes approximately linear. The slope of the interpolation line (estimated using the least mean squared procedure) is the FD (Mesin et al., 2009a). Therefore, the following expression defines the FD:

$$FD = \frac{\log N}{\log \frac{1}{L}}$$

where N is the number of boxes required to cover the signal and L is the box side, with the ratio indicating the slope of the interpolation line.

Calculation of performance fatigability

Performance fatigability was quantified indirectly as the slopes of ARV, MNF, CV and FD. Please refer to the single studies for more details.

Statistical analyses

Extraction of the relevant data from the raw files was performed in MATLAB R2014b (The MathWorks, Massachusetts, USA). Statistical analyses of data were then performed using SPSS Version 26.0 (IBM, Chicago, IL, USA), with specific procedures detailed in each experimental study. Statistical significance was accepted at $\alpha = 0.05$ level.

STUDY 1 Reliability of surface electromyography in estimating muscle fiber conduction velocity: A systematic review.

Introduction

Muscle fiber conduction velocity (CV) is defined as the propagation velocity of action potentials along the membrane of a muscle fiber. As an important physiological parameter, CV is correlated with muscle fiber membrane properties, e.g., ion concentration, pH, muscle temperature and motor unit (MU) firing rate (Andreassen and Arendt-Nielsen, 1987; Arendt-Nielsen and Zwarts, 1989; Brody et al., 1991; Farina, 2001). Moreover, muscle fiber CV depends on the muscle fiber diameter, which is related to the fiber type (Del Vecchio et al., 2018; Hakansson, 1956). Therefore, changes in CV have been associated with the recruitment of different types of MUs (Del Vecchio et al., 2017; Masuda and De Luca, 1991; Sbriccoli et al., 2009). Moreover, alterations in muscle fiber CV are related to gradation of muscle force (Sbriccoli et al., 2003), local muscle fatigue (Merletti et al., 1990), and neuromuscular disorders (Zwarts and Arendt-Nielsen, 1988).

The increased investigation of and interest in muscle fiber CV is probably linked to the fact that it can be determined non-invasively and from a large number of concurrently detectable MUs, using surface electromyography (sEMG), during normal muscle function. The classic method of calculating CV involves estimating the delay between signals recorded at fixed distance along the direction of propagation, and the ratio between such measured distance and the estimated delay (Merletti and Farina, 2016). As two sEMG signals detected at different points along a fiber are usually not identical, there is no strict mathematical definition of the delay between them. Thus, several methods for CV estimation from sEMG recordings have been proposed (for a review, see Farina and Merletti, 2004b). The methods are all based on the assumption that signals are propagated along the muscle fibers from the innervation zone (IZ) to the tendon regions (i.e., in muscles with fibers parallel to the skin). As not all muscle fibers end in the same place, operators need to be able to identify the channels where unidirectional propagation is observed. In addition, it is important to note that when multichannel sEMG techniques are used for CV estimation, CV refers to the mean value of the different MU action potentials (MUAPs) propagating under the electrodes at different velocities (Farina and Merletti, 2004b). The CV of individual MUs can be estimated using MUAP templates obtained via spike-triggered averaging based on the firing instants identified by decomposition of intramuscular EMG recordings (Farina et al., 2002a) and sEMG recordings (Keenan et al., 2006). Recently, Negro et al. (2016) proposed the convolutive blind source separation method, which allows the identification of

tens of MUs detected using high-density sEMG (for a comprehensive review, see Farina et al., 2016). Estimation of the CV from a MUAP template is relatively simple, but it is considerably more complex for an interferential signal, which is the sum of the contributions of different asynchronously appearing MUs (Farina and Merletti, 2000).

Many factors other than the physiological phenomena under study bias the estimation of CV during voluntary contractions. In fact, the detection system's features, such as the electrode positioning, interelectrode distance (IED), number of electrodes, and algorithm used (Farina et al., 2002b; Farina and Merletti, 2000; 2003; 2004b; Farina et al., 2001b), directly influence the CV estimations. Furthermore, to reduce the effect of cross-talk from nearby muscles or to "isolate" the investigated muscle from the central nervous system, selective electrical stimulation of a nerve branch or of the motor point may be applied. Electrically evoked myoelectrical signals allow easier estimation of the muscle fiber CV from a MU pool, which is likely to lead to a more stable estimation than an estimation based on voluntary contractions (Botter et al., 2009; Merletti et al., 1992a).

In addition, the estimation of muscle fiber CV during dynamic exercise has become possible due to the development of multichannel adhesive arrays of electrodes (Pozzo et al., 2004) and a novel data processing algorithm that allows CV estimation based on short signal epochs (Farina et al., 2004b). This method has helped to overcome the problems previously associated with CV estimation during dynamic exercise (which were exhaustively discussed in a study by Merletti and Farina, 2016), such as movement artifacts (Clancy et al., 2002) and signal non-stationarity (Merlo et al., 2005). Moreover, the evaluation of muscle fiber CV has gained the attention of researchers and clinicians interested in understanding the neuromuscular system modifications caused by disease (Allen et al., 2008a; Bazzichi et al., 2009; Blijham et al., 2006; Boccia et al., 2016; Butugan et al., 2014; Campanini et al., 2009; Meijer et al., 2008; Minetto et al., 2011), pain (Falla and Farina, 2005; Klaver-Krol et al., 2012) and fatigue (Gonzalez-Izal et al., 2012). For instance, the CV value may be used to supplement information obtained at the muscle fiber level with intramuscular EMG, which is the clinical standard for neurological assessments, allowing the firing pattern of single MUs to be reliably studied and the shape of intramuscular potentials to be investigated, which is critical for the diagnosis of several neuromuscular diseases (Drost et al., 2006). Recently, several studies in the field of sport science have focused on possible relationships between CV and cardiorespiratory responses during dynamic exercise (e.g., Kilen et al., 2012; Lenti et al., 2010; Pereira et al., 2013; Stewart et al., 2011).

The assessment of the reliability of CV (measured using sEMG) is of considerable relevance, as it is important to be confident that changes in estimated muscle fiber CV are associated with real physiological events and not with measurement errors. Thus, the aims of this systematic review were

(1) to verify whether muscle fiber CV may be reliably estimated during voluntary and electrically elicited contractions using sEMG and (2) to identify the experimental conditions that allow highly reliable CV estimation.

Methods

A systematic review of studies reporting on the reliability and/or reproducibility of sEMG for assessing muscle fiber CV was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) statement (Moher et al., 2009). A detailed protocol was written a priori and is available at https://www.crd.york.ac.uk/prospero/ (CRD42018092421).

1. Identification of studies

On April 20, 2018, a comprehensive search of the databases MEDLINE (PubMed) and Web of Science was conducted. The search string for MEDLINE involved the following medical subject heading (MeSH) terms and free-text terms: [[Reproducibility of Results (MeSH) AND Electromyography (MeSH)] AND conduction velocity], whereas Web of Science was searched using the following search string: [surface electromyography OR surface-electromyography OR surface EMG OR sEMG] AND [conduction velocity] AND [reliability OR reproducibility OR agreement]. No restrictions were applied regarding the publication date or the language of the articles. All hits obtained using the search strategies were exported to EndNote X8 (Clarivate Analytics, Philadelphia, PA, USA), and duplicates were then removed.

2. Study selection

Two reviewers (MBP and CC) independently screened the titles and abstracts of the resulting studies and identified those that satisfied the inclusion and exclusion criteria (see Section 2.3). If it was not clear whether an article should be included based on the title and abstract, the full text was inspected. Moreover, controversies between the reviewers regarding the eligibility of titles/abstracts or full texts were solved in a consensus meeting involving the two reviewers. If a consensus could not be reached, a third reviewer (MB) was asked to make the final decision.

3. Eligibility criteria

Studies that fulfilled all of the following inclusion criteria were eligible for inclusion in this review: (1) full-text article published in peer-reviewed journal; (2) longitudinal study with a repeated-measures experimental design; (3) investigated reliability or reproducibility of sEMG for assessing

CV; (4) used sEMG to estimate CV. In addition, we excluded studies that fulfilled at least one of the following criteria: (1) used mathematical models; (2) used needle/intramuscular EMG; (3) estimated CV of nerve signal; (4) used animal models. Studies that met the eligibility criteria formed the final sample, and two reviewers (MBP and CC) independently assessed the reporting quality.

4. Reporting quality assessment

Each included study was assessed using the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) checklist, which is a 15-item checklist designed to determine the reporting quality of reliability studies. The GRRAS checklist was developed in 2011 by Kottner et al. (2011) to improve the quality of reporting in reliability and agreement studies in the healthcare and medical field, as no established standards were previously available. The items overlap with the Standards for Reporting of Diagnostic Accuracy (STARD) (Bossuyt and Reitsma, 2003) and the Standards for Educational and Psychological Testing (American Educational Research Association et al., 1999).

Studies were not given an overall numeric quality score. Instead, each item was considered separately, and the page number of the page containing an appropriate description was noted, if applicable. A chart involving three categories (reported, not reported or inapplicable) was then constructed.

5. Data extraction

The following information related to sEMG methodology was extracted from the studies: sEMG electrode description (type, size, inter-electrode distance and electrode positioning); a priori identification of the muscle IZ or motor point; muscle contraction type (voluntary or electrically elicited) and intensity; signal type (interferential or single potential); sEMG signal detection derivation (monopolar or single differential); CV estimation method and interval of acceptance of the physiological range of CV values; extracted CV parameters (i.e., initial value, slope [rate of change] and area ratio). Based on the study by Merletti et al. (1990), the initial CV value and slope were defined as follows. First, a regression line of CV over time was estimated using the CV estimates obtained from each signal epoch. Thereafter, the intercept with the *y*-axis and rate of change of the regression line were used to define the initial CV value and slope, respectively.

Next, the following study characteristic data were extracted: test-retest period, whether the electrodes were repositioned and relative and absolute reliability values. The criteria used for the interpretation of the relative reliability correlation coefficients were as follows:

I. Intraclass correlation coefficient (ICC): 0.00–0.25: very low; 0.26–0.49: low; 0.50–0.69: moderate; 0.70–0.89: high; 0.90–1.00: very high reliability (Munro, 2005).

II. Pearson's correlation coefficient (r): 0.00–0.19: very weak; 0.20–0.39: weak; 0.40–0.59: moderate; 0.60–0.79: strong; 0.80–1.00: very strong correlation (Evans, 1996).

The data were extracted by a reviewer (MBP) and double-checked for accuracy by another reviewer (CC).

6. Grouping of studies

A previous literature analysis showed that terms such as "reliability," "repeatability," "reproducibility," "consistency" and "agreement" have been used interchangeably (Atkinson and Nevill, 1998). For this reason, we defined three categories of reliability study: (1) test-retest reliability (i.e., repeated measurements within one day, without electrode replacement); (2) intrasession reliability (i.e., repeated measurements within one day, with electrode replacement); and (3) intersession reliability (i.e., repeated measurements separated by at least one day, with electrode replacement).

Results

1. Literature search

Figure 16 shows a flowchart of the processes regarding study retrieval, screening and eligibility assessment. The literature search yielded 89 potentially eligible articles on CV assessment using EMG. Of these, 72 were excluded based on the title/abstract or full text, leaving 17 articles that met all the eligibility criteria (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; Falla et al., 2002; Farina et al., 2004c; Harba and Teng, 1999; Hogrel et al., 1998; Linssen et al., 1993; Macaluso et al., 1994; Macdonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Merletti et al., 1998; Merletti et al., 1995; Ollivier et al., 2005; Rainoldi et al., 2001; Rainoldi et al., 1999). A single discrepancy between reviewers about the inclusion of one of the studies (Macaluso et al., 1994) was resolved by discussion. Table 2 shows the study details, with the studies listed in chronological order from 1993 to 2018. The most frequent reasons for exclusion were: muscle fiber CV was not assessed; lack of reliability data or appropriate reliability study design; and needle EMG or mathematical simulations were used to evaluate CV (Figure 16).

2. Study characteristics

a) Subjects, muscles and contractions

The number of participants in the studies varied from three to 40. The following muscles were investigated to assess CV reliability during isometric constant force contractions: biceps brachii (BB; n=7), vastus medialis, vastus medialis obliquus and/or vastus lateralis (VM, VMO and VL; n=5), tibialis anterior (TA; n=2), sternocleidomastoid and anterior scalene muscles (SCM and AS; n=1) and anterior temporal muscle (ATM; n=1). The study by (Macdonald et al., 2008) investigated CV reliability during cyclic movements of the vastii muscles (Table 2).

b) Electrode characteristics, positioning and CV estimation

In 11 studies, myoelectric signals were detected using linear electrode arrays in single differential (SD) configuration, whereas in the other six, bi-dimensional arrays in SD or monopolar configuration were used. Electrode positioning, except in the study by (Macaluso et al., 1994), involved considering anatomical landmarks and, in 12 studies, the IZ (or motor point) position on the muscle belly was defined. Electrode repositioning occurred in all the studies except the study by (Macaluso et al., 1994). However, electrode repositioning only occurred partially in the studies by Hogrel et al. (1998) and Beretta-Piccoli et al. (2018), which both used multiple experimental designs. In eight out of 16 studies, to improve the reliability and reduce the displacement error, the position of the electrode array was marked on the skin.

CV was computed with the fast Fourier transform algorithm (Linssen et al., 1993), the cross-correlation method (Harba and Teng, 1999; Hogrel et al., 1998; Macaluso et al., 1994; McIntosh and Gabriel, 2012; Ollivier et al., 2005), the discrete Fourier transform-based alignment algorithm developed by McGill and Dorfman (1984) (Falla et al., 2002; Merletti et al., 1998; Merletti et al., 1995; Rainoldi et al., 2001; Rainoldi et al., 1999) or the multichannel maximum-likelihood algorithm developed by Farina et al. (2004c) (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; Farina et al., 2004c; Macdonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017) (Table 3).

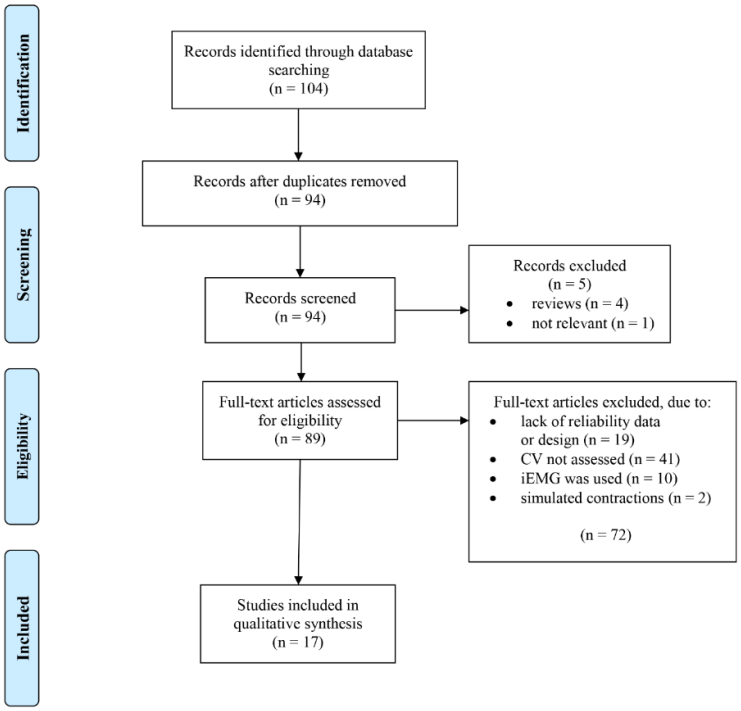

Figure 16 PRISMA Flowchart of study retrieval, screening and eligibility

3. Quality of reporting

Studies were classified depending on how compliant they were with respect to the GRRAS checklist. The reviewers classified items 4, 6, 7, 9 and 12 (out of 15) as inapplicable to the included studies, as the number of raters and their characteristics are usually only relevant in clinical studies. Additionally, information about the sample size (item 6) was disregarded in all the studies, as the all investigated reliability in relatively small groups, with the number of participants varying from three to 40. Although none of the studies satisfied all the relevant criteria for reporting quality (Figure 17), most were compliant with many of the checklist items: in 15 studies, it was possible to locate an appropriate description of up to five items out of 10. The studies by Merletti et al. (1995) and Rainoldi et al. (1999) had the best quality, whereas the studies by Harba and Teng, (1999) and Beretta-Piccoli et al. (2018) had the lowest.

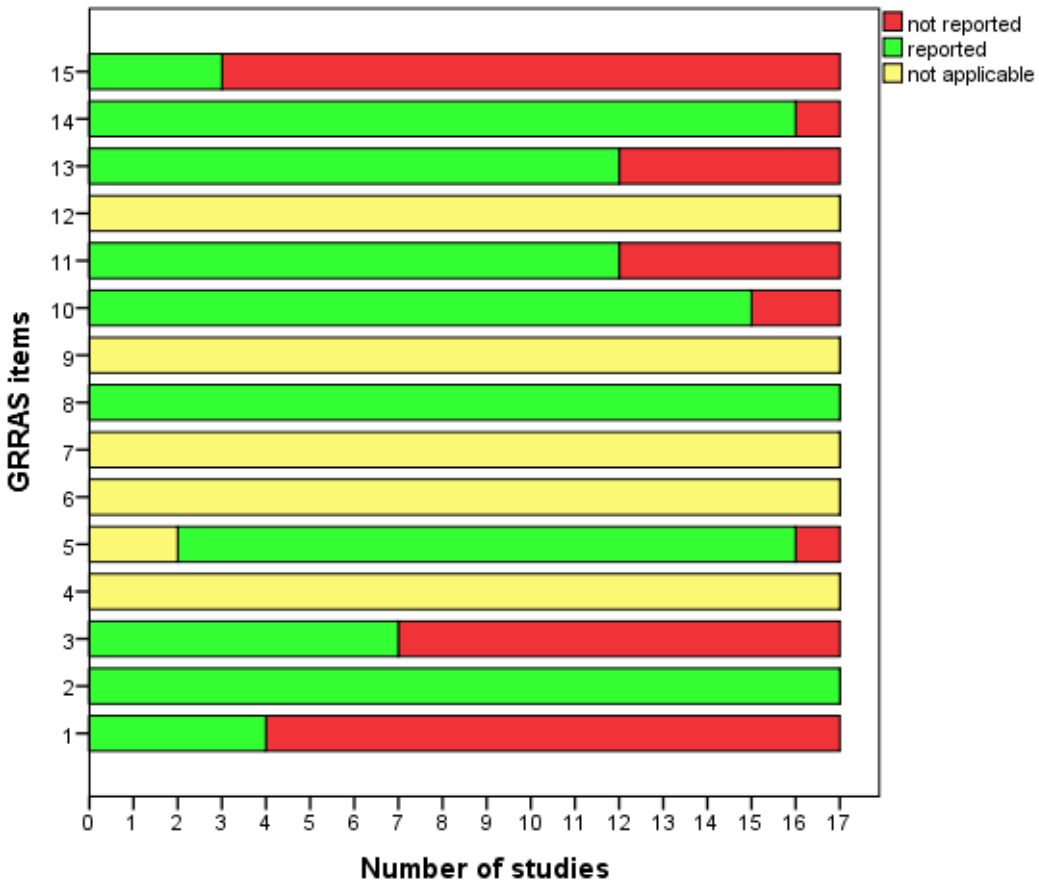

Figure 17 Stacked bar chart representing reliability reporting quality of the reviewed studies (n=17*)*

Several studies were lacking regarding one or more of the following minor points:

- Item 1: 13 studies did not mention the type of reliability (i.e., test-retest, intrasession or intersession) investigated in the title/abstract (Beretta-Piccoli et al., 2018; Falla et al., 2002; Farina et al., 2004b; Harba and Teng, 1999; Linssen et al., 1993; Macaluso et al., 1994; MacDonald et al., 2008; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Merletti et al., 1998; Ollivier et al., 2005; Rainoldi et al., 2001; Rainoldi et al., 1999).
- Item 3: as the included studies were conducted with healthy subjects only, 10 studies did not specify the subject population of interest, leaving it implied (Beretta-Piccoli et al., 2018; Farina et al., 2004b; Harba and Teng, 1999; Hogrel et al., 1998; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi et al., 2001).
- Item 15: only three studies (Martinez-Valdes et al., 2017; Rainoldi et al., 2001; Rainoldi et al., 1999) provided supplementary materials in an appendix.

Several studies were lacking regarding the following items, which were considered to represent major issues:

- Item 10: in two studies (Harba and Teng, 1999; Macaluso et al., 1994), the statistical approach used to evaluate the reliability of CV was not described sufficiently to allow repetition of the study by other researchers.
- Item 11: five studies (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017) did not mention, in the Results section, whether it was possible to estimate CV in all the participants.
- Item 13: five studies (Beretta-Piccoli et al., 2018; Harba and Teng, 1999; Hogrel et al., 1998; Macaluso et al., 1994; Ollivier et al., 2005) did not report a combination of reliability coefficients, which makes it difficult to form a detailed impression of the degree of reliability.

4. Reliability results

The diversity among the included studies precludes a simple synthesis of the results. Thus, the studies were grouped according to the reliability design (Table 4):

1) Hogrel et al. (1998) reported good within-location *test-retest reliability* (i.e., without electrode replacement) for CV estimates at L_0 (the electrode location where CV was minimal, when averaged over all contraction conditions) in the VL.

Martinez-Valdes et al. (2016) reported high to very high *test-retest* and *intersession reliability* (ICC up to 0.97; SEM $\leq$ 0.11) for the initial MU CV value, estimated using bi-dimensional arrays, monopolar EMG derivation and MU decomposition techniques. High reliability was found at all the isometric contraction levels in the VL and VM. Furthermore, the study by Beretta-Piccoli et al. (2018) evaluated *test-retest reliability* (1-hour delay between measurements, without electrode repositioning) and *intersession reliability* (1-week delay between measurements) in the BB. They obtained very high ICC values for *test-retest reliability* (>0.9). However, for *intersession reliability*, the ICC values regarding the initial CV value were much lower (0.04–0.79), and the ICC value depended on the contraction level (the higher the maximum voluntary contraction [MVC, %], the lower the ICC). Unfortunately, Macaluso et al. (1994) did not succeed in measuring the CV in the ATM.

Table 2 Study characteristics of included articles

Authors (y)	Subjects	Muscle	Task	Aims
Linssen et al (1993)	n=26 n=13*	BB	intermittent isometric flexions of the forearm at 80% MVC	To evaluate the inter- and intra-individual repeatability of the muscle fiber CV during fatiguing isometric ischemic intermittent exercise with a contraction rate of 30/min.
Macaluso et al (1994)	n=6	ATM	clenching as hard as possible	To evaluate the behavior of muscle fiber CV and mean power spectrum during fatiguing contractions and to investigate their repeatability.
Merletti et al (1995)	n=6	TA	isometric contractions elicited by electrical stimulation	To investigate test-retest reliability of CV estimates of electrically evoked myoelectric signal shape (M-waves) in isometric conditions.
Hogrel et al (1998)	n=5	VL	isometric knee extensions at 1, 20, 40, 60, 80, 100% MVC	To evaluate reproducibility, sensitivity and variation of CV depending on the electrode location with respect to various contraction modalities.
Merletti et al (1998)	n=9	VM	isometric contractions elicited by electrical stimulation	To investigate test-retest reliability of CV estimates of electrically evoked myoelectric signal shape (M-waves) in isometric conditions.
Harba and Teng (1999)	n=3	BB	isometric flexion of the forearm at 100° joint angle, without weights.	To investigate the reproducibility of measurement of CV from surface EMG using a multi-electrode unit and parallel correlators.
Rainoldi et al (1999)	n=10	BB	isometric flexions of the forearm at 10, 30, 50, 70% MVC	To evaluate the repeatability of estimates and rates of change of muscle fiber CV during voluntary contractions sustained for 30 s at different torque levels.
Rainoldi et al (2001)	n=9	VMO VL	isometric knee extensions at 50% MVC	To evaluate the repeatability of EMG signal muscle fiber CV during voluntary, isometric contractions sustained for 50 s.
Falla et al (2002)	n=9	SCM AS	isometric cervical flexion at 50% MVC	To examine the repeatability and reliability of sEMG-derived indices of muscle fatigue during fatiguing contractions.
Farina et al (2004)	n=10	BB	isometric flexion of the forearm at 50% MVC	To investigate the effect of varying the number of surface EMG signals and the distance between detection points on the standard deviation, sensitivity to electrode displacements over the muscle, and reproducibility of CV estimates.
Ollivier et al (2005)	n=10	BB	isometric flexions of the forearm at 20, 40, 60, 80, 100% MVC	To assess the repeatability of two sEMG recording techniques, the classical bipolar configuration and a Laplacian configuration to assess muscle fiber CV during isometric contractions.
Macdonald et al (2008)	n=20	VL VM	incremental cycling at 20, 40, 60, 80% PPO	To determine the test-retest reliability of muscle fiber CV during incremental and fatiguing cycling using multichannel surface EMG.
McIntosh and Gabriel (2012)	n=40	TA	isometric dorsiflexions of the foot at 30% and 100% MVC	To examine the reliability of CV while using twitch contractions and evoked potentials to orient the surface electrodes with respect to the longitudinal axis of the muscle fibers.
Martinez-Valdes et al (2016)	n=10	VL VM	isometric knee extensions at 10, 30, 50, 70% MVC	To assess the intra-and inter-session reliability of estimates of CV derived from high-density surface EMG.
Beretta-Piccoli et al (2017)	n=40	BB	isometric flexions of the forearm at 20% and 60% MVC	To determine the test-retest reliability of muscle fiber CV obtained from multichannel sEMG recordings.
Martinez-Valdes et al (2017)	n=17	VL VM	isometric knee extensions at 10, 30, 50, 70% MVC	To determine the test-retest reliability of motor unit CV extracted from high-density sEMG signals using a novel decomposition approach.
Beretta-Piccoli et al (2018)	n=28	BB	isometric flexions of the forearm at 10, 20, 30, 40, 50, 60, 70, 80, 90% MVC	To evaluate the relationship between force and several EMG variables during isometric contractions and to examine their reliability.

Abbreviations: MVC, maximal voluntary contraction; AS, anterior scalene; ATM, anterior temporal muscle; BB, biceps brachii; CV, conduction velocity; EMG, electromyography; PPO, peak power output; SCM, sternocleidomastoid, VL, vastus lateralis; VM, vastus medialis, VMO, vastus medialis obliquus.

* only 13 subjects participated in the intraindividual reliability analysis.

2) Harba and Teng (1999) and Merletti et al. (1998, 1995) investigated the *intrasession reliability* of CV. Harba and Teng evaluated the reliability of CV measured at different locations in the BB, with a custom-made linear array, using the relative variance (R%), which is defined as the squared coefficient of variation. Nevertheless, their results suggest that it was not possible to obtain the exact same CV value using the cross-correlation technique. The authors found CV variations over time of up to 5.4% (in terms of R%), when the electrode location, IED and signal epoch were fixed. When different electrode locations were investigated, and the IED was fixed, the CV variations were up to $\pm$ 0.75 ms^{-1}. An increase in the time delay (when the electrodes were more widely spaced) resulted in more consistent CV estimates. The studies by Merletti et al. (1998, 1995) examined the reliability of muscle fiber CV estimates, slope and area ratio after electrically eliciting contractions in the VM and

TA muscles. Their results showed the low reliability of CV based on the ICC values although, in general, the Pearson's r values were high.

Table 3 Characteristics of sEMG electrodes and signal acquisition.

Authors (y)	sEMG electrodes				IZ / motor point identification	voluntary contraction	signal type	sEMG signal acquisition		Conduction Velocity	
	type (number)	size (mm)	IED (mm)	location				type	visual channel selection	estimation method	physiological range (m/s)
Linssen et al (1993)	linear array (4)	2	12	anat land	✓	✓	interferential	SD	×	on SD, FFT-based A	NA
Macaluso et al (1994)	linear array (3)	2.75	15	muscle belly	×	✓	interferential	SD	×	on SD, c-c function	NA
Merletti et al (1995)	linear array (4)	10x1	5	anat land	✓	×	single potential	SD	×	on DD, DFT-based AA	2-8
Hogrel et al (1998)	linear array (3)	4	13	anat land	×	✓	interferential	SD	×	on SD, c-c function	NA
Merletti et al (1998)	linear array (4)	10x1	10	anat land	✓	×	single potential	SD	×	on DD, DFT-based AA	2-8
Harba and Teng (1999)	linear array (6)	7.5	7.5, 15	anat land	×	✓	interferential	SD	×	on SD, c-c function	NA
Rainoldi et al (1999)	linear array (4)	4x1	10	anat land	✓	✓	interferential	SD	×	on DD, DFT-based AA	NA
Rainoldi et al (2001)	linear array (4)	4x1	10	anat land	✓	✓	interferential	SD	×	on DD, DFT-based AA	NA
Falla et al (2002)	linear array (4)	5x1	10	anat land	✓	✓	interferential	SD	✓	on DD, DFT-based AA	2.5-6.5 [2]
Farina et al (2004a)	2D array (61)	1.27	5	anat land	✓	✓	interferential	SD	✓	on DD, multichannel maxlike [1]	2-7 [1]
Ollivier et al (2005)	linear array (4)	4	11	anat land	✓	✓	interferential	SD	×	on SD, c-c function	NA
	2D array (11)	4	10							Laplacian configuration	
Macdonald et al (2008)	linear array (4)	7	10	anat land	✓	✓	interferential	SD	×	on DD, multichannel maxlike [3]	2-8
McIntosh and Gabriel (2012)	linear array (3)	10x1	5	anat land	✓	✓	interferential	SD	×	on SD, c-c function	2-10
Martinez-Valdes et al (2016)	2D array (64)	1	8	anat land	✓	✓	single potential	monopolar	✓	on DD, multichannel maxlike [1]	2-6
Beretta-Piccoli et al (2017)	2D array (64)	3	10	anat land	×	✓	interferential	monopolar	✓	on SD, multichannel maxlike [1]	3-8
Martinez-Valdes et al (2017)	2D array (64)	1	8	anat land	✓	✓	single potential	monopolar	✓	on DD, multichannel maxlike [1]	2-6
Beretta-Piccoli et al (2018)	2D array (64)	3	10	anat land	×	✓	interferential	monopolar	✓	on SD, multichannel maxlike [1]	3-6.5

Abbreviations: IED, inter-electrode distance; anat land, anatomical landmarks; IZ, innervation zone; SD, single differential; DD, double differential; c-c, cross-correlation; NA, not available; maxlike maximum likelihood; FFT-based, fast Fourier transform based algorithm; DFT-based AA, discrete Fourier transform alignment algorithm.

[1] according to Farina et al., 2001
[2] according to Rainoldi et al., 2001
[3] according to Farina et al., 2004

3) Finally, the other 10 studies (Beretta-Piccoli et al., 2017; Falla et al., 2002; Farina et al., 2004b; Linssen et al., 1993; MacDonald et al., 2008; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi et al., 2001; Rainoldi et al., 1999) investigated the *intersession reliability* of CV. Moderate to high reliability scores (ICC 0.7–1), of either CV or slope estimates, were reported by six studies (Beretta-Piccoli et al., 2017; Farina et al., 2004b; Linssen et al., 1993; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi et al., 2001). Rainoldi et al. (2001) also evaluated the reliability of CV using the Fisher test (F) ratio between the mean squared error (MSE) due to the subject's differences and the sum of the MSE due to trial and day variations, and they obtained similar results.

Furthermore, both relative and absolute reliability of CV were evaluated in 12 out of 17 studies (of these 12, one assessed test-retest reliability, one assessed intrasession reliability and 10 assessed intersession reliability). The following coefficients were used to assess relative and absolute reliability:

- relative reliability: ICC (n=13 studies); Pearson's r (n=3); analysis of variance (ANOVA)-based Fisher test, F (n=1) (Atkinson and Nevill, 1998);
- absolute reliability: standard error of the mean, SE (n=5); standard error of measurements, SEM (n=5); coefficient of variation, CoV (n=4); square CoV (n=1); minimal detectable change, MDC_{95} (n=1); Bland & Altman 95% limits of agreement, LoA (1986) (n=2).

In the study by Hogrel et al. (1998), median reliability was assessed, without using a particular coefficient (Table 4).

Generally, a higher degree of reliability was found more often in the more recent studies, in association with the use of bi-dimensional arrays, reduced IED, multichannel algorithms and, in particular, in association with the use of the initial CV value.

Discussion

In this systematic review, we aimed to synthesize the evidence concerning the reliability of sEMG for assessing muscle fiber CV. Seventeen studies met the inclusion criteria and were reviewed. Despite several methodological flaws identified in the included studies, which are discussed later on, the results of this review indicate that sEMG is a reliable tool for estimating CV in muscles with relatively long fibers that are parallel to the skin surface. There was great variability in the experimental conditions (e.g., isometric or dynamic conditions in various muscles, with various acquisition systems and CV estimation methods using interferential signals or single potentials), and in the test-retest periods (ranging from 4 minutes to 2 weeks). The more recent studies showed a higher degree of reliability (ICC up to 0.9; Table 4). Moreover, the results suggest that the mean and initial CV values are generally the most reliable extracted parameters (compared to the slope and area ratio), with higher ICC and lower absolute reliability values, suggesting that the mean and initial CV values are sufficiently accurate for clinical applications.

Nevertheless, a major aspect of using sEMG to estimate CV is the operator-dependent nature of the CV estimates, though this was not considered in any of the 17 studies. For instance, muscle fiber CV estimation during isometric contractions and, in particular, dynamic contractions is strongly affected by the electrode positioning. Before placing the electrodes, attention should be paid to the guidelines suggested by the "Surface EMG for a Non-Invasive Assessment of Muscles" (SENIAM) project (Hermens et al., 2000) and to descriptions of the IZ locations in superficial muscles (Barbero et al., 2012; Beretta Piccoli et al., 2014). Moreover, due to the anatomical variability of the IZ, while an

optimal or highly reliable method regarding electrode placement may exist for specific muscles and specific subjects, there is no optimal method for the same muscle in different populations or different muscles in the same subject.

Table 4 Reliability analysis

STUDY	TEST-RETEST PERIOD	ELECTRODES REPOSITIONING	EXTRACTED PARAMETER	% MVC	MUSCLE	RELATIVE RELIABILITY		ABSOLUTE RELIABILITY			
Macaluso et al (1994)	1 hour	×	ND		ATM	NA					
Hogrel et al (1998)	1 hour	✓, × random	median value	1 20 40 60 80 100	VL	Reliability of the median estimate was assessed by verifying that its 95% confidence interval was within the limits of the system resolution					
			slope	80		good test-retest reliability					
Martinez-Valdes et al (2016)	T1-T2: 15' T2-T3: 30' T1-T3: 45'	×	mean value	10 30 50 70	VL	test-retest ICC 0.89 0.85 0.92 0.93	inter ICC 0.85 0.86 0.92 0.97	CoV (%) test-retest 1.5 1.7 1.8 1.6	CoV (%) inter 2.0 1.8 1.8 1.7	SEM test-retest 0.07 0.09 0.1 0.11	SEM inter 0.09 0.09 0.1 0.09
				10 30 50 70	VM	0.94 0.88 0.95 0.97	0.95 0.91 0.96 0.94	1.3 1.9 1.5 1.5	1.3 1.6 1.4 1.6	0.07 0.09 0.09 0.09	0.06 0.09 0.08 0.11
	1 week	✓ the skin was marked									
Beretta-Piccoli et al (2018)	1 hour	×	initial value	10 20 30 40 50 60 70 80 90	BB	test-retest ICC 0.95 0.97 0.98 0.97 0.96 0.96 0.90 0.91 0.96	inter ICC 0.79 0.76 0.77 0.68 0.39 0.59 0.21 0.04 0.22				
	1 week	✓									

Merletti et al (1995)	T1-T2: 12' T2-T3: 20' T1-T3: 32'	✓ the skin was marked	initial value n slope area ratio	ES	TA	ICC 0.11 0.44 0.44	Pearson's r 0.25; 0.12; 0.26 0.45; 0.88; 0.62 0.47; 0.82; 0.56		
Merletti et al (1998)	T1-T2: 4' T2-T3: 4' T1-T3: 8'	✓ the skin was marked	initial value n slope area ratio	ES	VM	ICC 0.36 0.42 0.43	Pearson's r 0.83 0.88 0.70 0.41 0.89 0.35 0.68 0.94 0.60	CoV (range) 9.1 (5.1-12) 49.5 (16-82) 44.9 (23-76)	
Harba and Teng (1999)	few seconds	✓	initial value	ND	BB			relative variance R (squared CoV) 5.4%	
Linssen et al (1993)	1 week	✓	initial value slope	80	BB	Pearson's r = 0.81 Pearson's r = 0.80		SE = 0.37 SE = 0.62	
Rainoldi et al (1999)	3 consecutive days	✓ the skin was marked	initial value slope	10 30 50 70 BB 10 30 50 70		ICC 0.40 0.26 -0.22 -0.27 -0.05 0.03 0.14 0.30		(n)SE within (%) 1.88 2.52 3.09 3.15	(n)SE between (%) 2.21 2.47 1.50 1.10
Rainoldi et al (2001)	3 consecutive days	✓ the skin was marked	initial value slope n slope	50	r VL r VMO l VL l VMO r VL r VMO l VL l VMO r VL r VMO l VL l VMO	ICC 0.80 -0.10 0.40 -0.08	Fisher test F ratio 12.44 0.63 2.61 0.76 0.83 0.58 0.12 1.15 0.83 1.01 0.25 1.49	(n)SE within (%) 4.60 7.96 5.57 5.99	(n)SE between (%) 9.39 5.38 5.19 5.21
Falla et al (2002)	3 non-consecutive days	✓	initial value slope initial value slope	50	SCM AS	ICC 0.42 0.086 0.28 0.031		(n)SE within (%) 2.99 14.47 3.23 54.90	(n)SE between (%) 2.84 8.53 2.61 24.10

Farina et al (2004)						method **	ICC	(n)SE within (%)		(n)SE between (%)	
						[2,5]	-0.05	7.60		4.39	
						[2,10]	0.27				
						[2,15]	0.52				
						[2,30]	-0.03	6.19			
						[3,5]	0.25				
						[3,10]	0.70				
						[3,15]	0.43	5.48			
			initial value			[4,5]	0.60	2.34		4.54	
						[4,10]	0.75	3.82			
						[5,5]	0.63	2.72			
						[6,5]	0.72	2.21		4.60	
	3 non-consecutive days	✓ the skin was marked		50	BB	[7,5]	0.74				
						[2,5]	0.01				
						[2,10]	0.08				
						[2,15]	0.19				
						[2,30]	0.04				
						[3,5]	0.10				
			slope			[3,10]	0.33				
						[3,15]	0.06				
						[4,5]	0.22				
						[4,10]	0.34				
						[5,5]	0.22				
						[6,5]	0.35				
						[7,5]	0.38				
Ollivier et al (2005)						ICC (bipolar)	ICC (Laplacian)	CoV (range)			
	3 non-consecutive days	✓ the skin was marked	initial value	20		0.75	0.37				
				40		0.64	0.60				
				60	BB	0.60	0.50				
				80		0.71	0.16	10-15%			
				100		0.80	0.11				
			slope	60		0.44	0.08				
			area ratio	60		0.50	-0.07				
Macdonald et al (2008)						ICC		CoV (%)	SEM	Bias	LofA
	5 consecutive days	✓		20		0.44		7.1	0.13	0.06	0.35
				40	VL	0.69		5.1	0.31	0.06	0.87
				60		0.59		6.3	0.43	0.09	1.18
				80		0.65		6.2	0.38	0.12	1.05
			mean value								
				20		0.42		9.4	0.64	-0.11	1.78
				40	VM	0.58		8.3	0.57	-0.17	1.58
				60		0.55		8.6	0.63	-0.20	1.75
				80		0.57		8.8	0.63	-0.14	1.75
McIntosh and Gabriel (2012)	3 non-consecutive days	✓	initial value	30	TA	ICC=0.84		SEM=0.10 m s^{-1}			
				100		ICC=0.83		SEM=1.65 m s^{-1}			
Beretta-Piccoli et al (2017)	1 week	✓	initial value	20; 60	BB	ICC: 0.70; 0.68		SEM: 0.04; 0.05		B&A mean 0.050	LofA (-1.26 – 1.36)
								MDC95: 0.12; 0.14		-0.015	(-1.61 – 1.56)
			n slope	20; 60		ICC: (-0.09); 0.78		SEM: 0.009; 0.02		-0.028	(-0.35 – 0.29)
								MDC95: 0.02; 0.06		0.009	(-0.57 – 0.61)
Martinez-Valdes et al (2017)	2 weeks T1-T3	✓ the skin was marked	mean value	ramps 0-10-0		ICC for matched MUs, averaged across all contraction levels		SEM, averaged across all contraction levels			
				0-30-0							
				0-50-0	VL	0.88 (0.84-0.99)		0.12 (0.07-0.12)			
				0-70-0	VM	0.84 (0.83-0.87)		0.18 (0.16-0.21)			

Abbreviations: MVC, maximal voluntary contraction; AS, anterior scalene; ATM, anterior temporal muscle; BB, biceps brachii; SCM, sternocleidomastoid, VL, vastus lateralis; VM, vastus medialis, VMO, vastus medialis obliquus; NA, not available; ND, not defined; ICC, intraclass correlation coefficient; MU, motor unit; n slope, normalized slope; (n)SE,

(normalized) standard error of the mean; (n)SEM, (normalized) standard error of measurement; CoV, coefficient of variation; LofA, level of agreement; B&A, Bland and Altman; MDC95, minimal detectable change; T1, trial 1; ES, electrical stimulation; intra, intrasession; inter, intersession.

** see text for explanations.

1. Reporting quality assessment

The overall reporting quality of the included studies (five GRRAS checklist items were considered inapplicable) was in general between moderate and good. Although the majority of the item results are self-explanatory, the following points are notable:

Item 1: 13 studies did not mention in the title/abstract which types of reliability (test-retest, intrasession and/or intersession) were selected to investigate CV. This lack only partially affects the MEDLINE search as, in the hierarchical classification of MeSH terms, the entry term *reliability* refers to the MeSH term "reproducibility of results" (which was used in the search string), which also includes "test-retest reliability," but not "intrasession" or "intersession." Moreover, in the Web of Science database, the terms "test-retest,", "intrasession" and "intersession" are not indexed. Therefore, since the primary resources for searching evidence are internet and the bibliographic resource, authors should use the MeSH terms explicitly in the title/abstract as suggested in (Kottner et al., 2011).

Item 5: the reviewers agreed to consider item 5 (which requires the information that is already known about reliability and agreement to be described) as inapplicable to the studies by Macaluso et al. (1994) and Martinez-Valdes et al. (2017), as the two studies were pioneer investigations and thus were not required to provide an overview of existing reliability evidence.

Item 10: the statistical methods selected to analyze reliability were heterogeneous, ranging from Pearson's r to the Bland & Altman plot. Moreover, the early studies assessed only one type of reliability (relative or absolute). Additionally, two studies (Harba and Teng, 1999; Macaluso et al., 1994) did not accurately describe their statistical methods or the reason why a certain approach was chosen.

Item 13: Due to the very broad spectrum of statistical approaches that can be adopted, the GRRAS recommends reporting at least one combination of reliability coefficients, e.g., measures of relative and absolute reliability, to allow a better interpretation of the calculated values. In five studies (Beretta-Piccoli et al., 2018; Harba and Teng, 1999; Hogrel et al., 1998; Macaluso et al., 1994;

Ollivier et al., 2005), the Results sections provided only limited information about the reliability tests, making the reliability results more difficult to comprehend and interpret.

2. Reliability assessment

In the medical literature, at least two critical issues regarding reliability studies have been identified:

1) The term "reliability" has been used interchangeably with "repeatability," "reproducibility," "consistency," "agreement," "concordance" and "stability," with varying degrees of consistency (Atkinson and Nevill, 1998; Bartlett and Frost, 2008).

2) Many statistical tests have been used to assess reliability, and no single approach can be regarded as standard (Dunn et al., 2004). Despite this, the recommendations in the GRAAS (Kottner et al. 2011) suggest reporting at least one combination of coefficients (e.g., ICC and SEM), which should allow the reader to form a more detailed impression of the degree of reliability. For instance, the reliability results expressed using Pearson's r in this systematic review should be treated carefully, as this coefficient only gives information about the degree of association between repeated measures. Moreover, it cannot detect systematic errors: high correlation does not mean high reliability (Bruton et al., 2000).

2.1 Test-retest reliability

Very high test-retest reliability (ICC > 0.9) of initial CV values estimated using interferential signals in the BB was reported in the study by Beretta-Piccoli et al. (2018). Moreover, the study by Martinez-Valdes et al. (2016) also reported mostly very high levels of reliability (ICC > 0.8) for MU CV estimated using surface multichannel single potentials in the vastii muscles. High reliability was independent of force intensity in both studies.

2.2 Intrasession reliability

Two studies reported low intrasession reliability of initial CV values, normalized slopes and area ratios (ICC < 0.45) estimated from compound potentials in muscles of the lower limb (Merletti et al., 1995; 1998). A third study, by Harba and Teng (1999), also reported intrasession reliability but, as reliability was not expressed in terms of ICC, or Pearson's r, it was not possible to compare the reliability results with those of the other studies.

2.3 Intersession reliability

Seven out of 12 studies (including studies with multiple designs for assessing reliability) showed high intersession reliability of CV estimated from compound potentials (ICC > 0.8) and interferential signals (ICC > 0.7) in lower and upper limb muscles, mainly at force levels ≥ 50% MVC. Low absolute reliability estimators for CV and slope values (within-subjects normalized SE < 10% and SEM ≤ 0.11) suggest that these parameters are sufficiently accurate and suitable for clinical applications (Table 4).

3. Conditions for reliable CV estimation

The authors of the included studies identified several factors that may affect the reliability of the CV estimation, ranging from the muscle architecture to the algorithm used. In this section, the most relevant conditions for highly reliable CV estimation are summarized.

a) Muscle characteristics and contraction type

The muscles from which CV was reliably estimated were limited in number, but included the VL and VM/VMO (Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017; Rainoldi et al., 2001), BB (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; Farina et al., 2004b; Ollivier et al., 2005) and TA (McIntosh and Gabriel, 2012). The common anatomical features of these muscles are the presence of relatively long fibers arranged in a plane parallel to the skin, with IZs concentrated in a small muscle region. In addition to isometric contractions, these features are particularly relevant when dynamic contractions are performed (e.g., MacDonald et al., 2008) as, during movement, the IZ shift and muscle shortening limit the portion of fiber semi-length in which propagating signals can be detected (Farina et al., 2004b). Notably, in pinnate or fusiform muscles with multiple IZs, the CV cannot be estimated reliably (Barbero et al., 2012).

b) Electrode locations and positioning

The issue of electrode locations (as well as repositioning before each experiment) was identified as the most critical factor influencing the reliability of CV estimation in all the included studies. For instance, in the study by Hogrel et al. (1998), the authors identified two “muscular critical zones” that must be avoided. A systematic overestimation of CV occurred in these regions, i.e., when myotendinous junctions and/or neuromuscular junctions were in the detection volume. Moreover,

reliable CV estimation requires careful orientation of the electrodes along the muscle fibers. This issue was investigated by McIntosh and Gabriel (2012), who proposed a novel procedure to help operators orient the electrodes, which resulted in highly reliable CV estimates. Furthermore, the development of accurate electrode positioning criteria (based on the localization of the IZs) is recognized as vital for achieving standardization of sEMG methodology (Falla et al., 2002). The identification of these criteria represent the main goal of the standardization process initiated with European Concerted Action – "Surface EMG for a Non-Invasive Assessment of Muscles" (SENIAM) and continued with the publication of "Atlas of Muscle Innervation Zones" (Barbero et al., 2012), which suggests appropriate electrode positions when a single electrode pair is used.

In addition, when using electrode arrays, visual inspection is needed to select the channels between the IZ and tendons, where the MUAPs appear similar in shape and shifted in time (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; Farina et al., 2004c; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017).

c) IED and number of channels

The selection of the distance between detection points is critical, and it greatly depends on the semi-fiber length. Thus, it is not possible to suggest an optimal IED that applies to all muscles. Nevertheless, the reliability of CV estimates increases with an increasing number of signals and an increasing IED, between 5 and 10 mm (Farina et al., 2004c; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi, 2001). As, an IED of 5 mm may be more affected by minor electrode displacements and local tissue dishomogeneities (Merletti et al., 1995), small electrodes (diameter < 3 mm) with small IED (< 10 mm) have to be recommended in order to avoid the spatial aliasing (Afsharipour et al., 2019; Afsharipour et al., 2015). Therefore, using multichannel sEMG with between four and eight electrodes and 5 mm IED, can help to increase the reliability of CV (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; Farina et al., 2004c; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017).

d) CV estimation method

Among the factors affecting the reliability of CV estimates, the estimation method has an impact. In fact, before the study by Farina et al. (2004c), in which bi-dimensional arrays of electrodes and the multichannel maximum-likelihood algorithm were used, a highly reliable estimation of CV was achieved only once, in the study by Rainoldi et al. (2001). This was done using the discrete Fourier

transform-based algorithm (McGill and Dorfman, 1984) and a four-bar linear electrode array. Furthermore, the cross-correlation analysis of two delayed signals (Naeije and Zorn, 1983) may be applied to reliably estimate muscle fiber CV when using a three- or four-electrode system (McIntosh and Gabriel, 2012; Ollivier et al., 2005), anyway this method requires interpolation, which requires a spatial sampling frequency is above the Nyquist rate (Afsharipour et al., 2019).

Nonetheless, the most reliable and robust methods are those based on maximum-likelihood estimation in the frequency domain (Farina et al., 2001), which provide higher velocity resolution and lower variance than other approaches (Farina and Merletti, 2004b). Further developments and adaptations of the maximum-likelihood algorithm (Farina and Merletti, 2004a; Farina et al., 2004c) have allowed highly reliable estimates of muscle fiber CV and CV associated with single MUs to be obtained during isometric and dynamic contractions, using multichannel sEMG involving interferential and single potential signals (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017).

e) CV parameters

Initial and mean CV values were the most reliable parameters in eight studies (Beretta-Piccoli et al., 2018; Farina et al., 2004c; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017; McIntosh and Gabriel, 2012; Ollivier et al., 2005; Rainoldi et al., 2001), whereas the slope was associated with a high degree of reliability only once, during fatiguing isometric contractions of the BB (Beretta-Piccoli et al., 2017). The estimation of the muscle fiber CV slope may be useful to characterize the peripheral components of muscle fatigue (Arendt-Nielsen et al., 1989; Bigland-Ritchie et al., 1981). Furthermore, if the MU pool is stable, this variable correlates with fiber size and type (Sadoyama et al., 1988).

In seven studies (Falla et al., 2002; Farina et al., 2004c; Merletti et al., 1998; Merletti et al., 1995; Ollivier et al., 2005; Rainoldi et al., 2001; Rainoldi et al., 1999), CV slopes were not as reproducible as the initial CV values, reflecting the high sensitivity of CV slope to electrode repositioning in retest sessions. Notwithstanding, the promising result of Beretta-Piccoli et al. (2017), regarding the high degree of reliability of slope estimates, may be related to the reduction in noise associated with the use of bi-dimensional arrays with a 10-mm IED (which allows a larger number of electrodes and an optimal distance between detection points, and which lowers the sensitivity to electrode displacement).

However, it should be noted that as CV slopes depend on fatigability, their reproducibility also depends on the reproducibility of the fatiguing protocol and of the subject's fatigability conditions. Good reproducibility of such variables is very difficult to achieve. Additional studies are needed to investigate whether muscle fiber CV slope calculated using multichannel recordings in various superficial muscles with fibers parallel to the skin is a reliable parameter.

f) Operator

Importantly, the issue is not simply the reliability of sEMG itself for assessing CV, but also the training and expertise of the operator regarding the use of this technique to correctly detect, process and interpret sEMG signals (Barbero et al., 2012). For example, visual inspection to select the appropriate number of channels of a bi-dimensional electrode array, to be used for CV estimation, is still needed. Operators should be able to identify movement artifacts, missing channels or the presence of large sinusoidal components at 50 Hz, which must be dealt with before the analysis.

Study limitations

i) The lack of standardized reporting across the reliability studies (probably partly because the GRRAS checklist was only published in 2011) combined with the poor statistical analysis descriptions (particularly in the early studies) limited the data extracted for use in this systematic review. Adequate reporting regarding the methodology used in reliability studies should be encouraged. Reporting can often be affected by the word count restrictions imposed by journals at the time of publication. However, inadequate reporting affects the comparison of results between studies and restricts the synthesis of evidence.

ii) The included studies considered only six muscles when investigating the reliability of CV: BB (n=7), VL and VM (n=5), TA (n=2), SCM (n=1) and AS (n=1). Therefore, the conclusions of this systematic review may only be applicable to these muscles and may not be generalizable to other muscles.

iii) Most studies analyzed the reliability of CV estimation during isometric contractions as opposed to functional activities (only one study analyzed dynamic contractions, which involved cyclic movements of the vastii muscles (MacDonald et al., 2008)). Therefore, the results of this review are not generalizable to dynamic conditions other than cyclic.

Conclusions

Since the publication of the recommendations by Farina et al. (2004c) regarding the appropriate number of electrodes and IED, along with visual channel selection and the use of the maximum-likelihood algorithm, the results of studies investigating the reliability of CV estimation in muscle fibers parallel to the skin exhibited an increase in reliability. In addition, trained expert operators should be encouraged to use a standardized electrode location and possibly to identify the IZ location prior to positioning. In these conditions, muscle fiber CV estimates (as an important physiological parameter) and slope (as an indicator of muscle fatigue), are suitable for use in sport science, rehabilitation and interventional studies with multisession longitudinal designs. To continuously increase the reporting quality of reliability studies, a critical requirement for future studies is to follow the GRRAS guidelines.

STUDY 2 Relationship between isometric muscle force and fractal dimension of surface electromyogram

Introduction

The relation between electromyography (EMG) and force has been a controversial topic for more than four decades. The surface EMG (sEMG)/force relationship strongly depends on motor units (MUs) control by the central nervous system (CNS) and by the peripheral features of muscle. The CNS modulates the force expressed by the muscle by controlling two parameters: the recruitment of MUs and the firing rate of active MUs (Erim et al., 1996). These two parameters are directly connected with the generation of electrical activity inside the muscle and also influence the sEMG signal (Fuglevand et al., 1993a). Indeed, the sEMG signal is a result of the interferential summation of MU action potentials (MUAPs) detected by electrodes and thus it is of interest to understand the role played by the neural parameters in driving the sEMG-force relationship (Keenan and Valero-Cuevas, 2007). The shape of this relationship has been explored in experimental and simulation studies, with conflicting results ranging from linearity to non-linearity (Al Harrach et al., 2017; Basmajian, 1978; Bigland-Ritchie, 1981; Botter et al., 2011; Lawrence and De Luca, 1983; Milner-Brown and Stein, 1975; Moritani and deVries, 1978; Zhou and Rymer, 2004). The shape of this relationship might also depend on the muscle investigated, on muscle fiber composition and muscle fiber size (Alkner et al., 2000; Zhou and Rymer, 2004).

Inconsistent results in the literature may also reflect that muscles are not necessarily uniformly activated at increased loads in a specific action. For this reason, sEMG varies spatially over the muscle belly (Holtermann et al., 2005; Staudenmann et al., 2009; Staudenmann et al., 2014). Applying multichannel array electrode systems in sEMG recordings have been demonstrated to improve the extraction of reliable sEMG/force relationship increasing the representability of the measured sEMG signal (Rantalainen et al., 2012; Staudenmann et al., 2006; Staudenmann et al., 2005).

Great interest has been given in the literature to the non-linear feature of the sEMG signal, such as recurrent quantification analysis, percentage of determinism, sample entropy, normalized mutual information and fractal dimension (FD) (Bingham et al., 2017; Eckmann et al., 1987; Felici et al., 2001; Gitter and Czerniecki, 1995; Richman and Moorman, 2000). Non-linear analysis offers a powerful approach for the investigation of physiological time series because it provides a measure of the signal complexity. In particular, the FD of the signal is a measure of self-similarity over multiple time scales. Several studies (Anmuth et al., 1994; Gitter and Czerniecki, 1995; Gupta et al., 1997;

Shields, 2006; Xu and Xiao, 1997) have applied box-counting methods to estimate the FD of the sEMG signal and a recent investigation, showed a good reliability of FD during isometric contractions in the BB muscle (Beretta-Piccoli et al., 2017).

Non-linear feature of the sEMG has been widely applied to monitor the myoelectric manifestations of fatigue during the course of isometric contractions (Gazzoni et al., 2017). Indeed, during sustained submaximal contractions, the alterations in the activity of muscles undergoing fatigue can be quantified, using linear or non-linear methods, prior to task failure (Gazzoni et al., 2017). Mesin and colleagues (2009a) computed a combination of both linear and non-linear analysis to synthetic and experimental sEMG signals. They found that FD was the most related to the level of synchronization and least related to the changes of muscle fiber conduction velocity (CV). Consequently, they proposed the combination of FD and CV as a bi-dimensional index providing information about the central and peripheral adjustments occurring during fatigue (Mesin et al., 2009a). In a more recent simulation study, Mesin and colleagues (2016) found that beyond synchronization level, the FD of the EMG signals increased with the average firing rate of the active MUs. For this reason, recently, the combined monitoring of muscle fiber conduction velocity (CV) and FD parameters during continuous contractions was applied in the evaluation of myoelectric manifestations of fatigue (Boccia et al., 2016; Beretta-Piccoli et al., 2015; Meduri et al., 2016). However, to fully understand the applicability of FD analysis in the study of myoelectric manifestations of fatigue, it is crucial to determine if the FD is also affected by the level of force exerted by muscles.

Some studies found that the FD of sEMG was linearly but weakly related to the contraction level (% of maximal voluntary contraction, MVC) in simulated and experimental conditions (Gitter and Czerniecki, 1995; Anmuth et al., 1994; Gupta et al., 1997). However, recent investigations showed that FD is not related to the intensity of muscle contraction (Arjunan and Kumar, 2014; Troiano et al., 2008): therefore, the relationship between force and the FD of sEMG is still controversial. Thus, the aim of this study was to evaluate the relationship between force and FD of sEMG during isometric contractions of the BB.

Methods

Participants

Twenty-eight healthy recreationally active volunteers (14 women and 14 men) aged between 20 and 36 years (25±4 yrs) from a university setting were recruited to participate in the study.

Experimental procedure

The subjects participated in three experimental sessions ("trials 1-3"): the first two trials were conducted within the same day, with four minutes of rest in between, without repositioning the electrodes. The third trial was performed a week apart under the same environmental conditions.

Initially, two isometric MVCs were performed, separated by 2 min rest. During each contraction of the trial, the force trace was displayed to participants on a computer monitor as visual feedback. Participants were instructed to increase the force up to their maximum, and to hold it for 2-3 s. Participants were given strong verbal encouragement.

Next, after 4 min rest, the subjects performed a sequence of nine short contractions, from 10 to 90% of their MVC in steps of 10% MVC in randomized order, lasting 5 s, with 20 s of rest in between. After each contraction, the subjects were asked to provide a value of the perceived exertion on a visual Borg scale, ranging from 6 to 20 (Borg, 1982). In the first day of measurement, after the first session (trial 1), a second sequence of contraction, constituting the trial 2, was performed.

The methods used for the setup of the ergometer, sEMG and force measurements and signal processing are described in the General Methods.

Signal processing

For each signal, a 3-s lapse was identified, where the force level was stable within the 10% boundaries of the target force requested to the subjects. Signals were then divided in epochs of 1-s and CV was computed using a multichannel algorithm (Farina and Merletti, 2003) on the selected channels. The three obtained values were then averaged. Next, each of the three epochs of each signal were used for the estimation of ARV, MNF and FD. Estimates obtained from single channels were averaged over the channels previously selected by visual analysis and over the three signal epochs. Therefore, for each contraction level one value for ARV, MNF and FD was obtained.

In addition, ARV, MNF, CV, and FD data, as well as Borg scale values were normalized for each subject according to their values at 70% MVC and expressed as percentages. The force level of 70% was selected after the completion of data collection, since many of the subject could not perform

80 and 90% MVC contraction. The 70% value was the maximum force level, which all the subjects could reach.

Statistical analysis

Intra- and inter-session reliability was examined using the Intraclass Correlation Coefficient ($ICC_{(2,1)}$) on averaged measures (Weir, 2005), since its use has been recommended in reliability studies (Bruton et al., 2000, Rankin and Stokes, 1998). The criteria used for the interpretation of the ICCs were as follows: 0.00–0.25: no correlation; 0.26–0.49: low correlation; 0.50–0.69: moderate correlation; 0.70–0.89: high correlation; 0.90–1.00: very high correlation (Munro, 2005).

To test the relationship between EMG variables and force, only the first session, i.e. trial 1, was considered. A Shapiro-Wilk test revealed that all the estimated EMG variables were not normally distributed across subjects and thus, the non-parametric Kruskal-Wallis test was performed on the sEMG variables for each contraction at difference force levels. Considered factors were trial and force level. When the Kruskal-Wallis test indicated significant variations, a post-hoc Dunn-Bonferroni test (Dunn et al., 2004) was applied on pairwise comparisons; statistical significance was accepted at the $p < 0.001$ level.

The epsilon-squared estimate of effect size was calculated using the following equation (Tomczak and Tomczak, 2014):

$$E_R^2 = \frac{H}{n-1}$$

where H is the value obtained in the Kruskal-Wallis test (the K-W H-test statistics) and n the total number of observations. The E_R^2 coefficient assumes values between 0 (indicating no relationship) and 1 (perfect relationship).

Statistical analyses were performed using SPSS Version 22.0 (SPSS Inc, Chicago, IL, USA), and significance was set to $\alpha=0.05$. Results are reported as median and interquartile range.

Results

Reliability analysis

Table 5 documents the results of $ICC_{(2,1)}$ analysis for the initial values of CV, FD, MNF and ARV during the short isometric contractions, with force levels between 10 and 90% MVC. According to the classification of (Munro, 2005), high to very high levels of intra-session reliability were identified

for all the parameters (ICC between 0.86 and 0.97), whereas the intersession reliability was considerably lower. The most reliable parameter across experimental sessions was indeed ARV, followed by FD and MNF. Initial values of CV showed higher ICC values at lower contraction levels, whereas at force levels between 70% and 90% MVC, CV displayed a very low inter-subject variability, demonstrating dependence on days and trials larger than dependence on subjects (Farina et al., 2004c; Rainoldi et al., 2001).

Table 5 Results of the reliability analysis of initial values of CV, FD, MNF and ARV at 10 to 90% MVC. Intra- and intersession ICC scores are reported.

MVC	ICC			ICC			ICC			ICC	
	intra	inter		intra	inter		intra	inter		intra	inter
10%		0.95	0.79		0.86	0.74		0.94	0.76	0.92	0.83
20%		0.97	0.76		0.86	0.78		0.96	0.86	0.86	0.74
30%		0.98	0.77		0.91	0.81		0.97	0.78	0.96	0.87
40%		0.97	0.68		0.94	0.85		0.97	0.75	0.90	0.85
50%	CV	0.96	0.39	FD	0.91	0.81	MNF	0.97	0.59	ARV 0.88	0.83
60%		0.96	0.59		0.89	0.90		0.96	0.79	0.89	0.81
70%		0.90	0.21		0.87	0.70		0.96	0.77	0.96	0.87
80%		0.91	0.04		0.94	0.82		0.96	0.73	0.96	0.89
90%		0.96	0.22		0.90	0.81		0.96	0.76	0.96	0.81

Note: MVC, maximal voluntary contraction; ICC, intra-class correlation coefficient intra- and inter-session.

Relation with force

Kruskal-Wallis test did not reveal any statistical dependence of the variables on trials. Distributions of FD, ARV, MNF and Borg ratings were similar for all contraction levels, as assessed by visual inspection of boxplots (Figure 18 and 19). Median scores of these parameters were statistically different across the nine levels of force ($p < 0.0001$). Only the increasing trend of CV versus force was not statistically significant, for this reason no post-hoc analysis was performed for CV.

To allow a better visualization of the parameters trend, a boxplot for each normalized parameter with respect to their values at 70% MVC was added to Figure 18. Effect size analysis, i.e. the percentage of the variability of the considered parameters which is really accounted for by the level of force, revealed very high scores for ARV and Borg values (epsilon-squared estimates respectively 87% and 70%), whereas smaller effect size was found for FD and MNF (epsilon-squared estimates

respectively 37% and 17%). The post-hoc analysis revealed statistically significant differences in the considered parameters obtained at low force levels (respectively 10-40% MVC for ARV and Borg ratings and 10-30% MVC for FD and MNF) with respect to high force levels (50-90% MVC) (Figures 18 and 19).

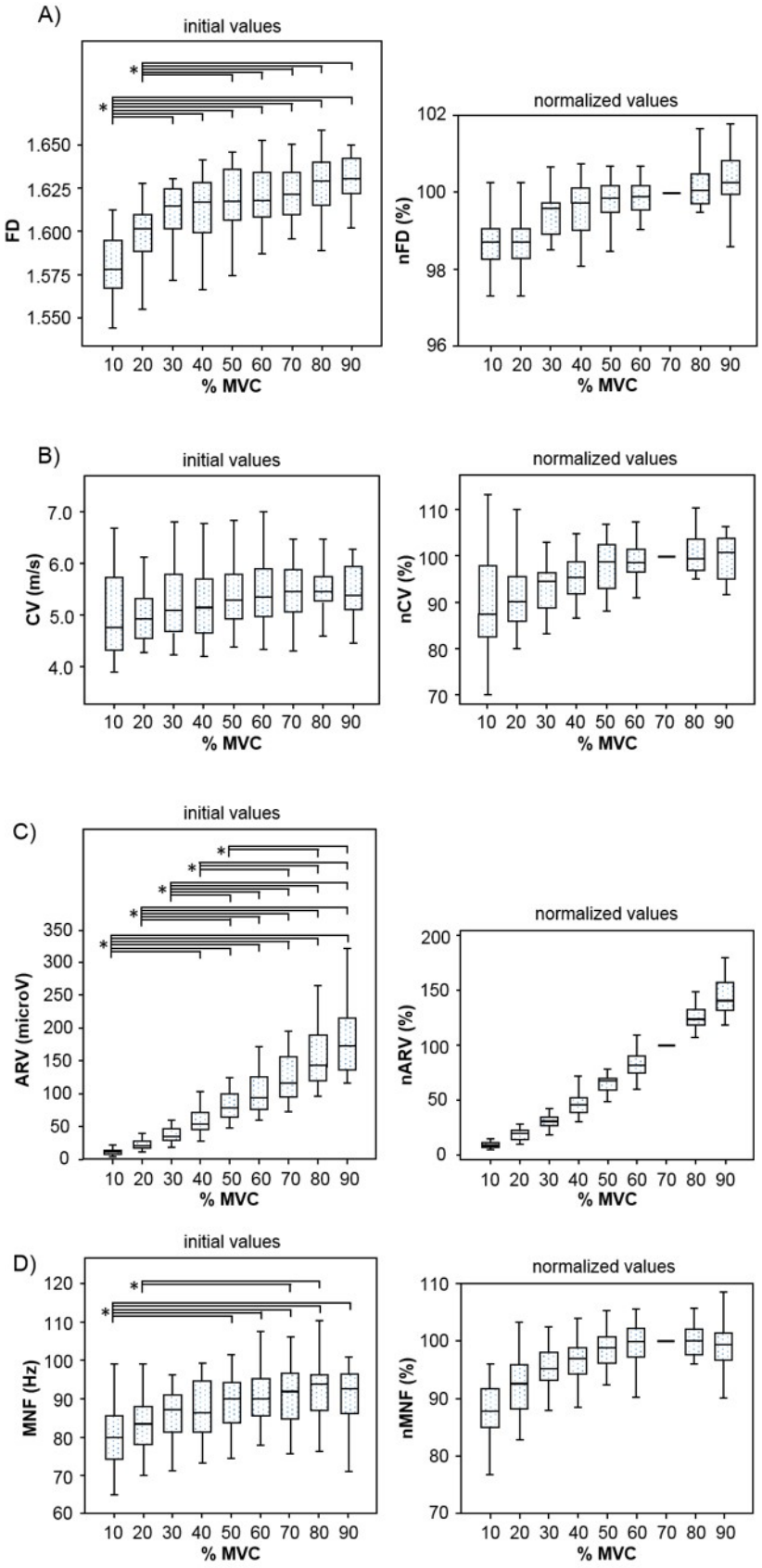

Figure 18 Box-and-whisker plots of initial and normalized values (with respect to their values at 70% MVC) of fractal dimension (FD) conduction velocity (CV), average rectified value (ARV), and mean frequency (MNF) during short isometric 10-90% maximal voluntary contractions (MVCs) of the biceps brachii. Statistically significant results of the Dunn-Bonferroni post-hoc test are indicated (* $p < 0.001$)

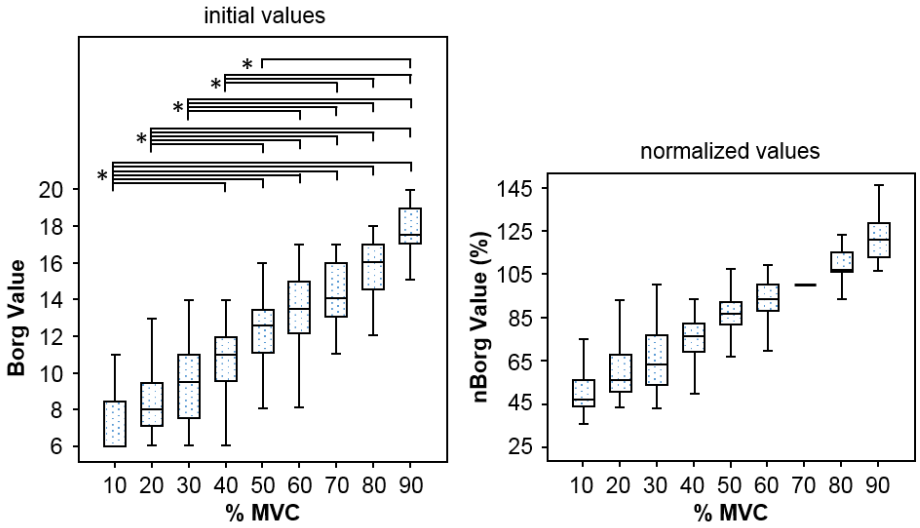

Figure 19 Box-and-whisker plots of the initial and normalized values (with respect to their values at 70% MVC) of Borg ratings during short isometric 10-90% maximal voluntary contractions (MVCs) of the biceps brachii. Statistically significant results of the Dunn-Bonferroni post-hoc test are indicated (* $p < 0.001$)

Discussion

1. *Intra- and intersession reliability*

FD, MNF and ARV showed high intra- and intersession reliability, in accordance with previously published studies (e.g. Arnall et al., 2002; Beretta-Piccoli et al., 2017; Falla et al., 2002; Farina et al., 2004c; Lee et al., 2011; Rainoldi et al., 2001); the intersession reliability of CV at contraction levels higher than 60% MVC, was very low. This result might be explained by the fact that the variability of CV between subjects decreases as the level of contraction increases over 60% MVC (Rainoldi et al., 1999).

2. *Relation between EMG parameters, Borg ratings and force*

In the present study, FD and MNF were the variables least influenced by the level of exerted force (Figures 18A and D). In fact, both variables showed a trend, increasing from 10% to 30% MVC, but thereafter reaching a plateau beyond 30% of MVC (confirmed by the results of the post-hoc analysis, as well). The little or even independence of FD and MNF on the level of muscle force, was reported also in two previous investigations in other muscles and with different methods (Bilodeau et al., 2003; Troiano et al., 2008). In particular, in Troiano et al. (2008) the upper trapezius muscle was investigated, which compared to the BB, presents a much more complex architecture and an heterogeneous distribution of the muscle activity (Gallina et al., 2013a).

As already reported in literature, FD is sensitive to the presence of large active MUAPs that usually appear in the signal due to synchronization at high force levels, during fatiguing contractions (Mesin et al., 2009a). Nevertheless, a similar phenomenon happens also at low force levels, whenever larger MUs and with low firing frequency, are recruited according to the Henneman's size principle. Moreover, in simulated EMG signals, FD was positively correlated to the firing rate of the active MUs, and negatively correlated to the level of MU synchronization (Mesin et al., 2016). Since the level of synchronization is not expected to change in non-fatiguing contractions, it was reasonable to hypothesize that FD could somehow increase with increasing force levels. Thus, it is possible to speculate that FD might be a reliable indicator of MU synchronization, less dependent from the firing rate.

Muscle fiber CV, seems to be the most affordable variable for relating EMG signals modifications and MUs pool recruitment (Farina et al., 2004a). Since CV increases gradually when larger MUs are recruited (Blijham et al., 2006), it was expected to increase with contraction intensity (Andreassen and Arendt-Nielsen, 1987). Contrary to the expectation, the average CV did not increase significantly with increasing force levels although we could observe a trend in that direction in our data set (see Figure 18B). There are two main confounding factors that could have affected CV estimates: (1) the subcutaneous tissue and (2) the alignment of the electrode row along the direction of muscle fibers. Indeed, a high thickness of subcutaneous tissue and malalignment of electrode grids might both produce an overestimation of CV and consequently affect the trend of CV across force levels. Since the CV values were relatively high (>4.5 m/s) even at the lowest force levels (i.e. 10% of MVC), this explanation seems to be plausible. Anyway, an overestimation of CV, if present, would be visible at all contraction levels, thus normalized values would not be affected by this bias.

The amplitude of the EMG signal (ARV) was the variable most dependent on the level of force exerted (Figure 18C). This was an expected result, since many previous studies demonstrated a direct relationship between EMG and force (Al Harrach et al., 2017; Basmajian, 1978; Bigland-Ritchie, 1981; Botter et al., 2011; Lawrence and De Luca, 1983; Milner-Brown and Stein, 1975; Moritani and deVries, 1978). In particular, ARV values obtained at the highest force, i.e. the 90% of the MVC, were greater than those lower or equal to the 50% of MVC. Whereas, between 60% and 90% of MVC, no increase in ARV was found. Thus, EMG amplitude seemed to be sensitive to the increase of force only from low (10% of MVC) to medium (50% of MVC) force levels, but not from 50% to 90%. Even this was an expected result because Troiano and colleagues previously reported the same pattern (Troiano et al., 2008). The recruitment of motor units and the firing rate of active motor units progressively increase at increasing force exertion (Erim et al., 1996), and this leads to increasing electrical activity inside the muscle (Fuglevand et al., 1993a). Consequently, increasing amplitude of

EMG signal would be expected throughout the whole range of forces. However, our results showed that the EMG amplitude was not consistently affected by the increase in force after 50% of MVC. This can be explained by the fact that the amplitude cancellation influenced the measures of EMG amplitude mostly at high force levels. Indeed, the amplitude cancellation has been proven to increase with increasing number of active motor units (Keenan et al., 2005).

Finally, the present study found a relation between ratings of perceived exertion (Borg ratings) and force levels (Figure 19) in line with previous published studies, where a linear relationship, during isometric contractions, was found (Stevens and Cain, 1970; Timmons et al., 2009; Troiano et al., 2008). Interestingly, as occurred with ARV, no statistically significant increase in perceived exertion was found between 60% and 90% of MVC. Together, these results furthermore support previous findings indicating the relationship between muscle activation and perceived exertion (Lagally et al., 2002).

The limitations of this study are mainly related to technical constraints. Firstly, we investigated only one muscle, which, of course, does not represent the behavior of all the muscles. Secondly, to our knowledge, literature is currently lacking studies on validity of FD in estimating MU synchronization. If future studies will overcome this gap, FD will provide a valid and robust measure of MU synchronization during fatiguing contractions.

Conclusions

The present study showed that FD is a reliable EMG parameter at all contraction levels and has little dependency from muscle force, in the BB muscle above 30% MVC. In such conditions, FD can be applied in experimental studies focusing on fatigue or on motor unit synchronization, independently from the force exerted.

STUDY 3 Increased resistance towards fatigability in patients with facioscapulohumeral muscular dystrophy

Introduction

Fatigue is recognized as a common symptom in muscular dystrophies (Kalkman et al., 2005), although little is known about the pathophysiology of this disabling condition. Among dystrophies whose genetic defects have been molecularly identified, facioscapulohumeral muscular dystrophy (FSHD), shows the most peculiar mutation: in fact, the FSHD genetic defect does not reside in any protein-coding gene (Wijmenga et al., 1990). Instead, the disease has been associated in the majority of patients with the contraction a polymorphic region known as D4Z4 (4q35 chromosome) that is characterized by an array of tandemly repeated units of 3.3 kb (van Deutekom et al., 1993; Wijmenga et al., 1992). In normal conditions, the D4Z4 array varies from 10 to 100 repeated units, whereas FSHD patients have less than 10 (Larsen et al., 2015). Clinically, FSHD is characterized by slowly progressive weakness of the facial and shoulder girdle muscles, followed by leg and trunk muscles. The wide variability of clinical outcomes, linked to a large heterogeneity of severity (Mul et al., 2017), allows the identification of at least nine patient subcategories, including classic FSHD phenotype/category A and facial sparing phenotypes/category B1 (Ricci et al., 2016). Overall, clinical examination and family studies suggest that FSHD is a complex disease, in which several factors, including genetic, epigenetic or environmental variables, can influence the onset and development.

In FSHD, fatigue appears as an early leitmotif of the disease and a disabling symptom in common daily activities. In a recent qualitative study by Schipper et al. (2017), patients described fatigue as “an overwhelming and unpredictable experience”, without recognizing the actual causes, which makes it hard to deal with and thus causing a huge impact on participation, social contacts and their quality of life. Moreover, in a survey involving 328 participants with FSHD, one of the symptoms with the highest prevalence was fatigue (93.8%) (Hamel et al., 2019).

For the purpose of this study, fatigue will be discussed within the taxonomy proposed by Kluger et al. (2013). Specifically, fatigue is defined as a symptom or percept, characterized by feelings of tiredness and weakness, in which physical and cognitive functions are limited by interactions between performance fatigability and perceived fatigability. Performance fatigability refers to the decline in an objective measure of performance, such as the production of maximal voluntary force, the ability to provide an adequate signal to voluntary activate muscle, or the involuntary twitch response to

stimulation (Enoka and Duchateau, 2016). In addition, indexes of fatigability may be extracted from the surface electromyogram (sEMG), such as muscle fiber conduction velocity (CV) or non-linear parameters (see Rampichini et al. 2020 for a review).

For instance, Schulte-Mattler et al. (2003) described excessive fatigability in different neuromuscular disorders, though only four patients with FSHD were recruited. Later, Schillings et al. (2007) used the twitch interpolation technique on the BB of patients with myogenic or neurogenic disorders (FSHD, myotonic dystrophy and hereditary motor and sensory neuropathy type I), and described an increased voluntary activation at rest and a reduced performance fatigability following a 2-min sustained isometric MVC, in comparison with age/sex matched controls. However, reduced performance fatigability may be linked to lower strength production caused by an impaired neural drive. Finally, these results agreed with the observations of Di Lazzaro et al. (2004) showing an increased resting motor threshold in FSHD using transcranial magnetic stimulation that, in contrast, was found unchanged by Liepert et al. (2004) in a group of 6 FSHD patients. More recently, Bachasson et al. (2014) using femoral nerve magnetic stimulation showed higher voluntary activation of VL at baseline in FSHD patients compared to control, in contrast to the large activation failure in BB reported by Schillings et al. (2007). Overall, available data do not allow drawing safe conclusions on the relative contribution of central and peripheral factors to performance fatigability in FSHD patients, thus the main purpose of this study was to determine, using fatigability indices extracted from the sEMG signal, whether patients fatigued differently with respect to healthy controls. Moreover, based on the above considerations, we aimed at exploring possible associations between fatigability and clinical parameters of the patients.

Methods

Participants

The study was part of a crowdfunding project (#Sport4therapy) carried out at the CRIAMS-Sport Medicine Centre Voghera promoted by the University of Pavia, aiming at identifying the correct sport therapy approach in patients affected by rare neuromuscular diseases (Siciliano et al., 2019), including FSHD (Berardinelli and D'Antona, 2019). Data collection started in 2013 and was completed in 2019. Inclusion criteria were: age of ≥16 years, a clinical or genetic diagnosis of FSHD, and enrollment in the Italian National Registry for FSHD (INRF). Exclusion criteria were wheelchair bound at selection, use of corticosteroids, severe cardiac and respiratory dysfunction, and psychological-psychiatric disorders. A diagnosis of FSHD had to be confirmed by DNA testing

(Lemmers et al., 2012) at the University of Modena and Reggio Emilia (Italy). Nineteen patients with FSHD were enrolled in the study. Disease severity was assessed through the FSHD clinical score (Lamperti et al., 2010). The FSHD score ranges from 0, when no objective sign of functional impairment is present, to 15, when all tested muscle groups are severely impaired and patient is wheel-chair dependent. Functional alterations of scapular girdle muscles was determined through the specific subscale (scored from 0 to 3). Main participants' characteristics are listed in Table 6. Patients were allocated to the four clinical categories according to the Comprehensive Clinical Evaluation Form (Ricci et al., 2016). Seventeen participants were selected among the FSHD group to be compared with 17 healthy controls, matched for age and sex. All subjects gave their written informed consent to participate in this study, which was conducted according to the Declaration of Helsinki with approval from the local Ethics Committee of the University of Pisa.

Experimental procedures

Perceived and performance fatigability was assessed only once, when the participants attended the Sports Medicine Center Voghera.

1. Perceived fatigability

Trait levels of perceived fatigability were assessed before the fatiguing task, with the fatigue subscale of the Checklist Individual Strength (CIS-fatigue). This scale consists of 8 questions regarding fatigability experienced during the previous 2 weeks; each question was scored on a 7-point Likert scale (Vercoulen et al., 1994). A total score ≥ 35 indicates severe fatigue (Vercoulen et al., 1996). The CIS-fatigue has good internal consistency (Cronbach a 0.83–0.92), high discriminative validity, and high sensitivity to change in patients with FSHD (Kalkman et al., 2007).

2. Performance fatigability

The selected protocol has been shown to produce fatigue in the elbow flexors in healthy subjects and patients (Beretta-Piccoli et al., 2017). Briefly, participants were asked to perform two maximal voluntary contraction (MVC), separated by 2 min rest, followed by a 20% MVC contraction lasting 2 min and finally a 60% MVC held until the force level decreased below 90% of the target (endurance time, i.e. the time for which a subject is able to maintain the requested mechanical task). The two submaximal contractions were separated by 5 min rest.

The methods used for the setup of the ergometer, sEMG and force measurements and signal processing are described in the General Methods.

Statistical analysis

Descriptive statistics were used to present the variables included in both the comparative and the explorative analyses; the categorical variables were described through frequency distributions, while the continuous variables were described using synthetic indicators (median and interquartile range, IQR). At the bivariate level, the analyses were conducted using nonparametric statistical indicators and tests to account for the small sample size and the generalized non-normality of the distributions.

The Wilcoxon signed-rank test was used to evaluate the differences between 20% and 60% MVC for the initial values and the slopes of MNF, CV and FD. Differences in the sEMG measures between FSHD patients and healthy controls were assessed using the Mann-Whitney U test.

For the explorative analysis, the relations between continuous variables were investigated using Spearman correlation coefficients. Finally, differences in the continuous variables related to FSHD categories and asymmetry were assessed using the Mann-Whitney U test. The statistical significance was set at $\alpha = 0.05$. All statistical analyses have been carried out with Stata/IC 16.0 (StataCorp, College Station, Texas, USA).

Table 6 Descriptive statistics of the socio-demographic and clinical variables

		n	**Median**	**IQR**
Socio-demographic variables				
Gender	Woman	10	-	-
	Man	9	-	-
Age		-	33.50	31.25
Clinical variables				
FSHD categories	A	14	-	-
	B	3	-	-
	D	2	-	-
FSHD asymmetry	Right > Left	10	-	-
	Right = Left	6	-	-
	Right < Left	3	-	-
D4Z4 contraction (kb)		-	27.00	11.50
Checklist individual strength[1]		-	26.00	15.00
Severity of FSHD (clinical score)		-	4.00	6.25
Scapular girdle involvement score			2.00	1.00

[1] Variable with 3 missing values (n=17)

FSHD, facioscapulohumeral muscular dystrophy; IQR, interquartile range

Results

Socio-demographic and clinical variables

Fourteen patients out of 19 belonged to category "A", according to (Ricci et al., 2016), presenting facial and scapular girdle muscle weaknesses. A more accentuated muscle weakness on the right side was observed in 10 patients, nine of whom were right-handed (Table 6, category "Right > Left"), while for three patients it was more accentuated on the left side and for six of them it was equally distributed. The median length of the D4Z4 contraction was 27 kb [IQR=11.5]. The median clinical score assessing the severity of FSHD was 4 [IQR=6.25], while the median scapular girdle involvement score was 2 [IQR=1].

Perceived fatigability, as measured with the CIS fatigue-subscale, was reported as mild (26, [IQR=15]).

sEMG variables

Significant changes between the two levels of isometric contractions, were observed in the initial values of CV ($p<0.01$) and in the negative slopes of all the considered sEMG parameters ($p<0.001$; data not shown).

Comparative analysis between the FSHD patients and matched healthy controls

No statistically significant variations were observed in patients' category "A" with respect to the full sample of patients with FSHD. Thus, the results of the comparative analysis are referred to the entire sample of patients. Both groups were composed by 9 women and 8 men. The difference in the median age was not statistically significant (Table 7). The hypothesis test highlighted some statistically significant differences between the two groups: (1) both the MNF and the FD initial values at 20% MVC resulted higher in the FSHD group, while the negative MNF and FD slopes at 60% MVC were less steep. (2) The CV negative slope at 60% MVC resulted less steep in the FSHD patients' group.

Finally, the exerted force resulted lower in the FSHD group, whereas the endurance time was longer compared to the healthy subjects.

Table 7 Descriptive statistics of the variables for the FSHD patients' group and the healthy controls and results of the test

	% MVC	FSHD patients		Healthy controls		Mann-Whitney U test result
		Median	IQR	Median	IQR	
Socio-demographic variables						
Gender	(Men; Women)	(9 women; 8 men)		(9 women; 8 men)		
Age		33.0000	31.2500	24.000	7.7500	z=-0.856
Electromyographic variables						
MNF initial value	20%	97.8149	23.1978	81.1161	14.0275	z=-2.784**
	60%	85.2488	7.4633	86.8530	18.3018	z=-0.380
MNF slope	20%	-0.0271	0.0886	-0.0461	0.1184	z=-0.063
	60%	-0.3611	0.4637	-0.6962	0.5111	z=-3.006**
CV initial value[2]	20%	4.0234	0.7594	4.3410	0.6002	z=1.898
	60%	4.5405	1.2137	4.7504	1.0464	z=1.070
CV slope[2]	20%	-0.0095	0.1792	-0.0469	0.1208	z=-1.311
	60%	-0.2873	0.3570	-0.6526	0.4602	z=-2.139*
FD initial value	20%	1.6503	0.0352	1.5828	0.0493	z=-4.208***
	60%	1.6115	0.0854	1.6167	0.0571	z=-1.107
FD slope	20%	-0.0050	0.0164	-0.0101	0.0304	z=-0.664
	60%	-0.0673	0.0754	-0.1471	0.0834	z=-2.942**
MVC[3]		20.0806	11.3750	34.2736	15.6047	z=3.533***
Endurance time	60%	82	32	43	19	z=-3.260***

* p<0.05, ** p<0.01, *** p<0.001

[2] Variables with 2 missing values in the FSHD patients' group (n=15)
[3] Variable with 4 missing values in the FSHD patients' group (n=13)
MNF, mean frequency; CV, conduction velocity; FD, fractal dimension; MVC, maximal voluntary contraction; IQR, interquartile range.

Correlation analysis

No significant correlations were observed between socio-demographic and clinical variables. The length of D4Z4 fragment resulted not significantly correlated to any variables extracted from the sEMG signal. The score of the scapular girdle involvement showed a significant correlation to the slope of muscle fiber CV during the 20% MVC (Spearman's rho = 0.52, p = 0.03).

Differences in the continuous variables related to FSHD category and asymmetry

The length of the D4Z4 contraction did not show any statistically significant differences among the FSHD categories, which were recoded into binary variables as "A" and "not-A".

Patients with muscle weakness more accentuated on the right side (category "Right>Left") reported a significantly lower FSHD severity (clinical score) and MNF initial value at 20% MVC, while they had significantly higher CV initial values (Table 8).

Table 8 Statistically significant differences in the continuous variables related to FSHD category and asymmetry

	FSHD asymmetry		Mann-Whitney U test result
	Right>Left	Not Right>Left	
Severity of FSHD (Median)	3.0000	5.0000	z=-2.156*
MNF initial value 20% (Median)	86.8923	105.1850	z=-2.849**
CV initial value 20% (Median)	4.1765	3.6348	z=2.252*

* p<0.05, ** p<0.01, *** p<0.001

Discussion

This study measured investigated sEMG parameters known to be indirect indices of performance fatigability, in the BB of FSHD subjects and healthy controls matched for age and sex. We also assessed whether socio-demographic and clinical variables of the patients correlated with several sEMG parameters known to be indirect indices of performance fatigability, in a group of FSHD patients. Results indicated that in FSHD category A, significant differences in all the considered parameters were detectable in comparison with the healthy controls. In particular, at higher contraction intensity, a lower rate of change in fibers conduction velocity and a longer time of task revealed a condition of reduced fatigability, most probably sustained by a fast to slow transition in skeletal muscle composition as a common phenotype evolution in presence of the disease.

Comparative analysis between FSHD patients and healthy controls

In line with previous studies, the results showed a rather consistent picture, characterized by the fact that patients with FSHD were not able to exert a comparable force with respect to healthy controls (Bachasson et al., 2014; Doix et al., 2017; Kalkman et al., 2007; Schillings et al., 2007; Turki et al., 2012). As a direct consequence, the patients' endurance time was significantly longer than healthy

controls, suggesting lower fatigability, likely for several reasons. First, patients with FSHD may have fibrosis and lipid infiltration (Friedman et al., 2012) and strong alteration of the sarcomeric contractile properties, preferentially of type II fibers (D'Antona et al., 2007; Lassche et al., 2013). Second, weaker participants are shown to be less fatigable than stronger ones (Hunter and Enoka, 2001); as the intramuscular pressure is lower, the blood occlusion will also be lower (Zwarts and Arendt-Nielsen, 1988) and the negative feedback from afferent groups III and IV will be reduced. Interestingly, an increased resistance towards fatigability was also confirmed from the results of the sEMG parameters. In fact the initial values of MNF and FD during the low level contractions were higher in patients with FSHD. Furthermore, although not statistically significantly different, the CV initial values were lower (both at 20% and 60% MVC) in the FSHD group probably related to the fast-to-slow muscle fiber shift. A similar reduction in CV, was reported also by Naumann and Reiners (1996) in several muscular dystrophies.

However, during the low level contraction, the slopes of MNF, CV and FD did not change significantly in patients with FSHD over healthy controls, possibly due to larger recruitment of type I muscle fibers. On the contrary, a significant reduction in the considered fatigability parameters (MNF, CV and FD slopes) during the high-level fatiguing contraction was observed, suggesting that patients are less prone to get fatigued, probably due to the fast-to-slow muscle fiber shift. In literature there are several studies which assessed fatigability in patients with FSHD, using the twitch interpolation technique or electrical neurostimulation combined to MVC protocols (e.g. Bachasson et al., 2014; Schillings et al., 2007; Schulte-Mattler et al., 2003); however, the results of these studies are contradictory and difficult to compare, due to different stimulation procedures, muscle groups, fatiguing tasks and disease severity. For instance, Bachasson et al. (2014) showed similar levels of performance fatigability in patients compared to healthy controls, whereas Schillings et al. (2007) described lower levels of BB fatigability in patients with FSHD than in healthy controls. Arguably, one could question that this procedure (electrical stimulation and MVC) may not be representative of fatigability after activities in daily living, where mainly submaximal contractions are performed.

Correlations between socio-demographic, clinical variables and performance fatigability parameters

i. Scapular girdle involvement score

Importantly the scapular girdle involvement score showed a relevant correlation with the slope of muscle fiber CV measured at 20% MVC ($p<0.05$). This evidence suggests that the clinically relevant scapular girdle involvement links to the level of peripheral fatigue arising when the BB contraction

is held at low percentage of MVC. Considering that, in FSHD, scapular girdle dysfunction generally precedes the involvement of other muscular districts, this evidence identifies the CV slope change as a sensitive outcome measure for the early identification of scapular girdle involvement in apparently asymptomatic subjects carrying a genetic defect and for the study of the clinical evolution in these subjects. In addition, if we consider that a low level of contraction is mainly sustained by slow or intermediate muscle fibers, the correlation between clinically relevant dysfunction and peripheral fatigue seems to suggest the presence of a state of dysfunction of slow/intermediate muscle fibers even in the presence of a fast to slow transition, or a defect in the motor unit rotation and central drive during sustained contraction.

ii. Age

In healthy subjects it is well known that a correlation between muscle fiber CV and age, characterized by a tendency of delay due to age, both during MVC and submaximal contractions, exists (Bilodeau et al., 2001; Hara et al., 1998; Mase et al., 2006; Merletti et al., 2002; Merletti et al., 1992b; Yamada et al., 2002). This phenomenon was interpreted as a consequence of the selective atrophy of fast-twitch fibers and decrease of central drive (Merletti et al., 1992b). In addition, an age-related decrease in FD was demonstrated by Arjunan and Kumar (2013) in 96 healthy subjects. The authors suggested that the reduction in FD may be an indicator of the reduced number of motor units (De Luca et al., 1996; Merletti et al., 2002; Roos et al., 1997). Since FSHD is characterized by selective muscle fibers atrophy (Kalkman et al., 2006), our results seem to suggest that young patients (median age 33.5) behave as healthy elderly with a similar age-related decrease in CV and FD.

iii. D4Z4 contraction

A rather discordant picture emerges from the literature: Wang et al. (2012) provided evidence of a negative correlation between muscle computed tomography grade values and D4Z4 fragment size, whereas Olsen et al. (2006) showed that radiological severity was not related to the D4Z4 array. Esnault et al. (2018) found a correlation between the number of D4Z4 repetitions and trunk extensors and flexors isokinetic peak torque. Recently, Sacconi et al. (2019) found significant correlations between D4Z4 repeat size and manual muscle testing sum score. A possible explanation for this heterogeneity in the correlation results could be related to the mean deletion length, as noted by Scionti et al. (2012). Evidence of the absence of a correlation between a “border-line” number of the D4Z4 repeats (8 to 10) and the severity of clinical manifestations was made evident in Zernov and Skoblov (2019). Since the FSHD patients in our study had a median D4Z4 contraction of 27 kb (or 8.2 repeats), our results seem to agree with what has been reported in the literature.

iv. Perceived fatigability

In line with previous findings in patients with FSHD (Schillings et al., 2007) and in other neuromuscular disorders such as multiple sclerosis, no correlation with any of the indices of performance fatigability was determined (e.g. Beretta-Piccoli et al., 2020; Dodd et al., 2011; Severijns et al., 2016; Wolkorte et al., 2015a). An hypothesis may be that, in the current study, patients were not severely fatigued (CIS-fatigue <35). In addition, Wolkorte et al. (2015b) suggested that at least age and maximal voluntary contraction values, may affect positively and negatively performance fatigability. These parameters also interact with each other and will interfere with the association between perceived and performance fatigability, as established also in a study with a large sample size (Romani et al., 2004).

Influence of FSHD category and asymmetry over fatigability

Surprisingly, no statistically significant differences in the continuous variables were detected across the categories of FSHD, which were considered as A or not-A, suggesting that the two groups are comparable from a clinical point of view. In particular, the fact that the D4Z4 array is not different across the categories, seems to underline a labile relationship with the clinical picture and even that the size of the deletion is not sufficient to tell if a patient with 8.2 repeated units belongs to category A or not-A. The two considered categories share common pathophysiological traits, at least from the point of view of fatigue. For instance, the most conclusive shared event is the loss of strength and a fast-to-slow shift of muscle fiber-type composition.

The results concerning asymmetry, which is a very common feature in FSHD, unexpectedly showed that patients with muscle weakness more accentuated on the right side have a better clinical picture, compared to those with symmetric or greater left side involvement. Interestingly, the right-sided initial value of muscle fiber CV during the 20% MVC contraction was higher, probably due to a recruitment of both slow and fast muscle fibers. On the contrary, the left-sided CV was much lower, suggesting a deficit in fast muscle fibers, probably due to a more pronounced fast-to-slow shift of muscle fiber composition. Moreover, right-sided MNF initial value at 20% MVC is lower in those patients with a lower clinical score and this corresponds to a higher initial CV (Table 8).

The prevalence of involvement on the right side, is in accordance with previous findings and has been put in relation with mechanical factors and in particular with preferential use of the right side by right-handed people (Brouwer et al., 1992; Tasca et al., 2014).

Limits

This study has some limitations. First, since the assessment of fatigability is task dependent (Enoka, 1995; Enoka and Stuart, 1992), it is known that protocol specifications affect the findings and the underlying mechanisms of fatigue. We used submaximal isometric contractions, which may not be related to patients' daily living activities. Moreover, the majority of the studies on fatigability in FSHD patients were conducted using electrical stimulation, thus the results may not be comparable. Second, we evaluated fatigability in the dominant BB only, which may not be representative of the disease condition on the entire individual.

Conclusions

We reported impaired neuromuscular function due to muscle weakness and selective muscle atrophy, which caused patients with FSHD to exert a smaller MVC, yield a longer endurance time and perform a reduced fatigability compared to healthy participants. Unexpectedly, this study also showed that FSHD patients with muscle weakness more accentuated on the right side had a better clinical picture, characterized by a probable less pronounced fast-to-slow shift of muscle fiber composition. Considering these results, this study identifies the sEMG variables of peripheral fatigue as strong predictors of skeletal muscle involvement and its temporal evolution in FSHD patients. In particular, results open new avenues onto the identification of early muscular involvement and its evolution in presence of asymptomatic subjects carrying a contraction of the polymorphic region D4Z4.

Further studies must be conducted to assess performance fatigability in the FSHD subcategories and to investigate the patients' fatigue induced by functional exercise unrelated to individual MVC (e.g. walking, sit-to-stand transfer) in order to clarify the impact fatigue on their daily living activities.

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GENERAL DISCUSSION

The sEMG signal undergoes several changes of features during sustained isometric and dynamic muscle activities. These features reflect both central and peripheral phenomena leading to mechanical fatigue and are detectable much earlier than mechanical failure (Merletti and Farina, 2016). For instance, linear and non-linear parameters have been obtained for monitoring changes in the sEMG signal due to fatigue, and may be considered as indirect indices of performance fatigability (Gazzoni et al., 2017; Gonzalez-Izal et al., 2012; Rampichini et al., 2020). This thesis focused on several parameters, including classical amplitude and spectral parameters, muscle fiber CV (as a unique physiological parameter) and FD of the sEMG (as a non-linear parameter). We investigated whether muscle fiber CV may be reliably estimated through sEMG (study 1), the relationship between the force exerted by healthy subjects and the considered parameters (study 2) and how they changed during fatigue in patients with FSHD.

Main findings

I. ***Muscle fiber CV can be reliably estimated through sEMG if certain conditions are met (Study 1)***.

The first study of this thesis was a systematic review sought to determine if sEMG is a reliable tool for estimating CV of muscle fibers as well as CV associated to single MUs. The results obtained suggested that, after the publication of the study of Farina et al. (2004c) the reliability of sEMG in estimating CV in muscles with relatively long fibers that run parallel to the skin surface, has extremely increased. In fact the combination of multi-channel electrodes, visual channel selection, and the maximum likelihood algorithm (Farina et al., 2001b) has proven to be a highly reliable method for the estimation of CV. Furthermore, high to very high relative reliability values combined to low absolute reliability estimators suggested that CV is sufficiently accurate and suitable for clinical applications.

Several conditions to be met were identified:

a) *The operator-dependent nature of the CV estimates*. Importantly, the issue is not simply the reliability of sEMG itself for assessing CV, but also the training and expertise of the operator regarding the use of this technique to correctly detect, process and interpret sEMG signals (Barbero et al., 2012).

b) *Electrode locations and positioning*. The issue of electrode locations was identified as the most critical factor influencing the reliability of CV estimation in all the included studies. The myotendinous and neuromuscular junctions must be avoided to obtain physiological values of CV.

c) *IED and number of channels*. The use of multi-channel sEMG with between four and eight electrodes and 5 mm IED, can help to increase the reliability of CV.

d) *Estimation method*. The most reliable and robust methods are those based on maximum-likelihood estimation in the frequency domain (Farina et al., 2001b). Further developments and adaptations of the maximum-likelihood algorithm (Farina and Merletti, 2004a; Farina et al., 2004b) have allowed highly reliable estimates of muscle fiber CV and CV associated with single MUs to be obtained during isometric and dynamic contractions, using multichannel sEMG (Beretta-Piccoli et al., 2018; Beretta-Piccoli et al., 2017; MacDonald et al., 2008; Martinez-Valdes et al., 2016; Martinez-Valdes et al., 2017).

e) *CV parameters*. Initial and mean CV values were the most reliable parameters.

II. MNF and FD were the parameters least influenced by the level of the exerted force (Study 2).

The second study sought to determine the relation between fatigability parameters (ARV, MNF, CV and FD), perceived fatigability (measured through the Borg scale) and force during short isometric contractions (from 10% to 90% MVC) of the BB muscle of healthy subjects using multi-channel sEMG. It was demonstrated, as expected, that ARV was the most force-dependent parameter, showing an high sensitivity to force, particularly between 10% and 50% MVC (similar to Troiano et al., 2008). The fact that ARV beyond 50% MVC did not increase significantly could be motivated by the fact that the amplitude cancellation influenced the measures of EMG amplitude mostly at high force levels. Indeed, the amplitude cancellation has been proven to increase with increasing number of active MUs (Keenan et al., 2005). In addition, geometrical aspects, such as a preferential distribution of large MUs deep into the muscle, could have contributed to the result.

Unexpectedly, muscle fiber CV did not increase with increasing force level, probably due to a technical constraint caused by a malalignment of the electrode grids along the direction of the muscle fibers, which caused an overestimation of CV. Consequently, the relation between CV and force was apparently biased.

MNF and FD increased with the increasing force level, but only between 10% and 30%. Thereafter, they reached a 'plateau' beyond 30% MVC, similar to the study of Gitter and Czerniecki et al. (1995),

where an almost linear increase of FD with force was determined for force values below 50% MVC. Conversely, above this level the FD rise declined, deviating from the linear increase. However, it should be noted that the relationship between the FD and force could also depend on the method of calculation of FD (box-counting, Katz's or Higuchi's algorithms), the muscle where the experiment was conducted and the sample size.

Finally, the rate of perceived exertion showed an almost linear relationship with the force level (Stevens and Cain, 1970; Timmons et al., 2009; Troiano et al., 2008). Similarly, to the behavior of ARV, between 60% and 90% MVC, no statistically significant increase was found, supporting previous findings that perceived exertion is related to muscle activity (Lagally et al., 2002).

III. Patients with FSHD showed lower levels of fatigability compared to healthy subjects (Study 3).

The third study was conducted in a group of patients with facioscapulohumeral muscular dystrophy (FSHD) and a matched population of healthy participants, to evaluate the behavior of MNF (as parameter sensible to global performance fatigability), CV (as peripheral descriptor) and FD (as central descriptor of performance fatigability). The results showed that patients with FSHD exerted lower MVCs compared to healthy participants. As a direct consequence, the patients' endurance time was significantly longer, suggesting lower fatigability. Similarly, all the fatigability indices exhibited a significant reduction during the 60% MVC contraction, indicating that patients are less prone to get fatigued. A key finding of this study was that the initial values of MNF and FD during the 20% MVC contraction were higher in patients compared to the controls. However, during the same contraction level, the slopes of MNF, CV and FD did not change significantly in the patients' group.

A second key finding was that the score of the scapular girdle involvement showed a significant relatively strong correlation to the slope of muscle fiber CV during the 20% MVC ($r_s = 0.52$, $p = 0.03$).

Discussion of the results

From the literature that was reviewed in this thesis, it seems evident that the majority of the mathematical descriptors that have been used in the past decades to track changes in the EMG signal during a fatiguing task, show several limitations. In particular, global signal features extracted from the interference EMG signal (such as amplitude and spectral parameters) are influenced by both central and peripheral properties of the neuromuscular system. Thus, it is not possible to extract information related either to the inputs from the CNS or to peripheral MU properties (Gazzoni et al., 2017). Nevertheless, complexity analysis may still provide useful information to detect changes in the sEMG signal during a fatiguing task, even though more works remain to be done to elucidate the relationship between complexity indices with the physiologic phenomena underlying performance fatigability (Rampichini et al., 2020). In addition, the estimation of CV slope using multi-channel EMG, might be useful to characterize the peripheral components of fatigue (Merletti and Farina, 2016) and this physiological variable may be considered as a robust index of performance fatigability.

Study 1 Reliability of muscle fiber CV

The aim of the systematic review, in which seventeen studies were included, was to synthesize the evidence concerning the reliability of sEMG for assessing muscle fiber CV. Different types of reliability, including test-retest, intrasession and intersession were considered. The most peculiar results concern the very high levels of test-retest reliability, which were reported for initial CV values estimated using interferential signals and for MU CV estimate using multichannel single potentials (Beretta-Piccoli et al., 2018 and Martinez-Valdes et al., 2018, respectively). Moreover, high intersession reliability estimated from compound potentials and interferential signals were reported in seven out of twelve studies. This result combined to low absolute reliability suggested that CV parameters (initial values and slope) are sufficiently accurate and suitable for clinical applications.

In addition, we identified several conditions for reliable CV estimation, such as the presence of relatively long muscle fibers arranged in a plane parallel to the skin, with an IZ concentrated in a small region (e.g. in VL, VM, BB and TA); accurate electrode positioning criteria (based on the SENIAM and on the “Atlas of Muscle Innervation Zones”, Barbero et al., 2012) when a single electrode pair is used, or accurate visual inspection to select the channels between the IZs and tendons, when electrode grids are used; the use of multi-channel sEMG with between four and eight electrodes and 5 mm IED; a CV estimation method based on the maximum-likelihood algorithm (Farina and Merletti, 2004b; Farina et al., 2001b); the use of initial and mean CV values, which appeared to be

the most reliable parameters in the considered studies; and finally, a trained and expert operator, able to identify movement artifacts, missing channels or the presence of large sinusoidal components at 50 Hz.

As regards CV slope, good reliability is very difficult to achieve, since it depends not only on fatigability, but also on the reproducibility of the fatiguing protocol and on the subject's fatigability conditions. The study of Beretta-Piccoli et al. (2017) was the only one which showed high reliability, probably related to the reduction in noise associated with the use of an electrode grid with a 10-mm IED (which allows a larger number of electrodes and an optimal distance between detection points, and which lowers the sensitivity to electrode displacement).

Study 2 Relation between force and FD

Since in literature the relation between the FD of the sEMG signal and the exerted force is controversial (some studies found a linear, though weak, relation (Gitter and Czerniecki, 1995; Anmuth et al., 1994; Gupta et al., 1997), others showed no relation to the intensity of muscle contraction (Arjunan and Kumar, 2014; Troiano et al., 2008), we aimed at evaluating this relationship during isometric contractions of the BB.

The results showed that MNF and FD were the parameters least influenced by the force level, at least above 30% MVC. The little or even independence of FD and MNF on the level of muscle force, was reported also in previous investigations in other muscles and with different methods (Troiano et al., 2008; Bilodeau et al., 2003). Conversely, Gitter and Czerniecki (1995) showed that FD increased almost linearly with the force intensity for force values below 50% MVC, whereas above this level the FD rise declined.

As already reported in literature, FD is sensitive to the presence of large active MUAPs that usually appear in the signal due to synchronization at high force levels, during isometric fatiguing contractions (Mesin et al., 2009a). Nevertheless, a similar phenomenon happens also at low force levels, whenever larger MUs and with low firing frequency, are recruited according to the Henneman's size principle. Moreover, in simulated EMG signals, FD was positively correlated to the firing rate of the active MUs, and negatively correlated to the level of MU synchronization (Mesin et al., 2016). Since the level of synchronization is not expected to change in non-fatiguing contractions, it was reasonable to hypothesize that FD could somehow increase with increasing force levels. Thus, it is possible to speculate that FD might be a reliable indicator of MU synchronization, less dependent from the firing rate.

Study 3 Performance fatigability in facioscapulohumeral muscular dystrophy.

The third study was conducted in a population of patients with FSHD. The objective of this study was to verify whether fatigability indices extracted from the sEMG signal (MNF, CV and FD) were suitable to differentially characterize performance fatigability in the patients compared to matched healthy controls. FSHD is marked by slowly progressive weakness of the facial and shoulder girdle muscles, followed by leg and trunk muscles. Moreover, patients with FSHD may have fibrosis and lipid infiltration (Friedman et al., 2012) and strong alteration of the sarcomeric contractile properties, preferentially of type II fibers (D'Antona et al., 2007; Lassche et al., 2013). In addition, one of the symptoms with the highest prevalence is fatigue.

The results confirmed previous studies on FSHD, which suggested that the patients were unable to exert similar forces compared to the controls (e.g. Bachasson et al., 2014; Doix et al., 2017). Furthermore, results indicated that in FSHD, significant differences in all the considered sEMG parameters were detectable in comparison with the healthy controls. In particular, at higher contraction intensity (60% MVC), a lower CV slope and a longer endurance time revealed a condition of reduced fatigability, most probably sustained by a fast to slow transition in skeletal muscle composition, as a common phenotype evolution in the presence of the disease. In addition, weaker participants are shown to be less fatigable than stronger ones (Hunter and Enoka, 2001): as the intramuscular pressure is lower, the blood occlusion will also be lower (Zwarts and Arendt-Nielsen, 1988) and the negative feedback from afferent groups III and IV will be reduced.

However, during the low level contraction, the slopes of MNF, CV and FD did not change significantly in patients with FSHD over healthy controls, possibly due to larger recruitment of type I muscle fibers.

The correlation analysis between clinical variables and indices of performance fatigability revealed a significant relatively strong correlation between the scapular girdle involvement score and the slope of muscle fiber CV during the intensity contraction. This evidence suggests that the clinically relevant scapular girdle involvement links to the level of peripheral contributions to performance fatigability, arising when the BB contraction is held at low percentage of MVC. Considering that, in FSHD, scapular girdle dysfunction generally precedes the involvement of other muscular districts, this evidence identifies the changes in CV slope as a sensitive outcome measure for the early identification of scapular girdle involvement in apparently asymptomatic subjects carrying a genetic defect and for the study of the clinical evolution in these subjects. In addition, if we consider that a low level of contraction is mainly sustained by slow or intermediate muscle fibers, the correlation between

clinically relevant dysfunction and peripheral factors affecting performance fatigability, seems to suggest the presence of a state of dysfunction of slow/intermediate muscle fibers even in the presence of a fast to slow transition, or a defect in the MU rotation and central drive during sustained contractions. Beyond the significant correlation just described, no other correlation was found. Possible reasons are explained in the discussion of the third manuscript.

Finally, the behavior of MNF and FD over the two groups of participants will be discussed a little further. As regards the initial values, MNF and FD showed the most significant changes ($p<0.01$) in the FSHD group compared to the healthy controls at 20% MVC. Moreover, similar changes were described also for their slopes (normalized with respect to their initial values) at 60% MVC ($p<0.01$). Furthermore, in the correlation analysis, a strong significant positive correlation between FD and MNF slopes both at 20% and at 60% MVC in the FSHD group ($p<0.001$).

Changes in MNF during fatigue were initially related to changes in muscle fiber CV, and in the IAP duration (Bigland-Ritchie et al., 1981) and later to the modifications in the MUAP shape, MU firing rate and synchronization (Brody et al., 1991; Bigland-Ritchie and Woods, 1984; Dimitrova and Dimitrov, 2003; Gabriel and Kamen, 2009). Therefore, MNF may be considered as a parameter sensitive to global performance fatigability. As far as FD is concerned, Mesin et al. (2009a) showed no association with changes in muscle fiber CV, supporting the idea that FD was more sensible to central factors, such as MU synchronization during fatiguing contractions. Later, Mesin et al. (2016) evidenced during simulated contractions, the existence of an inverse relationship between FD and MU synchronization and a positive relation with MU firing rate. Alternatively, a reduction in FD during fatigue may be seen also as a decrease in the geometrical complexity of the sEMG signal (Gitter and Czerniecki, 1995).

The results of the third study seems to indicate that the origin of performance fatigability affecting FSHD patients is related to both central and peripheral factors, and unless the methodology do not include MU decomposition techniques, it is not possible to separate the contribution of the single factors. However, three major limitations affect the applicability of MUs decomposition: the muscle anatomy, the volume conductor and the contraction intensity (reviewed in Del Vecchio et al., 2020). In particular, to identify a greater number of MUs by decomposition, muscles with fibers that are not all parallel to each other should be preferred, such as the tibilias anterior. Therefore, to evaluate central and peripheral factors affecting performance fatigability in muscles such as the BB, during contraction intensities above 50% MVC, may not be favorable for using sEMG decomposition techniques. Moreover, FD may be easier to implement in clinical settings than decomposition.

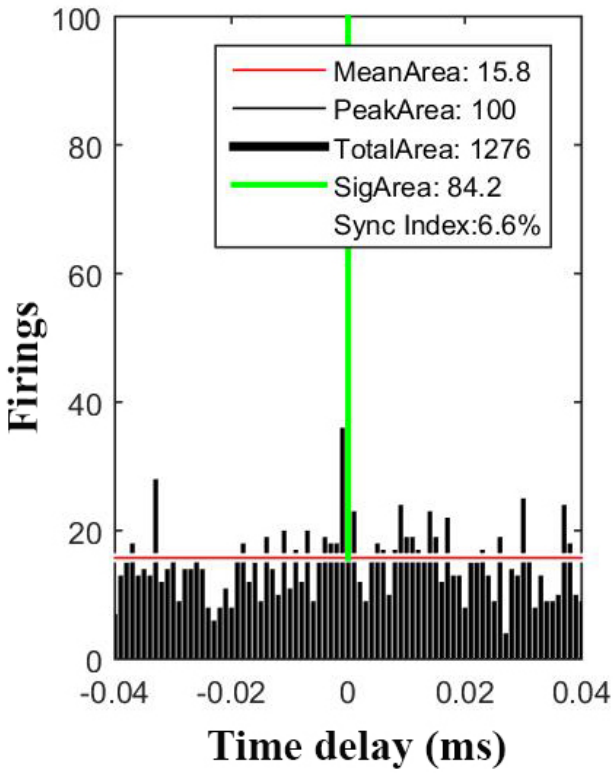
DIRECTIONS FOR FUTURE RESEARCH

The results of the present thesis have demonstrated that the considered parameters are useful to monitor changes in the sEMG signal during fatiguing contractions, both in healthy subjects and in patients. Moreover, recent studies have demonstrated that the EMG signal also exhibits many complexity characteristics deserving to be evaluated, particularly to understand if these features have a different sensitivity to the changes in the sEMG signal due to fatigue, compared to the classical parameters. Consequently, it is evident that classical parameters (such as ARV and MNF) are not able to differentiate between central and peripheral factors affecting performance fatigability, even when normalized. On the contrary, FD seems to be more sensible to central factors, whereas muscle fiber CV is a physiological parameter. However, the sensitivity of FD to MU synchronization and firing rate was confirmed during simulated contractions, only (Mesin et al., 2009a and 2016). Hence, the priority is first and foremost, to conduct a study to verify *in vivo* whether the FD of the sEMG signal is related to MU synchronization.

In 2019 we set up an experimental protocol to determine if a relation between the MUs synchronization index based on the study of De Luca et al. (1993) and the FD does exist. Briefly, participants performed two isometric maximal voluntary extensions of the quadriceps muscle (MVC_pre), interspersed by 120 s, followed by a 5% MVC lasting 300 s. Immediately after the contraction, participants performed another MVC (MVC_post), which was later used to calculate a fatigue index (Enoka and Duchateau, 2008). The EMG signal was detected using fine-wire needle electrodes and two couples of bipolar adhesive surface electrodes. The iEMG signal was decomposed into its constituent MUAP trains by the use of an interactive decomposition algorithm, EMGLAB (McGill et al., 2005), which includes a user interface for manually editing and verifying the accuracy of the spike trains. Once the automatic discrimination was completed, each MU spike train was manually edited by an experienced operator and inspected for checking potential discrimination errors.

Calculation of FD is reported in the General methods section.

The ‘synchronization index’ was calculated based on De Luca et al. (1993) as follows:



$$Synch\ Index = \frac{Peak\ Area - Mean\ Area}{Total\ Area/2} \times 100$$

Figure 20 Definition of the synchronization index. The green area shows synchronous occurrences that are beyond what would be expected if motor units fired independently.

Peak Area-Mean Area refers to the area under the cross-interval histogram in the peak region that surpasses the expected level. *Total Area* refers to the total area under the histogram between ± the mean interfering interval of the alternate MU, whereas *Total Area/2* represents the total number of firings of the reference MU that were considered in the histogram (De Luca et al., 1993).

Unfortunately, due to the COVID-19 emergency, we were not able to conclude the data analysis by the submission deadline of this thesis. We expect to submit a manuscript by the end of 2020.

Secondly, Coelho and Lima (2014) demonstrated that the normalized version of the Katz' algorithm (Castiglioni et al., 2010) and the Hurst exponent (Hurst, 1951) significantly outperformed the other FD methods as feature extractors from the sEMG signal. Consequently, a study should be conducted initially in simulated conditions, and later on *in vivo*, to identify the algorithm most sensitive to changes in MU synchronization and firing rate during fatiguing isometric contractions.

Furthermore, it has been demonstrated that a reduction in the complexity of the sEMG interference signal, is not only a characteristic of fatiguing contractions, but also of aging (Arjunan and Kumar, 2013) and pathological conditions (Rampichini et al. 2020), and is associated with a loss of system

control and increased dysfunction (Lipsitz and Goldberger, 1992; Peng et al., 2009). For instance, Meigal et al. (2013) found differences in complexity indices between Parkinson's disease (PD) patients, whose MU synchronization is known to be increased, and controls. The authors suggested that sEMG signal in PD is less complex, more predictable and regular. Thus, they concluded that non-linear parameters may be used in pre-clinical diagnosis as they were able to differentiate healthy participant from PD patients'. However, research into complexity is very much in its infancy, and further research is undoubtedly warranted to expand the findings of this thesis. Thus, FD as complexity parameter of the EMG signal, may be used in future studies during dynamic contractions (such as cycling, or concentric and eccentric contractions), to investigate the recruitment pattern of the MUAP trains and their firing patterns (Chakraborty and Parbat, 2017), to quantitatively assess muscle activity and fatigue.

Finally, the combined use of CV and FD, as performance fatigability indices should be further explored and investigated in other muscles and different experimental conditions. Recently, we conducted a study in a group of persons with multiple sclerosis, which showed the inability of FD and CV in detecting changes in the sEMG signal due to fatigue in VL and VMO. On the contrary, the same parameters succeeded in detecting performance fatigability in the BB (Beretta-Piccoli et al., 2020). This unexpected behavior, was hypothetically explained by clinical issues related to the non-physiological MUs recruitment or to methodological constraints. However, to better understand what lies behind these results, we should consider to analyze the complexity of the sEMG signal (which was not explored), and to compare it with healthy controls.

Limits and generalization of the results

Several limitations were identified in the three studies, and were reported in the end of the discussions of each individual study. The experimental studies presented in this thesis investigated only the changes in the sEMG signal during fatiguing isometric elbow flexions.

Future studies on changes in fatigability and complexity parameters extracted from the sEMG interference signal, in other muscle groups and as a result of dynamic exercise would extend and enhance the present findings.

CONCLUSIONS

In literature, it is commonly reported that the progress of performance fatigability may be indirectly assessed through the changes in features of the sEMG signal. In particular, during isometric constant force contractions, changes in the sEMG signal are caused by several physiological factors, such as a decay in muscle fibers CV; an increase of the degree of synchronization between the firing times of simultaneously active MUs, by the CNS; a reduction of the recruitment threshold and a modulation of MUs firing rate (Farina et al., 2014). Amplitude and spectral parameters may be used to characterize the global contributions to performance fatigability, such as MU control properties and fiber membrane properties, or central and peripheral factors, respectively. In addition, being CV a physiological parameter, its estimation is of marked interest to the study of fatigue both in physiological and in clinical studies.

As regards the FD of the sEMG several studies suggested that it may be related to central factors, in particular MU synchronization and firing rate (Mesin et al., 2009a and 2016). In addition, direct evidence of a fatigue-induced increase in MU synchronization was reported by McManus et al. (2016) using a sEMG decomposition technique. Nevertheless, it should be noted, that sEMG decomposition is still affected by limitations associated to muscle and subject anatomy (Del Vecchio et al., 2020). For instance, to investigate central factors affecting performance fatigability in muscles like the BB, VL and VM, non-linear parameters extracted from the interference signal, such as the FD, seems to be a valid alternative.

The present thesis aimed at investigating the reliability of sEMG in the estimation of muscle fiber CV, the relationship of fatigability parameters to force, and the applicability of these parameters in differentiating performance fatigability in patients and healthy subjects. The promising results suggested that the analysis of features extracted from the interference sEMG signal, in particular CV and FD, may be still considered as useful to investigate peripheral and central factors affecting performance fatigability. It is indeed well known that for separating the neural information (MU recruitment/de-recruitment and discharge characteristics) from the peripheral information (membrane properties and MU anatomy) in the surface EMG signal, the use of multi-channel detection systems along with more advanced processing techniques are needed.

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APPENDIX

Guidelines for Reporting Reliability and Agreement Studies (GRRAS)-checklist, based on Table I in Kottner et al. (2011)

Section	Item #	Checklist item	Reported on page #
Title/Abstract	1	Identify in title or abstract that interrater/intrarater reliability or agreement was investigated.	
Introduction	2	Name and describe the diagnostic or measurement device of interest explicitly.	
	3	Specify the subject population of interest.	
	4	Specify the rater population of interest (if applicable).	
	5	Describe what is already known about reliability and agreement and provide a rationale for the study (if applicable).	
Methods	6	Explain how the sample size was chosen. State the determined number of raters, subjects/objects, and replicate observations.	
	7	Describe the sampling method.	
	8	Describe the measurement/rating process (e.g. time interval between repeated measurements, availability of clinical information, blinding).	
	9	State whether measurements/ratings were conducted independently.	
	10	Describe the statistical analysis.	
Results	11	State the actual number of raters and subjects/objects which were included and the number of replicate observations which were conducted.	
	12	Describe the sample characteristics of raters and subjects (e.g. training, experience).	
	13	Report estimates of reliability and agreement including measures of statistical uncertainty.	
Discussion	14	Discuss the practical relevance of results.	
Auxiliary material	15	Provide detailed results if possible (e.g. online).	