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TITOLO TESI

**TECNICHE E METODI PER UN'ANALISI MULTI-SCALA DEL
FENOMENO DELLA FATICA MUSCOLARE**

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*One equal temper of heroic hearts,
Made weak by time and fate, but strong in will
To strive, to seek, to find, and not to yield.*

(Ulysses, Lord Alfred Tennyson)

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INTRODUCTION

If to understand it is difficult, to know it is necessary (Primo Levi)

The term *fatigue* refers to a really complex and not yet well-defined phenomenon encompassing different causes, mechanisms and forms of manifestations. It develops as a result of metabolic, structural and energetic changes in the muscles due, in general, to a change in the efficiency of the Central Nervous System (CNS), which reflect insufficiency of oxygen and energy supplies in blood (Cifrek, & al., 2009). The excitation given from supraspinal centers to motoneurons might be impaired during prolonged contractions, causing an increase in the effort associated with a task, a decrease in voluntary activation of the muscles and the recruitment of accessory muscles in order to maintain the desired motor output (Enoka, 2008a). Different definitions have been provided through the literature around the more general term *fatigue*: some referring to the related-phenomena taking place at the CNS level (central component) (Bigland-Ritchie & Woods, 1984; Nybo, 2003; Bell, 1994) some others directly connected to the impairment in the muscle and in the mechanisms leading to muscle contraction (Hickie, & al. 1996). Some others concern the psychological point of view, in terms of experienced fatigue and perception of effort (Zwarts, & al., 2008; Friedman, & al., 2007), and finally some focus mainly on the mechanical outcome of the occurrence of fatigue, i.e. the decrease of force or muscle soreness (Edwards, 1981; Booth & Thomason, 1991). Since there are many confounding definitions around *fatigue*, it seems very hard to classify the phenomenon just from one point of view, e.g. just as metabolic, or

electro-physiological or biomechanical. *Fatigue* itself, in fact, is not a physical variable and it cannot be measured: its assessment depends on the definition of some indicators based on physical variables that can be effectively measured, such as force/torque, power, variables associated to the single motor unit such as firing rate, conduction velocity or degree of synchronization (Merletti & Parker, 2005). Physiologists consider the force output of a muscle as an index of muscular fatigue and the point at which the muscle is considered fatigued, i.e. the failure point, is the one at which a contraction can no longer be sustained (De Luca, 1997). This concept introduces the view of fatigue as a point process, hypothesis not consistent with the widespread concept of fatigue as a result of continuous processes mentioned above. Moreover, relying on the concept of failure point, fatigue could be detected only after its occurrence and cannot be used, for example, for ergonomics or training purposes, where timely and possibly predictive information is useful.

Continuous monitoring of muscle fatigue while performing a motor task is possible by measuring different variables: time to endurance of a certain work, lactate concentration, indicators extracted from the myoelectric signals recorded either invasively or on the surface of the skin above the muscles involved (Cifrek, & al., 2009). Each of these variables refers to a particular level of analysis of the phenomenon that might be intracellular, electrophysiological or biomechanical. However, considering each of these variables alone leads to a lack of knowledge and does not clarify the mechanisms involved in the phenomenon. For example, spinal motor neurons receive direct excitatory inputs from supra-spinal sources and peripheral sensory receptors, such as the group Ia afferents of the muscle spindle. The motor neurons can also receive inhibitory input from group Ib afferents

(tendon organ) and excitatory input from group II (muscle spindle) afferents, but it is delivered indirectly through interneurons, and the influence of inputs from these sources during fatiguing contractions is still unclear (Gandevia, 2001). The time to endurance is known to be a mechanical manifestation of muscle fatigue and, although it is easy to determine, it depends also on environmental and psychological factors such as motivation or heat/cold in the room (Enoka & Duchateau, 2008). Lactate concentration in a muscle while performing a motor task is determined by means of blood samples taken at predefined time intervals (Cifrek, & al., 2009). Due to this way to take samples and to the way the concentration is then calculated, it is not possible to monitor the phenomenon of fatigue in real-time. Moreover, this kind of measurement gives an estimation of the global fatigue of the organism, without focusing on the particular muscles involved in the motor task.

The continuous monitoring of the phenomenon is, instead, possible by measuring biomechanical and physiological changes occurring in the muscles. The surface electromyographic signal (sEMG), in this context, is the most useful tool. It provides an attractive way to examine the role of the CNS, and its properties are related to biochemical and physiological changes in skeletal muscles during fatiguing contractions. The overall changes exhibited by EMG features have been demonstrated to reflect muscle properties and fiber constituency (Felici, & al., 1997; Gerdle, & al., 1990; 1991; Kupa, & al., 1995; Merletti, & al., 1992; Sadoyama, et al & al., 1988) and recruitment strategies (Solomonow, & al., 1990; Westbury, 1987; Bernardi, & al., 1995; Sanchez, & al., 1993).

Several sEMG-related characteristics have been used for the assessment of peripheral fatigue in many studies: in particular the root mean square (RMS) and the average rectified

value (ARV) have been used as amplitude estimators (Devries, 1968; Kwatny, et al., 1970; Petrofsky, & al., 1982) while the mean and median frequency (MNF or MDF) and the normalized higher order spectral moments have been used as spectral indicators (Petrofsky, & al., 1982; Moritani, & al., 1982; Arendt-Nielsen & Mills, 1988; Gerdle, et al., 2000; Potvin & Bent, 1997; Duchêne & Goubel, 1990). Also the muscle fiber action potential conduction velocity (CV) can be included in the features extracted from (multi-channel) EMG suitable for fatigue analysis.

The amplitude of the sEMG signal has been shown to increase during sustained isometric contractions performed at sub-maximal levels (Sadoyama & Miyano, 1981; Maton, 1981; Arendt-Nielsen & Mills, 1988) up to the endurance point, and this behavior has been attributed to the recruitment of additional large and superficial motor units (Lippold, & al., 1960; Viitasalo & Komi, 1977; Moritani & Muro, 1987; Christova & Kossev, 1998) necessary to maintain or increase the required level of force (BiglandRitchie, & al., 1983). After the endurance point EMG amplitude has been shown to fall (Stephens & Taylor, 1972; Komi & Tesch, 1979; Bigland-Ritchie & al., 1983; Moritani, & al., 1986) depending on the task to be accomplished (Dideriksen, & al., 2010). The occurrence of fatigue during maximal and submaximal contractions is reflected also in a spectral shift towards lower frequency bands (Piper, 1912; Viitasalo & Komi, 1977; Moritani, & al., 1986; Arendt-Nielsen & Mills, 1988) even if the underlying physiological mechanisms (Bigland-Ritchie, 1981; Solomonow, & al., 1990; Hagg, 1992; Vøllestad, 1997; Dimitrova & Dimitrov, 2003) has not been clarified yet (Karlsson & Gerdle, 2001; Farina, 2008).

Together with the increase in EMG amplitude and drop of EMG spectral moments, it has been reported a slowing in CV as soon as a submaximal endurance contraction progresses (Bigland-Ritchie, & al., 1981; Stulen & De Luca, 1981; Naeije & Zorn, 1982; Eberstein & Beattie, 1985; Arendt-Nielsen & Mills, 1985; Arendt-Nielsen & Mills, 1988; Farina, & al., 2002 ; Farina & Merletti, 2004). This decrease in CV has been correlated with the shift in the power spectrum of EMG towards low frequencies observed during fatiguing contractions (Eberstein & Beattie, 1985; Arendt-Nielsen & Mills, 1985; Krogh-Lund & Jørgensen, 1993). Eberstein and coworkers found that fatiguing contractions of the Biceps Brachii muscle performed at 60% and 70% MVC induced a progressive slowing of the CV which in turn brought to an increase of the duration of the motor unit action potential (MUAP) responsible of the compression of the EMG power spectrum. The relationship that they build up was linear (Eberstein & Beattie, 1985) and was also confirmed by Arendt Nielsen et al. in contractions performed by the Vastus Lateralis muscle at forces ranging from 10% to 90%MVC (Arendt-Nielsen & Mills, 1985). However, other studies claim that other factors apart from changes in the wave form of the MUAP are responsible for the shift of EMG spectrum (Bigland-Ritchie, & al., 1981; Naeije & Zorn, 1982).

A typical method to evaluate and compare the rate of change of EMG-related features is the *fatigue plot*, originally proposed by Knaflitz et colleagues, obtained by normalizing each time graph with respect to the intercept of the regression line of the variable in question (ARV/RMS, CV, MDF/NF) (Knaflitz, & al., 1996; Farina, & al., 1999). In this way, the fatigue plot is thought to provide information suitable for the classification of muscle behavior. However, amplitude and spectral parameters do not change only afterwards the occurrence of muscular fatigue but their variation depends also on force production. Experimental

studies have demonstrated that, during fatiguing conditions, RMS-EMG increases linearly with force (Moritani & Muro, 1987; Bilodeau, & al., 2003; Castronovo, & al., 2012), while novel computer simulations have led to an overestimation of muscle force from EMG amplitude indicators (Dideriksen, & al., 2010), leaving the question quite unsolved. Because of the lack of a unique and clear behavior shown by the above-mentioned indicators during fatiguing contractions, due in turn, to a unclear definitions of the phenomenon underlying and mechanisms leading to fatigue (central and peripheral), a multi-scale approach is proposed as a novel way to better comprehend the evolution of the phenomenon and of all implications.

The thesis framework will be the following: first, the definitions given through the last century around the concept of *fatigue* will be reported. Central and peripheral components will be extensively described, highlighting the weakness, the strength and the relevance of both. From that point on, the focus of the thesis will be on peripheral mechanisms and on techniques and methods that help to better understand the fatigue phenomenon. A special attention will be devoted to the surface EMG and to the indicators extracted as a tool to assess the manifestations of fatigue in isometric and dynamic motor tasks. In particular, the limitations offered will act as a springboard to introduce and justify the multi-level approach that is the overall issue of this thesis. This kind of approach to the study of this phenomenon could help in overcoming the main contradictions present in literature and defining the fatigue from a global point of view. In the second chapter, three levels will be proposed as necessary to obtain a more complete view on fatigue: *metabolic*, *electrophysiological* and *biomechanical*. The biochemical processes involved in the arising of fatigue will be extensively discussed according to what is reported in literature. Then, the

electrophysiological level will concern the modifications occurring at the muscle level and the use of sEMG features for fatigue assessment. The biomechanical level, instead, will concern the adaptations induced by the manifestation of fatigue during the execution of a motor task. In particular, the latter two levels, electrophysiological and biomechanical, will be then examined via experimental protocols involving isometric and dynamic motor tasks that have been developed during these years. Most of these results have been published on international journals or presented at international conferences. In Chapter 3 some preliminary observations about neuromuscular adaptations occurring while performing a cycling task will be presented. In order to overcome as far as possible the limitations met in this study a novel bi-dimensional parameter extracted from sEMG signals will be introduced (Chapter 4). The simultaneous use of temporal and spectral features to classify the muscular status proposed previously by (Luttmann, & al., 2000; Lin, & al., 2004; Potes, 2009) is applied and compared with the results provided through the novel indicator applied to sEMG recordings during isometric contractions in a leg extension task. Chapter 5 will, instead, present an attempt to clarify physiological mechanisms underlying muscle fatigue and, in turn, quantify the information that is possible to extract from sEMG signals. In order to do this, a sEMG decomposition technique together with a coherence analysis on motor unit spike trains will be applied to investigate the changes in neural shared drive and force fluctuations. The last Chapter, finally, will report an example of application of multi-level approach to analyze sport performance. This case, in fact, represents an example of how a “multi-factorial” analysis could help in better understanding all the phenomena concurring and to design an approach for researchers and trainers that could consider all of them. A multi-level model is, therefore, proposed. Conclusions will then follow.

CHAPTER 1:

What is fatigue?

There are no facts, just interpretations (F.W. Nietzsche)

DEFINITIONS OF FATIGUE

"Fatigue: a reduction in the efficiency of a muscle or organ after prolonged activity" (Oxford Dictionaries, 2013).

"At first sight might appear an imperfection of our body, is on the contrary one of its most marvelous perfections. The fatigue, increasing more rapidly than the amount of work done, saves us from the injury which lesser sensibility would involve for the organism" (Mosso, 1904).

"Fatigue: state of temporary lowered capacity to perform work of a certain intensity, caused by the work itself" (Heimer, 1987).

"Fatigue is probably the most common symptom of illness affecting sufferers of both acute and chronic conditions. However, confusion surrounds the definition and use of the term fatigue" (Ream & Richardson, 1996).

"Fatigue is a common symptom in neurology and occurs in the diseases of the central and peripheral nervous system. In order to understand the mechanism of fatigue, it is important to distinguish symptoms of peripheral neuromuscular fatigue from the symptoms of physical and mental fatigue characteristic of disorders like Parkinson's disease or multiple sclerosis" (Chauduri & Behan, 2000).

“Although everyone knows *fatigue* personally, it is a difficult concept to define. For muscular fatigue, one must know the aspect of performance affected. The most obvious demonstrations are decreased maximal force and slowed muscular answer. Fatigue can have a central origin, by reducing cognitive performance or lowering excitation of motoneurons. The word *fatigue* covers many concepts: sometimes, mental fatigue not produced by exercise, or fatigue after an exhaustive exercise. According to the dictionary “Trésor de la langue française informatisée”, *fatigue* is a “reduction of organism forces following excessive work, too long a duration of work, or a defective functional state [...] We must take into account not only the modality of the exercise that has led to the fatigue but also the ambiance in which the exercise was done: the same work in extreme ambiance can lead to different sensations than under normal conditions. We must also compare the difference between the feeling of exhaustion and the real changes in muscular function” (Sesbouè & Guincestre, 2006).

“In everyday language, the term *muscle fatigue* is used to denote a variety of conditions that range from an exercise-induced impairment of motor performance through the sensations of tiredness and weakness that accompany some neurological disorders. Although convenient for casual conversation, such broad usage precludes the systematic study of the underlying mechanisms.” (Enoka, 2008b)

“I am a 74 year old man. I have had Parkinson’s disease (PD) for three years. I can live with the PD (not great, but livable), but the *fatigue* is unbearable. As all my life I was full of superman energy, now I’m good for about an hour or so at a time and between the lightheadedness and the fatigue I’m wasted [...] I’ve been told to go see a shrink and start on

anti-depressants. I don't think I'm depressed." (HT; e-mail to J.H.F. 12/05) (Friedman, et al., 2007)

From the definitions above, it is quite clear that even if *fatigue* is a seemingly simple word its meaning is not necessarily understood in the same way by the lay public and physiologists and there is at least agreement on its meaning among physiologists and clinicians.

When applied to muscle exercise, *fatigue* can refer to "failure to maintain the required or expected force" (Edwards R, 1981) or failure to "continue working at a given exercise intensity" (Booth & Thomason, 1991), stressing the fact that fatigue in muscles begins only at the point of task failure when subject exercises until exhaustion (Gandevia, 2001). In fact, the maximal force-generating capacity of muscles starts to decline once exercise begins so that fatigue would begin almost at the onset of the exercise and would develop progressively before the muscle fails to perform the required task. A more realistic definition of fatigue was given by Bigland-Ritchie et colleagues, who defined the phenomenon as "any exercise-induced reduction in the ability to exert force or power, regardless of whether or not the task can be sustained" (Bigland-Ritchie & Woods, 1984). Later, a meeting of physicians in 1990 formally defined *fatigue* as "a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest" (NHLBI Workshop summary , 1990). Finally, *fatigue* refers "not only to a physical or pathological state in which muscles perform below their expected maximum, but also to a symptom reported by subjects in whom there may be no obvious defect in muscle performance" (Hickie, & al., 1996; Bültmann, & al., 2002).

Central fatigue	A progressive reduction in voluntary activation of muscle during exercise.
Maximal evocable force	Maximal force that can be produced by a muscle or muscle group; it would occur when twitches interpolated during a maximal voluntary contraction add no more force.
Maximal voluntary contraction	A maximal contraction that a subject accepts as maximal and that is produced with appropriate continuous feedback of achievement.
Muscle fatigue	Any exercise-induced reduction in the ability of a muscle to generate force or power; it has peripheral and central causes.
Peripheral fatigue	Fatigue produced by changes at or distal to the neuromuscular junction.
Supraspinal fatigue	Fatigue produced by failure to generate output from the motor cortex; a subset of central fatigue.
Task failure	Cessation of a bout of exercise. This may be accompanied by peripheral fatigue, central fatigue, or both.
Twitch interpolation	A method to measure voluntary activation in which one (or more) stimulus is delivered to the motor axons innervating the muscle during a voluntary effort.
Voluntary activation	Level of voluntary drive during an effort. Unless qualified, the term does not differentiate between drive to the motoneuron pool and that to the muscle. Maximal voluntary activation can be measured using twitch interpolation during a maximal voluntary contraction.

Figure 1: Definitions of ‘fatigue’ as reported by (Gandevia, 2001)

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Because peripheral force-generating capacity usually declines early during exercise and because Central Nervous System (CNS) changes occur before muscles fail to perform a task, the most useful definition of muscle fatigue is “any exercise-induced reduction in force generating capacity” (Gandevia, 2001). This definition, however, neglects the intramuscular mechanisms that potentiate force during fatiguing exercise and focuses just on the net reduction in performance that develops at the end (Gandevia, 2001).

CENTRAL AND PERIPHERAL FATIGUE

Despite the large number of definitions on *fatigue* that have been provided during the last century, researchers focused on determining if the mechanisms responsible for fatigue are located in the exercising muscle (“*peripheral fatigue*”) or in those parts of the nervous system that activated the involved muscles (“*central fatigue*”) (Enoka, 2008b). In fact, muscle performance can be limited by the muscle and also by the neural machinery that drove it (Gandevia, 2001).

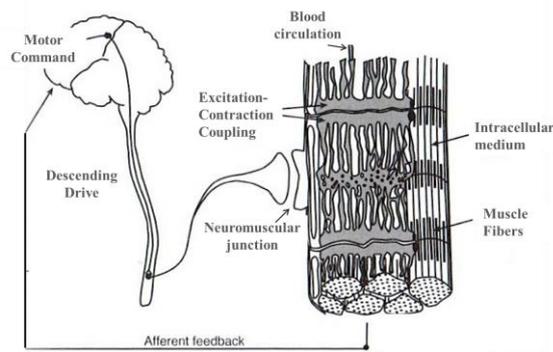


Figure 2: Central and peripheral sites that contribute to the development of neuromuscular fatigue. 1) activation of the primary motor cortex; 2) propagation of the neural drive to motor neurons; 3) activation of motor units and muscles; 4) excitation-contraction coupling; 5) neuromuscular propagation; 6) availability of metabolic substrates; 7) state of the intracellular medium; 8) performance of the contractile apparatus; 9) blood flow.

Adapted from Boyas & Guevel, 2011

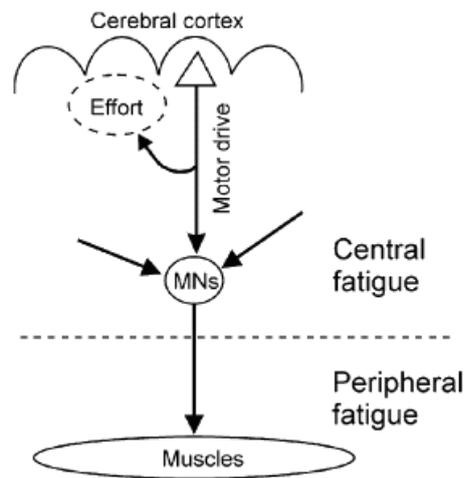


Figure 3: The descending motor drive from the cerebral cortex to muscles via the motor neuron during fatigue

From Kernell, 2009. © Copyright 2009 Oxford University Press

CENTRAL FATIGUE

Central fatigue can be defined as a “*progressive, exercise-induced degradation of the muscle voluntary activation*” (Gandevia, & al., 1995). One of the causes of this weakness in central command during prolonged exercise could be the decreased excitation supplied by the motor cortex. Furthermore, central command may be limited by changes in the brain concentration of other substances, such as ammonium ions and glycogen (Boyas & Guevel,

2011). Voluntary activation is defined as a progressive reduction in voluntary activation during exercise. Voluntary activation refers to a notional level of “drive” to muscle fibers and motor neurons. This term is often used without distinction between the drive to the motor neuron pool and that to the muscle. These drives are not the same: one recruits motor neurons and increases their firing, and the other relies on muscle fibers to translate the motor neuron firing into force. As applied to motor neurons, the term voluntary activation becomes poor since the source of excitation is not well defined (from descending motor paths and associated spinal circuits). During maximal isometric efforts the motor unit firing rates decline, initially rapidly and then reach a plateau after about 30s. This decline is accompanied by changes in the properties of muscle receptors and, in turn, of the reflexes they are normally able to evoke (Gandevia, 2001). The maximal rates vary for subjects and muscles, due to the type of motor neurons (Grimby, & al., 1981; Bellemare, & al., 1983; Bigland-Ritchie, & al., 1983; Gandevia, & al., 1990; Peters & Fuglevand, 1999). Another indicator of a central failure of voluntary activation can occur when initially submaximal isometric contraction (e.g. 30% MVC) is held until it cannot longer be continued (Loescher, & al., 1996). Development of central fatigue can also be inferred from the increasingly obvious fluctuations in force (Taylor, & al., 2003; Moritz, & al., 2005; Contessa, & al., 2009; Missenard, & al., 2008, 2009) and variations in motor unit firing rates (Grimby, & al., 1981; Kernell & Monster, 1982; Bigland-Ritchie, & al., 1986; Garland, & al., 1994; Moritz, & al., 2005) as the voluntary force declines. The natural decline in motoneuron firing rate during isometric MVCs is however, beneficial because of the hysteresis in the force-frequency relationship and the stability of its descending path. This kind of discharge pattern, in fact, efficiently maintains force. As the precise pattern of MU firing can enhance force production and

minimize fatigue, a failure of these strategies impairs voluntary activation and might contribute to the development of fatigue. However, the CNS is involved not only in driving motor neurons but also in the increased sense of effort (Aniss, & al., 1988), the increased tremor of the exercising limb (Lippold, & al., 1957; Lippold, 1981; Ebenbichler, & al., 2000), the recruitment of muscle initially not involved (Fallentin, & al., 1993; Green, 1997) in the task and the subjective increase in effort. In particular, after severe exercise, tremor increases across a wide frequency range and this phenomenon persists for many hours (Buchtal & Madsen, 1950; Lippold, 1970, 1981). Moreover, a small but statistically detectable synchronization exists between the firing probabilities of motor units within a muscle during voluntary contractions, suggesting some excitatory/inhibitory presynaptic drives (Buchtal & Madsen, 1950; Nordstrom, & al., 1990; De Luca, & al., 1993; Arihara & Sakamoto, 1999; Carpentier, & al., 2001; Contessa, & al., 2009). The relationship between synchronization and fatigue-induced tremor is still unclear (Nordstrom & Miles, 1991). Some of these central features may disrupt performance more than reduction in maximal muscle force. The increased perceived '*effort*' reflects the need to recruit more motoneurons and muscles and to drive them harder (Gandevia, & al., 1996; Gandevia, 1998).

Thanks to the evidence obtained by means of isometric contractions made by different muscles, it is possible to state that central fatigue is likely to limit performance. In fact, performance can deteriorate due to (1) the central fatigue development in muscles working almost at maximal level; (2) a different motor limits implying the deterioration of the capacity to coordinate the contraction to be accomplished; (3) a tolerance limit to avoid catastrophic consequences for the whole organism.

PERIPHERAL FATIGUE

The term peripheral fatigue usually refers to “exercise-related changes which are distal to the site of stimulation and which are seen as a decrease in twitch or tetanic force generated by the stimulus” (Taylor, & al., 2000). It largely occurs within the muscle but may present components related to neuromuscular junction or terminal branches of the motor axon (Taylor, & al., 2000). At the core of the peripheral fatigue is the energy-carrying molecule adenosine triphosphate (ATP), which is the primary molecule used in the body as an energetic intermediate. In the 1920s, A.V. Hill and his colleagues postulated that fatigue arose from the development of oxygen deficiency and the accumulation of lactic acid due to anaerobiosis (Hill, 1932). The factors involved in peripheral fatigue include impairment in the transmission and propagation of muscle action potentials, excitation-contraction coupling and others related contractile mechanisms. Neuromuscular transmission is defined as transformation of the nerve action potential (AP) into a muscle action potential and takes place at the neuromuscular junction. During fatigue this mechanisms can be altered by: (i) insufficient propagation of the nerve potential at the nerve ending; (ii) failure of the coupling between excitation and neurotransmission; (iii) neurotransmitter depletion; (iv) reduced neurotransmitter release; (v) decrease in the sensitivity of the acetylcholine receptors and the post-synaptic membrane. The sEMG seems to be the most appropriate tool to examine the role of the CNS and its properties related to biochemical and physiological changes in skeletal muscles during fatiguing contractions. In fact, it provides a quantitative approach to the analysis of fatigue based on the definition of some indexes relying on the time evolution of its features throughout the contraction. In this way, it is possible to monitor the evolution

of the phenomenon from the very beginning of the muscle effort (Merletti & Parker, 2005). Surface EMG is however influenced by several factors, hereby reported, that influence the fatigue assessment.

Factors That Influence the Surface EMG	
Nonphysiological	
Anatomic	Shape of the volume conductor Thickness of the subcutaneous tissue layers Tissue inhomogeneities Distribution of the motor unit territories in the muscle Size of the motor unit territories Distribution and number of fibers in the motor unit territories Length of the fibers Spread of the endplates and tendon junctions within the motor units Spread of the innervation zones and tendon regions among motor units
Detection system	Presence of more than one pinnation angle Skin-electrode contact (impedance, noise) Spatial filter for signal detection Interelectrode distance Electrode size and shape Inclination of the detection system relative to muscle fiber orientation Location of the electrodes over the muscle
Geometrical	Muscle fiber shortening Shift of the muscle relative to the detection system
Physical	Conductivities of the tissues Amount of crosstalk from nearby muscles
Physiological	
Fiber membrane properties	Average muscle fiber conduction velocity Distribution of motor unit conduction velocities Distribution of conduction velocities of the fibers within the motor units
Motor unit properties	Shape of the intracellular action potentials Number of recruited motor units Distribution of motor unit discharge rates Statistics and coefficient of variation for discharge rate Motor unit synchronization

Figure 4: Factors that influence the sEMG as reported by Farina, et al., 2004.
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Already in 1912, Piper reported, in one of his study, a slowing of surface myoelectric signals during static contractions (Piper, 1912). Given to the random nature of the voluntary EMG signal, this slowing cannot be easily quantified in the time domain (Merletti & Parker, 2005). Some years later, Cobb and Forbes (Cobb & Forbes, 1923) noticed an increase in the signal amplitude during fatiguing static motor tasks that was later confirmed by Knowlton and

colleagues and Lippold and colleagues (Knowlton & Bennet, 1952; Lippold, & al., 1960). Bigland-Ritchie et al. (Bigland-Ritchie & Lippold, 1954) established that integrated surface EMG increased linearly with force. There is an obvious increase in the low-frequency content of the signal during fatigue (Kranz, & al., 1983; Mills, 1982), but this can be fully explained by changes in the compound muscle action potential, and thus, the ongoing signal provided no certain clue about central changes in motor neuron firing frequency. Changes in frequency spectrum of EMG accompany muscle fatigue, but they do not definitively cause it at a peripheral level, nor do necessarily signify altered neural drive (Gandevia, 2001). The increase in concentration of lactate leads to changes in intracellular pH which, in turn, is responsible for the drop of muscle fiber conduction velocity (CV). The latter directly influences the MU action potential (MUAP) waveform and, finally, the properties of sEMG as interference signal (Cifrek, & al., 2009). The lowering of CV is one of the causes for the shift of the sEMG spectrum towards lower frequencies and of the increase of the amplitude (De Luca, 1984). Others, by analyzing sEMG signals coming from Rectus Femoris muscle during static contractions, found out an increase in the amplitude and duration of the averaged MUAP and a compression of the spectrum, even if these results are in contradiction with results coming from intramuscular recordings (Viitasalo & Komi, 1977).

The amplitude of the EMG also seemed simple to interpret in terms of *neural drive* during fatigue, but the size and propagation velocity of the intracellular muscle fiber action potential (Dimitrova & Dimitrov, 2003) and a possible compromise at the neuromuscular junction affects the signs. Although the consensus is that blocking at the neuromuscular junction does not occur significantly with natural rates of motor unit firing, activity-induced changes in the single fiber action potential, and activation of the muscles Na^+/K^+ pump to

degrees which vary between fiber types and the degree of local ischemia seriously limit the surface EMG as a measure of voluntary activation of motoneurons. The sEMG, in fact, comprises the sum of all the electrical contributions made by the active MUs as detected by electrodes placed on the skin overlying the muscle. The information extracted from the sEMG is often considered as a global measure of MU activity, because of the inability of the traditional bipolar recording configuration to detect activity at the level of single MUs. The global characteristics of the sEMG, such as amplitude and power spectrum depend on the membrane properties of the muscle fibers as well as on the timing of MU action potential. Thus, the sEMG reflects both peripheral and central properties of the neuromuscular system.

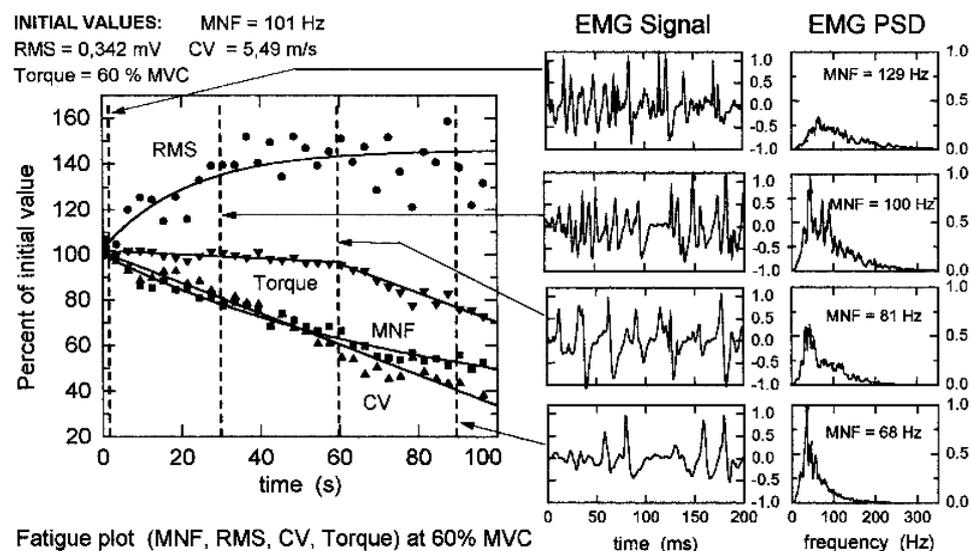


Figure 5: Fatigue plot of a voluntary isometric submaximal (60% MVC) contraction of the Tibialis Anterior muscle. From Merletti & Lo Conte, 1997. © Copyright 1997 Elsevier Science

The alternative to measures of global EMG during fatigue was to record the discharge of single MU in voluntary contractions. Although the principle of motor neuron recruitment and frequency modulation had been known for decades, few had succeeded in recording unitary activity during sustained strong contractions due to the interference pattern caused

by the discharge of many muscle fibers. Motor units were believed to be recruited in a relatively stable order according to Henneman's size principle, from those with slow conduction velocity producing small forces to those with fast conduction velocity producing large forces (Henneman, & al., 1965; Henneman & Mendell, 1981; Mendell, 2005). This principle links motor neuron properties (i.e. small size, long after hyperpolarization, and low axonal conduction velocity) with properties of muscle fibers (small twitch force, long contraction times, slow fiber conduction velocity and low fatigability). During fatigue, exceptions to Henneman's principle seem to occur (Enoka, & al., 1989). An additional effect of the distribution of motor unit size and fatigability across the pool is that during sustained maximal contractions, the decline in force will be dominated by fatigue in the large motor units, those recruited late in voluntary contractions. Furthermore, there is probably an increased difference among the thresholds of the motor neurons recruited close to maximal force, so that greater effort will be needed to generate the final part of the force in the maximal effort (Gandevia, 2001). Another observation key on motor unit behavior during fatigue was made by Marsden and colleagues (Marsden, & al., 1983). During sustained maximal contractions they observed a decline in firing rates of motor units in the FDI muscle. This decline was termed *muscular wisdom* because it matched the firing of the motor neuron to the altered contractile properties of the muscles. This behavior was subsequently confirmed by (Bellemare, & al., 1983; Bigland Ritchie, & al., 1983; Marsden, & al., 1983; Gandevia, & al., 1990). If the voluntary contraction continues for more than a few seconds discharge rates decline and approach a plateau towards 60s (Grimby, & al., 1981) (Bigland-Ritchie, & al., 1983). This decline in rate assists the output from the muscle because relaxation times for whole muscle usually lengthen during strong isometric contractions and thus lower

discharge frequencies produce the same fraction of maximal force (Bigland-Ritchie, & al., 1983).

CHAPTER 2:

The multi-level approach to better understand mechanisms of muscular fatigue

When we try to pick out anything by itself, we find it hitched to everything else in the Universe.
(J. Muir)

In the past century, Mosso (Mosso, 1904) tried to replicate the variability of performance during a voluntary task. If performance deviated from that expected, then he inferred that the changes represented an influence of CNS. He considered not only the physical activity but also the mental work since mental excitement or agitation could improve voluntary endurance and concluded that performance variations reflected central factors which, in turn, alter directly the peripheral function of the muscle. If the muscle were the only significant limit to voluntary performance, then it's natural to check how the CNS drives the muscles.

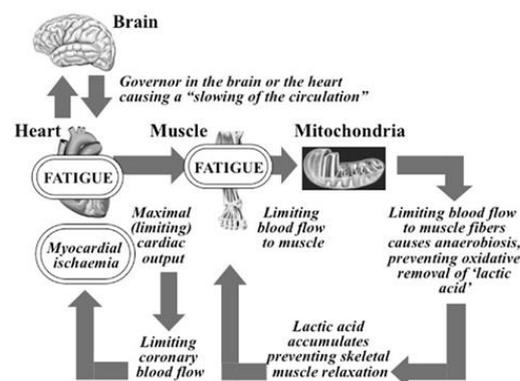


Figure 6: The complete A. V. Hill Cardiovascular/Anaerobic/Catastrophic Model of Human Exercise Performance. From Noakes, 2012

It is commonly thought that many neurophysiological mechanisms are perturbed before the body feels the effect of fatigue. In fact, the state of the neuromuscular system (i.e. the energy reserves, the ion concentrations and the arrangement of contractile proteins) is altered soon as the exercise starts and fatigue develops until the muscle is no longer able to perform the requested task (Boyas & Guevel, 2011). It is thus, not simple to determine the onset and development of muscular fatigue and, in particular, to distinguish between central and peripheral factors. This is a crucial topic since it highlights the necessity of a multi-level approach to better understand the mechanisms leading to the manifestation of muscle fatigue. In fact, the activation of the muscle from the CNS has its effect not only at a metabolic level but it reflects also in changes in electro-physiological environments and biomechanical observations.

BIOCHEMICAL LEVEL

Much of the knowledge about the mechanisms of muscle contraction comes from biochemical and functional studies performed in the early 50s. During this period, some methods for isolating specific muscle proteins were developed as well as the methods for measuring their physiochemical and biochemical properties. In order to better understand them and the mechanisms that are believed to cause the impairment that lead to fatigue at the cellular level, a brief overview about muscle structure and mechanisms of contraction will follow.

THE STRUCTURE OF THE SKELETAL MUSCLE CELLS

As Sir Charles Sherrington said in 1924, “to move things is all that mankind can do, for such the sole executant is muscle, whether in whispering a syllable or in felling a forest” (Sherrington, 1924). The major consequence of the elaborate information processing that takes place in the brain is the contraction of the skeletal muscle. This is composed by many identifiable elements. The cells that compose it, the *muscle fibers*, are linked together by a three level network of connective tissue¹: *epimysium*, *endomysium* and *perimysium*.

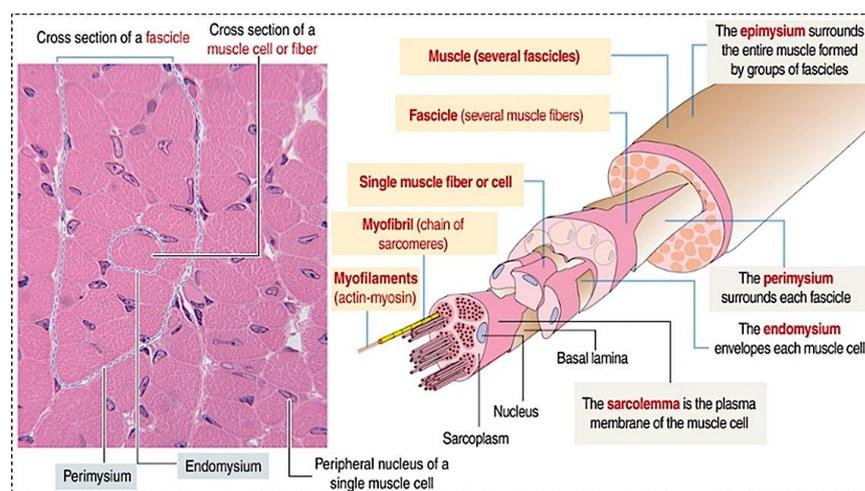


Figure 7: General organization of the skeletal muscle. From Blaustein, et al., 2012.
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The first covers the outside surface of a muscle and separates it from the surrounding muscles. Muscles producing small or very fine movements have smaller fascicles containing relatively few fibers and a larger proportion of connective tissue. Arteries and veins run through the endomysium (Gowitzke & Milner, 1988). The *perimysium* collects bundles of fibers into fascicles in a cross-pattern adding stability to the structure. At the end, the *endomysium* is made up of collagen fibers 60 to 120 nm in diameter and surround each

¹ *Connective Tissue* is a kind of tissue that support, connect or separate different kind of tissues and organs of the body. (Connective Tissue, 2013)

muscle fiber (Stone, & al., 2007). This connective tissue matrix connects muscle fibers to tendon and hence to the skeleton (Huijing, 2003). The cell membrane that composes a muscle fiber is a 7.5 nm thick excitable membrane called *sarcolemma*. The fluid enclosed between the fiber and the sarcolemma is then known as *sarcoplasm* (Enoka, 2008c). The latter contains nourishing sources (such as lipid or glycogen), organelles (such as nuclei, mitochondria etc.), enzymes (such as myosin adenosine triphosphate [ATPase], etc.) and the contractile apparatus (such as bundle of myofilaments arranged into myofibrils). The sarcoplasm also contains a membranous system that assists muscle in the conduction of the signals coming from the CNS. This system encompasses the *sarcoplasmic reticulum* (SR), lateral sacs and transverse tubules (*T-tubules*). The SR runs longitudinally along fibers and, at specific locations long the myofibril, bulges into *lateral sacs*. Orthogonal to the SR and associated with sacs are the T-tubules, which are branched invaginations of the sarcolemma. The rapid transmission of the activation signal from the sarcolemma to the contractile apparatus is facilitated by the connection between the SR and T-tubules. The basic contractile unit of the muscle is the *sarcomere* and it comprises a set of thick and thin contractile proteins (Enoka, 2008). Skeletal muscles, under the microscope, show an alternance of low and high electronic density regions, named A-band (anisotropic) and I-band (isotropic) respectively. These differences are due to the disposition of thin (actin²) and thick (myosin³) filaments, in part aligned and in part overlapped.

² *Actin* is the main component of the thin filament. In the monomeric form, it is a globular protein and presents two isoforms: α -skeletal and α -cardiac differing by only four amino acids encoded by two different genes.

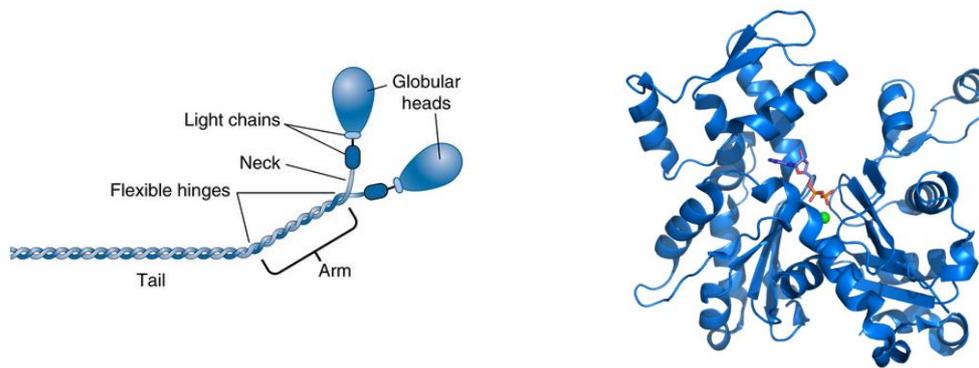


Figure 8: (Left) Structure of myosin. Myosin is composed of two identical heavy chains and two different pairs of light chains. *From Blaustein, et al., 2012;* (Right) Structure of the actin. *From Wikipedia, 2013.*

The I-band is the region with thin filaments, while the A-band contains thick filaments. In the middle of the I-band there is a thin structure, called Z-line, orthogonal to the filaments. Also the A-band has a thin central structure, the M-line, with a high electronic density. The sarcomere includes, then, the zone of myofibrils from one Z band to the next one. Repeating sections of sarcomere compose the *myofibrils* (Nelson & Cox, 2004).

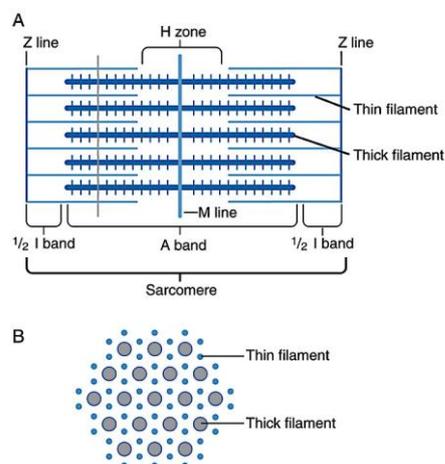


Figure 9: Thick and thin filaments are arranged in regular arrays in the myofibril. **A)** Schematic drawing of a longitudinal section of a single sarcomere, which is the region of myofibril between two adjacent Z lines. **B)** Diagram of a cross section of the myofibril through the A band at the position indicated by the *thin gray line* in **A**. In this region, thick and thin filaments overlap, and each thick filament is surrounded by a hexagonal array of thin filaments. *From Blaustein, et al., 2012. © Copyrighty 2011 Elsevier*

³ *Myosin* is the most abundant muscle protein and is the molecular motor of muscle contraction. Myosin present in the sarcomere is composed of two heavy chains (MHCs) and four light chains (MLCs). In lower limb muscles of healthy adults, MHC-I is the most abundant isoform followed by the MHC-IIA. In upper limb muscles the proportion of the fast MHC isoforms is generally higher.

CLASSIFICATION OF MUSCLE FIBERS AND MOTOR UNITS

Muscle fibers possess distinct mechanical and energetic properties and their heterogeneity has its origin in the molecular composition of the intracellular structure where the molecular motor of the muscle contraction converts chemical into mechanical energy, i.e. the myofibrils. Therefore, large differences are exhibited by the skeletal cells, and several efforts have been made to classify them. One is based on the myofibrillar ATPase activity (Engel, 1962), leading to the first separation in type I and type II fibers. The human trunk and the limb muscles, for example, exhibit three main groups, I, IIA and IIB and four intermediate groups, IIC, IC, IIAC, IIAB (Staron & Hikida, 1992). A parallel classification is based on the determination of metabolic enzymes (Ogata & Mori, 1964). Fibers identified with this method present different content of mitochondrial enzymes and can be related with oxidative potentials. Based on observation of the contractile properties of motor units (force, velocity and fatigability), Burke and coworkers (Burke, Levine, & Zajac, 1971) classified four motor unit/fibers types: *slow* (S or SO), characterized by long duration twitches, low peak force and high resistance to fatigue, high oxidative enzymes concentration and low glycolytic markers concentration and low ATPase activity; *fast fatigue resistant* (FOG or FR), present high oxidative and glycolytic enzymes concentration and ATPase activity; *fast fatigable* (FG or FF), display high contraction rates and extremely large forces but for very short time, high ATPase and glycolytic activity and low oxidative capacity.

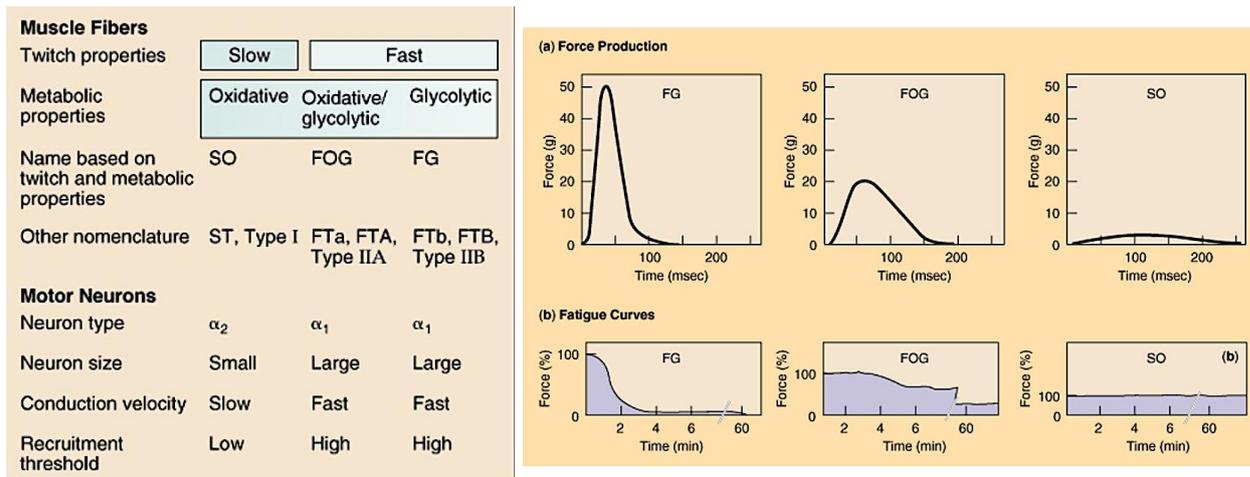


Figure 10: (Left) Muscle fibers and Motor Neurons classification and properties; (Right) Trend of force production and fatigue curves in the three types of muscle fibers (FG, FOG and SO). Source web

However, the major determinant of muscle fibers properties (i.e. the maximum shortening velocity, ATPase activity, maximum power output and rate of force developed) is the MHC (Myosin Heavy Chain) isoforms. This classification has a high relevance in the context of fatigue since a fast isoform consume ATP at faster rate that the slow isoform (Allen, & al., 2008).

MECHANISMS OF MUSCLE CONTRACTION

At the neuromuscular junction, the acetylcholine⁴ takes less than 100 μ s to diffuse across the synaptic cleft and attach to receptors on the post-synaptic membrane (i.e. the muscle fiber). This attachment results in the opening of the Na^+/K^+ channels and the influx of Na^+ into and the efflux of K^+ from the muscle fiber. The movement of these ions results in the development of the end-plate potential that can trigger the generation of a muscle fiber action potential (AP). This process is referred to as *neuromuscular propagation* (Enoka, 2008c). Once an AP has been generated, several processes, known as *excitation-contraction coupling*,

⁴ *Acetylcholine* (ACh) is a small molecule neurotransmitter used in both the brain and peripheral nervous system. From Enoka, 2008a.

contribute to the generation of force. Seven major processes are involved in this conversion phenomenon and in particular: (1) propagation of the action potential along the sarcolemma; (2) propagation of the AP along the T-tubule; (3) coupling of the AP to the change in Ca^{2+} conductance of the SR; (4) release of Ca^{2+} from SR; (5) reuptake of Ca^{2+} by the SR; (6) Ca^{2+} binding to troponin⁵ (Tn); (7) interactions of the contractile proteins. Under normal circumstances, the thick and thin filaments are prevented to interact by the regulatory action of TnT and tropomyosin⁶ (TM), and Ca^{2+} is stored largely in the SR. The events, known as *Ca²⁺ disinhibition*, enable the muscle fibers AP to trigger the release of Ca^{2+} from SR. In a resting state, the potential across the sarcolemma is negative on the inside with respect to the outside and most of the Ca^{2+} is stored in cisternae, i.e. enlargements of the SR near the T-tubules. When the AP as it is propagated along the sarcolemma down the T-tubules and into the interior of muscle fiber, it triggers an increase in Ca^{2+} conductance, which corresponds to an opening of ryanodine receptors⁷ (Bastian & Nakajima, 1974). When the Ca^{2+} concentration in the sarcoplasm is above a threshold level, Ca^{2+} binds to Tn that is attached to the actin filament. This binding probably determines a structural change in the thin filament such that the myosin-binding site is uncovered and the two filaments become able to interact (Enoka, 2008c).

⁵ *Troponin*: is a three component molecule that forms part of the actin filament and is involved in the regulation of interaction between actin and myosin. TnC is the component of Tn that binds Ca^{2+} ; TnI is the component of Tn that inhibits G-actin from binding to myosin; TnT is the component of Tn that binds to tropomyosin (Enoka, 2008a).

⁶ *Tropomyosin*: is a thin-filament protein involved in the regulation of interaction between actin and myosin (Enoka, 2008a)

⁷ *Ryanodine receptors*: calcium releasing channel in the SR of the muscle fiber (Enoka, 2008a)

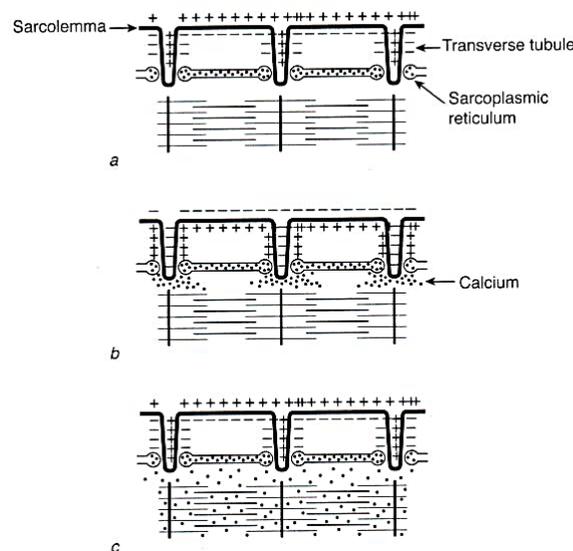


Figure 11: Role of Ca^{2+} in the excitation-contraction coupling. (a) Ca^{2+} is stored in the SR at rest; (b) Ca^{2+} is released into the sarcoplasm in the presence of an AP; (c) Ca^{2+} remains in the sarcoplasm after the AP has passed until it is returned in the SR. From Enoka, 2008a.

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The change in Ca^{2+} conductance is, however, transient: once the AP passed, the conductance returns to a resting state and Ca^{2+} is absorbed again into the SR. The rate at which this reuptake happens determines the rate of decline of force after the cessation of AP. Fatigued muscle, for example, exhibits a reduction in the rate of return which produces a decline in the rate of relaxation time. The reuptake then lowers the concentration of Ca^{2+} in the sarcoplasm, which inhibits the activity of the enzyme that regulates the interaction of actin and myosin (Enoka, 2008c).

The Ca^{2+} disinhibition phenomenon causes the interaction between the two contractile proteins, actin and myosin, known as cross-bridge cycle. This process involves the use of ATP by the globular head of myosin to attach actin and comprises several steps that can be distinguished based on the absence or presence of Ca^{2+} bounded to Tn (Gordon, & al., 2001). For each globular head the cycle involves a detachment phase, an activation phase and an attachment phase (Enoka, 2008c). Hydrolysis of ATP into ADP and P_i causes a rotation of the

head of the protein toward the tail and the pulling on the compliant arm of the cross-bridge. The direct consequence of this pull is a relative movement of thin and thick filaments and the shortening of the sarcomere. Since the displacement of the myosin head occurs while actin and myosin are connected, both the filaments slide one with respect to the other and exert a force on the cytoskeleton. The sliding of the filaments gave rise to the *sliding filament theory of muscle contraction* (Huxley & Niedergerke, 1954). In detail, ATP binds to myosin and then, in presence of Ca^{2+} , to Troponin (TnT) and Tropomyosin (TM). A myosin-binding site is exposed on the thin filament and a physical link is formed between actin and myosin (Kitamura, & al., 1999). Since the molecules of the myosin are oriented in opposite directions at the opposite ends of the thick filament, each of them pulls its adjacent thin filament toward the center of the sarcomere and then the sarcomere shortens. The cycling of cross-bridges continues as long as there are sufficient amounts of Ca^{2+} and ATP in the muscle cell. The force exerted by the muscle is usually explained as a consequence of the concurrent, but not synchronous, cycling of many cross-bridges following Ca^{2+} disinhibition. This phenomenon is referred to as cross-bridge theory of muscle contraction (Huxley, 2000).

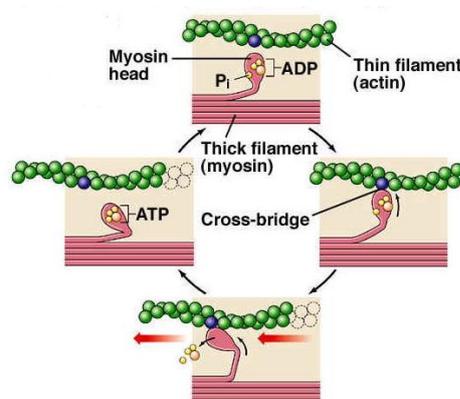


Figure 12: Cross-bridge cycle. *From Burkett, 2005.*

METABOLIC PROCESSES UNDERLYING MUSCLE FATIGUE

From the biochemical point of view, two are the main causes that determine fatigue and that have to be analyzed: **changes in intracellular environment** (accumulation of lactate and hydrogen ions; accumulation of ammonia or heat) and **changes within the muscle fibers** (accumulation of inorganic phosphate, hydrogen, magnesium (Mg^{2+}) and calcium ions (Ca^{2+}) in the sarcoplasm; inhibition of Ca^{2+} release by the sarcoplasmic reticulum (SR); decrease in glycogen reserves and drop in blood glucose; drop in nerve action potential speed; increase in the efflux of potassium (K^+) ions in the muscle fibers). Each of the processes of the cross-bridge cycle can be influenced by different factors: e.g. the opening of Ca^{2+} release channels in the SR is mediated by ATP, inhibited by Mg^{2+} and altered by P_i and pH. The impairment of excitation-contraction coupling, however, does not contribute to the initial decline of force during a fatiguing contraction (Allen, Lamb, & Westerblad, 2008), but, rather, as fatigue progresses the availability and efficacy of Ca^{2+} as activation signal decreases and so does the force exerted by each cross-bridge cycle (Enoka, 2008). These phenomena are referred to as *activation failure* and *myofibrillar fatigue*. The first one occurs generally later than the other during high tension contractions and it is believed to be caused by a decrease in the sensitivity of myofibrils to Ca^{2+} and a reduction in the release of the same by SR, but also by a reduction in the release of Ca^{2+} from the SR. Myofibrillar fatigue, instead, corresponds to an impairment of cross-bridge function highlighted from a decrease in isometric force and shortening velocity (Edman, 1995). During prolonged exercises there is an increase of P_i due to the dissociation of phosphocreatine (PCr) into P_i and Cr and the hydrolysis of ATP (Westerblad, & al., 1998). The increase of $[H^+]$ for a long time has been considered

responsible for the decrease in force generation since it is associated with a diminished intracellular pH, which, in turn, leads to an increase in acidosis (Sahlin, 1985; Sahlin, & al., 1981; Lamb & Stephenson, 2006).

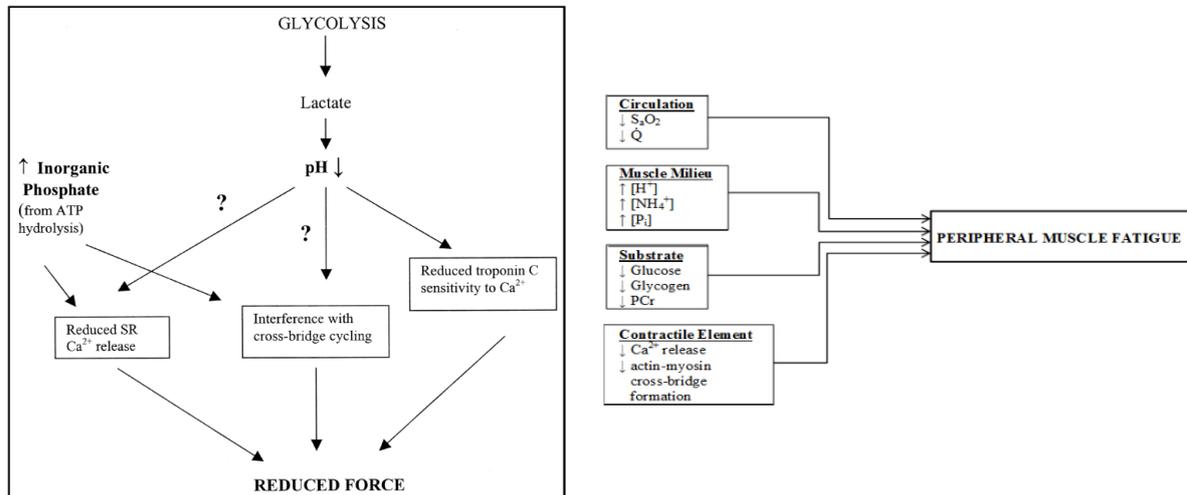


Figure 13: (Left) Potential mechanisms through which decreasing pH and increased P_i could cause fatigue. From Stackhouse, et al., 2001; (Right) Summary of peripheral factors contributing to the development of fatigue. From Shei & Mickleborough, 2013. © Copyright 2001 American Physical Therapy Association

ELECTROPHYSIOLOGICAL LEVEL

The mechanisms related to the manifestation of neuromuscular fatigue during submaximal contraction are a combination of an increase in MU recruitment and modulation of their discharges. The increase the amplitude level of EMG, estimated by variables as Average Rectified Value (ARV) and Root Mean Square (RMS) has been assumed to be due to failure in the muscle contractile properties, so that with sustained tasks the active fibers exert progressively less force. In order to compensate for this, new motor units are recruited and the already active will fire with a decreased frequency (Lippold, & al., 1960; Viitasalo & Komi, 1977). The equation representing amplitude estimators of EMG are shown below:

$$ARV = \frac{1}{T} \int_0^T |x(t)| dt \quad (\text{continuous time})$$

$$RMS = \sqrt{\frac{1}{T} \int_0^T x^2(t) dt} \quad (\text{continuous time})$$

$$ARV = \frac{1}{N} \sum_{i=1}^N |x_i| \quad (\text{discrete time})$$

$$RMS = \sqrt{\frac{1}{N} \sum_{i=1}^N x_i^2} \quad (\text{discrete time})$$

Equation 1: Definition in continuous and discrete time of EMG amplitude estimators.

From *Hermens H., et al., 1999. ISBN 90-75452-17-9.*

Different types of MU types are distinguished according to their mechanical response: the ones with fast fibers (FT) produce short time to peak forces while the one made by slow fibers (ST) need more time to reach the maximum. Generally, ST-MU encompass fibers with slower conduction velocity (CV) while FT-MU comprises of fibers with higher CV. Fiber composition is generally investigated by biopsy or histochemical analysis even if the information carried out is not representative of the whole muscle. The variables potentially useful for this purpose are the spectral characteristics of the sEMG, i.e. mean frequency (MNF), median frequency (MDF), or normalized higher spectral moments. The MNF (Mortimer, & al., 1970; Kwatny, & al., 1970; Komi & Tesch, 1979; Arendt-Nielsen & Mills, 1985; Eberstein & Beattie, 1985) represents the ratio between the spectral moment of order 1 and the one of order 0 (Lindstrom, & al., 1977). The MDF, instead, is the frequency dividing the spectrum into two regions of equal power (Stulen & De Luca, 1981; Mannion & Dolan, 1994; Kupa, & al., 1995; Merletti & Roy, 1996). Finally, the index normalized index FI_{nsmk} , proposed by Dimitrov and colleagues is expressed by the ratio between the spectral moments of order (-1) and the spectral moment of order k, with k ranging from 1 to 5 (Dimitrov, & al., 2006). The formulations of the MNF, MDF and FI_{nsmk} are reported in Equation 2.

$$MNF = \frac{\int_0^{\infty} f * P(f) df}{\int_0^{\infty} P(f) df} \quad \int_0^{MDF} P(f) df = \int_{MDF}^{\infty} P(f) df = \frac{1}{2} \int_0^{\infty} P(f) df \quad FI_{nsmk} = \frac{\int_{f_1}^{f_2} f^{-1} * P(f) df}{\int_{f_1}^{f_2} f^k * P(f) df}$$

Equation 2: (Left) Mean frequency of the EMG power -frequency spectrum P(f). (Middle) Median Frequency of the power-frequency spectrum P(f). From *Stulen & De Luca, 1981*. © Copyright 1981 IEEE (Right) New spectral index proposed by *Dimitrov, et al., 2006*. © Copyright 2006 by the American College of Sports Medicine

The MDF and MNF provide some basic information about the spectrum of the signal. They coincide if the spectrum is symmetric with respect to its center line, but are usually different reflecting the skewness of the power spectrum. The standard deviation of the estimated MDF is higher than the one of MNF (Stulen & De Luca, 1981, 1982), even if it has been demonstrated that in presence of additive noise and fatigue (spectrum more skewed) the MDF leads to better estimates (Merletti & Parker, 2005), and that's why it is the preferred parameter for measuring frequency compression as a function of conduction velocity of the muscle fibers. These spectral parameters have been used several times as an indirect measure of the relative changes in muscle fiber CV (Merletti, & al., 1990) or to infer about MU recruitment strategies and fiber type composition (von Tscharnner & Nigg, 2008; Wakeling, 2008). Actually, it is true that each MU has a specific force-generating capacity but task specificity of each MU is not a physiological variable. Muscle fibers of different MU, within a muscle, have a complex distribution and they are locked among themselves, making thus, difficult to assign a filtering weight to the AP propagating along each fiber (Bawa, 2008). Furthermore, the possible inference about motor unit recruitment strategies is based on the rationale that higher threshold (FT) MU produce AP with larger energies at higher frequencies than lower threshold ones (ST). This is not completely true (Farina, 2008). First of all because the two types of fibers do not have distinct CV but rather CV shows a continue

single-peak distribution (Troni, & al., 1983), second of all because CV of muscle fibers AP can differ among population of MU (Blijham, & al., 2006) and then because the number of fibers innervated by a MU presents a skewed distribution. Moreover, the CV of muscle fiber can vary of about 20% with changes of discharge rate (Staalberg, 1966), and the range of firing rates vary with recruitment threshold (Barry, & al., 2007).

BIOMECHANICAL LEVEL

From the biomechanical point of view, the evaluation of muscular fatigue comes from the observation of another phenomenon, the *task failure*, intended as the time instant when the exercise cannot longer be continued (Hunter, & al., 2004; Maluf & Enoka, 2005). This point is often termed *exhaustion* by exercise physiologists (Gandevia, 2001). The extent to which any single process contributes to task failure depends on the relative demand placed on each of the processes that contribute to the force exerted by the muscle during the execution of a motor task (Maluf & Enoka, 2005). Exercises that are completed after a certain time, distance or number of repetitions removes the variance due to subjectivity from the point of termination, but makes clear that performance can be limited by central factors. Some circulatory, respiratory or metabolic events lead to task failure (Gandevia, 2001).

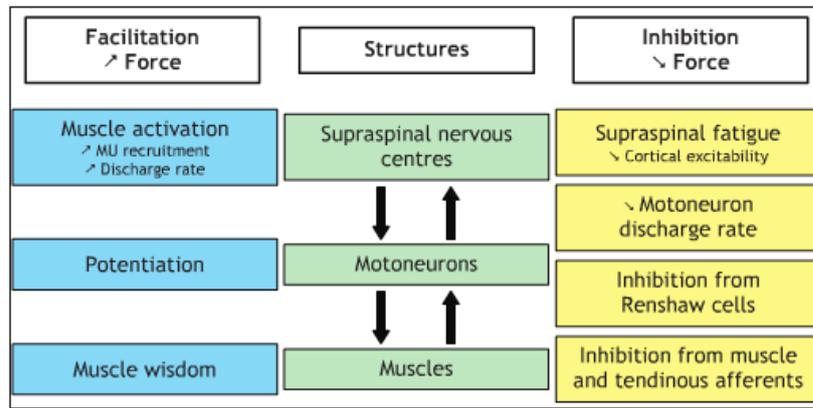


Figure 14: Mechanisms influencing the maintenance of submaximal force. From *Boyas & Guevel, 2011*.
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CHAPTER 3:

Neuromuscular Adaptations during Submaximal Prolonged Cycling

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This study was carried out to evaluate the neuromuscular adaptations occurring during dynamic motor tasks. In this context, several efforts have been made in the last century in order to find the best technique to adopt to evaluate the signs of fatigue from surface electromyographic signal (sEMG) (Knaflitz & Bonato, 1999; Karlsson, & al., 2000; Bonato, & al., 2001; Zory, & al., 2011). The protocol to be accomplished was designed basing on time to failure exhibited by the subjects. Task failure was defined as the instant the participant was no longer able to maintain the required task. Electromyographic activity was recorded from eight muscles of the dominant leg and burst characteristics of sEMG signals were analyzed in order to assess the changes in muscle activity level produced by the occurrence of neuromuscular fatigue. In particular, three features were extracted from the sEMG signal for each burst: amplitude, location of the maxima and mean profile of the burst envelope. A speculation is presented about changes induced by neuromuscular fatigue essentially in the mono-articular muscles, which produce power. This phenomenon is highly correlated with the adopted pedaling strategy which, being not constrained, induces subjects

¹ Castronovo, A. M., De Marchis, C., Bibbo, D., Conforto, S., Schmid, M., & D'Alessio, T. (2012, August). Neuromuscular adaptations during submaximal prolonged cycling. In *Engineering in Medicine and Biology Society (EMBC), 2012 Annual International Conference of the IEEE* (pp. 3612-3615). IEEE. Copyright © 2012, IEEE

to express the maximal power in the downstroke phase, related to knee extension and involving mainly mono-articular muscles.

INTRODUCTION

The possibility of tracking the athletes' muscles status when performing any sport is very attractive. In particular, surface electromyography (sEMG) could be a powerful non-invasive tool for this kind of analysis. The sEMG signal is generated by skeletal muscles and, reflecting the electrical activity of muscle cells, provides an insight into physiological mechanisms at the basis of any movement (Merletti & Parker, 2005). sEMG is related to the neural descending drive and to the number of active motor units and relative discharge rate and the pattern of muscle activation can reflect these characteristics (Farina, & al., 2010) even if the amplitude cancellation phenomenon has to be taken into account (Keenan, & al., 2006). In the field of cycling science, electromyographic analysis has been used for the assessment of muscle coordination changes, in particular those occurring with incoming fatigue (Bini, & al., 2008; Hug & Dorel, 2009; Dorel, & al., 2009; Turpin, & al., 2011; Theurel, & al., 2012). In order to characterize the patterns of muscle activation, the analyzed features are essentially two: muscle activity level and activation timing with respect to the crank angle and pedal revolution. Timing parameters are determined considering signal onset and offset instants, which identify the duration of the single burst and they are generally calculated by fixing a threshold of about 1, 2 or 3 times the standard deviation (Hodges & Bui, 1996). The activation timings of muscles involved in the pedaling gesture with respect to the pedal revolution have been extensively reported in literature (Jorge & Hull, 1986; Li & Caldwell, 1998; Bibbo, & al., 2006; Dorel & Couturier, 2008; Wakeling & Horn, 2009). Gluteus Maximus

is activated from the Top Dead Center (TDC) to the Bottom Dead Center (BDC), while Vastii are active just before the TDC until the first quarter (nearly 90°) of the pedal revolution cycle. The onset of Rectus Femoris precedes, on the other hand, the onset of Vastii even if the offset is just the same. Tibialis Anterior is activated during the upstroke phase (second half of the pedal revolution) and terminates just after TDC. The Gastrocnemii are activated after Tibialis activation and their activity stops just before the onset of the Tibialis itself. Finally, the Soleus is activated during the downstroke phase (first half of the pedal revolution). The different timings of activation reflect differences in muscles roles: the production of power is attributed to mono-articular muscles (Gluteus Maximus, Vastii, Tibialis and Soleus), while, on the contrary, bi-articular ones (Biceps Femoris, Rectus Femoris, Gastrocnemii) deal with the transfer of energy between joints in specific sectors of the crank revolution (van Ingen Schenau, & al., 1992). This information is a matter of importance when considering muscle coordination and the relative changes occurring with incoming neuromuscular fatigue. The latter is defined as an impairment in the force generation capacity of the neuromuscular system during sustained activities (Edwards, 1981; Bigland-Ritchie, 1981) and many studies, conducted during prolonged cycling exercises, reported muscle fatigue as the major limitation for athletes' performance (Lepers, & al., 2000, 2002; Billaut, & al., 2005). Moreover, it has been hypothesized that neuromuscular fatigue induces changes in muscle activity level and activation timings (Turpin, & al., 2011) and in modular organization and muscle coordination also (Forestier & Rougier, 1998; Hufnuss, & al., 2006). The aim of this chapter is to cover, in a descriptive way, the changes of the sEMG signal at a higher detail level considering the single bursts in order to assess if incoming neuromuscular fatigue produces

changes in the single burst characteristics, and, as a result, in the characteristics of the whole signal.

MATERIALS AND METHODS

Participants - Six male subjects (age: 26.2 ± 1 yr.) voluntarily participated to the experimental protocol after being informed about the aim of the study and possible risks. All participants reported no previous experience of training in cycling exercises. The study was performed according to the Declaration of Helsinki.

Experimental Design - The experimental protocol consists of a submaximal cycling test performed until voluntary exhaustion and at a constant power output on a cycle-simulator. Forces applied to each pedal have been acquired using a novel system based on instrumented pedals (Bibbo, & al., 2008, 2009; Conforto, & al., 2009). At the beginning of the session each participant performed a 10 s all-out trial in order to determine the peak power output (PPO) and the maximal value of RMS-EMG reached in correspondence of this point. Subjects were asked to remain seated throughout the exercise. Power output was fixed at 20% of PPO. Throughout the entire exercise, subjects used a visual feedback to maintain the power output at a constant level. They were also instructed to maintain the pedaling cadence in the range 65-75 rpm and to stop when they started to feel tired and painful at muscular level. In this way, the exhaustion was defined as the moment when the power output exerted by the participant fell below 18% of PPO

Muscle Activity - Surface electromyographic signals were obtained from eight muscles of the dominant leg: Gluteus Maximus (GMax), Biceps Femoris (BF), Rectus Femoris (RF), Vastus

Lateralis (VL), Vastus Medialis (VM), Gastrocnemius Medialis (GAM), Tibialis Anterior (TA), Soleus (SOL).

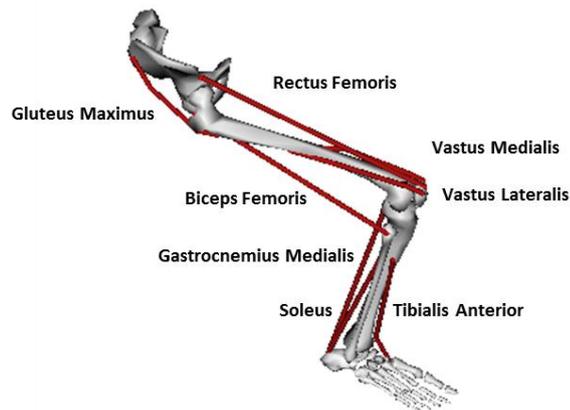


Figure 15: Main muscles involved in the pedaling gesture and analyzed for this study.
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sEMG data were recorded using a wireless system (FREEEMG 300, by BTS Bioengineering S.p.A.) provided with eight bipolar wireless channels, sampled at 1000 samples/s and digitized with a 14 bit A/D converter. Skin was shaved and cleaned in order to decrease skin impedance. Pre-gelled Ag/AgCl surface electrodes were placed over the surface of the muscle, parallel to muscle fibers orientation according to SENIAM recommendations (Hermens H. , Freiks, Disselhorst-Klug, & Rau, 2000). sEMG signals were recorded throughout the entire trial and then an off-line analysis was performed.

Instrumented Pedals - Two instrumented pedals fitted on the cycle-simulator allowed recording the three components of force applied on the pedal with an accuracy of 0.1%. The full scale value is 2000N. The pedal, designed to maintain the same characteristics of a commercial clipless pedal, using a strain gauge based load cell, permits the measurement of the three force components. Strain gauges are connected according to a full Wheatstone bridge configuration. Moreover, an encoder, placed between the pedal frame and the pedal

spindle, permits to obtain the relative angle between the pedal and the crank. Signals are recorded and converted on board by means of a microcontroller and digitized data are transmitted via Bluetooth™ to a remote PC. The use of this novel system allows to calculate the power output profile throughout each pedal cycle and to separate the contribution of the two legs, not possible in commercial systems (Bibbo, & al., 2012). The total power output is, in fact, calculated as the sum of the separate contribution of power output for the left and right leg and in accordance with the formulas:

$$P_{tot} = F_{tg} * l_{ped} * \omega$$

$$F_{tg} = F_x * \cos(\mathcal{G}_p) + F_z * \sin(\mathcal{G}_p)$$

where P_{tot} is the total power output, F_{tg} is the tangential force to the crank, l_{ped} is the length of the crank arm, ω is the angular velocity, F_x and F_z are the components of force in the pedal reference system and \mathcal{G}_p is the angle between pedal and crank.

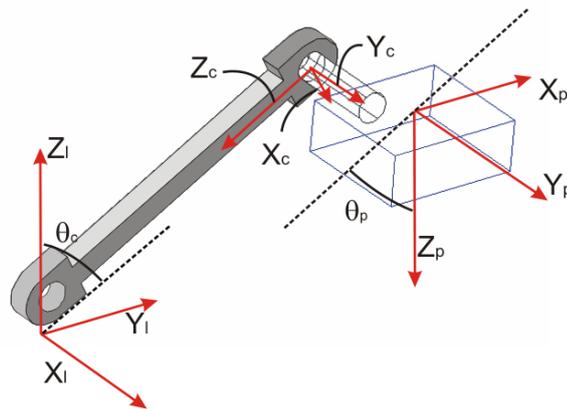


Figure 16: Representation of pedal forces as they are fixed in the reference system of the pedal and of the crank.
From *Castronovo, et al., 2012*. Copyright © 2012, IEEE

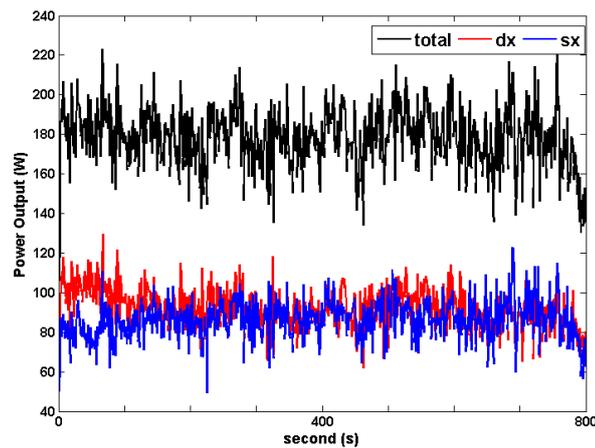


Figure 17: The black line represents the total power output profile, the blue line the power output profile calculated over the left leg and the red line the power output profile calculated over the right leg. As can be noticed the total power output profile is kept constant.

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sEMG Data Analysis - Each raw sEMG recording was filtered using a band-pass Butterworth digital filter in the band 20-350 Hz and normalized to the peak RMS sEMG value reached during all-out task (Rouffet & Hautier, 2008). Linear sEMG envelopes were estimated by an adaptive algorithm optimized for dynamic conditions (D'Alessio & Conforto, 2001). The onset and offset of muscle activity were detected by means of the double threshold detector (Bonato, & al., 1998). For each burst the envelope profile was extracted and the position of its maximum value was calculated as a percentage of the burst duration. Mean envelopes were then derived by averaging the envelopes of the burst resulting in each phase. An amplitude parameter was also calculated as the area under the envelope profile between onset and offset of each burst.

Statistical Analysis - Data were visually inspected and descriptive statistics was calculated for each data set. For each subject and each muscle data were compared between the initial and the final phase of the task. In particular, the initial phase corresponds to the first 100 s of

the task, while the final one encloses the last 100 s before the exhaustion point. The extracted parameters were compared by means of statistical tests. In particular, a paired t-test analysis was performed in order to assess differences among location of the maxima with respect to the burst duration in the comparison initial phase vs. final phase. A one-way ANOVA was performed for the assessment of differences among amplitude of the envelopes of each burst of the sEMG signal using both phases (initial vs. final) as factors. Finally, the Pearson coefficient between mean envelope profiles was determined for the assessment of similarities among them. The level of significance was set at 0.05 for all data analysis. The signal processing and statistical analysis was performed using MATLAB (MathWorks, Natick, MA, USA).

RESULTS

Burst Amplitude - The envelope amplitude of each burst for each muscle and each subject were statistically compared between initial phase and final phase of the task. The results coming from one-way ANOVA reported a significant difference for only Vastii when comparing the initial and the final phase. Other muscles, and in particular bi-articular ones, reported a high variability among subjects (Table I) resulting in a not significant difference.

Location of the Maxima (ML) - A shift of the maximal value of the mean envelope towards the final stage of the burst for the bi-articular muscles (BF, RF, GAM) and a shift to the initial stage for mono-articular muscles (VL, VM, TA, GLU, SOL) are present when the final phase is compared to the initial phase of the task. The results of the t-test confirmed this behavior for only BF and SOL.

Envelope Profile - In order to assess similarities between shapes of burst envelopes in the comparison initial vs. final phase of the task, a cross-correlation analysis was performed. The Pearson coefficient values showed a moderate correlation only for BF and TA (around $r = 0.66$ for BF and $r = 0.69$ for TA), while other muscles reported a high correlation between shapes of the burst profiles (mean values across subjects: $r = 0.92$ for RF, $r = 0.94$ for VL, $r = 0.92$ for VM, $r = 0.94$ for GLU, $r = 0.83$ for GAM and $r = 0.89$ for SOL). Envelope profiles are however all positively correlated, except for one muscle of one subject.

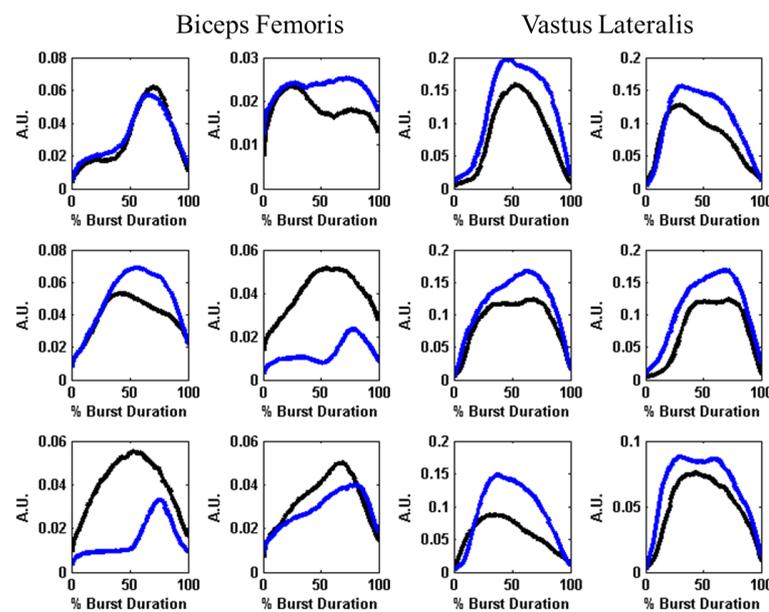


Figure 18: In the left side the mean envelope of BF for all subjects with respect to the percentage of the burst duration are depicted. In the right side of the figure the mean envelope of VL is represented. The black line is representative of the envelope profile at the beginning of the task, while the blue line depicts the envelope profile at the end of the exercise.

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TABLE I
 MEAN \pm STANDARD DEVIATION ACROSS SUBJECTS FOR EA AND F
 AT THE BEGINNING AND AT THE END OF THE EXERCISE, FOR THE TWO LOAD CONDITIONS AND FOR ALL MUSCLES.
 ALSO p VALUES COMING FROM T-TEST HAS BEEN REPORTED
 †n.s, * P<0.05, ** P <0.001

<i>Muscles</i>	<i>BA</i>			<i>ML</i>		
	IN	END		IN	END	
BF	3.6 \pm 1.2	3.2 \pm 0.9	†	52 \pm 17.3	71.6 \pm 8.7	*
RF	3.9 \pm 0.9	4.8 \pm 1.1	†	42.8 \pm 9.9	47.5 \pm 7.7	†
VL	7.5 \pm 1.5	7.5 \pm 1.5	*	50.3 \pm 17.1	45.5 \pm 17.3	†
VM	7.7 \pm 1.5	9.9 \pm 1.9	*	51.6 \pm 13.5	52.8 \pm 15.2	†
TA	5.2 \pm 1.5	4.7 \pm 1.3	†	39.3 \pm 16.8	37.8 \pm 20.7	†
GLU	1.9 \pm 0.6	2.4 \pm 0.5	†	69.6 \pm 6.3	67 \pm 15.3	†
GAM	3.8 \pm 0.8	3.3 \pm 0.6	†	60.3 \pm 12.1	68 \pm 11.9	†
SOL	3.3 \pm 0.6	4.3 \pm 0.7	†	62.5 \pm 6.1	52.8 \pm 9.8	*

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DISCUSSION AND CONCLUSIONS

The present chapter aims at describing what happens at the burst level of the sEMG signal when subjects perform a submaximal pedaling exercise at a constant power output until voluntary exhaustion. Three features were extracted from the sEMG signal: amplitude, location of the maxima and profile of the mean envelopes of the bursts. All these parameters were considered for both initial and final phase. We found an increase in the amplitude of the mean envelope for all subjects only for VL and VM muscles. This could be due to the primary role of power producers of Vastii muscles in the pedaling gesture. It can, in fact, be inferred that VL and VM are the muscles mainly affected by neuromuscular fatigue and the increase of amplitude of the sEMG signal is just a sign of this (Petrofsky, 1979). The location

of the maximal value of the mean envelope of the bursts was found to change when considering bi-articular or mono-articular muscles. In particular, a statistical difference was found for BF and SOL, accounting for a shift to the right and to the left respectively. Despite these changes the shape of the envelope of the burst at the end of the task is highly correlated with the one at the beginning of the exercise, indicating the absence of structural alterations in the burst profile. The obtained results permit to hypothesize that neuromuscular fatigue induces changes observable essentially in the Vastii, which are involved especially in the downstroke phase. The typical pedaling strategy consists of pedaling by exerting the major part of force in the first half of pedal revolution without pulling up during the second phase. It seems clear that, pushing down the pedal involves the knee extensors (i.e. Vastii) that have the role of power producers. Other muscles, especially the bi-articular ones, have the main role of transferring energy between joints, and thus, in this kind of task, they are not corrupted by neuromuscular fatigue. This was confirmed by the high variability in the amplitude of the envelope. This variability is reflected also in the moderate correlation of the shape of the mean burst envelope and location of maximal values. So, we can speculate that neuromuscular fatigue influences the power-producing muscles, which are furthermore constrained by the imposed constant power output, more than other muscles that present high variability in the sEMG signal, not only for burst amplitude but also for shape and location of maximal values. The pedaling strategy adopted by all subjects is, in fact, concentrated mainly in the propulsive phase of the pedal revolution. Therefore, we can speculate that changing the pedaling technique with external constraints could lead to a decreased variability in bi-articular muscles. For example, the use of an external feedback for the pull-up action could imply a major involvement of bi-articular muscles that could result

in a common behavior across all muscles also for amplitude feature when neuromuscular fatigue occurs.

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CHAPTER 4:

Bi-dimensional analysis of parameters from sEMG recordings during exhausting isometric contractions under different biomechanical demands

In the previous chapter the focus was on the assessment of neuromuscular adaptations induced by the occurrence of neuromuscular fatigue during dynamic tasks by using indicators extracted from sEMG only in the temporal domain. The aim of this chapter, instead, is to overcome the limitations arisen from the previous study and assess and characterize the occurrence of neuromuscular fatigue by introducing a new parameter more reliable and robust for fatigue analysis. Partial results of this work have been presented during the XIII Mediterranean Conference on Medical and Biological Engineering and Computing (Conforto, & al., 2014)¹.

INTRODUCTION

Muscle fatigue is generally referred to as a task-induced phenomenon reducing the force generation during sub-maximal or maximal contractions (BiglandRitchie, & al., 1983). This phenomenon is known to cause changes in muscular activities that are typically assessed by

¹ The Fatigue Vector: A New Bi-dimensional Parameter for Muscular Fatigue Analysis. Conforto S.; Castronovo AM.; De Marchis C.; Schmid M.; Bertollo M.; Robazza C.; Comani S.; D'Alessio T. XIII Mediterranean Conference on Medical and Biological Engineering and Computing 2013 IFMBE Proceedings 41:149-152, 2014. Copyright © 2013 | IFMBE.

monitoring the surface EMG signal, both in the temporal and spectral domains (Vøllestad, 1997). Several sEMG-related characteristics have been used for the assessment of peripheral fatigue in many studies: in particular the root mean square (RMS) and the average rectified value (ARV) have been used as amplitude estimators while the mean and median frequency (MNF or MDF) and the normalized higher order spectral moments have been used as spectral indicators (Petrofsky, & al., 1982; Moritani, & al., 1982; Arendt-Nielsen & Mills, 1988; Duchêne & Goubel, 1990; Potvin & Bent, 1997; Gerdle, & al., 2000). Also the muscle fiber action potential conduction velocity (CV) can be included in the features extracted from (multi-channel) EMG suitable for fatigue analysis. It has been, in fact, reported a slowing in CV as soon as a submaximal endurance contraction progresses (Bigland-Ritchie, & al., 1981; Stulen & Luca, 1981; Naeije & Zorn, 1982; Eberstein & Beattie, 1985; Arendt-Nielsen & Mills, 1985; Arendt-Nielsen & Mills, 1988; Farina, & al., 2002; Farina & Merletti, 2004). The amplitude of the sEMG signal has been shown to increase during sustained isometric contractions performed at sub-maximal levels (Sadoyama & Miyano, 1981; Maton, 1981; Arendt-Nielsen & Mills, 1988) up to the endurance point, and this behavior has been attributed to the recruitment of additional large and superficial motor units (Lippold, & al., 1960; Viitasalo & Komi, 1977; Moritani & Muro, 1987; Christova & Kossev, 1998) necessary to maintain or increase the required level of force (Bigland-Ritchie, & al., 1983). After the endurance point, EMG amplitude has been shown to fall (Stephens & Taylor, 1972; Komi & Tesch, 1979; Bigland-Ritchie & al., 1983; Moritani, & al., 1986) depending on the task to be accomplished (Dideriksen, & al., 2010). The occurrence of fatigue during maximal and submaximal contractions is reflected also in a spectral shift towards lower frequency bands (Piper, 1912; Viitasalo & Komi, 1977; Arendt-Nielsen & Mills, 1988; Moritani, & al., 1986)

even if the underlying physiological mechanisms (Bigland-Ritchie, 1981; Solomonow, & al., 1990; Hagg, 1992; Vøllestad, 1997; Dimitrova & Dimitrov, 2003) has not been clarified yet (Karlsson & Gerdle, 2001; Farina, 2008). However, amplitude and spectral parameters do not change only afterwards the occurrence of muscular fatigue but their variation depends also on force production. The relation between the sEMG amplitude and the muscular force has been demonstrated to be linear during isometric contractions in both non-fatiguing (Inman, & al., 1952; Milner-Brown & Stein, 1975) and fatiguing protocols (Edwards & Lippold, 1956; BiglandRitchie, & al., 1983). This linear trend could be due either to the intrinsic nature of EMG signal as the summation of motor unit action potentials (MUAP) which, at higher contraction levels are highly overlapped (Person & Libkind, 1970; Milner-Brown & Stein, 1975), or to the uniformity of fiber composition of the analyzed muscles (BiglandRitchie, & al., 1983). It has been also proposed that the changes occurring during fatiguing contractions, related to the twitch force of single motor unit and the shape of action potentials, may alter the EMG-force relation during fatiguing contractions, making it non-linear (Fuglevand, & al., 1993; Carpentier, & al., 2001; Dideriksen, & al., 2010). It is however difficult to define a correct trend for the EMG-force relation, due to the high dependency of fatigue on the performed tasks. Experimental studies have demonstrated that, during fatiguing conditions, RMS-EMG increases linearly with force (Moritani & Muro, 1987; Bilodeau, & al., 2003), while novel computer simulations have led to an overestimation of muscle force from EMG amplitude indicators (Dideriksen, & al., 2010), leaving the question quite unsolved. On the contrary, EMG-force estimates during dynamic contractions are misleading due to the effects of the variation of muscle length and contraction velocity during the motor task, leading to

the necessity of combined experimentally and modeling conditions to achieve correct predictions (Staudemann, & al., 2007).

Due to the lack of an unique and clear behavior shown by the above-mentioned indicators during fatiguing contractions, some authors proposed different methods for the classification of muscular status during static or quasi-static motor tasks, relying on the use of temporal and spectral features simultaneously (Luttmann, & al., 2000; Lin, & al., 2004; Potes, 2009). Nevertheless, the previous studies did not assess the behavior of the proposed parameters with respect the variations of factors such as biomechanical load or force production mechanisms in a systematic way.

Starting from this point, this chapter aims at evaluating if a bi-dimensional approach can facilitate the assessment of the electrical signs of neuromuscular fatigue. To this aim, data recorded during a fatiguing protocol were processed to extract temporal and spectral parameters that were then used individually or combined in a bi-dimensional space (firstly following the JASA approach and then in the new proposed space) to clarify the existence of a more effective methodology in the revelation of signs of muscular fatigue.

MATERIALS AND METHODS

Participants - Thirteen subjects (9 males, age: 29 ± 5 yr., and 4 females: 34 ± 7 yr) volunteered for the study. They were informed about the aim of the study and possible risks and they agreed by means of a written informed consent. All participants did not report previous history of knee pathology or surgery. The study was carried out in accordance with the Declaration of Helsinki.

Experimental Protocol - Participants were seated on a leg-extension machine (Leg Extension ROM, Technogym) in an upright position, and the axis of the knee was identified by aligning the axis of rotation of the moving machine arm with the lateral femoral condyle. Subjects came in the laboratory for three times. The first visit was dedicated to the familiarization with the instrumentation in the laboratory and to the determination of the maximal tolerable load (ML) that the subjects were able to sustain for 5 seconds with the leg fully extended. In the exercises performed in the following visits, a fixed percentage of this ML was set i.e. 20% and 70% respectively. From now on, in this paper we will refer to Low Intensity Exercise (LIE) for the session conducted at the 20% of ML and to High Intensity Exercise (HIE) for the session conducted at the 70% ML. The task, the same for both experimental sessions (Figure 1), consisted of a sequence of 10 dynamic knee extensions repeated until voluntary exhaustion. In order to evaluate the progression of fatigue each pair of consecutive dynamic flexion-extension sequence was spaced by a 7-seconds isometric contraction with the knee in a fully extended position. A 5-seconds rest period was allowed between isometric and dynamic contractions (Fig. 1). In order to completely recover from the fatiguing protocol, at least 3 days of rest were allowed between two consecutive visits to the laboratory. Moreover, in order to avoid any kind of muscle soreness, subjects were asked not to perform any intensive physical activity in the days before each experimental session.

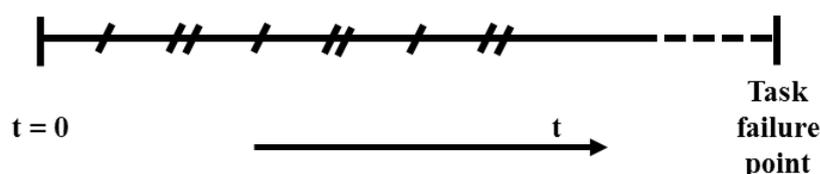


Figure 19: Schematically representation of the protocol. The single slash indicates the isometric contraction, while the double slash indicates the dynamic bout (1 bout = 10 dynamic knee extensions). This sequence is repeated until the task failure point is approached.

Electromyographic data - sEMG data were recorded from three of the quadriceps muscles of the right leg: Rectus Femoris (RF), Vastus Lateralis (VL) and Vastus Medialis (VM), throughout the entire exercise duration, by means of a wireless system (FREEEMG 300, by BTS Bioengineering S.p.A.). Data were sampled at 1000 samples/s, digitized via a 14 bit AD converter and stored for post-processing. Before placing the sensors, the skin was shaved and cleaned in order to decrease the electrode/skin impedance. Pre-gelled Ag/AgCl surface electrodes (ARBO, Kendall, Neustadt/Donau, Germany), were placed over the surface of the muscle following the European Recommendations for Surface Electromyography (Hermens, & al., 2000).

sEMG data Processing - All the recorded data were analyzed off-line and processed with custom-written codes in Matlab environment (MATLAB, Natick, MA). sEMG recordings, for each muscle, were band-pass filtered in the range [20-450] Hz by means of a 3rd order Butterworth digital filter. The processing for movement artefacts removal was accomplished with (Conforto, & al., 1999). The SNR-independent detector, proposed by (Severini, & al., 2012), was then used to isolate the isometric contraction bursts from the rest of the signal: in particular, the first and the last isometric bursts were considered for the further analysis (Mannion & Dolan, 1996; Ebenbichler, & al., 1998; Pincivero, & al., 2006).

Features Extraction - For both load conditions (LIE and HIE), the Root Mean Square (RMS) was calculated with a moving window of 0.25 s and then time-averaged within the burst period, thus obtaining a single amplitude value, **EA**. The power spectral density was also estimated using an autoregressive model of order $p = 10$ and a rectangular window (Farina & Merletti, 2000) and then the mean frequency **F** was calculated as the ratio between the first

order moment and the energy of the signal (Conforto & D'Alessio , 1999). In this way, for each burst a pair of parameters was available and was graphically represented as a point on the Cartesian plane with coordinates $\{EA, F\}$, where EA represents the amplitude and F the mean frequency of the burst of the sEMG signal, by averaging the trend of each indicator over each burst.

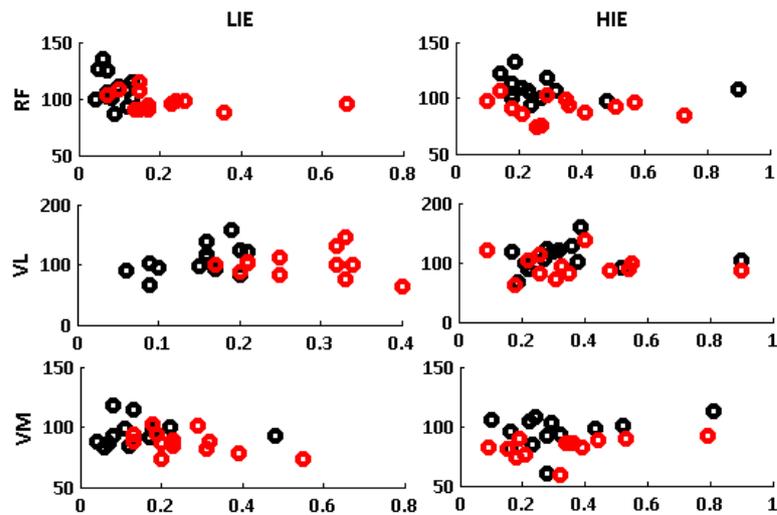


Figure 20: $\{EA, F\}$ points for all subjects and all muscles for both experimental sessions (LIE in the left column and HIE in the right one). Black circles represents the initial condition while red ones represents the end.

The final parameters $\{EA_e, F_e\}$ were later normalized with respect to the beginning values $\{EA_i, F_i\}$ (Mathur, Eng, & MacIntyre, 2005), obtaining $\{\Delta EA, \Delta F\}$, and then expressed as percentage values $\{\Delta EA\%, \Delta F\%\}$ (Luttmann, & al., 2000).

$$\begin{cases} \Delta EA\% = \frac{EA_e - EA_i}{EA_i} * 100 \\ \Delta F\% = \frac{F_e - F_i}{F_i} * 100 \end{cases}$$

Equation 3: Normalized values for EA and F

The new fatigue indicator: the fatigue vector

In order to assess the improvements in the fatigue analysis that we think may occur while using a combination of parameters, each of the $\{\Delta EA\%, \Delta F\%\}$ points was expressed in the polar domain. Thus, we obtained a vector, the '*fatigue vector*', which will be characterized by means of its magnitude ρ and phase ϑ , calculated accordingly to the following equations:

$$\rho_i = \sqrt{\Delta EA\%_i^2 + \Delta F\%_i^2}$$

$$\vartheta_i = \frac{2 * \text{atan}\left(\frac{\Delta F\%_i}{\rho + \Delta EA\%_i}\right)}{\pi} * 180$$

Equation 4: magnitude and phase of the '*fatigue vector*' representative of the $\{\Delta EA\%, \Delta F\%\}$ point in the polar domain.

In particular, the magnitude ρ takes into account the simultaneous variation of $\Delta EA\%$ and $\Delta F\%$ and so quantifies how much the muscular status is varying throughout the motor task. ϑ instead, takes into account how and with which weight each indicator contributes to the overall fatigue. Thus, two vectors having same magnitude ρ but different values of ϑ will exhibit different weight of the involved parameters, e.g. amplitude increases more than fatigue drops or vice versa, leading to different physiological implications.

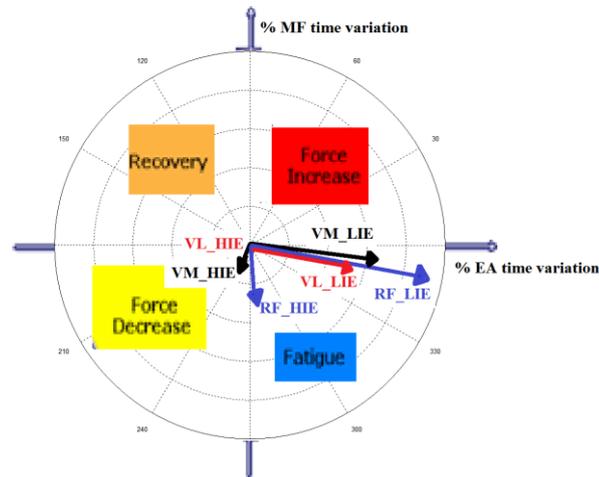


Figure 21: Example of how the fatigue vectors are distributed in the polar domain.
From Conforto, et al., 2014 © Springer International Publishing Switzerland 2014

Statistical analysis - All extracted features underwent descriptive statistical analysis. Normal distribution of extracted features was assessed by means of a Lilliefors test. Both parameters EA and F, for the three muscles (RF, VL and VM), were initially compared between the phases of the exercise ('*phase*': beginning vs. end) for the two biomechanical load conditions tested ('*load*': LIE vs. HIE) with a Wilcoxon rank sum test. .

Also the characterization of the normalized indicators ($\Delta EA\%$ and $\Delta F\%$) and of the new parameters (ρ and θ), was performed using a non-parametric statistic test (Wilcoxon rank sum test).

RESULTS

All subjects completed both test sessions. In particular we recorded 24 ± 14.2 (mean \pm standard deviation) isometric bursts for the LIE session and 4.2 ± 1.1 for the HIE one.

Characterization of single parameters - In TABLE II mean \pm standard deviation values exhibited from EA and F at the beginning and at the end of the exercise for both LIE and HIE are listed. The amplitude of the sEMG signal tends to increase in all muscles during LIE (RF, $P=0.002$; VL, $P=0.0001$; VM, $P=0.0003$) and HIE, but in this case the variation was not significant. The starting level of amplitude is however greater during HIE, depending on the higher force requirements (RF, $P=0.00003$; VL, $P=0.00006$; VM, $P=0.0001$). This behavior was not consistent at the end of the exercise.

Unlike amplitude, the higher force requirement does not affect F since the starting levels were not different, except for RF ($P=0.04$). The frequency indicator tends to drop during both LIE and HIE, but variations were not significant, except for RF during HIE ($P=0.005$).

TABLE II
MEAN \pm STANDARD DEVIATION ACROSS SUBJECTS FOR EA AND F
AT THE BEGINNING AND AT THE END OF THE EXERCISE, FOR THE TWO LOAD CONDITIONS AND FOR ALL MUSCLES.
ALSO p VALUES COMING FROM T-TEST HAS BEEN REPORTED
†n.s, * $P<0.05$, ** $P<0.001$

<i>Muscles</i>	<i>EA</i>			<i>F</i>			<i>Load</i>
	IN	END		IN	END		
RF	0.1 \pm 0.05	0.3 \pm 0.2	*	104.3 \pm 15.4	95.5 \pm 6.7	†	LIE
	0.4 \pm 0.2	0.5 \pm 0.3	†	104.5 \pm 11.8	89.2 \pm 10.8	*	HIE
VL	0.2 \pm 0.05	0.3 \pm 0.08	**	104.4 \pm 21	100.4 \pm 18.3	†	LIE
	0.4 \pm 0.3	0.5 \pm 0.3	†	105.5 \pm 21	92.5 \pm 18.2	†	HIE
VM	0.1 \pm 0.05	0.3 \pm 0.08	**	93 \pm 8.2	86.5 \pm 7.9	†	LIE
	0.4 \pm 0.2	0.5 \pm 0.2	†	91.9 \pm 13.4	81.2 \pm 9.5	†	HIE

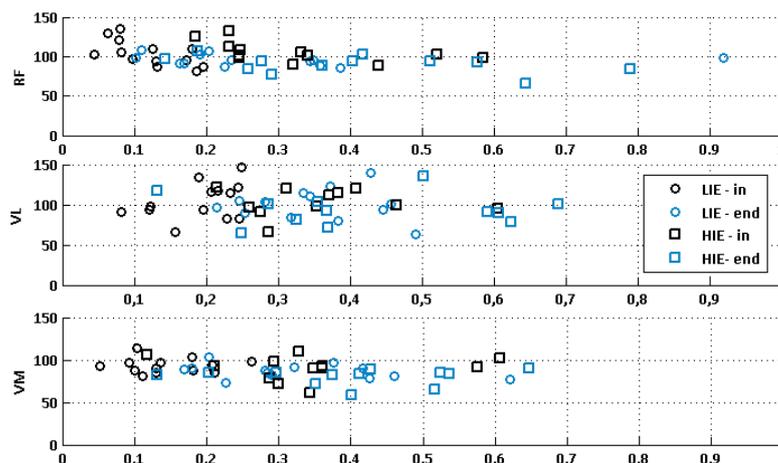


Figure 22 – {EA, F} points for all subjects and all muscles for both experimental sessions (LIE, ‘circles’ and HIE ‘squares’) at the beginning (‘black’) and at the end (‘blue’).

Normalized Parameters - $\Delta EA\%$ was different when compared among loads in all muscles (RF, $P=0.001$; VL, $P=0.001$; VM, $P=0.0005$), while $\Delta F\%$ did not exhibited significant variations for all muscles (TABLE III).

TABLE III

NORMALIZED PARAMETERS $\Delta EA\%$ AND $\Delta F\%$ EXPRESSED AS MEAN \pm STANDARD DEVIATION FOR THE TWO LOAD CONDITIONS AND FOR ALL MUSCLES.

† n.s, * $P<0.05$, ** $P<0.001$

<i>Muscles</i>	<i>$\Delta EA\%$</i>	<i>$\Delta F\%$</i>	<i>Load</i>
RF	139.3 \pm 103.2	-6.9 \pm 11.3	LIE
	26.1 \pm 46.5	-14.5 \pm 7.3	HIE
VL	100.4 \pm 86.4	-3.27 \pm 7.4	LIE
	14.2 \pm 39.7	-11.9 \pm 7.1	HIE
VM	152.2 \pm 123.3	-6.7 \pm 7.3	LIE
	22.9 \pm 43.7	-11 \pm 5.9	HIE

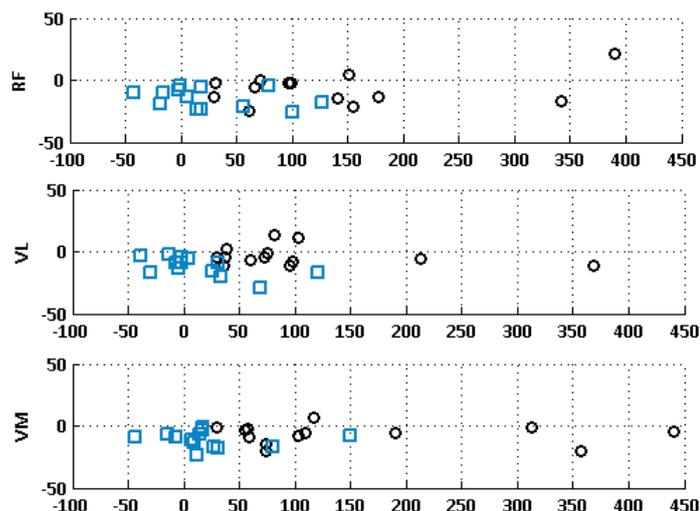


Figure 23: Normalized indicators $\{\Delta EA\%, \Delta F\%\}$ in the JASA plane for all muscles and for both load conditions LIE ('black circles') and HIE ('blue squares').

The fatigue vector characterization - The magnitude ρ of the fatigue vector was substantially reduced passing from LIE to HIE for all muscles (RF, $P=0.002$; VL, $P=0.004$; VM, $P=0.001$). In particular, the values exhibited from VL were the lowest for both LIE and HIE. Regarding to the phase ϑ , we observed that the reduction was significant for all muscles (RF, $P=0.0004$; VL, $P=0.000033$; VM, $P=0.00024$).

TABLE IV

MEAN \pm STANDARD DEVIATION VALUES FOR ρ AND ϑ ; T-TEST RESULTS,
† n.c, * $p < 0.05$, ** $p < 0.001$

<i>Muscles</i>	ρ		ϑ			
	<i>LIE</i>	<i>HIE</i>	<i>LIE</i>	<i>HIE</i>		
RF	140.24 \pm 102.8	43.57 \pm 35.8	*	-5.7 \pm 7.7	-69.5 \pm 52.7	**
VL	100.89 \pm 86.3	33.16 \pm 30.4	*	-3.1 \pm 6.5	-85.9 \pm 56.6	**
VM	152.74 \pm 123.1	36.60 \pm 36.1	*	-3.9 \pm 4.6	-57.7 \pm 53.1	**

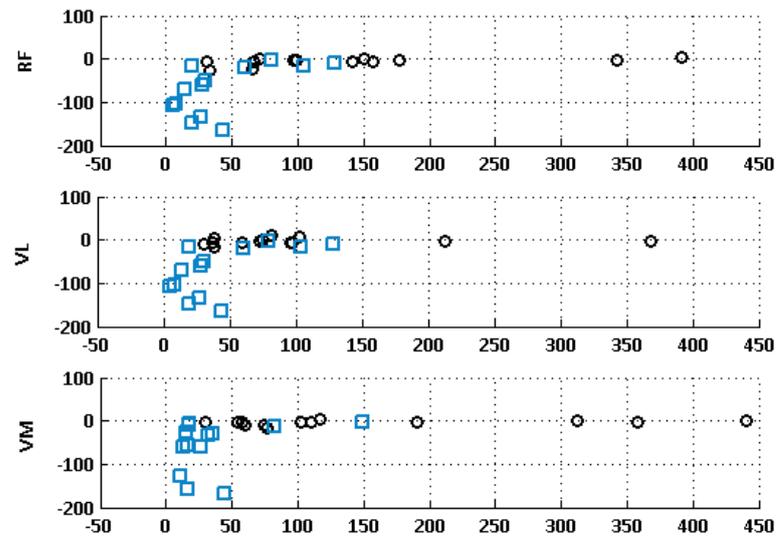


Figure 24: Fatigue vector characterization for all muscles and for both load conditions LIE ('black circles') and HIE ('blue squares').

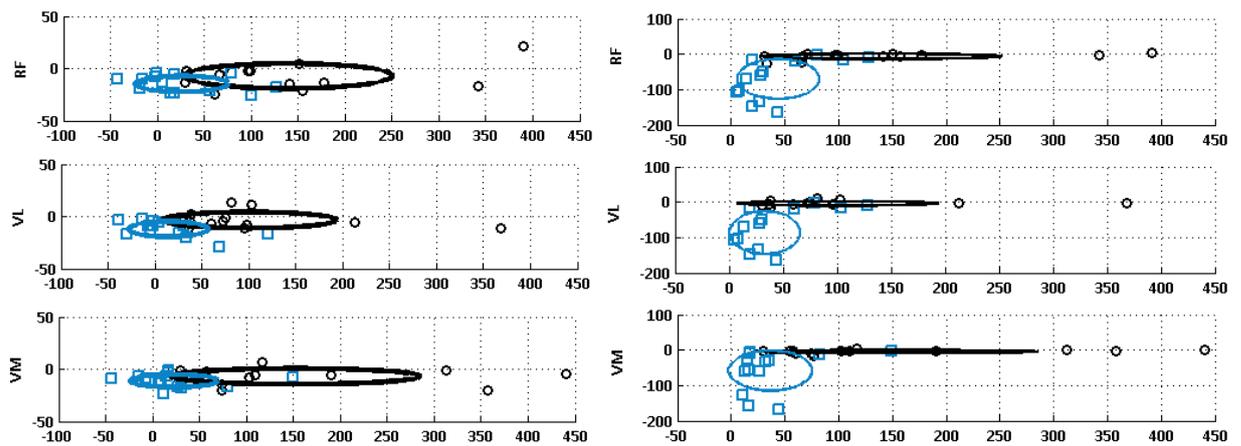


Figure 25: Clustering ellipses for normalized parameter ($\Delta EA\%$, $\Delta F\%$) distributions and for fatigue vector indicators (ρ and θ). Black lines represents the LIE condition and blue lines the HIE one.

DISCUSSION

The major goal of this study was to investigate the occurrence of localized neuromuscular fatigue during a leg extension task performed under two different biomechanical loads, by

means of a bi-dimensional electromyographic analysis of quadriceps main muscles RF, VL and VM. Three electromyographic measures have been extracted to determine a fatigue pattern of all three muscles: initial and final amplitude (EA) and mean frequency (F), normalized percentage amplitude (ΔEA %) and mean frequency (ΔF %) and magnitude (ρ) and phase (θ) of the novel fatigue vector. These measures were derived for all muscles in order to have a complete characterization of the synergistic action which determines the knee extension. As reported also in other studies, the fatigue pattern displayed by RF, VL and VM is not the same. Ebenbichler and colleagues (Ebenbichler, & al., 1998) reported a decline of the amplitude indicator for the RF for a task performed at 70% of MVC, outcome of a dualistic synergy among mono- and bi-articular muscles in a simple mono-articular fatigue task. Also other studies reported a different behavior of RF with respect to VL and VM because of the differences in the distribution of fiber types. A greater proportion of type I fibers (more fatigue resistant) in VM with respect to VL and of type II (fast fatigable) has been demonstrated by histochemical studies to occur in RF (Edgerton, & al., 1975; Rainoldi, & al., 2001). In particular, type I fibers are recruited when the required force is low, while for higher contraction levels, type II fibers are recruited to maintain the target force (Yoneda, & al., 1986). In our study, the dichotomy behavior between mono- and bi-articular muscles was not so enhanced. The EA was significantly higher for all muscles during LIE but not during HIE. This result is accordance with study from (Fallentin, & al., 1993), stating that the motor unit recruitment patterns during submaximal isometric contractions are influenced from the contraction level and thus from required force. In particular, during prolonged low level isometric contraction the EMG amplitude tends to increase due to an increased number of fresh motor units recruited. The differences in starting activation level between LIE and HIE

could be thus ascribed to different recruitment strategies, since for the LIE task the required level of force is very low and there is no necessary to recruit fiber as type II, which are fundamental during HIE for the higher force target to be maintained. As the task approaches the breakdown point the amplitude of the EMG increases for all the muscles. The contractile failure is compensated by an increase in neural drive to muscles, which is reflected in the sEMG signal into an increase of amplitude (i.e. increase in summation of motor unit action potentials) (BiglandRitchie, & al., 1983, 1986). The difference between initial and final phases during HIE resulted not significant, maybe due to the lowered number of repetitions necessary to determine the failure point, given such a high force level.

No differences in starting level between LIE and HIE were found for the F indicator. The drop of frequency was significant just for the RF in the HIE condition ($P < 0.05$). These results are in accordance with that of (Ebenbichler, & al., 1998) and (Mathur, & al. 2005) who reported a significant shift of EMG spectrum in the lower frequency band larger for bi-articular muscles with respect to mono-articular ones. This shift in EMG spectrum has also been correlated to the proportion of a certain type of muscle fiber and relative occupied area. For example (Karlsson & Gerdle, 2001) reported a negative correlation between RMS and type I fiber area, and a positive one between F and type IIa and IIc fiber area. Since in our study, a histochemical integration was not expected, we cannot speculate on the physiological mechanisms underlying spectral compression of EMG.

Since an analysis on single parameters was not sufficient to classify the occurrence of fatigue we normalized the indicators extracted from sEMG signals to their correspondent initial values and we analyzed them. In this case we reported a significant sensitivity of EA with

respect to the biomechanical load ($P < 0.05$) which was not showed from F. This procedure is in accordance with hypothesis of (Mathur, & al., 2005) but our results displayed a higher value (in absolute terms) of $\Delta EA\%$ during LIE for all muscles and of $\Delta F\%$ during HIE.

The main result of this work however concerns the use of a bi-dimensional analysis in order to discriminate different fatigue status. We have introduced the fatigue vector and characterized it in terms of magnitude and phase obtained from a transposition in polar coordinates. As detailed in the Materials and Methods section, the meaning of these two parameters is different and helpful in fatigue studies since they permit a global view about the phenomenon, including both temporal and spectral domains. The magnitude ρ is the square sum of amplitude and frequency indicators, that is, it takes into account the simultaneous variation of EA and F indicators, but not the direction of the vector, which is in charge of the phase ϑ , which defines the direction. Thus, the fatigue vector phase permits to discriminate among different muscular status (e.g. force increase, force decrease, fatigue and adaptation) and, depending on its magnitude to state how much it belongs to a particular one. As can be noticed from our results, the use to the bi-dimensional analysis permits to better characterize the influence of different force levels on the type of muscular fatigue developed and on different muscular status. Both magnitude and phase showed significant differences among LIE and HIE for all muscles ($P < 0.05$). The magnitude of the vector was higher for VM during LIE but for RF during HIE. In particular, the magnitude decreased for all muscles during HIE, maybe due to the lower number of repetitions necessary to reach the failure point. We can speculate that different values of magnitude among LIE and HIE could be indicators of different types of developed fatigue: the first, during LIE, more central and more importantly due to a slow reduction of motor drive from the CNS (Bigland-Ritchie, &

al., 1983, 1986). The second, during HIE, more peripheral, quicker and maybe due to a failure in mechanisms of electrical transmissions, given the high force requirement. This is reflected also in the variation of the ϑ indicator. The absolute value tends to increase considerably from LIE to HIE, defining a strong contribute of F more than EA. This could mean that the load, i.e. the different force requirements may not influence the F alone, but has a noteworthy effect on fatigue evaluation when both indicators are considered together.

CONCLUSIONS

An analysis of neuromuscular fatigue based on electromyographic signals have been prevalently based on two features extracted one from the temporal and one from the spectral domain, most of the time considered separated. The findings of this study suggest that this kind of analysis can be strengthened by using a bi-dimensional parameter, expressible in terms of magnitude and phase, obtained from amplitude and mean frequency of the sEMG signals, considered jointly, normalized and transformed in polar coordinates. The results have demonstrated that by means of the bi-dimensional parameter it has been possible to determine the influence of the different force levels on the fatigue development. Further studies are needed to test the reliability of this analysis in discriminate other muscular status and its application in a dynamic context.

CHAPTER 5:

Increases of common synaptic input to motoneurons during fatigue

This chapter aims at investigating the physiological mechanisms underlying the occurrence of neuromuscular fatigue focusing, in particular, on motor unit response during submaximal isometric contraction of the Tibialis Anterior muscle held at three different force levels. Several studies have been conducted in the last century concerning the phenomenon. However, all of them show some limitations. First of all, the literature around the topic is quite unclear and does not define both a clear and unique behavior for MU during the occurrence of fatigue and the causes that lead to the drop of force during prolonged sustained contractions. Another limitation can be found in the recording technique mostly used such as the invasive EMG, which leads to an observation of really few motor units. Furthermore, this kind of technique is very difficult to apply in experimental sessions including high contraction level and fatigue manifestations.

In this chapter a technique for motor unit extraction from surface EMG recordings decomposition is applied to overcome the classical limitations. Preliminary results of this

work have been presented at the Annual Meeting of the Society for Neuroscience (SfN) held in San Diego in November 2013¹.

INTRODUCTION

The human central nervous system (CNS) drives motor neurons causing a common modulation in the firing of these neurons (Myers, Erim, & Lowery, 2004) and EMG recordings can be used to reflect this common modulation of MU within muscles (Myers, & al., 2003; Semmler, & al., 2013). The common modulation of motor unit discharges can be quantified by different techniques in the temporal domain via peaks of the cross-correlation histogram (Sears & Stagg, 1976; Contessa, & al., 2009) normalized with different indexes such as Common Input Strength (CIS) (Nordstrom, & al., 1992) or in the spectral domain by means of coherence analysis (Rosenberg, & al., 1989; Farmer, & al., 1993). In particular, these two measures of correlation, i.e. synchronization and coherence, even if related to the same physiological event, concerns different features of the same input shared by motoneurons (Semmler, & al., 2003). Synchronization is a measure of the power of common input via corticospinal path (Farmer, & al., 1993). Coherence, instead, quantifies the strength of common oscillatory input to motoneurons originated either in the motor cortex or in the brainstem (Conway, et al., 1995; Baker & Baker, 2003). Thus, coherence tends to emphasizes only those frequencies where there is a constant phase relationship between two signals (McClelland, & al., 2012). In particular, the discharge rates of motor units resonate in particular frequencies ranging from 0 up to 30-40 Hz. The first, spanning from 0 to 3-4 Hz

¹ Castronovo AM, Negro F., D'Alessio T., Farina D. Coherence between motor unit spike trains increases during submaximal fatiguing contractions of the Tibialis Anterior muscle, *Proceedings of the Annual Meeting of the Society for Neuroscience*, San Diego, California, USA, November 8-13th, 2013.

(Myers, & al., 2004; Christou, & al., 2007), usually termed '*common drive*', refers to a common excitation to the motoneuron pool responsible for concurrent fluctuations in the firing rates of the pool (Myers, & al., 2004). The second frequency interval ranges from 5-8 up to 12 Hz and represents a neurogenic oscillations with spinal and supra-spinal components related to the rhythmic activity of motor unit (Vallbo & Wessberg, 1993; Farmer, 1999; Groß, et al., 2002) and also physiological tremor (Conway, & al., 1995). Finally, a corticospinal modulation in the 15-30 Hz (beta band) mainly driven by the presynaptic activity from motor cortex (Farmer, & al., 1993) with less influential contributions from supplementary and premotor cortices (Grosse, & al., 2002) has been noticed. The variations in the firing of motor units are believed to be responsible for the increase in force fluctuations observed during sustained fatiguing contractions (Taylor, & al., 2003; Contessa, & al., 2009). In general, two are the main mechanisms responsible for force generation and gradation depending on the main role of the muscle involved: progressive recruitment of motor units from the smaller to the larger ones according to Henneman's size principle (Henneman, 1981) and the modulation of the firing rate of the active motor units referred to as '*rate coding*' or '*neural coding*' (Fuglevand, & al., 1993). In small muscles required for precision in movements motor units are fully recruited at relatively low force levels so that neural coding is the main mechanism responsible of force gradation. In larger muscles, instead, recruitment process operates over most of the force range since neuronal excitability thresholds are largely distributed (Fuglevand, & al., 1993). During fatiguing contractions, in order to compensate for the loss of force more motor units are recruited as also observed by (Contessa, & al., 2009). Edwards et al. proposed that the reduced endurance time noticed during electrically elicited contraction of the quadriceps femoris at 15-30% MVC was caused by an increase in

low frequency fatigue determined by a decrease in discharge frequency of the already active motor units (Edwards, & al., 1977; Dundon, & al., 2008). Due to a decrease in discharge the compensation for force loss may be due to an increase in recruitment process (Dartnall, & al., 2009). The degree of correlation between motor unit's activity and force increases with the size of the motor unit and decrease with the number of active motor units in the muscle (DeLuca, & al., 1982). As soon as the force level increases, the motor unit recruitment and the firing rate increase and the discharges become more synchronized (Milner-Brown & Lee, 1975). Furthermore, this phenomenon is more evident during fatigue (Perry & Bekey, 1981). Recruitment and neural coding are not the only mechanisms influencing the force output and its decrease of steadiness during an isometric contraction: some authors have referred also to the synchronization between motoneuron discharges (Taylor, 1962; Laidlaw, & al., 2000; Yao, & al., 2000; Faisal, & al., 2008) even if this process is still under debate (Semmler & Nordstrom, 1998; Semmler, & al., 2000; Semmler, & al., 2002; Contessa, & al., 2009). However, up to now, there are no studies in literature focusing on the influence of synchronization between motor unit discharges during fatigue and under different force levels. Thus this chapter aims at evaluating if an increase in the excitability of motoneurons due to fatigue lead to an increased synchronization between motor units and in turn of synaptic input. In fact, an increase in neuronal excitabilities should lead to an increase recruitment of motor units throughout the entire contraction in order to maintain the force level. Increasing the number of motor units, then, the common synaptic input should increase up to a saturation level. An ad hoc experimental protocol has been designed to accomplish the above mentioned goals. In particular the fatiguing exercises will be performed at three different contraction levels

MATERIALS AND METHODS

Participants - 10 healthy males (age: 31.6 ± 3.4) volunteered for the study. No one of the participants reported previous history of knee or ankle pathology or surgery. An informed written consent approved by the Ethical Committee of the Universitätmedizin Göttingen was signed by all the subjects before participate the experiments.

Experimental Protocol – Participants accomplished a motor task consisting of isometric contractions of the Tibialis Anterior (TA) muscle, held up until voluntary exhaustion, at three different force levels: 20%, 50% and 75% of the Maximal Voluntary Contraction (MVC) produced by each subject. The tasks, performed in separate and not consecutive days were also randomized in order. At the beginning of each experimental session participants were asked to progressively increase their ankle dorsiflexion force up to their maximal and sustain it for 3 s for three times. Verbal encouragements were also given to subjects. The maximum among the three was taken as reference value for MVC. The MVC was also repeated after the end of the isometric contraction in order to verify the occurrence of muscular fatigue. Participants were provided with a visual feedback of force during the execution of each trial. This feedback consisted in a bold red line of 60 s in duration displayed on a monitor placed in front of the subjects at a distance of nearly 1,5 m. A black cursor moving left-to-right over the course of the red line represented the level of force exerted by the subject. Participants were instructed to follow force trajectories by apply isometric ankle dorsiflexion force on the transducer (Tracy, 2007).

Force and EMG recordings – Participants were seated in an upright position on a force dynamometer with the right leg fully extended and with the foot restraint onto a force

transducer (Biodex Multi Joint System 3, Biodex Medical systems, Shirley, NY). The angle of the ankle was nearly 20° with respect the reference system (0°) identified in the ideal full dorsiflexion of the ankle joint. The leg was fastened to the machine by means of Velcro straps in order not to alter the position of the ankle throughout the entire task. Force measures were normalized to percent MVC recorded for each subject and each contraction level. Force signals were sampled at 5000 samples/s. Surface EMG signals were recorded from the TA muscle through a semi-disposable adhesive matrix with 64 channels with 8 mm of inter-electrode distance (OT Bioelettronica, Torino, Italy) attached on the surface of the muscle with adhesive foam. The skin was shaved and lightly abraded with abrasive paste (Meditec-Every, Parma, Italy) and cleansed afterward before placing the electrodes. The EMG signals were amplified (EMG-USB, LISiN - OT Bioelettronica, Torino, Italy), sampled at 2048 samples/s and digitized via a 12 bit A/D converter. Data recorded were analyzed off-line using custom written codes developed in MATLAB environment (Mathworks, Natick, MA) in order to compare the non-fatiguing phase of each contraction with the fatiguing one, we focused on the first 25 s and the last 25 s before the drop of the force of each signal corresponding to each contraction level.

Force Data Analysis - Force traces were low pass filtered with a 4th order Butterworth filter with a cutoff frequency of 20 Hz. Data were then down-sampled at 2048 Hz to synchronize them with EMG traces. The coefficient of variation of force (CV-Force) was calculated as the ratio between the standard deviation and the average of the first 10 s and the last 10 s of the signal within a moving window of 1s.

sEMG Data Analysis - sEMG signals were band-pass filtered in the range [20-500] Hz with a 4th order Butterworth filter and then the amplitude was estimated by means of root mean square index. sEMG signals were also decomposed into MU spike trains using a state-of-the-art technique developed by Holobar et al. (Holobar & Zazula, 2007). This algorithm relies on blind source separation and guarantees an accuracy of about 84%-87% in the identification of motor unit discharge in TA muscle (Holobar, & al., 2010). Each identified motor unit was also visually inspected and inter-spike intervals longer than 250 ms and shorter than 20 ms were removed. Spike trains of single motor units were then obtained with a sampling frequency of 1000 samples/s and then the discharge rate (DR) was computed: the instantaneous firing rate of each of the decomposed MU was smoothed using a 1 s Hanning window, and then averaged across the pool in order to characterize the global discharge rate (DR). The degree of correlation and synchronization between spike trains was calculated by means of coherence analysis, computed using the MATLAB function *mscoherence* on composite spike trains (CST), intended as the linear sum of single spike trains (Negro & Farina, 2011, 2012). Values obtained (magnitude squared coherence values (C)) were then converted to Fisher's z values (FZ) and then to standard z scores (Z) by using the following formula, according to (Amjad, & al., 1997; Rosenberg, & al., 1989):

$$Z = (a \tanh(\sqrt{C})) / (1/2L) = FZ / (1/2L)$$

where L is the number of segments used to estimate the coherence. From each coherence profile was then subtracted an intrinsic bias, determined empirically as the maximum value of coherence for frequencies higher than 100 Hz because no significant correlated activity exists in this range (Baker, & al., 2003). Coherence values exceeding 95% confidence level in

each of the three frequency bands of interest were considered significant. Finally, each average coherence profile was integrated to obtain an index of total shared input over all frequencies and a Student's T test was used to assess if integrated coherence was affected by fatigue.

Statistics - Only the first 10 s ('Beginning') and the last 10 s ('End/Fatigue') of the contraction for all force levels underwent descriptive statistical analysis. An initial Lilliefors test was performed to check if the considered variables were normally distributed. Then, a Student's paired t-test was used to quantify the decline in MVC, and the changes induced by fatigue onto the CV-Force, DR and z-coherence values. A non-parametric Wilcoxon rank sum test was implemented to assess the sensitivity of RMS to fatigue for all contraction levels. A one-way analysis of variance (ANOVA) was performed to evaluate the influence of the increasing contraction level on force variability, DR and z-coherence, both at the beginning and at the end of the contraction. A Kruskal-Wallis analysis of variance was also performed on RMS features to check the sensitivity to increasing contraction level. Values were considered significant if the resulted P values were less than 0.05.

RESULTS

Maximal Voluntary Contraction - The maximal voluntary contraction decreased significantly for all subjects and for all contraction levels due to fatigue. In particular, it dropped of about 18% at 20% MVC (P=0.0004), of about 19% at 50% MVC (P=0.00003) and of about 10% at 75% MVC (P=0.0001).

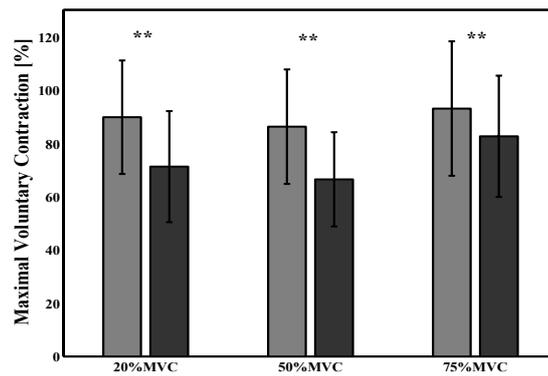


Figure 26: Maximal Voluntary Contraction for all force levels performed before (grey bars) and after (black bars) each contraction (* $P < 0.05$, ** $P < 0.001$)

Force Variability - The coefficient of variation of force (CV-Force) exhibited a significant increase at the end of the contraction. It varied from $0.7 \pm 0.2\%$ to $3.3 \pm 1.9\%$ at 20% MVC ($P = 0.004$) and from $0.8 \pm 0.3\%$ to $2.7 \pm 1.7\%$ at 50% MVC ($P = 0.01$). At 75% MVC it varied from $1.6 \pm 0.7\%$ to $1.9 \pm 1.3\%$ but this was not significant ($P = 0.22$).

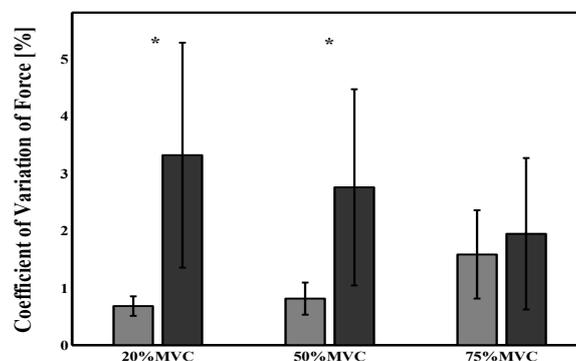


Figure 27: Coefficient of variation of force for all force levels for the first 10 s (grey bars) and the last 10 s of each contraction.

Motor Unit Discharges - The global discharge rate (DR) of the pool of decomposed motor units exhibited dissimilar trend for the three different force levels. It varied from 11 ± 0.8 pps

to 11.3 ± 3.4 pps at 20% MVC, from 14.7 ± 1.9 pps to 14.1 ± 2.9 pps at 50% MVC and from 15.8 ± 2.6 pps to 17.8 ± 4.3 pps at 75% MVC. However these did not result significant.

TABLE V

MEAN \pm STANDARD DEVIATION OF THE DISCHARGE RATE
AT THE BEGINNING AND AT THE END OF THE CONTRACTION.
†n.s, * P<0.05, ** P<0.001

CONTRACTION LEVEL	DISCHARGE RATE [pps]		
	BEGINNING	END	
20% MVC	11 ± 0.8	11.3 ± 3.4	†
50% MVC	14.7 ± 1.9	14.1 ± 2.9	†
75% MVC	15.8 ± 2.6	17.8 ± 4.3	†

Amplitude of EMG - The root mean square values increased for all contraction levels: from 0.05 ± 0.02 to 0.08 ± 0.04 at 20% MVC (P=0.02), from 0.1 ± 0.02 to 0.2 ± 0.05 at 50% MVC (P=0.01) and from 0.2 ± 0.05 to 0.2 ± 0.08 . The latter change did not result significant (P=0.06).

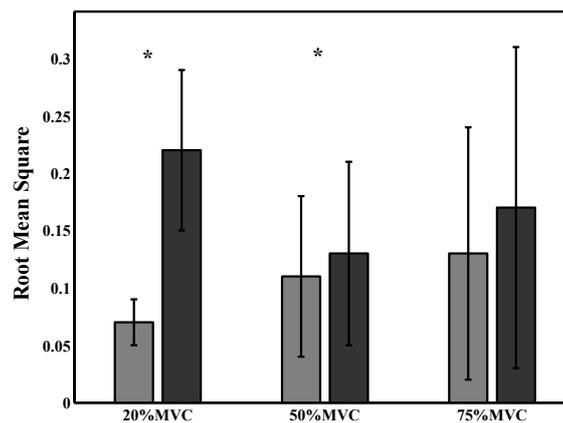


Figure 28: Amplitude of sEMG signals. (* P<0.05, ** P<0.001)

Coherence Analysis – The z-coherence computed in the range [1-5] Hz, reflecting a common modulation of motoneurons, showed an increase at the end of the contraction for all force

levels (20% MVC, $P=0.04$; 50% MVC, $P=0.0001$; 75% MVC $P=0.01$). The rhythmic activity of motor units, reflected in the [5-10] Hz band, instead, showed a significant increase only at 20%MVC ($P=0.01$). Ultimately, the z-coherence in the frequency band of the corticomuscular coupling did not show any significant change.

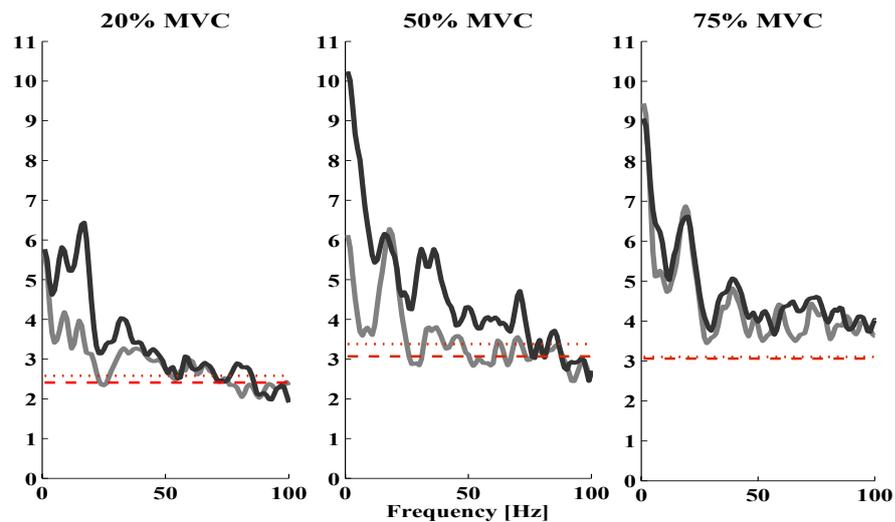


Figure 29: Z-Coherence profiles averaged across all subjects for both the beginning (grey thick line) and the end (black thick line) of the task and for all contraction levels. Lines in red represent the confidence levels for each coherence profile (dash-dot lines: beginning and dot lines: end)

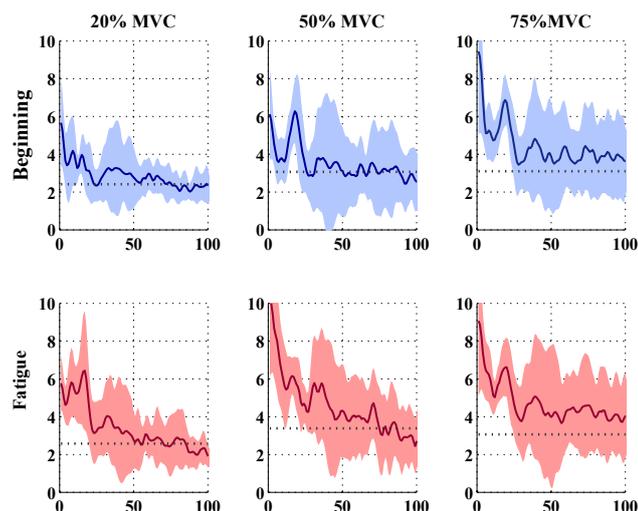


Figure 30: Z-coherence profiles depicted as mean (thick line) and standard deviation (color shade around line) for all contraction levels at the beginning and at the end of the motor task.

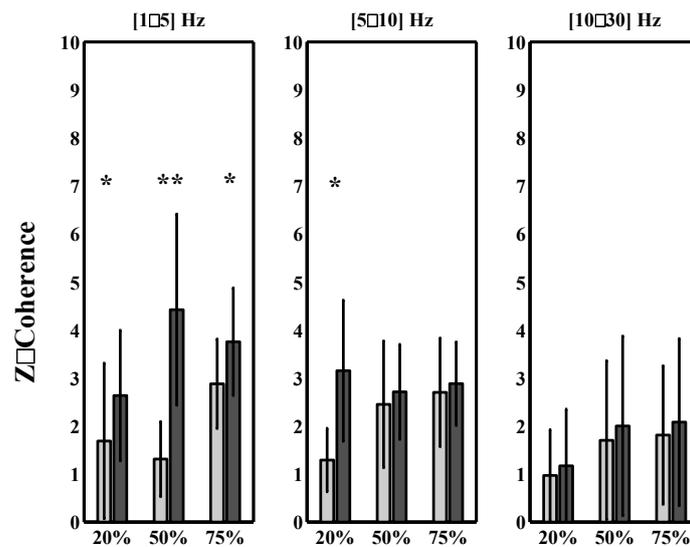


Figure 31: Histogram representation of the averaged z-coherence values in the comparison between beginning and end of the contraction in three frequency ranges for all force levels (* $P < 0.05$; ** $P < 0.001$)

DISCUSSION

The main purpose of this work was to investigate the physiological mechanisms induced by an increased net excitation to motorneurons due to the occurrence of fatigue, by coherence analysis between motor unit spike trains.

The maximal voluntary force decreased after the endurance contraction and this was interpreted as a sign of the occurrence of neuromuscular fatigue (Merton, 1954; Bigland-Ritchie, & al., 1983) for all the contraction levels tested. Together with a decrease in maximal voluntary activation, we reported an intensification of the central drive due to fatigue gathered from the increase of sEMG amplitude at the end of each motor task for all force levels tested. Our results show a significant increase of RMS-EMG in the last 10 s of the contraction for both 20% MVC ($P=0.02$) and 50% MVC ($P=0.01$) but not for 75% MVC. This increase has been reported several times in literature for studies involving fatigue and the

major cause has been attributed to the recruitment of newer and larger motor units following the Henneman's size principle (Edwards & Lippold, 1956; Viitasalo & Komi, 1977; Henneman, 1981; Petrofsky, & al., 1982; Fallentin, & al., 1993), or to the increased firing frequency (Hultman & Sjöholm, 1983) or to the synchronization of MU (Buchtal & Madsen, 1950; Jørgensen, & al., 1988; Yao, & al., 2000; Gandevia, 2001). During a submaximal prolonged contraction in fact it has been observed that motor units are recruited progressively from the smaller (producing smaller twitches of force) to the larger ones (producing instead larger twitches) until the maximal excitation threshold is reached and the pool is all recruited (Hannerz, 1974). The recruitment of more motor units and the modulation of their firing rate are the main mechanisms underlying force generation and gradation throughout an entire contraction (Fuglevand, & al., 1993). The two processes are selected depending on the main role of the muscle involved: in small muscles like the ones in the hand, involved in precise movements, motor units are fully recruited at relatively low force levels so that neural coding is the main mechanism responsible of force gradation. In larger muscles, such as the ones in the leg or in the trunk, neuronal excitabilities are largely distributed and in this case the recruitment process operates over most of the force range (Fuglevand, & al., 1993). During fatiguing contractions, in order to compensate for the loss of force, more motor units are recruited as also observed by Contessa et al. (Contessa, & al., 2009). Edwards et al. proposed that the reduced endurance time noticed during electrically elicited contraction of the quadriceps femoris at 15-30% MVC was caused by an increase in low frequency fatigue resulting in an excessive loss of force generated by the already active motor units discharging at low frequencies (Edwards, & al., 1977; Dundon, & al., 2008). According to the authors, this was the cause of an increase in recruitment and discharge rate

of the already active motor units to compensate for force loss (Dartnall, & al., 2009). In our study we observed that the increase in sEMG amplitude was not accompanied by a change in discharge frequency of motorneurons leading to the assumption that recruitment was the main process underlying the intensification of neural drive to muscle during fatigue. However, the intervention of fatigue may compress the range of motor unit recruitment during isometric contractions (Carpentier, & al., 2001). This may explain why at 75% MVC the increase of sEMG amplitude was not significant: we believe that at a high level such as 75% MVC larger motor units are recruited from the very beginning of the contraction so that the recruitment process is not that involved. The intervention of fatigue influenced also the CV of force which increased significantly for all levels except for 75% MVC where the value did not change from the very beginning of the contraction. The values increased at the end of the contraction of about 375% for 20% MVC ($P=0.004$), of about 235% for 50% MVC ($P=0.01$) and of 18% for 75% MVC ($P=0.22$). The smoothness of force that a MU exerts depends on the rate at which the motor neuron discharges action potentials (Thomas, & al., 1991; Macefield, & al., 1996). When the discharge frequency is lower than the one essential for a fused tetanus, the force exerted by the MUs will fluctuate around an average value (Christakos, 1982; Taylor, & al., 2003). However, since tetanus is hardly achieved (Enoka & Fuglevand, 2001), the force exerted by a muscle will always fluctuate due to the submaximal activation of many motor units (Taylor, & al., 2003) and this influences the performance in a task (Harris & Wolpert, 1998; Jones, & al., 2002). It has been demonstrated that as the force produced by a subject increases, the same does the standard deviation (Schmidt, & al., 1979; Galganski, & al., 1993) and this can be explained by the presence of signal-dependent noise in the input of the process of force generation whose standard deviation increases with the mean value

(Hamilton, & al., 2004; Stein, & al., 2005). This noise is linearly scaled over a wide range of motor output and results from peripheral neuromuscular noise due either to orderly recruitment or discharge variability (Jones, & al., 2002; Moritz, & al., 2005; Tracy, & al., 2005). Simulative studies have instead demonstrated that the signal dependent noise, except for low contraction levels, is due to features of control signal produced from CNS and thus, for level of forces higher than 20% MVC, differences in force variability have to be related to the stability of neural drive coming from CNS rather than to differences in synaptic noise or motor unit intrinsic properties (Dideriksen, & al., 2012). The contribution of a motor unit to the fluctuations in force changes as a function of contraction intensity (Taylor, & al., 2003) and in particular the standard deviation of the force (i.e a measure of force fluctuations) increases together with the amplitude of force (Rudroff, & al., 2005). As the level of voluntary activation increases, so does the discharge rate of motor units (Person & Kudina, 1972). Milner-Brown et al. have demonstrated that during isometric fatiguing contractions of the FDI muscle, the largest contribution of motor unit recruitment occurs at low force levels while the contribution of increased firing rate becomes more important at force levels higher than 75% MVC (Milner-Brown & Stein, 1975). The discharge rate of motor units tended to increase, decrease and keep constant throughout the entire duration of the contraction depending on the contraction level: it passed from 11 ± 0.8 to 11.3 ± 3.4 pps for 20% MVC, it decreased from 14.7 ± 2 to 14 ± 3 pps for 50% MVC and increased from 16 ± 3 to 18 ± 4 pps for 75% MVC. However, these changes did not result significant were the motor units were grouped in a pool and all the subjects were pooled together. This effect has been reported previously in literature: during fatiguing contractions, in fact, the firing rate to motor units exhibits different behaviours. Gantchev et al. reported an increase of the variability of

discharge during fatiguing contractions (Gantchev, & al., 1985) confirmed also by Garland et al. (Garland, & al., 1994). Maton et al. during a fatiguing isometric contraction of the biceps brachii at different levels noticed that for forces < 30% MVC the discharge rate could either increase, decrease or remain stable, while, for 30% MVC < forces < 75% MVC the discharge rate increased with time (Maton, 1981). Carpentier et al. found out that during the hold phase of a ramp-and-hold contraction performed by the FDI at 50% MVC, the discharge rate tended to decrease progressively for motor units activated from the very beginning of the contraction, while it firstly increased and then drop for the newly recruited ones (Carpentier, & al., 2001). Also Bigland-Ritchie et al. registered a significant decrease during fatiguing contraction performed at 50% MVC.

The degree of correlation between motor units' activity and force output increases with the size of the motor unit and decrease with the number of active motor units in the muscle (DeLuca, & al., 1982). As soon as the force level increases, the motor unit recruitment and the firing rate increase and the discharges become more synchronized (Milner-Brown & Lee, 1975). Moreover this phenomenon is more evident during fatigue (Perry & Bekey, 1981). The recruitment and rate coding mechanisms are not the only ones influencing the steadiness of force. Some authors have referred also to the synchronization between motoneuron discharges (Taylor A. , 1962; Laidlaw, & al., 2000; Yao, & al., 2000; Faisal, & al., 2008) even if this process is still under debate (Semmler & Nordstrom, 1998; Semmler, & al., 2000; Semmler, & al., 2002; Contessa, & al., 2009). Motor unit synchronization is defined as the tendency of two motoneurons to discharge with dependent times "*relative to each other more often than it would be expected if the motor units were to fire randomly but independently*" (De Luca, & al., 1993). This behaviour has been ascribed either to the common input delivered to

motorneurons in the spinal cord by the axons of the motorneurons located in the brain stem (Sears & Stagg, 1976) or to the presynaptic synchronization between efferent fibres from the motor cortex (Baker, & al., 2001). De Luca et al. postulated that all motor units belonging to one pool active during a constant force isometric contraction receive the same net excitation at any given time: any input received by an individual motor unit and not shared by the others of the pool figures out as uncorrelated noise (DeLuca, & al., 1982) (DeLuca & Erim, 1994).

The correlated discharges of motor units have been extensively examined not only in the temporal domain (De Luca, & al., 1982; Nordstrom, & al., 1992) but also in the spectral one (Rosenberg, & al., 1989). In our work we focused on the spectral domain features and in particular on the coherence spectrum of motor units spike trains pooled together. According to Christou et al. (Christou, & al., 2007) the coherence spectrum of motor unit discharges should be analysed in three different bands, each one with its own physiological meaning: [0-4] Hz, [8-12] Hz and [16-32] Hz. The first one represents the common drive to motor units that is a slow modulation of the net excitatory drive to the motorneuron pool (De Luca, & al., 1982). The second one seems to reflect the rhythmical activity of motor units in voluntary contractions (Vallbo & Wessberg, 1993; Farmer, 1999; Groß, & al., 2002). Lastly, the [16-32] Hz seems to reflect the interaction between the rhythms of the motor cortex and the spinal motorneurons (Farmer, & al., 1993) (Salenius, & al., 1997). In our study we focused on three particular frequency ranges: [1-5] Hz, [5-10] Hz, [10-30] Hz. We firstly noticed a significant increase of the coherence content in the low-frequency band for all contraction levels due to fatigue and these results are in accordance with the ones from Contessa et al. who found the common drive altered during fatiguing contractions of the Vastus Lateralis muscle

performed at 20% MVC (Contessa, & al., 2009) and partially with the ones from Gandevia who stated that the excitation delivered to motoneurons coming from supraspinal center is impaired during prolonged contractions but not during high-force fatiguing ones (Gandevia, 2001). The variations due to the increasing force level both at the beginning and at the end of the contraction were instead significant at [5-10] Hz. On the contrary the effect of fatigue was found to be significant only at 20% MVC ($P=0.01$) but not for the other levels. Erimaki et al. found tremor like oscillations in the analysis of FDI motor units ranging in the [6-10] Hz accompanied by corresponding motor unit firing synchrony. This component seems to have a supraspinal origin and to be a peripheral manifestation of rhythmical motor control (Erimaki & Christakos, 1999, 2008). In our study we noticed a significant increase of the coherence component related to tremor oscillation only for 20% MVC level ($P=0.01$) at the end of the contraction due to fatigue. These results are in accordance with the ones of Erimaki et al. who stated that the [6-10] Hz component varied widely among contraction levels but they also found that these changes did not depend on motor unit firing statistics (Erimaki & Christakos, 2008). The authors concluded that the [6-10] Hz synaptic input seemed to be a distinct component, additional to the common drive that underlies the voluntary force variations through the recruitment and neural coding mechanisms (Freund, 1983; De Luca & Erim, 1994). Ultimately we did not report any significant variation of coherence due to fatigue maybe due to a saturation of the net excitation input.

CONCLUSIONS

The main purpose of this work was to investigate the physiological mechanisms induced by an increased net excitation to motoneurons due to the occurrence of fatigue, by coherence

analysis between motor unit spike trains. We demonstrated that fatigue is the result of an increase of net excitation to motoneurons as reported by an increase of motor unit recruitment at the end of each task for all contraction levels. The manifestation of fatigue is, however, more evident during low force contractions with respect to the higher contraction levels tested where impairment in force generation and holding occurs very quickly. Further studies will be needed to test if the decrease in force steadiness observed for all contraction levels is better explained either by an increase in the variation of discharge frequencies or in the synchronization of motor units. Another step-forward in the knowledge of fatigue could be achieved by following motor units firing throughout the entire contraction duration and, in turn, by analysing the contribute of single motor unit to the overall increase in synaptic noise.

CHAPTER 6:

How to assess performance in cycling: the multivariate nature of influencing factors and related indicators

This chapter represents an example of the application of a multi-level approach in a sport context, i.e. the assessment of cycling performance. This study will highlight the lack of an actual method for the evaluation of cycling performance and aim at giving a suggestion to researchers and/or trainers for a better understanding of the motor task and of the factors that (might) influence it. Finding an optimum for the cycling performance is not a trivial matter, since the literature shows the presence of many controversial aspects. In order to quantify different levels of performance, several indexes have been defined and used in many studies, reflecting variations in physiological and biomechanical factors. In particular, indexes such as Gross Efficiency (GE), Net Efficiency (NE) and Delta Efficiency (DE) have been referred to changes in metabolic efficiency (Eff_{Met}), while the Indexes of Effectiveness (IE), defined over the complete crank revolution or over part of it, have been referred to variations in mechanical effectiveness (Eff_{Mech}). All these indicators quantify the variations of different factors (i.e. muscle fibers type distribution, pedaling cadence, setup of the bicycle frame, muscular fatigue, environmental variables, ergogenic aids, psychological traits), which, moreover, show high mutual correlation. In the attempt of assessing cycling performance, most studies in the literature keep all these factors separated. This may bring to

misleading results, leaving unanswered the question of how to improve cycling performance. This chapter provides an overview on the studies involving indexes and factors usually related to performance monitoring and assessment in cycling. In particular, in order to clarify all those aspects, the mutual interactions among these factors are highlighted, in view of a global performance assessment. Moreover, a proposal is presented advocating for a model-based approach that considers all factors mentioned in the survey, including the mutual interaction effects, for the definition of an objective function E representing the overall effectiveness of a training program in terms of both metabolic efficiency and mechanical effectiveness. This work has been published in *Frontiers in Physiology* in 2013¹.

INTRODUCTION

The optimization of athletic performance is a matter of importance for all sports and, for a specific field such as cycling, is mainly related to the measurement of time or distance. Depending on the task (i.e. sprint, track time trial, endurance), cyclists are asked either to cover a fixed distance as fast as possible or to go as far as possible in a fixed amount of time. Then a performance improvement takes place when an athlete increases her/his previous results and hopefully manages to win the race. The improvement of performance is based on “efficacious” training, which aims at the development of motor strategies, including aspects such as energy expenditure and mechanical implementation.

From the analysis of the literature focusing on the development and implementation of such kind of trainings, different points of view emerge. For instance, most of the papers dealing

¹ Castronovo AM., Conforto S., Schmid M., Bibbo D., D’Alessio T. (2013). How to assess performance in cycling: the multivariate nature of influencing factors and related indicators. *Frontiers in Physiology*, 4(116): 1-10. doi:[10.3389/fphys.2013.00116](https://doi.org/10.3389/fphys.2013.00116)

with this issue use the terms i) cycling efficiency (Coyle, & al., 1991, 1992; Chavarren & Calbet, 1999; Hansen, & al., 2002; Cannon, & al., 2007; Korff, & al., 2007, 2011; Leirdal & Ettema, 2011), ii) muscular efficiency (Whipp & Wasserman, 1969; Gaesser & Brooks, 1975; Neptune & Herzog, 1999; Zameziati, & al., 2006; Hansen & Sjøgaard, 2007; Carpes, & al., 2010), iii) mechanical efficiency (Umberger, & al., 2006; Wakeling, & al., 2010; Theurel, & al., 2012) and iv) mechanical effectiveness (Zameziati, & al., 2006; Korff, & al., 2007; Ettema, & al., 2009; Mornieux, & al., 2010) with an almost equivalent meaning. This confusion is due to the fact that the terms “efficient”, “effective”, and “efficacious” share a common etymology but, since the real meaning is slightly different (Haynes, 1999), some clarifications are needed:

- ❖ the term “*effective*” means that a method produces a decided, decisive, or desired effect (in the reality) (Merriam Webster, 2003). It focuses on whether something either achieves the required objective or has a noticeable effect (e.g. “homework is an effective mean to let a student learn the topics of the lesson”);
- ❖ the term “*efficient*” focuses on speed, ease, and convenience with which an objective is achieved: something is efficient if it works well without wasting time, money or energy (Merriam Webster, 2003). Something can be thus effective (i.e. it does the job) without being efficient;
- ❖ ultimately, the term “*efficacious*” applies to things that are used for a certain purpose, such as medicines, or treatments: something is efficacious if it has the power of producing the desired effect (Merriam Webster, 2003).

From now on we will refer to a cycling performance as the objective to be reached by using a specific training. This latter one can focus on a parsimonious use of the metabolic resources, thus aiming at the metabolic efficiency, and/or can deal with the improvement of the cycling gesture, thus improving the mechanical effectiveness as well. The crucial point is that, in order to achieve a desired level of performance, there are different ways to develop and implement motor strategies, which depend, in turn, on the optimization of either the metabolic efficiency or the mechanical effectiveness (Neptune & Herzog, 1999; Korff, & al., 2007; Sarre & Lepers, 2007; Mornieux, & al., 2010). These two quantities are influenced from, and mutually connected to, several factors, even different in nature, that have not been yet completely investigated, and whose cross-effects are still far from being fully understood. Among these factors it is worth citing the following: **muscle fibers distribution** (Staron & Pette, 1986; Coyle, & al., 1991, 1992; Ahlquist, & al., 1992; Hansen, & al., 2002; Umberger, & al., 2006; Hansen & Sjøgaard, 2007; **pedaling cadence** (Chavarren & Calbet, 1999; Neptune & Herzog, 1999; Neptune & Hull, 1999; MacIntosh, & al., 2000; Lucia, & al., 2001; Hansen, & al., 2002; Umberger, & al., 2006; Bieuzen, & al., 2007; Hansen & Sjøgaard, 2007; Mornieux, & al., 2008; Ettema, & al., 2009; Abbiss, & al., 2009; Vercruyssen & Brisswalter, 2010; Leirdal & Ettema, 2011), **biomechanical characteristics** (Davis & Hull, 1981; Coyle, & al., 1991; Neptune & Herzog, 2000; Bibbo, & al., 2006; van Sickle & Hull, 2007; Cannon, & al., 2007; Sarre & Lepers, 2007; Korff, & al., 2007, 2011; Romanov, 2008; Mornieux, & al., 2008; Carpes, & al., 2010; Mornieux, & al., 2010; Wakeling, & al., 2010; Theurel, & al., 2012), **ergogenic factors** which include dietary supplements and psychological strategies (Morgan, 1973; Foster, & al., 1985; Morgan, 1985; Dietary Supplement Health and Education Act of 1994; Hulmer, 1996; Garcin, & al., 1998; Berger, & al., 1999; Raglin, 2001; Williamson, & al., 2001,

2004,2005; Albertus, & al., 2004; Tucker, & al., 2006; MacRae & Mefferd, 2006; Bishop, 2010; Waterhouse, & al., 2010) and, last but not least, **muscular fatigue** (Coast & Welch, 1985; Neptune & Hull, 1999; Abbiss & Laursen, 2005; Lepers, & al., 2002; Theurel, & al., 2008, 2012; Bini, & al., 2010).

When focusing on metabolic efficiency, muscle fiber type, pedaling cadence, oxygen consumption, ergogenic aids and training can be listed as influencing factors. When shifting the attention to mechanical effectiveness, some factors are maintained (pedaling cadence and training), and new ones are introduced (e.g. power output and mechanical setup of the bicycle). Moreover, the contribution of muscular fatigue cannot be underestimated, since it is a disturbing element for both efficiency and effectiveness leading to a general decline of performance. Muscular fatigue, due to its physiological genesis, is directly linked to dietary supplements and psychological factors, which, in some way influence the performance of athletes, either beneficially or detrimentally.

Most of the studies in literature keep metabolic efficiency and mechanical effectiveness separated, and they use heterogeneous parameters (e.g. cycling velocity, oxygen consumption, neuromuscular efficiency, energy expenditure or force exertion) as performance indicators. These are generally associated with the metabolic cost of the task, the biomechanics of the gesture, and the time and/or the race distance. In this way, the most efficacious treatment, intended as the optimum with respect to an objective function depending on both metabolic efficiency and mechanical effectiveness, cannot be directly determined, and the general question of how to improve the cycling performance cannot be answered.

A proposal will follow in the direction of finding an optimum for the function evaluating the efficacy of training, by revising the literature of the field and highlighting those controversial aspects still present. A review of the indexes used as quantitative estimators of performance in cycling is presented as well, together with a report of physiological, psychological and biomechanical factors influencing cycling gesture and its correlates. Attention is devoted to the analysis of cross-effects regarding all these parameters, and to the way these indexes and factors are correlated with the motor strategies leading to that performance, more than assessing the performance *per se*. A computational model is then evoked as a prospective solution for integrating all the factors that affect the performance assessment in this field, possibly overcoming the difficulty to take them into account simultaneously in experimental protocols.

For the authors, the development of a computational model, trying to overcome the difficulties associated with setting up an experimental protocol for multifactorial analysis, is considered as a possible implementation of the multivariate analysis of the performance. This approach is justified by the outcomes of the literature review presented in this work

INDEXES FOR CYCLING PERFORMANCE ASSESSMENT

Metabolic Indexes - Endurance sports rely on aerobic metabolism for energy demand. Thus, the main factors related to oxygen consumption, i.e. VO_2 and $\text{VO}_{2\text{Max}}$, are assumed as determinants of endurance exercise performance. VO_2 is a measure of the O_2 volume used in the energy conversion process into ATP molecules, needed by the muscles to continue working during exercise. $\text{VO}_{2\text{Max}}$ is related to the maximum exercise intensity that a subject

can withstand without further increases in VO_2 , and is often used as an indicator of performance *per se* (Cerretelli & Di Prampero, 1987).

The concept of metabolic efficiency (Eff_{met}), defined as the ratio between the exerted work and the expended energy, is based on the assessment of VO_2 kinetics. This ratio has been quantified by indexes such as 'Gross Efficiency' (GE) (Whipp & Wasserman, 1969), 'Net Efficiency' (NE) (Gaesser & Brooks, 1975) and 'Delta Efficiency' (DE) (Coyle, & al., 1992; Zameziati, & al., 2006). GE represents the overall metabolic expenditure and is expressed by the following equation:

$$GE(\%) = 100 * \left(\frac{W_{ext}}{VO_2 \times \kappa} \right)$$

Equation 5: Gross Efficiency. From Castronovo, et al., 2013.

where W_{ext} is the accomplished work per minute, VO_2 ($l \cdot s^{-1}$) is the oxygen consumption at steady state and $\kappa = 20.9 \text{ kJ} \cdot l^{-1}$ is the energetic equivalent for O_2 .

NE is similar to GE, from which it differs because the contribution of oxygen consumption at rest is subtracted:

$$NE(\%) = 100 * \left(\frac{W_{ext}}{(VO_2 - VO_{2rest}) \times \kappa} \right)$$

Equation 6: Net Efficiency. From Castronovo, et al., 2013.

Finally, DE does not take into account the influence of those metabolic processes that do not contribute to the accomplished work, and thus is not an integral parameter; rather it represents an incremental ratio measure:

$$DE = \frac{\Delta W_{ext}}{\Delta VO_2}$$

Equation 7: Delta Efficiency. From *Castronovo, et al., 2013*.

For this reason it has been considered as a more viable performance indicator than both GE and NE (Gaesser & Brooks, 1975; Coyle, & al., 1992; Zameziati, & al., 2006).

Mechanical Indexes - The mechanical effectiveness (Eff_{mech}) is mainly related to the way forces are applied on the pedal (Gaesser & Brooks, 1975; Romanov, 2008; Mornieux, & al., 2010). It can be ranked in terms of ratio between the *useful* component of force, that is the one tangential to the crank (F_t) and the overall one (F_{tot}), applied by the foot to the pedal load plane surface (Bibbo, & al., 2008).

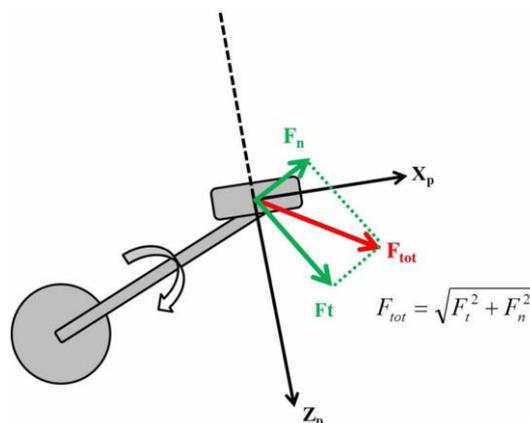


Figure 32: Forces applied to the pedal in the pedal reference system $\{X_p, Y_p\}$. From *Castronovo, et al., 2013*.

This ratio has been expressed through different indicators (that share the same notation “Index of Efficiency”, IE), expressed as a percentage evaluated over the pedal cycle.

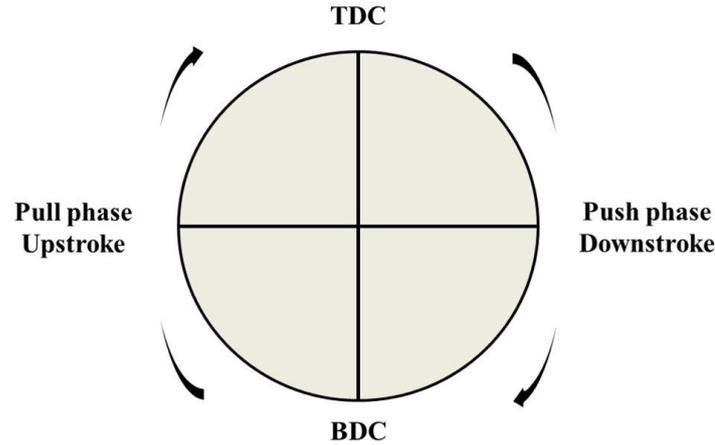


Figure 33: Pedal cycle. Push phase: from the Top Dead Center (TDC) to the Bottom Dead Center (BDC); Pull phase: from the BDC to the TDC. From *Castronovo, et al., 2013*.

In particular, IE_{360° is defined over the entire pedal cycle (Davis & Hull, 1981), while $IE_{180^\circ Desc}$ and $IE_{180^\circ Asc}$ are defined over the descending (i.e. downstroke) and the ascending (i.e. upstroke) phases, respectively (Coyle, & al., 1991; Zameziati, & al., 2006).

$$IE_{360^\circ}(\%) = \frac{\int_0^{2\pi} F_t(\vartheta) \times d\vartheta}{\int_0^{2\pi} F_{tot}(\vartheta) \times d\vartheta} \times 100$$

$$IE_{180^\circ Desc}(\%) = \frac{\int_0^{\pi} F_t(\vartheta) \times d\vartheta}{\int_0^{\pi} F_{tot}(\vartheta) \times d\vartheta} \times 100$$

$$IE_{180^\circ Asc}(\%) = \frac{\int_{\pi}^{2\pi} F_t(\vartheta) \times d\vartheta}{\int_{\pi}^{2\pi} F_{tot}(\vartheta) \times d\vartheta} \times 100$$

Equation 8: Indexes of Efficiency: (1) for the entire pedaling cycle (IE_{360}), (2) for the first half of the pedaling cycle ($IE_{180Desc}$) and (3) for the second half of the pedaling cycle (IE_{180Asc}). From *Castronovo, et al., 2013*.

FACTORS RELATED TO CYCLING PERFORMANCE

Muscle Fibres - Since muscle fibers produce energy from ATP (Kushmerick, 1983; Staron & Pette, 1986; Coyle, & al., 1991; Ahlquist, & al., 1992; Hansen, & al., 2002; Umberger, & al., 2006), they are directly connected to cycling efficiency. The way the ATP reaction occurs, depending on the myosin heavy chain forms (MHC-I or II) (Staron & Pette, 1986), allows a classification of the human skeletal muscle fibers into the following types: i) *slow twitch* (ST) or type I fibers, characterized by slow firing rates and a good resistance to muscular fatigue (M_{Fat}); ii) *fatigue-resistant* (FR) or type IIA fibers, resistant to muscular fatigue and able to produce high force levels; iii) *fast twitch* (FT) or type IIB fibers, producing short-time peaks of force and not fatigue-resistant.

The distribution of the muscle fibers can influence the cycling performance in terms of VO_2 response, which can be quantified by Eff_{met} . This distribution, in the muscles, is quite heterogeneous, and the percentage of ST and FT fibers heavily influences the indexes assessing the performance, as confirmed also by quantitative models (Coyle, & al., 1992; Umberger, & al., 2006).

Peddaling Cadence - The existence of an optimal Pedaling Cadence (PC) has not been demonstrated yet, because there are some PCs optimizing the metabolic efficiency and other ones improving the neuromuscular one (Faria, & al., 2005).

With respect to the neuromuscular efficiency, the optimum PC is the one minimizing the overall muscle activation and the muscular fatigue (Abbiss, & al., 2009; Theurel, & al., 2012). Neptune and co-workers (Neptune & Herzog, 2000) have demonstrated that a 90 rpm PC

asks the muscles for the minimal levels of force, while 93.5 rpm (Bieuzen, & al., 2007) is the value that maximizes the neuromuscular efficiency in well-trained cyclists. On the contrary, higher PCs are responsible for increased metabolic costs (Chavarren & Calbet, 1999).

It was demonstrated that triathletes, during prolonged exercises, tend to choose a PC close to the energetically optimal one, as determined analytically based on the relation between cadence and oxygen uptake variations (Brisswalter, & al., 2002), by changing the muscle activity pattern, and this may explain the shift towards higher PCs (Brisswalter & Hausswirth, 2000). The energetically optimal PC has been found at around 63.5 rpm in (Bieuzen, & al., 2007), at about 50 rpm in (Chavarren & Calbet, 1999; Zameziati, & al., 2006), and in the range 90-105 rpm for professional riders in (Lucià, & al., 2001). These rather contrasting results need to be mingled with the values reported by Vercruyssen and co-workers (Vercruyssen & Brisswalter, 2010), who showed that the energetically optimal PC falls in a range (i.e. 55-65 rpm) different from the freely chosen PC range (i.e. 80-95 rpm). Marsh and Martin tried to associate the preferred pedaling cadence to the rate of perceived exertion in professional cyclists, runners and non-cyclists (Marsh & Martin, 1998). They found out that, irrespective of Borg's scale (Borg, 1970), the pedaling rates minimizing RPE were lower than the selected preferred ones for all subjects, concluding that the changes in RPE are not critical for cadence selection during submaximal cycling.

Biomechanics - Several biomechanical factors affect the pedaling performance, first of all the Pedaling Technique (PT). It can be regarded as the way the cyclist pedals, and thus it includes the type of pedaling gesture (mash, circular or triangular; right vs. left leg dominance), the geometry of the bike frame, the saddle position and its height, the crank

length. These factors heavily affect the way the main muscles involved in the cycling task are used, and have been extensively investigated in literature, both studying the specific contribution of each of them (Jorge & Hull, 1986; Bibbo, & al., 2006) and analyzing how they work in synergy (DeMarchis, & al., 2012). Muscles are activated in different phases of the crank cycle, according to their principal functions (Bibbo, & al., 2006), and, while mono-articular muscles are mainly power producers, the bi-articular ones act mainly to transfer energy between joints during the pedal revolution (Van-Ingen-Schenau, & al., 1992).

Since the force demands drive the energy requests, it appears that reducing muscular forces at a given power output may improve the performance. One way to decrease the force developed by the ankle plantar-flexors is to move the foot anteriorly on the pedal, thus balancing the moment about the ankle caused by the reaction force of the pedal. This force reduction could be translated into a better Eff_{met} (Coyle, 1995; van Sickle & Hull, 2007), even if the relationship between GE and IE is not direct. The mechanical energy produced by multiple muscles is, in fact, transferred via the body segments to the crank and this could make Eff_{mech} not resulting in an improved Eff_{met} .

Several studies assessed the influence of technical factors on performance: the pedal type (Mornieux, & al., 2008), the rider position and the pedaling technique (Romanov, 2008; Korff, & al., 2011), different chain rings (Kautz, 1994), the inclination of the seat (Leirdal & Ettema, 2011). Shoe-pedal interfaces do not influence the pedaling pattern and VO_2 during submaximal cycling, but during uphill cycling, where maximal power is required, wearing of clip-less pedals may be advantageous (Mornieux, & al., 2008). Regarding the position of the rider on the seat, the "Pose" method (Romanov, 2008) was introduced to define a PT

improving Eff_{met} : this method benefits from the gravitational contribution to pedal power, and corresponds to a lower seat height and a more upright body position. These factors lead to a GE increase when compared to subject's preferred bicycle position, even if aerodynamic conditions are disregarded. The combined effect of changing bicycle setup and PT showed no effect on GE, and only small effects on pedaling mechanics (Leirdal & Ettema, 2011). Different chain rings affect the angular velocity of the crank-arm and, in turn, the mechanical work produced to move the legs (Kautz, 1994).

Muscular Fatigue - The onset of Muscular Fatigue (M_{Fat}), intended as an exercise-induced reduction of voluntary force (Coast & Welch, 1985; Lepers, & al., 2002), is an important factor affecting cycling performance. Different cause-and-effect models have been developed to address this topic without, however, fully explaining the phenomenon (Abbiss & Laursen, 2005). The reduction in force levels, concomitant with the increasing exercise duration, leads to a drop of the muscle activation level at the end of the exercise, because of an impairment of contractile properties and of an alteration of both excitability and central drive (Lepers, & al., 2002; Castronovo, & al., 2012). During prolonged cycling, changes in PT can influence the occurrence of M_{Fat} and also the energetic demand. In contrast with studies showing occurrence of M_{Fat} after prolonged exercises (Coast & Welch, 1985; Lepers, & al., 2002) some authors have reported its onset just after 15 min of exercise, irrespective of PT (Theurel & Leperd, 2008). The influence of M_{Fat} on performance can be better evaluated during cycling until voluntary exhaustion, by introducing also biomechanical data, such as the net joint moment distribution or the joint forces and kinematics (Bini, & al., 2010). For example, the ankle joint contribution to the net joint moment decreases with increasing M_{Fat} (Lepers, & al., 2002).

ERGOGENIC AIDS

Dietary Supplements - With the term dietary supplement (Diet) it is intended “any product taken by the mouth in addition to common foods, which has been proposed to have a performance-enhancing effect” (Bishop, 2010). Several studies have been dedicated to the evaluation of the improvement of cycling performance via dietary supplements (MacRae & Mefferd, 2006) (Williams, 2004, 2005). Many athletes need a correct nourishment to improve their physiological performance, preventing them to use pharmacological agents as steroids or amphetamines (Williams, 2004). The Dietary Supplement Health and Education Act (DSHEA) in 1994 (Dietary Supplement Health and Education Act of 1994) clarified the dietary supplements that can be assumed by athletes, helping them in improving their sport performance: vitamins, minerals, amino acids, herbs and botanicals, metabolic constituents. For example it has been demonstrated that B vitamins are necessary for physiological mechanisms as carbohydrate and fats processing for ATP production, but also C and E vitamins act as antioxidants preventing cellular and subcellular damages during exercise training. Mineral, instead, have been demonstrated to be unnecessary for athletes with an already well-balanced nutrition, as well as vitamin C supplementation. Vitamin E enhances the oxygen utilization during exercise at higher altitude levels but it has been revealed ineffective during cycling at sea level condition (Williams, 2004, 2005). Moreover, the antioxidant supplementation combined with flavonoids as Quercetin (FRS), which can be found in blueberries, cranberries, crowberries and grapes but also in red onions or apples, has been found to improve average power and %peak power during 30 km cycling trials, without significant amelioration of %HR max or VO₂ (MacRae & Mefferd, 2006). Also

caffeine effects have been evaluated in several studies focused on cycling activities (Jeukendrup & Martin, 2001; Foad & Coleman, 2008). In fact, it has been reported that the use of caffeine improves the performance and endurance capacity when it does not exceed the threshold concentration defined by the International Olympic Committee (12 mg/l) (Jeukendrup & Martin, 2001; Foad & Coleman, 2008).

Psychological Traits - The performance of an athlete may change also in relation of his/her perceived exertion, which “integrates various information, including many signals elicited from the peripheral working muscles and joints, from the central cardiovascular and respiratory functions, and from the central nervous system” (Borg, 1982). In order to assess the perceived exertion, the scale developed by Borg (Borg, 1970), referred to as Ratings for Perceived Exertion (RPE) scale (Borg, 1970, 1982), has been used in different application fields, including cycling.

O’Sullivan in her review on perceived exertion mentioned all the physiological and psychological variables which showed a correlation with RPE (O’Sullivan, 1984), and several studies have considered the RPE in relation to cycling exercises (Borg & Linderholm, 1967; Skinner, & al., 1970; Noble, & al., 1973; Garcin, & al., 1998; Tucker, Marle, Lambert, & al., 2006). In particular, the variance that could not be explained with physiological variables (around 33%), such as HR, force produced, ventilatory and oxygen response or gender, was estimated as coming from psychological factors (Morgan, 1973). In fact, the effect that a particular mental status can have on athletes’ performance is not to be underestimated: subjects that are depressed, neurotic or anxious tend to process information related to muscular work more unlikely than individuals without this pathology (Raglin, 2001). As of

now, the focus of sports medicine is upon maintaining the physical health of the athletes, and this means including psychological variables as well, because of their impact on performance and general status (Raglin, 2001). For example, well-trained athletes resort to 'pacing strategies' to optimize performance during cycling or running, which is a subjective way to use and distribute own sustainable power output (i.e. effort) in a wise way during the overall duration of the race (Foster, Shrager, Snyder, & al., 1985). One remarkable work including psychological aspects predicting performances of athletes focused on a Mental Head Model (MHM) (Morgan, 1985) and thus included psychological traits (Psych_T) as neuroticism, confusion, anxiety stress and fatigue which are likely to invalidate the performance of the athlete (Morgan, 1973; Raglin, 2001). Changes in mood upon athletic performance in response to high intensity training or to the introduction of music during training have also been evaluated by other studies (Berger, & al., 1999; Waterhouse, & al., 2010). For example, it has been demonstrated that fast music tempo positively influences the performance acting on motivation and distracting effects compared to slow music during low and moderate intensity cycling exercise (Waterhouse, & al., 2010). Another topic to be mentioned concerns the use of acting drugs or hypnosis, which may result in an alteration of psychological responses (Albertus, & al., 2004). Some studies are focused upon hypnotic manipulation of effort, and, in particular, on the physiological responses following this psychological treatment during cycling. Morgan et al. evaluated metabolic responses during various and different hypnotic suggestions either at a constant workload and rpm and they did find out that hypnosis modified physiological outcomes (Morgan, & al., 1973). As a matter of fact, some subjects thought that the duration of the exercise, and not its intensity, was reduced under hypnosis; the HR was higher during suggestion of heavy work and

lower during the suggestion of light work, so following individual suggestion, even if the workload did not actually changed at all. Williamson et al. (Williamson, & al., 2001) evaluated cardiovascular and cerebral responses, in an attempt to separate the descending signals originating from the brain in response to afferent inputs coming from peripheral pathways, and thus to determine whether cortical structures involved in cardiovascular modulation are activated during hypnotic suggestion of downhill or uphill cycling. The hypnotic suggestion, according to the authors, should not involve central commands, which rely on a feed-forward mechanism of activation of both motor and cardiovascular centers. The authors concluded that cerebral cortical structures (right insular cortex and right thalamic region), during hypnosis, are activated by an increased sense of effort, thus reflecting an augmented cardiovascular response; this is not paralleled by a corresponding reduction of their activation, when a decreased sense of effort is generated.

Including these aspects into a global performance assessment is not a trivial matter since they are mostly based on scores lying on different scales (i.e. Borg's Scale (Borg, 1982), Barber Suggestibility Scale (BSS) (Barber, 1971), Eysenck Personality Inventory (Eysenck & Eysenck, 1962), Spielberger's state-trait inventory (Spielberger, & al., 1969), Somatic Perception Questionnaire (Landry & Stern, 1971), Lubin's Depression Adjective Checklist (Lubin, 1967)). Moreover the psychological influence on biomechanical variables, intended as index of effectiveness of produced forces, is still lacking in literature and an integration of these three aspects is really needed.

ENVIRONMENTAL VARIABLES

In the cycling field, as it happens in many motor tasks, the influence of the environment on the execution of the gesture has to be taken into account. This aspect can be explained by considering if and how the performance of a cyclist can be affected by stimuli coming from the environment (Marsh & Sleivert, 1999; Kay, & al., 1999; O'Brien & O'Conner, 2000; Waterhouse, & al., 2010). Biofeedback techniques (Sanderson & Cavanagh, 1990; Sveistrup, 2004; Hasson, & al., 2008) aim at improving the pedaling performance by stimulating different sensorial channels of the athlete: through a set of recording devices, processing algorithms and graphical user interfaces (GUIs) it is possible to extract information to be presented in real-time to the athlete (Sanderson, 1987). In this way, biomechanical or metabolic information return to the subject's brain as a feedback. Thus, the peripheral input and the way it is integrated in the central paths influences the subsequent plan of exercise intensity (pacing strategy) (Ulmer, 1996). Different representations are used to provide information to the athlete, and some studies in the literature focus on the use of visualization techniques (Aris, & al., 2005). The aim of letting cyclists learn to pull up on the pedal and thus increase Eff_{Mech} , has driven some authors to present visual feedback to the riders in rather different ways (Sanderson, 1987; Mornieux, & al., 2010; Bibbo, & al., 2012). The above-mentioned studies reported a significant variation of pedaling gesture with the use of visual feedback confirming the hypothesis that, independently of the particular rendering scheme, the use of biofeedback allows riders to improve Eff_{Mech} . No evidence exists concerning the improvement of Eff_{Met} in biofeedback-based training, evidencing the lack of convergence on a global assessment of cycling performance.

The place where the cyclist lives and practices is strictly connected to the cycling performance. In order to maximize adaptations to altitude and minimizing its influence to training, a hybrid approach, named Live High Train Low (LHTL) has been developed, consisting in living at moderate altitudes and training at sea level or low altitudes (Hahn & Gore, 2001). An exposure to moderate altitude seems to enhance sport performance at sea level ground since the benefit of reduced aerodynamic drag overcomes the decrease in maximum aerobic power, estimated as VO_{2Max} . Training at moderate altitudes, thus breathing with higher levels of oxygen than those experienced in the lifetime, determines an increase in aerobic power, more than a higher aerobic capacity. Other studies have demonstrated that aerobic exercise performance decreases upon ascent to altitude whereas anaerobic performance remains unchanged (Burtscher, & al., 2006). On the other hand, training in a hyperoxic environment may lead to higher training intensities, which result in a significant improvement in maximal steady state power output (Morris, & al., 2000). The exposure to altitude has been demonstrated to be related also to time trial performance: there was no change in the 5-min cycling performance but the 50-min cycling performance improved after 45 hours of altitude acclimatization (Burtscher, & al., 2006).

MUTUAL INTERACTIONS AMONG FACTORS AFFECTING THE PERFORMANCE

A complete characterization of athletes' performance could be misleading, if the factors mentioned above are kept separated. Focusing on single factors may determine results different from those obtained when all the factors are considered together.

PC is affected simultaneously by several factors, such as the power output (Coast & Welch, 1985) and the changes in the fiber muscle recruitment pattern (Ahlquist, & al., 1992;

Umberger, & al., 2006), but also by the rider's skill (Umberger, & al., 2003) and the workload (Coast & Welch, 1985; Hansen, & al., 2002; Foss & Hallen, 2004). No influence is reported when considering the relationship between VO_2 (and its kinetics) and PC, irrespectively of fiber type distribution. The prevailing presence of MHC-I is related to high pedaling rates but not to maximum values of GE (Hansen, & al., 2002). In particular, the correlation between MHC-I and GE is positive when subjects pedal at preset pedal rates, and becomes negative when a freely PC is chosen (Chavarren & Calbet, 1999; Hansen, & al., 2002; Zameziati, & al., 2006). Musculoskeletal models and computer simulations confirmed those experimental values (Seabury, & al., 1977; Coast & Welch, 1985; Neptune & Hull, 1999; Umberger, & al., 2003, 2006). It has also been reported that low PC (around 50 rpm), for the same metabolic cost, causes augmented muscular forces when compared to higher PCs (Ahlquist, & al., 1992). Supporting this hypothesis, the required level of force was found as the factor determining the decrease of PC during an endurance exercise (Coast & Welch, 1985; Lepers, & al., 2000). This fall in cadence, concomitant with an increase in exercise duration and occurrence of muscle fatigue, is interpreted as an adaptation of the movement pattern in order to minimize the energy cost rather than the neuromuscular one. If, instead, we focus on submaximal workloads, a unique PC was found to minimally activate the muscles (MacIntosh, & al., 2000). During submaximal exercises with constant PCs, IE was found to increase, especially in the downstroke phase, resulting as an important factor for changes in Eff_{met} (Zameziati, & al., 2006).

PC affects, on the other hand, the muscular activations and patterns, making thus impossible to estimate the timing of muscular internal forces from the forces applied on the pedal. Muscle power, as soon as the cadence increases, is generated at a later crank angle, making

the choice of the preferred PC dictated not only by metabolic costs. Higher PCs increase the inertial non-muscular component of the pedal forces, which is closely related to fluctuations of the kinetic energy.

In this perspective, PC seems to affect both GE and IE but no causal relationship between the two indexes emerges: inertial forces, in fact, do not have any metabolic cost, and the increase of cycling economy cannot be linked to the decrease of IE.

Moreover, PT is a further controversial aspect for performance evaluation. Changing PT modifies the number of muscles involved and their activation timing, and may lead to a variation (that may be detrimental) of some physiological variables: for example, switching to a dorsi-flexed PT increases metabolic costs (Zameziati, & al., 2006). The activity of each muscle involved in the task depends on the mechanical demand (Bibbo, & al., 2006; Wakeling, 2010), which drives the generation of forces applied to the pedals. When forces are not applied correctly, as it can be monitored by the variations of IE_{360° , $IE_{180^\circ Desc}$, $IE_{180^\circ Asc}$, inefficient muscular work is produced (Neptune & Herzog, 1999; Zameziati, & al., 2006; Mornieux, 2010). These findings are, however, in contrast with those showing an increase of IE with a concomitant worsening of metabolic behavior under different PTs (Korff, & al., 2011). The latter results are supported and well explained by the analysis of the pull-up action on Eff_{mech} and Eff_{met} (Mornieux, & al., 2008): this action is thought to be responsible for an augmented Eff_{mech} during the upstroke phase (which causes a higher IE_{360°), but it is also associated with an increased muscular work and co-activations, leading to augmented VO_2 (Mornieux, & al., 2008). This has been correlated to the training volume affecting the pedaling pattern: any induced alteration, such as an active pulling-up action, could impair

the physiological response. Different is the case of non-experienced cyclists who, not exhibiting an intrinsic pedaling pattern due to the absence of previous training, can improve Eff_{mech} without altering VO_2 (Mornieux, & al., 2010).

All these counteracting aspects, when considered together, lead to a wide range of PTs, each with its optimal PC, eliciting similar levels of Eff_{met} , and these findings are at odds with the hypothesis of having just one optimal PC, as discussed before (Korff, & al., 2007).

A PERSPECTIVE MODEL

What may thus be considered the best way to evaluate a cyclist's performance?

So far, the effectiveness of the training in cycling and, in turn, the goodness of the performance, has been assessed in several ways, mainly focusing on physiological factors (through the Eff_{met}) or on the pedaling technique and its correlates (through the Eff_{mech}). Each of these two approaches takes into account muscular variables, pedaling cadence, biomechanical factors, environmental variables, and also the occurrence of MFat. These factors are often analyzed separated from each other or, in some cases, considered as sub-sets by looking at the effects of one or two of them on both efficiency and effectiveness. We are under the impression that this way does not lead to a complete evaluation of the performance. If e.g. we want to find the pair of variables {cadence PC, seat height H_s } that optimizes the performance, by using the metabolic efficiency as the objective function, we may probably find an optimum set $\{PC_{eff}, \hat{H}_s\}$ which is different from the one obtained by using mechanical effectiveness as the objective function. In addition to that, these input variables interact with other status variables. For example, repetitive training with a changed

It will determine variations in muscular timing patterns so affecting the muscle fiber distribution. This change in distribution will modify in a recursive manner the shape of the objective function taken into consideration.

In an attempt to suggest a solution that could help in highlighting the relationships between the different parameters, we propose here to use a computational model. The idea of using models to optimize performance is not new in literature (Neptune & Hull, 1988; Martin, & al., 1988; Olds, & al., 1993; Swain, 1997; Olds T. , 2001; Jeukendrup & Martin, 2001; Abbiss & Laursen, 2005), because the mathematics allows to simplify the problem by reducing it to an expression of the type $y = f(x)$, where x represents a combination of biomechanical and/or physiological parameters and y is a performance variable (Olds, 2001). The studies published in literature used different combinations of input variables, and defined different performance variables. Some of those based the performance on the effect of physical variables on the athlete (i.e. altitude, aerodynamic setup, altitude variations upon energy supply) (Olds, 2001), others tried to predict the power output with respect to aerodynamic resistance, wheel rotation, rolling resistance, and changes in potential and kinetic energy (Martin, & al., 1988). Jeukendrup and coworkers, starting from the work of Martin et colleagues (Martin, & al., 1988), synthesized the factors that can influence cycling performance and divided them into internal (i.e. training, altitude training, carbohydrate and caffeine) and external factors (i.e. body weight, body position, clothing, bicycle and wheels) (Jeukendrup & Martin, 2001). A model of this kind, even if considering several factors among biomechanical and physiological, lacks of integration of information about muscular status and muscular fatigue or psychological variables. Neptune and Hull also developed a model and an optimization framework to simulate a pedaling exercise at submaximal power, with a

main focus on kinetic, kinematic and activation timing quantities, but without considering all the physiological correlates (Neptune & Hull, 1999). A model to optimize cycling performance varying power on uphill and windy conditions was also developed by Swain and pointed attention on time saving and its relation to VO_2 variations, but without considering the technical frame of the bike or the muscular status (Swain, 1997).

None of these models integrates all the factors that affect the performance, and so limits the multivariate analysis of the phenomenon. In this review, it is suggested to put together design specifications derived by experimental outcomes, with mathematical techniques already developed to solve multi-variables optimization problems. Typically, solving an optimization problem means locating the value set that corresponds to the maximum value of the objective function. If the objective function is the overall effectiveness (E) of a training program, we may consider the following equations:

$$\begin{cases} E = f(\text{Eff}_{\text{Met}}, \text{Eff}_{\text{Mech}}) \\ \text{Eff}_{\text{Met}} = g(m_{\text{prop}}, \text{PC}, \text{Biomech}_{\text{set}}, \text{MFat}, \text{Diet}, \text{Psych}_{\text{Tr}}) \\ \text{Eff}_{\text{Mech}} = h(\text{Biomech}_{\text{set}}, m_{\text{prop}}, \text{Envir}_{\text{var}}) \end{cases}$$

Equation 9: The overall effectiveness (E) is a function of Metabolic Efficiency (Eff_{Met}) and Mechanical Efficiency (Eff_{Mech}). These, in turn, are functions of several other parameters. *From Castronovo, et al., 2013.*

where the underlying hypothesis is that E is a function of both Eff_{Mech} and Eff_{Met} . According to our literature survey, we may consider that Eff_{Mech} is only directly dependent on biomechanical configuration $\text{Biomech}_{\text{set}}$, muscular properties m_{prop} and environmental variables $\text{Envir}_{\text{var}}$, while Eff_{Met} adds to these factors pedaling cadence PC and muscular fatigue MFat and Diet and Psych_{Tr} as well. In this simplified scheme, the mutual interactions

are not highlighted, but the interdependency can be listed as well according to the following equations that summarize the relations found in the literature:

$$\begin{cases} MFat = u(m_{prop}, Diet, Envir_{var}, Psych_{Tr}, Biomech_{set}, PC) \\ m_{prop} = v(MFat, Diet) \\ Biomech_{set} = w(m_{prop}, Envir_{var}) \end{cases}$$

Equation 10: Muscular Fatigue is a function depending on several factors as muscular properties and biomechanical set up. *From Castronovo, et al., 2013.*

where the fatigue variable depends on the specific biomechanical configuration and may depend on the environmental variables; the muscular properties, and their activation patterns, are dependent on fatigue, and their change may determine a variation in the biomechanical configuration that is used. Also the psychological traits, as we said before, have an influence on physiological performance and thus on metabolic efficiency and may change the muscular fatigue variable, but since the connection with the mechanical outcomes is lacking, a computational model could help estimate this relation.

By applying mathematical laws to express the mutual interactions and recursions, we are confident that it could be possible to find a solution for the maximization of the effectiveness function E, by considering all its dependencies on the listed variables. Moreover, by using suitable instrumentation (Bibbo, & al., 2008, 2012) and objective processing techniques, respectively to measure the biomechanical properties and to estimate the activation patterns in terms of amplitude (D'Alessio & Conforto, 2001) and timing (Bonato, & al., 1998) (Vannozzi, & al., 2010, Severini, & al., 2012) and spectral characteristics (Conforto & D'Alessio, 1999), also the model validation appears as a feasible operation.

Further researches are needed in order to define the mathematical relationships explaining mutual interactions and thus, ultimately, defining the model. In our opinion, the latter is one of the few viable solutions for understanding the multiple factors affecting a performance and thus, in perspective, for the development of training techniques based on the reported scientific evidences.

CONCLUSIONS

But if the kinds of causes had been infinite in number, then also knowledge would have been impossible; for we think we know, only when we have ascertained the causes, that but that which is infinite by addition cannot be gone through in a finite time.
(Aristotele, *Metaphysics*, II, 2, 994b)

In 350 B.C., Aristotle in his book *Metaphysics* wrote that knowledge would be impossible if the mechanisms that generate a phenomenon were infinite. Fortunately for us, once ascertained the causes, we can be sure to have understood something. Following this concept, this thesis proposes an integrated holistic approach to the study of neuromuscular fatigue in order to encompass all the causes and all the consequences underlying the phenomenon with the main goal of an increased and more complete knowledge. Starting from the metabolic processes occurring at the cellular level, the reader is guided toward the physiological changes at the motorneuron and motor unit level and from this to the more general biomechanical alterations. In this context, pedaling will be the gesture mainly considered, since much attention has been devoted in the literature to the influence of fatigue on cycling performance. However, it is not possible to study a phenomenon without firstly defining it. In the first chapter (*"Definitions of Fatigue"*) a list of the various meanings for fatigue has been reported spanning several contexts. It is not easy to find an unique description for this phenomenon: many factors have to be taken into account and this is why most of the studies focus more on the effects rather than on causes. Occurrence of fatigue produces a decrease of force, muscle soreness, sense of effort and tiredness. But what are the causes of neuromuscular fatigue is still an unclear topic. Several studies have focused on the metabolic processes (Westerblad, & al., 1998, 2002) occurring in the cellular compartments,

some others on the central (spinal and supra-spinal) or peripheral mechanisms (Bigland-Ritchie & Woods, 1984; Gandevia, & al., 1995; Gandevia, 2001; Enoka & Duchateau, 2008, Boyas & Guevel, 2011). The electrophysiological changes in terms of motor unit behavior and descending neural drive to the muscle have been studied extensively (DeLuca, 1984; Taylor, & al., 2000; Contessa, & al., 2009) as well as the biomechanical adaptations induced (Theurel & Lepers, 2008; Bini, & al., 2010). However, an integration of all these mechanisms in a simple and clear flow is still missing. In the attempt to relate the occurring physiological and biomechanical events, the sEMG has been the most largely used tool. However, indicators extracted from this signal have shown different behaviors leading to confounding inferences (Solomonow, & al., 1990; Farina, & al., 2004, 2008; vonTscherner & Nigg, 2008). The approach proposed in this thesis is based on the rationale that a possible clear view and understanding of the phenomenon can be obtained only when all the contributing variables are put together. In order to test this hypothesis, some experimental studies have been designed and conducted. In the third chapter a preliminary study based on the observation of temporal features extracted from sEMG signals, recorded during a dynamic task, has been reported. This study has been presented at the *34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society* held in San Diego (CA) in 2012. However, the need of a/some more robust and reliable indicator(s) during fatiguing tasks has emerged from this work. Therefore, a novel bi-dimensional parameter has been then proposed trying to reply to this requirement in chapter four. This parameter relies on the simultaneous use of temporal and spectral features, put together in a polar domain, and allows tracking the evolution of muscular status during the execution of a fatiguing motor task. The preliminary results of this work have been presented to the *XIII Mediterranean Conference on Medical and Biological*

Engineering and Computing held in Seville in September 2013. An extended journal paper is also going to be submitted. The study on sEMG-based indicators opened a scenario also on neurophysiological mechanisms underlying fatigue. For this purpose, an ad hoc protocol has been designed for the analysis of physiological parameters, such as MU recruitment strategies, MU discharge rate and synchronization as well as decrease of force steadiness during prolonged fatiguing contractions. In particular, two methodologies have been applied to multichannel sEMG recordings of isometric contractions of the Tibialis Anterior muscle: the state-of-the-art technique for sEMG decomposition (Holobar & Zazula, 2007) and a coherence analysis on MU spike trains (Rosenberg, & al., 1989). The use of these techniques allowed also the observation of the changes induced by fatigue not only on MU behavior but also on the neural drive shared by motorneurons and on the force variability. We found out that the neural excitation to motorneurons, which increases during fatigue, leads to an increased common synaptic input and synchronization of MU. In particular, the synchronization of MU was found to be the major determinant of decrease of force steadiness observed throughout the entire contraction. The preliminary results of this study have been presented at the *Annual Meeting of the Society for Neuroscience (SfN)* held in San Diego (CA) in November 2013. An extended journal paper is also going to be submitted. The importance of a multi-scale approach has been finally highlighted in the context of the evaluation of cycling performance, where fatigue is one of the limiting factors. In particular, the last chapter of this thesis can be considered as a paradigm: physiological, metabolic, environmental, psychological and biomechanical factors influence the performance of a cyclist and only when all of these are kept together in a novel integrative way it is possible to

derive a clear model and make correct assessments. This work has been published on the international journal *Frontiers in Physiology* in 2013.

Summarizing, this thesis tries to enhance and recommend the use of a multi-scale methodology in the assessment of the physical and psychological phenomena, such as muscular fatigue, and makes it by reporting several examples of studies designed during these years. These studies, presented at International Conferences or published on International Journals, report different aspects of the same phenomenon trying to highlight the limitations and the original outcomes with respect the techniques used trying to better clarify the phenomenon of neuromuscular fatigue.

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