universität wien

MASTERARBEIT

Titel der Masterarbeit

Analysis of surface electromyography (sEMG) signals at and above the maximal lactate steady state (MLSS) during ergometer cycling

verfasst von

Bojan Makivic

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Betreuer:	Dipl. – Sporting. Dr. Mario Heller

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ABSTRAKT (Deutsch)

Zielsetzung: Das Ziel der Masterarbeit ist es, oberflächenelektromyographische Signale ausgewählter Muskeln bei Ausdauerbelastungen im maximalen Laktat-Steady-State (MLSS) sowie leicht über dem MLSS (aMLSS) zu untersuchen.

Methode: 32 Freizeitsportler (Alter: 29.3±5.4 Jahre; Körpermasse: 75.3±7.2 kg; Körperhöhe: 178.6±5.4 cm) führten 3-6 Dauerbelastungstests (30 Minuten) durch, um die Bereiche des individuellen MLSS und aMLSS zu bestimmen. Die Aktivität von 6 Muskeln der unteren Extremitäten wurde aufgezeichnet (Gluteus medialis (GM), Biceps femoris (BF), Rectus femoris (RF), Vastus medialis (VM), Vastus lateralis (VL) und Gastrocnemius medialis (GastM). Die Medianfrequenz (MDF) und der quadratische Mittelwert (RMS) jedes Muskels wurden für drei Zeitpunkte (2.5, 10 und 30 Minuten) bestimmt, mit dem jeweils höchsten vorkommenden Wert innerhalb des Tests normalisiert und mit dem linearen gemischten Modell statistisch analysiert.

Ergebnisse: Die mittlere mechanische Leistung betrug im MLSS 226.9 W und im aMLSS 235.9 W. Die MDF stieg im Laufe der Belastung bei 5 von 6 Muskeln (außer VM) in beiden Belastungsbereichen signifikant an (MLSS: 10% und aMLSS: 8%, beide $p<.001$). Unterschiede zwischen MLSS und aMLSS (VL) bzw. Interaktionseffekte (GastM und VM) waren signifikant, ($p<.05$), eine Posthoc- Analyse ergab jedoch keine einzelnen Mittelwertunterschiede.

Der RMS fiel im Laufe der Belastung ab der 10. Minute für alle Muskeln ab ($p<.001$). Unterschiede zwischen MLSS und aMLSS (RF) bzw. Interaktionseffekte (RF) waren signifikant ($p<.05$), die Post-hoc-Analyse ergab einen signifikanten Unterschied ($p<.05$) von 18% bei Minute 2.5.

Schlussfolgerung: Bei einem mittleren Unterschied der mechanischen Leistung von 9 Watt zeigten die oberflächenelektromyografischen Parameter MDF und RMS beim Vergleich der Ausdauerbelastungen im MLSS sowie aMLSS mit Ausnahme des RMS vom RF zu Beginn der Belastung kein unterschiedliches Verhalten in den einzelnen Messzeitpunkten. Die Ergebnisse der Studie deuten darauf hin, dass beim Überschreiten des maximalen Laktat-Steady-States (aMLSS) durch einen geringfügigen Anstieg der Belastung im Bereich von weniger als 10 Watt im Vergleich zu MLSS keine systematischen Unterschiede in MDF und RMS oberflächenelektromyografischer Signale ausgewählter Muskeln auftreten.

ABSTRACT (English)

Purpose: The aim of this master thesis is to investigate surface electromyographic (sEMG) signals of chosen muscles between two power outputs defined as maximum lactate steady state (MLSS) and above MLSS (aMLSS) power output.

Methods: 32 recreational cyclists (mean ± SD: age 29.3±5.4 years; weight: 75.3±7.2 kg; height: 178.6±5.4 cm) have performed 3-6 constant-load tests (30 min) to determine MLSS and aMLSS power outputs. Muscle activation was recorded from six lower limb muscles: gluteus medialis (GM, bicpes femoris (BF), rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL) and gastrocnemius medialis (GastM). The median frequency (MDF) and root mean square (RMS) were normalized to the highest single value in the data set for each muscle separately. In addition values at three time points (2.5^{th}, 10^{th} and 30^{th} minute) were statistically analyzed with the linear mixed model (LMM).

Results: The mean power outputs for MLSS and aMLSS were 226.9 and 235.9 Watts, respectively. The MDF increased significantly from the beginning toward the end of tests in five from six muscles, except VM, (MLSS: 10% und aMLSS: 8%, both $p<.001$). Significant mean difference between MLSS and aMLSS (VL MDF) as well as interaction (VM and GastM) were observed, respectively ($p<.05$). However, the following post hoc analysis has not found significant difference between MLSS and aMLSS power outputs at any of three time points separately. The RMS decreased from 10^{th} minute toward the end of tests for all muscles ($p<.001$). The mean difference between MLSS and aMLSS as well as interaction were significant for RF RMS ($p<.05$). Subsequent post hoc analysis has revealed significant difference ($p<.05$) of 18% at the 2.5^{th} minute.

Conclusion: With the mean difference of 9 Watt between MLSS and aMLSS power outputs the sEMG parameters have demonstrated unchanged behavior between aforementioned workloads at the single time points, except for RF RMS at the beginning of the test. The results of present study indicate that the significant systematic difference in MDF and RMS parameters of sEMG of chosen lower limb muscles is not taking place by overstepping MLSS power output by less than 10 Watt.

Content

1 Introduction

Metabolic and physiological changes during exercise at and above the level of Maximal Lactate Steady State (MLSS) have been detailed elaborated in research literature (Baron et al., 2008; Ralph Beneke, Hütler, & Leithäuser, 2000; Dekerle, Baron, Dupont, Vanvelcenaher, & Pelayo, 2003; Pringle & Jones, 2002; Smekal et al., 2012; Van Schuylenbergh, Vanden Eynde, & Hespel, 2004).

Different study methodologies of testing that concerns electromyographic (EMG) alternation during prolonged cycling exercise at and above MLSS makes difficult to mark off different neuromuscular factors (inter-muscular and intra-muscular coordination, motor unit (MU) recruitment, etc…) that could underline the performance difference between these two exercise intensities.

Therefore, standardized methodology in terms of exercise intensity (workload), duration of tests, subject's gender and age, etc., has to be priority of future studies which are intended to study sEMG activity during constant-load cycling on MLSS and above MLSS (aMLSS) workload.

Well known axiom in science literature has approved that bearable duration of high-intensity exercise demonstrate hyperbolically decreasing trend of time to exhaustion as a function of the power output during whole-body exercise such as cycling (Poole, Ward, Gardner, & Whipp, 1988; Pringle & Jones, 2002).

It has been stated that cycling on power output below the level of highest stable value of lactate accumulation could be sustained for longer periods of time without apparent signs of fatigue (> 60 min.), whereas cycling on power output above highest stable lactate value leads to the progressive accumulation of blood lactate and depletion of the stored energy sources until its exhaustion ,which in turn significantly reduce the time of exercise (Moritani, Nagata, & Muro, 1982; Pringle & Jones, 2002).

This master thesis is divided into two parts: introduction and empirical part. Introduction will depict concept of maximal lactate steady state (MLSS) and related subjects considering stable and unstable states of metabolism: aerobic and anaerobic interaction. Furthermore, the basics of surface electromyography (sEMG) will be discussed followed by analysis and interpretation of sEMG signals. In addition, biomechanics of cycling will be explained together with important aspects which are influencing the cycling performance. The end of

introduction part contains brief and comprehensive overview of studies which investigated sEMG during cycling at different constant-loads. The empirical part consists of methodology implemented to investigate neuromuscular behavior of lower limb muscles during cycling at MLSS and above MLSS. Finally, the results will be discussed and elaborated altogether with similar studies.

1.1 Maximal Lactate Steady State (MLSS)

Maximal Lactate Steady State (MLSS) has gained the more importance during the last decade. It has been established as a gold standard for estimation of the anaerobic threshold during laboratory tests (Billat, Sirvent, Py, Koralsztein, & Mercier, 2003; Dekerle et al., 2003; Ferreira et al., 2007; Goodwin, Harris, Hernandez, & Gladden, 2007). Furthermore, this chapter will first provide a basic introduction to aerobic and anaerobic metabolism as well as aerobic to anaerobic transition during exercise and at the end it will discuss anaerobic threshold (AT), lactate threshold (LT) and MLSS. Furthermore, implementation of different methods in evaluation of AT and MLSS will be discussed.

1.1.1 Aerobic metabolism

During aerobic metabolism, body requires a steady supply of oxygen in order to re-synthesize adenosine triphosphate (ATP) from aerobic processes and namely from aerobic glycolysis and fat oxidation (Kenney, Wilmore, & Costill, 2012, p. 58) (cf. Fig 1). It has to be emphasized that at the very beginning of an activity regardless of intensity all energy substrates are used as an energy sources with the predomination of ATP-CP pathways followed immediately by anaerobic glycolysis (Birch, MacLaren, & George, 2005). As the physical activity progress with the time, the anaerobic mechanisms are suppressed by aerobic processes with predomination of fat and aerobic glycolysis energy supply (Kenney et al., 2012; Nikolić, 1995). During the aerobic energy processes, ATP re-synthesis is taking place in the special cell organelles called mitochondria (Kenney et al., 2012, p. 58). Positive correlation between aerobic capacity and the numbers of mitochondria in the muscles has been confirmed (Coen et al., 2013; U. F. Rasmussen & Rasmussen, 2000; Ulla F. Rasmussen et al., 2001). Unlike the anaerobic ATP production, aerobic system is slower in energy

supply, but it has a larger energy capacity and therefore aerobic system is the primary energy supplier during long –term exercise activities (Kenney et al., 2012, p. 58)

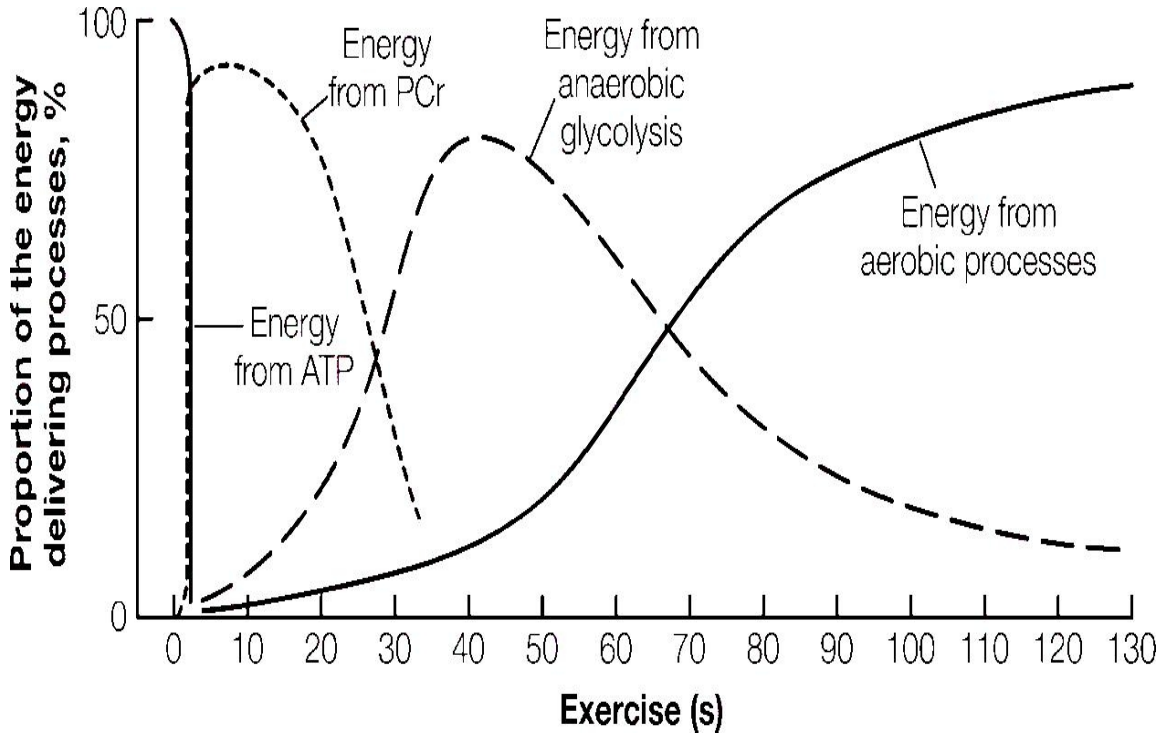

Fig. 1: *Schematic of the energy continuum. (It is important to note that each of these mechanisms of energy production occur simultaneously) (Shaw, Gorely, & Corban, 2004, p. 12)*

1.1.2 Anaerobic metabolism

High-intensity exercise requires the rapid re-synthesis of ATP to provide energy for musclar contraction. While the oxidative pathway provides a relatively efficient but slow re-synthesis of ATP, the anaerobic pathways are relatively fast. Unlike the aerobic production of ATP that takes place in the mitochondria of the cells, anaerobic production of ATP occurs within the cytoplasm of the cells (Kenney et al., 2012, p. 58). Anaerobic endurance is determined by the capacities of the two anaerobic energy pathways: (1) the hydrolysis of intramuscular stores of ATP and CP (together known as high-energy phosphates or the ATP-CP system); (2) anaerobic glycolysis. Together, these pathways provide large energy contributions to short-duration, high-intensity exercise (Whyte, 2006, pp. 86-87).

1.1.3 Anaerobic pathways

ATP-CP Pathway: This pathway is often considered the immediate system that provides energy at the start of high-intensity exercise. It is however relatively short term in duration. The hydrolysis of intramuscular stores of the high-energy phosphates ATP and CP provide energy for muscular contraction during maximal exercise. The store of ATP in skeletal muscle is very small (5 $mmol.l^{-1}$ of muscle) and can fuel exercise for just a couple of seconds (cf. Fig. 2). Rather than completely depleting this reserve of ATP, however, the CP system offers an immediate supply of energy for the rapid re-synthesis of ATP (Whyte, 2006, pp. 87-88).

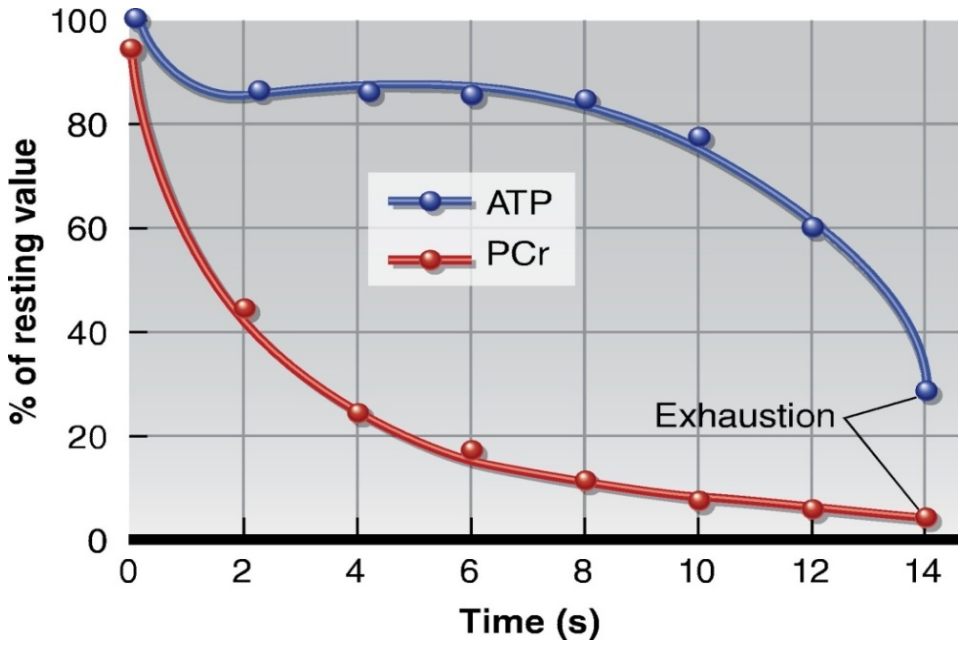

Fig. 2: *Changes in type II (fast twitch) skeletal muscle adenosine triphosphate (ATP) and phosphocreatine (PCr) during 14s of maximal muscular effort (Kenney et al., 2012, p. 55)*

Anaerobic Glycolysis: During high-intensity exercise it is almost entirely muscle glycogen rather than extracellular glucose that is metabolized. Although not quite as rapid as the ATP-CP system, glycolysis synthesizes ATP at a faster rate than achieved through the oxidative pathway (Whyte, 2006, p. 89) (cf. Tab. 1).

Tab. 1: *Characteristics of the various energy supply systems*

Energy system	Oxygen necessery?	Overall chemical reaction	Relative rate of ATP formed per second	ATP formed per molecule of substrate	Availabele capacity
ATP-PCr	No	PCr to Cr	10	1	<15s
Glycolysis	No	Glucose or glycogen to lactate	5	02. Mrz	~ 1 min
Oxidative (from CHO)	Yes	Glucose or glycogen to CO_2 and H_2O	2.5	36-39	~ 90 min
Oxydative (from fat)	Yes	FFA or triglycerides to CO_2 and H_2O	1.5	>100	days

Source: Adapted from (Kenney et al., 2012, p. 65)

1.1.4 Lactate Threshold (LT)

The lactate threshold (LT) is the point at which blood lactate concentration begins to substantially accumulate above resting values during exercise of increasing intensity (Kenney et al., 2012). In the research literature mainly are used two LTs, namely LT1 and LT2. LT1 corresponds to aerobic threshold and it is categorized as the first increase in [La] whereas LT2 is defined as second increase in [La] and corresponds to anaerobic threshold (Faude, Kindermann, & Meyer, 2009). During a progressive incremental exercise test, there is a range of mild workloads in which there is only a minimal or no increase in blood (and presumably muscle) lactate concentration. With further increases in work rate, a "threshold" is reached beyond which there is an abrupt or rapid increase in blood lactate concentration as workload increases further (Tipton, 2003) (cf. Fig. 3). The LT2 has been considered to reflect the interaction of the aerobic and the anaerobic energy system. However, blood lactate concentration is achieved not only by the production of but also by the removal of lactate from the blood by the liver, skeletal muscle, cardiac muscle, and other tissues in the body. Thus, LT2 is best defined as that point during exercise where the rate of lactate production exceeds the rate of lactate clearance or removal (Kenney et al., 2012).

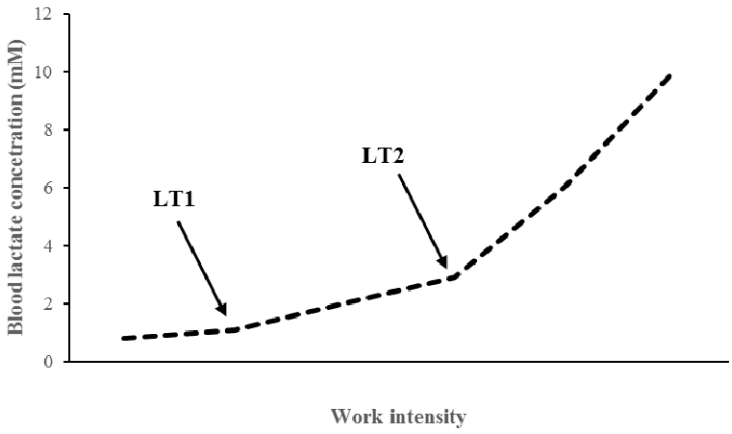

Fig. 3: *LT1 and LT2 during incremental ergometer cycling. Adapted from Fauede et al. (2009) (Faude et al., 2009)*

1.1.5 The interplay between aerobic and anaerobic metabolism

One of the fundamental facts in physiology of sports performance states that during activities that involve whole body movements such as running, cycling, swimming and rowing, adenosine triphosphate (ATP) production is the outcome of an interaction between aerobic and anaerobic metabolism.

It is well known that marathon runners may have near-resting lactate level at the end of the race, despite their exhaustion (Wilmore & Costill, 2004). This implies that a basic component of their metabolism includes predominantly aerobic energy supply, although the pace intensity of marathon runners, for some other individuals may be predominantly covered with anaerobic energy supply.

The major energy source at the very beginning of exercise are carbohydrates from muscle and blood, glycogen and glucose, respectively, but as the exercise proceed it has been observed a greater dominance of free fatty acids (FFA) either from intramuscular triglyceride stores or from adipose tissue (Birch et al., 2005).

Some authors have (S. P. Brown, Miller, & Eason, 2006) stated that primary energy source for exercise below 30% to 40% of VO_2max are fat and carbohydrates (aerobic pathway), whereas primary energy source for exercise above 60% to 70% of VO_2max is carbohydrates (anaerobic pathway). At the exercise intensity between 40% to 60% of VO_2max, the energy demands are balanced between aerobic and anaerobic energy pathways (S. P. Brown et al., 2006) (cf. Fig. 4).

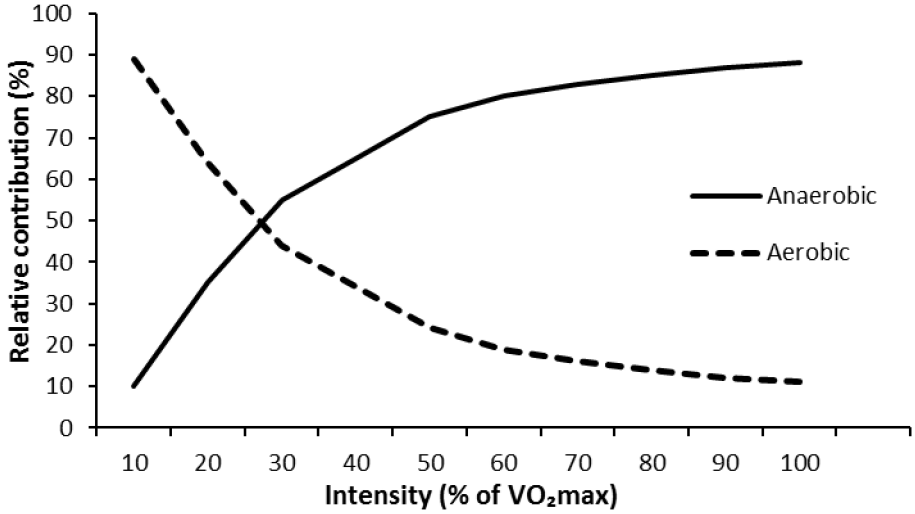

Fig. 4: *Relative contribution of aerobic vs. anaerobic energy sources as exercise intensity increases. Adapted from Arthur T. Johnson (1991) (Johnson, 1991)*

A similar pattern of relative contribution of aerobic vs. anaerobic energy supply, herewith can be observed during constant light to moderate exercise intensity that has been performed over a longer period of time (cf. Fig. 5), emphasizing that shift from aerobic to anaerobic metabolism is a time dependent.

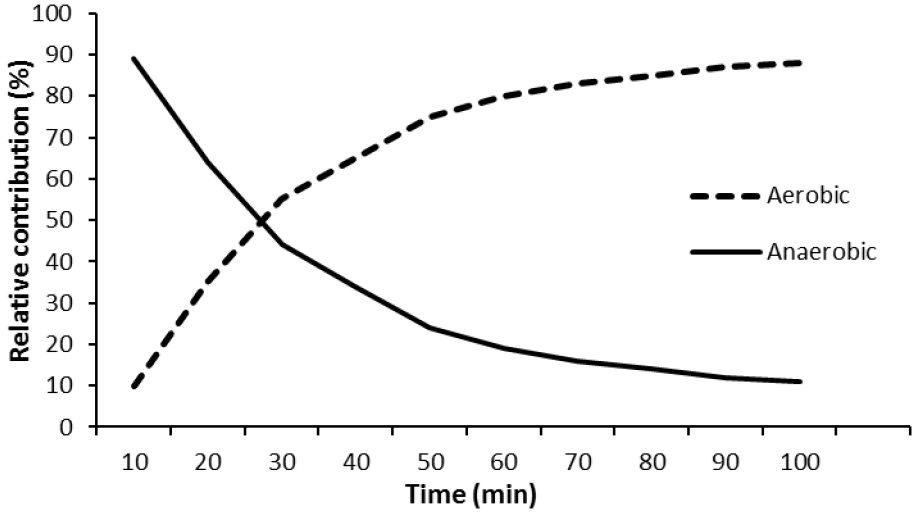

Fig. 5: *Relative contribution of aerobic vs. anaerobic energy sources during constant light to moderate exercise intensity as a function of the time. Adapted from Arthur T. Johnson (1991) (Johnson, 1991)*

In this case aerobic energy supply is dominant over anaerobic energy pathways as the time of exercise increases.

It considers factors such as gender, age, state of training, nutritional status, physiologic make up of skeletal muscle (fast twitch-slow twitch fibers) and hormonal status (S. P. Brown et al., 2006).

1.1.6 Maximal Lactate Steady State (MLSS)

Maximal lactate steady state (MLSS) has been defined as the highest constant power output that can be maintained without a progressive increase in blood lactate concentration [La] over a period of time (R. Beneke & von Duvillard, 1996; Heck et al., 1985; Jones & Doust, 1998) (cf. Fig. 6). Practically, this is maximal intensity for which [La] increases less than ≤1 mM during the last 20 minutes of a 30 minute constant workload test (Goodwin et al., 2007).

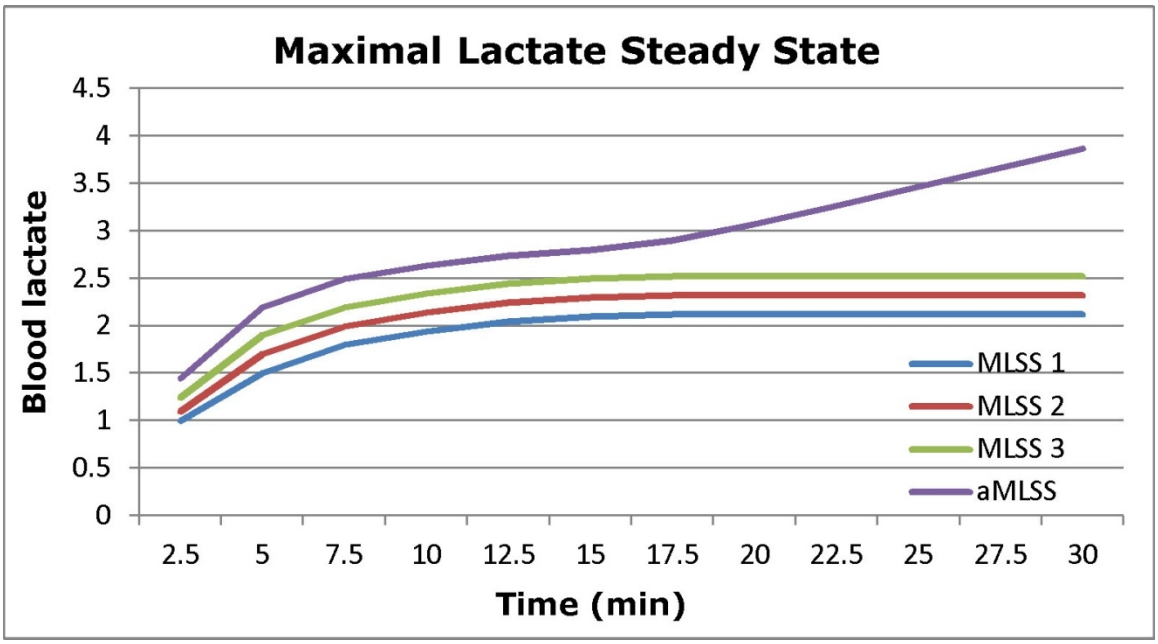

Fig. 6: *Maximal Lactate Steady State (MLSS) test performed on different days and the four different workloads: 220 W, 230 W, 240 W and 245 W respectively.*

High individual variability in terms of lactate concentration at the MLSS level exists between different subjects (Tomasits & Haber, 2011). Nevertheless, blood lactate concentration [La] at MLSS is independent from the endurance performance level, whereas no significant difference has been found between genders regarding MLSS [La] (Smekal et al., 2012).

Anaerobic metabolism has an increasingly important contribution to net energy demand of exercise when the intensity of exercise goes above MLSS level (Pringle & Jones, 2002). It has been demonstrated that MLSS intensity can be sustained for even more than 60 minutes, whereas just a small increase in workload above MLSS intensity has led to a dramatic exhaustion and reduction in time of exercise (Baron et al., 2008; Pires et al., 2011).

1.2 Surface Electromyography (sEMG)

Electromyographic (EMG) testing involves evaluation of development, recording and analysis of myoelectric activity of a muscle (Konrad, 2005, p. 4; Massó et al., 2010, p. 128; Weiss, Weiss, Silver, & Weiss, 2004, p. 41). Electrical muscle activation pattern can be recorded with the two main EMG methods: intramuscular electromyography and surface electromyography. Within the first method, a thin wire is implemented directly into muscle. Second method is using two electrodes that are placed on the top of the muscle belly of interest and it measures the difference in potential at the two electrodes (Latash, 2012, p. 297)

Surface electromyography (sEMG)[1], is mostly performed in studies of human locomotion that involve large muscles with the muscle bellies close to the surface of a body segment, whereas intramuscular electromyography is mainly used in clinical studies (Latash, 2012, p. 297).

1.2.1 Basics of Surface Electromyography (sEMG)

The use of sEMG has many advantages. Surface EMG recording provides a safe, easy, and noninvasive method that allows objective quantification of the electrical signals that reach the muscle (Cram & Criswell, 2011, p. 5). Since the sEMG method provide us information

[1] For more information cf. Latash (2012), Enoka (2008)

about changes of action potential of sarcolemma, which is generated through activity of motor neurons, it also provide insight into condition of nervous system of the body (Ilic & Mrdakovic, 2009, p. 189).

In order to record the differences of myoelectric activity of muscle fibers, specific electrodes have to be applied above muscle belly and on the surface of the skin. For sEMG use, silver/silver chloride pre-gelled bipolar electrodes have demonstrated the best reliability and they are recommended for the general use from Surface Electromyography for Non-Invasive Assessment of Muscles (SENIAM) (Ilic & Mrdakovic, 2009, p. 190; Konrad, 2005, p. 15). The most recommended size of bipolar electrodes intended for muscle fibers should not exceed 10 mm in diameter with inter-electrode distance (IED) that does not exceed 20 mm (H.J. Hermens et al., 1999, pp. 109-110; Ilic & Mrdakovic, 2009, p. 193)

1.2.2 Nature of sEMG signal

Excitability of muscle fiber plays very important role in muscle physiology. The muscle fibers have intracellular resting potential of approximately –80 to – 90 mV that is similar to nerve fibers (Weiss et al., 2004, p. 41). If the Na^+ influx through the cell membrane exceeds the specific threshold level, depolarization of the membrane occur which in turn evoke change in action potential from –80 /– 90 mV up to + 30 mV. The action potential from a motor neuron is passing through the neuromuscular junction and then propagates along the sarcolemma. Depolarization will be further followed by spreading of action potential through the system of T-tubules which causes the release of calcium from the sarcoplasmic reticulum. Calcium enables contact between actin and myosin filaments that in the end results with the shortening of the sarcomere (Weiss et al., 2004, p. 41).

The action potential forms an electrical dipole (positive and negative wave form) which travels along the muscle fiber (cf. Fig. 7).

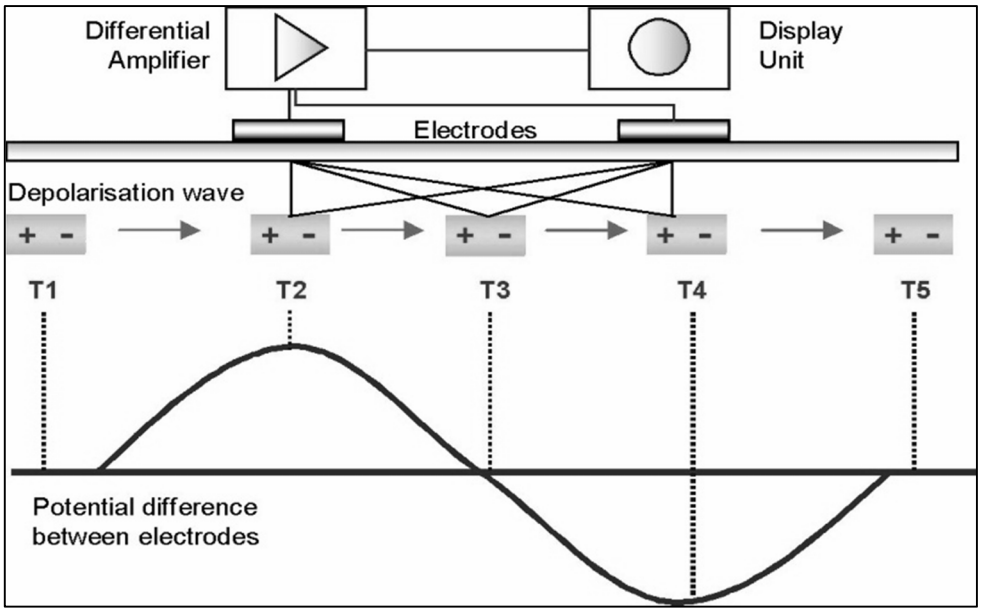

Fig. 7: *Electrical dipole of action potential, which travels through muscle fiber between two electrodes (Konrad, 2005, p. 8)*

Hence, electrodes records changes of action potential as positive when positive ions are moving out from the cell and vice versa (Ilic & Mrdakovic, 2009). The final result is the interference EMG signal (iEMG) that represent the superposition of all active motor unit action potentials (MUAP) that are summed in an bipolar signal with proportional distribution of positive and negative amplitudes (cf. Fig. 8) (Konrad, 2005, p. 9).

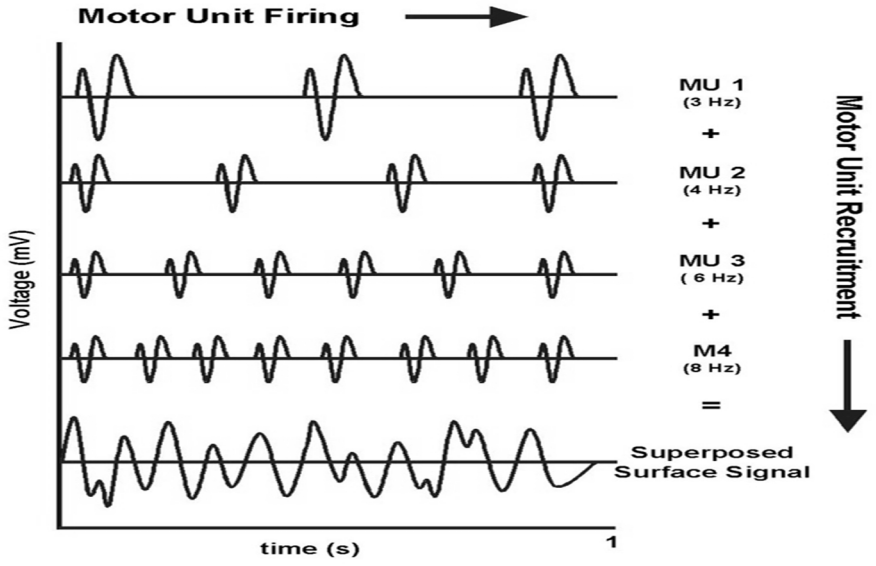

Fig. 8: *Superposition of MUAPs (Konrad, 2005, p. 9)*

1.2.3 External Factors influencing the sEMG Signal

Several external factors alter shape and characteristics of sEMG signal on its way through muscle fibers (Ilic & Mrdakovic, 2009, p. 193; Konrad, 2005, p. 12). External factors that have influence on sEMG signal can be generally grouped in several factors:

Tissue characteristics

The electrical conductivity of human body is influenced by tissue type, thickness, physiological changes and temperature (Konrad, 2005, p. 9). One of the most influential tissue factors on sEMG signal represent amount and size of subcutaneous fat tissue i.e. its thickness (cf. Fig 9.)

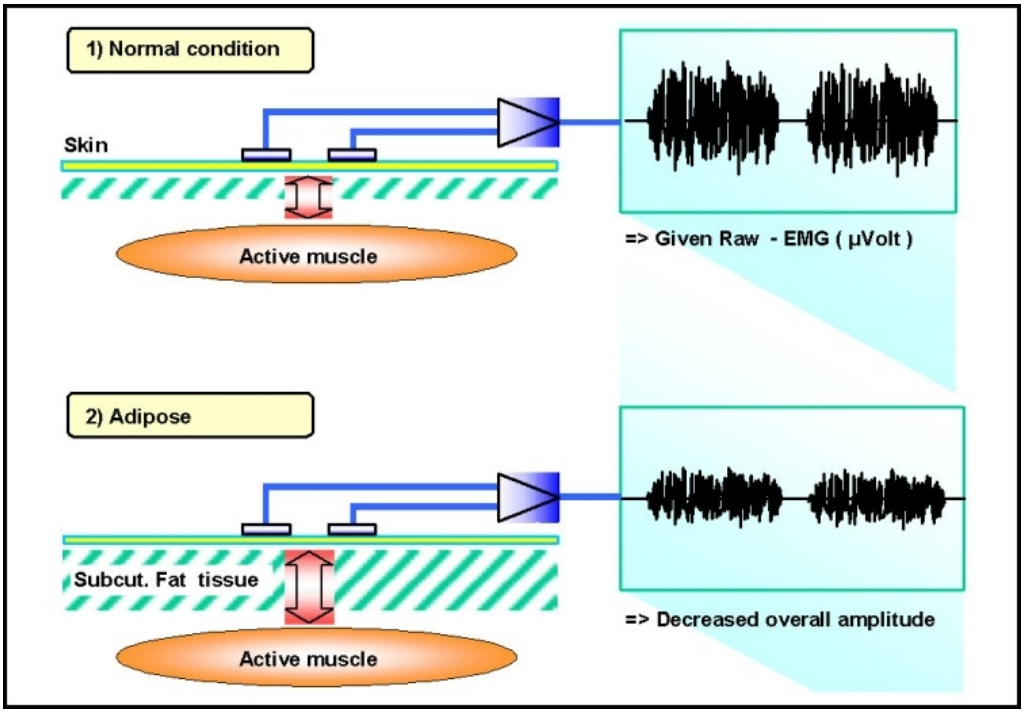

Fig. 9: *Different thickness of fat tissue and its influence on amplitude of raw sEMG signal (Konrad, 2005, p. 12)*

Cross talk

Cross talk is a phrase that indicates the amount of sEMG that is detected by neighboring muscles (10-15 % of overall signal). Electrocardiography (ECG) signals can also interfere with the regular sEMG signal, especially when upper body recording is performed (Konrad, 2005, p. 12).

Movement artifacts

Any change in geometry and distance between muscle belly and electrode site i.e. between signal origin and receiver will evoke alteration of the sEMG recording. Special caution has to be taken in terms of motion of electrodes during dynamic movement. Therefore electrodes have to be located in a central position over the muscle belly (Konrad, 2005, p. 12).

Ambient electromagnetic fields and power line noise

Ambient electromagnetic fields exist in the vicinity of AC 120 V (or 230 V) power lines and electric equipment such as monitor, computer, light bulbs and etc. Whenever possible, EMG, recording should be performed far away from big electronic devices, cell phones, cables, etc.

Electrode and amplifiers

The appropriate selection of electrodes and internal amplifier noise is of great importance because they may add signal contents to EMG baseline. Internal amplifier noise should not exceed 5 Vrms (Konrad, 2005, p. 12).

1.2.4 Processing of sEMG Signal

Surface EMG (sEMG) signal is basically a function of time and is measurable in terms of its amplitude, frequency and phase (Reaz, Hussain, & Mohd-Yasin, 2006, p. 11). Mathematical representation of classic model of EMG signal can be described by the following equation:

$$x(n) = \sum_{r=0}^{N-1} h(r)e(n-r) + w(n)$$

where *x(n)*, modeled EMG signal, *e(n)*, point processed, represents the firing impulse, *h(r)*, represents the MUAP, *w(n)*, zero been addictive white Gaussian noise and *N* is the number of motor unit firings.

However, whenever an EMG signal is being recorded various types of noises contaminate it with artifacts and therefore one of the major problems in surface electromyography is how to remove artifacts and noise from raw sEMG data, but in the same time to preserve as much as possible signal data from the original recordings (Chowdhury et al., 2013; Reaz et al., 2006). Nevertheless, the raw EMG data already contains valid information that may serve

as the first step in evaluation of muscle mechanics such as onset and offset of myoelectric activity (Konrad, 2005).

Hardware filters such as notch filters should not be used with the exception of the amplifier bandpass (10-500 Hz) filters that are necessary in order to avoid anti-aliasing effects within sampling (Konrad, 2005, p. 27).

Before being evaluated, the signal can be processed with a post hoc method to eliminate low-frequency or high-frequency noise or artifacts (Konrad, 2005; Reaz et al., 2006). Mostly, the amplitude of the signal is frequently researched in studies. Result of that signal is often rectified and averaged with specific method to indicate EMG amplitude (Reaz et al., 2006). It seems that that mostly used averaging and spectral analysis methods in kinesiological studies are root mean square (RMS) and median frequency (MDF) respectively, which will be in detail elaborated in the following chapters.

1.2.5 Full Wave Rectification

Surface EMG signal envelops the information regarding the activation pattern of muscle fibers and therefore provides the concrete insight into fire timing and amplitude of motor units (Enoka, 2008, p. 201). Even though the fire timing and amplitude parameter of the sEMG signal can be evaluated from interference EMG, the rectification and smoothing of the raw sEMG signal has to be performed in order to minimize as much as possible the random nature of the interference (raw) EMG (iEMG) signal (Basmajian, 1978, p. 95). Protocol of rectification involves utilization of only the positive deflection of the signal. This can be done either by pure elimination of negative deflection o (half wave rectification) or by converting the negative amplitudes to positive amplitudes (full wave rectification) (cf. Fig. 10). The second protocol is often used because it retains all the power of the signal (Basmajian, 1978, p. 95).The rectification of sEMG signal, besides easier visualization, assures that basic amplitude parameters such as mean, peak/max value and area trends can be calculated and displayed as a curve as a function of the time (Konrad, 2005, p. 27). The rectified EMG signal is further filtered in order to obtain smoothed pattern. The extent of smoothing is variable with regard to the protocol and purpose of the study (Enoka, 2008, p. 201).

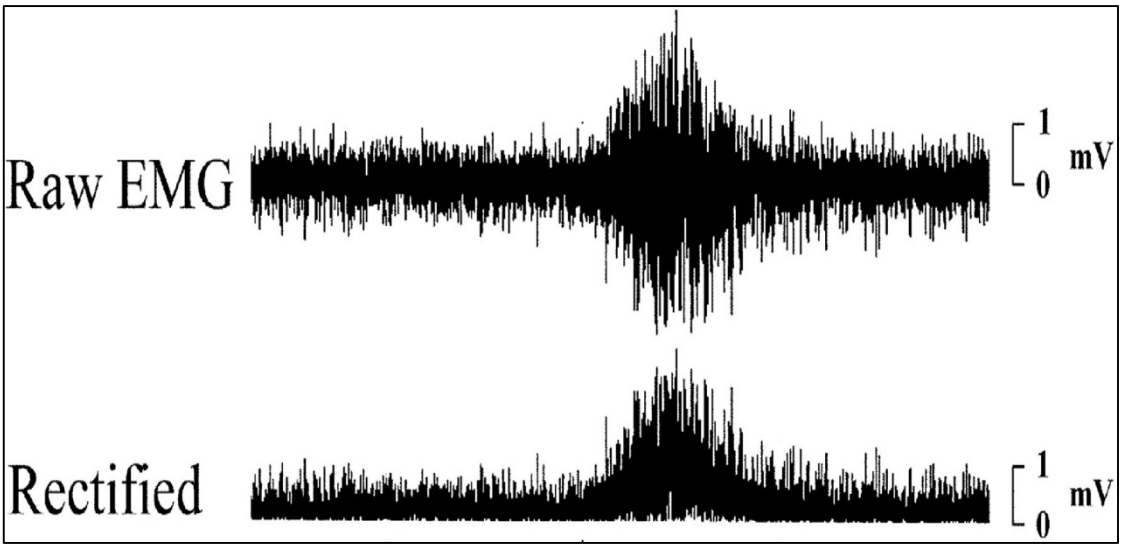

Fig. 10: *The full wave rectified EMG signal. Adapted from Lang et al. (Lang, Medda, & Shaker, 2001, p. 1249)*

1.2.6 Smoothing

The rectified EMG signal still possesses a stochastic nature and as a result of that it is impossible to reproduce the raw EMG burst for the second time in exactly the same shape (Konrad, 2005, p. 28). In order to reduce this stochastic nature of the iEMG signal and to improve its reproducibility, two typical algorithms are established for smoothing the interference EMG: moving average (MOV) and root mean square (RMS) (Enoka, 2008; Konrad, 2005). Since the root mean square is more commonly used algorithm in kinesiology for estimation of the amplitude of MUAPs, it will subsequently be elaborated in detail within the next chapter.

1.2.7 Root Mean Square (RMS)

Root mean square generally represents the strength of the signal i.e. its amplitude and reflects the mean power of EMG signal (Konrad, 2005, p. 28; Massó et al., 2010, p. 131). RMS can be represented by the following mathematical equation:

$$RMS = \left\{ \frac{1}{s} \sum_{1}^{s} f^2(s) \right\}^{½}$$

It squares the data, summing the squares, dividing this sum by the number of observations, and finally taking the square root (Cram & Criswell, 2011, p. 48). It is known as a time domain variable because the amplitude of the signal is measured as a function of time. In scientific literature it can be found significant positive correlation between RMS and muscle force (Alkner, Tesch, & Berg, 2000; Hug & Dorel, 2009; Luca, 1997; Milner-Brown & Stein, 1975).

1.2.8 Frequency Spectral Analysis (FSA) of sEMG

Spectral analysis represents indirect estimation of muscle fiber conduction velocity (Farina, 2006, p. 124) and reflects the fatigue state of muscles during static and dynamic contractions (Cram & Criswell, 2011). CRAM's introduction to surface electromyography has summarized frequency spectral analysis in the following definition:

"FSA is a type of analysis in which the sEMG signal is recorded and submitted for a decomposition of the energy into its frequency components, typically using a Fast Fourier Transform (FFT). It may be used in verifying that the sEMG is being "clean" of 60Hz noise, as well as for the assessment of muscle fatigue" (Cram & Criswell, 2011, p. 389).

FSA encompasses power distribution of different frequency content (cf. Fig. 11) and as a result of that analysis Total Power Spectrum (TPS) graph of different frequency powers can be estimated (cf. Fig. 12) (Konrad, 2005, p. 41).

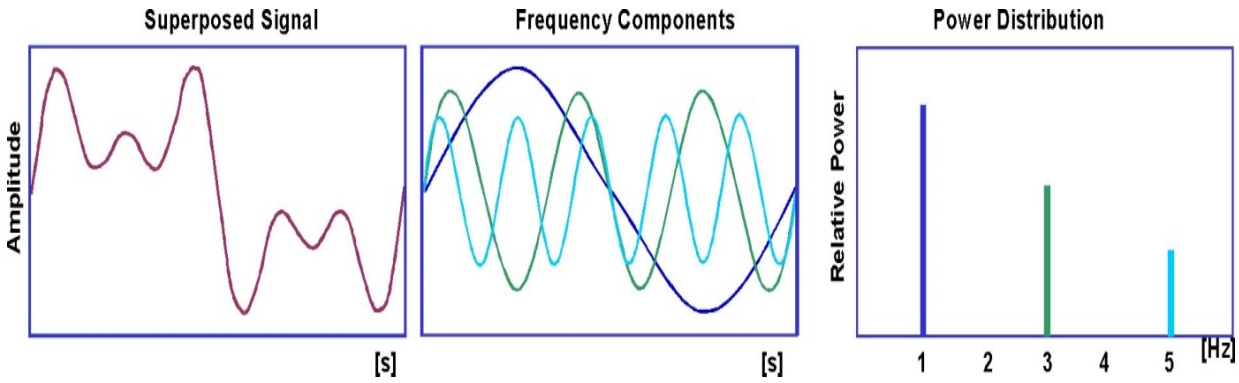

Fig. 11: *Power spectrum analysis and distribution of different frequency contents (Konrad, 2005, p. 41)*

1.2.8.1 Median Frequency (MDF)

Within Total Power Spectrum, besides MDF, it is possible to calculate other frequency parameters such as peak power, mean frequency and total power (TP) (cf. Fig. 12). In the movement studies MDF is mostly used as a parameter for spectrum analysis and as an indicator of muscle fatigue (Cifrek, Medved, Tonković, & Ostojić, 2009). Median frequency is representing the value (line) that divides TP area into two equal parts with the same amount of power (Konrad, 2005, p. 41).

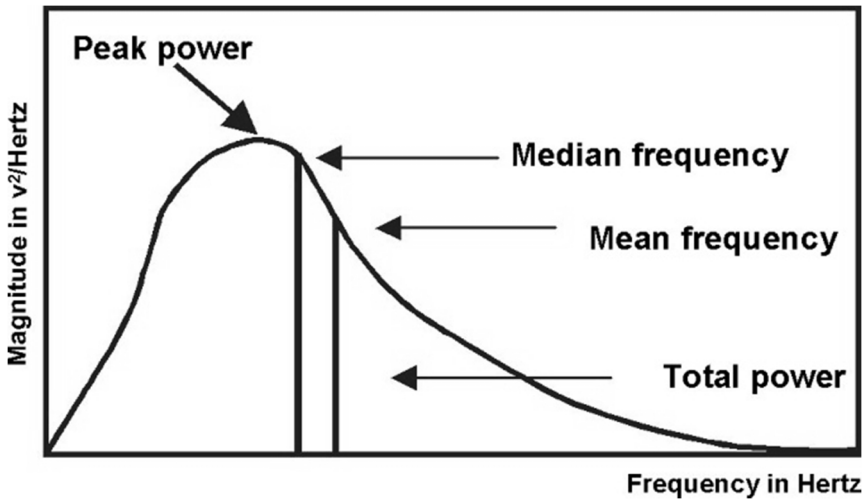

Fig. 12: *Frequency parameters estimated with the means of FFT (Konrad, 2005, p. 41)*

1.2.9 Normalization of the sEMG signal

Normalization is necessary to overcome inter and intra-subject variability caused by different electrode sites on the same muscle or on two different muscles, subcutaneous fat of subjects and different pattern of muscle activation during exercise task execution done by subjects (A. M. Hunter, St Clair Gibson, Lambert, & Noakes, 2002; Lehman & McGill, 1999).

In order to perform any reliable comparative analysis of sEMG signals the normalization of iEMG is prerequisite (Lehman & McGill, 1999).

Lehman et al. (1999, p.444-445) have defined the sEMG normalization using the following definition: „EMG normalization is the process by which the millivolts of activity are expressed as a percentage of that muscle's activity during a calibrated test contraction".

Different normalization methods and protocols are developed for dynamic and static movement analysis (Albertus-Kajee, Tucker, Derman, & Lambert, 2010; Fernández-Peña, Lucertini, & Ditroilo; Rouffet & Hautier, 2008). Nevertheless, one of the most employed normalization protocols is the maximal voluntary contraction (MVC) method (Burden & Bartlett, 1999; A. M. Hunter et al., 2002; Lehman & McGill, 1999; Nicholson, 2000; Rouffet & Hautier, 2008).

Alternatives to MVC normalization protocol were proposed (Albertus-Kajee et al., 2010; Burden & Bartlett, 1999; Konrad, 2005; Rouffet & Hautier, 2008). The common normalization protocols used as a substitute to MVC protocol are averaging the amplitude of rectified smoothened EMG signal to the mean or the maximum amplitude value (Konrad, 2005, p. 35). The main outcome of such normalization approach is diminished variability i.e. lower values of coefficient of variance (CV).

1.3 Biomechanics of Cycling

Cycling activity has gained more attention in research literature during the last decades. This is due to its simplicity and comprehensive utility in research areas such as performance, rehabilitation and testing of human locomotion. On the other hand subsequent consequence of intensive and extensive cycling activity has resulted in the growing number of injuries and other complications of body system. This has led to increased rate of research publications in order to understand the influences of different cycling workloads, body positions and complex muscle mechanic on human body during cycling (Fonda & Sarabon, 2010).

Varieties of biomechanical, anthropometrical, physiological and environmental factors affect the mechanic and technique (kinetic and kinematic) of cycling (anthropometry of rider, height of seat and handlebars, pedals, wind, temperature, humidity, friction force of wheels, structure of terrain, fitness level, etc.).

However, lots of environmental factors such as wind resistance, outdoor temperature and structure of terrain that affects mostly the mechanic of cycling, are not of interest in laboratory tests. Therefore, there is distinct difference between ergometer cycling and cycling in real outdoor conditions. Nonetheless, in the following chapters the nature of cycling will be elaborated regardless of the environmental factors whose influence on cycling mechanic has been shown.

1.3.1 Basic concepts and terminology in cycling

The terminology used in cycling community is mainly unknown to the general population and therefore it will be outlined in this chapter in order to ease the understanding of basic concepts of cycling activity.

The saddle's (seat) height has shown significant influence on muscle's activation pattern during the cycling and it is defined as a distance between top part of the saddle and the pedal axle, with the pedal in the lowest position (Fonda & Sarabon, 2010). Saddle's position can be additionally adjusted with regard to tilt and forward/back position.

The handlebar position can be positioned according to distance from ground and saddle (Fonda & Sarabon, 2010). Today are known two types of handlebars that have demonstrated different effects on cycling technique and muscle mechanic: triathlon and road bike handlebars.

Saddle and handlebar position are of high importance in studies of ergometer cycling and therefore they always have to be always standardized individually for every test.

The position of pedals during one revolution is crucial for understanding of biomechanical aspects of cycling and therefore two main pedal positions are identified as:

- Top dead center (TDC)
- Bottom dead center (BDC)

As a function of pedal's angle these two main positions are corresponding to 0° and 180° of crank angle for TDC and BDC respectively (Castronovo, Conforto, Schmid, Bibbo, & D'Alessio, 2013; Fonda & Sarabon, 2010).

Phases that can be distinguished during one pedal's revolutions are (cf. Fig. 13) (Castronovo et al., 2013):

- The downstroke phase (from 0° to 180°)
- The upstroke phase (from 180° to 360°)
- Two transitional phases (± 5° from TDC and BDC)

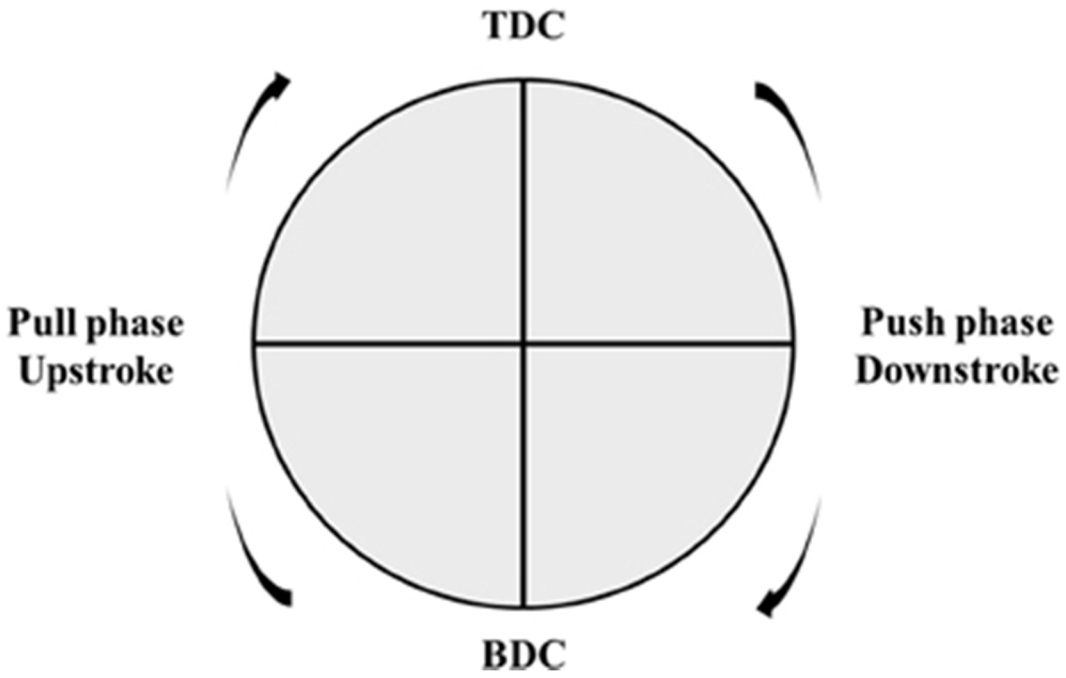

Fig. 13: *Phases of one revolution during cycling (Castronovo et al., 2013, p. 3)*

1.3.2 Biomechanical aspects of cycling

Several biomechanical factors such as seat and handlebar position and height, bike frame and crank length affect the pedaling performance and a way the main muscles are involved in the cycling task, but above all the pedaling technique has demonstrated the highest impact on cycling efficiency and performance (Castronovo et al., 2013).

Mechanical efficiency of cycling task is mainly related to the distribution of forces applied on the pedal (cf. Fig. 14). As result of leg muscle activity on the pedal, three main force components can be distinguished. Deeper observation into interplay between those three force components can give explanation about mechanical efficiency of cycling. Resultant force (Ftot) in the Fig. 14 is a superposition of two forces, tangential force (Ft) which is perpendicular to the crank and centrifugal force (Fn) which is perpendicular to Ft and goes alongside the crank (Fonda & Sarabon, 2010). Tangential component (Ft) solely contribute to mechanical work during cycling while Fn does not and generally represent dissipation of energy applied on pedals. The highest value of Ft can be observed at the crank angle of 90° and 270° whereas the highest Fn occur at the angle between 120° and 190° (Ericson & Nisell, 1986, 1987).

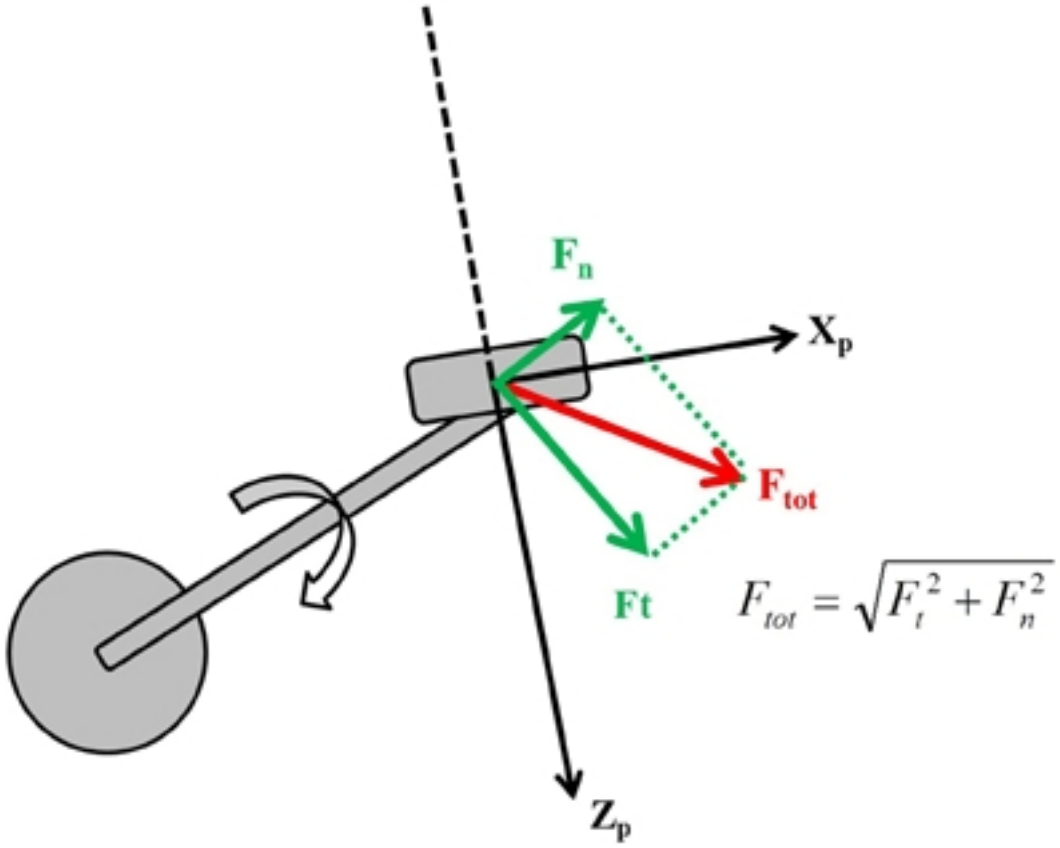

Fig. 14: *Diagram of forces as a result of leg activity on the pedal (Castronovo et al., 2013, p. 3)*

There are contrasting results in scientific literature in terms of optimal paddling cadence (Castronovo et al., 2013). The studies of Abbiss and Laursen (2009) and Theurel et al. (2012) have demonstrated that optimal pedaling cadence with respect to neuromuscular efficiency is the one which decreases the overall muscle activity and muscular fatigue (Abbiss & Laursen, 2005; Theurel, Crepin, Foissac, & Temprado, 2012). Some studies are recommending approximately 90-110 revolutions per minute (RPM) in order to maximize neuromuscular efficiency (F. Bieuzen, Vercruyssen, Hausswirth, & Brisswalter, 2007; Neptune & Herzog, 2000), however, some authors stated that extremely high cadence increases the metabolic cost of cycling and propose lower RPM (<90) that are more convenient for beginners and recreational athletes, achieving during lower workload i.e. intensity (F. Bieuzen et al., 2007; Castronovo et al., 2013; Chavarren & Calbet, 1999; Timmer, 1991; Vercruyssen & Brisswalter, 2010).

1.3.3 Electromyography (EMG) during cycling activity

Very important utilization of sEMG technique, among all other things, is to define onset and offset of muscle activity during specific motor tasks, namely in this case during the one crank revolution. Muscles typically involved in a cycling activity are presented in the Fig. 15.

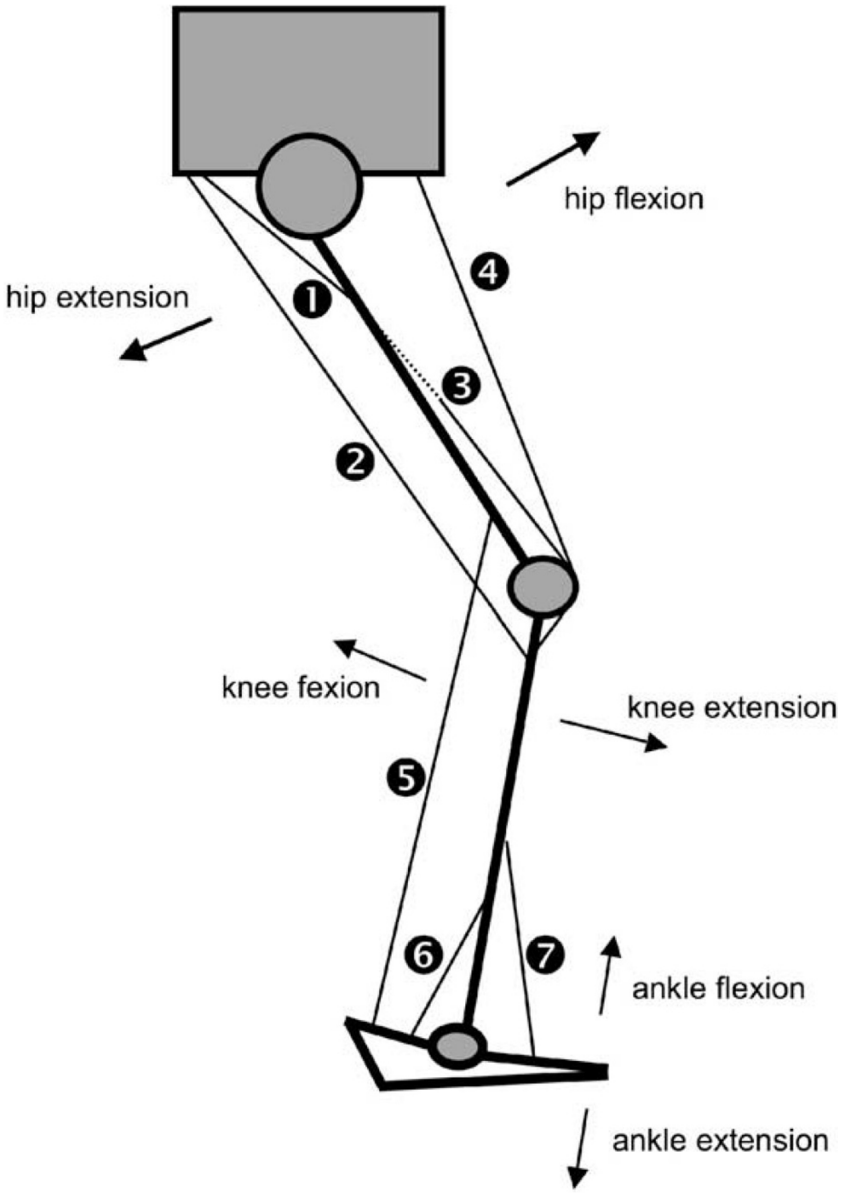

Fig. 15: *Schematic description of involved muscles during cycling activity:1) Gluteus Maximus (GM), 2) Biceps Femoris (BM), 3) Vastus Lateralis (VL) and Vastus Medialis (VM), 4) Rectus Femoris (RM), 5) Gastrocnemius (Gast), 6) Soleus (SOL) and Tibialis Anterior (TA) (Hug & Dorel, 2009, p. 187)*

It has to be emphasized that 8 fundamental muscles from Fig. 15 are mostly studied during sEMG protocol, while activation pattern of deeper musculature of lower limbs are only possible to be evaluated with the intramuscular electrodes (Hug & Dorel, 2009).

Various authors reported difference in a function between mono-articular and bi-articular muscles (Hug, Bendahan, Le Fur, Cozzone, & Grélot, 2004; Ryan & Gregor; G. J. van Ingen Schenau, Boots, de Groot, Snackers, & van Woensel, 1992). In the study of Ryan and Gregor (1992) has been noted that mono-articular muscles (GM, VL, VM, TA and SOL) are the main power producers. Contrary, the muscles that produce force over two joints (bi-articular muscles: BF, RF and Gast) have demonstrated greater performance and activation pattern variability (Hug, Bendahan, et al., 2004; Ryan & Gregor, 1992). The role of bi-articular muscles can be partly explained by their ability to transfer the energy between joints at the critical moment during cycling (G. J. van Ingen Schenau et al., 1992). On the other hand the role of bi-articular muscles as controllers of the direction of force production on the pedal is well established in the literature (Hug, Bendahan, et al., 2004; G. J. van Ingen Schenau et al., 1992; G J van Ingen Schenau et al., 1995; Gerrit Jan van Ingen Schenau, Pratt, & Macpherson, 1994).

In terms of co-activations of agonist/antagonist muscle groups over leg joint during cycling is well researched and elaborated in research literature (Hirokawa, 1991; Hug & Dorel, 2009; Lombard, 1903; Sanderson, 1991; Solomonow, Baratta, Zhou, & D'Ambrosia, 1988; G. J. van Ingen Schenau et al., 1992). This co-activation pattern was first explained by Lombard (1903) during extension of the knee. Several mechanisms can be responsible for agonist/antagonist co-activation, however the most reasonable explanations are joint stability (Hirokawa, 1991) and equalizing the pressure distribution in the articular surface (Solomonow et al., 1988).

In the Fig. 16 is presented activation pattern of leg muscles and their corresponding mean onset, offset and duration of EMG activity during one crank revolution according to the Hug and Dorel (2009, p.190ff.).

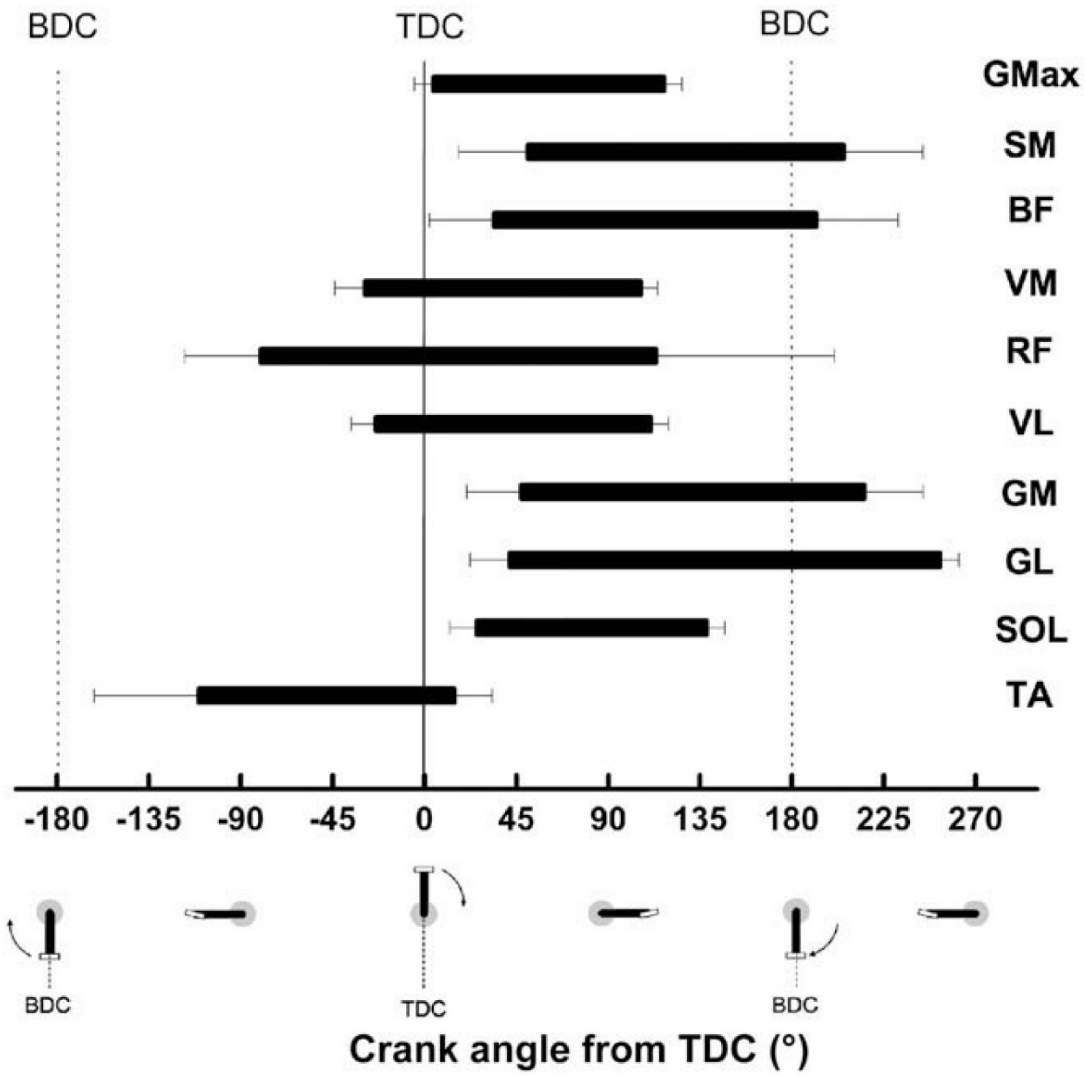

Fig. 16: *Muscle activity timing in lower limb muscles during one pedaling: Gluteus maximus (GMax), Semimembranosus (SM), Biceps femoris – long head (BF), Vastus medialis (VM), Rectus femoris (RF),Vastus lateralis (VL), Gastrocnemius medialis (GM), Gastrocnemius lateralis (GL), Soleus (SOL), Tibialis anterior (TA) (Hug & Dorel, 2009, p. 190)*

Onset and offset of muscle activity in different muscles of the leg are listed in Table 2[2] (values are presented after Hug and Dorel, 2009).

[2] For more information cf. Hug and Dorel (2009)

Tab. 2: *Onset and offset of muscle activity during on crank revolution and their corresponding crank angles*[3]

Muscle	Onset (crank angle)	Offset (crank angle)
Gluteus Maximus	0° (TDC)	**130°**
Biceps Femoris	45°	**-15° (195°)**
Vastus Medialis	-30° (330°)	**110° - 115°**
Rectus Femoris	-80° (280°)	**115° - 120°**
Vastus Lateralis	-25° (335°)	**115° - 120°**
Gastrocnemius Medialis	45°	**-30° (210°)**
Soleus	20° - 25°	**135°**
Tibialis Anterior	-115° (245°)	**15°**

Source: Adapted from Hug & Dorel (2009)

[3] For the better understanding angle values in the table 3 are described as positive or negative i.e. positive angle values represent full circle of one crank revolution from 0° (TDC) to 360° (BDC), whereas negative angle values are from -180° (BDC) to 0° (TDC)

1.4 Surface electromyographic (sEMG) behavior during ergometer cycling at MLSS and aMLSS: overview of literature

Surface electromyography (sEMG) as a non-invasive method for assessment of muscle activity is well established and accepted in the sport scientific and clinical community. Uniqueness of cycling, unlike other cyclical movements (e.g., running or swimming), can be explained by postulate that the cycling constraints lower body muscles to preserve a somewhat repeatable kinematic and mechanic pattern (Ryan & Gregor; Suzuki, Watanabe, & Homma, 1982). This is generally expected when the environmental conditions are stable (workload, cadence, body position, seat height and handlebar position) (Baum & Li, 2003; François Bieuzen, Lepers, Vercruyssen, Hausswirth, & Brisswalter, 2007; Chapman et al., 2008; S. Dorel, Couturier, & Hug, 2009). Any deviations of muscle activation pattern, obtained by the sEMG signals, during steady state conditions should be reflected by either magnitude or the timing of the sEMG signal (Briscoe, Forgach, Trifan, & Malek, 2013; Pringle & Jones, 2002; Ryan & Gregor, 1992; Theurel et al., 2012).

Metabolic changes as well as physiological responds from the body during maximal lactate steady state (MLSS) and above maximal lactate steady state (aMLSS) intensities of exercises has been already elaborated in detail in the scientific literature (R. Beneke & von Duvillard, 1996; de Lucas, de Souza, Costa, Grossl, & Guglielmo, 2013; Dekerle et al., 2003; Smekal et al., 2012), but there is not much to be found in existing literature about electromyographic activity of a muscles during the constant-load cycling at MLSS and aMLSS. Anaerobic metabolism has an increasingly important contribution to net energy demand of exercise when the intensity of exercise goes above MLSS level (Pringle & Jones, 2002). It has been demonstrated that MLSS intensity can be sustained for even more than 60 minutes, whereas just a small increase in workload above MLSS intensity has led to dramatic exhaustion and reduction in time of exercise (Baron et al., 2008; Pires et al., 2011).

The study of Pringle and Jones (2002) has demonstrated increase in normalized iEMG over time for cycling workloads at MLSS and aMLSS, however this increase in iEMG was not statistical significant for both power outputs (Pringle & Jones, 2002). Their results have been explained due to the large inter-subject variability in iEMG response and the time needed to end exercise. An additional reason for non-significant increase in iEMG in the study of Pringle and Jones (2002) might be due to investigation of only of one muscle, namely vastus lateralis (VL).

Briscoe et al. (2013) have been investigating the relationship between normalized iEMG amplitude of vastus lateralis (VL) muscle at the three different power outputs (low, EMG_{FT} and high power output) that are related to the power output obtained at the EMG fatigue threshold (EMG_{FT}), defined as 70%, 100% and 130% of the estimated EMG_{FT}, respectively (Briscoe et al., 2013). The authors determined EMG_{FT} by incremental cycle ergometer test with 2 min stage and load increments of 25 Watts. The sEMG slopes at each stage were afterward compared. The highest power output with non-significant sEMG slope increase and the lowest power output with significant slope increase were used for EMG_{FT} determination. Average value (W) between those two workloads was taken as EMG_{FT}. The results of Briscoe et al. (2013) study indicated significant increase in slope of iEMG for 130% EMG_{FT} power output compared to iEMG slope of 100% EMG_{FT} power output. Again lack of investigation of more than one lower extremity muscle in the study might be limiting factor.

Electromyorgaphic activity (particular iEMG) has been examined at the level of 10% below ventilatory threshold (VT) and 30% above VT in the study of Shinohara and Moritani (1992) (Shinohara & Moritani, 1992). The iEMG amplitude was normalized by the iEMG value obtained at 4^{th} minute since the data revealed that the amplitude was lowest at 4^{th} min by most subjects when they have cycled above VT. The difference in workload between the tests was around 40 W. Normalized iEMG increased to 106% in above VT test, while it did not significantly change (98.6%) in the below VT test.

All this studies have demonstrated different methodologies of tests in terms of exercise intensities (workload), duration of tests and number of investigated muscles which in turn make them hard to be comparable regarding sEMG activity during constant-load cycling.

Moreover, Correlation between VT2, EMG_{FT} and MLSS is disputed in the research literature (Pringle & Jones, 2002; Van Schuylenbergh et al., 2004) but, nevertheless, it can be accepted that there is no big difference between workloads which correspondent to those three anaerobic thresholds and therefore all three parameters could be used in order to distinguish heavy from severe exercise intensity (Dekerle et al., 2003).

1.5 Aim of the study

The purpose of this study was to investigate behavior in sEMG signals over time (time effect) for six lower extremity muscles during the cycling at two different constant workloads (MLSS and aMLSS). In addition, analysis between MLSS sEMG and aMLSS sEMG was carry out for every single muscle in order to observe neuromuscular behavior between corresponding workloads. We have hypothesized that amplitude or/and frequency of sEMG might demonstrate significant change over time. Moreover, some/all muscles might exhibit significant statistical difference in sEMG signal between two cycling tests defined by different workloads. This study might be unique, because to author's knowledge there is no study that has (to the point) investigated differences in sEMG signals during constant-workload cycling at and above MLSS workload with such small differences between two corresponding power outputs (~ 9 Watts).

The following study was a part of a long-term project focusing on sEMG and cycling which has been conducted in the Institute of Sport Science at the University of Vienna in the period from April 2009 to June 2014.

2 Methods

2.1 Subjects

Thirty two healthy male volunteers (mean ± SD: age = 29.3 ± 5.4 years; height = 178.6 ± 5.4 cm; weight = 75.3 ± 7.2 kg) have agreed to sign written informed consent to participate in this study. All subjects were free of any known diseases, injuries or use of medications. They were physically active and all of them have fulfilled two main criteria in order to be eligible to participate in the study: (i) They have to have a past of regular cycling physical activities (road cycling, mountain biking or triathlon and (ii) they have to be experienced in cycling with the clipless pedals. The biological, anthropometric and physiological characteristics of the subjects are presented in Table 3.

During first visit all subjects were familiarized with the test procedures, design of the study and they have been instructed to avoid any strenuous physical activity prior the each visit to laboratory. They have been encouraged to preserve healthy life style and eating habits during

the whole course of the study. Each subject has visited the laboratory on 3-7 occasions separated by 3-7 days.

Tab. 3: *Subject characteristics (BMI body mass index, VO_2max maximal oxygen uptake, MLSS maximal lactate steady state, aMLSS above maximal lactate steady state, Wmax maximal workload in Watts, Wmax/kg maximal workload relative to body weight, RCPW workload at respiratory compensation point, RCPWmax% workload at respiratory compensation point as a percentage of maximal workload, CV coefficient of variation)*

Subjects (n=32)	Mean (SD)	Range	CV
Age (years)	29.3 (5.4)	21 - 44	0.18
Height (cm)	178.6 (5.4)	163 - 190	0.03
Mass (kg)	75.3 (7.2)	57.5 - 89	0.09
BMI (kg/m²)	23.6 (1.8)	19.9 - 28	0.07
VO_2max (ml/kg/min^{-1})	54.7 (7.4)	42.6 - 70.1	0.13
MLSS (W)	226.9 (25.7)	170 - 275	0.11
aMLSS (W)	235.9 (26)	180 - 290	0.11
Wmax	353.1 (36.3)	280 - 425	0.10
Wmax/kg	5.3 (2)	3.5 - 11.1	0.37
RCPW (W)	250.4 (35.1)	193 - 322.5	0.14
RCPWmax% (% from Wmax)	70.8 (5.4)	58.5 - 78.2	0.07

2.2 Design of the study

Before conducting each exercise test, the equipment and procedures prepared for this study have been explained to each subject. Furthermore, all subjects have received and signed informed consent and agreed that their data can be used for further investigations.

In addition to these first steps subjects have been asked to adjust ergometer to their optimal body proportions (height of saddle and handlebars, type of clipless pedals and orientation of upper body: road bike body position or triathlon body position). The chosen body position was instructed to be maintained stable as much as possible during the whole exercise test.

Incremental ergometer test to exhaustion was first completed in order to determine the anaerobic threshold (AnT), respiratory compensation point (RCP), maximal workload (Wmax) and VO_2max. In the following weeks after incremental test several maximal lactate steady state (MLSS) test procedures has been conducted (cf. Fig. 17).

Moreover, the room temperature was maintained constant at 23°C throughout the entire study.

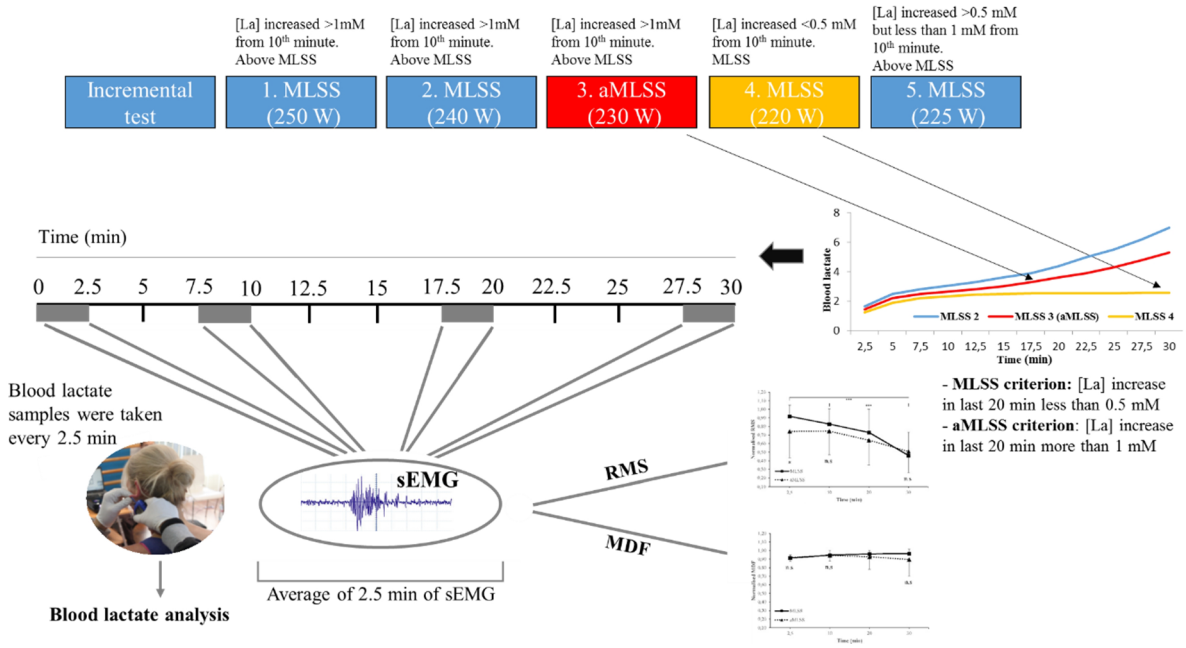

Fig. 17: *Study design and testing procedure. The value of sEMG at the 20th minute has not been used for analysis, but for the purpose of better visual presentation of graphs.*

Testing equipment

All tests were conducted on an electronically braked cycle-ergometer (Lode Excalibur Sport, Lode, Groningen, NL). Ventilation, oxygen consumption and carbon dioxide output were determined using an open air spirometry method (Jäger Oxycon Alpha, Würzburg, Germany). Blood lactate samples were taken from hyperemic earlobe for further analysis of blood lactate concentration [La] (Eppendorf ESAT 6666, Hamburg, Germany). During the tests the heart rate (HR) was monitored from ECG recordings. The surface electromyographic (sEMG) activity was recorded by a Delsys® EMG system (Delsys Inc. Boston, MA, USA).

Incremental exercise test

Prior to the incremental cycling test, resting electrocardiogram, blood pressure and pulmonary functions were measured in order to assess health status of each subject. The initial workload and stage increments were determined individually based on body mass and fitness (activity) level (cf. Tab. 4). The stage workload was increased every minute until voluntary exhaustion while the cadence of pedaling was preserved on 80 repetitions per minute (RPM).

Blood samples (20 µl) were collected in the following order: at rest, after each stage and at end of the test and afterward immediately analyzed for blood lactate concentration.

Maximal power output (Wmax), maximal oxygen uptake (VO_2max) and respiratory compensation point (RCP) were obtained for each subject (cf. Tab. 3) as described earlier (Smekal et al., 2012). The RCP was assessed according to Wasserman (Wasserman, 1984).

The following criteria were used for termination of incremental cycling test: (i) voluntary termination because of subjective exhaustion, or (ii) inability of subject to maintain cadence at 80 rpm cadence despite verbal encouragement.

Tab. 4: *Protocol for stage increments during the incremental cycling test (BM body mass)*

Incremental cycling test protocol (Watt/min)	
Untrained	(BM x 3) / 15
Trained	(BM x 4) / 15
Highly trained	(BM x 5) / 15

Determination of workload at maximal lactate steady state (MLSS) and above MLSS

After the completion of the incremental cycling test, each subject has performed several constant load cycling tests (30 min) in order to determine the MLSS and aMLSS workload. Six subjects (mean ± SD: time = 22.3 ± 3.2) have not succeeded to complete entire aMLSS test resulting a missing values for those subjects (cf. Tab. 5). The constant-load cycling test was performed immediately after a 5 min warm-up procedure at 0.6 $W \cdot kg^{-1}$ (Smekal et al., 2012). Initial workload for the first MLSS test was set at 10% under RCP workload (<10% RCPW), and the workload for the each subsequent MLSS test was increased or decreased (5-20 Watts), dependent on [La] temporal trend, until the criterion for determination of MLSS was accomplished.

Blood samples for [La] analysis were taken every 2.5 minute. For the sake of more accurate MLSS assessment, MLSS was defined as a maximal intensity for which [La] increases less than ≤ 0.5 mM during the last 20 minutes of a 30 minute constant load test (Almarwaey, Jones, & Tolfrey, 2004; Aunola & Rusko, 1992; Bacon & Kern, 1999a; P. Brown, Sharpe, & Johnson, 2012). Contrary to MLSS definition, above MLSS (aMLSS) workload was

defined as a first workload above MLSS where [La] was increasing more than ≥ 1 mM during the last 20 minutes of a test (cf. Fig. 17).

Each subject has performed between 3-6 constant load cycling tests separated by a period of 3-7 days.

Electromyography, data acquisition and processing

Surface electromyography (sEMG) was recorded using Delsys® EMG system (Delsys Inc. Boston, MA, USA) and EMGworks® Acquisition V 4.1.7 software. The data were stored afterwards in the personal computer for subsequent analysis. Furthermore the sEMG signals were analyzed by the software EMGworks® Analysis V 4.1.1 (Delsys Inc. Boston, MA, USA). The sEMG activity during constant load cycling was recorded and analyzed from six left lower limb muscles: gluteus maximus (GM), biceps femoris (BF), rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL) and gastrocnemius medialis (GastM).

Single differential surface EMG sensors (Delsys®, DE-2.1) with the contacts made from Ag and of dimension 10 x 1 mm spaced 10 mm apart were placed with respect to the muscle fiber arrangement according to recommendation of SENIAM (Hermie J. Hermens, Freriks, Disselhorst-Klug, & Rau, 2000; H.J. Hermens et al., 1999). The EMG preamplifier gain integrated in sensor was 10 V/V ± 1% with the bandwidth cut-off frequency of 20-450 Hz, noise 1.2 µV (RMS, R.T.I.), common mode rejection ration (CMRR) of -92 dB and input impedance $>10^{15}$ Ω//0.2pF. The signals were analog-to-digital converted at the sampling rate of 1000 Hz (1 kHz). If a muscle electrical signal was low or invisible during a test the sampling frequency was increased to 10 kHz. Prior to the sEMG sensor application, the skin was shaved, cleaned and abraded with abrasive paste to minimize impedance. In order to reduce intra-subject variability in terms of test-to-test signal differences, the sites of attached sensors were marked with a black water-resistant paint marker and the subjects were instructed to not remove the painted marks until the subsequent test.

Movement induced artifacts were minimized by cable fixation to the skin with the adhesive tape applied away from the sEMG sensors. A reference electrode was placed over the tip of the 7^{th} cervical spine. The functionality of sensors was checked before each test within the 30 sec test trial which assessed baseline activity of signals (no contraction), amplitude of signals during static muscle contractions and pedaling.

All raw sEMG data were further visually inspected for suspicious artefacts and noise. Signals or part of the signal which were contaminated with various noise and artefacts were excluded from analysis. The raw sEMG signals were further full wave rectified and processed by calculating the root mean square in mV (RMS, mV) and median frequency in Hz (MDF, Hz), with a window size of 150 sec (200 crank revolutions) without overlapping. The MDF power spectrum was calculated using Fast Fourier Transform (FFT) algorithm. The RMS was used as an indicator of muscle activity level (Sylvain Dorel, Couturier, & Hug, 2008; Duc, Bertucci, Pernin, & Grappe, 2008) while MDF is considered as an indicator of muscular fatigue and muscle fiber conduction velocity (Cifrek et al., 2009; Farina, Fattorini, Felici, & Filligoi, 2002; K. Masuda, Masuda, Sadoyama, Mitsuharu, & Katsuta, 1999; T. Masuda et al., 2001). In addition to reduction of inter-subject variability, the linear envelops were computed and normalized to the highest single value in the data set for each subject, muscle and test separately (Chapman, Vicenzino, Blanch, Knox, & Hodges, 2006, 2010; Sylvain Dorel et al., 2008).

Moreover, the differences between MLSS and aMLSS were analyzed at the three time frames: at the beginning of the test, at the 10^{th} minute and at the end of the test (each time frame represents average value of a sEMG with a window size of 150 sec).

Dietary and exercise recommendation

During the whole course of study, each subject had to pursue prescribed dietary and nutritional recommendations. Day before each test and on all test days, protocols for food and fluids consumption were same in order to achieve comparability of energy and nutritional intake. Subjects were required to avoid any strenuous and moderate exercise activity on the day prior to cycling tests. All tests were performed in the identical time of the day between 08:00 a.m. and 12:00 o'clock noon.

Statistical analysis

Due to ANOVA's limitation to incorporate missing values in repeated measurement analysis and therefore lowering the statistical power of a sample, analysis of repeated observation using linear mixed model (LMM) was introduced (Krueger & Tian, 2004; Kwok et al., 2008). Percentage of missing values in present study was 5% what is acceptable and considered as inconsequential according to Schafer (1999) and Bennett (2001) (Bennett,

2001; Schafer, 1999). Relevance of linear mixed models in EMG studies is well established (Hosseinzadeh et al., 2014; Martins et al., 2008; Racinais, Buchheit, & Girard, 2014; van der Houwen, Scholtes, Becher, & Harlaar). Linear mixed model (LMM) analysis was performed separately for MDF and RMS values and for each muscle (12 times in total). Normality of the data was confirmed with Shapiro-Wilk test. Two factor analysis between MLSS and aMLSS parameters in respect to time was performed. Post-hoc comparison with Bonferroni correction was used for multiple comparisons between each variable. The values of MLSS and aMLSS were used as a between-subject factor. Level of significance was accepted at $p<.05$. Results are presented as mean (SD). The statistical software SPSS was used for LMM analysis of the data (IMB SPSS Statistics V 20, Armonk, NY, USA).

3 Results

The mean difference in power (W) between two corresponding workloads was 9 Watts (mean ± SD, MLSS 226.9 ± 26, aMLSS 235.9 ± 25.7) (cf. Tab. 3). The inter-subjects variability in terms of subjects' performance level was observed (VO_2max range, 42.6 – 70.1 ml/kg/min^{-1}). Large inter-subject variability was in addition observed in lactate response during cycling at MLSS workload ([La] mean range, 1.38 - 7.38 mM). Missing values, as a result of noise, artefacts and inability of some subjects to finish whole aMLSS test were mostly observed in the second part of the 30 min of tests (cf. Tab. 5).

Tab. 5: *Number of data which are excluded as a result of noise and artefacts (missing values). The values in the table represent exclusion per muscle for all subjects per time interval in both tests (GM Gluteus Medius, BF Biceps Femoris, RF Rectus Femoris, VM Vastul Medialis, VL Vastus Lateralis, GastM Gastrocnemius Medialis).*

Time (min)	**Nr. of values excluded per muscle for each time interval**					
	GM	BF	RF	VM	VL	GastM
2.5	2	-	1	-	-	3
10	2	-	1	-	-	3
30	13	6	7	6	8	8

The Bonferroni corrected pairwise comparison depicts difference between each pair of means for MDF and RMS parameters separately (time effect) (cf. Tab. 6 and Tab. 7, respectively).

Tab. 6: *Time effect for MDF parameter. Table represents statistical significant difference between three time points for individual muscles (GM Gluteus Medius, BF Biceps Femoris, RF Rectus Femoris, VM Vastul Medialis, VL Vastus Lateralis, GastM Gastrocnemius Medialis). Bonferroni corrected pairwise comparisons was performed for each pair of means*

Time		**Level of significance per single muscle**					
		GM	BF	RF	VM	VL	GastM
1	2	.001	.05	.119	.180	.001	1.00
	3	.001	.001	.001	1.00	.001	.019
2	1	.001	.05	.119	.180	.001	1.00
	3	.001	.01	.001	1.00	.418	.016
3	1	.001	.001	.001	1.00	.001	.019
	2	.001	.01	.001	1.00	.418	.016

Tab. 7: *Time effect for RMS parameter. Table represents statistical significant difference between three time points for individual muscles (GM Gluteus Medius, BF Biceps Femoris, RF Rectus Femoris, VM Vastul Medialis, VL Vastus Lateralis, GastM Gastrocnemius Medialis). Bonferroni corrected pairwise comparisons was performed for each pair of means.*

Time		**Level of significance per singel muscle**					
		GM	BF	RF	VM	VL	GastM
1	2	.079	1.00	.940	.690	.399	.068
	3	.001	.001	.001	.001	.001	.001
2	1	.079	1.00	.940	.690	.399	.068
	3	.001	.001	.001	.001	.001	.001
3	1	.001	.001	.001	.001	.001	.001
	2	.001	.001	.001	.001	.001	.001

Tables 8 and 9 show estimates of fixed effects of median frequency (MDF) and root mean square (RMS) values for individual muscles (cf. Tab. 8 and Tab 9.) respectively. The results in Tab. 8 and Tab. 9 represent statistically significant difference for three following effects: difference over time (Time – time effect), difference between means of all three values of MLSS and aMLSS (P – power effect) and a difference between MLSS and aMLSS at each of three time points separately (Time*P – interaction effect).

Tab. 8: *Estimates of fixed effects of median frequency (MDF) for single muscles. Presented fixed effects are: Time (change of mean of both values over time), P (difference between means of all three values of MLSS and aMLSS) and Time*P (interaction between MLSS and aMLSS); if the Time*P was significant that post hoc analysis was performed.*

Muscle	Gluteus Medius	Biceps Femoris	Rectus Femoris	Vastus Medialis	Vastus Lateralis	Gastrocnemius Medialis
Time	.001	.001	.001	.170	.001	.007
P	.207	.092	.331	.066	.016	.883
Time*P	.941	.135	.639	.025	.216	.036

Tab. 9: *Estimates of fixed effects of root mean square (RMS) for single muscles. Presented fixed effects are: Time (change of mean of both values over time), P (difference between means of all three values of MLSS and aMLSS) and Time*P (interaction between MLSS and aMLSS); if the Time*P was significant that post hoc analysis was performed.*

Muscle	Gluteus Medius	Biceps Femoris	Rectus Femoris	Vastus Medialis	Vastus Lateralis	Gastrocnemius Medialis
Time	.001	.001	.001	.001	.001	.001
P	.075	.663	.048	.670	.936	.070
Time*P	.762	.882	.045	.381	.924	.789

If an interaction between MLSS and aMLSS reveals a presence of significant difference for a corresponding muscle then the post hoc analysis was performed in order to find a time points where the difference between MLSS and aMLSS is statistically significant (cf. Tab. 10).

Tab. 10: *Post hoc analysis between MLSS and aMLSS at single time points for Rectus Femoris (RF), Vastus Medialis (VM) and Gastrocnemius Medialis (GastM); (RMS root mean square, MDF median frequency).*

Muscle	RF	VM	GastM
Parameter	RMS	MDF	MDF
2.5 min	.050	.523	.449
10 min	.165	.882	.210
30 min	.484	.060	.079

Differences between normalized sEMG signals have been presented in Figure 18 and Figure 19 for each muscle separately and for MDF and RMS parameters, respectively[4].

[4] Normalized MDF and RMS are presented in Appendix (cf. Tab. 13 and 14)

3.1 Median frequency (MDF) analysis

When pairwise comparisons was analyzed over time a significant difference was observed in all muscles (cf. Tab. 6), except VM (p=.17), (cf. Fig. 18). The increase of normalized MDF from 2.5th to 10th minute was highest for GM (6% for both power outputs) and VL (6% for both power outputs), while increase between 10th and 30th minute was highly significant in GM (MLSS=5%, aMLSS=6%), BF (MLSS=8%, aMLSS=3%) and RF (MLSS=10%, aMLSS=7%) (cf. Tab. 11). The highest increase of normalized MDF over whole time was observed in GM (MLSS=11%, aMLSS=12%), BF (MLSS=11%, aMLSS=8%), RF (MLSS=13%, aMLSS=11%) and VL (MLSS=10%, aMLSS=6%) (cf. Tab. 11).

Gastrocnemius medialis (GastM) values changed moderate over whole period of time (p<.05). However, pairwise comparisons over time for vastus medialis (VM) have not resulted in statistically significant change (p>.05) (cf. Tab. 8).

The vastus lateralis (VL) has demonstrated statistically significant difference between means of all three values of MLSS and aMLSS (p<.05). Nevertheless, no significant difference for same analysis could be found for all other muscles (p>.05) (cf. Tab. 8).

Although the LMM analysis has shown that the interaction between MLSS and aMLSS for VM (p=.025) and GastM (p=.036) was not same over all three time points (cf. Tab. 8), post hoc analysis did not observed significant difference between MLSS and aMLSS values at any of three time points individually (p>.05) (cf. Tab. 10).

	GM		BF		RF		VM		VL		GastM		Mean	
Time (min)	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS
2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	6↑	6↑	3↑	5↑	3↑	4↑	4↑	3↑	6↑	6↑	0	0	4↑	5↑
30	11↑	12↑	11↑	8↑	13↑	11↑	6↑	3↓	10↑	6↑	0	10↑	10↑	8↑
10 - 30	5↑	6↑	8↑	3↑	10↑	7↑	2↑	6↓	4↑	0	0	10↑	6↑	3↑

***Tab. 11**: MDF increase over time for each muscle expressed as percentage. Data are presented for MLSS and aMLSS power outputs separately and are related to the difference from the minute 2.5 to 10 (min 10) and from 2.5th to 30th (min 30) minute. Differences from 10th to 30th minute are presented in last row. ↑indicate increase, ↓indicate decrease*

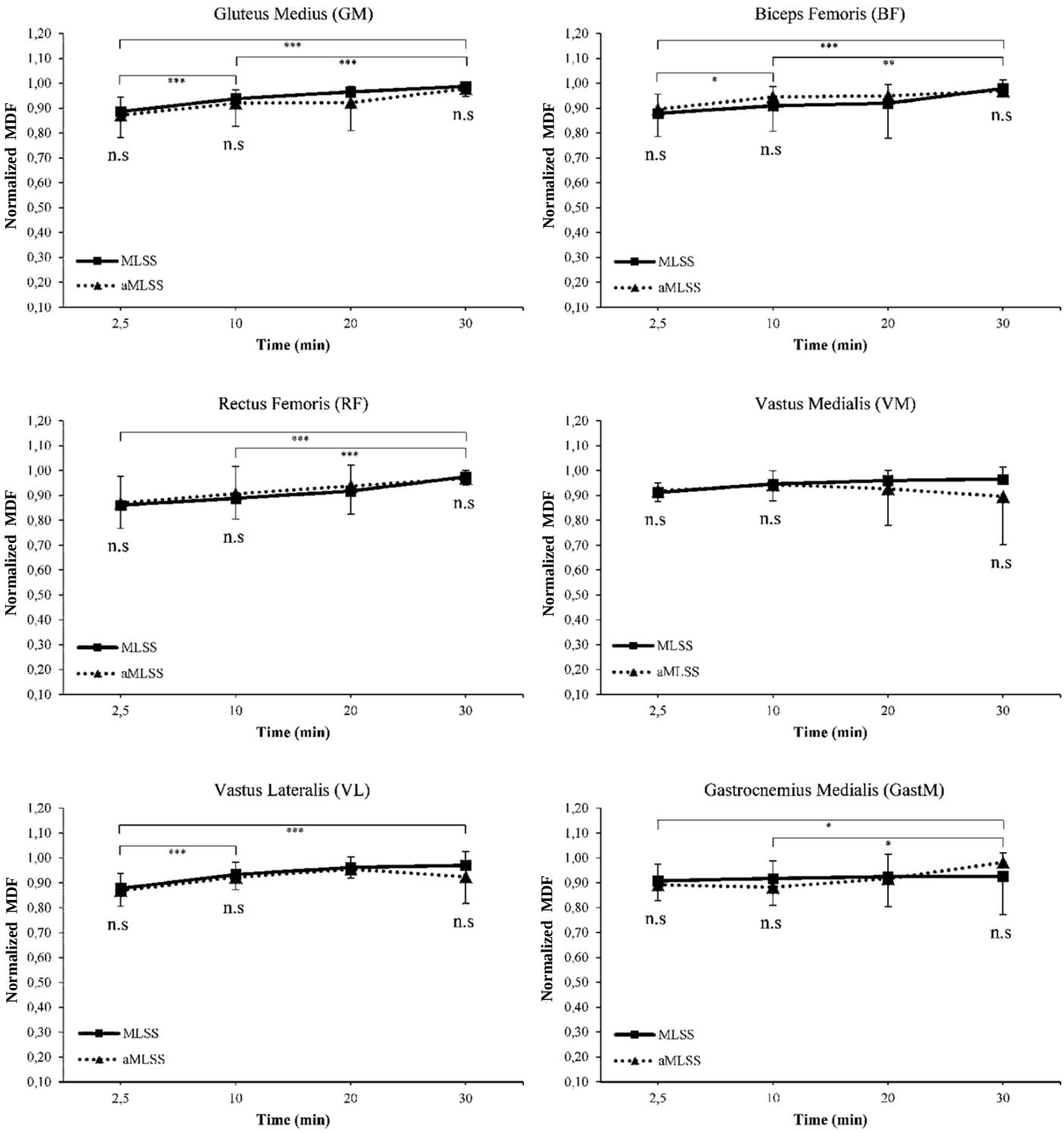

Fig. 18: *Normalized median frequenca (MDF) of two power outputs (MLSS and aMLSS) for a individual muscles. Values represent average median frequency (MDF) of all subjects normalized to the highest single value in the data set for each subject and test separately (mean ± SD). Normalized sEMG values used for analysis of linear mixed model between variables are at the time points which are corespodending to 2.5th, 10th and 30th minute. The value at 20th minute has been not included in analysis and serve for better visual overview of graphs.*

*(n.s = non-significant difference, * = p<.05, ** = p<.01, *** = p<.001) Bonferroni corrected*

3.2 Root mean square (RMS) analysis

The time effect has resulted in statistically significant difference over time (cf. Tab. 9) for all muscles ($p<.001$) (cf. Fig. 19). None of the muscles have demonstrated a significant change in normalized RMS of sEMG from 2.5th to 10th minute ($p>.05$). However, pairwise comparisons of the time effects has revealed that the RMS of all muscles was significantly decreased from 10th to 30th min (MLSS mean = 32%, aMLSS = 30%) as well as from 2.5th to 30th minute (MLSS mean = 40%, aMLSS = 37%) (cf. Tab. 12).

Difference between means of all three values of MLSS and aMLSS ($p<.05$) was found to be significant for the rectus femoris (RF) (cf. Tab. 9).

In addition, analysis of interaction between MLSS and aMLSS for the rectus femoris ($p<.05$) was not same at all three time points (cf. Tab. 9). Furthermore, the following post hoc analysis between MLSS and aMLSS values at each single time point has found that the normalized RMS values of MLSS and aMLSS significantly differ at the 2.5th minute ($p<.05$) (cf. Tab 10).

	GM		BF		RF		VM		VL		GastM		Mean	
Time (min)	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS
2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	5↓	9↓	5↓	1↓	10↓	0	4↓	9↓	7↓	7↓	14↓	9↓	8↓	7↓
30	35↓	37↓	40↓	38↓	50↓	32↓	34↓	25↓	36↓	39↓	46↓	51↓	40↓	37↓
10 - 30	30↓	28↓	35↓	37↓	40↓	32↓	30↓	16↓	29↓	32↓	32↓	42↓	32↓	30↓

Tab. 12: *RMS decrease over time for each muscle expressed as percentage. Data are presented for MLSS and aMLSS power outputs separately and are related to the difference from the minute 2.5 to 10 and from 2.5th to 30th minute. Difference from 10th to 30th minute is presented in last row.* ↑*indicate increase,* ↓*indicate decrease*

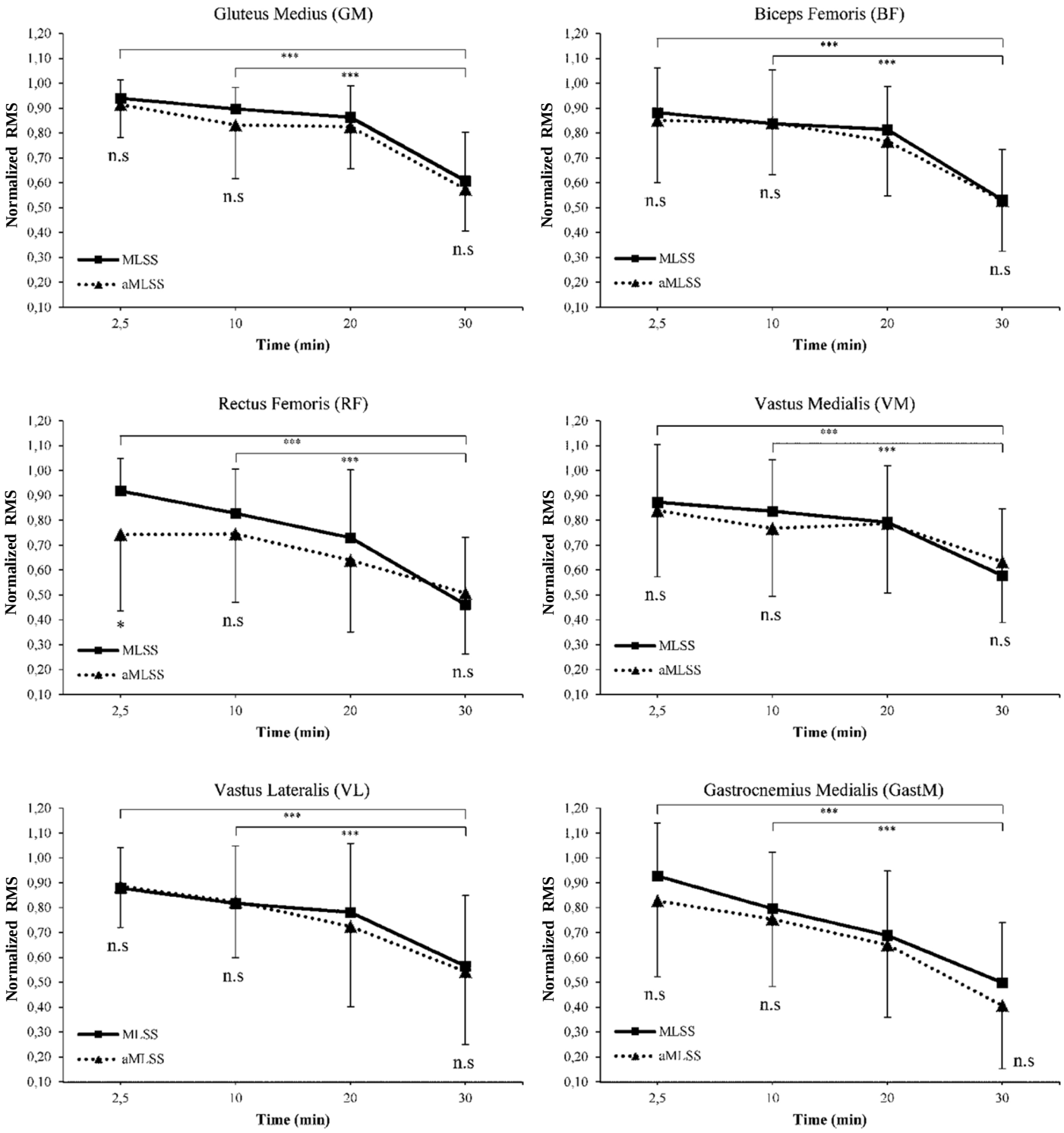

Fig. 19: *Normalized root mean square (RMS) of two power outputs (MLSS and aMLSS) for a individual muscles. Values represent average root mean square (RMS) of all subjects normalized to the highest single value in the data set for each subject and test separately (mean ± SD). Normalized sEMG values used for analysis of linear mixed model between variables are at the time points which are corespodending to 2.5th, 10th and 30th minute. The value at 20th minute has been not included in analysis and serve for better visual overview of graphs.*

*(n.s = non-significant difference, * = p<.05, ** = p<.01, *** = p<.001) Bonferroni corrected*

4 Discussion

The main aim of the present study was to investigate whether neuromuscular activity differs between maximal lactate steady state (MLSS) and above maximal lactate steady state (aMLSS) power outputs over 30 min constant-load cycling trials. We have hypothesized that increasing metabolic demands of the body during cycling at aMLSS workload (increase of [La], pH, Ve, CO_2 etc.) should be associated with the observable changes at the neuromuscular level. In addition, we hypothesized that neuromuscular activity at steady state metabolism (MLSS) should be stable during the whole cycling trial.

The findings of the present study have revealed that significant difference in neuromuscular activity (RMS) between MLSS and aMLSS workloads was only seeable in rectus femoris (RF) at the beginning of the test at the 2.5^{th} minute ($p<.05$) (cf. Fig. 19). The RF RMS at the MLSS power output was from the very beginning significantly higher (18%) compared to same muscle at the aMLSS power output. This difference decreased toward the end of the tests ($p>.05$). The difference of root mean square (RMS) between MLSS and aMLSS in all other muscles was non-significant ($p>.05$).

The difference between MLSS and aMLSS of vastus medialis MDF ($p=.060$) and gastrocnemius medials MDF ($p=.079$) was close to the level of significance ($p<.05$) at 30^{th} minute (cf. Tab. 10). However, both muscles have not succeeded to demonstrate statistically significant difference even though it was observed that the MDF trend demonstrated continuous increase in difference between two power outputs toward the 30^{th} min (cf. Fig. 19). In all other muscles the MDF difference between two power outputs could not be observed.

The power spectral component (MDF) of sEMG signal has significantly increased over time for all muscles in both tests. Our results are in line with findings of Saunders et al. (2000) who reported increase of spectral component of sEMG during constant-load cycling above lactate threshold (Saunders et al., 2000). In contrast, other studies have not found changes in power density spectrum of sEMG during constant-load cycling or changes were less than significant (Duc, Betik, & Grappe, 2005; J. S. Petrofsky, 1979; Scheuermann, Hoelting, Noble, & Barstow, 2001). In the studies of Scheuermann et al. (2001) and Duc et al. (2005) no changes were observed in power density spectrum during high intensity constant-load cycling bouts suggesting stable synchronization of motor units (MUs). However, Petrofsky (1979) has found after initial elevation of frequency of sEMG (first 20 min) a subsequent

decrease in a power spectrum of sEMG during prolonged cycling (80 min) at power outputs corresponding to 60, 80 and 100% of VO_2max. The initial elevation was related to increased temperature of the working muscles (J. Petrofsky & Lind, 1980; J. S. Petrofsky, 1979) while subsequent reduction in frequency of sEMG was associate with blood lactate [La] accumulation (Horita & Ishiko, 1987; Tesch, Komi, Jacobs, Karlsson, & Viitasalo, 1983). We assume that during our tests the blood lactate concentration [La] has not reached critical level in order to tremendously influence MDF ([La] mean ± SD, MLSS 4.3 mM ± 1.29, aMLSS 6.1 mM ± 2.1). Thus, usefulness of power spectral analysis to assess muscle fiber recruitment pattern or indices of fatigue is not fully understood and might be argued (Briscoe et al., 2013; Enoka, 2008; Priego Jose, Bini Rodrigo, Lanferdini, & Carpes Felipe, 2014; Pringle & Jones, 2002).

The present data have shown highly significant decrease in RMS of surface electromyographic signal over time in all muscles during a constant-load cycling for both tests. This continuous decrease in RMS amplitude was not observed in some earlier studies (Fontes et al., 2010; Hug, Decherchi, Marqueste, & Jammes, 2004; J. S. Petrofsky, 1979; Pringle & Jones, 2002), suggesting that extent of mechanical failure which takes place in most powerful fibers might mask increase in MUs recruitment or frequency charging of units lower in the recruitment hierarchy (Pringle & Jones, 2002). The rate of sweating is significant factor which has influence on sEMG amplitude. Correlation between decline of sEMG amplitude and sweat thickness has been detailed elaborated and confirmed (Abdoli-Eramaki, Damecour, Christenson, & Stevenson, 2012; Roy et al., 2007). Other explanation could be large inter-subject variability in sEMG response during cycling (Pringle & Jones, 2002), due the fact that some subjects did demonstrate continuous increase in RMS amplitude from the beginning toward the end of the test and some subjects did not.

Progressive increase of RMS has been expected during continuous contraction of submaximal intensity as a result of additional recruitment of previously inactive MUs in order to reimburse the decrease in contractility of exhausted MUs (Arabadzhiev, Dimitrov, Dimitrova, & Dimitrov, 2010; Gregor, Broker, & Ryan, 1991). However, the amplitude characteristics or MUs recruitment strategies and discharge rates can hardly be explained and understood solely by the use of sEMG (S. K. Hunter, Duchateau, & Enoka, 2004) due the fact that sEMG amplitude could increase (S. K. Hunter, Ryan, Ortega, & Enoka, 2002; Löscher, Cresswell, & Thorstensson, 1996; Smith et al., 1998), decrease (Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002; Stephens & Taylor, 1972) or remain unchanged

(Hautier et al., 2000) as a consequence of fatigue (Arabadzhiev et al., 2010; Dimitrova & Dimitrov, 2003).

Pringle and Jones (2002) have investigated response in integrated electromyographic (iEMG) signal of vastus laterlais (VL) at MLSS and aMLSS. However, they have not reported significant increase in iEMG during cycling at both workloads although the mean workload difference between MLSS and aMLSS was higher (20 W) compared to our study (9 W). Secondly, in their study the iEMG at aMLSS workload has initially non-significantly increased toward the 10^{th} minute (11%) and then subsequently decreased toward the end of the test. We have observed continuously decrease in sEMG amplitude from the beginning toward the end of the test. To our knowledge the present study is the only one which has investigated electromyographic activity with the smallest difference in power output between MLSS and aMLSS. Thus, that could be one of possible reasons why the sEMG signals were unchanged between corresponding power outputs except for rectus femoris (RF). In present study above MLSS workload was defined as power output during [La] has to increase more than 1 mM. It has been suggested that if blood lactate increase above the level where production and removal of [La] is in equilibrium it can cause considerable metabolic changes as well as higher [La] in working muscles (Bacon & Kern, 1999b; Green, Hughson, Orr, & Ranney, 1983; Noble, Borg, Jacobs, Ceci, & Kaiser, 1983). However, we did not observed significant alteration in neuromuscular activity in more than one muscle despite the fact that [La] has increased more than >1 mM after the 10^{th} minute. It is interesting that in our study 18% of subjects could not complete the full 30 min for the aMLSS trials while in the study of Pringle and Jones (2002) 50% of subjects canceled the aMLSS test before the end was reached due to exhaustion. This suggests that aMLSS power output in current investigation was somehow "stable" and not able to provoke critical disturbance at neuromuscular level.

Study of Briscoe et al. (2013) has reported significant increase in VL sEMG amplitude slope during cycling at the 130% of EMG fatigue threshold (EMG_{FT}). Nevertheless, they have not observed significant increase in sEMG slope for the 100% of EMG_{FT} which correspondents to our aMLSS power output. The results have additionally indicated statistically significant difference between sEMG slope of 130% and 100% of EMG_{FT} power outputs. Moreover, the difference between corresponding power outputs in study of Briscoe et al. (2013) was 57 Watts (mean ± SD, 100% EMG_{FT} = 193W ± 24, 130% EMG_{FT} = 250W ± 18). Considering that they used EMG_{FT} as an indicator of metabolic and neuromuscular steady state, the results

are less comparable to our study methodology. Anyway, the 130% EMG_{FT} corresponded to 85% of the maximal power output (Wmax) while 100% EMG_{FT} to 67% of Wmax. In our study the MLSS workload corresponded to 64% of maximal power output obtained from incremental cycling test while aMLSS was corresponded to 67% Wmax what is far less than %Wmax at 130% EMG_{FT} in the study of Briscoe et al.

In the present study neuromuscular activity of RF RMS only exhibited significant difference between two power outputs. The difference was observable at the beginning of the tests i.e. during the first 2.5 minute followed by the stabilization of neuromuscular activity. Inconsistence of RF is already elaborated (Ryan & Gregor, 1992). While single joint muscles are designated as power generator the bi-articular muscles are considered as power distributors (Broker & Gregor, 1994). Considering that majority of investigated muscles have demonstrated quite consistent neuromuscular activity patterns during both power outputs further investigations are suggested. The tasks of further investigations should be to define possible reasons behind neuromuscular consistency that we have observed and to discover more distant functional role of two-joint muscles during constant-load cycling.

Limitations of present study are related to analysis of sEMG signals. Most important limitation was missing values which might decrease power of statistical analysis depending on number of missing values. In our study less than 5% of data were missing which is considered as acceptable. Because of large inter-subject variability we normalized the data for each subject to the highest single value in the data set. Although such normalization procedure is acknowledged and proposed frequently in sEMG studies it has to be emphasized that other normalization procedures exist and are also recognized and valid. It should be noted that other frequency and amplitude parameters of sEMG are important source of additional information about neuromuscular activity.

5 Conclusion

In summary, to author's knowledge this is the only study which has investigated neuromuscular behavior between two constant-load power outputs with such a small differences between them (mean difference = 9W). All other studies had implemented larger difference between investigated cycling power outputs (>20W).

Despite the fact that in the present study six muscles were investigated, compared to previous studies where only one or two muscles were examined, significant difference in neuromuscular response between two power outputs could not be observed in sEMG parameters, except by RMS of rectus femoris (RF) at the beginning of the test. The results indicate that increase of less than 10 Watts above MLSS power output did not result in significant systematic change in sEMG parameters.

Uniform electromyographic activity of all muscles, except the rectus femoris RMS, imply to several causes which could possibly have influence on it: (i) inter-subject variability in neuromuscular response, (ii) small difference between power outputs, (iii) amount of sweat and (iv) difference in performance level of subjects.

Further investigations, however, are needed to examine if the neuromuscular response between MLSS and aMLSS power outputs can be influenced by performance level, sport, gender or age of subjects.

References

Abbiss, C., & Laursen, P. (2005). Models to Explain Fatigue during Prolonged Endurance Cycling. *Sports Medicine, 35*(10), 865-898. doi: 10.2165/00007256-200535100-00004

Abdoli-Eramaki, M., Damecour, C., Christenson, J., & Stevenson, J. (2012). The effect of perspiration on the sEMG amplitude and power spectrum. *Journal of Electromyography and Kinesiology, 22*(6), 908-913. doi: http://dx.doi.org/10.1016/j.jelekin.2012.04.009

Albertus-Kajee, Y., Tucker, R., Derman, W., & Lambert, M. (2010). Alternative methods of normalising EMG during cycling. *Journal of Electromyography and Kinesiology, 20*(6), 1036-1043. doi: 10.1016/j.jelekin.2010.07.011

Alkner, B. A., Tesch, P. A., & Berg, H. E. (2000). Quadriceps EMG/force relationship in knee extension and leg press. *Medicine and science in sports and exercise, 32*(2), 459-463. doi: 10.1097/00005768-200002000-00030

Almarwaey, O. A., Jones, A. M., & Tolfrey, K. (2004). Maximal lactate steady state in trained adolescent runners. *Journal of Sports Sciences, 22*(2), 215-225. doi: 10.1080/02640410310001641520

Arabadzhiev, T. I., Dimitrov, V. G., Dimitrova, N. A., & Dimitrov, G. V. (2010). Interpretation of EMG integral or RMS and estimates of "neuromuscular efficiency" can be misleading in fatiguing contraction. *Journal of Electromyography and Kinesiology, 20*(2), 223-232. doi: http://dx.doi.org/10.1016/j.jelekin.2009.01.008

Aunola, S., & Rusko, H. (1992). Does anaerobic threshold correlate with maximal lactate steady-state? *Journal of Sports Sciences, 10*(4), 309-323. doi: 10.1080/02640419208729931

Bacon, L., & Kern, M. (1999a). Evaluating a test protocol for predicting maximum lactate steady state. *The Journal of sports medicine and physical fitness, 39*(4), 300-308.

Bacon, L., & Kern, M. (1999b). Evaluating a test protocol for predicting maximum lactate steady state. *Journal of Sports Medicine and Physical Fitness, 39*(4), 300-308.

Baron, B., Noakes, T. D., Dekerle, J., Moullan, F., Robin, S., Matran, R., & Pelayo, P. (2008). Why does exercise terminate at the maximal lactate steady state intensity? *British Journal of Sports Medicine, 42*(10), 828-833. doi: 10.1136/bjsm.2007.040444

Basmajian, J. V. (1978). *Muscles alive, their functions revealed by electromyography*: Williams & Wilkins.

Baum, B. S., & Li, L. (2003). Lower extremity muscle activities during cycling are influenced by load and frequency. *Journal of Electromyography and Kinesiology, 13*(2), 181-190. doi: http://dx.doi.org/10.1016/S1050-6411(02)00110-4

Beneke, R., Hütler, M., & Leithäuser, R. M. (2000). Maximal lactate-steady-state independent of performance. *Medicine & Science in Sports & Exercise, 32*(6), 1135-1139.

Beneke, R., & von Duvillard, S. P. (1996). Determination of maximal lactate steady state response in selected sports events. *Medicine and science in sports and exercise, 28*(2), 241-246. doi: 10.1097/00005768-199602000-00013

Bennett, D. A. (2001). How can I deal with missing data in my study? *Australian and New Zealand Journal of Public Health, 25*(5), 464-469. doi: 10.1111/j.1467-842X.2001.tb00294.x

Bieuzen, F., Lepers, R., Vercruyssen, F., Hausswirth, C., & Brisswalter, J. (2007). Muscle activation during cycling at different cadences: Effect of maximal strength capacity. *Journal of Electromyography and Kinesiology, 17*(6), 731-738. doi: http://dx.doi.org/10.1016/j.jelekin.2006.07.007

Bieuzen, F., Vercruyssen, F., Hausswirth, C., & Brisswalter, J. (2007). Relationship between Strength Level and Pedal Rate. *Int J Sports Med, 28*(07), 585-589.

Billat, V., Sirvent, P., Py, G., Koralsztein, J.-P., & Mercier, J. (2003). The Concept of Maximal Lactate Steady State. *Sports Medicine, 33*(6), 407-426. doi: 10.2165/00007256-200333060-00003

Birch, K., MacLaren, D., & George, K. (2005). *Sport and Exercise Physiology*: BIOS Scientific Publishers.

Briscoe, M. J., Forgach, M. S., Trifan, E., & Malek, M. H. (2013). Validating the EMGFT from a Single Incremental Cycling Test. *Int J Sports Med, 35*(07), 566-570. doi: 10.1055/s-0033-1358672

Broker, J. P., & Gregor, R. J. (1994). Mechanical energy management in cycling: source relations and energy expenditure. *Medicine & Science in Sports & Exercise, 26*(1), 64-74.

Brown, P., Sharpe, G., & Johnson, M. (2012). Inspiratory muscle training abolishes the blood lactate increase associated with volitional hyperpnoea superimposed on exercise and accelerates lactate and oxygen uptake kinetics at the onset of exercise. *European Journal of Applied Physiology, 112*(6), 2117-2129. doi: 10.1007/s00421-011-2185-8

Brown, S. P., Miller, W. C., & Eason, J. M. (2006). *Exercise Physiology: Basis of Human Movement in Health and Disease*: Lippincott Williams & Wilkins.

Burden, A., & Bartlett, R. (1999). Normalisation of EMG amplitude: an evaluation and comparison of old and new methods. *Medical Engineering & Physics, 21*(4), 247-257. doi: http://dx.doi.org/10.1016/S1350-4533(99)00054-5

Castronovo, A. M., 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*.

Chapman, A. R., Vicenzino, B., Blanch, P., Knox, J. J., Dowlan, S., & Hodges, P. W. (2008). The influence of body position on leg kinematics and muscle recruitment during cycling. *Journal of Science and Medicine in Sport, 11*(6), 519-526. doi: http://dx.doi.org/10.1016/j.jsams.2007.04.010

Chapman, A. R., Vicenzino, B., Blanch, P., Knox, J. J., & Hodges, P. W. (2006). Leg muscle recruitment in highly trained cyclists. *Journal of Sports Sciences, 24*(2), 115-124. doi: 10.1080/02640410500131159

Chapman, A. R., Vicenzino, B., Blanch, P., Knox, J. J., & Hodges, P. W. (2010). Intramuscular fine-wire electromyography during cycling: Repeatability, normalisation and a comparison to surface electromyography. *Journal of Electromyography and Kinesiology, 20*(1), 108-117. doi: http://dx.doi.org/10.1016/j.jelekin.2008.11.013

Chavarren, J., & Calbet, J. A. L. (1999). Cycling efficiency and pedalling frequency in road cyclists. *European Journal of Applied Physiology and Occupational Physiology, 80*(6), 555-563. doi: 10.1007/s004210050634

Chowdhury, R. H., Reaz, M. B., Ali, M. A. B. M., Bakar, A. A., Chellappan, K., & Chang, T. G. (2013). Surface Electromyography Signal Processing and Classification Techniques. *Sensors, 13*(9), 12431-12466.

Cifrek, M., Medved, V., Tonković, S., & Ostojić, S. (2009). Surface EMG based muscle fatigue evaluation in biomechanics. *Clinical Biomechanics, 24*(4), 327-340. doi: http://dx.doi.org/10.1016/j.clinbiomech.2009.01.010

Coen, P. M., Jubrias, S. A., Distefano, G., Amati, F., Mackey, D. C., Glynn, N. W., . . . Goodpaster, B. H. (2013). Skeletal Muscle Mitochondrial Energetics Are Associated With Maximal Aerobic Capacity and Walking Speed in Older Adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 68*(4), 447-455. doi: 10.1093/gerona/gls196

Cram, J. R., & Criswell, E. (2011). *Cram's Introduction to Surface Electromyography*: Jones & Bartlett Learning.

de Lucas, R. D., de Souza, K. M., Costa, V. P., Grossl, T., & Guglielmo, L. G. A. (2013). Time to exhaustion at and above critical power in trained cyclists: The relationship between heavy and severe intensity domains. *Science & Sports, 28*(1), e9-e14. doi: http://dx.doi.org/10.1016/j.scispo.2012.04.004

Dekerle, J., Baron, B., Dupont, L., Vanvelcenaher, J., & Pelayo, P. (2003). Maximal lactate steady state, respiratory compensation threshold and critical power. *Eur J Appl Physiol, 89*(3-4), 281-288. doi: 10.1007/s00421-002-0786-y

Dimitrova, N. A., & Dimitrov, G. V. (2003). Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. *Journal of Electromyography and Kinesiology, 13*(1), 13-36. doi: 10.1016/s1050-6411(02)00083-4

Dorel, S., Couturier, A., & Hug, F. (2008). Intra-session repeatability of lower limb muscles activation pattern during pedaling. *Journal of Electromyography and Kinesiology, 18*(5), 857-865. doi: http://dx.doi.org/10.1016/j.jelekin.2007.03.002

Dorel, S., Couturier, A., & Hug, F. (2009). Influence of different racing positions on mechanical and electromyographic patterns during pedalling. *Scandinavian Journal of Medicine & Science in Sports, 19*(1), 44-54. doi: 10.1111/j.1600-0838.2007.00765.x

Duc, S., Bertucci, W., Pernin, J. N., & Grappe, F. (2008). Muscular activity during uphill cycling: Effect of slope, posture, hand grip position and constrained bicycle lateral sways. *Journal of Electromyography and Kinesiology, 18*(1), 116-127. doi: http://dx.doi.org/10.1016/j.jelekin.2006.09.007

Duc, S., Betik, A. C., & Grappe, F. (2005). EMG Activity does not Change During a Time Trial in Competitive Cyclists. *Int J Sports Med, 26*(02), 145-150. doi: 10.1055/s-2004-817922

Enoka, R. M. (2008). *Neuromechanics of Human Movement*: Human Kinetics.

Ericson, M. O., & Nisell, R. (1986). Tibiofemoral joint forces during ergometer cycling. *The American Journal of Sports Medicine, 14*(4), 285-290.

Ericson, M. O., & Nisell, R. (1987). Patellofemoral Joint Forces During Ergometric Cycling. *Physical Therapy, 67*(9), 1365-1369.

Farina, D. (2006). Interpretation of the Surface Electromyogram in Dynamic Contractions. *Exercise and Sport Sciences Reviews, 34*(3), 121-127.

Farina, D., Fattorini, L., Felici, F., & Filligoi, G. (2002). Nonlinear surface EMG analysis to detect changes of motor unit conduction velocity and synchronization. *Journal of Applied Physiology, 93*(5), 1753-1763.

Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate Threshold Concepts. *Sports Medicine, 39*(6), 469-490. doi: 10.2165/00007256-200939060-00003

Fernández-Peña, E., Lucertini, F., & Ditroilo, M. (2008). A maximal isokinetic pedalling exercise for EMG normalization in cycling. *Journal of Electromyography and Kinesiology, 19*(3), 162-170.

Ferreira, J. C. B., Rolim, N. P. L., Bartholomeu, J. B., Gobatto, C. A., Kokubun, E., & Brum, P. C. (2007). Maximal lactate steady state in running mice: effect of exercie training *Clinical and Experimental Pharmacology and Physiology*, *34*(8), 760-765. doi: 10.1111/j.1440-1681.2007.04635.x

Fonda, B., & Sarabon, N. (2010). Biomechanics of Cycling *Sport Science Review* (Vol. 19, pp. 187).

Fontes, E. B., Smirmaul, B. P. C., Nakamura, F. Y., Pereira, G., Okano, A. H., Altimari, L. R., . . . Moraes, A. C. d. (2010). The Relationship Between Rating of Perceived Exertion and Muscle Activity During Exhaustive Constant-Load Cycling. *Int J Sports Med, 31*(10), 683-688. doi: 10.1055/s-0030-1255108

Goodwin, M. L., Harris, J. E., Hernandez, A., & Gladden, L. B. (2007). Blood lactate measurements and analysis during exercise: a guide for clinicians. *J Diabetes Sci Technol, 1*(4), 558-569.

Green, H. J., Hughson, R. L., Orr, G. W., & Ranney, D. A. (1983). Anaerobic threshold, blood lactate, and muscle metabolites in progressive exercise. *Journal of Applied Physiology, 54*(4), 1032-1038.

Gregor, R. J., Broker, J. P., & Ryan, M. M. (1991). 4 The Biomechanics of Cycling. *Exercise and Sport Sciences Reviews, 19*(1), 127-170.

Hautier, C. A., Arsac, L. M., Deghdegh, K., Souquet, J., Belli, A., & Lacour, J.-R. (2000). Influence of fatigue on EMG/force ratio and cocontraction in cycling. *Medicine & Science in Sports & Exercise, 32*(4), 839-843.

Heck, H., Mader, A., Hess, G., Mücke, S., Müller, R., & Hollmann, W. (1985). Justification of the 4-mmol/l Lactate Threshold. *Int J Sports Med, 06*(03), 117-130. doi: 10.1055/s-2008-1025824

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., & Rau, G. (2000). Development of recommendations for SEMG sensors and sensor placement procedures. *Journal of Electromyography and Kinesiology, 10*(5), 361-374. doi: http://dx.doi.org/10.1016/S1050-6411(00)00027-4

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., . . . Hägg, G. (1999). *SENIAM Project - European Recommendations for Surface Electromyography*. Netherlands: Roessingh Research and Development.

Hirokawa, S. (1991). Three-dimensional mathematical model analysis of the patellofemoral joint. *Journal of Biomechanics, 24*(8), 659-671. doi: http://dx.doi.org/10.1016/0021-9290(91)90331-G

Horita, T., & Ishiko, T. (1987). Relationships between muscle lactate accumulation and surface EMG activities during isokinetic contractions in man. *European Journal of Applied Physiology and Occupational Physiology, 56*(1), 18-23. doi: 10.1007/bf00696370

Hosseinzadeh, M., Andersen, O. K., Arendt-Nielsen, L., Samani, A., Kamavuako, E. N., & Madeleine, P. (2014). Adaptation of local muscle blood flow and surface electromyography to repeated bouts of eccentric exercise. *The Journal of Strength & Conditioning Research, Publish Ahead of Print*. doi: 10.1519/jsc.0000000000000745

Hug, F., Bendahan, D., Le Fur, Y., Cozzone, P., & Grélot, L. (2004). Heterogeneity of muscle recruitment pattern during pedaling in professional road cyclists: a magnetic resonance imaging and electromyography study. *European Journal of Applied Physiology, 92*(3), 334-342.

Hug, F., Decherchi, P., Marqueste, T., & Jammes, Y. (2004). EMG versus oxygen uptake during cycling exercise in trained and untrained subjects. *Journal of*

Electromyography and Kinesiology, 14(2), 187-195. doi: 10.1016/s1050-6411(03)00081-6

Hug, F., & Dorel, S. (2009). Electromyographic analysis of pedaling: A review. *Journal of Electromyography and Kinesiology, 19*(2), 182-198.

Hunter, A. M., St Clair Gibson, A., Lambert, M., & Noakes, T. D. (2002). Electromyographic (EMG) normalization method for cycle fatigue protocols. *Medicine & Science in Sports & Exercise, 34*(5), 857-861.

Hunter, S. K., Duchateau, J., & Enoka, R. M. (2004). Muscle Fatigue and the Mechanisms of Task Failure. *Exercise and Sport Sciences Reviews, 32*(2), 44-49.

Hunter, S. K., Ryan, D. L., Ortega, J. D., & Enoka, R. M. (2002). Task Differences With the Same Load Torque Alter the Endurance Time of Submaximal Fatiguing Contractions in Humans. *Journal of Neurophysiology, 88*(6), 3087-3096.

Ilic, D., & Mrdakovic, V. (2009). *Neuromehaničke osnove pokreta*. Beograd: Samostalno izdanje autora.

Johnson, A. T. (1991). *Biomechanics and Exercise Physiology*. the University of Michigan: Wiley.

Jones, A. M., & Doust, J. H. (1998). The validity of the lactate minimum test for determination of the maximal lactate steady state. *Med Sci Sports Exerc, 30*(8), 1304-1313.

Kenney, W. L., Wilmore, J., & Costill, D. (2012). *Physiology of Sport and Exercise, 5th Ed.*: Human Kinetics.

Konrad, P. (2005). *The of ABC of EMG - A Practical Introduction to Kinesiological Electromyography*: Noraxon U.S.A., Inc.

Krueger, C., & Tian, L. (2004). A Comparison of the General Linear Mixed Model and Repeated Measures ANOVA Using a Dataset with Multiple Missing Data Points. *Biological Research For Nursing, 6*(2), 151-157. doi: 10.1177/1099800404267682

Kwok, O.-M., Underhill, A. T., Berry, J. W., Luo, W., Elliott, T. R., & Yoon, M. (2008). Analyzing longitudinal data with multilevel models: An example with individuals living with lower extremity intra-articular fractures. *Rehabilitation Psychology, 53*(3), 370-386. doi: 10.1037/a0012765

Lang, I. M., Medda, B. K., & Shaker, R. (2001). Mechanisms of reflexes induced by esophageal distension. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 281*(5), G1246-G1263.

Latash, M. L. (2012). *Fundamentals of Motor Control*: Elsevier Science.

Lehman, G. J., & McGill, S. M. (1999). The importance of normalization in the interpretation of surface electromyography: A proof of principle. *Journal of Manipulative and Physiological Therapeutics, 22*(7), 444-446.

Lepers, R., Maffiuletti, N. A., Rochette, L., Brugniaux, J., & Millet, G. Y. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *Journal of Applied Physiology, 92*(4), 1487-1493.

Lombard, W. P. (1903). The Action of Two-Joint Muscles. *American Physical Education Review, 8*(3), 141-145. doi: 10.1080/23267224.1903.10649915

Löscher, W. N., Cresswell, A. G., & Thorstensson, A. (1996). Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. *The Journal of Physiology, 491*(1), 271-280. doi: 10.1113/jphysiol.1996.sp021214

Luca, C. J. D. (1997). The use of surface electromyography in biomechanics. *Journal of applied biomechanics, 13*(2), 135-163.

Martins, J., Tucci, H. T., Andrade, R., Araújo, R. C., Bevilaqua-Grossi, D., & Oliveira, A. S. (2008). Electromyographic Amplitude Ratio of Serratus Anterior and Upper Trapezius Muscles During Modified Push-Ups and Bench Press Exercises. *The*

Journal of Strength & Conditioning Research, 22(2), 477-484. doi: 10.1519/JSC.0b013e3181660748

Massó, N., Rey, F., Romero, D., Gual, G., Costa, L., & Germán, A. (2010). Aplicacions de l'electromiografia de superfície a l'esport. *Apunts. Medicina de l'Esport, 45*(166), 127-136.

Masuda, K., Masuda, T., Sadoyama, T., Mitsuharu, I., & Katsuta, S. (1999). Changes in surface EMG parameters during static and dynamic fatiguing contractions. *Journal of Electromyography and Kinesiology, 9*(1), 39-46. doi: http://dx.doi.org/10.1016/S1050-6411(98)00021-2

Masuda, T., Kizuka, T., Zhe, J. Y., Yamada, H., Saitou, K., Sadoyama, T., & Okada, M. (2001). Influence of contraction force and speed on muscle fiber conduction velocity during dynamic voluntary exercise. *Journal of Electromyography and Kinesiology, 11*(2), 85-94. doi: http://dx.doi.org/10.1016/S1050-6411(00)00048-1

Milner-Brown, H. S., & Stein, R. B. (1975). The relation between the surface electromyogram and muscular force. *The Journal of Physiology, 246*(3), 549-569.

Moritani, T., Nagata, A., & Muro, M. (1982). Electromyographic manifestations of muscular fatigue. *Medicine & Science in Sports & Exercise, 14*(3), 198-202.

Neptune, R. R., & Herzog, W. (2000). Adaptation of muscle coordination to altered task mechanics during steady-state cycling. *Journal of Biomechanics, 33*(2), 165-172.

Nicholson, W. R. (2000). The importance of normalization in the interpretation of surfaceelectromyography: A proof of principle. *Journal of Manipulative and Physiological Therapeutics, 23*(5), 369-370.

Nikolić, Z. (1995). *Fiziologija fizičke aktivnosti (Physiology of physical activity)*. Beograd: Fakultet fizičke kulture.

Noble, B. J., Borg, G. A. V., Jacobs, I., Ceci, R., & Kaiser, P. (1983). A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Medicine & Science in Sports & Exercise, 15*(6), 523-528.

Petrofsky, J., & Lind, A. (1980). The influence of temperature on the amplitude and frequency components of the EMG during brief and sustained isometric contractions. *European Journal of Applied Physiology and Occupational Physiology, 44*(2), 189-200. doi: 10.1007/bf00421098

Petrofsky, J. S. (1979). Frequency and amplitude analysis of the EMG during exercise on the bicycle ergometer. *European Journal of Applied Physiology and Occupational Physiology, 41*(1), 1-15. doi: 10.1007/bf00424464

Pires, F. O., Noakes, T. D., Lima-Silva, A. E., Bertuzzi, R., Ugrinowitsch, C., Lira, F. S., & Kiss, M. A. P. D. M. (2011). Cardiopulmonary, blood metabolite and rating of perceived exertion responses to constant exercises performed at different intensities until exhaustion. *British Journal of Sports Medicine, 45*(14), 1119-1125. doi: 10.1136/bjsm.2010.079087

Poole, D. C., Ward, S. A., Gardner, G. W., & Whipp, B. J. (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics, 31*(9), 1265-1279. doi: 10.1080/00140138808966766

Priego Jose, I., Bini Rodrigo, R., Lanferdini, F. J., & Carpes Felipe, P. (2014). Effects of workload level on muscle recruitment in cycling *Human Movement* (Vol. 15, pp. 45).

Pringle, J., & Jones, A. (2002). Maximal lactate steady state, critical power and EMG during cycling. *European Journal of Applied Physiology, 88*(3), 214-226. doi: 10.1007/s00421-002-0703-4

Racinais, S., Buchheit, M., & Girard, O. (2014). Breakpoints in Ventilation, Cerebral and Muscle Oxygenation, and Muscle Activity During an Incremental Cycling Exercise. *Frontiers in Physiology, 5*. doi: 10.3389/fphys.2014.00142

Rasmussen, U. F., & Rasmussen, H. N. (2000). Human skeletal muscle mitochondrial capacity. *Acta Physiologica Scandinavica, 168*(4), 473-480. doi: 10.1046/j.1365-201x.2000.00699.x

Rasmussen, U. F., Rasmussen, H. N., Krustrup, P., Quistorff, B., Saltin, B., & Bangsbo, J. (2001). Aerobic metabolism of human quadriceps muscle: in vivo data parallel measurements on isolated mitochondria. *American Journal of Physiology - Endocrinology and Metabolism, 280*(2), E301-E307.

Reaz, M. B. I., Hussain, M. S., & Mohd-Yasin, F. (2006). Techniques of EMG signal analysis: detection, processing, classification and applications. *Biological Procedures Online, 8*(1), 11-35. doi: 10.1251/bpo115

Rouffet, D. M., & Hautier, C. A. (2008). EMG normalization to study muscle activation in cycling. *Journal of Electromyography and Kinesiology, 18*(5), 866-878.

Roy, S. H., De Luca, G., Cheng, M. S., Johansson, A., Gilmore, L. D., & De Luca, C. J. (2007). Electro-mechanical stability of surface EMG sensors. *Medical & Biological Engineering & Computing, 45*(5), 447-457. doi: 10.1007/s11517-007-0168-z

Ryan, M. M., & Gregor, R. J. (1992). EMG profiles of lower extremity muscles during cycling at constant workload and cadence. *Journal of Electromyography and Kinesiology, 2*(2), 69-80.

Sanderson, D. J. (1991). The influence of cadence and power output on the biomechanics of force application during steady-rate cycling in competitive and recreational cyclists. *Journal of Sports Sciences, 9*(2), 191-203. doi: 10.1080/02640419108729880

Saunders, M. J., Evans, E. M., Arngrimsson, S. A., Allison, J. D., Warren, G. L., Currenton, & J., K. (2000). Muscle activation and the slow component rise in oxygen uptake during cycling. *Medicine & Science in Sports & Exercise, 32*(12), 2040-2045.

Schafer, J. L. (1999). Multiple imputation: a primer. *Statistical Methods in Medical Research, 8*(1), 3-15. doi: 10.1177/096228029900800102

Scheuermann, B. W., Hoelting, B. D., Noble, M. L., & Barstow, T. J. (2001). The slow component of O2 uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *The Journal of Physiology, 531*(1), 245-256. doi: 10.1111/j.1469-7793.2001.0245j.x

Shaw, D., Gorely, T., & Corban, R. (2004). *BIOS Instant Notes in Sport and Exercise Psychology*: Taylor & Francis.

Shinohara, M., & Moritani, T. (1992). Increase in neuromuscular activity and oxygen uptake during heavy exercise. *Ann Physiol Anthropol, 11*(3), 257-262.

Smekal, G., von Duvillard, S. P., Pokan, R., Hofmann, P., Braun, W. A., Arciero, P. J., . . . Bachl, N. (2012). Blood lactate concentration at the maximal lactate steady state is not dependent on endurance capacity in healthy recreationally trained individuals. *Eur J Appl Physiol, 112*(8), 3079-3086. doi: 10.1007/s00421-011-2283-7

Smith, D. B., Housh, T. J., Johnson, G. O., Evetovich, T. K., Ebersole, K. T., & Perry, S. R. (1998). Mechanomyographic and electromyographic responses to eccentric and concentric isokinetic muscle actions of the biceps brachii. *Muscle & Nerve, 21*(11), 1438-1444. doi: 10.1002/(sici)1097-4598(199811)21:11<1438::aid-mus11>3.0.co;2-x

Solomonow, M., Baratta, R., Zhou, B. H., & D'Ambrosia, R. (1988). Electromyogram coactivation patterns of the elbow antagonist muscles during slow isokinetic movement. *Experimental Neurology, 100*(3), 470-477. doi: http://dx.doi.org/10.1016/0014-4886(88)90032-5

Stephens, J. A., & Taylor, A. (1972). Fatigue of maintained voluntary muscle contraction in man. *The Journal of Physiology, 220*(1), 1-18. doi: 10.1113/jphysiol.1972.sp009691

Suzuki, S., Watanabe, S., & Homma, S. (1982). EMG activity and kinematics of human cycling movements at different constant velocities. *Brain Research, 240*(2), 245-258. doi: http://dx.doi.org/10.1016/0006-8993(82)90220-7

Tesch, P. A., Komi, P. V., Jacobs, I., Karlsson, J., & Viitasalo, J. T. (1983). Influence of lactate accumulation of EMG frequency spectrum during repeated concentric contractions. *Acta Physiologica Scandinavica, 119*(1), 61-67. doi: 10.1111/j.1748-1716.1983.tb07306.x

Theurel, J., Crepin, M., Foissac, M., & Temprado, J. J. (2012). Effects of different pedalling techniques on muscle fatigue and mechanical efficiency during prolonged cycling. *Scandinavian Journal of Medicine & Science in Sports, 22*(6), 714-721.

Timmer, C. A. W. (1991). Cycling Biomechanics: A Literature Review. *Journal of Orthopaedic & Sports Physical Therapy, 14*(3), 106-113.

Tipton, C. M. (2003). *Exercise Physiology*: Elsevier Science.

Tomasits, J., & Haber, P. (2011). *Leistungsphysiologie*: Springer Wien.

van der Houwen, L. E. E., Scholtes, V. A., Becher, J. G., & Harlaar, J. Botulinum toxin A injections do not improve surface EMG patterns during gait in children with cerebral palsy—A randomized controlled study. *Gait & Posture, 33*(2), 147-151. doi: 10.1016/j.gaitpost.2010.11.001

van Ingen Schenau, G. J., Boots, P. J. M., de Groot, G., Snackers, R. J., & van Woensel, W. W. L. M. (1992). The constrained control of force and position in multi-joint movements. *Neuroscience, 46*(1), 197-207.

van Ingen Schenau, G. J., Dorssers, W. M., Welter, T. G., Beelen, A., de Groot, G., & Jacobs, R. (1995). The control of mono-articular muscles in multijoint leg extensions in man. *The Journal of Physiology, 484*(Pt 1), 247-254.

van Ingen Schenau, G. J., Pratt, C. A., & Macpherson, J. M. (1994). Differential use and control of mono- and biarticular muscles. *Human Movement Science, 13*(3–4), 495-517. doi: http://dx.doi.org/10.1016/0167-9457(94)90051-5

Van Schuylenbergh, R., Vanden Eynde, B., & Hespel, P. (2004). Correlations Between Lactate and Ventilatory Thresholds and the Maximal Lactate Steady State in Elite Cyclists. *Int J Sports Med, 25*(EFirst), 403-408. doi: 10.1055/s-2004-819942

Vercruyssen, F., & Brisswalter, J. (2010). Which factors determine the freely chosen cadence during submaximal cycling? *Journal of science and medicine in sport / Sports Medicine Australia, 13*(2), 225-231.

Wasserman, K. (1984). The anaerobic threshold measurement to evaluate exercise performance. *The American review of respiratory disease, 129*(2 Pt 2), S35-40.

Weiss, L., Weiss, L. D., Silver, J. K., & Weiss, J. (2004). *Easy EMG: a guide to performing nerve conduction studies and electromyography*: Butterworth-Heinemann.

Whyte, G. (2006). *The Physiology of Training*: Churchill Livingstone Elsevier.

Wilmore, J. H., & Costill, D. L. (2004). *Physiology of Sport and Exercise*: Human Kinetics.

Appendix

Tab. 13: *Normalized MDF. The values are normalized to the highest single value in the data set.*

	GM		BF		RF		VM		VL		GastM		Mean	
Time (min)	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS
2.5	.89	.87	.88	.90	.86	.87	.91	.92	.88	.87	.91	.89	.74	.74
10	.94	.92	.91	.95	.89	.91	.95	.94	.93	.92	.92	.88	.77	.77
30	.99	.98	.98	.97	.97	.97	.97	.90	.97	.93	.93	.98	.80	.79

Tab. 14: *Normalized RMS. The values are normalized to the highest single value in the data set.*

	GM		BF		RF		VM		VL		GastM		Mean	
Time (min)	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS	MLSS	aMLSS
2.5	.94	.92	.88	.85	.92	.74	.87	.84	.88	.89	.93	.83	.90	.85
10	.90	.83	.84	.84	.83	.75	.84	.77	.82	.82	.79	.76	.84	.80
30	.61	.57	.53	.52	.46	.51	.58	.63	.56	.54	.50	.41	.54	.53

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Abbreviations

aMLSS	Above Maximal Lactate Steady State
AT	Anaerobic Threshold
ATP	Adenosine Triphosphate
BDC	Bottom Dead Center
BF	Biceps Femoris
CP	Creatine Phosphate
CV	Coefficient of Variance
ECG	Electrocardiography
EMG	Electromyography/Electromyographic
FFT	Fast Fourier Transform
FSA	Frequency Spectral Analysis
GastM	Gastrocnemius Medialis
GM	Gluteus Maximus
Hz	Hertz
iEMG	Interference EMG signal
[La]	Blood Lactate Concentration
LT	Lactate Threshold
LT1	Lactate Turn Point 1
LT2	Lactate Turn Point 2
MDF	Median Frequency
MLSS	Maximal Lactate Steady State
mm	Millimeter
mM	Millimole
MOV	Moving average

MU	Motor Unit
MUAP	Motor Unit Action Potential
mV	Millivolt
MVC	Maximal Voluntary Contraction
PSD	Power Spectrum Density
RCP	Respiratory Compensation Point
RM	Rectus Femoris
RMS	Root Mean Square
sEMG	Surface Electromyography
SOL	Soleus
TA	Tibialis Anterior
TDC	Top Dead Center
TP	Total Power
TPS	Total Power spectrum
VL	Vastus Lateralis
VM	Vastus Medialis
VO_2max	Maximal oxygen consumption
W	Watt

Erklärung über die persönliche Urheberschaft

Ich erkläre, dass ich die vorliegende Arbeit selbstständig verfasst habe und nur die ausgewiesenen Hilfsmittel verwendet habe. Diese Arbeit wurde weder an einer anderen Stelle eingereicht noch von anderen Personen vorgelegt.

Wien, März 2014

Bojan Makivic

CURRICULUM VITAE

Personal information

Name:	Bojan Makivic, BSc.
Date of birth:	21.09.1979
Place of birth:	Virovitica, Croatia

Education

Since 09/2013	Master study program in training therapy, University of Vienna, Austria
09/2011-09/2013	Magister study program in sport science, University of Vienna, Austria
09/2000-12/2007	Bachelor study program in Sport and Physical Education, University of Belgrade, Serbia
09/1994-06/1998	11th Belgrade Gymnasium, natural science

Professional and career experiences

Since 09/2013	Co-founder and co-owner of PROFEX OG Institute for Health and Sport, Vienna, Austria
04/2011-06/2013	Fitness trainer in “In Good Shape”, Vienna, Austria
09/2008-09/2010	Founder and owner of Urban Body fitness studio (self-employed), Belgrade, Serbia
06/2006-09/2008	Personal and fitness trainer in DnD Fitness Center, Belgrade, Serbia

Scientific experience

Lectures

- First international fitness trainer course. Mansoura, Ägypten, November 2014

Conference attendances

- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrade, Serbia, December 2013.
- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrade, Serbia, December 2011.
- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrade, Serbia, December 2007.

Publications

- Makivic B. (2015) in Press. Heart rate variability (HRV) analysis in sport: utility, practical implementation and future perspective. Aspetar Sport Medicine Journal
- Makivic B, Djordjević-Nikić M, Willis MS. (2013). *Heart rate variability (HRV) at the level of anaerobic threshold measured by the blood lactate concentration*. Conference proceedings, Belgrade, Serbia
- Makivic B, Djordjević-Nikić M, Willis MS. (2013). *Heart rate variability (HRV) as a tool for diagnostic and monitoring performance in sport and physical activities*. Journal of Exercise Physiology Online 06/2013; 16(3):103-131
- Makivic B., Willis M., Djordjevic-Nikic M. (2011) *Heart rate variability (HRV) as a diagnostic and monitoring tool in Sport*. Conference proceedings, Belgrade, Serbia
- Makivic B., Djordjevic-Nikic M., Macura M., Stojiljkovic S. (2007). *Woman’s recreational workout in gym*. Conference proceedings, Belgrade, Serbia

LEBENSLAUF

Persönliche Daten

Name, Vorname:	Makivic Bojan, BSc.
Geburtsdatum:	21.09.1979
Geburtsort:	Virovitica, Kroatien

Ausbildung

Seit 09/2013	Masterstudim Trainingstherapie, Universität Wien
09/2011 - 09/2013	Magisterstudium Sportwissenschaft, Universität Wien
09/2000 - 12/2007	Bachelorstudium Sportwissenschaft, Universität Belgrad
09/1994 - 06/1998	Matura, 11. Naturwissenschaftliches Gymnasium Belgrad

Berufliche Erfahrungen

Seit 09/2013	Mitbegründer und Geschäftsteilhaber von PROFEX OG, Institut für Gesundheit und Sport, Wien, Österreich
04/2011 - 06/2013	Fitnesstrainer in “In Good Shape”, Wien, Österreich
09/2008 - 09/2010	Begründer und Inhaber von Urban Body Fitness Studio (Selbstständig), Belgrad, Serbien
06/2006 - 09/2008	Personal- and Fitnesstrainer in DnD Fitness Zentrum, Belgrad, Serbien

Wissenschaftliche Erfahrungen

Vorträge

- First international fitness trainer course. Mansoura, Ägypten, November 2014

Konferenzteilnahme

- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrad, Serbien, Dezember 2013.
- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrad, Serbien, Dezember 2011.
- International Scientific Conference ‘’Effects of physical activity application to anthropological status with children, youth and adults ’’, Belgrad, Serbien, Dezember 2007.

Publikationen

- Makivic B. (2015) in Vorbereitung. Heart rate variability (HRV) analysis in sport: utility, practical implementation and future perspective. Aspetar Sport Medicine Journal
- Makivic B, Djordjević-Nikić M, Willis MS. (2013). *Heart rate variability (HRV) at the level of anaerobic threshold measured by the blood lactate concentration*. Tagungsband, Belgrad, Serbien
- Makivic B, Djordjević-Nikić M, Willis MS. (2013). *Heart rate variability (HRV) as a tool for diagnostic and monitoring performance in sport and physical activities*. Journal of Exercise Physiology Online 06/2013; 16(3):103-131
- Makivic B., Willis M., Djordjevic-Nikic M. (2011) *Heart rate variability (HRV) as a diagnostic and monitoring tool in Sport*. Tagungsband, Belgrad, Serbien
- Makivic B., Djordjevic-Nikic M., Macura M., Stojiljkovic S. (2007). *Woman’s recreational workout in gym.* Tagungsband, Belgrad, Serbien