Neuromuscular Functions in Sportsmen and Fibromyalgia Patients

A Surface EMG Study in
Static and Dynamic Conditions

Ewa Klaver-Król

**NEUROMUSCULAR FUNCTIONS IN SPORTSMEN
AND FIBROMYALGIA PATIENTS**

A SURFACE EMG STUDY IN STATIC
AND DYNAMIC CONDITIONS

Ewa Klaver-Król

Dissertation Committee:

Prof. dr. K.I. van Oudenhoven-van der Zee (University Twente, Chairman/
secretary)

Prof. dr. J.J. Rasker (University Twente, Promoter)

Dr. M.J. Zwarts (Centre for Epilepsy Kempenhaeghe, Heeze; Assistant-
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Dr. M.J. Nederhand (Rehabilitation Center Het Roessingh, Enschede)

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**A SURFACE EMG STUDY IN STATIC
AND DYNAMIC CONDITIONS**

PROEFSCHRIFT

ter verkrijging van
de graad van doctor aan de Universiteit Twente,
op gezag van de rector magnificus,
prof. dr. H. Brinksma,
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door

Ewa Grażyna Klaver-Król
geboren op 1 juli 1943
te Lublin, Polen

Dit proefschrift is goedgekeurd door de promotor
Prof. dr. J.J. Rasker

en de assistent-promotor
Dr. M.J. Zwarts

I can't speak for elsewhere,
But here on Earth we've got a fair supply of everything.
Here we manufacture chairs and sorrows,
Scissors, tenderness, transistors, violins,
Teacups, dams, and quips.

Wisława Szymborska (1923 - 2012)

To the memory of my teachers, Professor Jan Droogleever-Fortuyn and my family
To Bożena, Krystyna and Krzysztof
To Marten, Peter and Igor

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Abbreviations

ACR = American College of Rheumatology

ARV = average rectified voltage = amplitude of the sEMG signal

BB = biceps brachii (muscle)

CC = cross-correlation method

CV = mean muscle fibre conduction velocity, muscle conduction velocity

CV-cc = CV obtained by the cross-correlation method

CV-ipl = CV obtained by the inter-peak latency method

EMG = electromyography

FF type = fast-twitch fatigue-sensitive muscle fiber type
= fast glycolytic, FG = type IIb

FIQ = Fibromyalgia Impact Questionnaire

FM = fibromyalgia, fibromyalgia syndrome

FR type = fast-twitch fatigue-resistant muscle fiber type
= fast oxidative glycolytic, FOG = type IIa

IPL = inter-peak latency method of measuring CV

$\text{m} \cdot \text{s}^{-1} = \text{m/s}$

MU = motor unit (a compound of one motoneurone, one axon and many belonging muscle fibres)

MUP = motor unit (action) potential (an action potential generated in one motor unit)

MUP-V, PV = MUP velocity/peak velocity = propagation velocity of an MUP/peak

MVC = maximum voluntary contraction force = maximum force

PF = peak frequency = MUP frequency = number of peaks/MUPs per second

sEMG = surface electromyography

S type = slow-twitch fatigue-resistant muscle fiber type
= slow oxidative, SO = type I

Sk, Sk-PV = skewness of the peak/MUP velocities obtained within-subject

SD-mup, SD-PV = standard deviation of the peak/MUP velocities obtained within-subject

TP = tender point

Part I

General Introduction

Chapter 1

**Surface electromyography:
its goals, conditions and variables**

In everyday life, every movement of the body is a result of a highly organized cooperative action between the muscles and the controlling neural system. Investigating muscle electrical activity has been proved to be a good way to gain information about the physiological properties of a muscle itself and of the governing system. In particular, noninvasive methods for obtaining muscle activity, known as surface electromyography (sEMG), are successful in research situations. In sEMG, muscular activity is produced through voluntary contractions by the subject and is recorded from the skin surface. Since the procedure is not painful, there are no issues with repeated or long experiments. sEMG has been applied in revalidation, in ergonomics, and in fundamental research on muscular function. However, it is seldom used in a clinical-diagnostic setting.

In general, sEMG strategies have tended to evolve from an investigation into large amounts of muscle activity in rigid procedures, with robust results, towards the investigation of less activity in more refined procedures, and with refined results.

Properties of contractions

To obtain muscular activity that is feasible to analyze using sEMG, the way in which force and movement are exerted during an experiment must be strictly standardized. Contractions can vary in different ways, such as in the force level, in the dynamics of the effort, and in the manner of controlling the effort.

Force level

Force level is expressed in terms of the required force as a percentage of the maximum force that the subject is able to produce in a given situation (maximum voluntary contraction force = MVC). The required force can vary from 100% of MVC down to the minimum force that still renders any muscular activity.

Isometric and dynamic contractions

In an experiment, the mobility of a muscle (the mobility of an extremity) can vary. The extremity can be immobile (known as an isometric contraction or isometric condition) or it can move (a dynamic contraction or dynamic condition). To date, many studies have been performed in isometric conditions, and relatively few in dynamic conditions.

Force-controlled and position-controlled contractions

The way in which the exerted force is controlled by the subject can also differ. There are experiments with force-controlled contractions and others with position-

controlled contractions. In a force-controlled contraction (also called a force task), the subject is required to maintain a given force level that is usually measured and displayed using a force transducer. Force control can be applied under either isometric or dynamic conditions. In an isometric force task, the extremity is firmly fixed. Experimental designs involving isometric force tasks have been popular and there are many classical publications, such as on the biceps brachii muscle (Zwarts et al. 1987), on the tibialis anterior (Andreassen and Arendt-Nielsen 1987), or on the vastus lateralis (Arendt-Nielsen et al. 1989). In recent decades, several force-controlled experiments under *dynamic* conditions have been reported; these include exercising on an ergometric bicycle with an sEMG registration from the leg muscles (Pozzo et al. 2004) and shoulder girdle exercise on a isokinetic dynamometer with sEMG measurement of the neck and shoulder musculature (Elert et al. 1992).

In a position-controlled contraction (also known as a position task), the subject is required to maintain a targeted limb position, while a force is applied using a fixed inertial load (Hunter et al. 2002, Rudroff et al. 2005). To date, experiments involving position control have mostly been isometric in nature, traditionally known as static contractions (Krogh-Lund and Jorgensen 1993). In recent decades, standardized *dynamic position experiments* have sporadically been published (Potvin 1997). In dynamic position-controlled exercises, the extremity is moved between two well-defined positions.

Analyzing sEMG: types of sEMG variables

Depending on what one is looking for, sEMG activity can be analyzed in different ways. In fact, the variable one uses determines the feature that will be extracted from sEMG. Historically, there has been a trend away from global and robust analysis to more refined analyses or domains. In the following paragraphs, the properties of several sEMG variables will be presented: those that are commonly used and those less commonly used but with specific or interesting features.

Integrated EMG (IEMG)

The measurement of the global amount of sEMG activity, called an integrated EMG (IEMG), provides a well-established variable. An IEMG is expressed by the amplitude or surface of an sEMG signal. An IEMG depends on the number of activated motor units (MUs) and their firing rates. This relative variable can present increases or decreases in sEMG such as those due to changing force levels or as an effect of time during fatigue tests (Arendt-Nielsen et al. 1989, Edwards and Lippold 1956). An IEMG can also show differences in activity between the right and left

site of a body with paralysis or posttraumatic weakness, and therefore this variable is often used in revalidation and ergonomic activities (Krabben et al. 2011, van der Hulst et al. 2010, Wentink et al. 2012). It is easy to obtain an IEMG from practically any muscle, and registration requires only two electrodes (a recording electrode and a reference electrode) (Hermens et al. 1999).

Power spectrum frequency and zero-crossing rate

The power spectrum frequency distribution (SF) is probably the oldest and still a commonly used sEMG variable. The SF was, we believe, first described by Kopec and Hausmanowa, and denoted as “harmonic analyze” (Kopec and Hausmanowa-Petrusewicz 1966). Many publications followed, including several classical papers such as those by Lindstrom (Lindstrom et al. 1970). SF is expressed as a mean power frequency (Lindstrom et al. 1977) or the median frequency (Stulen and DeLuca 1981) of the signal’s spectrum. As with IEMG, the registration requires only two electrodes (Hermens et al. 1999). The SF is a relative measure and it has to be normalized to the primary or average value. It is also an indirect measure in the sense that it is not clearly embedded in a specific physiological phenomenon (Stulen and DeLuca 1981). SF is primarily (positively) related to the propagation velocity of an action potential along muscle fibers (conduction velocity = CV) (Eberstein and Beattie 1985)

During fatigue, the SF shifts towards lower frequencies, which is mainly due to the decrease in the CV (peripheral fatigue) (Lindstrom et al. 1977). Another factor contributing to these shifts are the changes in the so-called “firing statistics” (MU recruitment along with MU firing rates) (Broman et al. 1985, Hägg 1991b). As such, the SF is especially valuable as a fatigue parameter; a shift to lower SF values during fatigue is known as a sign of fatigue.

Besides the SF, another fatigue parameter is the zero-crossing rate (ZC). This formula, introduced by Hägg, calculates how often the signal crosses a zero-line (Hägg 1981). The ZC decreases during muscular fatigue and the main underlying factor is the decrease in the CV (Hägg 1991b).

Muscle fiber conduction velocity (CV) variables: different methods and various levels of refinement

The CV obtained from sEMG directly shows the propagation velocity of the muscle membranes of the active motor units. It is expressed in absolute values of meters per second, and is typically around 4 m/s. The CV reflects physiological properties of muscle membranes, the bioelectric signal messengers to the contractile mechanism (Kernell 2006a)

CV measurements can be categorized by the electrodes applied and the calculation formula used. The development of CV measurements in research has reflected the desire of physiologists and clinicians to resolve pathophysiological problems, and the endeavors of technicians and software specialists to develop newer and more sophisticated devices (Andreassen and Arendt-Nielsen 1987, Drost et al. 2004, Hermens et al. 2000).

A CV measurement requires an array of at least three electrodes, which form two electrode pairs (Hermens et al. 1999). When such an electrode-set is placed on the skin in the direction of the muscle fibers, and a muscle is activated by the subject, two identical differential electromyographic signals can be derived, with a time delay between the two. Knowing the distance between the electrodes and having measured the time delay, the velocity at which the muscle action potential was propagated along the muscle membrane can be calculated, i.e. the CV.

The average CV

The most frequently applied CV variable has been the average CV, and the most popular calculation method has been that of cross-correlation (Naeije and Zorn 1983). The principle is that a cross-correlation is determined between the signals obtained along a muscle. The CV is then derived from the shift (time delay) in the peak of the correlogram between the signals, and the distance between the electrodes. The average CV found using the cross-correlation method thus reflects a global velocity of an sEMG signal. Since an sEMG signal is composed of motor unit potentials (MUPs), the method reports the mean velocity of the MUPs produced by the motor units that are actually active. However, the CV obtained by the cross-correlation method is biased toward the large, most pronounced (and generally faster propagating) MUPs.

CV of individual motor units

When two or more parallel electrode arrays are used, or a longitudinal electrode array is paired with another array placed perpendicular to the muscle fibers, thus making a cross, additional spatiotemporal information can be gained from sEMG (Yamada et al. 1987). The shape and spatial distribution of the MUPs can then be followed in two dimensions across a muscle. This allows the MUPs to be distinguished and then assigned to the individual specific motor units from whence they originate (Disselhorst-Klug et al. 1999, Zwarts and Stegeman 2003).

Such spatially extending electrodes have seen further development. The electrode combinations currently applied are in a form of a matrix electrode that may cover large parts of a muscle and contain over a hundred small-faced electrodes with short

inter-electrode distances (Beck et al. 2005, Kleine et al. 2007). Using the technique of CV estimation in conjunction with matrix electrodes, known as high density – sEMG (HD-sEMG), enables the scrutinizing of large muscle surfaces in order to identify individual motor units (Kleine et al. 2000, Stegeman et al. 2000). For every individual motor unit, a CV can be calculated. Individual motor units can only be discerned at low force levels, from 5 to 20 % of MVC force (Kleine et al. 2000). To date, we believe that only isometric and force-controlled contraction types have been applied in CV measurements using HD-sEMG.

CV of motor unit potentials/peaks

To the best of our knowledge, Lange et al. were the first to obtain large numbers of MUP propagation velocities from sEMG and to analyze them as a set of values (Lange et al. 2002). In this method, named the inter-peak latency (IPL) method, MUPs are often called ‘peaks’ in order to avoid confusion with the propagation velocities of *individual* motor units. In the IPL method, as many MUPs/peaks as possible are selected from an sEMG signal and then a velocity is calculated for each MUP/peak. The mean CV in the IPL method (CV-ipl) is the average of all the peak velocities (peak-CVs) found. CV-ipl is a more refined measure than CV obtained by the cross-correlation method because it does not represent a global signal’s velocity but is a result of various velocities attributed to the discharging motor units. This will be discussed in detail in Chapter 8. However, one must remember that the peak-CVs are not motor unit-specific. In other words, the IPL method cannot, in contrast to the HD-sEMG method, distinguish between individual motor units.

Chapter 2

Introduction to the presented Method

The basic philosophy: natural movement

This thesis describes a complete method for the examination of the functions of the biceps brachii muscle in a cooperative human using sEMG.

We strived to create a situation where the standardized contractions would, as far as possible, represent a natural situation in daily life. A characteristic feature of the study design was a combination of *dynamic* exercise with *position control* of the limb, rather than the usually applied isometric contractions with force control. During the sEMG measurement, the lower arm was swung between two defined positions over an angle of 45°. The movements were rhythmical, at a rate of 40 cycles per minute, dictated by a metronome. Low force levels were used, no more than 20% of the MVC. The load was a small bag, filled with a mixture of sand and lead, held in the palm. During such movements, the biceps brachii is repeatedly shortened and lengthened while sEMG output is continuously registered from the muscle over periods of several minutes.

A position task is far more natural than a force task. In our daily lives, we are always dragging inertial loads and controlling the position of our body. Force control is not a normal function in everyday existence. Apart from the natural aspect of a position control experiment, it also has a methodological benefit in that it renders more electromyographic activity than force-controlled tasks (Maluf et al. 2005). The physiological background to this phenomenon is that position control requires an extra input from the central nervous system (Mottram et al. 2005b). A practical consequence is that, during a position-controlled task, one is able to obtain considerable electromyographic activity at very low force levels, even when a muscle is unloaded. In contrast, during a force-controlled performance, there is no activity in an unloaded muscle. Trying to maintain any position for a lengthy period is difficult, and one tends to somehow support or move the body. If we do not, we risk falling over or even fainting, as sometimes happens to the guards in front of presidential or royal palaces. This additional challenge in a position-controlled, as against a force-controlled, task is exactly what leads to an “extra input” from the higher neural centers.

Similarly, dynamic situations are more common in daily life than static situations. Moreover, dynamic exercises not only better reflect everyday situations, they also have methodological benefits. That is, during a long-lasting activity, the dynamic conditions better maintain blood flow in an extremity because the lower intramuscular pressure, allowing undisturbed tissue oxidation (Crenshaw et al. 1997, Petrofsky et al. 1981, Radegran 1997, Sjogaard et al. 1988). Another benefit of dynamic conditions is that the muscular activity is more variable due to its cyclic character.

There are cycles of recruitment and de-recruitment of motor units and, moreover, in different parts of a cycle, different motor unit types are activated (Barnes 1980, Milner-Brown et al. 1973a, Nardone et al. 1989). Having such variability can be important in some types of sEMG analyses (Klaver-Król et al. 2010a). This issue will be discussed in more detail in the chapters that follow.

Refined features of sEMG analysis. The goal of the thesis

Among the numerous studies on muscular functions using sEMG, little has been investigated under dynamic conditions. In particular, research is lacking that addresses continual and prolonged measurements of muscle fiber conduction velocities during dynamic processes.

In this thesis, a method is presented for examining the biceps brachii muscle by sEMG under both static and dynamic conditions. A refined analysis of the sEMG signal has been developed based on the measurement of MUP/peak velocities. Besides extracting the mean CV, new aspects were deduced from the peak velocities by transforming them into their statistical parameters. Here, the skewness and the standard deviation of peak velocities, as measured within an individual, were calculated. Finally, the intensity of the electromyographic activity was found from the peak velocity (the number of MUPs/peaks per second).

In Chapter 3, the proposed method is described as it was used in healthy subjects. In the following chapters, applications of the method in two different groups are presented: in sportsmen, and in patients with fibromyalgia (FM). With sportsmen, we were searching for sEMG differences between sprinters and endurance athletes - groups with genetically different muscle fiber compositions (Chapters 4 and 5). With FM, which is a disorder characterized by generalized muscular pain, we were investigating possible abnormalities in the function of the muscular membrane and in central neural regulation (Chapters 6 and 7).

The aim of the thesis is to show what one can usefully learn from sEMG under static conditions, and under dynamic conditions with position control.

Part II

Presentation of the Method

Chapter 3

Distribution of motor unit potential velocities in short static and prolonged dynamic contractions at low forces: use of the within-subject's skewness and standard deviation variables

E.G. Klaver-Król, N.R. Henriquez, S.J. Oosterloo, P. Klaver, J.M. Bos, M.J. Zwarts.

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Abstract

Behaviour of motor unit potential (MUP) velocities in relation to (low) force and duration was investigated in the biceps brachii muscle using surface electromyography (sEMG).

Short static tests of 3.8 s (41 subjects) and prolonged dynamic tests (prolonged tests) of 4 min (30 subjects) were performed as position tasks, applying forces up to 20% of maximal voluntary contraction (MVC). Four variables were extracted from the sEMG signal using the inter-peak latency technique: the mean muscle fibre conduction velocity (CV); the proportion between slow and fast MUPs expressed as the within-subject skewness of MUP velocities; the within-subject standard deviation of MUP velocities [SD-peak velocity (PV)]; and the amount of MUPs per second (peak frequency = PF).

In short static tests and the initial phase of prolonged tests, larger forces induced an increase of the CV and PF, accompanied with the shift of MUP velocities towards higher values, whereas the SD-PV did not change. During the first 1.5 – 2 min of the prolonged lower force levels tests (unloaded, and loaded 5% and 10% MVC) the CV and SD-PV slightly decreased and the MUP velocities shifted towards lower values; then the three variables stabilized. The PF values did not change in these tests. However, during the prolonged higher force (20% MVC) test, the CV decreased and MUP velocities (skewness) shifted towards lower values without stabilization, while the SD-PV broadened and the PF decreased progressively.

It is argued that these combined results reflect changes in both neural regulatory strategies and muscle membrane state.

Introduction

Diverse laboratory conditions have been used in surface electromyography (sEMG) studies in order to gain insights into the neural regulatory strategies and muscle membrane alterations. The influence of force load on sEMG can be investigated by using force tasks or position tasks. The majority of studies have been performed as force tasks, which means that the subject controls the effort by maintaining a target force while the limb position is fixed. During the position tasks, in contrast, an inertial load is applied while the subject controls a target limb position. Both force and position tasks can be performed in static or dynamic conditions. Examples of the static force tasks are the well-known isometric experiments, with higher and lower force levels. In the recent past, force tasks in dynamic conditions have been performed sporadically, such as the cycling experiments by Pozzo et al. (2004) and Farina et al. (2004). Since Hunter et al. (2002) found that, with the same load torque, position tasks resulted in a shorter endurance time than force tasks, suggesting different regulatory mechanism for the both type of tasks, the position task studies gained field. Most *position task* experiments have been performed *in static conditions*, evaluating the underlying physiological phenomena during force versus position tasks (Hunter et al. 2002, 2003; Hunter and Enoka 2003 and Rudroff et al. 2005, 2007). Potvin (1997) has described the changes in the sEMG during *position tasks in dynamic conditions*. Previous findings suggest that, as compared with force tasks, position tasks induce greater synaptic input into the motor neurons (Mottram et al. 2005a) and greater adaptation in the motor unit discharge (MacGillis et al. 2003).

Changes in muscle activity during (static and dynamic) position tasks have been assessed using two of the three traditional sEMG parameters, the power spectrum and the global sEMG amplitude. However, the third parameter, the mean muscle fibre conduction velocity (CV), has been lacking. Spectral estimates are generally accepted in fatigue experiments as equivalents of CV because they highly correlate with the CV's changes (Bigland-Ritchie 1981; Eberstein and Beatie 1985; Arendt-Nielsen and Mills 1985). But this correlation holds true only for the constant forces and isometric conditions; thus, a replacement of CV by power spectrum assessments does not always seem feasible (Farina et al. 2002; Broman et al. 1985). The favour of CV above power spectrum is, furthermore, that it renders direct and absolute values of conduction velocity, and is less sensitive to the anatomical local relationships, such as depth of the motor unit (MU) in relation to the muscle and skin surface (Farina et al. 2002). Yet the limitation of a global CV measurement remains the lack of sensitivity to the changes at the level of an individual motor unit potential/ motor unit.

To accommodate with the limitation of a global CV, researchers have recently been trying to disentangle the propagation velocities of individual motor unit potentials (MUPs) from sEMG. One of the methods is the inter-peak latency (IPL) method proposed by Lange et al. (2002). The principle comprises calculating conduction velocities of the MUPs from the latencies between paired MUPs of two differential sEMG signals obtained parallel to the muscle fibres, and the distance between the recording electrodes. The negative peaks of two paired MUPs are then the elements determining the IPL. As MU propagation velocity reflects the intrinsic physiological properties of a MU, such as fast-twitch or slow-twitch type (Buchthal et al. 1973; Andreassen and Arendt-Nielsen 1987), the IPL method renders many diverse MUP velocities. Lange et al. (2002) proposed using the standard deviation of MUP velocities as an additional measure that offers information about muscle fibre properties. Changes in these velocities during prolonged effort may indicate, for example, slowing/fatigue of the activated MUs and/or appearance of fast/newly recruited MUs. Such shifts in the activated MUs' populations were shown by Houtman et al. (2003) by eliciting the MUP velocities with the IPL method and presenting their distribution in histograms. The IPL method has not been applied much. It yields insights into the diversity of MUP velocities and thereby the underlying changes in the MU activity. The method is simple and does not require expensive apparatus or software. When compared with techniques that assess the propagation patterns of MUPs by multi-channel/spatial resolution sEMG (Masuda and Sadoyama 1986; Rau et al. 1997), the IPL method is unable to distinguish and follow individual MUPs belonging to the specific MUs.

In the present study, the sEMG signal was described with four variables derived using the IPL method: (1) the mean muscle CV which was the average of the obtained MUP velocities; the two statistical distribution variables, which were: (2) the within-subject MUP velocities' skewness [Sk-peak velocity (PV)] and (3) the within-subject MUP velocities' standard deviation (SD-PV); and (4) the peak frequency (PF), a variable expressing the amount of MUP activity (number of MUPs/peaks) per second.

The aim of the present study was to investigate, with the four variables, the changes in MUPs' velocities of the biceps brachii (BB) muscle during *prolonged dynamic position tasks*, in relation to (low) force and duration. It is chosen for the *dynamic position tasks* as a study design because their physiology promised the finding of a large variety of MUP velocities (great amount of activity due to the position task character, and diversity because of the recruitment/derecruitment changes within the dynamic cycle). Whole cycles of movement with their concentric and eccentric phases were analyzed in order to evaluate a total of the sEMG activity

with its evolution over time. To highlight the initial changes with the effect of force on it, the changes during the first 14.4 seconds of the dynamic tasks were evaluated separately. Additively, *short static position tasks* were performed in order to show the (early) changes on force, without any influence of movements on the signal.

Methods

Subjects

The study involved short static and prolonged dynamic experiments. Forty-one healthy and physically active males (24.7 ± 6.7 years, from 16 to 48) (mean $\pm$ SD) volunteered for the first experiment and 30 randomly chosen subjects from that group (25.4 ± 7.6 years, from 18 to 42) participated in both experiments. Exclusion criteria were drug abuse and the practice of body building. Three from a total of 44 subjects were excluded because of the impossibility to obtain a required correlation coefficient between the sEMG signals used to estimate the variables' values. The experimental protocol was conducted according to the Helsinki Declaration and approved by the local ethics committee. All participants gave their written informed consent.

Experimental set-up

Maximal voluntary contraction (MVC) of the elbow flexors was measured at least five days before the experiment with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands). During the MVC measurements, the subjects were seated upright. The shoulder was slightly abducted and flexed at 45° , the elbow was firmly sustained and flexed at 90° , and the forearm was supinated. The dynamometer was applied to the wrist by the break method (van der Ploeg and Oosterhuis 1991). The peak hold was switched off and the force was kept for at least three seconds. The mean of three maximal values was taken as a MVC. The MVC was assessed with the elbow at 90° , although the tests were performed at the elbow angle of 135° (Philippou et al. 2004).

During the experiment, the subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45° of shoulder flexion; the forearm was free. When the elbow was stretched, the line of the upper arm-forearm was at 45° in relation to horizontal. When the elbow was flexed to the angle of 135° , the forearm was horizontal. The forearm was supinated during static and dynamic tests.

Static tests

Subjects were asked to hold the forearm horizontally (elbow angle was then 135°). A visual bar helped to maintain the correct (horizontal) position of the forearm. In the loaded tests, a sack filled with lead and sand was placed in the palm. Three levels of force were applied in blocks that were three min apart: unloaded, loaded 10 and 20% MVC. A block consisted of 3 tests (three repetitions at the same level of force); every test lasted for 3.8 s and was within a block separated 30 s from one another.

Dynamic tests

All participants of the dynamic tests underwent previously static tests, separated by 5 min. Subjects were asked to swing the forearm from the stretched (elbow angle 180°) to horizontal (elbow angle 135°) position, thus moving over an angle of 45°. They did it within a rate of 40 beats per minute (one up-and-down movement in one beat), given by a metronome sound. The visual bar indicated the horizontal position to which the lower arm returned after being stretched. Four force levels were applied: unloaded, and loaded 5, 10 and 20% MVC. The tests lasted for 4 min and were separated by 5 min.

EMG recording

Measurements were performed on the short head of the BB muscle of a dominant arm. A surface electrode array consisted of three gold-coated electrodes (Harwin, P25-3526), diameter 1.5 mm, insulated in synthetic material plate, with a 10 mm distance between the electrodes (Sadoyama et al. 1985). The skin was cleaned with 95 % ethanol. The electrode array was placed parallel to the muscle fibres (Sollie et al. 1985). The proximal electrode was positioned exactly on the distal one-quarter point of the upper arm, measured between the coracoid and the elbow crease. This place of the electrode was about halfway between the endplate zone and the tendon, securing a sufficient distance from the endplate (Sadoyama et al. 1985; Masuda and Sadoyama 1987). Bipolar derivation was made from the proximal to distal direction, producing two differential signals. The optimal electrode position was controlled by both the observation of the signal on the monitor and the estimation of a correlation coefficient (CC) between the two sEMG signals, which was accepted at $r > 0.7$ for unloaded, $r > 0.85$ for 5% MVC and $r > 0.9$ for higher loaded tests. (During the static and dynamic tests, the maximal CC for unloaded arm was usually lower than that for loaded arm, which was consistent with Hogrel et al (1998), who have estimated for an unloaded arm $r > 0.7$). The ground electrode was placed on the lateral upper arm, slightly proximal from the derivation electrode. The temperature sensor was medial on the upper arm. Two obtained signals were differentially amplified (gain 2000 to 10,000x) and band pass filtered at 2-250 Hz by EMG apparatus (Viking IV, US).

Data processing

The signals were simultaneously A/D converted (sampling 10 kHz, 12 bits acquisition). Data were stored on a personal computer. The signal was analyzed with LabVIEW (version 6.1) software that also facilitated a partial on-line analysis. The peak selection and the correlation coefficient assessments for both static and dynamic tests were performed on 0.2 s epochs. In the static tests, measurements were taken every second during 0.8 s (comprising 4 epochs of 0.2 s). A static test was of 3.8 s duration and was repeated three times for each force level. The statistical analyses were performed on the data of these three repeated tests taken together. In the dynamic tests, the data were assembled every 30 s during 14.4 s (comprising 72 epochs of 0.2 s). The test duration was 4 min.

Peak selection

The basic principle was that of Lange et al. (2002). The software was custom designed and written in LabView. The orientation of the signals is up-negative. *1st step*: finding the peak-to-peak amplitude of the largest MUP in an epoch of 0.2 s. *2nd step*: finding a peak-decline structure. The peak-decline is defined as a structure with a decline of $\geq 20\%$ compared with the largest MUP amplitude of a 0.2 s epoch, over ≤ 4 ms (≤ 40 sample points). *3d step*: finding a peak. A peak is the highest (most negative) point previous to the decline, and must be ≥ 10 μ V (the threshold of the noise level). *4th step*: finding a pair peak. A pair peak is a peak in the second signal with the properties as previous, found in a time window between 1.49 and 4 ms after the peak of the first signal. This window is chosen assuming the physiological CV values of 2.5 – 6.67 m/s. *5th step*: excluding double peak. If a peak from the first signal matches two different peaks from the second signal, then the first peak from the second signal is true. *6th step*: If during the fatiguing (20% MVC) tests the CV severely diminishes, then the low limit of CV is put at 1.3 m/s, making a window of 1.49 - 7.68 ms. As an objective pragmatic criterion to this change, a lowering of the PF with $\leq 30\%$ was assumed, compared with the first PF value of the 20% MVC test.

The variables

The following calculations were performed: (1) the basic calculation of peak velocities (PVs) following the IPL method; (2) the mean CV, expressed as an average value of the PVs; (3) the within-subject skewness of the peak velocities (Sk-PV), expressed as a skewness of a PVs' population of a subject; (4) the within-subject standard deviation of the peak velocities (SD-PV), expressed as a standard deviation of a PVs' population of a subject; and (5) the peak frequency (PF), expressed as a number of peaks per second.

Statistics

One-way analysis of variance (ANOVA) with repeated measures on force was used to compare the dependent variables for static tests (three levels) and for the initial values of the dynamic tests (four levels). A two-way ANOVA with repeated measures on force (four levels) and time (nine levels) was used to compare variables during the dynamic tests. In the case of interactions between force and time, to describe changes over time, when appropriate, four separated ANOVAs were performed with smaller time windows of 0 – 60, 60 – 120, 120 – 180 and 180 – 240 s. To be assured that the dependent variables met parametric assumptions, plots of residues were produced with SPSS software, model control as suggested by Kutner et al. (2005, p. 1157). No relevant deviations of model were detected. Pearson correlation coefficients were calculated to evaluate associations between variables. A level of $P < 0.05$ was used to identify statistical significance.

Results

Subjects' physical characteristics are presented in the Table 1. The force of the elbow flexors correlated positively with the upper arm circumference ($r = 0.484$, $P < 0.01$). No association was found between either force or upper arm circumference and the sEMG variables. The average skin temperature increased during the dynamic tests by 1.65°C ($P < 0.001$); it did not change during the static tests.

Table 1. Characteristics of the participants to the static tests

	N	Minimum	Maximum	Mean	SD
Age (years)	41	16	48	24.7	6.7
Height (cm)	41	166.0	197.0	183.2	8.0
Weight (kg)	41	60.0	95.0	74.3	9.2
Force (N) right	38	148.5	346.5	246.1	41.9
Force (N) left (for left-handed)	3	262.3	267.3	265.1	2.5
Skin thickness (mm)	41	1.4	4.2	2.4	.7
Circumference upper arm (cm)	41	24.0	31.0	27.5	2.0
Length of biceps (cm)*	41	30.50	39.50	35.4	2.0
Length lower arm/radius (cm) †	33	24.0	30.0	26.4	1.6
Length lower arm/palm (cm) ‡	33	30.0	36.0	33.1	1.6
Initial skin temp. ($^{\circ}\text{C}$)	41	30.8	33.8	32.3	.8
Temp. upper arm before tests ($^{\circ}\text{C}$)	30	30.8	34.2	32.3	.9
Temp. upper arm after tests ($^{\circ}\text{C}$)	30	32.2	35.7	33.9	1.1
Room temp. ($^{\circ}\text{C}$)	41	20.0	24.0	22.3	1.0

* From the coracoid to the elbow crease; † From the lateral epicondyle to the wrist crease; ‡ From the lateral epicondyle to the middle of the palm

Static tests

Mean muscle fibre conduction velocity (CV)

Histograms in Fig. 1 show an example of a PVs' population in one subject during the static tests at three levels of force: unloaded, 10% and 20% MVC. Figure 2a presents the averages of the CVs over 41 subjects, calculated from the subjects' PVs. The CV of the unloaded test was the lowest ($3.92 \pm 0.27 \text{ m} \cdot \text{s}^{-1}$) and it increased with augmenting force levels (effect of force, $P < 0.001$). A positive correlation existed between the CVs of unloaded test with 10% MVC, and 10% with 20% MVC (all $r > 0.505$, $P < 0.05$).

Skewness of peak velocities (Sk-PV)

Figure 2b presents the averages of the within-subject PVs' skewness over 41 subjects (see also the histograms of PVs in Fig. 1). In all the tests a moderate positive Sk-PV was found, which indicates a relative excess of lower PVs at the distribution scale. With increasing force the Sk-PV significantly diminished, indicating that the proportion of higher PVs increased (effect of force, $P < 0.05$). The PVs' population as a whole shifted towards higher values with increasing forces too, which can be seen on the histograms in Fig. 1.

Figure 1. Distribution of peak velocities of one subject during short static tests at three force levels: unloaded, and loaded 10 and 20% MVC. Note the shift of the velocities as a whole to the higher values with increasing level of force

Standard deviation of peak velocities (SD-PV)

The averages of the within-subject's standard deviations of PVs are shown in Fig. 2c. The SD-PV did not change when the force levels increased (effect of force, n.s.).

Peak frequency (PF)

Figure 2d shows the averages of PF in the static tests. The PF increased with increasing forces (effect of force, $P < 0.001$).

Figure 2. Behaviour of peak velocities (PVs) as effect of force, expressed with four variables. (a) Mean conduction velocity (CV); (b) skewness of within-subject PVs; (c) standard deviation of within-subject PVs (SD); and (d) number of peaks per second (peak frequency = PF). Averages and standard errors are given, obtained from 41 subjects in short static tests at three levels of force: unloaded, and loaded 10 and 20% of maximal voluntary contraction (MVC). With increasing forces, the CV and amount of activity (PF) increases, accompanied with augmenting proportion of fast peaks (the skewness value diminishes). However, the spread of peak velocities within an individual (SD) does not change

Dynamic tests

Muscle fibre conduction velocity (CV)

Histograms in Fig. 3 show the PVs estimated from one subject during dynamic tests at four levels of force: unloaded, and loaded with 5%, 10% and 20% MVC. Figure 4a shows the averages of CV calculated from the subjects' PVs over 30 subjects. As in the static tests, the initial CV of the dynamic tests increased with level of force (effect of force, $P < 0.001$). Further, a positive correlation was found between the CV of the static tests and the initial CV of the respective dynamic tests (all $r > 0.409$, $P < 0.05$).

The lowest initial CV was that of the unloaded test with about 4 (3.6 to 4.5) $\text{m} \cdot \text{s}^{-1}$ and the highest was that at 20% MVC with approximately 4.6 (4.0 to 5.15) $\text{m} \cdot \text{s}^{-1}$, increasing from the unloaded to 20% MVC test with $14 \pm 9.6\%$. During all the dynamic tests, the CV significantly declined (effects of time for the unloaded test, $P < 0.02$; for other tests, $P < 0.001$), whereby the decline was steeper with larger forces (interaction between force and time, $P < 0.001$). At the three lowest force levels (unloaded, and loaded 5% and 10% MVC), the CV had two phases: a decline phase lasting for about 120-150 s and a steady phase continuing to the end of a test.

During the 20% MVC test, however, the CV continued to decline, without a stable phase. Twelve of 30 subjects (40%) reported fatigue during the 20% MVC test and terminated the task prematurely between 90 and 210 s. The CV decreased for the fatigued subjects from 4.6 ± 0.3 (4.2 – 5.0) to 3.6 ± 0.3 (3.0 – 4.1) $\text{m} \cdot \text{s}^{-1}$ and for the continuing subjects from 4.5 ± 0.3 (4.0 – 5.15) to 3.8 ± 0.4 (3.0 – 4.7) $\text{m} \cdot \text{s}^{-1}$. Neither the absolute initial CV nor the end CV differed significantly between the groups ($P = 0.497$ and $P = 0.124$, respectively). However, the relative decline of the CV tended to be larger for the fatigued subjects than for the continuing subjects, for fatigued being about -20 (-7 to -35)% and for continuing -15 (+7 to -28)%; $P = 0.074$.

Skewness of peak velocities (Sk-PV)

Histograms in Fig. 3 show the distribution of PVs of one subject and Fig. 4b presents the averages of Sk-PV over 30 subjects. In the initial phase, consistent with the static tests, Sk-PV was most positive (= skewed in favour of lower velocities) in the unloaded test, and the Sk-PV diminished with increasing forces (effect of force, $P < 0.001$). That means that lower PVs dominated in the unloaded test and the proportion of higher PVs increased when forces augmented. Thus, in the 20% MVC test, the initial PVs approached a normal distribution. In addition, with increasing forces the PVs as a whole group seem to shift towards higher values, as can be seen in the histograms Fig. 3a-d at time zero. During the tests, the Sk-PV increased again,

except for the unloaded test, indicating a growing proportion of lower PVs over time and decreasing amount of higher PVs (histograms Fig. 3a-c). The larger the forces the steeper increase of the Sk-PV over time (for all tests together: effect of time, $P < 0.001$; interaction between force and time, $P = 0.005$; effect of time in unloaded test, $P = \text{n.s.}$; for the tests 5%, 10% and 20% MVC: interaction between force and time, $P < 0.001$). During the last 2 minutes of the 5% and 10% MVC tests, the Sk-PV stabilized. In the 20% MVC test, however, the Sk-PV still tended to increase up to the end of the test (over the time windows 120-180 and 180-240 s: effect of time for the 5%, 10% and 20% MVC tests, n.s. ; interaction between force and time, $P = 0.106$; for the 5% and 10% MVC tests effect of time, n.s. ; for 20% MVC test effect of time over 120-180s n.s. , over 180-240s $P = 0.052$). At the end of the 20% MVC test, the whole population of PVs appeared to shift towards the lower values too, as can be seen at the last two histograms in the Fig. 3d.

Taken together, in the initial phase of activity, the proportion of fast peaks increased with increasing force. In the prolonged tests loaded up to 10% MVC, the proportion of fast peaks declined again over the first 2 minutes and then stabilized at about the level of the unloaded test. During the 20% MVC test, however, the proportion of fast peaks still tended to decline up to the end of the test, accompanied with a growing amount of slow peaks.

Standard deviation of peak velocities (SD-PV)

The averages of SD-PVs of 30 subjects are presented in Fig. 4c. The initial SD-PV was for all force levels similar ($P = 0.65$), which resembled the static tests. The values in the dynamic tests were significantly higher compared with those of respective static tests (paired sample *t*-test for the unloaded, 10% and 20% MVC tests, respectively $P = 0.014$, $P = 0.027$ and $P = 0.001$). During the tests, the SD-PV changed significantly over time, depending on the force level (for all tests effect of time, $P = 0.011$, interaction between force and time, $P < 0.001$). The course of the SD-PV had two phases which were different for the three lower force levels (unloaded, 5% and 10% MVC) compared with 20% MVC. In the three lower force levels, the SD-PV first declined over about 90 s and then stabilized (interaction between force and time over 0-240 s, $P = \text{n.s.}$; effect of time over the time windows 0-60 s, $P < 0.001$; 60-120 s, $P = 0.075$; 120-180 s and 180-240 s for both $P > 0.343$). However, during the 20% MVC test, the decline, which lasted for approximately 60 s, was followed by an extreme increase (effect of time over 0-240 s, $P < 0.001$; effect of time over 0-60 s, $P = 0.019$; over 60-120 s, which was in opposite direction, $P = 0.019$; over 120-180 s and 180-240 s, $P < 0.05$). This pattern of results can also be seen in the histograms Fig. 3d.

Figure 3. Changes in the distribution of peak velocities (PVs) over time at different levels of force. The PVs are obtained from one subject (the same as in Fig. 1 for static tests) during prolonged dynamic contractions at four levels of force: unloaded, and loaded 5, 10 and 20% of maximal voluntary contraction (MVC). Every histogram represents a number of PVs within a period of 14.4 s. Initially (at time zero), a global shift of peak velocities is visible towards higher values when forces augment. During the unloaded, and loaded 5 and 10% MVC tests, the amount of the slower peaks is moderately increasing and of the faster peaks is diminishing. During the 20% MVC test, the peak velocities shift considerably as a whole towards lower regions, and the amount of peaks visibly diminishes

Peak frequency (PF)

Figure 4d shows averages of PF over 30 subjects. The initial PF rose with increasing force levels (effect of force, $P < 0.001$). Then, during the tests at three lowest force levels (unloaded, 5% and 10% MVC) the PF remained stable. But during the 20% MVC test, the PF significantly diminished, at the beginning gradually and from about 120 s steeply (interaction between force and time for all the four tests, $P < 0.001$; interaction between force and time for the three lowest force levels, $P = 0.234$, effect of time for the three lowest force levels, $P = 0.541$, effect of time for 20% MVC, $P < 0.001$). There was much variability among subjects in the size of decline in 20% MVC test. For those who were able to complete the test, the PF continued to decline up to the end, with exception of one subject in whom the PF increased instead. At 240th s the PF of the continuing subjects was reduced by -35 (-78 to +3)%.

Figure 4. Effects of force and time on the behaviour of peak velocities (PVs), expressed with four variables: (a) Mean conduction velocity (CV); (b) skewness of within-subject PVs; (c) standard deviation of within-subject PVs (SD); and (d) number of peaks per second (peak frequency = PF). Averages and standard errors are given, obtained from 30 subjects during prolonged dynamic tests at four levels of load: unloaded, and loaded 5, 10 and 20% of maximal voluntary contraction (MVC). Note the difference in the decline pattern of the CV and the PF: the CV declines over time for all levels of force, whereas the PF remains stable for the three lower force level tests (unloaded, and loaded 5 and 10% MVC). In the higher force level (20% MVC) test, the CV starts declining immediately, whereas the PF declines first gradually and later on steeply. Note the stable SD values from about 90 s for the three lower force tests, whereas the SD of the higher force (20% MVC) test clearly increases.

Discussion

Changes in the distribution of MUP velocities as an effect of (low) force and duration were described with four variables: (1) the global variable of mean CV; (2) the within-subject skewness of a population of MUP velocities; (3) the within-subject standard deviation of MUP velocities and (4) the amount of MUP activity, expressed as the MUP frequency. First we will comment on the four variables. Next, we will discuss the main findings, based on these variables.

The four variables

The CV variable renders a mean value of the motor unit potentials' propagation velocities. The CV will increase or decrease, depending on the type of the activated (fast-twitch and slow-twitch) motor units. It will also change with the alterations in muscle membrane potential, which influences the depolarisation/repolarisation processes. For example, CV decreases in muscular fatigue (Stalberg 1966; Milner-Brown and Miller 1986), and increases with a smaller interstimulus interval, such as that due to the rising discharge rate (Gydikov and Christova 1984; Radicheva et al. 1986; Nishizono et al. 1989). Because of the lack of studies, it is not possible to compare our CV results with those of any other *dynamic position task* experiment. However, the estimates are consistent with those of the studies using a *static force task*, especially with those of Lange et al. (2002), also obtained with the IPL method.

Skewness is used as an sEMG variable in the present study for the first time. This statistical measure of deviation from a normal distribution, in this case expresses the proportion between slower and faster MUPs within an individual. The Sk-PV will increase with the growing proportion of the activated slow/tonic/fatigue resistant MUs and will decrease with the augmenting proportion of fast/phasic/fatigable MUs. All the Sk-PV estimates were moderately positively skewed, which indicates a relative excess of lower MUP velocities.

The within-subject standard deviation of MUP velocities, a variable introduced by Lange et al. (2002), shows the spread of MUP velocities. For a fresh and healthy muscle, it will render information about diversity of the participating MUs. In a fatigued muscle, when membrane propagation is slowing, the SD-PV will broaden as a result of the temporal dispersion of velocities. Further, the SD-PV can be expected to narrow when the same velocities repeat, such as in a higher discharge rate of a certain group of MUs. In the short *static position tasks*, the SD-PVs were larger than those previously estimated by Lange and colleagues (2002) in the *static force tasks*, with 0.55 - 0.62 m s⁻¹ and 0.3 - 0.52 m s⁻¹, respectively. This difference can be due to the different type of a task, as data are available suggesting that different excitatory/inhibitory inputs to the motoneurons play a part in the position tasks and the force tasks (Rudroff et al. 2005).

In the present study, the SD-PVs of the dynamic tests were larger than those of the static tests. This difference can have different explanations. With every contraction of a dynamic cycle, the muscle fibres' diameter increases, leading to higher fibre propagation velocities (in a part of a cycle) (Arendt-Nielsen et al. 1992). This problem was partially restrained by using a small movement angle of 45°. However, the most important role in the increase of SD-PVs' during dynamic contractions might be played by the cyclic changes in the motor units' discharge characteristics. Several

studies deliver the supporting data. For example, during dynamic contractions the amount of activity differs between the concentric and eccentric phases, suggesting different regulatory strategies for the two phases (Potvin 1997). Previous studies have also shown that the rate of MU discharge is related to the movement's velocity, and the (angle) velocity alters depending on the elbow angle (Gillis 1972; Milner-Brown et al. 1973a; Potvin 1997). Thus, the discharge rates will alter through a cycle. In addition, eccentric movements are shown to further the activation of high-threshold (fast propagating) motor units (Komi and Tesch 1979; Nardone et al. 1989).

Peak frequency (MUP frequency) expresses the number of MUPs in a time. To our knowledge, it is used as sEMG variable in the present study for the first time. The PF is comparable with the zero crossings' number parameter (Lynn 1979; Masuda et al. 1982; Hägg 1981). It is argued that the diminishing zero crossings' number during prolonged exercises indicates a decrease in MU activity, as a sign of fatigue (Inbar et al 1986; Hägg and Suurküla 1991). Lange et al. (2002) mentioned a number of MUPs obtained during a 1.5 s measurement in *an isometric force task* experiment, which rendered the frequencies of about 4–5 MUPs/s for 10% MVC test, and about 8 MUPs/s for 20% MVC test. These values are much lower than ours with 37, 47 and 50 MUPs/s (for respectively unloaded, and loaded 10% and 20% MVC tests) in *static (= isometric) position tasks*. The findings are consistent with the interpretation that during position tasks more MUs are being recruited compared with force tasks, and the discharge rate of MUs is higher (Mottram et al. 2005a).

The initial changes on increasing force levels (in the static and dynamic tests)

The effects of force on the behaviour of MUPs in the short static tests and the initial phase of the prolonged dynamic tests were similar. With increasing forces the CV grew higher and MUP frequency increased. In the population of MUP velocities, not only the proportion of the fast MUPs increased (see the skewness in Fig. 2b), but also the MUP velocities as a whole shifted towards higher values (histograms in Figs.1, 3 a–d at time zero). Despite of the changes in the skewness, the standard deviation of MUP velocities remained unaltered.

The increases in the CV with increasing force are in accordance with the previous findings in *force tasks* (Lange et al. 2002, Naeije and Zorn 1983, Sadoyama and Masuda 1987, Zwarts and Arendt-Nielsen 1988). It is generally accepted that these increases are caused by activating high threshold/fast/phasic MUs when demands on the muscle are augmented (Gantchev et al. 1992, Henneman et al. 1965, Milner-Brown et al. 1973b). This explanation is supported by the increasing proportion of the fast MUPs found in the present study. However, the global shift of MUP velocities towards higher values may be induced by either replacing the slow MUs

by fast ones, or by increasing the propagation velocity of the muscle membrane due to the rising discharge rate (Van der Hoeven and Lange 1994). Little is known 4). Little is known about changes in the within-subject standard deviation of MUP velocities. Only Lange and colleagues (2002) found (*using a force task*), contrary to our results, increases in the SD-PV when increasing forces between 10% and 50% MVC, and no increases between 50% and 100%. The experiments of Lange et al. and the present *short static* experiments were both isometric, and the duration of the contraction did not differ much (our 3.8 s versus their 1.5 s). In fact, the two studies only differed in the type of a task (position tasks applied by us versus force tasks by Lange et al.). This task difference may play a part in the discrepancy of the standard deviation, as the excitatory and inhibitory inputs for the two tasks are supposed to be different (Rudroff et al. 2005). Thus, for the two tasks different types of motor units (with their different velocities) may be activated.

In short, increases of mean CV with increasing forces in the initial phase of muscle activity may be a result of both recruitment of the fast/phasic motor units, and faster membrane propagation.

Changes in the prolonged dynamic tests

Tests loaded below 20% MVC

The main feature of the prolonged tests loaded 5% and 10% MVC were changes in the CV, skewness and standard deviation over the first 90-120 seconds, followed by stabilizing. Thus, the CV first declined (with steeper decline for higher forces) and then stabilized at approximately the level of the unloaded test (Fig. 4a). The MUP velocities, which at the beginning of tests were shifted towards the higher values with increasing forces, re-shifted over time back to the lower values (skewness variable in Fig. 4b and histograms in Fig. 3 a-c). Subsequently, the MUP velocities stabilized nearly at the level of the unloaded test too. The standard deviation narrowed first, and later on stabilized at a new level (Fig. 4c). The MUP frequency held steady at the primary level, determined by the used force (Fig 4d). We suggest that this pattern of results may reflect an emerging equilibrium between phasic and tonic MU activity.

No comparison is possible between the variables used here and those of any other prolonged *dynamic position tasks* study. The decline of CV during contractions at low forces was in contradiction with the studies in *isometric force tasks*, which reported increases during sustained contractions at forces of 10-25 % MVC ((Arendt-Nielsen et al. 1989, Krogh-Lund 1993, Krogh-Lund and Jorgensen 1991, Krogh-Lund and Jorgensen 1993, Zwarts and Arendt-Nielsen 1988) This discrepancy may be caused by either/ or both the different contraction types (isometric versus dynamic)

or different tasks (force versus position tasks). Increases of the CV in prolonged isometric contractions at low force levels are supposed to be due to the recruitment of fast (anaerobic) MUs in response to the hindered blood flow (Crenshaw et al. 1997; Zwarts et al 1987). In the dynamic conditions, the blood supply is assumed to be undisturbed, so the aerobic MUs can be activated. The decline of the CV followed by stability, along with the changes in the skewness, suggest that, within the cyclically fluctuating activity, the amount of initially recruited fast/fatigable/anaerobic MUs may successively diminish and the proportion of slow/fatigue resistant/aerobic MUs may augment. The maintaining activity of slow MUs is in accordance with the hypothesis that tonic/fatigue resistant (aerobic) MUs remain active through the whole muscular action (Grimby and Hannerz 1968, Hägg and Suurkula 1991).

On the other hand, the *position character* of the present tasks may have contributed to the discrepancy between the decline of CV in the present experiments and increases in previous studies. Firstly, the discharge characteristics of the same motor unit differ between the force and position tasks, and secondly, motor units show greater discharge adaptation during the position tasks (Mottram et al. 2005a, 2005b; MacGilles et al. 2003). The evolution of the standard deviation variable, with its narrowing followed by stabilizing, fits in with the idea that the discharge rate may temporarily increase and consecutively adapt, resulting in a new balance.

The MUP frequency variable, expressing the amount of MU activity produced as a result of recruitment and discharge rate, did not change during these tests. This suggests that all the changes in recruitment and discharge rate do not, in principle, affect the total amount of MU activity.

All subjects were able to complete the tests, and the sEMG variables became stable over time as well. Thus, one can assume that the three tests at lowest force levels were non-fatiguing. Taken together, the results of these apparently non-fatiguing dynamic position tasks suggest that, following the initially increased activation of fast MUs, their proportion shifts after about 2 minutes in favour of slower MUs. The amount of activity seems to remain stable throughout the tests.

The test at 20% MVC

The changes encountered during the prolonged test at 20% MVC differed clearly from those at lower forces (Fig. 4). During the 20% MVC test, the CV dropped below the level of the unloaded test. At the same time, the proportion of low MUP velocities increased (skewness increased), and finally the velocities' population made a global move from higher towards lower values, while their standard deviation broadened clearly. The MUP frequency, in contrast with that of non-fatiguing tests, progressively diminished (histogram in Figs. 3d, 4d).

The CV's decreases were in accordance with those shown by Farina et al. (2004) during fatiguing *dynamic force tasks* of the vastus medialis muscle. The behaviour of the skewness and standard deviation suggests that a large majority of the MUPs became extremely slow at the end. This general slowness best matches the slowing of the muscle membrane propagation, which is generally accepted as sign of muscular fatigue (Milner-Brown et al. 1986; Miller et al. 1987). The diminishing MUP frequency can be due to the synchronisation of discharges (Datta and Stephens 1990; Semler and Nordstorm 1999) and diminishing motor unit activity (Hägg 1981; Hägg and Suurkulla 1991). The non-linear decline pattern of MUP frequency, first slow and later on steep, suggests successively exhausting available MUs' reserves, resulting in lower firing frequencies and failing recruitment. Accordingly, many subjects reported fatigue and stopped exercising.

In short, during these apparently fatiguing dynamic position tasks, a global slowing of MUP velocities appears, suggesting a fatigued muscle membrane. The amount of MU activity seems to diminish progressively and finally the recruitment stops.

Some aspects of the method must be explained. The load was applied to the palm, whereas the MVC was assessed from the wrist. By applying load to the palm, we intended to mimic natural circumstances, such as holding something in the hand. Assessment of the MVC from the palm was not feasible, however, due to the relative weakness of the wrist's flexors compared with the elbow flexors, which influenced the estimates. A reasonable alternative was to measure the MVC from the wrist. Distances measured over the forearm and the palm (Table 1) enabled calculation of the real exerted load torque, which was about 20-25 % larger than the used one. The MVC was assessed at the elbow angle of 90° despite performing the tests at the angle of 135°. The reason was that, when applying the dynamometer at the wrist with high forces, the elbow angle being at 135°, subjects tended to overstretch the wrist and experienced pain. This was not the case at the 90° angle. The MVC values at 135 and 90° were similar, which was consistent with the findings of Philippou et al. (2004), so it was chosen for assessments at 90°.

In conclusion, we present a set of variables derived from the interpeak latency method, which yields information about changes in MUP velocities' distribution and amount of MUP activity. Skewness, standard deviation and peak frequency variables appear to corroborate the results of a global muscle conduction velocity. Together they can contribute to quantifying the dynamics of motor unit activity and membrane fatigue. The interconnected results may be useful in ergonomics (for assessment of fatigue) and in sports (for eliciting specific capabilities, such as explosive or endurance capabilities).

(S) sprinter;

(D) distance runner

Part III

Application of the Method in sportsmen

Chapter 4

Distribution of motor unit potential velocities in the biceps brachii muscle of sprinters and endurance athletes during short static contractions at low force levels

E.G. Klaver-Król, N.R. Henriquez , S.J. Oosterloo, P. Klaver, H. Kuipers, M.J. Zwarts.

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Abstract

In surface electromyography (sEMG), the distribution of motor unit potential (MUP) velocities has been shown to reflect the proportion of faster and slower propagating MUPs.

This study investigated whether the distribution of MUP velocities could distinguish between sprinters and endurance athletes in not-specifically trained muscles (here the biceps brachii). sEMG results were acquired from 15 sprinters and 18 endurance athletes during short static contractions (3.8 s) at three force levels: unloaded, 10% and 20% of maximum voluntary contraction. The features extracted from the sEMG were: the mean muscle conduction velocity (CV) - estimated using both the inter-peak latency and the cross-correlation method, the within-subject skewness of MUP velocities (expressing the relative proportions of faster and slower propagating MUPs), and the within-subject standard deviation of MUP velocities.

Sprinters had a higher CV than endurance athletes using both methods. Sprinters also demonstrated a greater proportion of fast propagating MUPs, as indicated by the skewness.

In conclusion, the distribution of MUP velocities was able to demonstrate physiological differences between sprinters and endurance athletes during short contractions at low forces. The findings can be extrapolated to the motor unit level. Since the investigated muscle was not involved in sport-specific training, the differences seem to reflect inherited properties.

Introduction

In a recent study, we introduced a method that used the distribution of motor unit potential (MUP) velocities as a measure of the proportions of faster and slower propagating MUPs in surface electromyography (sEMG) (Klaver-Król et al. 2007). The present study investigates whether the method is able to distinguish between the muscle properties of sprinters and endurance athletes.

It is recognized that the physiological properties of the skeletal muscles of sprinters and endurance athletes differ (Brooks et al. 2005b, Gollnick et al. 1972). In simplified terms, the muscles of a sprinter rely more on anaerobic metabolism, and this is attributed to a preponderance of large, fast contracting, fatigue-sensitive type FF (= type IIb) muscle fibers. In contrast, the muscles of an endurance athlete are dominated by the aerobic metabolism associated with smaller, slow contracting, fatigue-resistant type S (= type I) fibers (Buchthal et al. 1973, Burke et al. 1973, Sadoyama et al. 1988). Muscle fibers (with their motor units), in a muscle, form a spectrum ranging from the FF to the S types, with many intermediate fibers/units (Kernell 2006d). Since FF fibers, belonging to the larger motor units (MUs), propagate an action potential faster along the membrane than S fibers, belonging to smaller MUs (Andreassen and Arendt-Nielsen 1987, Buchthal et al. 1973), the mean conduction velocity of a given muscle will depend on the proportions of the fiber/MU types within it. In line with this, Sadoyama et al. have found that the mean muscle conduction velocity (CV) correlates linearly with the area of anaerobic (FF) muscle fibers in the vastus lateralis muscle of sprinters and distance runners (Sadoyama et al. 1988).

The CV assessments can be refined by measuring the propagation velocities of individual motor unit potentials (MUPs). One technique is the inter-peak latency method (IPL) proposed by Lange et al. in which the MUP velocities are calculated from the time intervals between paired MUPs of two differential sEMG signals obtained parallel to the muscle fibers (Lange et al. 2002). In a previous study, we quantified the MUP velocities estimated by the IPL method using two statistical distribution parameters: the within-subject skewness and the within-subject standard deviation of MUP velocities (Klaver-Król et al. 2007). The skewness expresses the relative proportions of faster and slower propagating MUPs. The standard deviation reflects the spread of MUP velocities (Klaver-Król et al. 2007, Lange et al. 2002).

In order to optimize the estimation of MUP velocities, a muscle must be activated in such a way that many MUPs are produced, and that the smaller MUPs are not obscured by larger ones. Therefore, we have developed a study design in the form of a *position task at low force levels* (Klaver-Król et al. 2007). In position tasks (as

opposed to force tasks), a fixed inertial load is applied while the subject is required to maintain a target limb position (Hunter et al. 2002, Rudroff et al. 2005). Conversely, in force tasks, the limb position is fixed and the subject adjusts the effort to maintain a target force. A study on the biceps brachii muscle has shown that position tasks result in a greater number of recruited motor units than force tasks (Mottram et al. 2005b). A further study (this time involving an intrinsic hand muscle) has shown that position tasks render a greater amount of muscular activity (Maluf et al. 2005). In the present experiment, low force contractions, performed as an isometric position task (traditionally referred to as static contractions), yielded a mixed pattern EMG which enabled many individual MUPs to be identified allowing a statistical analysis.

To date, as far as we know, surface EMG in sprinters and endurance athletes has only been assessed in leg muscles (Goswami et al. 2001, Rainoldi et al. 2008, Sadoyama et al. 1988). We wanted to know whether the distinguishing characteristics between such sportsmen could also be found in other muscles, reflecting inherited rather than trained properties. Consequently, a muscle was chosen that was little influenced by specific training. The biceps brachii (BB), arm muscle was selected since both long-distance runners and sprinters mainly train their leg muscles. The experiments were performed as position tasks, involving brief isometric contractions and low applied forces. We applied the force load to the palm as we intended to mimic natural circumstances, such as holding something in the hand. The features extracted from the EMG signal were the mean muscle conduction velocity (CV), the skewness of the MUP velocities (Sk), and the standard deviation of the MUP velocities (SD-mup). In order to strengthen confidence in the results, the CV was measured not only using the IPL method but also by the more commonly used cross-correlation (CC) method.

The goal of the present study has been to investigate whether the distribution of MUP velocities obtained during short, low-force contractions of the biceps brachii muscle is able to discriminate between sprinters and endurance athletes. Our expectation was that sprinters would show a higher mean CV and a greater proportion of faster propagating MUPs than endurance athletes.

Methods

Subjects

Fifteen competitive male sprinters and 18 competitive male endurance athletes volunteered for the experiment (age: sprinters 23 SD 4, endurance athletes 26 SD 5 yr, $P = 0.052$; height: sprinters 182.5 SD 8.5, endurance athletes 187 SD 6.5 cm, $P = 0.12$; body mass: sprinters 77.0 SD 8.0, endurance athletes 74.5 SD 9.0 kg,

$P = 0.47$). Endurance athletes tend on average to be slightly older than sprinters and we were not able to exactly match the groups in terms of ages. The athletes were recruited from their sports clubs by personal contact with the clubs' trainers. The protocol was conducted according to the Helsinki Declaration and approved by the local ethics committee. All participants gave their written informed consent. The inclusion criteria were (a) participation for at least 3 years in either sprint events (100 – 400 meter running) or long distance sports; (b) being well-trained, with a training frequency over the last year of at least three times a week and a total duration of at least three and a half hours per week. Exclusion criteria were drug abuse and practicing body building, because in body building the primary focus is on increasing muscle mass, and muscle fiber diameter influences the CV (Blijham et al. 2006).

The sprinters were all runners. The group of endurance athletes consisted of 14 distance runners, two marathon skaters, one triathlon athlete and one survival athlete. Two sprinters and one endurance athlete were left-handed. The experiments took place outside the competition period. Five days before the experiment, any unaccustomed exercise with the arms or strenuous exercise in general was not allowed. After running a marathon, a recovery period of at least six weeks was required.

Experimental set-up

The methods used have been described previously (Klaver-Król et al. 2007). Maximum voluntary contraction (MVC) of elbow flexors was measured at least five days before the experiment with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands). During the MVC measurements the subjects were sitting upright. The set-up is shown in Fig. 1A. The shoulder was slightly abducted and flexed at 45° ; the elbow was firmly supported and flexed at 90° , while the forearm was supinated. The dynamometer was applied to the wrist. The force was kept for at least three seconds. The mean of three maximal values was taken as an MVC. The MVC was assessed at an elbow angle of 90° whereas the tests were performed at 135° (see below). The reason for not using an angle of 135° for the MVC assessments was that, when applying high forces at the wrist with this angle, subjects tended to overstretch the wrist and experienced pain, so influencing the results. This is not the case for a 90° angle. Further, 90° is the classic recommended position for MVC assessments (van der Ploeg and Oosterhuis 1991) and previous work has shown that MVCs obtained at 135° and 90° elbow flexions are similar (Philippou et al. 2004).

Figure 1. Set-ups used for the assessment of the elbow flexors' strength with a hand-held dynamometer (A) and for the surface electromyography (sEMG) measurements from the biceps brachii muscle (B). The strength is assessed using the classical method with an elbow angle of 90° , and a dynamometer applied to the wrist. However, sEMG measurements required a 135° elbow angle and the load applied to the palm. The maximum voluntary contractions (MVCs) obtained at the angles of 90° and 135° are known to be similar (Philippou et al. 2004).

During the experiment the subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45° of the shoulder flexion; the forearm was free. The forearm was supinated. The set-up is shown in Fig. 1B. Subjects were asked to hold the forearm horizontally (elbow angle 135°). An individually adjustable horizontal bar was used showing the subjects the position at which the lower arm was really horizontal (i.e., when the elbow angle was 135°). Three levels of force were applied in blocks that were three minutes apart: unloaded, 10% and 20% of MVC. We used fixed sequences of force levels because our object was to investigate differences between sprinters and endurance athletes and not the effects of different force levels. In the loaded tests a bag filled with lead and sand was placed in the palm. A block consisted of three tests (three repetitions at the same force level) that were within a block separated 30 s from one another. The position was held for ~ 5 s; the measurements were performed during 3.8 s. During the tests, the load was applied to the palm, whereas the MVCs were assessed from the wrist (Figs 1A and B). By applying the load to the palm, we were intending to mimic natural events, such as holding something in the hand. However, assessing the MVC from the palm was not feasible due to the weakness of the wrist's flexors relative to the elbow flexors, which would therefore influence the estimates. Distances measured from the elbow to the wrist and from the elbow to the palm enabled the true load torque exerted during the experiment to be calculated, and this was around 20-25 % larger than the applied load.

EMG recording

Measurements were performed on the short head of the BB of the dominant arm. A surface electrode array consisted of three gold-coated electrodes in a bipolar configuration, with a common center electrode. The electrodes were insulated in a rigid synthetic plate (Harwin, P25-3526). The diameter of the electrodes was 1.5 mm and the inter-electrode distance 10 mm. The skin was cleaned with 95 % ethanol. The electrode array was placed parallel to the muscle fibers following a system we described previously: the proximal electrode was exactly on the distal one-quarter point of the BB, measured between the coracoid (proximal tendon insertion) and the crossing of the distal tendon with the elbow crease found by palpation (Klaver-Król et al. 2007). This position of the electrode array was about halfway between the endplate zone and the tendon, securing a sufficient distance from the endplate and the tendon (Masuda and Sadoyama 1987, Sadoyama et al. 1985). Bipolar derivation was made from the proximal to distal direction. For the electrode position, a correlation coefficient (C-coef) of the sEMG signals was accepted at $r > 0.7$ (Hogrel et al. 1998, Merletti et al. 2001); the check was performed before and after the tests. The signals were differentially amplified (gain 2000 - 10,000) and band-pass filtered at 2 – 250 Hz by EMG apparatus (Viking IV, US). We used the relatively low cut-off frequency of 250 Hz to remove possible high frequency noise in the signal. This was done because we wanted well-defined peaks and was not interested in possible changes to the spectrum.

The skin temperature was measured with an electronic thermometer; the sensor was placed ~ 5 cm proximally from the derivation electrode. The average skin temperature was $32.2 \pm 0.85^\circ \text{C}$ and was similar between the groups ($t(31) = 1.14$, $P = 0.26$). The skin thickness (measured with callipers after performing tests, on the spot where the electrodes were placed) did not differ between sprinters and endurance athletes ($2.4 \pm 0.8 \text{ mm}$ and $2.6 \pm 0.7 \text{ mm}$, respectively), $t(31) = 0.96$, $P = 0.34$.

Data processing

The signals were simultaneously A/D converted (sampling 10 kHz, 12 bits acquisition). Data were stored on a personal computer and analyzed with LabVIEW 6.1 software.

Determining the CV with the CC method (CV-cc)

We used the method as introduced by Nishizono et.al. (Nishizono et al. 1979). The C-coef check was performed over periods of 0.8 s. The CV-cc calculations during the tests were over periods of 3.8 s.

The IPL method and its derived variables

The signal was described by three variables derived from MUP velocities (MUP-Vs = peak velocities): the muscle conduction velocity (CV-ipl), the skewness (Sk) and the standard deviation of MUP velocities (SD-mup). The MUP-Vs were obtained by the IPL method proposed by Lange and colleagues (Lange et al. 2002) and modified by the authors (Klaver-Król et al. 2007).

Peak selection and estimation of peak velocities

The software was custom-designed and written in LabView. The negative MUP deviations in the signal were up-directed (Figure 2). The procedure steps 1, 2 and 3 of the analysis were similar for the two sEMG signals. The steps were as follows:

- (1) Finding the largest positive-negative amplitude difference in an epoch of 0.2 s. This amplitude difference is denoted as 100%.
- (2) Finding declines. In a moving time window of ≤ 4 ms (≤ 40 sample points), declines (from negative to positive) are found of $\geq 20\%$ of the largest amplitude of an epoch of 0.2 s.
- (3) Finding peaks. A peak is the highest (most negative) point previous to a decline.
- (4) Finding pair-peaks in the second signal. A pair-peak is a peak in the second signal that satisfies the same requirements as those for the first signal's peak, and is found in a time window of 1.5 – 4.0 ms after the first signal's peak (this time interval corresponds with the CV values of 2.5 – 6.7 m·s⁻¹) (Houtman et al. 2003, Lange et al. 2002).
- (5) Excluding a double peak. If a peak from the first signal matches two different peaks from the second signal, then the second peak from the second signal is rejected.
- (6) Estimating peak velocities. Peak velocities (MUP-Vs) are estimated for each pair of peaks from the latencies between the paired peaks and the inter-electrode distance.

The values of the three variables (CV-ipl, Sk and SD-mup) were calculated from the gathering of all MUP-Vs obtained during the three tests (three repeated measurements of 3.8 s duration) at a given force level. The definitions of the variables were:

- CV-ipl: this was the average of the MUP-Vs.
- Sk: the within-subject skewness was the statistical measure of distribution of the MUP-Vs. The Sk indicated the relative proportion between the number of faster and slower propagating MUPs. When faster MUPs rise, and/or slower MUPs diminish, then the skewness becomes less positive. And conversely,

when slower MUPs rise and/or faster ones fall, the skewness becomes more positive.

- SD-mup: The within-subject standard deviation was the measure of spread of the MUP-Vs found.

Figure 2. Two differential electromyographic signals obtained from the biceps brachii muscle, with the subject's arm unloaded, the elbow flexed at 135° (position task). The negative peaks of the motor unit potentials have been identified using the inter-peak latency method (for clarity, only the peaks of the proximal signal are indicated). The large amount of motor unit potential activity produced despite the no load situation is attributed to the position task procedure.

Statistics

A repeated measures model of ANOVA was used (Kutner et al. 2005) that included within-subjects factor 'force' with 3 levels (unloaded, 10% MVC and 20% MVC) and between-subjects factor 'group' with two levels (sprinters and endurance athletes). If needed, an independent or paired samples *t*-test was applied. To evaluate association between variables, Pearson correlation coefficients were calculated. We used the SPSS statistical software. A level of $p < 0.05$ was used to identify statistical significance.

Results

No association was found between the age of the subjects and the CV (for all tests and for both the CV-ipl and the CV-cc: $r < 0.178$). Nor did an association exist between the age and the skewness (for all tests: $r < 0.1$). The MVC was in sprinters 265.0 SD 29.0 N and in endurance athletes 242.5 SD 35.0 N, $P = 0.056$. There was no significant association between the MVC and the CV, or the MVC and skewness (CV for all tests and both methods: $r < 0.299$; skewness for all tests: $r < 0.296$).

Mean muscle conduction velocity (CV-ipl and CV-cc)

The CV obtained by the IPL method (CV-ipl) correlated positively with the CV by the CC method (CV-cc), (for all force levels: $r > 0.89$). Comparing the CV values of the two methods, the CV-ipl was at all force levels significantly lower than CV-cc, the difference being around 0.1 to 0.2 m·s⁻¹ (for all force levels: $t(32) > 5.65$, $P < 0.001$), see Fig. 3.

Overall, augmenting forces raised the CV (main effect of force: for the CV-ipl $F(2,62) = 20.35$, $P < 0.001$; for the CV-cc $F(2,62) = 20.11$, $P < 0.001$). Both the CV-ipl and CV-cc were significantly higher in sprinters than in endurance athletes (between-subjects effects: CV-ipl $F(1,31) = 4.46$, $P = 0.043$; CV-cc $F(1,31) = 5.49$, $P = 0.026$), see Fig. 4a. The increases of the CV with forces were similar in sprinters and endurance athletes (force x group interaction: for IPL $F(2,62) = 0.08$, $P = 0.92$; for CC $F(2,62) = 0.56$, $P = 0.58$).

The within-subject skewness of MUP velocities (Sk)

Figure 4b shows the averages of the Sk over 15 sprinters and 18 endurance athletes; the histograms in Fig. 4 present the distribution of MUP velocities in these subjects. Overall, with increasing forces the Sk became less positive, indicating that the relative proportion of faster propagating MUPs rose (main effect of force $F(2,60) = 3.43$, $P = 0.039$). In sprinters, the Sk was significantly less positive than in endurance athletes, which means that they had a greater relative proportion of faster propagating MUPs (between-subjects effects: $F(1,30) = 8.55$, $P = 0.007$). Histograms in Fig. 5 show this difference in the proportion of faster and slower propagating MUPs in sprinters versus endurance athletes, assuming the velocity of 4 m·s⁻¹ as an arbitrary threshold. Expressed in percentages, sprinters had 53%, 65% and 69% MUPs with velocities > 4 m·s⁻¹ in the unloaded, 10% and 20% MVC tests, respectively; endurance athletes had 38%, 55% and 62%. Reaction to forces did not differ significantly between the groups (force x group interaction $F(2,60) = 2.49$, $P = 0.09$).

Figure 3. Mean muscle conduction velocity (CV) as obtained using the inter-peak latency method (CV-ipl) and the cross-correlation method (CV-cc) in short static tests unloaded and loaded with 10% and 20% of maximum voluntary contraction force (MVC). Means $\pm$ SE of the CV are given over 33 subjects. The CV-ipl is lower than the CV-cc.

The within-subject standard deviation of MUP velocities (SD-mup)

The SD-mup values, and the reactions to forces were similar in both groups (between-subjects effect $F(1,31) = 1.05$, $P = 0.31$; force $\times$ group interaction $F(2,62) = 0.19$, $P = 0.83$), see Fig. 4c.

Discussion

The present study showed that distribution of MUP velocities in surface EMG is able to distinguish between the muscle properties of sprinters and of endurance athletes during short static low force contractions. Below, we will discuss the findings and consider certain aspects of the method.

Inter-peak latency versus cross-correlation method in the CV measurements

The CVs obtained by the inter-peak latency method were highly correlated with the CVs found using the cross-correlation method, a finding in line with the study by Lange and colleagues (Lange et al. 2002). However, in the present study, the

CV values found by the IPL were significantly lower than those using the CC (Fig. 3). This difference can be explained by the fact that a broader range of MUP velocities, including slower propagating MUPs, contributed to the average CV in the IPL. In contrast, in the CC measurements, mainly large, faster propagating MUPs determined the CV (Sollie et al. 1985b).

Figure 4. Changes in surface EMG of sprinters and endurance athletes during short static tests at three force levels: unloaded, 10% and 20% of maximum voluntary contraction force (MVC). The changes are expressed by three variables derived from the motor unit potential (MUP) velocities: the mean muscle conduction velocity (CV) (a); the skewness of MUP velocities (skewness-mup) (b); and the standard deviation of MUP velocities (standard deviation-mup) (c). Mean $\pm$ SE are shown over 15 sprinters and 18 endurance athletes. The CV of sprinters is higher (a) and skewness-mup is less positive (b) compared with endurance athletes.

Differences between sprinters and endurance athletes

Sprinters showed a higher mean muscle conduction velocity than endurance athletes and a larger proportion of faster propagating motor unit potentials. This is presented in Fig. 4a (the CV in sprinters is higher) and Fig. 4b (the skewness in sprinters is lower). Skewness is a relative distribution measure. However, the combination of a

higher global CV, which is an absolute measure, with a greater proportion of faster propagating MUPs (lower skewness values) in sprinters shows where these 'higher' MUP velocities are really positioned on the absolute velocity scale. Such compound information about the absolute MUP velocities' distribution is presented in the form of histograms as shown in Fig. 5: sprinters compared with endurance athletes have a greater number of MUPs with velocities higher than $4 \text{ m} \cdot \text{s}^{-1}$, and/or smaller number with velocities lower than $\text{m} \cdot \text{s}^{-1}$ (arbitrary threshold).

Since type II fibers, belonging to large/fast MUs, propagate an action potential faster along a membrane than type I fibers, the conduction velocity of a given motor unit action potential will depend on the motor unit type (Andreassen and Arendt-Nielsen 1987, Buchthal et al. 1973). Consequently, the mean conduction velocity and the skewness depend on the proportions of activated MU types in a muscle. The findings suggest that sprinters activate a greater number of larger/faster MUs than endurance athletes.

Position task and motor unit recruitment

Can larger/faster MUs be activated when using low forces of up to 20% MVC? Following the size principle, the larger/faster MUs have higher discharge thresholds, i.e., they are not recruited until high force levels are reached (Henneman et al. 1965). However, most of the studies to date on the recruitment order and recruitment gradation have employed force tasks (De Luca et al. 1982, Gantchev et al. 1992, Henneman et al. 1965, Kukulka and Clamann 1981), whereas the present study was designed around a position task. Although the *recruitment order* of MUs is similar for both force tasks and position tasks (Tax et al. 1989), the *recruitment thresholds* differ. In position tasks, the thresholds are lower (Baudry et al. 2009, Tax et al. 1989) and the number of recruited MUs is greater (Mottram et al. 2005b). Since MUs tend to discharge earlier in a position task, it seems possible that a proportion of the faster MUs, especially the intermediate MUs, will still be activated even when only low forces are involved. Thus, the greater proportion of higher MUP velocities in sprinters may indeed reflect a greater proportion of *intermediate* MUs recruited. An alternative explanation for the results is that, at the low force level used, only small MUs (with type I fibers) are recruited. The diameter of these type I fibers may be larger in sprinters and, if so, this will lead to higher propagation velocities along the membrane (and thus to higher MUP velocities) (Blijham et al. 2006).

Figure 5. Distribution of motor unit potential (MUP) velocities in sprinters and endurance athletes in short static tests at three force levels: unloaded, and loaded 10% and 20% of maximum voluntary contraction force (MVC). The results are expressed in terms of the mean number of MUPs with their propagation velocities over 15 sprinters (filled bars) and 18 endurance athletes (empty bars). Assuming an arbitrary threshold between faster and slower propagating MUPs of $4 \text{ m} \cdot \text{s}^{-1}$, sprinters compared with endurance athletes show a greater number of fast and/or a smaller number of slow propagating MUPs.

Recruitment versus firing rate and velocity recovery function

Besides recruitment, the larger proportion of faster propagating MUPs/peaks (skewness) in sprinters could also be due to higher firing rates of the larger/faster propagating MUs. When the force increases, both the recruitment and firing rate of the activated MUs increase. However, in large muscles, such as biceps brachii, the force gradation relies more on recruitment than on firing rate (De Luca et al. 1982, Kukulka and Clamann 1981). Therefore, especially at the low force levels used, recruitment will play the dominant role. Further, there is no task-dependent preference for firing rates in a position task (as is the case with recruitment): at forces lower than 20% MVC, the initial firing rates are similar in both types of tasks (MacGillis et al. 2003, Mottram et al. 2005b).

In the present experiments, the firing rate was probably 10-12 Hz, as assessed on the base of other studies on the biceps brachii muscle in position tasks (Mottram et al. 2005a, Tax et al. 1989). The gain in the firing rate across the force range from unloaded to 20% MVC would be no more than 2-3 Hz (De Luca 1985). Consequently, the firing rates, in the present study, must have contributed to the *total number* of MUPs produced and thus strengthened the distribution results. Nevertheless, it is unlikely that they had a significant influence on the results.

Increases in the proportion of faster MUPs/peaks with increasing force might be influenced by higher propagation velocities along muscle fiber membrane as a result of augmented firing rates (velocity recovery function of muscle fiber, or VRF) (Morimoto and Masuda 1984, Nishizono et al. 1989). Farina and Falla have found that during contractions of the sternocleidomastoideus muscle, the conduction velocity of MUPs increases on average by 11% over the range of MU discharges from 7 Hz (minimum frequency) to 28 Hz (maximum frequency) (Farina and Falla

2008). Since, in the present study, the assessed gain in the firing rates over the force range from unloaded to 20% MVC was only 2-3 Hz, the increase in the MUP conduction velocity would be no more than 1-2%. The influence of the VRF on the present results thus seems negligible.

Genetics versus arm training

The between-group differences found may depend partly on inherited factors and partly on muscle training. During strength training of sufficient intensity and duration, the diameter of a muscle fiber increases (hypertrophy) (Bouchard et al. 1988, Brooks et al. 2005b), and this may result in an increase in the fiber conduction velocity (Blijham et al. 2006, Nandedkar et al. 1985). However, the present investigations were performed on the arm muscles of sportsmen who in their sports especially use their legs. Further, the experiments were done outside of competition periods, and the subjects did no more than routine training exercises for their arms. Furthermore, an analysis of the data showed no relationship between CV and arm training intensity. Arm training might thus have been only a small factor in the results. Studies have shown that muscle fiber composition (and thus also fiber thickness) is chiefly determined by genetics (Komi 1992, Thorstensson 1976). Also, the effects of training are dictated by the genetic make-up of the muscles (Bouchard et al. 1988, Thomis et al. 1998). Consequently, although some influence of training cannot be excluded, the differences found between the sportsmen seem for the greater part to be intrinsic/inherited.

In conclusion, in a muscle that had not been specifically trained (biceps brachii), sprinters showed a higher mean CV and a greater proportion of faster propagating MUPs than endurance athletes. These findings may reflect intrinsic/inherited properties of the sportsmen. The results can be explained by the sprinters' earlier recruitment of intermediate muscle fibers/motor units, which in the spectrum lie closer to the fast type II fibers. Alternatively, the recruited type I fibers/motor units may be thicker in sprinters, resulting in higher propagation velocities along the membrane. The physiological difference between the two types of sportsmen could still be seen during contractions at only low force levels, which is attributable to the use of a position task procedure. The inter-peak latency method, with its resulting MUP velocity distribution (skewness), appears valuable in distinguishing muscle properties. Findings based on the combination of mean CV and skewness of MUP velocities allow some extrapolation to the type of motor units.

Chapter 5

Distribution of motor unit potential velocities in the biceps brachii muscle of sprinters and endurance athletes during prolonged dynamic exercises at low force levels

E.G. Klaver-Król, N.R. Henriquez, S.J. Oosterloo, P. Klaver, H. Kuipers, M.J. Zwarts.

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Abstract

In surface electromyography (sEMG), the distribution of motor unit potential (MUP) velocities has been shown to reflect the proportion of faster and slower propagating MUPs.

This study investigated whether the distribution of MUP velocities could distinguish between sprinters ($n = 11$) and endurance athletes ($n = 12$) in not-specifically trained muscles (biceps brachii) during prolonged dynamic exercises at low forces. sEMG was acquired during 4 minutes' exercises, with the arm unloaded, and loaded at 5%, 10%, and 20% of maximal voluntary contraction (MVC). The features extracted from the sEMG were: the mean muscle conduction velocity – estimated using the inter-peak latency and cross-correlation methods, the within-subject skewness of MUP velocities (expressing the proportions of faster and slower propagating MUPs) and the within-subject standard deviation of MUP velocities (SD-mup).

Sprinters showed a greater proportion of faster propagating MUPs than endurance athletes. During fatigue, the SD-mup of sprinters broadened progressively, whereas that of endurance athletes did not.

The findings suggest that sprinters convey a greater proportion of faster motor units than endurance athletes and that motor unit behavior during fatigue differs between groups. Thus, the distribution of MUP velocities enables distinction between a muscle of sprinters and endurance athletes during prolonged dynamic exercises at low forces.

Introduction

The skeletal muscles of sprinters and endurance athletes differ in their physiological and metabolic properties (Brooks et al. 2005b, Gollnick et al. 1972). Essentially, the muscles of a sprinter rely mostly on anaerobic metabolism that is attributed to large, fast contracting, fatigue-sensitive type II muscle fibers. In contrast, the muscles of an endurance athlete are dominated by the aerobic metabolism associated with smaller, slow contracting, fatigue-resistant type I fibers (Buchthal et al. 1973, Burke et al. 1973, Sadoyama et al. 1988). Since type II fibers propagate an action potential faster along the membrane than type I fibers (Andreassen and Arendt-Nielsen 1987, Buchthal et al. 1973), the mean muscle conduction velocity (CV) of a given muscle will depend on the proportions of the fiber/motor unit (MU) types within it. Further, any decrease in the CV (a sign of fatigue) during prolonged effort might depend on the muscle composition.

Studies have demonstrated differences between the surface electromyography (sEMG) features of sprinters and endurance athletes. For example, CVs obtained at high forces (80% of maximum voluntary contraction (MVC)) correlated linearly with the area of anaerobic (type II) fibers in the vastus lateralis muscle of sprinters and distance runners (Sadoyama et al. 1988). Further, the rate of decline of power frequency, also a sign of fatigue, in the rectus femoris muscle during prolonged knee extension was more rapid in sprinters (Goswami et al. 2001). A recent study has shown a greater rate of decrease of individual motor unit potential (MUP) velocities in sprinters as against distance runners during fatiguing contractions of knee extensors (Rainoldi et al. 2008).

As far as we know, sEMG studies comparing sprinters and endurance athletes have only been performed on those muscles that are specifically trained (especially the knee extensors) (Goswami et al. 2001, Rainoldi et al. 2008, Sadoyama et al. 1988). They have been carried out at high force levels ($> 50\%$ MVC), in isometric conditions, and involved a force task procedure (as against a position task procedure) (Rudroff et al. 2005). In the present study, we investigated whether there were electromyographic differences between sprinters and endurance athletes in a muscle (biceps brachii) that was not-specifically trained. In so doing, we wished to investigate whether there were training-independent (i.e. inherited) distinguishing muscle characteristics. The experiments were carried out at low force levels, in dynamic conditions, and using a position task procedure (Klaver-Król et al. 2007). The use of low forces, combined with dynamic conditions and a position task, seemed to us to reflect better the natural muscle activities (Krogh-Lund and Jorgensen 1992, Potvin 1997).

Dynamic conditions are generally applied in fatigue studies that assess changes in sEMG variables such as the integrated EMG and power frequency. Recently, estimations of the CV have also been carried out during dynamic (both prolonged and short) contractions (Farina et al. 2004, Pozzo et al. 2004). Dynamic experiments have mostly been carried out as force tasks, whereas several of the isometric experiments have adopted a position task procedure (these isometric position tasks are traditionally referred to as ‘static contractions’) (Hunter et al. 2002, Mottram et al. 2006). In a force task, the target is to maintain a given force load. A position task involves using an inertial load with the goal of maintaining a target position (Rudroff et al. 2005). However, some *dynamic position* experiments have been reported sporadically (Klaver-Król et al. 2007, Potvin 1997, Tax et al. 1989). In the present *dynamic position* experiment, subjects were required to swing the arm, at a given rate, between two positions.

Using surface EMG, changes in the distribution of MUP velocities have been shown to reflect the changes in the MU recruitment strategies (Houtman et al. 2003). In the present study, the sEMG signal was described using the global CV and distribution variables of the MUP velocities. The MUP velocities were obtained by the inter-peak latency method (IPL) proposed by Lange et al (Lange et al. 2002); i.e. they were calculated from the time intervals between many paired MUPs of two differential sEMG signals obtained parallel to the muscle fibers. The IPL method is easy and cheap to use as it requires limited technical equipment. The drawback is that, compared with more sophisticated techniques such as e.g. high density – sEMG, one is unable to identify and trace specific motor units. If the number of MUPs rises or decreases, the IPL method cannot distinguish between changes in the recruitment versus changes in the firing frequencies.

The distribution variables derived from the MUP velocities were the within-subject skewness of the MUP velocities (Sk), which expresses the relative proportions of faster and slower propagating MUPs, and the within-subject standard deviation of MUP velocities (SD_{mup}). These distribution variables have not been used that often in the past. Skewness as an sEMG variable was first described by the present authors (Klaver-Król et al. 2007) and, as far as we know, has only recently been applied by Casale et al. who observed different proportions of faster and slower propagating MUPs in fibromyalgia patients compared to a control group, suggesting an aberrant central neural control (Casale et al. 2009).

The goal of the present study has been to investigate whether the distribution of MUP velocities (i.e. the within-subject skewness and the standard deviation of MUP velocities) in not-specifically trained muscles during prolonged dynamic exercises at low force levels can distinguish between sprinters and endurance athletes. Our

hypothesis was that sprinters would have a greater proportion of faster propagating MUPs than endurance athletes, and we also explored whether the distribution changes due to fatigue differed between the two groups of sportsmen.

Methods

Subjects

Eleven male sprinters aged 22 SD 4 (18 – 29) years and 12 male endurance athletes aged 27 SD 6 (18 – 36) years ($P = 0.045$) volunteered for the experiment. Endurance athletes tend on average to be slightly older than sprinters and we were not able to exactly match the groups in terms of ages. The athletes were recruited from their sports clubs by personal contact with the clubs' trainers. The protocol was conducted according to the Helsinki Declaration and approved by the local ethics committee. All participants gave their written informed consent. The inclusion criteria were a) participation for at least 3 years in either long distance sports or sprint events (100 – 400 meter running); b) being well-trained, with a training frequency over the last year of at least three times a week and a total duration of at least three and a half hours per week. Exclusion criteria were drug abuse and practicing body building because body building primarily increases muscle mass, and muscle fiber diameter affects the CV (Blijham et al. 2006).

The sprinters were all runners. The group of endurance athletes consisted of 8 distance runners, two marathon skaters, one triathlon athlete and one survival athlete. The experiments took place outside the competition period. Five days before the experiment, any unaccustomed exercise with the arms or strenuous exercise in general was not allowed. After running a marathon, a recovery period of at least six weeks was required.

Experimental set-up

The method has been described in the previous paper (Klaver-Król et al. 2007). Maximum voluntary contraction force (MVC) of elbow flexors was measured at least five days before the experiment with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands). During the MVC assessments the subjects were sitting upright. The shoulder was slightly abducted and flexed at 45°; the elbow was firmly supported and flexed at 90°, while the forearm was supinated. The dynamometer was applied to the wrist (van der Ploeg et al. 1991). The force was maintained for at least 3 s. The mean of three maximal values was taken as an MVC.

During the experiment, the subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45° of the shoulder flexion; the forearm was free.

The situation is presented on the Fig. 1. When the elbow was extended, the line of the upper arm/forearm was at 45° in relation to horizontal. When the elbow was flexed to the angle of 135° , the forearm was horizontal. The forearm was supinated. Subjects were asked to swing the forearm from the stretched (elbow angle 180°) to a horizontal (elbow angle 135°) position, thus moving over a range of 45° , at a rate of 40 beats/min (one up-and-down movement in one beat), dictated by a metronome sound. An adjustable horizontal bar was used showing to the subjects at which position the lower arm was really horizontal (i.e. when the elbow angle was 135°). Four force levels were applied in a fixed order: unloaded, 5%, 10% and 20% MVC. In the loaded tests a bag filled with lead and sand was placed in the palm. The tests lasted for 4 min and were separated by 5 min. Many subjects finished the 20% MVC test prematurely because of fatigue; the criterion to stop the exercise was that a subject was not longer able to lift the lower arm to the horizontal position.

Figure 1. Experimental set-up.

EMG recording

Measurements were performed on the short head of the BB muscle of a dominant arm. A surface electrode array consisted of three gold-coated electrodes (Harwin, P25-3526), diameter 1.5 mm, with a grooved contact surface for optimal contact. The electrodes were insulated in synthetic rigid plate, with a 10 mm distance between the electrodes (Sadoyama et al. 1985). The skin was cleaned with 95 % ethanol. The electrode array was placed distally from the innervation zone (IZ), between the IZ and the distal BB tendon, parallel to the fibers. Bipolar derivation was made from the proximal to distal direction, producing two differential signals. The optimal location of the electrodes was found by verifying that the two obtained signals from the adjacent

pairs of electrodes were monodirectionally propagating, i.e., were not affected by the vicinity of the IZ (Merletti et al. 2001). At a slight (unloaded) contraction, the signals must be delayed and similar in form and amplitude (Hermens et al. 1999). A correlation coefficient (C-coef) between the signals was checked and was accepted at $r > 0.7$ (Hogrel et al. 1998, Merletti et al. 2001). If the signals appeared disturbed by the IZ, the electrode array was replaced more distally. A possible misalignment between muscle fibers and the electrodes was checked by measuring the CV (by the CC method); the lowest CV indicates the optimal alignment. Subsequently, in order to secure a sufficient distance from both the IZ and the tendon *during movements*, subjects were asked to flex and extend the elbow slowly over an angle of around 60° (from 180 to ~ 100° of the elbow flexion). The signals must then remain similar in form and amplitude (Rainoldi et al. 2000). A correlation coefficient (C-coef) was checked before and after every test. During the tests the electrode position was continually controlled by the observation of the signals on the monitor. The signals were amplified (gain 2000 to 10,000 times) and band-pass filtered at 2-250 Hz by EMG apparatus (Viking IV, US). The ground electrode was on the lateral part of the upper arm, proximal to the derivation electrode.

Data processing

The signals were simultaneously A/D converted (sampling 10 kHz, 12 bits acquisition). Data were stored on a personal computer. The signal was analyzed with LabVIEW 6.1 software that also facilitated a partial on-line analysis. The C-coef check and the calculations of CV by the cross-correlation method (CV-cc) were performed on 0.8 s epochs. During the tests, which were of 4 min duration, the analyses were performed every 30 s during 14.4 s. This relatively long period contained several (~10) flexion and extension movements, thus enabling the eliciting of many MUP velocities. The first 14.4 seconds' period of every test was also analyzed separately in order to determine the effect of force apart from the effect of time; this is referred to as the 'initial phase'. Since many subjects finished the 20% MVC test prematurely, the time of the 20% MVC test was normalized to 100% (endurance time = 100%).

The IPL method and its derived variables. The signal was described by three variables derived from MUP velocities (MUP-Vs = peak velocities): the muscle conduction velocity (CV-ipl), the skewness (Sk) and the standard deviation of MUP velocities (SD-mup). The MUP-Vs were obtained by the IPL method proposed by Lange and colleagues (Lange et al. 2002) and modified by the authors (Klaver-Król et al. 2007).

Peak selection. The software was custom-designed and written in LabView. The negative MUP deviations in the signal are up-directed. The procedure steps 1, 2 and 3 of the analysis are similar for the two sEMG signals. The steps are as follows:

(1) Finding the largest positive-negative amplitude difference in an epoch of 0.2 s. This amplitude difference is denoted as 100%.

(2) Finding declines. In a moving time window of ≤ 4 ms (≤ 40 sample points), declines (from negative to positive) of $\geq 20\%$ of the largest amplitude of an epoch of 0.2 s are selected.

(3) Finding peaks. A peak is the highest (most negative) point previous to a decline.

(4) Finding pair-peaks in the second signal. A pair-peak is a peak in the second signal that satisfies the requirements as those for the first signal's peak, and is found in a time window of 1.5 – 4 ms after the first signal's peak (this time interval between the peaks corresponds with the CV values of 2.5 – 6.7 $\text{m}\cdot\text{s}^{-1}$) (Houtman et al. 2003, Lange et al. 2002).

(5) Excluding a double peak. If a peak from the first signal matches two different peaks from the second signal, then the second peak from the second signal is rejected.

(6) Estimating peak velocities. After selecting the peaks, latencies between the paired peaks are determined. Then, from these latencies and the inter-electrode distance (1 cm), the peak velocities (MUP-V) are estimated for each of the paired peaks.

(7) Including very slow propagating peaks. If during the fatiguing test of 20% MVC the peak velocities diminish below the primarily low limit of 2.5 $\text{m}\cdot\text{s}^{-1}$, then the time window for the detection of the pair-peaks is changed to 1.5 – 7.7 ms. The lower limit of MUP-V is then 1.3 $\text{m}\cdot\text{s}^{-1}$. A decrease of CV due to fatigue goes in parallel with a decrease of the number of peaks (Houtman et al. 2003). As an objective pragmatic criterion for the change of the lower MUP-Vs' limit, a decrease of the number of peaks per second is assumed to be $\geq 30\%$ compared with the first estimate of the 20% MVC test (Houtman et al. 2003).

The values of the three variables (CV-ipl, Sk and SD-mup) were calculated from the gathering of all MUP-Vs obtained over periods of 14.4 s. This relatively long period provided many MUPs, enabling statistical analysis. The variable definitions were as follows:

- *CV-ipl*: This was the average of all MUP-Vs elicited.
- *CV-cc*: This was the average of the CVs estimated by the cross-correlation method (cross-correlating over an epoch of 0.8 s) during 14.4 s.

- *Sk*: Skewness denoted the *within-subject* skewness of the MUP-Vs. The *Sk* expressed a relative proportion between amounts of faster and slower propagating MUPs. When faster MUPs rise, and/or slower MUPs diminish, then the skewness becomes less positive (the right tail becomes shorter). And reversely, when slower MUPs rise and/or faster ones become less, the skewness becomes more positive (the right tail becomes longer).
- *SD-mup*: The *within-subject* standard deviation of MUP-Vs.

Statistics

A repeated measures model of ANOVA was used (Kutner et al. 2005). The ANOVA included the within-subjects factor 'force' with 4 levels (unloaded, 5% MVC, 10% MVC and 20% MVC), the within-subjects factor 'time' with 9 levels (9 periods of 14.4 s), and the between-subjects factor 'group' with two levels (sprinters and endurance athletes). Every first measurement of a test was also analyzed separately, referred to as the 'initial phase'. This initial phase analysis had the within-subjects factor 'force' with 4 levels and the between-subjects factor 'group' with two levels. Univariate analyses were performed for each of the four dependent variables (CV-ipl, CV-cc, *Sk* and *SD-mup*). To evaluate association between variables, Pearson correlation coefficients were calculated. A level of $P < 0.05$ was used to identify statistical significance.

Results

No correlation was found between the age and endurance time ($r = -0.296$, $P = 0.17$). All subjects accomplished the unloaded, 5% and 10% MVC tests. However, the 20% MVC test was prematurely terminated by 8 of 11 sprinters and by 5 of 12 endurance athletes due to fatigue. The number of dropouts did not differ between the groups (Mann-Whitney test $z = 3.40$, $P > 0.05$).

Mean muscle fiber conduction velocity (CV-ipl and CV-cc)

The CV-ipl and CV-cc correlated very well throughout the tests at every force level (Table 1). The values of the CV-ipl were systematically about $0.15 - 0.35 \text{ m} \cdot \text{s}^{-1}$ lower than those of the CV-cc (Table 2 and Fig. 2).

Overall, the initial CV increased with forces (Fig. 2 and Fig. 3a) (initial effect of force for CV-ipl and CV-cc $F(3,63) > 17.57$, $P < 0.001$). Over time, the CV decreased again, with a steeper decline for higher forces (Fig. 4 a1 and a2) (CV-ipl and CV-cc: effect of time $F(8,168) > 41.15$, $P < 0.001$; force x time interaction $F(24,504) > 13.69$, $P < 0.001$). The CVs of the unloaded, 5% and 10% MVC tests stabilized after

about 2-3 min forming a plateau; the CV values were then similar between these tests (Fig. 4 a1 and a2) (CV-ipl and CV-cc: before 180 s effect of time for all $P < 0.001$; after 180 s effect of time n.s., effect of force n.s.). In the 20% MVC test, in contrast, the CV continued to decline throughout (CV-ipl and CV-cc: effect of time before 180 s $P < 0.001$; after 180 s $P < 0.003$) (Fig. 4 a1 and a2).

The initial CV values and the changes on forces in the initial phase were similar between sprinters and endurance athletes (Fig. 3a) (the initial phase: between-subjects effects CV-ipl $F(1,21) = 3.07$, $P = 0.094$ and CV-cc $F(1,21) = 0.49$, $P = 0.49$; force x group interaction CV-ipl and CV-cc $F(3,63) < 0.47$, n.s.). The CV values throughout the tests were also similar (between-subjects effects CV-ipl $F(1,21) = 3.41$, $P = 0.079$ and CV-cc $F(1,21) = 1.103$, $P = 0.31$). The time course did not differ between sprinters and endurance athletes either (Fig. 4 a1 and a2) (CV-ipl and CV-cc, time x group interaction $F(8,168) < 0.12$, n.s.), force x time x group interaction $F(24,504) < 0.3$, n.s.). At the end of 20% MVC test, after 180 s, when measured by the CC method, the CV of sprinters was higher than that of endurance athletes (CV-cc, between-subjects effects before 180 s n.s., after 180 s $F(1,21) = 5.02$, $P = 0.036$; CV-ipl before and after 180 s n.s.).

Figure 2. Mean muscle conduction velocities as obtained from the biceps brachii muscle by the cross-correlation method (CV-cc) (x), and the interpeak-latency method (CV-ipl) (■) during the first 14.4 seconds of the dynamic exercises. Measurements were taken when muscle was unloaded, and loaded with 5%, 10% and 20% of maximum voluntary contraction (MVC). Means and SE are shown over 23 subjects. The CV-cc is higher than the CV-ipl.

Table 1. Correlations between muscle conduction velocities by the cross-correlation method and the inter-peak latency method.

Force level*		Time†								
		0 s	30 s	60 s	90 s	120 s	150 s	180 s	210 s	240 s
Unloaded	<i>r</i>	0.532	0.671	0.581	0.673	0.706	0.666	0.562	0.708	0.734
	<i>P</i>	0.011	0.001	0.005	0.001	<0.001	0.001	< 0.01	<0.001	<0.001
5% MVC	<i>r</i>	0.784	0.886	0.812	0.724	0.715	0.577	0.749	0.746	0.786
	<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	<0.001	<0.001	<0.001
10% MVC	<i>r</i>	0.711	0.759	0.580	0.623	0.513	0.754	0.696	0.871	0.826
	<i>P</i>	<0.001	<0.001	0.004	0.002	0.012	<0.001	<0.001	<0.001	<0.001
20% MVC	<i>r</i>	0.718	0.662	0.746	0.895	0.889	0.930	0.965	0.927	0.905
	<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*The exercises have been performed with an unloaded arm, and loaded with 5%, 10% and 20% of maximum voluntary contractions (MVC). †Different time points during prolonged dynamic exercises. *r* = correlation coefficient; *P* = significance of the correlation. *N* = 23.

Table 2. Mean muscle conduction velocities (CV) by the inter-peak latency and cross-correlation methods.

Force level*	CV-ipl ($\text{m} \cdot \text{s}^{-1}$)†	CV-cc ($\text{m} \cdot \text{s}^{-1}$)‡	Correlation coefficient	Difference (paired <i>t</i> -test, <i>P</i>)
Unloaded	4.0 SD 0.21	4.13 SD 0.26	0.666	0.001
5 % MVC §	4.09 SD 0.2	4.28 SD 0.27	0.774	< 0.001
10 % MVC	4.12 SD 0.19	4.35 SD 0.27	0.722	< 0.001
20 % MVC	4.02 SD 0.28	4.36 SD 0.5	0.774	< 0.001

*Dynamic prolonged exercises at four force levels have been applied, designed in form of a position task. †CV obtained using the inter-peak latency method; ‡CV obtained using the cross-correlation method; The CV values for every force level are cumulative over a period of 4 min. §MVC = maximum voluntary contraction. *N* = 23.

The within-subject skewness of MUP velocities (Sk)

Overall, in the initial phase, the skewness diminished with increasing force (Fig. 3b) (initial effect of force $F(3,63) = 14.70$, $P < 0.001$). The Sk increased again throughout the tests, with greater increases for greater forces (Fig. 4 b1 and b2) (effect of time $F(8,168) = 15.12$, $P < 0.001$; force x time interaction $F(24,504) = 3.51$, $P < 0.001$).

The initial Sk values were lower (the right tail was shorter) in sprinters compared with endurance athletes, which means that initially, sprinters had a greater relative proportion of faster propagating MUPs (Fig. 3b and Figs. 5 and 6 column A: arrow F = faster MUPs, arrow S = slower MUPs) (between-subjects effects initial $F(1,21) = 5.78$, $P = 0.025$). The changes on forces in the initial phase were similar between the groups (initial force x group interaction $F(3,63) = 1.40$, $P = 0.25$).

The Sk values throughout the tests were lower in sprinters than in endurance athletes (between-subjects effects $F(1,21) = 4.56$, $P = 0.045$), indicating that over time, sprinters also had a greater relative proportion of faster propagating MUPs. The time course during the tests was similar between sprinters and endurance athletes (time x group interaction $F(8,168) = 0.49$, $P = 0.87$) (Fig. 4 b1 and b2).

Figure 3. Influence of force level on surface EMG variables in the initial 14.4 seconds of dynamic exercises. The variables are: (a) the mean muscle conduction velocity (by the interpeak latency method) (CV); (b) the skewness of motor unit potential (MUP) velocities (skewness-mup); and (c) the standard deviation of MUP velocities (standard deviation-mup). Four force levels have been applied: unloaded, and loaded with 5%, 10% and 20% of maximum voluntary contraction (MVC). Mean and SE are shown over 11 sprinters and 12 endurance athletes. With augmenting forces, the CV increases and skewness-mup decreases. The skewness-mup of the sprinters is lower as compared with endurance athletes.

The within-subject standard deviation of MUP velocities (SD-mup)

Overall, the initial SD-mup did not change with forces (initial effect of force $F(3,63) = 1.48$, $P = 0.23$). The SD-mup changed significantly over time (effect of time $F(8,168) = 3.28$, $P = 0.002$) (Figures 4 c1 and c2). The changes in the three lower forces' tests differed from those during the 20% MVC test (Figures 4 c1 and c2) (in the four tests: force x time interaction $F(24,504) = 4.76$, $P < 0.001$; in the three lower forces' tests: force x time interaction $F(16,336) = 1.39$, $P = 0.14$, and effect of time $F(8,168) = 5.63$, $P < 0.001$; in 20% MVC: effect of time $F(8,168) = 6.80$, $P < 0.001$).

In the initial phase, the values of the SD-mup in sprinters and endurance athletes were similar, and the reaction of the groups to forces did not differ significantly either (Fig. 2c), (initial between-subjects effects $F(1,21) = 1.59$, $P = 0.22$; force x group interaction $F(3,63) = 2.39$, $P = 0.08$).

The SD-mup values throughout the tests were similar between the groups (between-subjects effects $F(1,21) = 1.90$, $P = 0.18$). The time course of the SD-mup, however, differed between sprinters and endurance athletes depending on force level (force x time x group interaction overall, $F(24,504) = 2.23$, $P = 0.001$). These changes in the SD-mup course in sprinters versus endurance athletes were different in the three lower force levels as compared with the 20% MVC (Figs. 4 c1 and c2) (force x time x group interaction for the three lower forces' tests $F(16,336) = 0.65$, $P = 0.85$; time x group interaction for the three lower forces' tests $F(8,168) = 3.21$, $P = 0.002$; time x group interaction for 20% MVC test $F(8,168) = 3.11$, $P = 0.003$). In Fig. 4 c1, the SD-mup of sprinters during the three lower forces' tests seems to narrow gradually up to about 180 s, and to broaden at the end. However, the SD-mup of endurance athletes seems to narrow up to about 90 seconds, and subsequently to recover/stabilize (Fig. 4 c2). This impression was confirmed by ANOVA at the smaller time windows: no difference was found between the groups over the period of 0 – 120 s, and the difference appeared over the 120 – 240 s period (time window 0 – 120 s: effect of time $F(4,84) = 9.77$, $P < 0.001$; time x group interaction $F(4,84) = 0.90$, $P = 0.47$. Time window 120 – 240 s: effect of time $F(4,84) = 0.63$, $P = 0.65$; time x group interaction $F(4,84) = 3.25$, $P = 0.02$. The end values of SD-mup in the three tests tended to be greater in sprinters (between-subjects effects $F(1,21) = 4.17$, $P = 0.054$).

In the 20% MVC test, the SD-mup of sprinters first narrowed over about 60 seconds and then broadened progressively (Fig. 4 c1). However, the SD-mup of endurance athletes did not alter (Fig. 4 c2), (time x group interaction $F(8,168) = 3.11$, $P = 0.003$; effect of time for the groups separately: sprinters $F(8,80) = 7.52$, $P < 0.001$; endurance athletes $F(8,88) = 0.68$, $P = 0.71$). At the end of the test, a fraction of *very slow propagating MUPs* ($< 3 \text{ m} \cdot \text{s}^{-1}$) appeared in the majority of the

subjects, in both groups at the same time (sprinters 170 ± 49 s, endurance athletes 201 ± 48 s, $t(11) = 1.17$, $P = 0.27$) (arrow XS in Fig. 5 D-c and Fig. 6 D-c, for sprinters and endurance athletes, respectively).

Figure 4. Changes in surface EMG, as obtained from the biceps brachii muscle of sprinters ($n = 11$) and endurance athletes ($n = 12$) during prolonged dynamic exercises. The results are expressed by three variables derived from the motor unit potential (MUP) velocities: (a) the mean muscle conduction velocity (CV); (b) the skewness of MUP velocities (skewness-mup); and (c) the standard deviation of MUP velocities (standard deviation-mup). Means and SE are shown. Four force levels have been applied: unloaded, 5%, 10% and 20% of maximum voluntary contraction (MVC). The tests unloaded, 5% and 10% of MVC were accomplished by all subjects and the time of performance is presented in an absolute time scale (x-axis represents 240 s). The 20% MVC test was prematurely finished by around half of the participants due to fatigue, hence the changes are presented in a relative time scale, denoting the percentage of the endurance time (x-axis = 100% of endurance time). Note the stabilization of the CV that occurs in the three lower force level tests after about three minutes, and in contrast, a progressive decline of the CV in the 20% MVC test (a1 and a2). Note the progressive broader standard deviation-mup in sprinters during the 20% MVC test (c1).

Discussion

Using distribution parameters associated with motor unit potential velocities, differences were observed between sprinters and endurance athletes in the behavior of MUP velocities during prolonged dynamic exercises at low forces. Firstly, sprinters showed a relatively high proportion of fast propagating MUPs (as indicated by the within-subject skewness of MUP velocities). Secondly, during fatigue, in contrast to endurance athletes, sprinters showed an increasing spread of MUP velocities (as expressed by the within-subject standard deviation of MUP velocities). Below, we will discuss these results and the benefits of the method.

Figure 5. Distribution of motor unit potential (MUP) velocities in the biceps brachii muscle of sprinters during prolonged dynamic tests of 240 s duration. The results are expressed in terms of the mean number of MUPs with their MUP velocities over 11 sprinters. The tests of three force levels are shown: (a) unloaded, (b) 10% and (c) 20% of maximal voluntary contraction (MVC). Histograms present MUP velocities elicited over 14.4 s at times 0s, 60 s, 180 s and 240 s. The 20% MVC test was prematurely terminated by many subjects due to fatigue, hence the results of that test are shown in a relative time scale, the endurance time = 100%. Bin width = $0.25 \text{ m} \cdot \text{s}^{-1}$. Arrow S: slow MUP fraction; arrow F: fast MUP fraction; arrow XS: very slow MUP fraction appearing during fatigue. With increasing forces the number of faster propagating MUPs increases and that of slower propagating MUPs decreases; this is clearest in the initial phase of activation (column A, subplots a-c). Over time, the number of slower propagating MUPs augments and of faster propagating MUPs diminishes. At the end of the 20% MVC test (subplot D-c), a relatively small number of MUPs remains, with propagation velocities varying from fast (arrow F) to very slow (arrow XS).

Differences between sprinters and endurance athletes in the skewness of MUP velocities

Our previous study found that during *short* (≈ 4 seconds) *static* contractions at low (up to 20% MVC) force levels, sprinters produced relatively more fast propagating MUPs than endurance athletes, as indicated by the skewness variable (Klaver-Król et al. 2010b). In the present study, a larger proportion of fast propagating MUPs was again found in sprinters, this time during *prolonged dynamic* exercises at low forces of the same range. The results are consistent with the expectation that the muscles of an explosive-type sportsman will contain a greater proportion of fast/fatigable (FF) and/or fast/fatigue-resistant (FR) muscle fibers (Brooks et al. 2005b, Gollnick et al. 1972). Since faster propagating muscle fibers generally also contract faster, and belong to the fast/large motor units (Andreassen and Arendt-Nielsen 1987, Buchthal et al. 1973), the findings suggest that sprinters activate greater numbers of the faster/larger motor units than endurance athletes.

Following the size principle, at the low forces applied, we would expect especially small/slow MUs to be recruited (Henneman et al. 1965). However, the study was designed around a position task, rather than the more common force task. In position tasks, motor units have lower discharge thresholds than in force tasks. Tax and colleagues (1989) have found that the motor units of the biceps brachii are recruited at lower thresholds during both dynamic and static position tasks than during a force task. Recently, Baudry and colleagues (Baudry et al. 2009) have demonstrated, in the same muscle, that the decline in recruitment thresholds during fatigue is greater with a position task than a force task. Further, it has been found that the recruitment of large/fast (high-threshold) motor units is promoted during the active lengthening of a muscle, which is comparable with the eccentric phase of dynamic contractions (Komi and Tesch 1979, Nardone et al. 1989).

It therefore seems feasible that, at the low force levels used, a proportion of larger MUs will indeed be recruited. Compared to endurance athletes, sprinters will activate more intermediate units which, in the spectrum, lie closer to the fast type (Brooks et al. 2005b). Alternatively, at the low force level used, only small MUs (with type I fibers) are recruited. The diameter of these type I fibers may be larger in sprinters, resulting in higher propagation velocities along the membrane (and thus in higher MUP velocities) (Blijham et al. 2006).

In distinguishing between the two types of sportsmen, *skewness* appears a *more sensitive variable* than *mean CV*: the group differences were significant for skewness (in the initial phase $P = 0.025$, throughout the tests $P = 0.045$) but merely showed a trend for CV (in the initial phase $P = 0.094$, throughout $P = 0.079$). A comparable gradation in terms of sensitivity was previously found in short static tests (skewness:

$P = 0.007$; and CV: $P = 0.026$) (Klaver-Król et al. 2010b). Thus, when applying low force contractions in a position task, under either static or dynamic conditions, the relative proportion of faster and slower propagating MUPs (the skewness) is better able to distinguish muscle characteristics than the average muscle fiber propagation velocity (CV). The greater sensitivity of *skewness* underlines its value as a *surface EMG variable*, at least for *low force contractions* and *position tasks*. The high sensitivity of skewness is consistent with findings comparing fibromyalgia patients with healthy subjects (Casale et al. 2009).

Figure 6. Distribution of motor unit potential (MUP) velocities in the biceps brachii muscle of endurance athletes during prolonged dynamic tests of 240 s duration. The results are expressed in terms of the mean number of MUPs with their velocities over 12 endurance athletes. The presented tests are at three force levels: (a) unloaded, (b) 10% and (c) 20% of maximal voluntary contraction (MVC). The histograms show MUP velocities found over 14.4 s at times 0 s, 60 s, 180 s and 240 s. Many subjects ended the 20% MVC test prematurely due to fatigue, hence the results of that test are shown in relation to the endurance time (endurance time = 100% of time). Bin width = $0.25 \text{ m} \cdot \text{s}^{-1}$. Arrow S: slow MUP fraction; arrow F: fast MUP fraction; arrow XS: very slow MUP fraction. At the end of the 20% MVC test (subplot D-c), the great part of MUPs is slow propagating (arrow S).

Differences between sprinters and endurance athletes in the standard deviation of MUP velocities

Tests at three lower force levels (unloaded, 5%, and 10% MVC) were completed by all the subjects. In general, during these tests, the CV first declined but eventually stabilized. This pattern seems to reflect the altered status of the active motor units, whereby regulatory strategies apparently succeeded in achieving equilibrium in the MU activation (Grimby and Hannerz 1968, Hägg and Suurkula 1991). Within this equilibrium, the fatigued MUs (faster/more fatigable ones) were probably being substituted over time by fresh ones (Westgaard and de Luca 1999). Slower MUs (which are less fatigable) were possibly working over a period of several minutes (Hägg 1991a); although this must have involved a cyclic recruitment and derecruitment of these units, as dictated by the dynamics (Potvin 1997). There is a lack of published information on the relationship between recruitment and discharge rates in prolonged dynamic exercises, as involved here, since this relationship has mostly been investigated under isometric rather than dynamic conditions. It is known that the biceps brachii muscle, under *isometric conditions*, recruits MUs until almost the end of the fatiguing contraction and that the discharge rates play a relatively small part (Kukulka and Clamann 1981). However, the discharge rates under *dynamic conditions* are regulated differently; the differences mainly occurring between the concentric and eccentric phases (Potvin 1997). Further, the MU discharge rate is related to the movement's velocity, and the angular velocity depends on the elbow angle, which makes the rate changeable (Gillies 1972, Milner-Brown et al. 1973a, Potvin 1997).

The 20% MVC test was substantially more fatiguing and was terminated prematurely by around half of the participants. Further, the CV did not stabilize but decreased progressively (Fig. 4 a1 and a2). The most significant difference between sprinters and endurance athletes was in terms of the *spread (standard deviation) of MUP velocities* during this 20% MVC test. The spread of MUP velocities increased progressively in sprinters whereas it remained constant in endurance athletes (Fig. 4 c1 and c2, respectively). Also the histograms in Figures 5-c and 6-c illustrate the changes in MUP velocities during the 20% MVC tests for sprinters and endurance athletes respectively. The total number of elicited MUPs started to diminish after about three minutes in both groups, indicating a decrease in the number of activated MUs due to fatigue. In sprinters, at the end of the 20% MVC test (Fig. 5, histogram D-c), it can be seen that relatively few MUPs, with diverse MUP propagation velocities, remain. However, in endurance athletes (Fig. 6, histogram D-c), many of the MUPs propagate at slow (arrow S) or intermediate speeds and, further, the number of MUPs tends to be greater than in sprinters (~ 40% more MUPs than in sprinters,

$P = 0.105$). The findings for sprinters are compatible with the interpretation of a slower propagation of an action potential along active muscle fibers due to peripheral fatigue coupled with the recruitment of fresh, faster MUs. The intermediate velocities could be produced by slightly fatigued primary faster MUs, or by the newly recruited MUs lying, in the spectrum, near the slower MU types (Kernell 2006d). The results are in line with those obtained from the tibialis anterior muscle using multi-channel electrodes during fatiguing isometric contractions (Houtman et al. 2003). Their study has shown the simultaneous appearance of distinct groups of active MUs: very slow/fatigued and fast/newly recruited ones. In endurance athletes, the relatively greater amount of activity, along with a median MUP velocity value ($3.2 \text{ m}\cdot\text{s}^{-1}$) lying within the physiological range, suggests that the muscular activity might for the most part be generated by fresh MUs with slower, fatigue-resistant fibers. In endurance athletes, peripheral fatigue might play a less important role.

A recent study has shown a greater rate of decrease of individual MUP propagation velocities in sprinters than in distance runners during fatiguing isometric contractions of knee extensors (Rainoldi et al. 2008). The finding was ascribed to the earlier fatiguing of FF, compared with S, fibers/units, in line with the expected muscle fiber compositions of the athletes. The fatigue-associated broad spread of MUP velocities that we found in sprinters is in accordance with the findings of Rainoldi and colleagues in the sense that it expresses the activity of both newly recruited faster MUs and of fatigued MUs belonging to the faster (fatigable) type.

In the present study, we did not observe a steeper decline of the *mean CV*, which is seen as a sign of fatigue, in the sprinters over the endurance athletes during the 20% MVC test. Rather, the CV (obtained by the cross-correlation method) in the last minute of the 20% MVC test was higher in sprinters than in endurance athletes. This finding once again supports the idea of a dichotomy in the roles of the fast/fresh and the slowed/fatigued MU fractions in fatigue in sprinters.

On the method

The CV calculated by the IPL method correlated very well with that calculated by the CC method (Table 1), which was consistent with our earlier experiment under static conditions (Klaver-Król et al. 2010b). Both in the dynamic and the static experiments, the CV obtained by the CC method was systematically higher than that by the IPL method. The difference in the CV values can be explained by the fact that the CV calculated by the CC method depends mainly on the large MUPs in the sEMG signal, which are usually produced by MUs with faster propagating fibers (Sollie et al. 1985b, Wallinga-De Jonge et al. 1985). In the IPL method, the CV is averaged over a wider range of MUP velocities, thus resulting in a slower global CV.

Reliable analyses of the MUP velocity distribution require a significant quantity of varied MUP activity (i.e., many MUPs produced by diverse motor units). As such, a combination of a position task and dynamic conditions appears very suitable for distribution assessments. The recruitment thresholds of MUs are lower in a position task than in a force task (Baudry et al. 2009, Tax et al. 1989), resulting in the production of greater amounts of muscular activity (Maluf et al. 2005, Mottram et al. 2005b). Moreover, the thresholds are also lower in a dynamic position task than in an isometric position task (Tax et al. 1989). Further, MU diversity is probably promoted by the cyclic recruitment and derecruitment associated with dynamic procedure. For instance, eccentric movements and the lengthening of a muscle have been shown to promote the activation of high-threshold (hence fast) MUs (Komi and Tesch 1979, Nardone et al. 1989). So, several aspects of dynamic contractions favor the current method. However, within a concentric part of a cycle, the muscle fiber diameters will increase and the fibers shorten, which will result in higher fiber propagation velocities in the active fibers (Arendt-Nielsen et al. 1992, Schulte et al. 2004). As this could adversely affect results, the problem was minimized by using a small angular elbow movement (45°).

The arms of the subjects had not been specifically trained for force or endurance, with training focusing on the legs. Further, the experiments were done outside of competition periods. We could not, however, avoid the subjects doing routine arm training. So, an influence of arm activities on muscle properties (such as fiber diameter and enzymatic contents) cannot be excluded (Brooks et al. 2005a, Brooks et al. 2005b). Nevertheless, muscle fiber composition is chiefly determined by genetics (Komi 1992, Thorstensson 1976) and the effects of training are influenced by genetic abilities (Thomis et al. 1998). Thus, in terms of nature versus nurture, the findings seem for the greater part to be related to inherited muscle properties.

Concluding, the present study has demonstrated that, using the distribution parameters of MUP velocities, it is possible to identify differences between sprinters and endurance athletes during prolonged dynamic contractions at low forces, performed as a position task. Since the muscle studied was not-specifically trained, the sEMG features seem for the greater part to be intrinsic/inherited. The skewness variable showed a global difference between the two groups throughout the activity. The standard deviation variable was especially valuable in presenting the differences between the two types of sportsmen during fatigue. The findings can be extrapolated towards the motor unit level. The approach of examining the distribution of MUP velocities can be useful in observing the distinct activation patterns of motor units during fatigue.

(F) patient with fibromyalgia;

(C) controle subject

Part IV

**Application of the Method in patients with
fibromyalgia**

Chapter 6

Muscle fiber velocity and electromyographic signs of fatigue in fibromyalgia

E.G. Klaver-Król, J.J. Rasker, N.R. Henriquez, W.G. Verheijen, M.J. Zwarts

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Abstract

Fibromyalgia (FM) is a disorder of widespread muscular pain. We investigated possible differences in surface electromyography (sEMG) in clinically unaffected muscle between patients with FM and controls.

sEMG was performed on the biceps brachii muscle of 13 women with FM and 14 matched healthy controls during prolonged dynamic exercises, unloaded and loaded up to 20% of maximum voluntary contraction. The sEMG parameters were: muscle fiber conduction velocity (CV), skewness of motor unit potential (peak) velocities, peak frequency (PF) (number of peaks per second) and average rectified voltage (ARV).

There was significantly higher CV in the FM group. Although the FM group performed the tests equally well, their electromyographic fatigue was significantly less expressed compared with controls (in CV, PF and ARV).

Concluding, in the patients with FM, we clearly showed functional abnormalities of the muscle membrane which lead to high conduction velocity and resistance to fatigue in electromyography.

Introduction

Fibromyalgia (FM) is a syndrome characterized by chronic generalized pain, especially at the insertions of various muscles (tender points). Another major complaint is fatigue (Wolfe and Rasker 2006, Wolfe et al. 1990). FM is an affliction on a continuum with many related syndromes and disorders (Wessely and Hotopf 1999). The etiology of FM is unknown and elusive. Disturbances found in FM are diffuse, peripheral (i.e., neuromuscular) and central (i.e., in the central and autonomous nervous system, hormonal, or psychological) (Banic et al. 2004, Gracely et al. 2002, Griep et al. 1993, Nilsen et al. 2006).

Regarding the peripheral aspects of FM, a muscle biopsy of painful muscles in patients with FM shows only nonspecific changes, if any (Bengtsson et al. 1986). However, electrophysiological research on the muscles in FM and related disorders has revealed some functional disturbances which may be associated with symptoms. For example, the muscles of patients with FM appear hyperexcitable, which may lead to muscle cramps (Vitali et al. 1989). Furthermore, in clinically *affected* (i.e., painful) trapezius muscles of patients with FM (Gerdle et al. 2008) and patients with chronic neck pain (which is considered a syndrome related to FM (Falla and Farina 2005)), the muscle fiber conduction velocities (CVs) are shown to be higher than in muscles of healthy controls. These higher CVs in FM have been ascribed to supposed muscle fiber damage that changed the histopathology and microcirculation (Gerdle et al. 2008).

The higher CVs were also found in one study on an *unaffected* [biceps brachii (BB)] muscle of patients with FM when compared with healthy controls (Casale et al. 2009). This was noted only at higher and not at lower force levels. The investigators explained their finding as an altered central regulation in FM due to increased activation of the high-threshold motor units (MUs) with their fast propagating muscle fibers.

Regarding fatigue/fatiguability, some studies have shown increased clinical fatigue (earlier finishing of a task) in FM compared with controls (Maquet et al. 2002), whereas others have not (Elert et al. 1992). Fatigue can be assessed by changes in surface electromyography (sEMG) correlates of fatigue. Important features of electromyographic fatigue are a decrease in the CV and a spectrum frequency shift of the sEMG signal to lower frequencies (Arendt-Nielsen et al. 1989). Slower CV during fatigue is the most direct sign of muscle membrane disturbances due to muscle energy depletion, (Arendt-Nielsen et al. 1989) while changes in the spectrum frequency indirectly express the alterations in the CV (Eberstein and Beattie 1985). Findings with regard to electromyographic fatigue in FM are scanty and also

contradictory. For example, a recent study by Casale et al. showed reduced signs of fatigue in unaffected BB muscle of patients with FM, as measured by spectrum frequency (Casale et al. 2009). Conversely, a study by Falla and Farina described enhanced signs of fatigue in affected trapezius muscle of patients with chronic neck pain, as investigated by the CV parameter (Falla and Farina 2005).

The aim of this study was to determine, by means of sEMG, whether patients with FM and healthy controls differ in CV and electromyographic signs of fatigue in unaffected (i.e., not related to tender points) biceps brachii muscle during prolonged dynamic exercises at low force levels. We chose to use dynamic contractions and low forces, because they better represent activities of daily living.

Methods

Subjects

Thirteen FM patients who fulfilled the American College of Rheumatology (ACR) diagnostic criteria for primary FM (Wolfe et al. 1990, Yunus et al. 1981) and 14 healthy women volunteered for the experiment.

Patients were recruited from the Netherlands Association of Fibromyalgia Patients by personal contact and also through the Association's website. Control subjects were healthy sedentary women, friends or neighbors of the patients. The groups were matched for age, height and body mass. The *inclusion criteria* for patients were: primary fibromyalgia (i.e., no associated rheumatologic disease was allowed) (Yunus et al. 1981); female; age between 25 and 55 years; complaints for at least two years; and the presence of at least 11 tender points (from a maximum of 18). One experienced observer (a physical therapist) examined each participant by manual palpation of the 18 body sites of tender points defined in the ACR criteria for FM (Wolfe et al. 1990). *Exclusion criteria* were: severe FM requiring the use of a wheelchair; the use of orthoses; pain in the shoulder, elbow or wrist of the dominant arm; obesity (defined as body mass index > 30); co-morbidity with diabetes mellitus, hypothyroidism, hyperthyroidism, and polyneuropathy or myopathy (because these diseases can influence the CV results); and the use of medicines, drugs or tobacco (only sporadic use of paracetamol was allowed). Because we aimed to investigate clinically unaffected muscles, subjects were excluded when, on examination, palpation pain was elicited at the insertions of the BB muscle. Subjects were also excluded when the thickness of their upper arm skin layer at the spot where the electrodes were to be placed exceeded 10.0 mm, because skin thickness influences CV estimates (Hogrel et al. 1998). Finally, patients involved in legal procedures concerning disability or employment were also excluded.

The protocol was conducted according to the Declaration of Helsinki and was approved by the local ethics committee (Medics Spectrum Twente, Enschede, the Netherlands). All participants gave their written informed consent.

Experimental set-up

The method used has been described in detail in a previous study (Klaver-Król et al. 2007). Maximum voluntary contraction force (MVC) of the elbow flexors was measured at least 5 days before the experiment with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands).

The subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45° of shoulder flexion; the forearm was free and supinated. Subjects were asked to swing the forearm from the stretched (elbow angle 180°) position to the elbow flexion of 135°, thus moving over a range of 45°, at a rate of 40 beats per minute (one up-and-down movement in one beat), dictated by a metronome. Verbal encouragement was provided to help ensure that participants were producing maximal efforts during contractions. Four force levels were applied: unloaded, 5%, 10% and 20% MVC. In the loaded tests a bag filled with lead and sand was placed in the palm. The unloaded, 5% and 10% MVC tests lasted for 3 minutes, whereas the 20% MVC test lasted for 5 minutes. The tests were separated by 5 minutes. Many subjects finished the 20% MVC test prematurely because of fatigue. The criterion for stopping was that a subject was no longer able to lift the lower arm to the horizontal position.

EMG recording

Measurements were performed on the short head of the BB of the dominant (Mease et al.) arm. None of the participants were left-handed. A surface electrode array consisted of three gold-coated electrodes (Harwin, P25-3526), 1.5 mm in diameter, with a grooved surface for optimal contact. The electrodes were insulated in a synthetic rigid plate, with a 15-mm distance between the electrodes (Sadoyama et al. 1985). The electrode array was placed between the innervation zone and the distal BB tendon parallel to the muscle fibers. A bipolar derivation was made from the proximal-to-distal direction, producing two differential signals (similar signals with a time interval between them) (Hermens et al. 1999). A cross-correlation coefficient between the signals was checked and was accepted when $r > 0.7$ (Hogrel et al. 1998). Possible misalignment between muscle fibers and the electrodes was controlled by measuring the CV (by the cross-correlation method); the lowest CV indicates the optimal alignment (Sollie et al. 1985b). To secure a sufficient distance from both the innervation zone and the tendon during movements, subjects were asked to flex

and extend the elbow slowly over an angle of around 60° (from 180 to ~ 100°). The signals must then remain similar in form and amplitude (Rainoldi et al. 2000).

The signals were amplified (gain 2000 to 10,000 times) and bandpass filtered at 2-250 Hz by the EMG apparatus (Viking IV, US). The skin temperature was measured with an electronic thermometer (NTC type; Viking, US), placed ~ 5 cm proximally from the derivation electrode.

Data processing

The signals were digitized and stored on a personal computer (sampling 10 kHz, 12-bit acquisition). Data were analyzed with LabVIEW (version 6.1) software.

Motor unit potential/peak velocities and their derived parameters. Motor unit potential (MUP) velocity (MUP-V = peak velocity) data were obtained from the sEMG signal by the inter-peak latency (IPL) method, proposed by Lange et al (Lange et al. 2002) and modified by our group (Klaver-Król et al. 2007). The principle of the IPL method is to extract MUPs/peaks from the signals, and to calculate a propagation velocity for each peak. The MUP-Vs are calculated from the time intervals between the paired peaks of the two differential sEMG signals and the distance between the electrodes (15 mm). The algorithm of the peak selection was described in a previous study (Klaver-Król et al. 2007). Three parameters were derived from the MUP-Vs: the CV; the skewness of MUP-Vs (Sk); and the MUP/peak frequency (PF). The fourth parameter was the average rectified voltage (ARV).

The values of the parameters, as used for statistical analysis, were calculated from all MUP-Vs elicited over periods of 14.4 seconds every 30 seconds. The following definitions of parameters were used:

- CV: the average of the MUP-Vs.
- Sk: the within-subject skewness was the statistical measure of distribution of the MUP-Vs. The Sk indicated the relative proportion between the number of faster and slower propagating MUPs. When faster MUPs rise, and/or slower MUPs diminish, the skewness becomes less positive. Conversely, when slower MUPs rise and/or faster ones fall, the skewness becomes more positive.
- PF: the number of MUPs/peaks elicited per second. PF is comparable to the zero-crossing number parameter (Hägg 1981); it shows a number of elements (MUPs/peaks) in sEMG.

Statistics

To evaluate changes in the dependent sEMG variables (CV, Sk, PF and ARV), a repeated-measures model of analysis of variance (ANOVA) was used (Kutner et

al. 2005). The ANOVA included the within-subjects factor ‘force’ with 4 levels (unloaded, 5% MVC, 10% MVC and 20% MVC), the within-subjects factor ‘time’ with 7 levels (7 periods of 14.4 s), and the between-subjects factor ‘group’ with two levels (FM group and controls). Every first measurement of a test was also analyzed separately, referred to as the “initial phase”. This initial phase analysis had the within-subjects factor ‘force’ with 4 levels and the between-subjects factor ‘group’ with two levels. If significant interactions appeared between the three factors force x time x group, then post-hoc analyses were applied for every force level separately using the factors ‘time’ and ‘group’. Because the subjects finished the 20% MVC test at different individual endurance times, the results of this test were estimated using a relative time scale. In this relative scale, the endurance time was assumed to be 100%. To evaluate association between variables, Pearson correlation coefficients were calculated. If required, a student *t*-test or Mann-Whitney *U*-test were applied. The analyses were performed using SPSS (version 13.0) statistical software. $P < 0.05$ (two-tailed) was used to identify statistical significance.

Results

Characteristics of the subjects are shown in Table 1. The strength of the subjects in the FM group was significantly lower than that of controls ($P < 0.01$). The endurance did not differ between the groups; all subjects accomplished the unloaded, 5% and 10% MVC tests, whereas the 20% MVC test was prematurely terminated by 12 of the 13 subjects in the FM group and by 12 of the 14 controls ($P = 0.297$). The endurance time in the 20% MVC test was similar between the groups (Table 1).

Mean muscle fiber conduction velocity and motor unit potential velocity distribution (Skewness)

Mean muscle fiber conduction velocity

CV results are shown in Fig. 1A and D, and in Table 2. Overall, CV increased with increasing force level ($P < 0.001$). Over time, there was a gradual decline of the CV; the decline was steeper for greater forces (effect of time, $P < 0.001$; interaction force x time, $P < 0.001$).

In the FM group, CV was higher than in controls, both in the initial phase of the tests and throughout testing (Table 2). The decline in the CV over time also differed between the groups. It was generally less steep in the FM group ($P = 0.049$), and the difference between groups was clearest during the 20% MVC (most fatiguing) test (Table 2). The raw sEMG signals in Fig. 2 illustrate the difference in the propagation

of the MUPs/peaks between a patient with FM (A) and a control subject (B) during fatigue. The time intervals between the paired peaks of the sEMG signals are clearly shorter in the patient with FM, and thus the calculated CVs are higher.

The FM group tended to have thicker subcutaneous tissue than controls (Table 1). To ensure that this did not influence the CV results (Hogrel et al. 1998), we also analyzed CV in a subset of the entire sample with more strictly defined skin thickness ≤ 8 mm (FM group: $n = 9$, skin 5.9 ± 1.7 mm; controls: $n = 13$, skin 5.0 ± 2.2 mm; $P = 0.23$). Again, in the FM group the CV was significantly higher than in controls (throughout $P = 0.048$). The decline in CV over time was less steep ($P = 0.023$), and the reduced steepness was clearest in the 20% MVC test ($P < 0.001$).

Figure 1. Changes in the sEMG features over time. sEMG is obtained from the biceps brachii muscle of patients with fibromyalgia (FM, $n = 13$) and healthy women (controls, $n = 14$) during prolonged dynamic exercises with the arm unloaded, and loaded with 5%, 10%, and 20% of maximum voluntary contraction (MVC) strength. The extracted sEMG variables are mean muscle fiber conduction velocity (CV), peak frequency (PF), and average rectified voltage (ARV). Mean $\pm$ SE data are shown. The tests unloaded, 5%, and 10% of MVC are of 3-minute duration and were completed by all subjects; the x-axis represents 180 seconds. The 20% MVC test is of 5-minute duration and was prematurely terminated by all participants, except 1 patient with FM and 2 controls, due to fatigue; the x-axis is relative and represents 100% of the endurance time. CV in patients with FM (A); PF in patients with FM (B); ARV in patients with FM (C); CV in controls (D); PF in controls (E); and ARV in controls (F).

Table 1. Characteristics of the study subjects.

Characteristics	Fibromyalgia (<i>n</i> = 13)	Controls (<i>n</i> = 14)	<i>P</i> values
Age (years)	43 ± 5 (SD)	42 ± 7	0.77
Height (cm)	168.1 ± 7.0	171 ± 6.0	0.2
Body mass (kg)	69.3 ± 13.1	66.8 ± 7.1	0.54
MVC (N)	92.9 ± 20	114.2 ± 17.9	< 0.01
Duration complaints (y) (range)	9.1 ± 5.1 (2.5 - 20)	N.A.	N.A.
Number of tender points	14 ± 3	3 ± 2	< 0.001
Skin thickness (mm)	7.2 ± 2.3	5.6 ± 2.8	0.083
Skin temperature (°C)	30.4 ± 1.0	30.3 ± 0.8	0.84
Endurance 20% MVC (s)*	182 ± 81	178 ± 74	0.85

All subjects were women. All controls were healthy women. MVC, Maximum voluntary contraction; NA, not applicable.

* Endurance time of the elbow flexion during the dynamic test at 20% MVC.

Skewness

The Sk was significantly lower in the FM group than in controls, indicating that the FM group yielded a greater relative proportion of faster propagating MUPs/peaks ($P = 0.012$). These “relatively faster propagating MUPs/peaks” (as the skewness is a relative measure) contained many very fast MUPs in the FM group. Taking an arbitrary threshold for very fast MUPs of $5.5 \text{ m} \cdot \text{s}^{-1}$, the FM group showed a significantly greater proportion of very fast MUPs/peaks than controls (Table 2).

Overall, the Sk increased over time ($P < 0.001$), indicating that, during fatigue, the proportion of the slower MUPs/peaks increased or the proportion of faster MUPs/peaks decreased. The increases in Sk were less steep in the FM group, and this was clearest in the course of the 20% MVC test ($P = 0.001$). This indicates that the FM group tended to maintain a large proportion of the fast MUPs/peaks during fatigue.

Table 2. Differences in surface EMG between patients with fibromyalgia and healthy subjects.

sEMG parameter	Fibromyalgia (<i>n</i> = 13)	Controls (<i>n</i> = 14)	<i>P</i> values
Initial CV (m/s) *	4.8 ± 0.3 (SD)	4.5 ± 0.3	0.021
CV throughout (m/s) †	4.6 ± 0.37	4.3 ± 0.3	0.017
CV decline during fatigue ‡	smaller	greater	< 0.001
Number of very fast peaks (%) §	22 ± 12	12 ± 8	0.017
PF decline during fatigue ‡	smaller	greater	< 0.001
ARV increase during fatigue ‡	smaller	greater	0.002

sEMG, surface electromyography; CV, mean muscle fiber conduction velocity; PF, peak frequency (number of peaks per second); ARV, average rectified voltage.

*CV in the initial phase of the dynamic tests, cumulative;

† CV throughout the tests, cumulative;

‡ Decline or incline in parameter values during the (most fatiguing) test with 20% of the maximum voluntary contraction force;

§ Percentage of the very fast motor unit potentials / peaks ($> 5.5 \text{ m/s}$), cumulative.

Peak Frequency and Average Rectified Voltage.

Peak Frequency

PF data are shown in Fig. 1B and E. Overall, the PF increased with increasing force level and then declined over time, with only a small slope in the unloaded, 5% and 10% MVC tests and a steep decline in the 20% MVC test (effects of force, $P < 0.001$; effect of time in the four tests, $P < 0.001$). In FM group, the decline of the PF in the 20% MVC tests was significantly less steep than in controls (Table 2). This indicates that, during fatigue, the FM group tended to preserve more MUPs/peaks than controls.

Average Rectified Voltage

ARV data are shown in Fig. 1C and F. On the whole, the ARV increased with increasing forces and subsequently increased again over time, with greater increases at higher forces (effect of force, $P < 0.001$; effect of time, $P < 0.001$; interaction force $\times$ time, $P < 0.001$). The increase in the ARV over time during the 20% MVC test was significantly less steep in the FM group than in controls ($P = 0.002$).

Figure 2. Example of surface EMG (sEMG) signals of a patient with fibromyalgia (FM) (A) and a healthy control woman (B) during fatigue. The biceps brachii muscle is shown at an endurance point of an exercise at 20% of maximum strength. From each participant a pair of signals was obtained along muscle fibers, with a distance between recording locations of 15 mm. The delays between paired peaks of the signals are smaller in the patient with FM (A) than in the control subject (B), indicating that the muscle fiber conduction velocity is higher in the patient.

Discussion

Our main findings are that in an unaffected muscle of patients with FM the muscle fiber conduction velocity was higher, and electromyographic fatigue was significantly less expressed compared with controls.

Higher mean muscle fiber conduction velocity in patients with fibromyalgia

The CV in an unaffected muscle of patients with FM appeared to be higher than in controls, and this applied to both non-fatigued and fatigued muscles. Our findings are in line with those of other studies on an unloaded *affected* trapezius muscle of patients with FM (Gerdle et al. 2008) and of patients with chronic neck pain (Falla and Farina 2005). Our findings only partly corroborate those by Casale et al., who also studied unaffected BB muscle in patients with FM (Casale et al. 2009). In both studies, the mean CV was higher in patients with FM than in controls. However, a major difference was that Casale et al. found the high CV in patients with FM only at a higher force level (60% MVC), whereas we found it at very low forces (unloaded and loaded up to 20% MVC). Casale et al. explained their findings as being due to increased activation of the high threshold MUs with their fast propagating type II fibers. Such an explanation, however, does not apply to our results, because, following the size principle, at the low force levels that we used especially low threshold MUs with slow propagating type I fibers would be active (Henneman et al. 1965). Besides fiber type, also fiber diameter can influence CV; thicker muscle fibers propagate impulses faster (Nandedkar et al. 1985). However, the factor of the fiber diameter is not likely to play a role in the results because no larger fiber diameters have ever been observed in muscle biopsies of patients with FM (Kalyan-Raman et al. 1984).

A major factor influencing CV is the electrophysiological status of the muscle fiber membrane (Kernell 2006c, Stalberg 1966), and altered membrane properties in FM seem to provide a plausible explanation for our findings. A muscle fiber action potential is composed of a spike and after-potential. During the initial phase of the after-potential, that is, after-depolarization, the membrane typically discharges more easily (i.e., the excitability is increased) and its conduction is supernormal (Kernell 2006c, Stalberg 1966). Membrane excitability and membrane conductance (conduction velocity) are closely related phenomena, and both depend on the membrane potential and ionic permeability. The more consecutive impulses, the longer the after-depolarization (Gydikov and Christova 1984). After intermittent voluntary contractions, the supernormal CV may extend up to one hour or longer, suggesting a long-lasting after-depolarization (Van der Hoeven and Lange 1994).

It has been demonstrated by sEMG that patients with FM have sustained muscle activity between contractions, that is, they are unable to relax. It is suggested that continual muscle activation during ergonomic situations may be a major factor in developing FM (Elert et al. 1989). We propose that continual activation between contractions in patients with FM may lead to long-lasting after-depolarization, thus increasing excitability and conductance of muscle fibers (the CV). Evidence in support of the view of increased membrane excitability in FM was found by Vitali et al. in the form of long-lasting muscle discharges evoked by an ischemic test with a tourniquet (Vitali et al. 1989).

Altogether, increased muscle activation/activity in patients with FM during a supposed rest may raise both membrane excitability and CV. Because the findings concern an unaffected (i.e., not related to tender points) muscle in patients with FM, they are likely to represent a widespread property of voluntary muscles and their membranes.

Fatigue in fibromyalgia

Clinical fatigue (endurance time) was similar between groups. Reports about endurance in FM have been inconsistent in the literature. Some studies found lower endurance in patients with FM (Nilsen et al. 2006) whereas others did not (Elert et al. 1992). A notable finding from our study is that, despite the similar endurance time, *the electromyographic signs of fatigue* differed significantly between the groups; they were *less expressed in the patients with FM* than in controls. Overall, electromyographic fatigue is characterized by a decrease in the muscle fiber propagation velocity (the CV decreases) (Milner-Brown and Miller 1986), a reduction in the number of motor unit action potentials (the PF decreases) (Hägg 1981), and an increasing surface (or amplitude) of the EMG signal (the ARV increases) (Arendt-Nielsen and Mills 1988). In the patients with FM, the decrease in CV was less steep (Fig. 1A and D), the reduction in PF was smaller (Fig. 1B and E), and the increase in ARV was smaller (Fig. 1C and F) compared with controls.

The less steep decreases in CV of the patients with FM can be explained by a subtraction / competition between the features of fast propagating “fibromyalgic” membrane and slow propagating “fatigued” membrane (Milner-Brown and Miller 1986); the fibromyalgic feature appeared stronger.

The higher CV (fast propagating muscle membrane) in the patients with FM also seemed to be responsible for the behavior of the PF and ARV during fatigue. This is illustrated in Fig. 2, which shows sEMG signals of a patient with FM (A) and a control subject (B). In the control subject, the EMG elements (MUPs/peaks) melted together due to slower CV during fatigue (Fig. 2B). As a result, the number of

MUPs/peaks became smaller (PF decreased) and the surface of sEMG larger (ARV increased). In the signal of the patient, the EMG elements were more separated due to the higher CV. Many MUPs/peaks were still there, and the sEMG surface remained small (Fig. 2A).

If the MVC force estimates would have been too low in respect to the “real” strength, then the demands on the muscles would have also been relatively too low, thus preventing fatigue. MVC estimates may be reduced due to, for example, pain or diminished motivation of the subjects (Kent-Braun and Le Blanc 1996, Schillings et al. 2007). The MVCs of the patients with FM were around 20% lower than those of the controls. However, we are convinced that these MVCs correspond to the patients’ “real” strength. The subjects, both patients and controls, had no pain and were well motivated during experiment. Furthermore, our MVC results are in line with those of another study, which measured the strength more objectively by electrically evoked response (Norregaard et al. 1995). In this latter study, the strength of patients with FM was 30% lower than that of healthy controls. Thus, the MVC results and the demands on patients’ muscles in our study seem adequate.

On the methods

As some methodological pitfalls might lead to falsely high CV estimates, we took appropriate measures: (a) we selected participants with thin skin, and an additional analysis of sub-groups matched on skin thickness confirmed the CV results (Hogrel et al. 1998); (b) the electrodes were placed far from the innervation zone (Klaver-Król et al. 2010a, Nielsen et al. 2008, Rainoldi et al. 2000); (c) possible misalignments between electrodes and muscle fibers were checked by measuring the CV, (the lowest CV indicates the optimal electrode location) (Sollie et al. 1985b); and (d) the skin temperature in the vicinity of the electrodes was measured and was similar between patients and controls (Blijham et al. 2008).

During fatigue, we did not differentiate between the peripheral and central fractions of fatigue by, for example, using the interpolated twitch technique, which can be regarded as a methodological limitation. The reason for not doing so was that electrical stimulation did not fit with the dynamic design of the study. It would have been possible to apply the twitch technique after changing the set-up, but this would have caused a delay and affected the results.

Both the FM group and controls were sedentary women, but we do not know their exact activity level. It is often thought that patients with FM are less active. However, studies have shown that they are not less active – rather their fitness level is lower (Natvig et al. 1998). In addition, if the patients with FM in our study were more sedentary than controls, this would not change the interpretation of the results.

Less developed (sedentary) muscles would propagate the action potential slower and not faster, as we found in the patients.

Relevance of the study

The clinical relevance of our study is that clear muscle membrane disturbances are found in patients with FM, even in muscles that are not directly affected clinically. The findings may lead to new pathophysiological insights into this enigmatic affliction.

In conclusion, patients with FM showed higher CV in their unaffected biceps brachii muscle than healthy subjects. The fast muscle fiber conduction in FM may be related to an overall fast/hyperexcitable muscle membrane. The ‘fast membrane’ phenomenon seems to obscure the electromyographic signs of fatigue. Clearly, more research is needed to unravel the mutual relationship between fast muscle fiber conduction and central-neural disturbances and the clinical picture of fibromyalgia.

Chapter 7

Abnormal muscle membrane function in fibromyalgia patients and its relationship to the number of tender points

E.G. Klaver-Król, M.J. Zwarts, P.M. ten Klooster, J.J. Rasker.

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Abstract

Fibromyalgia (FM) is a disorder characterized by chronic widespread pain in soft tissues and, especially, in muscles. Previous research has demonstrated a higher muscle fibre conduction velocity (CV) in painful muscles of FM patients. The primary goal of this study was to investigate whether there is also a difference in CV in non-painful, non-tender point (TP) related muscles between FM patients and controls. The secondary goal was to explore associations between the CV, the number of TPs and the complaints in FM.

Surface electromyography was performed on the biceps brachii muscle of FM female patients (13) and matched healthy controls (13). Short static contractions were applied with the arm unloaded and loaded at 5% and 10% of maximum voluntary force. The CV was derived by cross-correlation method (CV-cc) and inter-peak latency method (CV-ipl). TP score and Fibromyalgia Impact Questionnaire (FIQ) were performed in all participants. Correlations were calculated between the CVs, TP score and items of the FIQ.

In FM patients, the CV was higher than in the controls (CV-cc $P = 0.005$; CV-ipl $P = 0.022$). The CV was correlated with the number of TPs in the FM patients ($r = 0.642$ and 0.672 for CV-cc and CV-ipl, respectively). No correlations were found between the CV and any aspect of health status on the FIQ.

The results demonstrate abnormally high muscle membrane conduction velocity in FM, even in non-TP muscles. In addition, a relationship has been found between the high membrane velocity and the number of TPs.

Introduction

Fibromyalgia (FM) is one of many unexplained disorders, with a high prevalence and large impact on the functioning of the patients. FM is characterized by chronic widespread pain, with painful spots known as tender points (TPs) (Mease et al. 2005, Wolfe and Rasker 2012, Wolfe et al. 1990). Although the causes are not clear, it is generally accepted that in FM and in its various related disorders dysregulation of control processes at several levels of the central nervous system plays an important role (Banic et al. 2004, Bazzichi et al. 2011, Desmeules et al. 2003, Emad et al. 2008, Staud 2011).

Since musculoskeletal pain is a major complaint, several studies have been dedicated to muscular physiopathology in FM. In biopsies of the painful muscles of FM patients only nonspecific changes have been found (Bengtsson et al. 1986, Kalyan-Raman et al. 1984). Electromyographic examination has revealed that a *painful* trapezius muscle of FM patients (Gerdle et al. 2008), as well as a *painful* trapezius of patients with chronic neck pain (Falla and Farina 2005) – which is considered a disorder related to FM – has higher muscle fibre conduction velocity (CV) than a muscle of healthy subjects. The investigators suggest that this difference might be due to local muscular changes in histopathology and microcirculation in FM (Gerdle et al. 2008).

The question arises whether the higher CV in FM patients could be a general rather than a local phenomenon, for example a property of muscle membrane. In the present study we tried to answer this question. Therefore we compared the CV of FM patients and healthy controls in a muscle that was clinically not painful and was not related to TP site (non-TP related). Such a muscle is the biceps brachii muscle (BB).

The primary goal of the present study was to investigate whether the CV in a non-TP related BB muscle of FM patients differs from that of healthy subjects. The second goal was to examine whether there is an association between CV and the symptoms and complaints, especially the number of TPs in FM patients.

Methods

Subjects

Thirteen female FM patients and 13 healthy women volunteered for the experiment. All fulfilled the 1990 American College of Rheumatology (ACR) diagnostic criteria (Wolfe et al. 1990), and had been diagnosed by a rheumatologist. We chose to apply the 1990 ACR criteria because they are useful in identifying and strictly defining

an FM group (Mease et al. 2005). Patients were recruited from the Association of Fibromyalgia Patients by personal contact and also through the Association website. Control subjects were healthy sedentary women, friends or neighbours of the patients. The groups were matched for age, height and body mass. The inclusion criteria for patients were: primary fibromyalgia (i.e., no concurrent rheumatologic disease was allowed) (Yunus et al. 1981); female; aged between 25 and 55 years; complaint for at least two years; and the presence of at least 11 TPs (out of the 18 specific sites) (Wolfe et al. 1990). Exclusion criteria were: severe FM requiring the use of a wheelchair; the use of orthoses; pain in the shoulder, elbow or wrist of the dominant arm (because such devices and pain locations would have influenced the subject's performance during the sEMG procedure); obesity (defined as a body mass index > 30); co-morbidity with diabetes mellitus, hypo- or hyperthyroidism, and polyneuropathy or myopathy (because these diseases can influence the CV results); and the use of medicines, drugs or tobacco (only sporadic use of paracetamol was allowed). Because we aimed to investigate clinically non-painful muscles, subjects were excluded when, on examination, palpation pain was stated at the insertions of the BB muscle. Subjects were also excluded when the thickness of their upper arm skin layer (at the spot where the electrodes were to be placed) exceeded 10.0 mm, because skin thickness influences the CV estimates (Blijham et al. 2006). Patients involved in legal procedures concerning disability or employment were also excluded.

Sample size. Since sEMG studies are usually performed using very small samples and there are no criteria available for defining clinically relevant differences, sample size was calculated based on the ability to detect at least a strong correlation between the CV and the symptoms and complaints of FM patients ($r = 0.6$, power = 80%, $\alpha = 0.05$, one-sided, required $N = 13$).

Ethics

The protocol was conducted according to the Declaration of Helsinki and was approved by the local ethics committee (Medisch Spectrum Twente, Enschede, the Netherlands). All participants gave their written informed consent.

Experimental set-up

In our protocol, we applied static contractions, also called position tasks (Hunter et al. 2002). We used low force levels because in the previously mentioned two studies on the *painful* muscles (see introduction), the higher CV in FM was found at low force levels (Falla and Farina 2005, Gerdle et al. 2008). In addition, low forces are more usual in daily living.

The method used has been described in detail in previous studies (Klaver-Król et al. 2007, Klaver-Król et al. 2010b). Maximum voluntary contraction force (MVC) of elbow flexors was measured with a hand-held dynamometer (Lameris Instruments, Utrecht, The Netherlands). During the experiment, the subjects were seated in a chair. The upper arm was slightly abducted and comfortably supported at 45° of shoulder flexion; the forearm was free and supinated. Subjects were asked to hold the forearm horizontally (elbow angle 135°). An individually adjustable horizontal bar was used showing the subjects the position at which the lower arm was really horizontal. The position was held for 6 – 7 seconds; the measurements were performed during 4 seconds. Three levels of force were applied: unloaded, 5% and 10% of MVC. Every test was repeated three times for each force level. In the loaded tests a bag filled with lead and sand was placed in the palm.

EMG recording and data processing

Measurements were performed on the short head of the BB of a dominant arm (Klaver-Król et al. 2007). A surface electrode array consisted of three gold-coated electrodes (Harwin, P25-3526), diameter 1.5 mm, with a 15 mm distance between the electrodes. The electrode array was placed parallel to the muscle fibres (Hogrel et al. 1998). Bipolar derivation was made from the proximal to distal direction, producing two differential signals. A correlation coefficient between the signals was accepted at $r > 0.7$ (Hogrel et al. 1998, Merletti et al. 2001). The signals were amplified (gain 2,000 to 10,000 times) and band pass filtered at 2-250 Hz by EMG apparatus (Viking IV, US). The signals were digitized and stored on a personal computer (sampling 10 kHz, 12 bits acquisition). Data were analyzed with LabVIEW version 6.1. In order to strengthen confidence in the results, the CV was measured using two methods, the CC method (Nishizono et al. 1979) and the IPL method (Klaver-Król et al. 2007, Lange et al. 2002), CV-cc and CV-ipl, respectively. The peak selection algorithm has been described previously (Klaver-Król et al. 2007). In both CC and IPL methods, the CV calculations were performed over 2.0 s signal epochs.

The skin temperature was measured with an electronic thermometer (NTC type, Viking, US), the sensor was placed ~ 5 cm proximally from the derivation electrode. The skin thickness was measured with callipers, on the spot where the electrodes were placed.

Tender points

The same experienced observer (physiotherapist) examined each participant by manual palpation of the 18 body sites defined in the 1990 ACR criteria for FM (Wolfe et al. 1990). The TP palpation is considered positive at a pressure of 4 Kg/cm² (see the 1990 ACR criteria). The TP score was calculated as the number of sites where the patient stated that the palpation was painful (range 0 to 18).

Fibromyalgia impact questionnaire (FIQ)

All participants completed a validated Dutch version of the FIQ (Burckhardt et al. 1991, Zijlstra et al. 2007). The FIQ is a ten-item questionnaire specifically designed to assess the current health status of people with FM. The first item contains ten items with 4-point rating scales about physical function. Items 2 and 3 ask for the number of days the individual felt well, and the number of days off work, during the past week. Further, the questionnaire contains seven visual analogue scales (VAS) for inability to do job, pain, fatigue, morning tiredness, stiffness, anxiety, and depression. The scores for each item were standardized so as to range from 0 to 10, with higher scores indicating greater impairment.

Statistics

For analyses of the CV, a repeated measures model of ANOVA was used (Kutner et al. 2005) that included the within-subjects factor ‘force’ with 3 levels (unloaded, 5% and 10% of MVC) and the between-subjects factor ‘group’ with two levels (FM group and controls). If significant interactions appeared between the factors force and group, then post-hoc analyses were applied for every group using the factor ‘force’. To evaluate associations between variables, Pearson’s correlation coefficients were calculated. If required, Student *t*-tests or Mann-Whitney *U*-tests were applied. The analyses were performed using SPSS 13.0 statistical software. $P < 0.05$, two-tailed, was used to identify statistical significance.

Results***Participant characteristics***

Characteristics of the subjects are summarised in Table 1. The strength was significantly lower in the FM group than in controls ($t(24) = 2.8$, $P = 0.011$). As it was expected, the number of TPs was higher in the FM group than in controls ($P < 0.001$) and FM patients scored significantly worse on all relevant FIQ items (Table 2). The distribution of TP sites in FM group and controls is shown in Table 3.

Table 1. Characteristics of the study subjects.

	FM * patients (n = 13)	Controls (n = 13)	P values
Age (years)	43 (5)	42 (7)	0.83
Height (cm)	168.1 (7.0)	171.8 (5.9)	0.15
Body mass (kg)	69.3 (13.1)	67.2 (7.1)	0.62
Voluntary maximum strength (Newton)	92.9 (20)	113.7 (18.6)	0.011
Duration of complaints (years)	9.1 (5.1)	N.A.	N.A.
Number of tender points	14 (3)	2 (2)	< 0.001
Skin thickness (mm)	7.8 (0.8)	5.5 (0.8)	0.057
Skin temperature (°C)	30.3 (1.0)	30.2 (0.8)	0.69

Values are mean (SD). All subjects are women.

* Fibromyalgia.

Table 2. Fibromyalgia impact questionnaire

	FM* patients (n = 13)	Controls (n = 13)	P values
Physical function	3.7 (2.6)	0.1 (0.2)	< 0.001
Days felt good	5.8 (3.4)	0.1 (0.4)	< 0.001
Work days missed ^a	4.0 (5.5)	0.0 (0.0)	N.A.
Job ability	5.7 (2.5)	N.A.	N.A.
Pain	5.6 (2.0)	0.0 (0.0)	< 0.001
Fatigue	5.3 (3.6)	0.4 (0.9)	< 0.001
Morning tiredness	6.5 (2.9)	0.8 (1.8)	< 0.001
Stiffness	5.2 (3.1)	0.1 (0.3)	< 0.001
Anxiety	2.7 (2.2)	0.3 (0.7)	0.002
Depression	2.1 (2.2)	0.0 (0.0)	0.001

Values are mean (SD). All subjects are women. * Fibromyalgia. ^an = 5 for FM.

Table 3. Frequency of occurrence of the tender points (TPs) in the fibromyalgia (FM) patients and controls.

Tender point location	FM patients (n = 13)		Controls (n = 13)	
	Right	Left	Right	Left
Suboccipital insertion	10 (77)	9 (69)	0	0
Mid upper trapezius	13 (100)	9 (69)	3 (23)	3 (23)
Lower sternocleidomastoid	10 (77)	11 (92)	2 (15)	2 (15)
Origin of the supraspinatus	11 (85)	8 (61)	2 (15)	1 (8)
Near the second costochondral junction	12 (92)	12 (92)	2 (15)	3 (23)
Upper outer quadrant of the buttock	9 (69)	8 (61)	2 (15)	3 (23)
Prominence of the greater trochanter	11 (92)	9 (69)	1 (8)	1 (8)
Lateral epicondyle	10 (77)	9 (69)	0	0
Medial fat pad of the knee	8 (61)	12 (92)	1 (8)	2 (15)
Mean number of TPs in a subject	7	7	1	1

Values are number (%) unless indicated otherwise. Number = a number out of 13 subjects in whom a given TP location occurred. % = % of the subjects in whom a given TP location occurred. All subjects are women.

Muscle fibre conduction velocity (CV)

The CV, as obtained by both the CC and IPL methods, was significantly higher in the FM group than in controls (between subjects effects: CV-cc $P = 0.005$; CV-ipl $P = 0.022$). The results are shown in Fig. 1. When measured by the IPL method, the CV changes on forces differed between groups: the CVs of the FM group did not alter whereas those of controls increased with increasing forces (interaction force $\times$ group $P = 0.049$; effect of force in FM group $P = 0.41$; in controls $P = 0.05$). This indicates that the FM patients tended to produce a high CV still at the lowest force levels, i.e. when the arm was unloaded.

CVs measured by the CC and IPL methods were highly correlated ($r = 0.83$, $P < 0.001$). However, the CVs measured by the CC method were higher than those measured by the IPL method; this applied to both groups (Fig. 1a and 1b) (paired samples t-test for all $P < 0.001$; FM group $P < 0.001$; controls $P < 0.001$).

Figure 1. Muscle fibre conduction velocity (CV) in fibromyalgia patients and healthy controls. The CV was obtained from the biceps brachii muscle using surface electromyography, the cross-correlation (CC) and the inter-peak latency (IPL) methods. Short (four-second) static contractions were applied at three force levels: with unloaded arm, and with 5% and 10% of maximum voluntary contraction strength (MVC). (a) Fibromyalgia female patients (13) and (b) matched controls (13). Means and standard errors are shown. In FM patients, the CV is higher than in controls, as measured by both methods.

Correlations between sEMG and signs and complaints

A positive association was found between the number of tender points and the CV in the FM group (CV obtained cumulatively from 3 force levels: CV-cc, $r = 0.642$, $P = 0.018$; CV-ipl, $r = 0.672$, $P = 0.012$) (Fig. 2). No correlation was found between the duration of complaints and the CV or between the duration of complaints and the number of TPs. Neither was a correlation found between

the strength and the CV. No significant correlation was found between the number of TPs in the FM group and the self-reported pain, as measured by the VAS-pain of the FIQ ($r = 0.395$, $P = 0.18$). Finally, no significant correlations were found between the CV and any other aspect of health status on the FIQ (r 's between -0.335 for physical functioning and 0.137 for stiffness).

Figure 2. Correlation between the number of tender points and the muscle fibre conduction velocity (CV) in fibromyalgia patients. The CV was obtained from a non-painful biceps brachii muscle of fibromyalgia patients ($n = 13$) during short (four-second) contractions at three force levels: the arm unloaded, and loaded with 5% and 10% of maximum voluntary contraction strength. The CV was measured by the inter-peak latency method. The CV values are cumulative over the three force levels. There is a positive correlation between the number of tender points and CV.

Discussion

In a non-painful and non-TP site related muscle of FM patients we found a higher muscle fibre conduction velocity than in healthy subjects. A second notable finding was that there was a clear positive correlation between the muscle conduction velocity and the number of TPs in the FM patients.

High muscle fibre conduction velocity in FM

In earlier studies, a higher CV was observed in TP related muscles of FM patients and of patients with related disorders as compared with controls. These findings were explained by supposed local muscle changes in histopathology and microcirculation (Falla and Farina 2005, Gerdle et al. 2008). Our results show, however, that a non-

painful, non-TP related muscle of FM patients also exhibits a higher CV. This can no longer be ascribed to local phenomena, but rather seems to point to a general feature of the musculature. CV measurements in non-TP related muscles of FM patients have rarely been performed. As far as we know, only a study by Casale et al. showed a larger proportion of fast propagating motor unit potentials in FM patients compared with controls, and the CVs of the patients tended to be higher, which is in line with our findings (Casale et al. 2009)

Several factors can influence CV. One of them is the type of motor units (MUs) participating in muscular activity: larger MUs with their fast twitch, fast propagating type II muscle fibres would produce higher CVs (Andreassen and Arendt-Nielsen 1987). Following the size principle, large MUs are in particular activated at higher force levels (Henneman et al. 1965). Since our study on non-TP related muscle, as well as the two studies discussed above on TP related muscle, involved low force levels, it seems unlikely that the activation of large MUs would have played a role. Another factor which the CV is dependent on is the muscle fibre diameter: thicker fibres propagate the action potential faster (Blijham et al. 2006). However, the diameter of muscle fibers would not seem to play a part in explaining the present findings since there is no evidence of thicker fibres in FM (Bengtsson et al. 1986, Kalyan-Raman et al. 1984).

A more plausible explanation for the higher CV in FM could be functional alterations of the muscular membrane. FM patients produce excessively muscular activity between intended contractions; i.e. they are unable to relax (Elert et al. 2001). Physiologically, after having produced an action potential, a membrane develops a short after-depolarization (AD), a period during which the membrane is hyperexcitable and the membrane conduction (the CV) is increased (Bergmans 1971, Kernell 2006c). Following a prolonged stimulation or voluntary contraction, the AD becomes longer and may last for several minutes or even hours (Gydikov and Christova 1984, Van der Hoeven and Lange 1994). In FM patients, the inability to relax may induce such a prolonged AD, with a higher CV as a result.

Alternatively, the muscle membrane in FM may be facilitated by the direct effect of the excitatory transmitter adrenaline (or noradrenaline) on it. Adrenergic hormones are being released in situations of stress and physical exercise (Kernell 2006c). Apart from the well-known adrenergic effects on the sarcolemma resulting in stronger contractions (Bowman and Nott 1969, Roatta and Farina 2010), adrenergic activity stimulates the K^+ / Na^+ pump of the skeletal membrane (Clausen and Nielsen 2007, Dockry et al. 1966). Activation of the K^+ / Na^+ pump leads to higher membrane excitability (Buchanan et al. 2002) and increased CV (Rongen et al. 2002). A muscle in FM seems to be over-activated; it resembles a muscle under adrenergic conditions.

Little is known about the production of adrenergic hormones in FM. However, one study has found relatively high plasma levels of noradrenalin in FM while the levels of adrenalin were relatively low (Hamaty et al. 1989).

In FM and related disorders, the overall neural processes are often over-activated and the central regulation is misbalanced. Examples are the mechanism of sensitization of pain processes in FM (Gracely et al. 2002), and the presentation of complaints in the fibromyalgia-related disorders such as irritable bowel syndrome (Mayer et al. 2002), tension headache, migraine (Bansevicius et al. 1999) or hyperventilation syndrome (Barsky and Borus 1999, Lewis and Howell 1986). The over-activated muscle membrane in FM might be regarded as part of the overall neural hyperactivity in the central and peripheral nervous system (Yunus 2008).

Tender points

TPs are painful spots not specific for FM (Wolfe et al. 1990, Yunus et al. 1981). They are found to occur on a continuous spectrum throughout the population (in particular female). However, the number of TPs is clearly higher in FM patients than in healthy women (Katz et al. 2006). In our study, the FM patients had on average 13 to 15 out of the potential 18 TPs whereas healthy women had 2 to 3 TPs. According to another study by Jacobs et al (Jacobs et al. 1996), we found no correlation between the number of TPs in the FM patients and their experienced pain. This finding suggests that there are other factors than only an increased pain experience or a higher sensitivity to pain (Staud 2006) that contribute to the development of TPs. In addition, previous studies have shown that the number of TPs is not stable. In the same person, the number of TPs may vary over time (Katz et al. 2006, Wolfe et al. 2010).

The variability in the number of TPs in the same subject (Wolfe et al. 2010), the presence of TPs across the population (Katz et al. 2006) and the lack of evidence for pathology in muscle biopsy (Bengtsson et al. 1986, Kalyan-Raman et al. 1984) suggest that TPs may have a functional character. We found a strong positive correlation between the number of TPs and the degree of muscle membrane disturbance (CV) in the FM patients. As far as we know, it is the first time that a relationship has been found between an objective physiological finding in a muscle (CV) and the number of TPs, i.e. the extent of peripheral signs in FM. A possible underlying cause of this relationship could be that the long-working muscles (i.e. especially the postural muscles) would – on the basis of the overactive, hyperexcitable membranes – contract continuously, excessively, and would thus become overworked. With those TPs which are located in muscles, an accumulation of metabolite substances, such as lactate, and changes in microcirculation would lead to pain (Lund et al. 1986, McIver et al. 2006).

Table 4. Pearson intercorrelations between study variables in the fibromyalgia patients.

	1	2	3	4	5	6	7	8	9	10	11	12
1. CV*												
2. TP	0.672											
3. Duration complaints	-0.126	0.153										
4. Physical function	-0.335	0.159	0.045									
5. Days felt good	0.077	0.467	0.045	0.692								
6. Work days missed	0.080	0.381	0.953	0.499	0.088							
7. Job ability	0.091	0.468	-0.115	0.481	0.612	0.842						
8. Pain	0.156	0.395	0.309	0.531	0.849	0.315	0.762					
9. Fatigue	0.192	0.627	-0.023	0.692	0.655	0.714	0.637	0.499				
10. Morning tiredness	-0.093	0.264	-0.017	0.592	0.652	0.668	0.848	0.757	0.516			
11. Stiffness	0.137	0.284	0.183	0.452	0.696	0.316	0.491	0.545	0.533	0.718		
12. Anxiety	-0.115	-0.129	-0.006	0.183	0.020	0.657	0.294	-0.025	0.294	0.164	-0.021	
13. Depression	-0.090	0.117	0.095	0.655	0.708	0.458	0.599	0.560	0.677	0.567	0.561	0.643

* muscle fibre conduction velocity.

On the methods

In this study, we deliberately applied very strict exclusion criteria for FM patients, including no other illnesses, no obesity, no medication, and no wheelchair use. Since FM patients often use medication and self-help devices, one can argue that our patients' sample may not have been representative of the FM population as a whole. We applied these strict criteria because we were interested in pure fibromyalgia and not in the effects of medication or bodily immobility.

The study groups were relatively small, which can be regarded as a limitation of the study. However, the groups were sufficiently large to show a significant difference in membrane function between patients and controls in sEMG, and a significant correlation between the sEMG finding and the number of TPs. For calculating mutual relationships between items of the FIQ, the patients' group was too small.

CVs obtained by the CC method were higher than those obtained by the IPL method (Fig. 1a and 1b). Such a difference was also found in a previous study (Klaver-Król et al. 2010b). The CVs in the CC method are probably biased towards higher values because in this method especially larger MUPs determine the CV (Sollie et al. 1985a).

Conclusions

1. In the non-TP related muscles of FM patients, functional muscle membrane disturbances have been found, suggesting an overall muscular membrane disorder.
2. The degree of membrane disturbances is correlated with the number of TPs; many of them are localized in muscles.
3. We suggest that both membrane disturbances and muscular signs might be caused by increased adrenergic excitatory action on the muscle membrane in FM.

(F) patient with fibromyalgia;

(S) sprinter;

(D) distance runner

Part V

General Discussion

Chapter 8

What can we learn from sEMG in static and dynamic conditions with position control? Values of the Method and its prospects

We have shown that the developed method (the ‘Method’) for investigating the biceps brachii muscle by sEMG under conditions of static and dynamic position tasks, combined with the application of a set of variables derived from peak velocities, renders a good deal of information about the fiber types in a muscle, the neural regulation strategies, and the muscle membrane function.

What is new in the Method presented?

There are several innovations in the Method we present here. The major novelty is that the CV was for the first time measured *continually* over lengthy cycles of *dynamic* exercises of a limb. To date, in dynamic situations, it has mostly been global sEMG variables that have been estimated such as the integrated EMG and the spectrum frequency. Direct CV measurements have particularly been made under isometric conditions. As far as we know, only a group based in Torino, Italy, has published on CV assessment (from knee extensors) in dynamic conditions. However, their study only addressed short fragments of a movement of no more than 150-200 ms (Farina et al. 2004, Pozzo et al. 2004). Another study by Masuda et al. also assessed CV linked to dynamic leg exercises, but here the measurement was made at the end of a movement (Masuda et al. 1999).

Another new feature of our Method is that, despite using only a simple three-electrode array, refined information can be extracted from sEMG. This was achieved through using an IPL formula for calculating the CV, rather than the commonly applied cross-correlation formula. IPL measurements render a large number of CVs, each attributed to a MUP in an sEMG signal. The IPL method for measuring CV was first described by Lange et al. (Lange et al. 2002); and we used an adapted algorithm (Klaver-Król et al. 2007). The basic principle is to select as many MUPs (here referred to as peaks) as possible from the signals obtained along muscle fibers, and then to calculate a propagation velocity for each of the peaks (peak-CV). Collecting many peak-CVs allows statistical analyses to be performed. Lange et al. (Lange et al. 2002) were the first in sEMG to apply a statistical variable in the form of the standard deviation of peak-CVs. They examined the biceps brachii muscle in a group of healthy subjects during short isometric contractions in a force-controlled procedure. The authors suggested that the standard deviation of peak-CVs might be a useful variable in evaluating myopathic and neuropathic diseases.

In our studies, besides the standard deviation, we have added a second statistical variable in analyzing peak-CVs, namely the skewness of the peak-CVs. Skewness is a measure of the relative proportion of higher and lower peak-CV values, as it were in the form of a histogram. In other words, skewness shows which peak-CVs

are more numerous, those with higher or lower propagation velocities. Calculating a mean CV along with a standard deviation and the skewness of the peak-CVs delivers a higher level of information.

We also proposed a new variable to represent the intensity of motor unit activation: the peak frequency (PF) (Klaver-Król et al. 2007). The PF expresses the number of MUPs/peaks per second in sEMG. In a fresh muscle, the PF is mainly related to the number of activated motor units and their discharge rates. During fatigue, however, as with the ‘number of zero crossings’ variable (Hägg 1981), the PF seems to be largely dependent on the muscle fiber conduction velocity.

sEMG in sportsmen

CV differences between sprinters and endurance athletes

During both static and dynamic tests, the mean CV of the muscles in sprinters was higher than that of the muscles in endurance athletes. Interestingly, our newly introduced variable, the skewness of peak-CVs, appeared even more sensitive than the mean CV in showing the differences between the two types of sportsmen: sprinters had a significantly greater proportion of fast propagating peaks than endurance athletes (Klaver-Król et al. 2010a, Klaver-Król et al. 2010b). This underscores the value of skewness as an additional sEMG variable. The higher mean CV and the greater proportion of the faster propagating peaks in sprinters can be explained by either a greater content of type II (large, glycolytic) muscle fibers, or by the fact that they tend to activate these fibers/motor units earlier (Brooks et al. 2005b, Komi 1992, Tax et al. 1989, Thorstensson 1976). Type II fibers propagate an action potential along their membranes faster than type I fibers (Andreassen and Arendt-Nielsen 1987, Buchthal et al. 1973).

In an earlier study by Sadoyama et al., the mean CV of the thigh muscles was found to be higher in sprinters than in distance runners, and the CV was positively correlated with the proportion of glycolytic (type II) fibers found in a muscle biopsy (Sadoyama et al. 1988). A new finding from the present study is that the differences in the CV (and the skewness) between the two types of sportsmen was also present in muscles that were not specifically trained. This suggests that the finding is likely to reflect an intrinsic hereditary property rather than to be an effect of training regime.

Different fatigue patterns between sprinters and endurance athletes

The main feature of peripheral fatigue is a decline in the propagation velocity of the muscle membrane (Milner-Brown and Miller 1986) and this is reflected in a decrease in CV.

During a prolonged submaximal effort, whether voluntary or electrically elicited in an experiment, or as part of daily life, fatigue does not simultaneously occur in all motor units of a muscle. Rather, pools of motor units are continually activated and de-activated (Knaflitz et al. 1990, Marsden et al. 1983, Westgaard and de Luca 1999). What we observe in sEMG are the MUPs produced by those motor units which are sufficiently fresh to be recruited. Some of these motor units are fully fresh; others are on the way to become fatigued.

A characteristic feature of the sprinters' fatigue was a smaller decline in mean CV than in the case of the endurance athletes. A second characteristic feature of the sprinters was that the standard deviation of the peak-CVs grew during fatigue, whereas it did not change in endurance athletes (Figs 4-c1 and 4-c2 in Chapter 5). Thus, the range of peak velocities in sprinters was greater, ranging from very slow to very fast peaks. This can be seen when comparing the histograms in Fig. 5c and Fig. 6c in Chapter 5, which represent sprinters and endurance athletes respectively. The findings suggest that, when a pool of active motor units becomes fatigued in sprinters, the newly activated, replacement motor units are of the fast propagating type: namely large motor units with type II (glycogenic, fatigable) fibers (Burke et al. 1973, Gollnick et al. 1972). When endurance athletes become fatigued, however, the peak-CVs are low and medium-low. This finding, combined with the fairly large amount of MUP activity produced, suggests that, in endurance athletes, especially smaller motor units with type I fibers (and probably also intermediate fiber types) are activated. Type I fibers propagate action potential more slowly, have oxidative metabolism, and are fatigue-resistant.

sEMG in patients with fibromyalgia***About fibromyalgia***

Fibromyalgia (FM) is an affliction characterized by chronic widespread pain, with painful spots at the insertions of various muscles (tender points), and other accompanying complaints, such as fatigue and sleep disturbance (Wolfe et al. 2010, Wolfe and Rasker 2012, Wolfe et al. 1990). FM is a disorder on a continuum with many related syndromes and disorders, such as irritable bowel syndrome (Mayer et al. 2002), tension headache, migraine (Bansevicius et al. 1999), and chronic fatigue syndrome (Fukuda et al. 1994). The etiology of FM and of the related disorders is

not clear. However, there is some evidence that dysregulation of control mechanisms on several levels of the central nervous system could play a role (Banic et al. 2004, Desmeules et al. 2003, Gracely et al. 2002). Tender points occur not only in patients with FM; they appear throughout the population, and especially among females. However, the number of tender points in patients with FM is clearly greater (Katz et al. 2006).

In biopsies of affected muscles of patients with FM, only aspecific features, if any, have been found (Bengtsson et al. 1986, Kalyan-Raman et al. 1984). Previous sEMG studies, performed on *painful* muscles in FM patients (Gerdle et al. 2008) and those with related disorders (Falla and Farina 2005), have shown higher CVs than those in control subjects. These higher CVs in FM patients have been ascribed to a local disturbance in tissue electrolytes or in the microcirculation (Gerdle et al. 2008).

Higher CVs in an unaffected muscle in patients with fibromyalgia

Our studies have demonstrated that the CV in patients with FM is abnormally high, even in an unaffected – i.e. one not associated with tender points and not painful – muscle (Klaver-Król et al. 2012a, Klaver-Król et al. 2012b). This finding suggests a membrane dysfunction in FM that seems to be a generalized rather than a focal phenomenon.

Two underlying mechanisms might play a role in producing this higher CV. An earlier study has shown that patients with FM (and patients with chronic neck pain, a disorder related to FM) tend to activate their muscles continuously, i.e. they cannot relax (Elert et al. 2001, Nederhand et al. 2000). It is possible that such an underlying (or foregoing) activity has a facilitatory effect on a muscle membrane, resulting in an increased propagation velocity (Gydikov and Christova 1984, Morimoto and Masuda 1984, Van der Hoeven and Lange 1994). A second possible explanation for the higher CVs in patients with FM could be an increased adrenergic activity on the membrane (or an increased sensitivity of the membrane to adrenergic activity). Adrenergic hormones, which are released in stress situations and during effort (Kernell 2006c), bind with various parts of a muscle and their effect is one of promoting muscle performance (Bowman and Nott 1969, Roatta and Farina 2010). Adrenergic hormones have been shown to bind with the muscle membrane and speed-up the sodium-potassium pump (Dockry et al. 1966), resulting in an increased CV (Buchanan et al. 2002, Rongen et al. 2002).

Resistance to electromyographic fatigue in patients with fibromyalgia

Despite the normally occurring clinical fatigue, we did not observe electromyographic signs of fatigue in our FM patient group (Klaver-Król et al. 2012a). Fatigue was

clearly absent in all three fatigue variables that we used: the mean CV (i.e. the membrane propagation velocity), the integrated EMG (i.e. the content of sEMG signal activity), and the PF (i.e. the number of MUPs in sEMG). Our analysis shows that the basic sEMG feature during fatigue in FM patients is a persistently higher mean CV, and that this is a cue for the results using the two other variables. In other words, the behavior of the high membrane propagation velocity during fatigue leads to the ‘lack of electromyographic fatigue’ in FM patients.

An alternative explanation for the lack of electromyographic fatigue could be a so-called “diminished central activation” (Gandevia et al. 1996). In an earlier study on patients with chronic fatigue syndrome (CFS) – a syndrome related to FM – there was a discrepancy between clinical fatigue (i.e. when patients stop the effort and declare themselves fatigued) and “real” fatigue (Schillings et al. 2007). In that study, “real” fatigue was determined by subtracting the voluntary maximum force from an electrically elicited maximum force after finishing a fatigue test. Such a force difference, a gradient in the central activation and a normally occurring phenomenon, appeared to be greater in patients with CFS than in controls, indicating their diminished central activation (Schillings et al. 2004). A lower central activation could be caused by factors such as pain, diminished motivation, or diminished concentration.

Although we did not apply an electrical stimulation-subtraction test, it seems unlikely that a diminished central activation would have caused the lack of electromyographic fatigue in our FM patients group. Here, the patients demonstrated a relatively high maximum voluntary force compared with other studies (Klaver-Król et al. 2012a, Norregaard et al. 1995), and the force levels applied were weighted to the maximum contraction force. Further, the endurance time was similar in both the patients with FM and the controls. Finally, the patients in our study did not complain of pain during the exercise and they were well motivated. Thus, the membrane property, with its fast propagation seems to be the only underlying cause of our findings.

The propagation speed of an action potential along the membrane and the membrane excitability are two phenomena with the same physiological background, namely the membrane’s readiness to depolarize (caused by the sodium-potassium pump) (Buchanan et al. 2002, Rongen et al. 2002). Hence, a higher CV in FM patients would result in a greater excitability and thus a lower discharge threshold for the membranes. Indeed, studies have shown an increased muscle membrane excitability in those suffering from FM and related disorders (Vitali et al. 1989). In various syndromes and disorders related to FM, including irritable bowel syndrome (Mayer et al. 2002), sleep disturbance (Barsky and Borus 1999), tension headache

(Bansevicius et al. 1999), hyperventilation syndrome (Lewis and Howell 1986), and atypical chest pain (Wessely and Hotopf 1999), there are features of widespread hyper-reactivity. Higher CVs in patients with FM might be part of such generalized hyper-reactivity in this large family of “unexplained syndromes”.

Association between CV and the number of tender points in patients with fibromyalgia

Surprisingly, we found a strong positive correlation between the CV measured in the biceps brachii – an unaffected muscle in patients with FM – and the number of tender points, i.e. painful spots in various muscles throughout the body. This finding suggests that the higher CV is somehow related to the extent of muscular signs in FM.

Tender points are mostly localized in postural muscles, such as the tensor fascia latae, neck, and buttock musculature, or in supporting muscles such as the extensor digitorum longus (Wolfe et al. 1990). These are precisely the muscles that work the longest in everyday life. The explanation for the development of tender points could be that, in a situation of hyper-activated membranes, the long-working muscles have no opportunity to relax and thus become over-worked. This would lead to an accumulation of metabolites or to aspecific damage, resulting in pain (Brooks et al. 2005c, Kernell 2006b).

Values and prospects of the presented Method

The presented sEMG method, especially its part involving dynamic arm exercise in a position-controlled procedure, appears to be able to identify several aspects of muscular function. Firstly, it is able to detect differences in muscle fiber composition, in principle a genetic property, such as we found in sportsmen. Secondly, the Method can, to some degree, distillate the patterns in central neural regulation. The changes in central regulation can be assessed both within and between individuals. Within an individual, the development of fatigue can be observed over time. Among individuals, differences can be found in the presentation of fatigue, such as between sprinters and the endurance athletes. The Method is also able to show disturbances at the muscular membrane level, such as the abnormal membrane function we found in the patients with FM.

Our Method is not difficult to apply. It can, in principle, be practiced in a standard clinical neurophysiological laboratory. The equipment is not expensive, and the software does not require very powerful computers. The short electrode array required is easy to place, and the method is not annoying for either the subjects or the investigators.

The presented Method may be suitable for diagnosing muscular complaints that are difficult to define, such as pain and cramp, and disorders on the border of neurology and internal medicine, such as hormonal diseases (muscular complaints in diabetes mellitus or Addison's disease), hepatic and renal disorders, and nutritional deficiencies. Further, chronic fatigue syndrome investigations could be extended to search for the "lack of electromyographic fatigue" as we found in patients with FM.

Part VI

Summaries

Chapter 9

Summary English

This thesis contains six parts covering the general introduction, the description of a novel method (referred to as the Method) for surface electromyography (sEMG) and the applications of the Method. The Method has been applied in studies of sportsmen and of patients with fibromyalgia syndrome (FM). The fifth part contains the general discussion. In this final (sixth) part, the chapters of the thesis are summarized, and this is done in three languages: English, Dutch and Polish. The goal of the thesis is to show that, in a relatively simple way, information can be extracted from sEMG that is relevant from the physiological and medical point of view.

Part I: General Introduction

In Chapter 1 of the introduction, the quintessence of the sEMG is presented, with its goals, conditions under which the experiments are carried out, and the ways of analyzing. sEMG is the measurement of electrical muscular activity that is produced through voluntary contractions by the subject and is recorded from the skin surface. sEMG has been applied in revalidation, in ergonomics, and in fundamental research on muscular function. Practically, there is no application in a clinical-diagnostic setting.

During sEMG measurements, contractions can vary in different ways. Firstly, the effort intensity (force level) can vary from the maximum force that the subject is able to produce down to the minimum force that still renders any activity. Secondly, the limb can be immobile (isometric contractions) or it can move (dynamic contractions). Thirdly, the way in which the exerted force is controlled by the subject can also differ. There are experiments with force-controlled contractions and others with position-controlled contractions. In a force-controlled contraction, the subject is required to maintain a given force level that is usually measured and displayed using a force transducer. In a position-controlled contraction, a force is applied using a fixed inertial load, while the subject is required to maintain a targeted limb position. During an isometric test, when it is performed under a position control (traditionally known as a static test), there is one position to be maintained. During a dynamic exercise under a position control, the extremity is moved between two well-defined positions.

The sEMG signal can be analyzed using various variables. In fact, the variable one uses determines the feature that will be extracted from sEMG. One well-established variable is the power spectrum frequency distribution (spectrum frequency) of the signal. The spectrum frequencies mainly depend on the velocity in which the action potentials are propagated along the muscle membranes (muscle fiber conduction

velocity, CV), so the changes in the spectrum frequency are considered as an indirect measure of the changes in the CV. Direct measurements of the CV are not often applied. This is probably because of their much cumbersome procedure. However, determining CV is especially of importance in fundamental research because of its absolute character. A CV can be measured in different ways. It can be measured as a global, average value. It can also be measured in a more refined way: as CVs of the individual motor units and as CVs of motor unit potentials (MUPs, peaks). In the first situation, the CVs are assigned to the individual specific motor units. In the latter situation, the CVs are attributed to an aspecific gathering of MUPs found in the signal. The first situation requires far more sophisticated equipment and software than the second one.

In Chapter 2 of the introduction, the principles of the new Method are briefly set out. We strived to create a situation where the standardized contractions would, as far as possible, represent a natural situation in daily life: low force levels were used and a characteristic feature of the study design was a combination of *dynamic* exercise with *position control* of the limb. In our daily lives, we are used to dragging inertial loads and controlling the position of our body. Force control is not a normal function in everyday existence. A refined analysis of the sEMG signal has been developed and applied.

Part II: The new Method

The sEMG measurements were performed on the biceps brachii muscle during short static contractions and prolonged dynamic exercises. The subjects were seated in a chair. The upper arm was supported, with shoulder flexion set at 45°, and the lower arm was free. The required effort was controlled by the subjects maintaining a given lower arm position while holding an inertial load in the palm of their hand. This way of controlling the effort differs from the conventional method where the arm position is fixed and the effort is controlled by a subject using a force transducer. The force levels used in our Method are low, ranging from an unloaded muscle up to 20% of the maximum exerted effort (MVC). During the static tests, the subjects were asked to keep their lower arm horizontal. During the dynamic tests, the lower arm was swung between two predetermined positions over a range of 45°, at a rate of 40 beats (oscillations) a minute. A short electrode array was attached, consisting of three electrodes from which one derived two differential sEMG signals.

The sEMG signal was analyzed using variables that were constructed from the MUP/peak conduction velocities (peak-CVs). The formula for the peak-CV is presented.

The variables used are:

- the mean CV (CV), which is an average of the peak-CVs;
- the within-subject skewness of the peak-CVs (skewness), which represents the relative proportions of faster and slower propagating MUPs;
- the within-subject standard deviation of peak-CVs, which is a measure of the spread of MUP velocities;
- the peak frequency (PF), which is a measure of the intensity of MUP activity in sEMG.

Part III: Studies in sportsmen

This study investigated whether the Method is able to distinguish between the not specifically trained muscles of sprinters and of endurance athletes.

In both short static (chapter 4) and prolonged dynamic (chapter 5) tests, sprinters showed higher CVs than endurance athletes. Skewness appeared to be more sensitive than CV in distinguishing between the types of sportsmen. The higher CVs and the greater skewness towards higher peak velocities in sprinters are in accordance with a greater proportion of type II muscle fibers. These fibers propagate an action potential faster along their membranes. Given that the muscle tested was not specifically trained, the Method seems to show genetic differences between the muscles of a sprinter and of an endurance athlete. During the fatiguing prolonged dynamic exercise at 20% MVC, the peak-CVs' standard deviation displayed different trends between the two types of sportsmen (chapter 5). The standard deviation became progressively broader in sprinters whereas it did not change in endurance athletes. In terms of MUP velocities, sprinters show both high and low MUP velocities, whereas endurance athletes show predominantly low velocities. This finding indicates that, during fatigue, the sprinters rely more on motor units with type II fibers, which are both fast propagating and fatigable. Endurance athletes seem mainly to use motor units with type I fibers, which are slower propagating and fatigue-resistant. Thus, the within-subject standard deviation of peak-CVs appears to be a sensitive variable in distinguishing fatigue patterns between types of sportsmen.

Part IV: Studies in patients with fibromyalgia

This study explored differences in sEMG findings in a clinically unaffected muscle between patients with fibromyalgia (FM) and healthy women. A second aim was to search for associations between sEMG results and patients' signs and complaints. FM is an unexplained affliction, characterized by widespread pain, in particular at the insertions of muscles, known as tender points (TPs). Measurements were performed on the biceps brachii, a muscle that is not associated with tender points and that was not found to be painful during physical examination.

In both short static and prolonged dynamic tests, the CV was abnormally high in patients with FM (chapters 6 and 7). Given that the findings refer to an unaffected muscle, they suggest a widespread disturbance of membrane function in FM patients. During prolonged dynamic tests, the muscles of the patients with FM appeared to be resistant to electromyographic fatigue (chapter 6). No evidence of fatigue was found in the variables used: the CV, the PF, and the sEMG amplitude (the average rectified voltage). Analyses show that a persisting high CV leads to the lack of fatigue signs in the other two variables. Another interesting finding was that the CV was correlated with the number of TPs in patients with FM (chapter 7). This suggests that membrane disturbance may play a role in the development of muscular pain. Possible physiological backgrounds to this are discussed.

Conclusions

The presented Method appears to be able to extract valuable features from sEMG. The information gained involves a range of functional levels.

1. The Method yields information on the state of the muscular membrane (as shown in the patients with FM).
2. The Method provides information about the fiber composition of a muscle (as demonstrated in the sportsmen tests).
3. The Method gives insight into central neural regulation (as in the differences found in the fatigue patterns of sprinters and endurance athletes).

Prospects

The method for sEMG analysis presented in this thesis could be applied in various study designs under more natural, dynamic conditions.

Chapter 10

Nederlandse samenvatting

Dit proefschrift bestaat uit zes delen. Deel 1 is een algemene introductie. In deel 2 volgt de beschrijving van een nieuwe methode (Methode) van oppervlakte electromyografie (sEMG). Hierna worden toepassingen van de Methode gepresenteerd. In deel 3 wordt de Methode toegepast bij sporters en in deel 4 bij patiënten met een fibromyalgie syndroom (FM). Deel 5 bevat een algemene discussie. Het afsluitende zesde deel bevat samenvattingen van het proefschrift in drie talen: het Engels, Nederlands en Pools. Het doel van het proefschrift is zichtbaar te maken dat op een relatief eenvoudige manier fysiologisch en medisch relevante informatie uit het sEMG gehaald kan worden.

Deel 1: Introductie

In hoofdstuk 1 van de introductie wordt in een notendop de essentie van sEMG beschreven, haar doelen, de omstandigheden waaronder het onderzoek wordt uitgevoerd en de wijze van analyseren. sEMG is een meting van elektrische spieractiviteit die ontstaat tijdens de aanspanning. sEMG wordt toegepast in de revalidatie, ergonomie en in het basaal wetenschappelijk onderzoek. Er zijn nagenoeg geen toepassingen in de klinische diagnostiek. Tijdens een sEMG meting kan de aanspanning in meerdere opzichten variëren. Ten eerste, kan de intensiteit van aanspannen, dat wil zeggen het krachtsniveau, variëren van zeer laag tot maximaal. Ten tweede, het ledemaat kan al dan niet in beweging zijn (het niet bewogen ledemaat kan al dan niet gefixeerd zijn). Dit noemen we isometrische en dynamische contracties. Ten slotte, kan de manier van toepassen van de belasting op het ledemaat en de controle hierover door de proefpersoon verschillen. Er bestaan testen gebaseerd op zgn. krachtscontrole en op positiecontrole. In de krachtscontrole opstelling wordt een bepaalde kracht met behulp van een transducer op het ledemaat uitgeoefend en de proefpersoon controleert op het scherm of hij/zij zich eraan houdt. Bij positiecontrole wordt de kracht opgelegd in de vorm van een gewicht (Drost et al.) dat rust op het ledemaat. De proefpersoon controleert vervolgens de correctheid van de aanspanning door het handhaven van de positie van het ledemaat. Tijdens de isometrische testen die onder een positiecontrole uitgevoerd worden (ook wel genoemd statische testen) wordt één positie gehandhaafd. Bij de dynamische testen onder een positiecontrole wordt het ledemaat bewogen tussen twee strikt bepaalde posities.

Het sEMG signaal kan geanalyseerd worden op grond van verschillende variabelen en, afhankelijk van de gekozen variabele, kunnen verschillende patronen uit het signaal worden gehaald. Een vaak gebruikte variabele is het frequentiespectrum van het signaal. Het frequentiespectrum is afhankelijk van de geleidingssnelheid

waarmee actiepotentialen tijdens een aanspanning langs de spiervezels voortgeleid worden (dit noemen wij spiervezelsnelheid, CV). Daarom worden de veranderingen in het frequentiespectrum gezien als een indirecte maat voor de veranderingen in de CV. Een *rechtstreekse* meting van CV wordt minder vaak gebruikt, waarschijnlijk omdat deze meting ingewikkelder en voor vele toepassingen niet noodzakelijk is. Echter in een basaal wetenschappelijk onderzoek is een CV meting van belang wegens haar absolute karakter. CV kan worden gemeten als een globale, gemiddelde waarde. Het kan ook meer verfijnd worden gemeten: als CVs van individuele motor units en als CVs van motor unit potentialen (MUPs), hier genoemd pieken. Het verschil tussen beide is dat in het eerste geval de CVs tot de geïsoleerde, specifieke motor units behoren en in het tweede geval betreffen ze een verzameling van de MUPs/pieken in het signaal, zonder het onderscheid van motor units. In het eerste geval is het type elektroden en software aanzienlijk complexer dan in het tweede geval.

In hoofdstuk 2 van de introductie wordt een voorzet gemaakt tot de nieuwe Methode. Het principe van de Methode is dat, met behoud van een strikte standaardisatie, de omstandigheden waarin sEMG wordt uitgevoerd zo goed mogelijk overeenkomen met een natuurlijke situatie. Dus, de krachtsniveaus zijn laag, de uitvoering is dynamisch en de aanspanning wordt gecontroleerd met een positiecontrole. In dagelijks leven wordt immers altijd met inerte gewichten gewerkt terwijl de lichaamspositie onder controle wordt gehouden. In de Methode worden verfijnde sEMG variabelen toegepast.

Deel 2: Een nieuwe Methode

Volgens de Methode worden sEMG metingen uitgevoerd op de biceps brachii tijdens korte statische contracties en langdurige dynamische oefeningen. De proefpersonen zitten tijdens de experimenten in een stoel. De bovenarm wordt ondersteund op 45° flexie van de schouder; de onderarm is vrij. Het vereiste inspanningsniveau wordt gehandhaafd door controle van de onderarmpositie terwijl een gewicht in de hand wordt vastgehouden. Tijdens statische testen houdt de proefpersoon de onderarm horizontaal. Tijdens dynamische testen zwaait de persoon met de onderarm tussen twee vastgestelde posities in het ritme van 40 per minuut (40 bewegingen naar boven en naar beneden), waarbij een hoek beschreven wordt van 45 graden. De gebruikte krachtsniveaus zijn laag, lopende van geen belasting tot 20% van de maximale ugeoefende contractie kracht (MVC). Er wordt een elektrodenreeks toegepast, samengesteld uit drie elektroden, die twee differentiële sEMG signalen opleveren. Uit de afstand tussen de elektroden en tijdsverschillen tussen de twee sEMG signalen

worden snelheden gemeten van vele motorunit potentialen (MUPs)/pieken; piek-snelheden (piek-CVs). Een formule voor het verkrijgen van de pieken en hun snelheden wordt gepresenteerd.

sEMG wordt verder geanalyseerd met behulp van de variabelen die afgeleid zijn van de piek-CVs. Hierbij worden door een statistische bewerking van de piek-CVs nieuwe variabelen ontwikkeld. De variabelen zijn:

- een gemiddelde CV (CV), wat de gemiddelde waarde is van de gevonden piek-CVs.
- een scheefheid (skewness) van de piek-CVs (binnen een persoon), die de verhouding weergeeft tussen de aantallen van snellere en tragere piek-CVs.
- een standaard deviatie (SD) van de piek-CVs (binnen een persoon), die de spreiding weergeeft van de piek-CVs.
- een piek frequentie (PF), (aantal pieken per seconde), die een maat is van intensiteit van de voorkomende MUP activiteit.

Deel 3: Onderzoek bij sporters

Het doel van deze studie was te onderzoeken of de Methode in staat is onderscheid te maken tussen een *niet specifiek getrainde* spier van een sprinter en van een duursporter.

Zowel in de statische (hoofdstuk 4) als dynamische (hoofdstuk 5) testen was de CV bij sprinters hoger dan bij duursporters. De skewness blijkt een gevoeliger variabele te zijn in het tonen van het verschil tussen de twee typen van de sporters dan CV. Zowel een hogere CV als een grotere scheefheid in de richting van hogere pieksnelheden bij sprinters komen overeen met het feit dat hun spieren meer type II spiervezels bevatten. Type II spiervezels geleiden de actiepotentialen sneller langs de membranen dan type I vezels. De Methode blijkt dus in staat te zijn genetische verschillen in de spiersamenstelling tussen sprinters en duursporters te detecteren. Gedurende de vermoeiende dynamische test met 20% van de MVC gedraagt de SD zich anders bij sprinters dan bij duursporters (hoofdstuk 5). Bij sprinters wordt de SD toenemend breder, terwijl de SD bij duursporters niet verandert. Vertalen we dit naar de MUP snelheden, dan hebben sprinters een brede spreiding van hoge naar lage MUP snelheden, terwijl duursporters vooral lage en middenlage MUP snelheden tonen. Deze bevinding suggereert dat sprinters tijdens vermoeidheid vooral grotere motor units met type II spiervezels activeren, die snel geleiden en vermoeibaar zijn. Duursporters lijken vooral gebruik te maken van traag geleidende, vermoeidheidsresistente type I vezels, aangestuurd door kleinere motor units. SD blijkt dus een gevoelige sEMG variabele te zijn in het onderscheiden van patronen van spiervermoeidheid tussen de twee onderzochte typen sporters.

Deel 4: Onderzoek bij patiënten met fibromyalgie

Het doel van deze studie was te onderzoeken of er verschillen zijn in sEMG van een *onaangedane* spier tussen patiënten met FM en gezonde groep vrouwen. Het tweede doel was zoeken naar verbanden tussen de sEMG resultaten en de klachten en symptomen van de patiënten. FM is een stoornis met een onbekende etiologie, waarbij diffuus pijn aanwezig is, vooral van de aanhechtingen van bepaalde spieren, bekend als ‘gevoelige punten’ (tender points = TPs). Volgens het protocol, werd de biceps brachii onderzocht, als spier die niet betrokken is bij de bekende TP locaties, en bij het onderzoek niet pijnlijk was.

Patiënten met FM toonden tijdens zowel korte statische als langdurige dynamische testen een abnormaal hoge CV, wijzende op een gestoorde membraanfunctie (hoofdstukken 6 en 7). Verder lieten patiënten met FM bij langdurige (vermoeidheids-) testen geen electromyografische vermoeidheid zien (hoofdstuk 6). Teken van vermoeidheid ontbraken in alle drie vermoeidheidsvariabelen: in zowel CV, als PF en de sEMG amplitude (average rectified voltage = ARV). Analyse wees uit dat een aanhoudende hoge CV tijdens vermoeidheid bij patiënten met FM de oorzaak was van het ontbreken van vermoeidheidskenmerken in de overige twee variabelen (PF en ARV). Een ander interessant resultaat van het onderzoek was dat CV sterk correleerde met het aantal TPs bij patiënten met FM (hoofdstuk 7). Deze bevinding suggereert dat de stoornis in de membraanfunctie een rol kan spelen bij het ontstaan van spierpijn bij FM. Mogelijke pathofysiologische achtergronden hiervan worden besproken.

Conclusies

De gepresenteerde Methode blijkt in staat te zijn waardevolle patronen uit het sEMG te halen. De informatie die uit het sEMG gedistilleerd kan worden betreft verschillende functionele niveaus.

1. De Methode levert informatie over eigenschappen van het spiermembraan (zoals gevonden bij FM).
2. De Methode geeft weer hoe de spiervezelsamenstelling is (zoals gevonden bij sporters).
3. De Methode geeft een inzicht in de centrale neuroregulatie van de spier tijdens activiteit (zoals het gevonden verschil in het patroon van electromyografische vermoeidheid tussen sprinters en duursporters).

Vooruitzichten ten aanzien van de toepassing

De gepresenteerde Methode van sEMG analyse kan toegepast worden in uiteenlopende studies en op verschillende spieren in meer natuurlijke, dynamische omstandigheden.

Chapter 11

Streszczenie

(Poolse Samenvatting)

Prezentowana praca składa się z sześciu części (Parts). Pierwsza część jest wstępem ogólnym. W drugiej części jest opisana nowatorska metoda elektromiografii powierzchniowej (surface electromyography, sEMG) – do której, w dalszej częściach pracy zastosowano odnośnik 'Metoda'. W celu potwierdzenia użyteczności Metody, została ona zastosowana u dwóch odrębnych grup ludzi: u sportowców (część trzecia) i u osób cierpiących na fibromialgię (FM) (część czwarta). W piątej części zostały zamieszczone rozważania ogólne. Część niniejsza (szósta) zawiera podsumowanie pracy w trzech językach: angielskim, holenderskim i polskim.

Celem pracy jest wykazanie, że w stosunkowo prosty sposób, przy pomocy Metody sEMG, można zdobyć bardzo istotne informacje z fizjologicznego i medycznego punktu widzenia.

Część I: Wstęp ogólny

W rozdziale 1 tego wstępu przedstawiona jest kwintesencja sEMG. Pokazane są cele, sposoby analizowania sEMG oraz warunki, w jakich przeprowadza się doświadczenia. sEMG polega na wykonywaniu pomiarów elektrycznej aktywności mięśni. Ta aktywność jest wywołana przez świadomą reakcję mięśni podmiotu badanego i następnie rejestrowana przez czujniki umieszczone na powierzchni skóry. sEMG znajduje zastosowanie w rehabilitacji, ergonomice i w podstawowych badaniach funkcji mięśni. W zasadzie nie ma zastosowania w diagnostyce klinicznej.

W trakcie wykonywania pomiarów sEMG, skurcze mięśniowe można klasyfikować z różnych punktów widzenia. Po pierwsze, intensywność wysiłku (force level) może się rozciągać od wartości maksymalnej, jaką podmiot badany potrafi osiągnąć w danych warunkach, do wartości minimalnej, przy której pojawia się jakakolwiek aktywność mięśnia. Po drugie, kończyna może zostać nieruchoma (skurcze izometryczne) lub może poruszać się (skurcze dynamiczne). Po trzecie, sposób kontrolowania poziomu wywieranego wysiłku przez osobę w czasie badania może być różny. Znane są doświadczenia, w których wysiłek regulowany jest przez obserwację intensywności siły (zadanie siłowe = force task) oraz takie, w których wysiłek regulowany jest przez obserwację położenia kończyny (zadanie pozycyjne = position task). W przypadku zadania siłowego od osoby badanej wymaga się, żeby utrzymywała dany poziom napięcia, które jest stale mierzone i pokazywane przy pomocy przetwornika siły (force transducer). W przypadku zadania pozycyjnego od osoby badanej wymaga się, żeby utrzymywała pewną pozycję kończyny,

podczas kiedy obciążenie jest niezmiennie (jest to bezwładny ciężar). W trakcie wykonywania zadania pozycyjnego izometrycznego osoba ma utrzymywać jedno stałe położenie (to jest tradycyjnie nazywane testem statycznym). W trakcie wykonywania zadania pozycyjnego dynamicznego osoba wykonuje ruchy pomiędzy dwoma dobrze zdefiniowanymi pozycjami.

Sygnal z sEMG można analizować przy pomocy różnych zmiennych. W rzeczywistości to, jaka zmienna zostanie użyta, określa właściwość, która zostanie pobrana z sEMG. Uznana zmienną jest rozkład widma częstotliwości mocy sygnału. Widmo częstotliwości głównie zależy od szybkości z jaką potencjały czynnościowe przenoszą się wzdłuż błony mięśniowej (szybkość przewodzenia we włóknie mięśniowym – CV). Zmiany widma częstotliwości są więc traktowane jako pośrednia miara zmian w CV. Rzadko stosuje się bezpośrednie pomiary CV, prawdopodobnie z tego powodu, że są kłopotliwe. Jednak określenie CV ma specjalne znaczenie w badaniach podstawowych, właśnie ze względu na to, że podaje szybkość przewodzenia w absolutnych jednostkach. CV można mierzyć różnymi sposobami. Najczęściej mierzy się jego wartość średnią globalną. CV można też mierzyć w bardziej wyrafinowany sposób: jako CV indywidualnych specyficznych jednostek motorycznych i jako CV (aspektycznych) potencjałów jednostki motorycznej (MUPs).

W rozdziale 2 tego wstępu została nakreślona w skrócie reguła nowej Metody. Staraliśmy się wytworzyć warunki, w których – o ile to możliwe – standardowy wysiłek mięśniowy występowałby tak jak w życiu codziennym. Zostały zastosowane niskie poziomy siły, a charakterystyczną cechą projektu była kombinacja ćwiczeń dynamicznych z kontrolą pozycji kończyn. W naszym życiu codziennym jesteśmy przyzwyczajeni do dźwigania ciężarów (bezwładnych obciążeń), a także do kontrolowania pozycji naszego ciała. Kontrolowanie siły nie jest normalną funkcją naszej codziennej egzystencji. W prezentowanej pracy została rozwinięta i zastosowana wyrafinowana analiza sygnału sEMG.

Część II: Metoda

Według protokołu stosuje się krótkie próby statyczne (kończyna jest nieruchoma) i próby dłużej trwające, wyczerpujące, dynamiczne (kończyna jest w ruchu).

W czasie eksperymentu zarówno statycznego jak i dynamicznego osoba siedzi na krześle. Ramię jest oparte, ze zgięciem stawu barkowego pod kątem 45°, podczas kiedy przedramię jest wolne. Osoba kontroluje wymagany wysiłek przez utrzymywanie wskazanej pozycji przedramienia, podczas kiedy ciężar

(czyli niezmienna masa) trzymany jest w dłoni. Ten sposób kontrolowania intensywności wysiłku przez utrzymywanie *pozycji* odróżnia się od tradycyjnej metody, kiedy pozycja kończyny jest niezmienna (kończyna jest umocowana) a osoba kontroluje wysiłek przez utrzymanie danej *siły*. Siła jest wtedy mierzona i wskazana przez przetwornik. Siły używane w Metodzie są niskie, od nieobciążonego ramienia do 20% maksymalnej siły którą osoba jest w stanie wyprodukować w danych warunkach (maximum voluntary contraction, MVC). W czasie prób statycznych osoba jest proszona o utrzymanie przedramienia poziomo (kąt pomiędzy ramieniem i przedramieniem jest wtedy 135°). W czasie prób dynamicznych osoba macha przedramieniem pomiędzy pozycją poziomą i pozycją, kiedy łokieć jest wyprostowany, zakreślając kąt 45° . Osoba robi to w rytmie 40-tu cykli na minutę. Dla otrzymania sEMG używa się rzędu trzech elektrod wtopionych w sztywną płytkę; odległość pomiędzy elektrodami 10 albo 15 mm, przekrój elektrody 1,5 mm. Elektrody zostają umieszczone wzdłuż włókien mięśniowych pomiędzy płytką ruchową i ścięgnem dystalnym. Robi się odprowadzenia bipolarne. Takie odprowadzenie daje dwa potencjały opóźnione w stosunku do siebie, co umożliwia mierzenie szybkości przewodzenia potencjału wzdłuż włókien mięśniowych.

W sEMG szuka się szczytów (pik) potencjałów jednostek ruchowych (motor unit potential, MUP). Następnie mierzy się szybkości przewodzenia indywidualnych pik po włóknach mięśniowych (peak conduction velocity, peak-CV, pik-CV). Z tych szybkości wyprowadza się zmienne (variables) służące do analizowania sygnału sEMG. Formuła dla pik-CV została podana. Zostały użyte następujące zmienne:

- przeciętna prędkość (CV), która jest średnią ze znalezionych pik-CV;
- statystyczna zmienna współczynnika skośności pik-CV (skewness), która przedstawia proporcję pomiędzy szybszymi i wolniejszymi pikami. (Współczynnik skośności jest analizowany dla każdej osoby osobno = wewnątrzsobniczo = within-subject);
- statystyczna zmienna odchyłki standardowej pik-CV (standard deviation), także wewnątrzsobniczo, która jest miarą rozproszenia prędkości;
- częstotliwość w jakiej piki występują (peak frequency, PF), oznaczająca ilość pik na sekundę.

Część III: Badania u sportowców

Celem tego badania było sprawdzenie czy Metoda jest w stanie ujawnić różnice w cechach (nie specyficznie ćwiczonych) mięśni pomiędzy biegaczem krótkodystansowym (KD) i długodystansowym (DD).

Zarówno w próbach statycznych jak i dynamicznych KD wykazywali wyższe CV niż DD. Współczynnik skośności okazał się bardziej czuły w wykazaniu różnicy pomiędzy grupami sportowców niż CV. Wyniki są zgodne z fizjologicznym faktem, że mięśnie u KD zawierają genetycznie więcej włókien typu II (grubsze, glikolityczne, podatne zmęczeniu) niż mięśnie u DD. Włókna typu II przewodzą potencjały wzdłuż błon mięśniowych szybciej niż włókna typu I. Ponieważ badany mięsień nie był specyficznie ćwiczony, rezultaty wskazują na genetyczne różnice w fizjologii pomiędzy typami sportowców.

W czasie wyczerpujących prób dynamicznych (z 20% MVC), standardowa odchyłka pik-CV zachowywała się inaczej u KD niż u DD. Standardowa odchyłka rosła u KD w miarę czasu, podczas kiedy nie zmieniała się u DD. Tłumacząc to na prędkości prezentujące się w mięśniach, to KD mieli zarówno bardzo szybkie jak i bardzo powolne piki, podczas gdy u DD występowały głównie średnio powolne piki. To sugeruje, że w zmęczonych mięśniach u KD uaktywniają się duże jednostki ruchowe kierujące typem II włókien (włókna szybkoprzewodzące, podatne na zmęczenie). Natomiast DD w zmęczeniu posługują się prawdopodobnie mniejszymi jednostkami ruchowymi z typem I włókien (wolniej przewodzących i nie wrażliwych na zmęczenie). Standardowa odchyłka pik-CV okazuje się więc czułą miarą różnicującą mięśnie u KD i DD w procesie zmęczenia.

Część IV: Badania u pacjentek z fibromialgią

Badanie miało dwa cele. Pierwszym celem było poszukiwanie za pomocą sEMG różnic w funkcji pomiędzy mięśniami u pacjentek z FM i u zdrowych kobiet (chodziło tu o mięśnie niezaburzone w sensie klinicznym). Drugim celem badania było poszukiwanie związków pomiędzy wynikami z sEMG i objawami, dolegliwościami pacjentek.

FM jest zaburzeniem o nieznannej etiologii, charakteryzującym się rozległymi bólami, szczególnie umieszczonymi na przyczepach pewnych mięśni, zwane czułymi miejscami (tender points). FM występuje szczególnie (ale nie tylko) u kobiet. Badania zostały dokonane na mięśniu dwugłowym ramienia (biceps brachii), w którym z reguły nie ma czułych miejsc i który przy badaniu wstępnym okazał się niebolesny.

Zarówno w czasie krótkich prób statycznych jak i długotrwałych dynamicznych, pacjentki z FM wykazywały nieprawidłowo wysokie CV. Ponieważ badania dotyczyły mięśnia klinicznie nienaruszonego, wyniki sugerują że w FM mamy do czynienia z ogólnym funkcjonalnym zaburzeniem błony mięśniowej.

W czasie długotrwałych prób dynamicznych mięśnie pacjentek z FM nie wykazywały, w przeciwieństwie do kobiet zdrowych, znaków zmęczenia w sEMG, chociaż ich zmęczenie kliniczne było normalne. Analiza wykazała, że za brak znaków zmęczenia w sEMG jest odpowiedzialna uporczywa wysoka prędkość przewodzenia mięśnia.

Inny interesujący wynik badań był taki, że wykazano pozytywną korelację pomiędzy CV i ilością czułych miejsc u pacjentek z FM. To powiązanie sugeruje, że zaburzenia w błonie mięśniowej mogą grać rolę w powstaniu czułych miejsc. Możliwe fizjologiczne podłoża tych wyników zostały omówione w pracy.

Wnioski

Zaprezentowana Metoda umożliwia otrzymanie za pomocą sEMG wartościowych patronów/ informacji, które dotyczą różnych poziomów funkcjonalnych.

1. Metoda pokazuje nam, w jakim stanie fizjologicznym znajduje się błona mięśniowa (tak jak to zostało stwierdzone w FM).
2. Metoda pokazuje, jaki jest skład mięśnia z punktu widzenia przeważających w nim typu włókien (tak jak to zostało stwierdzone u sportowców).
3. Metoda pokazuje nam podłoże centralnej regulacji nerwowej (tak jak różnice w prezentacji zmęczenia mięśni u krótko- i długodystansowców).

Perspektywy na przyszłość

Przedstawiona Metoda analizy funkcji mięśnia za pomocą sEMG może być stosowana także przy badaniu innych mięśni w bardziej naturalnych, dynamicznych okolicznościach.

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Dankwoord

Als het om promoveren gaat, solliciteert een academicus in de meeste gevallen op een promotieplaats en krijgt dan een al min of meer vast omschreven opdracht voor een te verrichten wetenschappelijk onderzoek. Indien het werk succesvol blijkt te zijn, volgt een bekroning in de vorm van een doctorstitel. Het doel is dan promoveren op zich, terwijl het voeren van het onderzoek dient als vehikel. Voor een kleine groep van promovendi ligt het anders. Dan is de academicus geïnteresseerd in een specifiek onderwerp. Hij of zij zet het onderzoek zelf op gang en zoekt een hoogleraar voor wetenschappelijke specifieke ondersteuning. Het primaire doel is dan het verrichten van het onderzoek wegens wetenschappelijke nieuwsgierigheid, terwijl het verkrijgen van doctorstitel alleen maar een logisch gevolg is en een aangename bijkomstigheid van de wetenschappelijke arbeid. Mijn promotieonderzoek behoorde tot deze tweede categorie. Logischerwijs had ik geen gespreid wetenschappelijk bedje tijdens mijn studies, toch werd ik geweldig gesteund door velen van dichtbij en veraf. Al deze mensen ben ik dan ook buitengewoon erkentelijk.

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Onderzoekers die deelnamen aan de studie waren gebonden aan verschillende universitaire centra in Nederland en zelfs daarbuiten: Universiteit Twente, Universitair Medisch Centrum Sint Radboud in Nijmegen, Universitair Medisch Centrum Maastricht, Universiteit van Zurich. Maar het onderzoek zelf werd uitgevoerd in het niet-akademisch ziekenhuis ZGT (destijds Streekziekenhuis Midden-Twente, SMT) in Hengelo. Dat dit mogelijk bleek, was bijzonder. Maar het Hengelose ziekenhuis was dan ook een bijzonder instituut door de heersende sfeer van vernieuwing, trots en saamhorigheid van medewerkers, en dan nog op een prachtige locatie.

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List of Publications

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Curriculum Vitae

Ewa Król was born in Lublin, Poland, in 1943. She became a physician at the University School of Medicine (Akademia Medyczna) in Lublin in 1967, and worked for two years as a general physician in a regional hospital in Giżycko, Poland. After she married Marten Klaver, a Dutchman, she emigrated to the Netherlands. In the early 1970s, she enrolled in a research program under the leadership of Professor J. Drooglever-Fortuyn, in the Department of Neurology, University of Groningen (now known as the University Medical Center Groningen), the Netherlands. That research involved the physiology of a non-understood fatigue (now known as Chronic Fatigue Syndrome) and chronic hyperventilation. Following this, she qualified in neurology and clinical neurophysiology from the University of Groningen. In 1980, she started a hospital practice in patient care as a neurologist and clinical neurophysiologist in the Streekziekenhuis Midden-Twente, Hengelo (now the Ziekenhuisgroep Twente, ZGT), the Netherlands. In the past decade, she has cooperated with Professor M. J. Zwarts, the then professor clinical neurophysiology at the Radboud University Medical Center Nijmegen, in developing, within the ZGT, a research line involving surface electromyography. She finished her patient care activities in 2004, and has since devoted most of her time to the research, while still associated with the ZGT. This thesis is the culmination of that research.

Ewa is married to Marten Klaver and they have two sons, Peter and Igor, and a grandson Tim.

Stellingen

1. Spiervermoeidheid uit zich bij een explosieve sporter anders in het oppervlakte EMG dan bij een duursporter. De onderscheidende parameter is de *standaard deviatie* van de snelheden van de pieken in het EMG.

Dit proefschrift

2. De parameter *scheefheid* van de snelheden van de pieken in het oppervlakte EMG geeft een indicatie over de verhouding tussen “snelle” en “langzame” spiervezels in een spier.

Dit proefschrift

3. Patiënten met fibromyalgie syndroom (stoornis met diffuse pijn in het lichaam, vooral in de spieren) hebben een functiestoornis van hun spiermembranen. De snelheid waarmee de activiteit voortgeleid wordt langs het membraan is hoger dan normaal.

Dit proefschrift

4. De gestoorde, te snel geleidende spiermembranen bij patiënten met fibromyalgie syndroom verdoezelen verschijnselen van vermoeidheid in het oppervlakte EMG.

Dit proefschrift

5. Een van de hoogste onkosten van dit onderzoek was de rekening van de Medisch-ethische Toetsingscommissie (METC). Een drastische aanpassing van de METC-tarieven is gewenst. Dit kan men realiseren door ongesubsidieerd onderzoek niet te belasten en voor een door de farmaceutische industrie geëntameerd onderzoek een dubbel tarief te rekenen.

Dit proefschrift

6. ...he knew so much that he thought otherwise. (A bullock talking about a mule)

R. Kipling (1865-1936) *‘The Jungle Book’*

7. Een van de meest heldere presentaties van de wetenschappelijke methode, met immense en duurzame gevolgen voor het menselijk denken is de volgende:

“...the fact that the same planets we one time see from a shorter distance and another time from a longer distance, compulsively suggests that the centre of the Earth is not the center of their orbits”.

Mikołaj Kopernik (Nicolaus Copernicus) (1473-1543) 'De revolutionibus orbium coelestium' (1543) 1st volume

8. Którzykiem siedzą z książkami,

Nie mogą być nigdy sami,

Aż kiedy w ciżbie siędą,

Tam dopiero sami będą.

(Wem Bücher die Zeit vertreiben,

Wird nie ohne Freunde bleiben,

Doch wer in der Menge weidet,

Das schlimmste Alleinsein leidet.)

Biernat van Lublin (1465-1529), een van de eerste Poolse dichters die niet in het Latijn maar in het Pools schreef

9. Met leren lezen op jonge leeftijd is het net als met leren eten van spijzen. Dwing kinderen niet, maar bied ze het aan als een grotemensenzaak. Des te gretiger pakken ze het op. Waarschijnlijk leert het kind ook liever zittend onder dan boven de tafel.

10. De volgende stelling van W.J. Snoek uit 1989 is nog steeds actueel:

Zolang het doen aanzienlijk beter gehonoreerd wordt dan het denken (in een doktersspreekkamer), zal van bezuinigingen in de gezondheidszorg weinig terecht komen.

W.J. Snoek, stelling bij het proefschrift "Het denken van de neuroloog" (1989)

Stellingen

behorende bij het proefschrift van Ewa Klaver-Król

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