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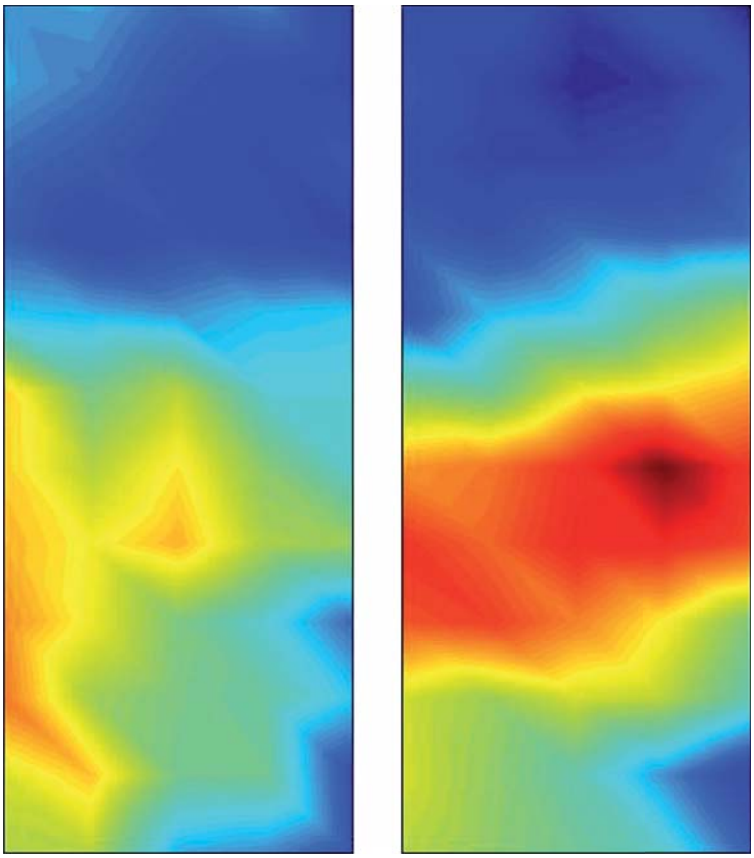
Harri Piitulainen

Functional Adaptation of Sarcolemma to Physical Stress



JYVÄSKYLÄN YLIOPISTO

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ABSTRACT

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It is known for a century that repetitive muscle activity where active lengthening of a muscle takes place with adequate intensity causes muscle soreness and prolonged loss of its force production capability. However, the exact mechanisms for this phenomenon have remained unclear. There is morphological evidence suggesting disruption in membrane structures of the muscle fibres after such an exercise. However, it is unclear how this is reflected in the function of the membrane structures, such as action potential propagation over sarcolemma, which is a crucial event for muscle fibre contractility. The aim of the present research was to find further knowledge for this issue by investigating the adaptation of sarcolemmal function after intensive exercise models with or without the active lengthening of the muscle *in vivo* in humans. The sarcolemmal action potential propagation properties were examined both in the whole muscle and individual motor unit level at wide range of isometric contraction forces. This research work included development, validation and application of novel high-density surface electromyography (sEMG) methodology. This methodology proved to be valid and consistent. The results indicated that both maximal eccentric (active lengthening) and concentric (active shortening) exercises cause a reduction of maximal force production capability, a delayed increase in muscle soreness and an increase in the permeability of the muscle sarcolemma to myoplasmic proteins. These symptoms were significantly higher after the eccentric exercise. Furthermore, greater impairments were observed in global whole muscle excitability and sarcolemmal action potential propagation after eccentric than concentric exercise. Moreover, distortion of control of motor units and slowing of action potential propagation were observed in individual motor unit level after the eccentric exercise. These findings were especially evident at high contraction levels and relatively early (< two days) after the exercise(s). For this reason, it seems that the early loss of force production could be partially explained by failure of the sarcolemmal action potential propagation, but the more prolonged effects are caused by impairment in the processes beyond the sarcolemma. Finally, it appears that the central nervous system attempts to compensate the early force loss by increasing rate coding of motor units at submaximal contractions after the maximal eccentric exercise.

Keywords: muscle cell membrane, surface electromyography, mean muscle fibre conduction velocity, exercise-induced muscle damage, eccentric exercise.

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Harri Piitulainen

Jyväskylä, June 2010

"What makes humans so special, is the desire to know why?"

ORIGINAL PAPERS

This thesis is based on the following original research articles, which will be referred to by their Roman numerals.

I Piitulainen H, Rantalainen T, Linnamo V, Komi PV, Avela J 2009. Innervation zone shift at different levels of isometric contraction in the biceps brachii muscle. Journal of Electromyography and Kinesiology 19, 667-675.

II Piitulainen H, Bottas R, Linnamo V, Komi PV, Avela J 2009. Effect of electrode location on surface electromyography changes due to eccentric elbow flexor exercise. Muscle and Nerve, 40, 617-625.

III Piitulainen H, Bottas R, Komi PV, Avela J 2009. Impaired action potential conduction at high force levels after eccentric exercise. Journal of Electromyography and Kinesiology, published online, doi:10.1016/j.jelekin.2009.10.001.

IV Piitulainen H, Holobar A, Avela J. Changes in motor unit characteristics after eccentric elbow flexor exercise. Scandinavian Journal of Medicine & Science in Sports, Submitted.

V Piitulainen H, Botter A, Merletti R, Avela J. Muscle fiber conduction velocity is more affected after eccentric than concentric exercise. European Journal of Applied Physiology, Submitted.

ABBREVIATIONS AND DEFINITIONS

AMP	Motor unit action potential peak-to-peak amplitude
AP	Sarcolemmal action potential
30MIN	30 minutes post-exercise measurement point
2H	Two hours post-exercise measurement point
2D	Two days post-exercise measurement point
4D	Four days post-exercise measurement point
BB	Biceps brachii muscle
BBM	Short head of biceps brachii muscle
BBL	Long head of biceps brachii muscle
BEF	Before exercise measurement point
CKC	Convolution kernel compensation
CoVISI	Coefficient of variation of inter spike interval
CV	Mean muscle-fibre conduction velocity
D1-1st	Validation group first measurement session at day one
D1-2nd	Validation group second measurement session at day one
D2	Validation group measurement session at day two
DHCP	Dihydropyridine receptor complex
DOMS	Delayed onset muscle soreness
EIMD	Exercise-induced muscle damage
EMG	Electromyogram
FT	Fast twitch fibres
IA	Immediately after exercise measurement point
MDR	Motor unit mean discharge rate
MNF	Mean frequency of power spectral density
MU	Motor unit
MUAP	Motor unit action potential
MVC	Maximal voluntary contraction
NMJ	Neuromuscular junction
RMS	Root mean square
pps	Pulses per second
sEMG	Surface electromyography
SNR	Signal-to-noise ratio
ST	Slow twitch fibres

CONTENTS

1 INTRODUCTION

It is commonly known that if an individual performs an unaccustomed bout of intensive exercise, he or she will experience delayed-onset muscle soreness (DOMS) or pain at the following day and lasting for several days. This phenomenon is characterized also with a prolonged loss of force production capability of the affected muscles. The muscle pain is often relieved or reduced by mild exercise or warm-up procedure, but the functionally important force deficit remains some days or weeks. The aforementioned phenomenon has been described more than a century ago by Hough (1900). However, the mechanisms behind the prolonged force deficit and muscle soreness or pain have still remained unknown. It is not even clear what is the rationale behind the evolving of the muscle pain. It is commonly associated to "micro injury" or damage of the muscle, but recent studies have suggested rather a remodelling process of the muscle tissue to meet the new demands of its mechanical environment (Yu et al. 2004). The "micro injury" theory was established in the eighties based on microscopy studies, where indeed observable changes were detected in the muscle tissue, which were then interpreted as damage (Friden et al. 1981, Lieber & Friden 1988). Therefore, it is customary to refer the aforementioned phenomenon as exercise-induced muscle damage (EIMD), although it might not be exclusively the correct expression.

Several researchers have paid attention especially to study the mechanisms behind the functionally important force deficit after the exercise-induced muscle damage. It seems that the problem is not only at the dysfunction or disruption of the contractile machinery responsible of force production in the muscle fibre, but a deficit in signal transmission of the excitable membranes of the muscle fibres may occur as well. This involves active propagation of muscle cell membrane action potential to structures responsible to activate the contractile machinery (see 2.1). The events occurring at the cellular level are challenging or currently impossible to examine *in vivo* in humans. Therefore, the role of muscle cell membrane in exercise-induced muscle damage has been examined mostly with single fibre or animal models. However, the muscle cell membrane is the outer boundary of the muscle fibre

and thus it interacts with its surrounding – the interstitial space of muscle tissue. The interstitial space volume is very limited and thus the single fibre experiments are done in environment which is dramatically different from *in vivo* conditions. One of the main aims in the current research was to overcome or compensate to this methodological constrain, with development and validation of electrophysiological measurements performed *in vivo*. This development work was done in collaboration with University of Maribor, Slovenia and Politecnico di Tornio, Italy. High-density surface electromyography was applied with novel decomposition algorithm to identify the electrophysiological properties of the muscle cell membrane at singe motor unit level. This method has been developed by Prof. Damjan Zazula and Dr. Aleš Holobar from University of Maribor. Dr. Aleš Holobar has conducted part of the development also in Politecnico di Torino. Although this method is unique and exceptionally good for non-invasive measurement of muscle cell membrane function and its adaptation in humans, *in vivo,* it has its limitations. For example, it is possible to measure the sarcolemmal part of muscle cell membrane only and the observations are limited to mean changes of all detected muscle fibres or groups of muscle fibres - the single motor units.

The present study investigated the adaptation of electrophysiological function of sarcolemma after intensive exercise models (studies II-V). The focus was on action potential propagation properties of the sarcolemma, which is one of the candidates for mechanism explaining the exercise induced prolonged loss of muscle force production. The investigations were done at a whole muscle and individual motor unit levels. For the latter purpose, development and validation of research methodology was conducted (studies I, II, IV). In addition, the aim was to examine the importance of contraction type applied in the exercise for the sarcolemmal function (study V).

2 REVIEW OF THE LITERATURE

2.1 Muscle cell membrane

Skeletal muscle cells are surrounded by excitable outer plasma membrane, which is consisted of phospholipid bilayer, imbedded with protein complexes such as ion channels (for references see Green 2004). The plasma membrane acts as boundary between intracellular space (myoplasm) and extracellular spaces. The extracellular spaces facing the plasma membrane can be further divided into interstitial space and intravascular space. The latter includes both blood and lymphatic vessels. Especially, the narrow (1 µm) interstitial space, which is located between muscle cell and intravascular space, is important for retaining excitability of the plasma membrane during exercise, since the excitability is dependent on ion concentration distribution over the plasma membrane between the myoplasm and interstitium (Sjøgaard 1996).

The plasma membrane can be divided into surface membrane, sarcolemma, and its invaginations deeper in the muscle cell referred as transverse tubular system (Edwards & Launikonis 2008). Both parts are essential for excitability and thus contractility. The contraction of muscle fibre precedes a series of events: 1) neurotransmitter mediated conversion of axonal action potential of motor neuron as end-plate potential at neuromuscular junction (NMJ) by opening of transmitter-gated Na^+-K^+ channels at sarcolemma, 2) initiation of sarcolemmal action potential (AP) by end-plate potential through opening of voltage-gated Na^+ channels, 3) conduction of sarcolemmal AP along the sarcolemma and the transverse tubular system by opening of the adjacent voltage-gated Na^+ channels 4) release of Ca^{2+} into the cytosol from the sarcoplasmic reticulum and 5) Ca^{2+} triggered myofilament interaction and consequent contraction of the muscle fibre. The sequential process from generation of the end-plate potential onwards is referred as excitation-contraction (E-C) coupling (Enoka 2001, Ruch & Patton 1965), where preceding steps must occur before the process can proceed. Therefore, impairment in any of the aforementioned steps may impair muscle fibre contractility.

2.1.1 Sarcolemma

The sarcolemma can be divided to NMJ and extrajunctional membrane (Milton & Behforouz 1995). In NMJ, where AP is generated, there is high density of voltage-gated Na^+ channels (Milton & Behforouz 1995) to enhance neuromuscular transmission. For the same reason, sarcolemma exhibits post synaptic folding in the NMJ with high density of acetylcholine receptors at the crest of the folds and high density of voltage-gated Na^+ channels in the troughs of the folds (Flucher & Daniels 1989b). NMJ junction demonstrates a large safety factor for generation of AP for both slow twitch (ST) and fast twitch (FT) fibres (Bigland-Ritchie et al. 1982). In FT fibres the neuromuscular transmission is guaranteed with higher density of Na^+ channels, although with lower synaptic area (Ellisman et al. 1976, Fahim et al. 1984) when compared to ST fibres. This structural difference is important, because the higher Na^+ channel density lowers the threshold potential for AP generation (Milton & Behforouz 1995, Ruff 1992, Ruff & Whittlesey 1993). Usually, NMJ is located at the middle region of the muscle fibre and the density voltage-gated Na^+ and thus Na^+ currents decreases rapidly away from NMJ and reaches steady background level for the extrajunctional part of the sarcolemma (Ruff & Whittlesey 1993). Anyway, the voltage-gated Na^+ channels are expressed throughout the sarcolemma (Colledge & Froehner 1998). The voltage-gated Na^+ channels overall architecture is similar to other ion channels on the sarcolemma, such as Ca2+-channels and voltage-dependent K^+-channels (for review see Marban et al. 1998).

Action potential propagation. After AP is generated at NMJ it must be propagated along the sarcolemma. In a resting state, the sarcolemmal resting transmembrane potential ranges between -60 to -80 mV, the inside of the cell being negative. This potential is maintained by active imbalance of different electrolytes across the sarcolemma, primarily Na^+, K^+ and Cl^-. During rest, the intracellular $[Na^+]_i$ and $[Cl^-]_i$ are low, while high at the extracellular side. For $[K^+]$ the opposite is true. During AP the ion concentrations are temporarily reversed. The depolarization is initiated by voltage-gated Na^+ channels and is followed with repolarization by delayed opening of the K^+ channels. With assistance of Cl^- channel opening and Na^+-K^+ pump activity the resting $[Na^+]$ and $[K^+]$ are restored and resting membrane potential is achieved. The sudden change in the local resting membrane potential on the sarcolemma then activates adjacent voltage-gated Na^+ channels. This way AP spreads from NMJ to tendon region of muscle fibre with velocity of about 2.6-5.3 m/s (Andreassen & Arendt-Nielsen 1987). To ascertain proper functioning of voltage-gated Na^+ channels and thus propagation of AP it is crucial that resting membrane potential ranges between -60 to -70 mV or lower (Stephenson et al. 1998). Otherwise, it is not possible to activate sufficient amount of voltage-gated Na^+ channels for AP propagation (Stephenson 2006) and excitability of the sarcolemma is lost. The velocity of AP can be measured with surface electromyography (sEMG) when the distances between recording sites (electrodes) remains constant (Sadoyama et al. 1985) (see 2.2.2).

Sarcolemmal excitability. During strenuous exercise or electrical stimulation the sarcolemmal excitability may be reduced, because of accumulation of K^+ in the interstitial space (Sjøgaard 1990). Sarcolemmal excitability can be defined as inward current that is required to depolarize the sarcolemma enough to reach the threshold potential that triggers sufficient amount of voltage-gated Na^+ channels to elicit an AP. Sarcolemmal excitability can be studied also by measuring the changes in amplitude of APs, e.g. during electrical stimulation. In resting conditions, FT fibres and ST fibres show more or less similar intracellular AP amplitudes (Wallinga-De Jonge et al. 1985). However, FT fibres are prone to decline in their AP amplitude in response to repetitive fatiguing electrical stimulation (Fitts & Balog 1996, Hanson 1974). It is demonstrated, that the activity of Na^+-K^+-pumps increases during muscle activity (Nielsen & Clausen 1997). This may oppose or relieve the accumulation of K^+ in the interstitial space and thus prevent or delay the loss of the sarcolemmal excitability (Nielsen & Clausen 2000). In ST fibres it has been observed, that repetitive electrical stimulation may lead even to hyperpolarisation of sarcolemmal resting potential and increase in muscle fibre AP amplitude probably due to protective increase in the activity of Na^+-K^+-pumps (Hicks & McComas 1989). The opposite was true for the responses to electrical stimulation in FT fibres (Fitts & Balog 1996, Hanson 1974). Therefore, it seems that the excitability of ST fibres is preserved much longer than in FT fibres. Sarcolemmal excitability can be studied with supramaximal electrical stimulation, where action potentials of all or most muscle fibres in the muscle are elicited simultaneously and a compound AP (M-wave) can be measured with sEMG. Since M-wave is a summation of all muscle fibre APs in the sEMG detection volume the M-wave amplitude or area represents the overall excitability of the whole muscle. Overgaard et al. (1999) showed with isolated muscle fibres, a linear relation between reduction in M-wave area and tetanic force when extracellular $[Na^+]_o$ and $[K^+]_o$ were altered resembling fatigued situation or the muscle fibres were fatigued with tetanic electrical stimulation (120 s, 30 Hz). They also noticed that increase in Na^+-K^+-pump activity, induced by β2-adrenoceptor agonist salbutamol, induced recovery of both M-wave area and tetanic force. This was associated with simultaneous recovery of the muscle fibre excitability. They concluded that loss of muscle fibre excitability is an important factor in fatigue induced by high frequency (30 Hz) stimulation. For this reason, M-wave area or amplitude may be valid indicator of muscle fibre excitability.

2.1.2 Tubular system

Huxley and Taylor (1958) were among the first ones to show evidence about structures capable to conduct sarcolemmal depolarization inwards to the muscle cells, although they suggested that this occurs passively. Later this network of structures were visualized with electron microscopy and were referred as transverse tubular system, since the tubules were orientated in transversal direction with respect the longitudinal axis of the muscle cell

(Peachey 1965) (Fig. 1). Page (1964) showed also that the transverse tubular system is directly connected to the sarcolemma and is formed of its invaginations. Soon after this, it was shown, that tubular system is excitable membrane and the process of AP propagation and generation in tubular system occurs similarly as is the case in sarcolemma (Adrian et al. 1969, Adrian & Marshall 1976, Posterino et al. 2000).

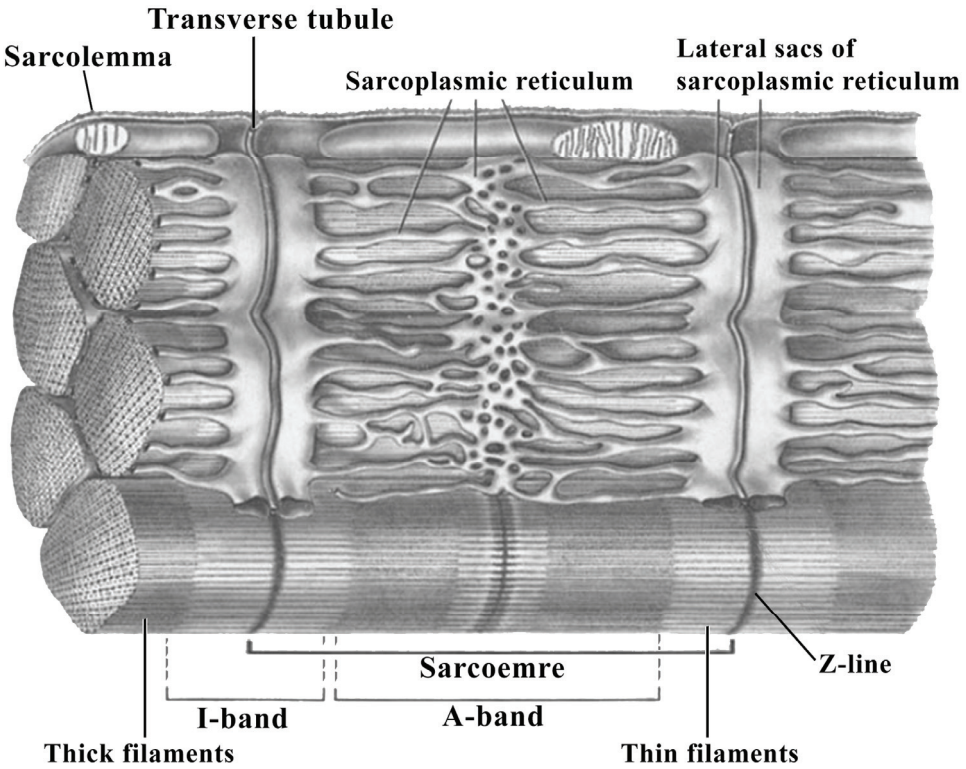

FIGURE 1 Alignment of excitable membrane structures of muscle fibre with respect to sarcomeres. Sarcolemma surrounds the myofibrils and its invaginations reach deeper to the fibre and connect with sarcoplasmic reticulum, which initiates interaction of thin and thick filament by releasing Ca^{2+} to myoplasm. Modified from Fawcett & McNutt 1969 with permission from the Journal of Cell Biology © Fawcett and McNutt, 1969. /J. Cell Biol./ 42:1--45.

The tubular system is highly organized structure as are the muscle fibres. There are two transverse tubular systems for each sarcomere located at the two A and I band junctions near the sarcoplasmic reticulum (Eisenberg 1983) (Fig. 1). Since major part of tubular system comprises of transverse tubules (Esenberg & Eisenberg 1968) less attention has been drawn towards longitudinal tubular system, which was visualized by Verratti (first published in 1902, see Veratti 1961) already in the early 20th century. The longitudinal tubules usually connect two adjacent transverse tubules, but in some cases several ones (Edwards & Launikonis 2008). Although, longitudinal tubules cover only less than 3% of total tubular system membrane area (Esenberg & Eisenberg 1968, Franzini-

Armstrong et al. 1988) it might be important structure in preventing total conduction block in the tubular system during strenuous muscular activity where propagation of AP in the transverse tubules may be impaired (Lamb 2002, Posterino et al. 2000). When part of the tubular network is blocked, the longitudinal tubules may serve as extra link between the adjacent transverse tubules.

The two main tasks of tubular systems include 1) rapid inward propagation of AP to voltage sensors, dihydropyridine receptor (DHCP) complex, imbedded into transverse tubular system membrane which then transfers the signal to the terminal cistarnae of the sarcoplasmic reticulum to release Ca^{2+} via Ca^{2+} release channels into myoplams (Melzer et al. 1995, Stephenson et al. 1998) and 2) transport of molecules in or out of the lumen of tubular system (Lannergren et al. 2000), since the tubular system is main interface between intra- and extracellular spaces. For this reason, tubular system seems to have important role in maintenance of normal membrane potential during muscular activity (Nielsen et al. 2004) and the longitudinal tubules may aid the muscle fibres to contract as fully as possible even in fatigued situation (Edwards & Launikonis 2008). It is also notable that the longitudinal tubules are the origin of swelling and formation of vacuoles in tubular system after eccentric contractions (Edwards & Launikonis 2008). Therefore, it seems that this part of tubular system takes part in transmission of force generated by the sarcomeres, and thus may be stressed especially during lengthening contractions. The swelling of longitudinal tubular system will increase its diameter and thus could help to prevent the loss of AP conduction velocity in fatigued situation (Krolenko & Lucy 2001). However, the AP conduction velocity (0.01-0.02 m/s versus 2.6-5.3 m/s) is much lower in tubular system as compared to sarcolemma mainly due to its low diameter (Andreassen & Arendt-Nielsen 1987, Posterino et al. 2000).

Total conduction block does not occur easily in tubular system. However, the conduction block is probably more likely event in tubular system than in the sarcolemma, because the ratio between the area of excitable membrane and the extracellular volume it faces is much larger for the tubular system than for the sarcolemma. Therefore, the alterations in $[K+]_o$ are more pronounced in the tubular system (Sejersted & Sjøgaard 2000). It has been shown with intact muscle preparations that marked change in extracellular $[Na^+]_o$ and $[K^+]_o$ are needed for failure of muscle fibre contraction (Cairns et al. 1997, Sejersted & Sjøgaard 2000). Therefore, it appears that even in situation where the AP amplitude in tubular system is reduced; Ca^{2+} release from sarcoplasmic reticulum will be preserved with relatively large safety factor. Furthermore, there is some evidence, although in frog muscle, to show that voltage sensor elements, DHPRs, on the tubular system membrane are not affected by fatigue (Gyorke 1993). However, the possible site for E-C coupling failure seem to involve some step(s) of the signal transmission from DHPRs to the Ca^{2+} release channels on the sarcoplasmic reticulum, since these channels are inhibited by rise in the myoplasmic $[Ca^{2+}]_i$ in FT fibres (Lamb et al. 1995). However, the exact mechanism(s) at this level still remain unclear.

During activity, intracellular K^+ is "leaking" to extracellular space. To counteract this, the activity of Na^+ - K^+ pumps in the tubular system increases markedly during exercise (Clausen 2003, Nielsen & Clausen 2000). It is estimated that increase in tubular or interstitial space [K^+] by 50% would reduce the twitch response by more than 25% and increase of 200% would be needed for major impairment in tubular system excitability (Stephenson 2006). A marked increase of K^+ applies only for strenuous exercise and fatigue models, such as high frequency electrical stimulation. In humans, it has been shown that, [K^+] in the interstitial space may increase ~2-3 folds during exercise and thus may hinder maximal muscle force production capability during fatiguing exercise (Juel et al. 2000).

2.2 Measurement of sarcolemmal function with electromyography

The sarcolemma has multiple functions, but the object of the current thesis includes only its electrophysiological function. In other words, the sarcolemmal function here is defined as the events related to AP propagation along sarcolemma from NMJ to transverse tubular system. The exact mechanism for AP propagation has been explained above (2.1.1 Sarcolemma). However, it involves the activation of adjacent voltage-gated Na^+ channels in chainlike manner, where resting membrane potential is temporarily reversed (Fig. 2). This fluctuation in potential can be measured with potentiometer, either as intra- and extracellular potential difference or as potential difference between electrodes placed above the sarcolemma along the length of muscle fibre (Fig. 2). The recording from the potentiometer is referred as electromyogram (EMG) and was first shown by Piper (1907) and Florence Buchanan (1908) (see de Barenne & Brevee 1926). These techniques are discussed in more detail below.

The *in vivo* measurements of EMG are restricted either to invasive intramuscular recordings with wire or needle electrodes or to non-invasive recordings above the muscle from the surface of the skin (Fig. 2). The latter technique is referred as surface electromyography (sEMG) and is restricted to superficial muscles only. The deeper muscles can be investigated with wire or needle electrodes. A distinct difference between intramuscular EMG and sEMG is the size of recording electrodes, which is often much smaller for the intramuscular recordings. This is beneficial technical feature especially if restricted detection volume is of interest, such as in the case of investigation of properties of individual motor units (MU). This makes the identification and discrimination of MUs much easier, since only small proportion of the total MUs active in the muscle are contributing to the recorded EMG signal (Buchthal et al. 1957). Therefore, intramuscular EMG recordings are mainly used for this purpose and have a long tradition with first recordings made by Adrian and Bronk (1929) . In sEMG recording the electrode size is much larger, since the aim of the standard sEMG recording is in most cases to observe the

compound activity of as many MUs as possible. Therefore, the sEMG recordings provide more global and presentable information about the activity of the whole muscle of interest when compared to the intramuscular EMG recordings (Holtermann et al. 2005).

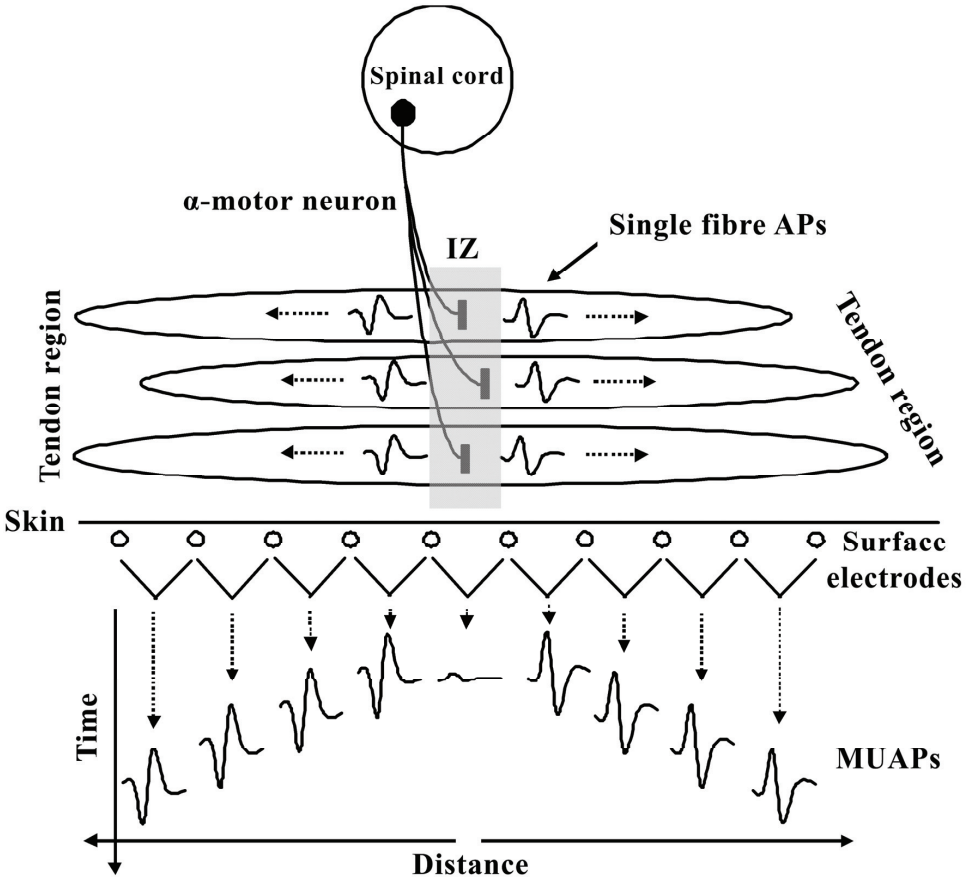

FIGURE 2 Schematic presentation of multichannel sEMG recording of propagating single fibre action potentials (APs), which form the compound motor unit action potential (MUAP). Note the low amplitude at innervation zone (IZ) region.

In addition, with sEMG it is possible to evaluate not only muscle activity, but also its morphological and anatomical features. With scanning sEMG it is possible to estimate the total number of MUs in the muscle (McComas 1998) and with high-density sEMG it is possible to measure spatial amplitude distribution of the muscle (Holtermann et al. 2005) and determine locations of innervation zones (IZ) and tendon regions. IZs corresponds to the area where individual NMJs are concentrated in the muscle, and can be located by identifying the origin of AP propagation with surface electrode arrays (Masuda & Sadoyama 1986) or grids (Masuda & Sadoyama 1988) (Fig. 2). IZ location

varies between different muscles (Rainoldi et al. 2004) and individuals (Masuda et al. 1985). Furthermore, Masuda et al. (1985, 1988) have reported multiple IZs in some muscles.

Masuda et al. (1985) have proposed recommendation that IZ should be located before placing the standard bipolar sEMG electrodes. It is also mentioned in European recommendations for sEMG (Hermens et al. 1999) that, standard bipolar sEMG electrodes should not be placed over IZ, because the recorded signals would be affected by two directional propagation patterns resulting in cancellation (Farina et al. 2001, Merletti et al. 1999, Mesin et al. 2008, Rainoldi et al. 2004, Sadoyama et al. 1985). In the case of high-density sEMG recordings the channels belonging to IZ or tendon region can be removed during analysis.

Another advance of high-density sEMG is the possibility to obtain the properties of sarcolemmal AP propagation, since multichannel surface electromyography systems enable the calculation of mean muscle fibre conduction velocity (CV) (Masuda & Sadoyama 1986). CV can be estimated in global level for whole muscle or part of it with simple electrode arrays placed over the muscle in the longitudinal direction of its muscle fibres (Masuda & Sadoyama 1986). With high-density sEMG it is possible also to estimate CV for individual MUs (Merletti et al. 2008). Similarly, other global muscle or individual MU level electrophysiological parameters can be calculated both in time (e.g. root mean square (RMS) amplitude of sEMG) and frequency domain (e.g. mean power frequency of sEMG signal power spectrum).

2.3 Extracting neural strategies from electromyography

Compound AP of the muscle fibres belonging to single individual MU can be referred as motor unit action potential (MUAP) (Fig. 2). Identification of MUAPs from EMG provides information about motor output of the central nervous system and especially from final common pathway rising from alpha-motor neuron pool located at ventral horn of spinal cord for most muscles and from brainstem (cranial nerves) for muscles of head and neck. For each firing of the alpha-motor neuron there is single MUAP generated in the muscle. In other words, the alpha-motor neuron functions with all or nothing principle (Kandel et al. 2000). Therefore, identification of individual MUAPs provides indirectly the information when alpha-motor neurons are activated.

As mentioned above, the traditional method to identify MUAPs is the use of intramuscular EMG recordings (Adrian & Bronk 1929, LeFever & De Luca 1982, LeFever et al. 1982). Furthermore, the methods for the decomposition of intramuscular EMG to individual MUAPs have been validated by several researchers and thus considered accurate and reliable tools for the physiological investigation of MUs (Stashuk 2001). With needle or wire electrodes, it is possible to record EMG in close vicinity of the source, whereas in the case of

sEMG the distance to the source is much longer. Recording at the proximity to the source is advantageous, since the tissues between the recording site and source can be considered volume conductor and functions as low-pass filter whose properties depends of type of tissue (Merletti et al. 2009) and distance between the source and recording electrodes (Lindstrom & Magnusson 1977). In general, the less conductive the tissue and the greater the distance between the source and recording electrodes, the greater the low pass filter effect on EMG. This will result as decrease in the selectivity of the EMG recordings, since the individual features of different MUAPs are filtered out at some degree (Dimitrov & Dimitrova 1974, Merletti et al. 1999).

In addition, the selectivity of the EMG recordings depends on number of observed sources, MUAPs (Merletti et al. 1999, Stegeman et al. 2000). In intramuscular EMG this number is considerably smaller due to lower electrode size and thus detection volume, when compared to sEMG. In such situation the MUAPs rising from the nearest muscle fibres dominate EMG. However, even in the case of intramuscular EMG, the measurements are often restricted to low contraction forces, typically at or below 30% of maximal voluntary contraction (MVC) force (Dartnall et al. 2009, Hedayatpour et al. 2009, Merletti et al. 2008). Since the detection volume is considerably larger in sEMG than in intramuscular EMG, the decomposition of sEMG signals to individual MUAPs is even more challenging task and interpretations of its results need to be made with extra caution (Farina et al. 2004). Despite of the difficulty of the task, several authors have developed methodology to identify MUAPs from sEMG (De Luca et al. 2006, Gazzoni et al. 2004, Hogrel 2003, Holobar & Zazula 2004, Kleine et al. 2007, Kleine et al. 2008, Rau et al. 1997, Zazula & Holobar 2005). There are still some benefits to use sEMG signals. When high-density (multichannel) sEMG is applied, it is possible to obtain not only firing patterns of individual MUs, but also electrophysiological properties of the identified MUs, such as MUAP amplitude and CV (Merletti et al. 2008).

2.3.1 Decomposition of high-density sEMG signals

Despite the limitations introduced above, the high-density sEMG systems have potential to extend the applicability of sEMG systems. One of the most significant features is the decomposition of high-density sEMG signals to its principal components – the individual MUAPs (Merletti et al. 2008). This allows investigation of motor control and its adaptation in more detail than previously.

At present, it seems that it is possible to indentify larger number of MUs from high-density sEMG (currently up to 20 MUs per contraction) than from intramuscular EMG (usually limited to few MUs only) (Holobar et al. 2009, Merletti et al. 2008). However, development of intramuscular EMG recordings are progressing also towards multichannel techniques (Farina et al. 2008). The advantage of multichannel EMG systems is the possibility to "observe" the muscle from multiple locations. This increases the total information of the MU activity and thus aids the discrimination of individual MUAPs (Zwarts et al. 2004). This aspect is referred as spatial sampling, where the spatially related

issues, such as location of IZs or MUAP conduction velocity can provide further information for the discrimination of MUAPs. Spatial filtering is another mean to increase selectivity of multichannel sEMG system, since use of certain physical organization of electrodes, such as Laplacian filter, can decrease the low-pass filtering effect due to subcutaneous tissue layer (Rau et al. 1997, Reucher et al. 1987).

In the current thesis a Convolution Kernel Compensation (CKC) algorithm for decomposition of individual MUAPs from high-density sEMG was applied (Fig. 3) (Holobar & Zazula 2007). The recent developments of CKC algorithm have made it possible to extract discharge characteristics of individual MUs at relatively high force levels. This method is developed by Holobar and Zazula (2007) and it has been experimentally validated against intramuscular EMG recordings (Holobar et al. 2009). With this methodology it is possible to assess CV of individual MUs (McGill & Dorfman 1984, Merletti et al. 2008), and thus extract information about sarcolemmal AP propagation of its muscle fibres.

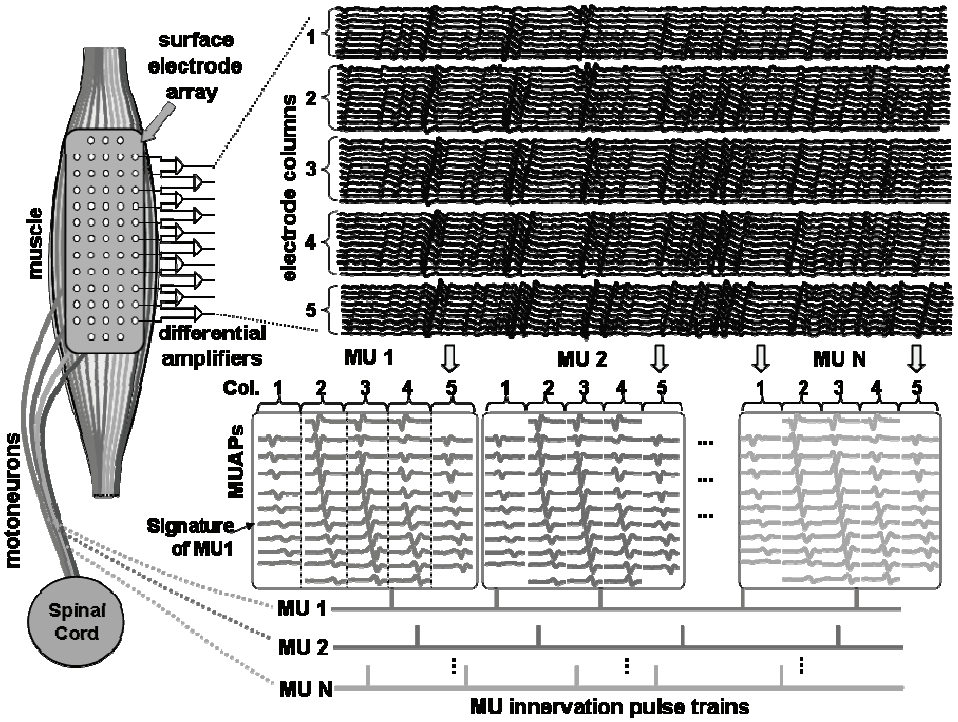

FIGURE 3 Principle of sEMG generation, acquisition and decomposition. In ideal conditions, the measured sEMG signals (upper right plot) can be decomposed into constituent MUAPs (central plot) and MU innervation pulse trains (lower plot). The innervation pulse trains carry information about discharges of corresponding alpha-motor neurons and can be used for investigations of central nervous system control strategies. From Merletti et al. 2008, with permission from Elsevier.

CKC decomposition technique belongs to the class of blind source separation techniques and is inherently capable of resolving MUAP superimpositions

(Holobar & Zazula 2004, Holobar & Zazula 2007). It does not rely on estimation of any morphological MUAP property, nor does it assume any particular MUAP shape. Instead, it focuses strictly on the properties of the innervation pulse trains, whereas the information about MUAPs is cancelled out during the decomposition process. This is not a serious limitation, because the MUAP shapes can always be estimated by averaging the measurements in the vicinity of the reconstructed innervation pulses, by using spike triggered averaging technique (Holobar et al. 2009).

CKC method has numerous advantages when compared to classical sEMG decomposition techniques. Firstly, it automatically resolves the MUAP superimpositions. Secondly, it is unbiased and has the minimal error variance of all the linear innervation pulse trains estimators (Holobar & Zazula 2004). Thirdly, it implicitly combines all the available information provided by all the measurements, what makes it an appealing candidate for the decomposition of high-density sEMG. Finally, by compensating the shapes of detected MUAPs, it directly estimates the innervation pulse trains (without reconstructing the detected MUAP shapes). See the further details from Holobar & Zazula (2004) and Holobar & Zazula (2007).

2.4 Exercise-induced muscle damage

Most if not all people are familiar with delayed onset muscle soreness (DOMS) and sensation of muscle stiffness some time after unaccustomed vigorous muscular activity, such as exercise or various work related tasks. DOMS was first introduced by Hough (1900). He also showed that if the exercise is repeated daily for some weeks, DOMS was gradually reduced. This protective effect of previous exercise is nowadays known as repeated bout effect (McHugh 2003). DOMS begins usually the day after exercise and peaks at about 48 hours post-exercise (Armstrong 1984, Newham et al. 1983b). It is usually most severe after exercise where the contracting muscle is forcibly lengthened (Proske & Morgan 2001). This type of muscle action is referred as eccentric contraction, whereas its opposite action is referred as concentric contraction. An example of eccentric exercise would be downhill running, descent of stairs or lowering a weight from table to floor. When studied with microscopy, the aforementioned and other types of eccentric loadings or exercises have been shown to cause observable changes in muscle tissue that has been interpreted as damage (Friden et al. 1981, Lieber & Friden 1988). Therefore, the phenomenon discussed in this chapter, has been referred as exercise-induced muscle damage (EIMD).

2.4.1 Symptoms and functional consequences

EIMD is characterized by delayed muscle pain or soreness (Armstrong 1984, Aasmussen 1956, Jones et al. 1989, Komi & Buskirk 1972, Newham et al. 1983b, Newham et al. 1987), increased joint stiffness (Stauber et al. 1990), muscle

swelling (Brendstrup 1962, Clarkson et al. 1992a) and inflammation (Fielding et al. 1993). However, there is no certainty if inflammation really exists after eccentric exercise due to methodological ambivalences (Malm 2001). The increased joint stiffness here refers to passive joint stiffness often described by resting arm angle of standing subject (Clarkson & Tremblay 1988) or passive resistance of joint primarily at the end of its range of motion (Howell et al. 1993, Jones et al. 1987). The joint stiffness can increase rapidly after eccentric exercise and remains elevated for days, whereas the muscle swelling tends to increase gradually and peak 3-4 days post-exercise (Chleboun et al. 1998). Therefore, the muscle swelling can not fully explain the increase in passive joint stiffness during first 48 hours post-exercise (Chleboun et al. 1998). Interestingly, the time course of the symptoms of EIMD: muscle pain, muscle swelling, joint stiffness and force production capability, can be different, although all of them persist for days (Howell et al. 1993).

The most important functional consequence related to EIMD is a prolonged reduction in maximal muscle force production (Davies & White 1981, Komi & Viitasalo 1977, Newham et al. 1987). Depending on exercise model and individual subject, the reduction in maximal force production can last in most severe cases up to 5-6 weeks (Howell et al. 1993). However, in most cases the recovery occurs in about a week (Clarkson et al. 1992b, Linnamo et al. 2000a). In addition, Katz (1939) observed in a classical experiment, that if isolated muscle is suddenly stretched during its activity, it becomes weaker and its optimum length in force-velocity curve shifts to longer lengths. Later Wood et al. (1993) confirmed these results with isolated muscle and Jones et al. (1997) observed a similar shift in the optimal joint angle for torque generation in humans after eccentric exercise.

Another functional consequence related to EIMD is a reduction in voluntary activation level (VAL) (Prasartwuth et al. 2005), which will naturally contribute to the reduction in maximal voluntary muscle force production. The measurement of VAL is based on principle introduced by Merton (1954), where maximal voluntary contraction is superimposed by brief (usually singe or double pulse) supramaximal electrical stimulation of muscle or the nerve innervating it. If the superimposed electrical stimulation induces increase in the force output of the investigated muscle, in other words observable twitch in force recording, VAL has not been maximal and is interpreted as a neural deficit. The superimposed twitch amplitude is usually normalized to resting (no background activity) twitch amplitude, which represents the maximal possible twitch force for the applied supramaximal electrical stimulation (Babault et al. 2001, Herbert & Gandevia 1999). There is evidence that neural deficit developed during muscle fatigue (usually referred as central fatigue) may occur either in cortical or spinal level after eccentric exercise (Prasartwuth et al. 2005).

There is some, but limited information available of the effect of EIMD on function or behaviour of single MUs, but unfortunately these studies have been limited to very low contraction levels only (≤ 26% of MVC) due to methodological limitations. However, Dartnall et al. (2008, 2009) have shown that after eccentric exercise, performed at submaximal eccentric force levels and

in continuous manner, may influence the control of MUs in BB muscle. They showed that MU synchronization and coherence were increased and mean MU recruitment threshold was reduced 24 hours post-exercise, while MU discharge rate variability and MU minimum discharge rate remained unchanged. Even though their observations were limited to low contraction levels, these results suggest that control of MUs may change towards more correlated and thus synchronized MU activity to counteract the fatigued situation in submaximal contraction levels.

2.4.2 Importance of contraction type

It has been shown that symptoms of EIMD are less pronounced after exercise which does not include eccentric contractions. For example, DOMS is not as high after concentric exercise when compared to eccentric one (Jones et al. 1989, Newham et al. 1983b). The same is true also for loss of muscle force production, which is greater both in magnitude and duration after eccentric exercise than after concentric one (Gibala et al. 1995a). In addition, there are less morphological changes visible in the muscle fibres after concentric exercise when compared to eccentric one (Gibala et al. 1995a, Jones et al. 1989, Newham et al. 1983a, Smith & Newham 2007). Moreover, it is know that single bout of eccentric exercise can diminish the symptoms of EIMD of the second bout of eccentric exercise performed some time after the first one (McHugh 2003). Interestingly, concentric exercise does not induce the protective repeated bout effect for eccentric exercise (Whitehead et al. 1998). It seems that the stretching of either active or passive muscle tissue is needed for repeated bout effect to occur, at least in mice, since passive stretching of muscle *in situ* has been show to prevent EIMD (Koh & Brooks 2001). The susceptibility of the muscle to eccentric exercise is often explained with higher mechanical stress to active muscle fibres during the eccentric contractions with respect to the concentric contractions (McHugh et al. 1999). It seems that there is lower neural drive to muscle and thus less MU activity during maximal eccentric contractions than concentric contractions (Westing et al. 1991), despite the fact that eccentric contractions often show higher maximal force values (Katz 1939, Westing et al. 1991).

Intensity of exercise affects the severity of EIMD. Nosaka & Newton (2002a) showed that maximal eccentric exercise causes greater symptoms of EIMD than submaximal one. However, the velocity of the eccentric contractions does not seem to be so important factor (Brooks & Faulkner 2001a), although Warren et al. (1993b) have observed that both higher contraction velocity and longer initial muscle length may be related to greater loss of muscle force production in isolated rat muscles.

2.4.3 Morphological findings

Morphological changes have been observed in muscle fibres after eccentric loading. These findings are mainly from animal experiments, but similar findings have been observed also in some human studies. There are several sites in muscle fibres where the morphological changes related to EIMD have been observed, such as 1) disruption and disorganization of sarcomeres (Brooks et al. 1995, Friden et al. 1981, Friden et al. 1983, Gibala et al. 1995a, Lieber et al. 1991, Newham et al. 1983a, Roth et al. 1999, Thompson et al. 1999, Vijayan et al. 2001), 2) disruption of the sarcolemma (Jones et al. 1986a, Lieber & Friden 1988, McNeil & Khakee 1992) and 3) swelling or disorganization of transverse tubular system (Takekura et al. 2001b). The trigger behind the observed morphological changes after eccentric loading is dependent of the mechanical stretching of the affected muscle fibres, but the damage of the cell structures is not necessarily purely mechanical in nature. Instead, increased proteolytic enzyme activity has been observed after eccentric loading of muscle (Belcastro 1993, Cannon et al. 1991, Salminen & Vihko 1981, Thompson & Scordilis 1994). Therefore, the actual initial "damaging" effect may occur through a chemical process (Belcastro et al. 1998).

Several different types of changes have been observed in the sarcomeres after eccentric loading. Roth et al. (1999) observed in human subjects a complete disruption of single or many adjacent sarcomeres. Some studies have shown somewhat milder damage or disorganization of sarcomeres with Z-line streaming and smearing, loss of some Z-lines, extension of Z-lines into adjoining A-bands and loss of A-bands (Friden & Lieber 1992, Newham et al. 1983a, Stupka et al. 2001, Thompson et al. 1999). The functional consequence of disruption of some of the sarcomeres after eccentric exercise is not clear. However, it may be that it is not very significant, since Yu et al. (2004) have noticed that there is not much sarcomere disruption present at the early (1 hour post-exercise) stage of EIMD. However, at later stages (2-8 days post-exercise) the amorphous sarcomeres start to appear. This suggests that the sarcomere alterations, considered to be sign of EIMD, may rather be associated with an adaptive remodelling of the myofibrils in the muscle fibres.

The morphological evidence suggests that FT fibres are more prone to disruption of their sarcolemma (Jones et al. 1986a, Lieber & Friden 1988) than ST fibres. The disruption of sarcolemma increases its permeability and thus causes muscle protein, such as creatine kinase and myoglobin, efflux to circulation (Clarkson et al. 1986, Jones et al. 1986b, McNeil & Khakee 1992, Newham et al. 1983b, Schwane et al. 1983, Warren et al. 1995) while exogenous markers enter the muscle fibres (McNeil & Khakee 1992, Warren et al. 1995). Furthermore, the increase in permeability of the sarcolemma seems to be higher in FT fibres than in ST fibres as evidenced by efflux of lactate dehydrogenase after eccentric loading in mice (Warren et al. 1994). The disruption of sarcolemma is associated with disruption of force-bearing proteins, such as subsarcolemmal dystrophin (Komulainen et al. 1998) and intermediate filament desmin (Barash et al. 2002, Friden & Lieber 1998). It is important to note that the

sarcolemma itself is an active force transmitter (Street & Ramsey 1965). Furthermore, Yeung et al. (2002b) noticed that pH regulation is distorted acutely after eccentric loading. They suggested that their finding indicates some loss of sarcolemmal function. In addition, McBride et al. (2000) showed acute and relatively long lasting (> 24 hours) reduction in resting membrane potential after eccentric loading due to opening of stretch-activated Na^+ ion channels. These findings suggest that the morphological changes in sarcolemma after eccentric exercise may lead to impairment of sarcolemmal function at least acutely after the exercise.

Vacuoles of tubular system appear approximately 1 hour after eccentric contractions possibly due to rupture of tubular system, because of increased osmotic load caused by leaking of Na^+ to tubular system (Yeung et al. 2002a). The vacuoles seem to appear predominantly in longitudinal tubular system, possibly due to its force transmitting role (Edwards & Launikonis 2008). Furthermore, the FT fibres are more prone to disorganization of transverse tubular system than the ST fibres (Takekura et al. 2001a). There is evidence that sarcoplasmic reticulum function is not impaired, at least not in the early phase (< 24 hours post-exercise) following eccentric loading, since Ca^{2+} uptake and Ca^{2+} release in sarcoplasmic reticulum was only minimally affected (Ingalls et al. 1998, Warren et al. 1999). For this reason, Warren et al. (1999) have suggested that the site of E-C coupling failure must lie above the level of the sarcoplasmic reticulum Ca^{2+} release channel in the EC coupling pathway.

2.4.4 Effect on sarcolemmal functioning

As mentioned above, especially eccentric exercise is likely to cause EIMD which is characterised with disruption of the sarcolemma occurring primarily in FT fibres (Jones et al. 1986a, Lieber & Friden 1988). However, it is not clear how this is reflected to the function of sarcolemma and especially its AP propagation, which is a crucial event for proper muscle force production. However, the sarcolemmal dysfunction is one of the possible candidates for neuromuscular fatigue among with the events of muscle activation occurring before and beyond the sarcolemma (Fig. 4). This is true especially in FT fibres, since reduction of sarcolemmal AP amplitude may cause reduction in force production (Fuglevand 1995). The sarcolemmal function has been studied in animal models and some attempts have been done also with human subjects. Some animal studies have shown reduction in resting membrane potential after eccentric loading (McBride et al. 2000), but some does not (Warren et al. 1993). Furthermore, strenuous eccentric animal models using tetanic electrical stimulation have not been able to show a major dysfunction in the sarcolemmal function after eccentric contractions based on RMS amplitude of EMG or its power spectral parameters (Warren et al. 1999) or sarcolemmal AP conduction velocity (Kano et al. 2008). Although Kano et al. (2008) did show a clear disturbance of AP waveforms in the presence of extensive muscle damage, conduction velocity remained intact. However, they only measured three days

post-exercise, and thus any acute reductions in the conduction velocity could not be detected.

In humans, there is some evidence of acute impairment of gross sarcolemmal function after eccentric exercise when studied with tetanic electrical stimulation (Piitulainen et al. 2008). Furthermore, acute (≤ 2 hours) reductions of mean frequency of power spectral density (MNF) (Linnamo et al. 2000a, Sbriccoli et al. 2001) has been detected after eccentric exercise when measured during maximal voluntary and electrically evoked contractions. In some cases, a reduction of MNF has been observed during MVC tests also after maximal concentric exercise (Linnamo et al. 2000a). In case of RMS, the previous experiments have shown either an acute reduction (< 1 day) in RMS (Hortobagyi et al. 1998, Michaut et al. 2002, Piitulainen et al. 2008), a more prolonged reduction (≥ 1 day) in RMS (Hortobagyi et al. 1998, Kroon & Naeije 1991), or no reduction in RMS (Chen 2003) after maximal eccentric exercise. There is some information available by Hedayatpour et al. (2009) about the effect of eccentric exercise on sarcolemmal AP propagation in vastus lateralis muscle, although their observations were limited to low contraction forces only (≤ 30% of MVC). They showed reduction in mean muscle fibre AP conduction velocity (CV) at 30% of MVC level in distal part of vastus lateralis muscle both 24 and 48 hour post-exercise, while proximal part of the muscle remained unaffected. Furthermore, they did not observe any changes at 10% of MVC level.

Although, sEMG has been widely used to study neuromuscular fatigue in eccentric exercise protocols leading to EIMD in humans, the results, and thus conclusions, have not always been consistent. These contradictory findings can be partly explained by differences in a) type, velocity and range of motion of the muscle contraction (Brooks & Faulkner 2001b, Gibala et al. 1995b, Talbot & Morgan 1998), b) intensity and volume of the exercise (Hunter & Faulkner 1997, Nosaka & Newton 2002b, Warren et al. 1993a), c) duration of resting periods during the exercise, d) muscle group and e) exercise history and gender of the subject group (Clarkson et al. 1992b, Stupka et al. 2000). Furthermore, dispersion of the results may be due to methodological issues related to sEMG measurements, such as a) positioning of sEMG electrodes with respect to the tendon regions and IZs (Farina et al. 2001, Hogrel et al. 1998, Rainoldi et al. 2000, Rainoldi et al. 2004, Roy et al. 1986, Sadoyama et al. 1985), b) inclination of sEMG electrodes with respect to the muscle fibres (Farina et al. 2002) and c) reproducibility of sEMG electrode placement from one measurement session to another. In addition, recent experiments have shown that different parts of a muscle can be affected more than others by EIMD (see e.g. Hedayatpour et al. 2008). For these reasons, and due the lack of experiments investigating sarcolemmal AP propagation after eccentric exercise, the effect of EIMD to sarcolemmal function remains still unclear.

3 PURPOSE OF THE STUDY

There are several possible sites which could explain the prolonged impairment of force production after intensive exercise (Fig. 4). The main purpose of the present research was to investigate the effects of intensive exercise on sarcolemmal function. In other words, sarcolemmal AP propagation properties were studied in whole muscle and individual MU level before and after exercise known to cause symptoms of EIMD. The aim was also to examine the importance of contraction type applied in the exercise for the sarcolemmal function. To achieve the aims, development work of novel sEMG methodology and its validation was required and thus constituted a significant part of the current research.

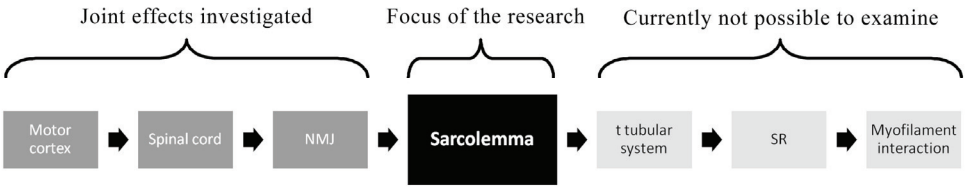

FIGURE 4 Possible sites for impairment of central and E-C coupling processes in EIMD. In humans it is possible to investigate the first four steps. The first three steps were all measured but could not be separated in the current research, while the main focus was on the sarcolemma.

The specific aims of the present studies were as follows:

1) To investigate repeatability of the applied high-density sEMG system for the extracted sEMG variables and for determination of innervation zone (IZ) location. In addition, the effect of stability of IZ and sEMG variables measured near IZ with increasing isometric contraction levels were determined from short head of biceps brachii muscle (BBM). (Original paper I).

2) To investigate whether electrode location with respect to the muscle fibres has an effect on the detection of exercise-induced changes in the time and

frequency domains of sEMG variables in the BBM muscle after intensive eccentric elbow flexor exercise in humans. In addition, the aim was to assess the extent to which the anatomical location of IZ(s) can affect sEMG variables after exercise. (Original paper II).

3) To extend investigation of the effect of maximal eccentric elbow flexor exercise on discharge rate and AP propagation of individual MUs up to relatively high contraction levels: 75% of maximal voluntary force. In addition, consistency analysis of the employed techniques for extraction of individual MU characteristics was also conducted on both synthetic sEMG and experimental sEMG signals from a validation group of healthy subjects. (Original paper IV).

4) To investigate whether sarcolemmal function is impaired in BBM muscle after intensive eccentric elbow flexor exercise both in whole muscle and individual MU level. An additional aim was to determine whether this impairment occurs across a wide range of isometric force levels and how it is recovered up to four days post-exercise. (Original papers III and IV).

5) To investigate whether the acute impairment of sarcolemmal function is limited only to repeated eccentric contractions or if exercise with concentric contractions will result in similar effects on the sarcolemmal AP propagation and sarcolemmal excitability. This was investigated both in voluntary and electrically evoked conditions for the BBM muscle. (Original paper V).

4 RESEARCH METHODS

4.1 Subjects

The study involved voluntary young adult male subjects. The subjects were recruited for the experiment by advertising in University of Jyväskylä notice boards and through email lists. Descriptive characteristics of the subjects are presented in table 1.

TABLE 1 Descriptive statistics (mean ± standard deviation).

Orginal paper	Groups	Age (years)	Weight (kg)	Height (cm)	Subcutaneous tissue (mm)
I	N=16	25,7 ± 3,4	73,0 ± 6,4	178 ± 5,1	2,22 ± 0,5
II, III, IV	ECC (N=9)	28,4 ± 5,5	77,1 ± 12	180 ± 5,4	3,43 ± 1,4
	VL (N=7)	25,4 ± 3,3	74,2 ± 4,7	178 ± 6,4	2,7 ± 0,6
V	CON (N=12)	27,2 ± 4,2	78,9 ± 14,0	183 ± 6,5	2,39 ± 1,1
	ECC (N=12)	27,8 ± 3,8	80,2 ± 11,6	183 ± 6,6	2,52 ± 1,0

ECC = eccentric exercise group, VL = validation group, CON = concentric exercise group.

No subject had any known symptoms of neuromuscular disorders. The subjects were right handed, non-smokers, did not drink caffeine rich drinks twelve hours before the measurements, and avoided strenuous exercise two days before the first measurement and throughout the experimental periods. The experiments were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä. All participants were aware of the possible risks and discomfort of the experiments, and signed a written informed consent form before inclusion. The subjects were aware that they were free to retreat from the measurements without justification at any point. Any subjects with multiple main IZs were excluded from further analysis to avoid misinterpretations in determination of IZ location and its shift, which could have affected also the calculation of the applied sEMG variables.

4.2 Experimental design

The study consisted of three principal experiments. The first one was conducted to strengthen and test reliability of the currently applied novel methodology (I, II, IV). The second experiment was designed to study responses of maximal eccentric exercise on sarcolemmal function in global and single MU level (II, IV). The last experiment was performed to study acute responses to both maximal eccentric and maximal concentric exercises (V). See the detailed experimental designs below.

4.2.1 Validation of the methodology (I, II, IV)

Innervation zone location. The measurements consisted of two sessions of identical measurements on two different days. The two sessions were separated by 2.8 ± 2.7 days (mean ± standard deviation).

In both sessions the MVC was first determined in the elbow flexors of the dominant arm at an elbow angle of 120° (180° corresponds to full extension) for each subject. The subjects then performed intermittent submaximal voluntary isometric contractions in the following order, at 10%, 20%, 30%, 40%, 50% and 75% of MVC (duration of 8 s each). Between each contraction, subjects were allowed a minimum of two minutes rest, to minimize the possibility of metabolic fatigue in the muscle.

All isometric contractions were performed twice in both days in order to analyze trial-to-trial reliability. Day-to-day reliability was assessed by comparing two contractions performed in different days. The warm-up protocol before both sessions consisted of three sets of submaximal isometric elbow flexions lasting 8 s each.

Decomposition of high-density sEMG signals. The consistency of the applied CKC decomposition algorithm has been proved to be consistent in lower isometric contraction levels (Holobar et al. 2009). However, this was the first time to apply the CKC algorithm in signals recorded at relatively high isometric contraction levels. Therefore, its day-to-day consistency was tested with experimental high-density sEMG signals measured from a separate validation (VL) group and with set of simulated synthetic EMG signals (see below).

The validation subjects were measured on three different sessions of identical measurements. Twice at day one (D1-1st and D1-2nd, separated by ~2 hours) and once at day two (D2). The measurements on different days were separated by 3.0 ± 3.4 days (mean ± standard deviation). In all sessions, MVC force was first determined for the elbow flexors of the dominant arm. Subjects then performed intermittent submaximal voluntary isometric contractions in the following order: 10%, 20%, 30%, 40%, 50% and 75% of MVC. This fixed order was selected to avoid the effect of muscle fatigue from the highest contraction levels on the lower contraction levels.

Simulation of synthetic high-density EMG signals. In the first step, capability of CKC algorithm to accurately extract the mean discharge rate (MDR) of individual MUs at higher isometric contraction levels was systematically tested on an extensive set of synthetic EMG signals (in total, 20 virtual muscles were simulated). The simulations were based on a model of recruitment of a population of MUs (Fuglevand et al. 1993) and a multilayer cylindrical volume conductor model (Farina et al. 2004). The volume conductor (Farina et al. 2004)comprised four layers (bone, muscle, fat, skin). Limb and bone radius were set equal to 50 mm and 25 mm, respectively. Thickness of skin and subcutaneous layer were 1 mm and 3 mm, respectively. A muscle with elliptical cross-section of 20 mm (transversal) × 8 mm (depth) was simulated (Fig. 5).

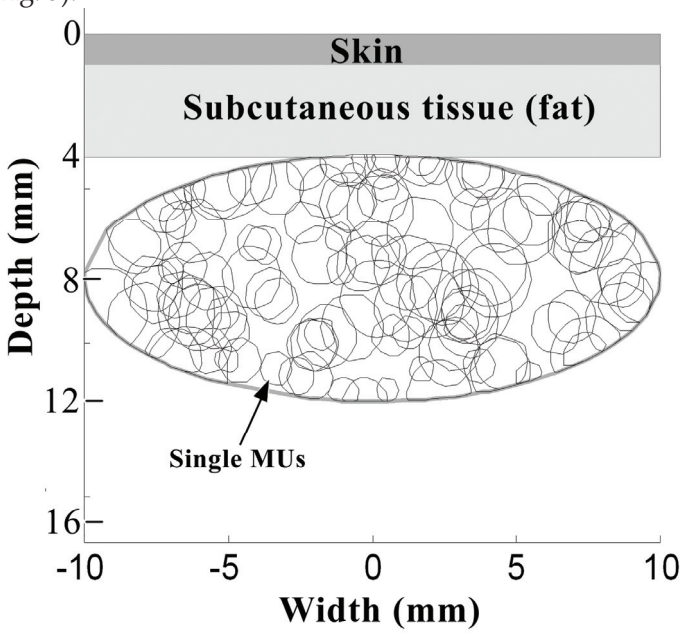

FIGURE 5 Basic structure of the modelled muscle showing the random distribution of the 120 individual MUs (grey circles). The synthetic sEMG signals were simulated similarly as were the experimental high-density sEMG measured (from skin surface with electrode grid of 5 columns x 13 rows).

The MU properties across the active MU pool were distributed according to the size principle (Henneman 1957), and included innervation number of 50- 400 fibres per MU, recruitment thresholds with range between 0% - 80% of maximal excitation level (Kukulka & Clamann 1981) and normally distributed conduction velocity values of MUAPs with mean of 4 ± 0.3 m/s. The muscle comprised a total of 120 MUs (McComas 1998, Miller et al. 1993) randomly distributed within its cross-section with a density of 20 fibres/mm^2 (Armstrong et al. 1988), and interdigitated with fibres belonging to many other units to yield a muscle fibre density of 200 fibres/mm^2 (Miller et al. 1993). Average fibre length was 130 mm with 10 mm spread of innervation and tendon zones.

Five different profiles of excitation level to the muscle were tested in the simulated 10-s long contractions: constant excitation levels at 20%, 30%, 40%, 50% and 75% of MVC. The distribution of recruitment thresholds for the motor neurons was modelled with an exponential function with many low-threshold neurons and progressively fewer high-threshold neurons, as described by (Fuglevand et al. 1993). The number of MUs active at 20% (30%, 40%, 50%, 75%) excitation level was 82 (93, 101, 107, 118) out of 120. Each MU discharged at 8 pps, once excitation exceeded the assigned recruitment threshold, and discharge rates increased linearly (0.3 pps/%) with excitation. The peak discharge rate was 35 pps for all simulated MUs. The last unit was recruited at 80% of maximal excitation (Kukulka & Clamann 1981). Discharge rate variability was modelled as a Gaussian random process with coefficient of variation of the interspike interval equal to 20%.

The MUAPs have been simulated as detected using a bidimensional detection system of 65 electrodes (5 columns, 13 rows) with radius of 1 mm and interelectrode distance of 8 mm. A bipolar recording was simulated for each longitudinal pair of adjacent electrodes, thus leading to 60 simulated detection points. The centre of the grid was over the centre of the muscle in the longitudinal and transverse direction. The surface-recorded MU potential was the sum of the APs of the muscle fibres belonging to the MU. EMG signals of length of 10 s were computed at 2048 samples/s. Coloured zero-mean Gaussian noise, with signal-to-noise ratio (SNR) 0 - 20 dB (5 dB increments) and bandwidth 20 - 450 Hz was added to the simulated recordings. Simulations were repeated over 20 Monte Carlo runs.

4.2.2 Prolonged responses to eccentric exercise (III, IV)

Experimental protocol. The experiment consisted of four sessions of identical measurements on four different days. Each session involved measurements of muscle soreness, muscle and subcutaneous tissue thickness, isometric MVC, isometric submaximal voluntary contractions, sEMG during the voluntary isometric contractions, passive twitch, superimposed twitch, and maximal M-wave. The subjects were measured before (BEF) the maximal eccentric elbow flexor exercise, and the follow-up measurements were performed two hours (2H), 2 days (2D) and 4 days (4D) after the exercise. The subjects were not measured immediately after the exercise, because skin temperature over the biceps brachii muscle was on average 1.8 ± 1.1 C° higher immediately after the exercise than BEF ($p < 0.05$). Increase in muscle temperature could cause bias to the applied sEMG variables (Stalberg 1966).

In all four sessions, muscle soreness and muscle and subcutaneous tissue thickness were always measured prior to the force measurements. MVC force was then determined for the elbow flexors of the dominant arm. Subjects then performed intermittent submaximal voluntary isometric contractions in the following order: 10%, 20%, 30%, 40%, 50% and 75% of MVC. The twitch responses were then measured after the submaximal contractions.

Exercise protocol. Subjects performed one set of 50 maximal eccentric contractions with the elbow flexors of the right arm, on a motorised isokinetic dynamometer (angular velocity of 1 rad/s) (Komi et al. 2000). Each contraction was divided into two phases. The first phase was from 65° to 120° and the second phase continued from 120° to 175° (180° corresponds to full extension). Thus, the full range of motion at the elbow joint was 110° (from 65° to 175°). The maximal eccentric contractions were performed with twenty second intervals and with maximal isometric pre-activation starting one second prior to the onset of the eccentric movement in both phases. The two phases were separated by three seconds. This form of eccentric contraction was chosen because previous evidence suggests that it may be difficult to maintain full activation throughout the range of the eccentric movement (Linnamo et al. 2006). Thus, the incorporation of two phases enabled higher muscle activation during each individual phase.

The half-supinated right forearm was attached to a strain gauge transducer, which was fixed to the horizontal lever arm of the dynamometer to record the force applied by the elbow flexors. While the lever arm returned to the initial position (at an angular velocity of 1 rad/s), subjects were instructed to relax their arm muscles. The exercise lasted for 16 minutes and 40 seconds with a total work time of 3 minutes and 20 seconds. Based on pilot tests and previous data from our laboratory, a rest period of 17 seconds between consecutive eccentric actions is an effective way of minimizing the accumulation of metabolites, since no increases have been observed in blood lactate levels (Piitulainen et al. 2008). Although, systemic changes may be difficult to detect due to small muscle mass involved.

4.2.3 Effect of contraction type on acute responses (V)

The study consisted of three primary sessions of identical measurements: 1) before (BEF) the exercise, 2) immediately after (IA) the exercise and 3) two hours (2H) after the exercise (Fig. 6). In addition to the aforementioned sessions, for tetanic motor point stimulation (see the details below) an additional measurement was conducted 30 minutes (30MIN) after cessation of the exercise (Fig. 6). Furthermore, follow-up for subjective perceived muscle soreness was continued daily up to seven days (BEF, IA, 2H and 1D- 7D) post-exercise. Moreover, a collection of venous blood sample was performed also one day (BEF, IA, 2H and 1D) post-exercise.

The three primary sessions (BEF, IA and 2H) involved measurements in the following order: 1) collection of venous blood sample, 2) evaluation of subjective perceived muscle soreness, 3) recording of train of maximal M-waves during the tetanic monopolar motor point stimulation and 4) tests for isometric, MVC (Fig. 6). Further measurements to the aforementioned were conducted at the beginning (at BEF) and at the end of the experiment (at 2H). These measurements included evaluation of 1) muscle and subcutaneous tissue thickness and 2) passive twitch force and superimposed twitch force during transcutaneous bipolar muscle stimulation (Fig. 6).

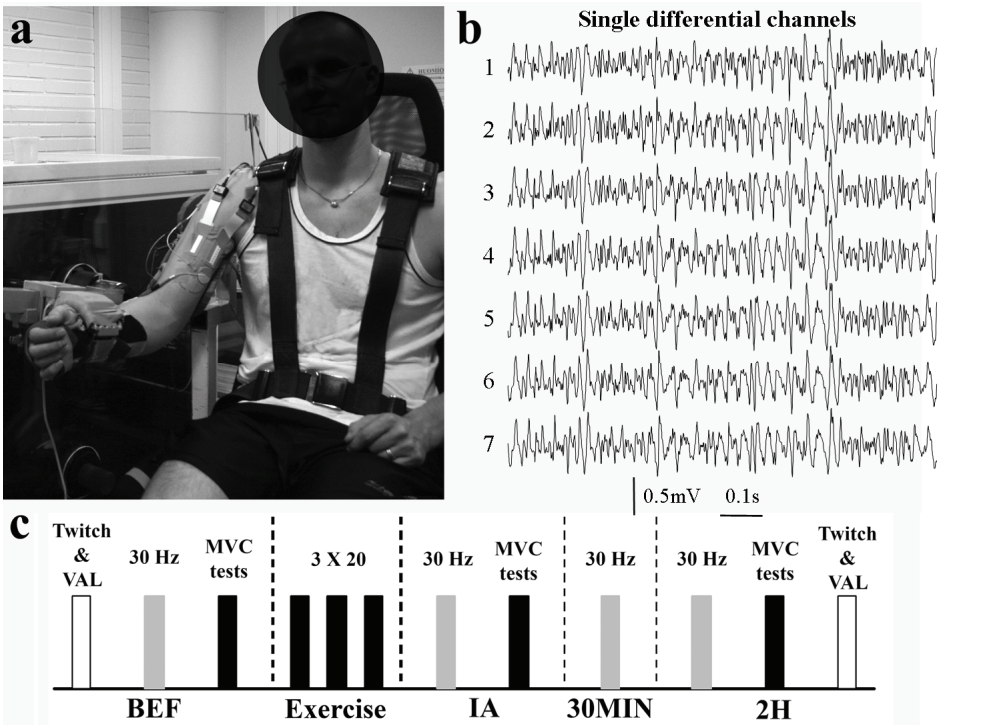

FIGURE 6 Measurement setup (a), raw signals during isometric MVC from BBM muscle in a single subject (b) and schematic presentation of experimental protocol (c). Functional measurements included evaluation of passive twitch forces (Twitch), voluntary activation level (VAL), monopolar motorpoint stimulation (30 Hz) and maximal voluntary contraction (MVC) tests, before (BEF) the exercise and immediately (IA), 30 min (30MIN) and two hours (2H) post-exercise.

Exercise protocol. Subjects performed either three sets of 20 maximal concentric or eccentric contractions with the elbow flexors of the right arm, on a motorised isokinetic dynamometer (angular velocity of 1 rad/s) (Komi et al. 2000). The range of motion at elbow joint was 110°, between 65° and 175° (180° corresponds to full extension). The maximal contractions were performed with fifteen second intervals and with maximal isometric pre-activation starting one second prior to the onset of the movement, giving a total duration of 2.8 seconds for each contraction.

During the exercises the subjects were seated and the half-supinated right forearm was attached to a strain gauge transducer, which was fixed to the vertical lever arm of the dynamometer to record the force applied by the elbow flexors. While the lever arm returned to the initial position (at an angular velocity of 1 rad/s), subjects were instructed to relax their arm muscles. The exercise lasted for 19 minutes with a total work time of 2 minutes and 50 seconds.

4.3 Measurements

For more details of the methods the original articles (I-V) should be consulted.

4.3.1 Force measurements

Isometric voluntary and electrically evoked contractions. Isometric submaximal and maximal voluntary elbow flexor forces and all electrically evoked maximal passive twitch forces were measured while subjects were seated in a custom-made chair (University of Jyväskylä, Finland), where the dominant arm was attached to the force transducer by applying straps to the wrist (Fig. 7). The chair dimensions were individually adjusted for each subject, and an arm support was used to set the elbow angle to 120°. Submaximal trials (duration of 8 s each, with 2 minute rest periods in between) were only accepted if the actual force level achieved was within ± 5% of the target level. The highest value of three MVC trials (duration of 4-5 s, with 2 minute rest periods in between) with less than a 5% difference from the second highest value was accepted as the true MVC value, and was used for further analysis.

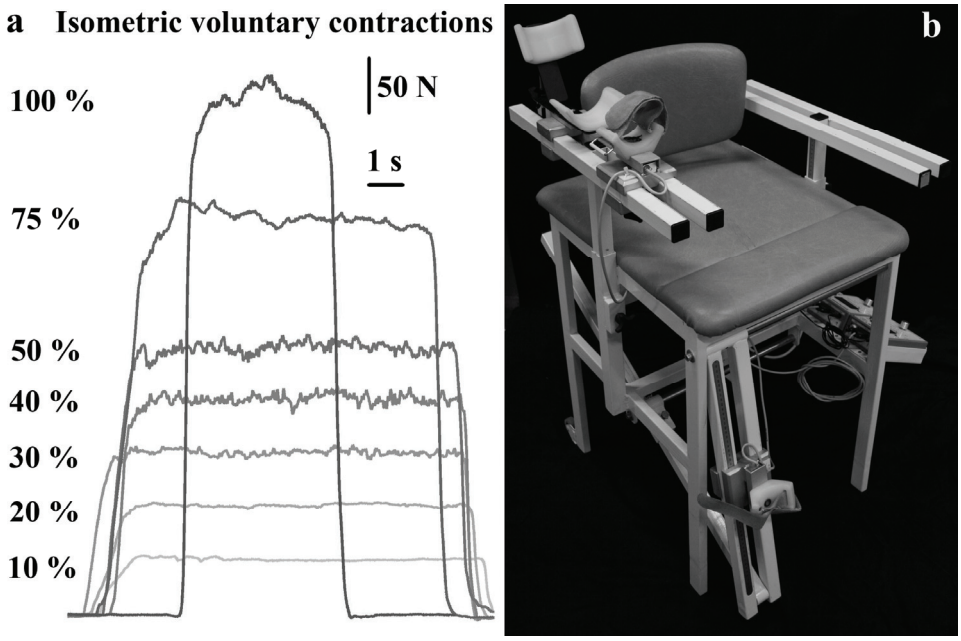

FIGURE 7 Example of elbow flexor force signals recorded from single subject during maximal and submaximal voluntary isometric contractions (a) and custom made chair where the isometric measurements were conducted (b).

In the last experiment (V), the isometric MVC was measured otherwise similarly, but the measurements were done in the motorised isokinetic dynamometer where the exercise was conducted (Fig. 6).

All force signals were sampled at 2048 samples/s, converted to digital data by a 12-bit analogue to digital converter (EMG-USB 128-channel sEMG amplifier, LISiN, Politecnico di Torino and OT Bioelectronica, Torino, Italy), stored on a computer hard disk and analysed in Matlab (The Mathworks Inc., Natick, MA, USA). The force signals were also displayed real-time on a computer screen as feedback to the subjects. For this purpose, an additional system was used where the force signals were sampled at 4000 samples/s and converted to digital data by a 16-bit analogue to digital converter (Power 1401, CED Ltd., Cambridge, England), displayed real-time (Signal software, CED Ltd.) on a computer screen.

4.3.2 Skin temperature

Skin temperature was measured with thermometer (YSI 4000A with YSI 427 stainless steel sensor disk, diameter 48 mm, YSI inc., Dayton, Ohio, USA) throughout all of the experiments from the central region of the muscle belly of biceps brachii (BB) muscle in order to monitor the effects of temperature on sEMG variables (Stalberg 1966).

4.3.3 Muscle and subcutaneous tissue thickness

Subcutaneous tissue thickness over BB muscle and muscle thickness of the BBM and underlying brachialis muscle were measured with ultrasonography (model SSD-5500, Aloka; Tokyo, Japan) BEF and after the exercise (II, III, IV: 2H, 2D and 4D and V: 2H) to control possible bias of the sEMG variables due to changes in muscle geometry. The probe was positioned along the midline of the muscle, two centimetres proximal to the distal tendon. The probe position was marked on the skin in the first measurement session. During the measurements, subjects stood upright with the right arm relaxed at the side of the body.

4.3.4 Muscle soreness

Subjective perceived muscle soreness of the right elbow flexors was assessed in each measurement session of the experiments that involved exercise (II, III, IV: 2H, 2D and 4D and V: BEF, IA, 2H and 1D). In the last experiment (V), the muscle soreness was self assessed on daily basis after the principal measurement sessions up to seven days post-exercise (2D-7D). The assessment involved the use of a visual analogue scale with a continuous line starting from 0 cm (no pain) and ending at 5 cm (worst possible pain) (Nosaka & Clarkson 1996). Each subject marked their subjectively perceived muscle soreness level on this line. The muscle soreness was assessed during voluntary muscle activity and during light palpation of the BB muscle.

4.3.5 Blood samples

In the last experiment (V), blood sample of 10 ml was drawn from the ulnar vein of non-exercised arm BEF, IA and 2h after the exercise. Biosen C Line Sport (EKF-Daignostic GmbH, Madgeburg, Germany) was used for analysing blood lactate concentration. Plasma electrolyte ($[Na^+]$ and $[K^+]$) and myoglobin concentrations were analysed with Konelab 20XTI (Thermo Electron Oy, Vantaa, Finland). Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan) was used to monitor blood haemoglobin concentration, which remained unchanged throughout the experiment.

4.3.6 Peripheral electrical stimulation

In all experiments same constant current stimulator (Digitimer Stimulator DS7, Digitimer Ltd., Hertfordshire, England) was used to stimulate either musculocutaneous nerve or BB muscle transcutaneously with monophasic rectangular pulses (100 μs) to measure the passive twitch force, superimposed twitch force of the elbow flexors and maximal M-wave properties of the BBM muscle.

Peripheral nerve stimulation (II, III, IV). First, a large positive adhesive electrode (diameter of 7cm; V-Trodes, Metler Electronics corp., Anaheim, CA, USA) was placed over the posterior part of the shoulder joint on the acromion, and a small negative electrode (diameter of 1cm) was placed midway between the epicondylus medialis and processus coracoideus between the short and long heads of the BB muscle. The positions of the stimulating electrodes were marked on the skin for accurate replacement. Then, musculocutaneous nerve was stimulated with single rectangular pulses to verify supramaximal electrical stimulation intensity, which was determined as the current level that was 20% above the level needed to elicit a maximal M-wave in the pre-exercise measurement session. In addition, it was ensured that this stimulation intensity produced maximal passive twitch force. After this, the passive twitch response, superimposed twitch of the elbow flexors and maximal M-wave properties of the BBM were measured.

Bipolar muscle stimulation (V). First, two (anode and cathode) large square shaped adhesive electrodes (diameter of 5 cm; V-Trodes, Metler Electronics corp., Anaheim, CA, USA) were placed over the proximal and distal end of the BB muscle and their positions were marked on the skin for accurate replacement for follow up at 2H session. Then, supramaximal double pulse electrical stimulation (with 10 ms of inter-pulse interval) intensity was determined as the current level that was 30% above the level needed to elicit a maximal passive twitch force in the first measurement session. After this, the BB muscle was stimulated with double pulses to measure 1) passive twitch force and 2) superimposed twitch force of the elbow flexors (at BEF and 2H).

Voluntary activation level (II, III, IV, V). Isometric MVC trial with superimposed twitch was used to calculate voluntary activation level (VAL) based on the principle introduced by Merton (1954). During a single VAL test,

two maximal stimulations were applied either to the musculocutaneous nerve (II, III, IV) or transcutaneously to the BB muscle (V): 1) during isometric MVC and 2) three seconds after relaxation from the isometric MVC. VAL was calculated with the following formula: VAL (%) = (1-ST / PT)*100 (Babault et al. 2001), where ST = superimposed twitch amplitude (1st stimulation) and PT = post-activity potentiated passive twitch amplitude (2nd stimulation). The peak force of the last (2nd) passive double stimulation was used to describe contractile properties of the BB muscle.

Monopolar transcutaneous motor point stimulation (V). In the last experiment (V) monopolar transcutaneous motor point stimulation was used to obtain information about maximal M-wave properties of BBM muscle during 30 s train of stimulations. First, the locations of the main motor points of BBM were identified by scanning the muscle's surface with a ball pointed (cathode, 8 mm diameter) pen electrode while a large round (anode, diameter 7cm) adhesive electrode (V-Trode, Mettler Electronics corp., Anaheim, CA, USA) was placed on the opposite side of the upper arm. The main muscle motor point was defined as the location of the negative electrode yielding the strongest mechanical response with the lowest pulse amplitude. After this, one small round (diameter 3.2cm) negative adhesive electrode (V-Trode) was placed over the main motor point. M-waves were detected with an 8-electrode array (see description below). The M-waves were monitored as the muscle was stimulated at 2 Hz with monophasic square pulses (duration of 100 μs) of increasing current intensity. The maximal current level was identified as the stimulation current where yielding the maximal M-wave amplitude took place (no clear increment of peak to peak amplitude was evident, even if the current level was further increased). The stimulation intensity was set at 20% above the maximal current level. Then, electrical stimulation train was applied for 30 s with a frequency of 30 Hz to measure maximal M-wave properties (at BEF, IA, 30MIN and 2H, Fig. 8).

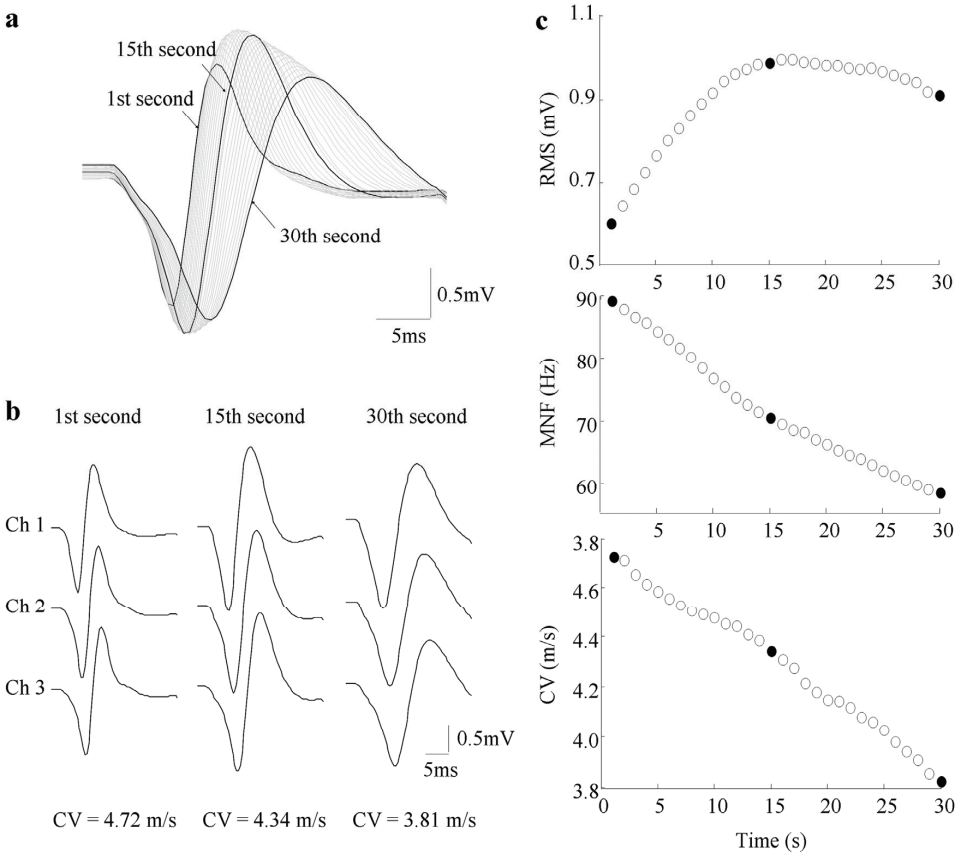

FIGURE 8 Single differential M-waves detected (from single subject) from BBM muscle (a). M-wave time evolution over 30 s of sustained stimulation at 30 Hz is shown: each curve is the average of the 30 responses obtained during one second epochs. The 1st, the 15th, and 30th (averaged) M-waves are highlighted. Single differential M-waves detected by three consecutive channels (corresponding to the 1st , the 15th and 30th second of stimulation) and relative conduction velocity (CV) estimates (b). Time course of the sEMG variable estimations during the 30 s electrically elicited contraction (c). Reported variables are: root mean square (RMS); mean muscle fibre conduction velocity (CV); mean frequency of the power spectral density (MNF). The black dots correspond to the highlighted M-waves of panel a.

4.3.7 sEMG recordings

All sEMG signals were amplified at a bandwidth of 10–750 Hz to reduce mechanical and electrical interference and with a gain of 200, 500, 1000 or 2000 depending of the contraction level. The signals were sampled at 2048 Hz, A/D converted at 12 bit resolution (EMG-USB 128 channel surface EMG amplifier, designed by LISiN at Politecno di Torino and manufactured by OT Bioelectronica, Torino, Italy) and stored on a hard disk for further analysis.

Before placement of the electrodes, the skin was abraded and cleaned with alcohol. In the first measurement session, the boundaries of bipolar electrodes, electrode arrays or electrode grids were marked on the skin to enable accurate replacement of the electrodes in the repeated measurement sessions (II, III, IV: 2H, 2D and 4D, V: IA, 30MIN and 2H).

Standard bipolar recordings. In the experiments where elbow flexor muscles were exercised (II, III, IV, V), standard bipolar sEMG was used to monitor antagonist activity and synergist activity for BB muscle. The bipolar electrodes (II, III, IV: Ag/AgCl miniature skin electrodes, NT615T, Nippon Koden, Tokyo, Japan. V: Blue Sensor N-00-S/25, Medicotest, Olstykke, Denmark) with 20 mm inter-electrode-distance were placed over the lateral head of the triceps brachii muscle and brachioradialis muscle.

Single 8-electrode-array sEMG recordings. In the last experiment (V), all sEMG signals were detected with two semi-disposable linear arrays consisting of 8-electrodes each (5 mm inter-electrode distance, model ELSCH008, designed by LISiN - Politecnico di Torino and manufactured by Spes Medica, Battipaglia, Italy) in single differential configuration. The recordings were done during isometric MVC test and monopolar transcuteneous motor point stimulation (analysis of M-wave properties). One 8-electrode-array was placed both over BBM muscle and one over long head of BB muscle (BBL). Furthermore, in the experiments II, III and V, the same 8-electrode-array was applied to detect maximal M-wave properties during maximal electrical nerve stimulation from the BBM muscle. Furthermore, the high-density sEMG grid was not used during the electrically elicited contractions, since single differential recording was not possible due to its complex electrode wiring and thus the applied monopolar recording mode turned out to be more sensitive for interference during electrical stimulation.

Before the 8-electrode-array electrode placement, the subject's main IZ was located individually prior to the 8-electrode-array placement with a special array of 16 electrodes (silver bar electrodes with 5 mm inter-electrode distance, LISiN, Politecno di Torino). During this search of optimal location for the 8-electrode-arrays, sEMG signals were recorded and displayed on-line on computer screen while the subject applied submaximal isometric contraction with elbow flexors. This was repeated from various parts of the BBM and BBL muscles, until the sites with clear muscle fibre AP propagation and main IZs were identified for the both heads. The 8-electrode-arrays were then placed on optimal part of the BBM and BBL muscles parallel to their fibres either proximally or distally from the main IZ location depending on anatomical features of the subject.

The 8-electrode-arrays were attached to the skin using double adhesive foam (1 mm thick), which included cavities under the electrodes between the skin and the electrode surface. To assure proper electrode-skin contact, each of the cavities was filled with 25 µl of conductive electrolyte gel (Spes-Medica, Battipaglia, Italy) with a pipette (Finnpipette 4540, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The reference electrode (NI-4560, Ag/AgCl, Unomedical

Ltd., Gloucestershire, Great Britain) was placed over ipsilateral acromion of scapula.

High-density sEMG recordings. In all isometric contractions of the first four experiments (I, II, III, IV), sEMG signals were recorded from BBM muscle with a semi-disposable grid of 64 electrodes (8 mm inter-electrode distance in both directions, model ELSCH064, designed by LISiN at Politecno di Torino and manufactured by OT Bioelectronica, Torino, Italy, Fig. 9). The aim was to cover as large surface area of the BBM muscle as possible. This electrode grid consisted of 13 circular electrodes (2 mm diameter, Ag/AgCl) in each of its five columns, except in the first column, which consisted of 12 electrodes. The missing electrode from the first column was located in the proximal-medial corner of the electrode grid. Double adhesive foam (1 mm thick), which included cavities under the electrodes, was then placed between the skin and the electrode surface. Each of the cavities was filled with 25 µl of conductive electrolyte gel (Spes-Medica, Battipaglia, Italy) with a pipette (Finnpipette 4540, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The middle column of the electrode grid was placed over the midline of the BBM muscle, with its distal end two centimetres proximal to the distal tendon. The columns of the electrode grid were positioned parallel to the longitudinal axis of the muscle. A reference electrode (strap, LISiN-OT Bioelectronica, Torino, Italy) was moistened and placed on the subjects' dominant wrist. Furthermore, one additional strap was placed on each arm and both were connected to a Driven Right Leg circuit to reject common mode interference in the applied monopolar acquisition mode.

4.4 Signal processing

All force and sEMG signals were analysed with Matlab software (R2007a, ver. 7.4.0.287, The MathWorks Inc., MA, USA). Before any further calculations, the sEMG signals were digitally band-pass filtered (20-450 Hz, 4th order Butterworth filter) and the force signals were low pass filtered with a cut-off frequency of 20 Hz (4th order Butterworth filter). In addition, all high-density sEMG signals were post-processed in MatLab from monopolar signals to single differential signals in the longitudinal axis (columns) of the electrode grid.

4.4.1 Standard bipolar sEMG signals

Standard bipolar sEMG was applied to monitor antagonist and synergist muscle activity. Therefore, only RMS values were analysed from these muscles similarly as in the case of the multichannel sEMG recording (see below).

4.4.2 Single array sEMG signals

Analysis of isometric MVC test (V). RMS and MNF were calculated separately for each bipolar channel in the 8-electrode-arrays. CV was estimated for the 8-

electrode-arrays with a method introduced by McGill and Dorfman (1984) based on three adjacent bipolar channels (triplets) in the longitudinal direction of the BBM and BBL muscles. This method computes the delay between two signals based on optimal cross-correlation of the signals. In the current experiment, the delay was computed for the first and second bipolar signal pairs (1st vs. 2nd and 2nd vs. 3rd) and average of these two estimates was applied. Then this delay estimate was divided by the physical distance (= inter electrode distance) between the recording sites of the corresponding bipolar signals. In the case of high-density sEMG signals, the same method was applied, but with double differential signals. Channels with high interference and triplets with CV values beyond the physiological range (2-6 m/s) were excluded. The values of RMS, MNF and CV were averaged among all the accepted channels or triplets (for CV estimation) in each array separately. The calculations were done within a fixed 1000 ms epoch corresponding to the section with the highest force value.

Analysis of motor point stimulation (V). For each single differential channel of the 8-electrode-arrays, the 30 electrically elicited responses (M-waves) corresponding to each epoch of 1s were averaged thus obtaining a sequence of 30 averaged M waves during the 30s long contraction (Fig. 8). Thereafter, a sequence of 30 sEMG variable estimates (RMS, MNF and CV) was calculated from each available triplet, defined as a group of three single differential signals provided by adjacent electrodes. Since for all the signals the stimulation artifact was almost completely separated from the M-wave, it was removed by offline blanking (Mandrile et al. 2003) which duration was defined by visual inspection of the signal (the average blanking window was 3ms).

SEMG estimates used for further analysis were obtained from the best of the five triplets, that is the one showing the highest correlation coefficient between the single differential signals (in any case greater than 0.70) and physiological estimates of CV (in the range 2–6 m/s). RMS and MNF were computed as the average among the estimates of each channel of the selected triplet

For all the subjects the time course of the sEMG variables showed a linear pattern for the first 10 s, therefore the first ten sEMG variable estimations were fitted with a least mean square regression line whose intercept with the Y axis (at time = 0) was defined as the initial value. The slope of the regression line was used as an estimate of rate of change over time and was adopted to assess myoelectric manifestations of fatigue.

Analysis of peripheral nerve stimulation (II, III, IV). In maximal peripheral nerve stimulation, peak-to-peak amplitude, MNF and CV of the maximal elicited M-wave were calculated from a fixed 35 ms epoch starting 5 ms after delivery of the stimulation. The peak-to-peak M-wave amplitude was determined as the difference between the maximum and minimum value separately in each of the seven bipolar channels of the 8-electrode-array. An average of all channels for peak-to-peak amplitude and MNF of the M-wave was included in the further analysis. Average CV was calculated as was the

case in analysis of motor point stimulation (see above Analysis of motor point stimulation).

4.4.3 High-density sEMG signals

Determination of innervation zone locations (I, II, III, IV). First, the adjacent channels in each of the five columns of the electrode grid were plotted to determine the location of the IZ in MatLab. The IZ location was determined individually for each column by visual inspection of these plots. This was based either on a reversal in signal polarity in two adjacent channels (IZ is located between these channels), or on the lowest amplitude in a single channel (IZ is located in this channel) (Fig. 9). With this procedure, 4 mm spatial resolution was obtained for IZ determination because of the inter-electrode distance of 8 mm. IZ location results were normalized with respect to 10% of MVC level to obtain the mean IZ shift of all five electrode columns collectively, and the IZ shift in each individual electrode column.

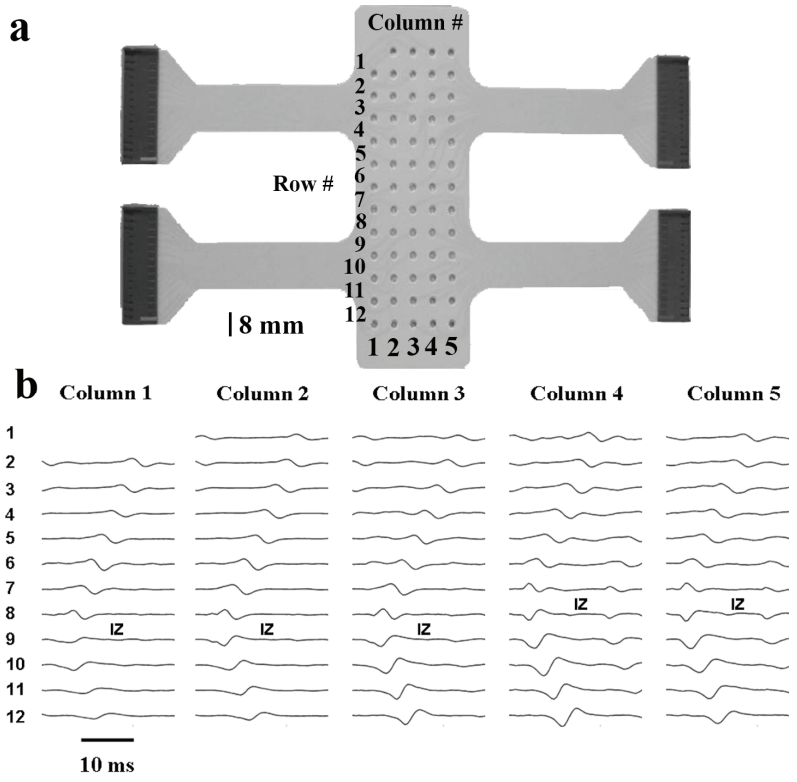

FIGURE 9 The multi-channel electrode grid, which consists of five columns of 13 circular electrodes (12 channels of single differential sEMG signals), except the first column, which consists of 12 electrodes (a) and example of the plotted raw single differential sEMG signals during isometric contraction at 30% of MVC. In this case, IZs are located between single differential channels 8 and 9 (columns 1-3) and between channels 7 and 8 (columns 4-5). IZ = innervation zone location.

Global sEMG variables during voluntary contractions (II, III, IV). For all subjects, RMS and MNF were calculated separately for each bipolar channel. A power spectral density estimate was used to acquire MNF, which was determined as a weighted average of the power spectrum. Similarly, CV was estimated with a method introduced by McGill and Dorfman (1984) based on two adjacent double differential channels in the longitudinal direction of the BB muscle. Channels belonging to IZs, channels with high interference and channels with values beyond the physiological range were excluded. The applied physiological ranges were: 0-5 mV for RMS, 40-180 Hz for MNF and 2-6 m/s for CV (Merletti & Parker 2004). Values of RMS, MNF and CV were then averaged among all the accepted channels.

During all isometric voluntary contractions, sEMG variables were calculated within a fixed 1000 ms epoch. In the isometric MVC trial, this epoch corresponded to the section with the highest force value. For the 8 s submaximal isometric contractions, the chosen 1000 ms epoch was set to the most stable part of the force signal (smallest variation).

Site-dependent changes in sEMG variables (II). The site-dependent changes were investigated in common sEMG variables, RMS, MNF and CV, during isometric MVC. For RMS and MNF, spatially dependent changes were investigated in three different ways: 1) by comparing individual values of each of the 59 bipolar channels, 2) by comparing the mean of all channels in each of the five longitudinal electrode columns, indicating site-dependency in the medial-lateral direction of BBM (COLUMNS) and 3) by comparing the mean of all channels in each of the 12 transversal electrode rows, indicating site-dependency in the proximal-distal direction of BBM (ROWS). In the case of CV, spatially dependent changes were investigated similarly, but the number of samples was lower due to the double differential configuration (see below). Therefore, level 1) included 49 different CV values and level 3) included ten values.

RMS and MNF values from the single differential channels of the electrode grid were plotted and colour coded in 2-dimensional maps (Fig. 13). In all 2-dimensional maps, the 1st channel in the 1st column (upper left hand corner) was always empty, but was computed for visualisation purposes by averaging the values in the adjacent channels (see also Fig. 11).

4.4.4 Decomposition of high-density sEMG signals

The acquired multi-channel sEMG signals were decomposed off-line with CKC algorithm (Holobar & Zazula 2007) (IV). The CKC-based decomposition of sEMG is non-parametric, fully automatic, and relies minimally on the anatomic properties of the investigated muscle (see 2.3.1 Decomposition of high-density sEMG signals). It implicitly combines all the available information provided by the multi-channel measurements and is not sensitive to superimpositions of MUAPs (see 4.2.1 Validation of the methodology).

The submaximal contractions were decomposed over their total duration, but only the discharge patterns from the stable plateau region of force (~8 s)

were included for further analysis (Fig. 10). The coefficient of variation (CoVISI) of inter spike interval was calculated as the standard deviation of inter spike interval divided by its mean. All MUs with an unstable discharge pattern or with CoVISI larger than 25% were discarded and excluded from further processing. The normal physiological upper limit for CoVISI of MU is ~30% (e.g. ()).

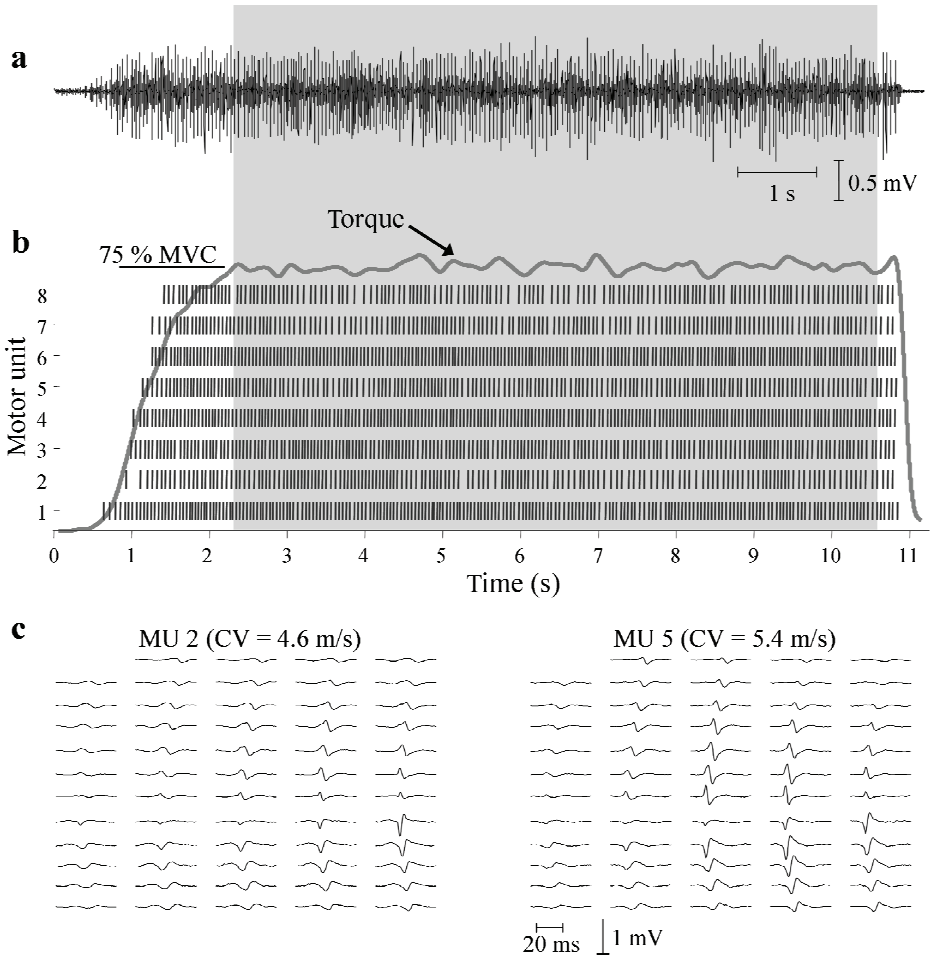

FIGURE 10 Raw sEMG from column# three and row# ten recorded with high-density electrode grid from BBM muscle during isometric contraction (75% of MVC) (a). Discharge patterns of identified MUs with each MU discharge denoted by a vertical line (b). Multi-channel surface MUAPs 2 and 5 with mean conduction velocity (CV) (c). The stable plateau region was used for calculation of MU characteristics (highlighted in light gray in panels a and b).

The mean multi-channel surface MUAP of each identified MU was obtained by averaging the multi-channel sEMG signals over 50 ms long rectangular windows, centred to all the MU discharge times identified at the stable force region (Fig. 10). Peak-to-peak amplitudes (AMP) of MUAPs were then calculated for each identified MU and averaged over all the matrix channels,

except the channels belonging to IZs and channels with high interference, as determined by visual inspection. On average, 6 ± 2 channels were discarded per contraction. Finally, CV of each identified MU was estimated from two adjacent double differential channels of the aforementioned MUAPs in the longitudinal direction with a method introduced by McGill and Dorfman (1984). Visual selection of the channels used to estimate conduction velocity was based on the criterion of a minimal change in shape of the MUAP. In addition, channels with values beyond physiological range (2 - 6 m/s) were excluded (Andreassen & Arendt-Nielsen 1987). For each MU, MDR was calculated as instantaneous discharge rate averaged over all the identified MU discharges during the stable plateau region of the force signal.

4.5 Statistical methods

All data in text, tables and figures are expressed as means ± standard deviation (SD) and all statistical tests were performed in SPSS (14.0, SPSS Inc. IL, USA) with a significance level of $P \leq 0.05$. When applicable, Holm-Bonferroni post hoc test was used for the multiple comparisons.

4.5.1 Shift and reliability of innervation zone locations (I)

General Linear Model ANOVA (GLM) was used to assess the effect of isometric contraction level on mean IZ shift, IZ shift in individual electrode columns and sEMG variables (RMS, MDF and CV) at fixed channels next to the respective IZ. The contrasts used were simple or repeated, where either the lowest contraction level was compared to the following higher contraction levels or successive contraction levels were compared to each other. In addition, paired-samples T-tests were used to determine the effect of IZ on sEMG variables in fixed channels as compared to average global values at different isometric contraction levels.

Trial-to-trial reliability of IZ location was tested by comparing two repeated contractions from the same session. Day-to-day reliability was tested by comparing two contractions performed in different days. Reliability was assessed by one-way random model of intraclass correlation coefficient (ICC) (Weir 2005) for each contraction level, by comparing the average of the IZ location values obtained from the five different electrode columns. The reliability was considered as acceptable if the ICC level was between 80-100%. Furthermore, repeated measures GLM was used to test whether any statistical differences existed in IZ location determined from different trials at the same day or different days at each of the contraction levels. The contrast used in GLM was simple, whereby the first trial was always compared to the following one.

4.5.2 Spatial dependent changes (II)

Repeated measures GLM was used to assess the effect of exercise on mean IZ location and sEMG variables in 1) global, 2) average single column (COLUMNS), 3) average single row (ROWS) and 4) single channel level. The paired comparisons were done using intra-individual contrasts between the pre-exercise values and the post-exercise values (BEF vs. 2H, BEF vs. 2D and BEF vs. 4D).

Moreover, nonparametric two-tailed Spearman's correlation coefficient was calculated for IZ shift and change in absolute force from 20% to 100% isometric MVC for each individual measurement session.

4.5.3 Exercise induced changes (II, IV, V)

Prolonged responses to eccentric exercise (III, IV). Repeated measures GLM was used to assess the effect of the eccentric exercise on isometric MVC, muscle soreness, muscle and subcutaneous tissue thickness, passive twitch amplitude, VAL, voluntary sEMG variables, maximal M-wave properties, antagonist muscle activity and skin temperature. Paired comparisons were performed using intra-individual contrasts between the pre- and post-exercise values (BEF vs. 2H, BEF vs. 2D and BEF vs. 4D).

Effect of contraction type on acute responses (V). The possible changes in the variables between the CON and ECC groups were compared with multivariate analysis of covariance (MANCOVA) for repeated measurements, with one between-subject factor (group: CON and ECC) and with one within-subject factor (time: BEF, IA, 30MIN and 2H). The values measured on BEF session were used as covariate. When applicable, the ANOVA for repeated measurements on one factor (time) and post hoc Holm-Bonferroni were used to determine the significant differences between study variables separately in the CON and ECC groups.

Single motor unit characteristics (IV). One-way analysis of variance was used to asses the effect of eccentric exercise on individual MU characteristics of MDR, CV and AMP in the exercise group. This test was applied instead of GLM, since the group and the number of identified MUs may vary from one measurement session to another. The validation group was tested with paired t-test (D1-1st vs. D2). Furthermore, a Z-test for parallelism (Kleinbaum et al. 1988) was used to compare the slopes of the linear regression lines separately for MDR, CV and AMP when plotted against relative force level between the pre- and post-exercise values (2H, 2D and 4D). In the validation group, the slopes of the linear regression lines were compared between D1-1st and D2.

5 RESULTS

The main findings of the present series of experiments are presented below. For more details the original articles (I-V) should be consulted.

5.1 Validation of methodology (I, II, IV)

5.1.1 Innervation zone location

Innervation zone shift with increasing contraction level. The average IZ shift in proximal direction was 0.6 ± 0.4 cm with increasing contraction level (10% vs. 100% MVC, $p < 0.001$) and the range was -0.8 cm and 2.4 cm depending on the subject and the investigated column (Table 2 and Fig. 11.) (I).

TABLE 2 Average IZ shift (cm) over all subjects (n=16) in individual columns with respect to the isometric contraction level. Column 1 is the most medial column.

MVC	Column 1	Column 2	Column 3	Column 4	Column 5
20 %	0,00 ± 0,24	0,17 ± 0,27	0,13 ± 0,60	0,03 ± 0,36	0,13 ± 0,36
30 %	0,13 ± 0,49	0,27 ± 0,43	0,17 ± 0,58	0,10 ± 0,35	-0,17 ± 0,50
40 %	**0,40 ± 0,57***	**0,40 ± 0,51***	0,37 ± 0,63	**0,27 ± 0,39***	0,00 ± 0,51
50 %	**0,40 ± 0,61***	**0,57 ± 0,55****	**0,43 ± 0,67***	**0,43 ± 0,52***	0,10 ± 0,64
75 %	**0,73 ± 0,70****	**0,83 ± 0,55*****	**0,53 ± 0,60***	**0,73 ± 0,68****	0,33 ± 0,56
100 %	**0,73 ± 0,51*****	**0,73 ± 0,45*****	**0,63 ± 0,83***	**0,73 ± 0,76****	0,3 ± 0,52

Mean ± SD, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

The individual columns showed a significant IZ shift with increasing contraction level from 40% or 50% up to the maximal MVC level in the four most medial columns ($p < 0.05 – 0.001$), but not in the most lateral column. Thus, the IZ shift demonstrated a spatial dependency in the medial-lateral direction of the muscle. Only two subjects showed a higher IZ shift in the most lateral column.

Reliability of innervation zone location measurement. The subjects exerted 432 ± 48 N and 418 ± 49 N of force in the isometric MVC test on day one and day two, respectively (n.s.). Determination of IZ location showed acceptable reliability, as the trial-to-trial ICC was more than 92.4%, and the day-to-day ICC was more than 91.0% at each individual contraction level (Table 3). In addition, there were no statistical differences in IZ location between repeated trials at any of the contraction levels.

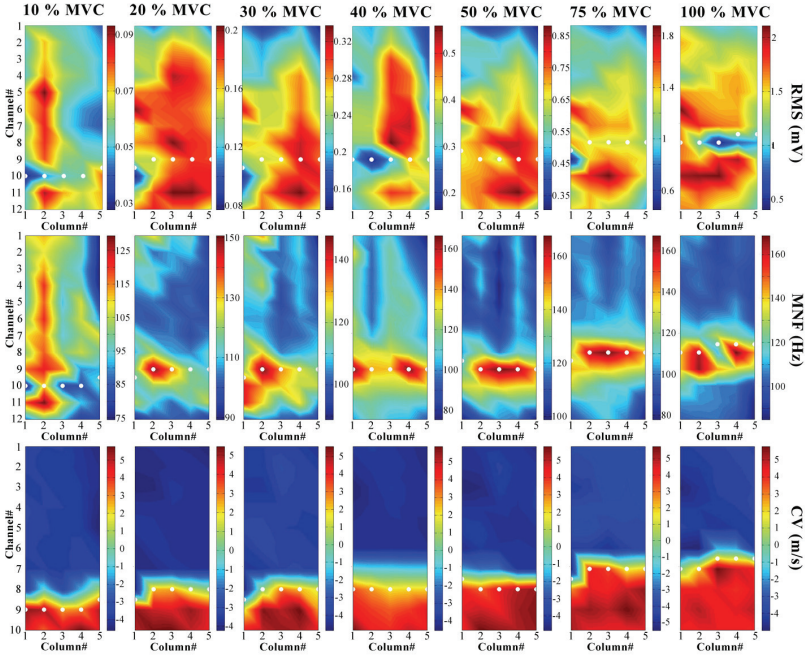

FIGURE 11 2-dimensional maps of root mean square (RMS), mean frequency of power spectral density (MNF) and AP conduction velocity (CV) values at different isometric contraction levels in one representative subject (results are calculated over a 1 s signal epoch). Each white dot corresponds to the IZ location in the individual electrode column. Note that the scale varies between some the different contraction levels.

TABLE 3 IZ location trial-to-trial and day-to-day ICC values (%) with respect to different isometric contraction levels. In general, 80-100% is considered as good value.

MVC	Trial to trial	Day to day
10 %	99,1	94,8
20 %	98,3	94,3
30 %	98,7	93,9
40 %	97,7	96,9
50 %	99,3	94,8
75 %	97,4	96,6
100 %	92,4	91,0

5.1.2 Spatially dependent changes

Spatially dependent sEMG variables – medial-lateral direction. The changes in RMS values of COLUMNS were uniform, indicating that there was no spatial dependency in RMS in the medial-lateral direction (Fig. 12a) (II). Some spatial dependency was observed in MNF of the COLUMNS (Fig. 12b). Average MNF in the most medial column (# 1) decreased 2H post-exercise ($p < 0.05$) and remained lower thereafter ($p < 0.05$). Column # 2 and # 5 decreased 2H post-exercise ($p < 0.05$) and remained lower at 2D ($p < 0.05$), but were recovered at 4D. Column # 3 only decreased at 2H ($p < 0.05$) and column # 4 exhibited no changes. Average CV decreased at 2H in column # 1 ($p < 0.05$). CV in this column recovered to the pre-exercise level at 2D (Fig. 12c).

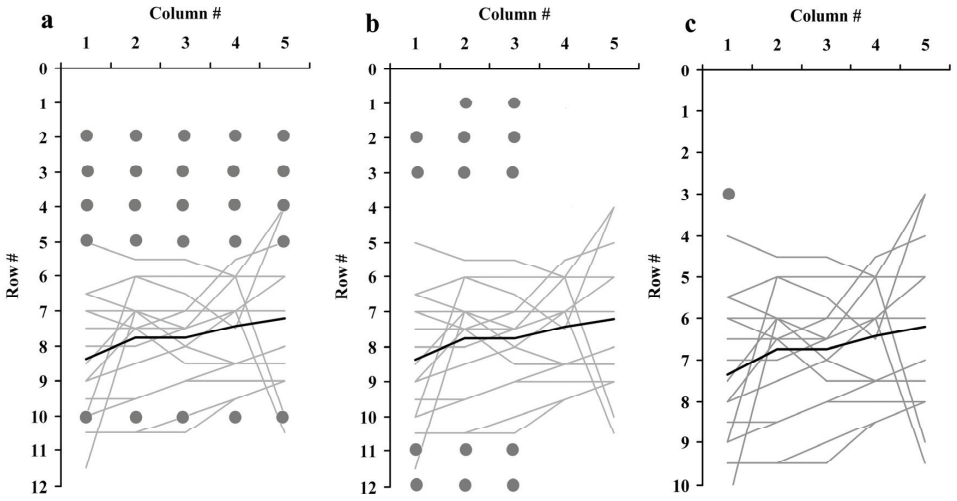

FIGURE 12 Average (n=9) maps of channels with significant changes ($p < 0.05$) in RMS (a), MNF (b) and CV (c) between pre-exercise and two hour post-exercise values. Dots indicate significant changes in both proximal-distal (significant change in the respective ROWS) and medial-lateral directions (significant change in the respective COLUMNS). Lines correspond to mean (black line) and subject's individual (gray lines) main innervation zone locations. Column# 1 is the most medial and column # 5 the most lateral column. Note that the row # in (c) corresponds to a grid of the double differential channels used for CV estimation and thus results in lower total number of rows.

Spatially dependent sEMG variables – proximal-distal direction. A spatial dependent decrease in RMS was observed in the proximal-distal direction at 2H, since RMS decreased in the ROWS (#2, #3, #4, #5 and #10, $p < 0.05$, Fig. 12a). Average RMS was unaffected in all ROWS at 2D and 4D.

Similarly, a spatial dependent decrease in MNF was observed in the ROWS at 2H (#1, #2, #3, #11 and #12, $p < 0.05$, Fig. 12b) and 2D (#3, #10 and #12, $p < 0.05$) post-exercise. Average CV showed a spatially dependent decrease in single row at 2H (#3, $p < 0.05$) and at 2D (#10, $p < 0.05$) post-exercise (Fig. 12c).

Interestingly, most of the ROWS that showed no significant decrease of sEMG variables due to exercise, were the same channels where the majority of main IZs were located in the current subject group. The mean channel number of IZs of the subjects at BEF and 2H was 7.8 ± 1.6 (range from 4 to 10.5) and 7.6 ± 1.5 (range from 4 to 11.5), respectively (Fig. 12 and 11).

Spatially dependent sEMG variables – individual bipolar channels. From 59 individual bipolar channels, only four individual channels showed significant differences ($p < 0.05$), and only in MNF (see Fig. 12 and 11). These channels showed a decrease in MNF at 2H compared to the pre-exercise values, but were recovered thereafter. However, in general, most of the individual bipolar channels followed the same trend as the global changes in RMS and MNF. However, some individual bipolar channels showed the opposite behaviour in RMS and MNF (Fig. 13). Furthermore, only one of the individual 49 CV estimates on column# 5 and row# 8 was significantly reduced from the pre-exercise value 4.78 ± 0.72 m/s to 3.83 ± 0.46 m/s at 2D ($p < 0.05$).

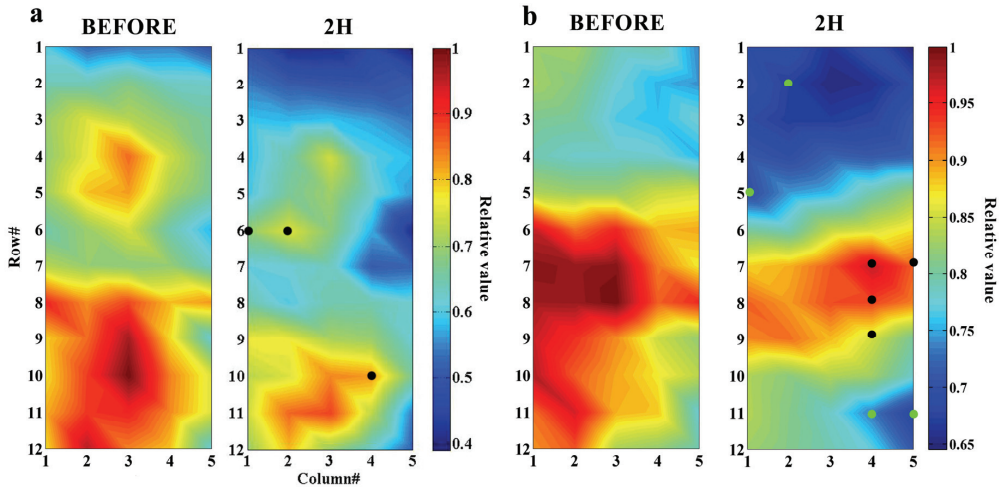

FIGURE 13 Average (n=9) 2 dimensional spatial plots of RMS (a) and MNF (b) before and two hours post-exercise (2H). Green dots correspond to bipolar channels with a significant ($p < 0.05$) decrease in MNF two hours post-exercise. Black dots correspond to bipolar channels that showed an opposite trend of behaviour in RMS or MNF compared to global RMS and MNF changes. Average IZ region, which often shows low amplitude and high frequency content, is located approximately between channels six and ten in the described experimental setup.

5.1.3 Decomposition of high-density sEMG signals

Simulated sEMG signals. At SNR of 20 dB and excitation levels of 20%, 30%, 40%, 50% and 75% of MVC, discharges of 10 ± 2, 11 ± 3 , 8 ± 3, 7 ± 2 and 5 ± 2 MUs were identified from synthetic sEMG, respectively, with CoVISI ≤ 25% (IV). With 10 dB SNR, the number of identified MUs decreased to 7 ± 3, 8 ± 3, 5 ± 2, 4 ± 2 and 3 ± 1, respectively.

With SNR of 20 dB (10 dB), the absolute error between the simulated and estimated MDR of all identified MUs was 0.1 ± 0.1 pps (0.1 ± 0.1 pps) at 20% and 30% excitation, 0.2 ± 0.1 pps (0.3 ± 0.1 pps) at 40% excitation, 0.3 ± 0.1 pps (0.3 ± 0.1 pps) at 50% excitation and 0.3 ± 0.2 pps (0.4 ± 0.2 pps) at 75% of maximal excitation. Although negligibly small, the absolute MDR error was in statistically significant relation with both SNR and excitation level. Methodology of MUAP conduction velocity and amplitude estimation has been validated in other studies, e.g. (McGill & Dorfman 1984, Merletti et al. 2008) and has been considered reliable.

Validation group. In the validation group, the total number of identified MUs did not differ significantly between the different days (D1-1st: 243 MUs and D2: 238 MUs, n.s.). Furthermore, no significant changes were observed in average values of MDR, CV or AMP (Table 4) or in the regression slopes between the different days (D1-1st and D2, Fig. 18d, e and f). In addition antagonist muscle activity remained at a constant low level throughout the experiment.

TABLE 4 Mean MU discharge rates (MDR), mean MU conduction velocities (CV) and mean peak-to-peak MUAP amplitude (AMP) in the validation group (n = 7) at different submaximal isometric contraction levels relative to maximal voluntary contraction (MVC) in two different sessions on day one and a single session on day two. No statistically significant changes were observed between the sessions at the same contraction levels.

	MDR (pps)			CV (m/s)			AMP (mV)		
MVC	Day 1-1st	Day 1-2nd	Day 2	Day 1-1st	Day 1-2nd	Day 2	Day 1-1st	Day 1-2nd	Day 2
10 %	14.1 ± 3.5	14.1 ± 4.1	14.6 ± 3.7	4.0 ± 0.4	4.0 ± 0.3	4.0 ± 0.4	0.23 ± 0.1	0.23 ± 0.2	0.28 ± 0.2
20 %	16.5 ± 3.3	16.0 ± 4.5	16.6 ± 3.7	4.1 ± 0.3	4.1 ± 0.4	4.1 ± 0.3	0.36 ± 0.3	0.36 ± 0.3	0.46 ± 0.6
30 %	15.0 ± 4.1	15.4 ± 6.0	15.6 ± 4.5	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.4	1.14 ± 1.1	0.90 ± 0.9	1.03 ± 1.0
40 %	17.5 ± 4.9	18.2 ± 4.4	18.3 ± 4.7	4.3 ± 0.4	4.5 ± 0.4	4.5 ± 0.4	1.36 ± 1.3	1.30 ± 1.1	1.31 ± 1.5
50 %	18.3 ± 4.7	18.3 ± 4.5	18.3 ± 4.5	4.3 ± 0.3	4.5 ± 0.4	4.5 ± 0.4	1.82 ± 1.4	1.82 ± 1.4	1.84 ± 2.1
75 %	22.8 ± 5.1	22.0 ± 3.0	23.5 ± 4.0	4.5 ± 0.3	4.5 ± 0.3	4.5 ± 0.4	2.01 ± 1.6	2.3 ± 1.7	2.17 ± 1.6

Mean ± SD.

5.2 Prolonged responses to eccentric exercise (II, III, IV)

5.2.1 Maximal force production capability

Isometric MVC test. After the eccentric exercise isometric MVC was 21.3 ± 5.6% ($p < 0.001$) lower at 2H and 12.6 ± 11.1% ($p < 0.05$) lower at 2D as compared to the pre-exercise values, but was recovered at 4D (Fig. 14, ECC group (II, III, IV)).

Passive twitch force. Maximal passive twitch force followed the changes of isometric MVC, whereby it decreased from an initial level of 69.7 ± 19.2 N to 33.8 ± 10.0 N at 2H ($p < 0.01$), and to 46.1 ± 9.4 N at 2D ($p < 0.05$) post-exercise, but was not statistically different from the baseline value at 4D (4D: 56.0 ± 7.5 N; Fig. 14).

Voluntary activation level. No significant changes were observed in VAL (BEF: 94.8 ± 4.6%; 2H: 87.0 ± 9.2%; 2D: 93.0 ± 5.8% and 4D: 90.6 ± 7.9, Fig. 14).

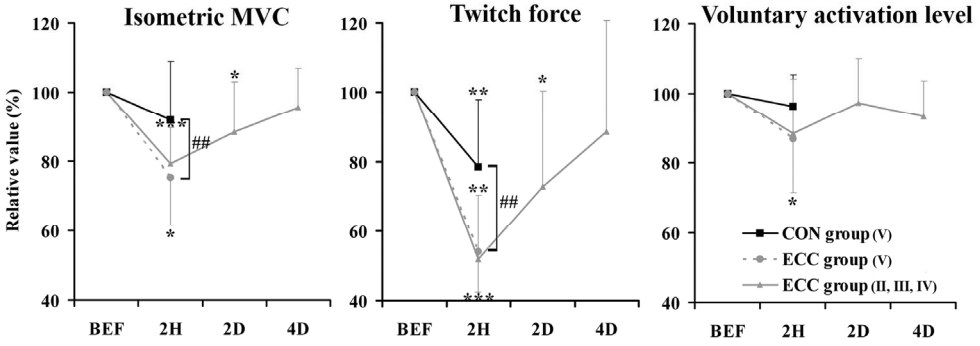

FIGURE 14 Relative values of isometric maximal voluntary contraction (MVC), maximal twitch force and voluntary activation level in concentric (CON) group (n=12), eccentric (ECC) group (n=12) of experiment V and eccentric group (n=9) of experiment II, III, IV. *** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$ with respect to the pre-exercise values. ## = $p < 0.01$ and # = $p < 0.05$ difference between the groups (CON and ECC, V). Error bars correspond to SD. Note that time axis is not continuous, but categorical.

5.2.2 Global high-density sEMG variables

Isometric MVC test. RMS and CV decreased from their initial levels (RMS: from 1.3 ± 0.5 mV to 1.1 ± 0.4 mV, $p < 0.05$; CV: from 4.1 ± 0.3 m/s to 3.8 ± 0.4 m/s, $p < 0.01$) at 2H, but were recovered at 2D (Fig. 15a and c). However, when RMS was normalized to peak-to-peak amplitude of the maximal M-wave no significant changes were observed. MNF decreased from an initial level of 92.6 ± 10 Hz to 85.2 ± 11 Hz at 2H ($p < 0.05$), remained lower at 2D (88.8 ± 10 Hz, $p < 0.05$), but was recovered at 4D (Fig. 15b).

Submaximal isometric contractions. Statistically significant reductions in RMS were observed at the three lowest submaximal force levels (10%, 20% and 30% of MVC) at 2D, and at the 10% and 30% MVC levels at 4D (Fig. 16a). The three highest submaximal force levels (40%, 50% and 75% of MVC) showed no significant time-dependent changes in RMS (Fig. 16a). MNF decreased markedly two hours post-exercise at 30%, 40%, 50% and 75% MVC levels (Fig. 16b). Furthermore, a prolonged reduction in MNF was observed at the 75% MVC level at 2D, and the 50% MVC level was lower at 4D (Fig. 16b).

CV changes were partly in line with the MNF findings in the submaximal isometric contractions. At 2H, CV showed a statistically significant decrease at the three highest submaximal force levels (40%, 50% and 75% of MVC), but not at the three lowest force levels (Fig. 16c).

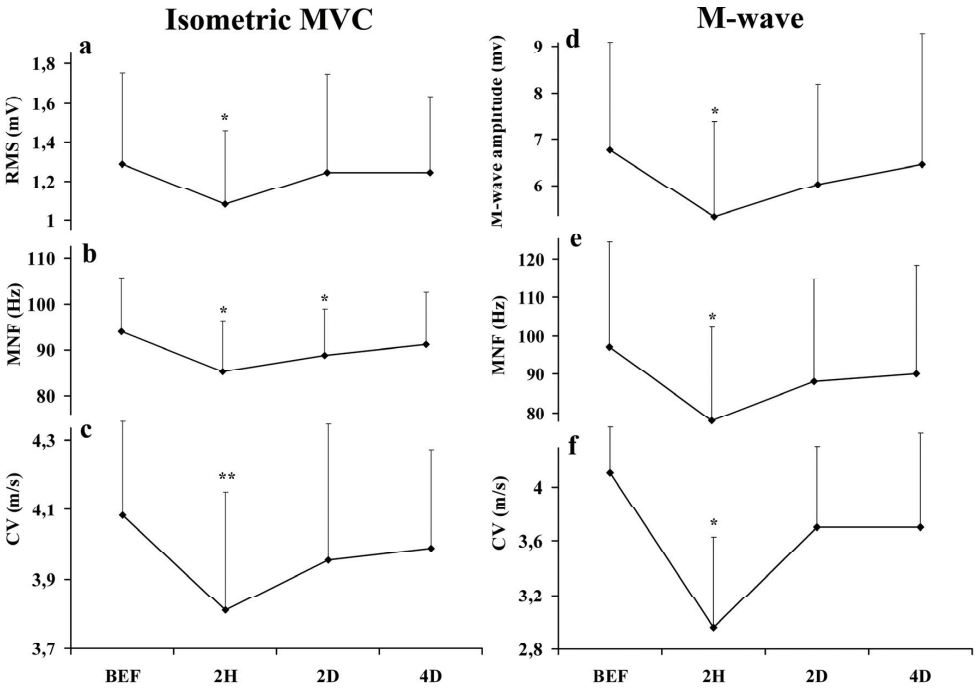

FIGURE 15 Surface EMG variables during isometric maximal voluntary contraction (MVC) test (a, b and c) and during maximal M-wave. ** = $p < 0.01$ and * = $p < 0.05$ with respect to the pre-exercise values. Note that time axis is not continuous, but categorical.

5.2.3 Maximal M-wave properties

Peak-to-peak amplitude of the maximal M-wave decreased from an initial level of 6.8 ± 2.3 mV to 5.3 ± 2.1 mV at 2H ($p < 0.05$), but was not statistically different from the baseline value at 2D (6.0 ± 2.1 mV) or 4D (6.5 ± 2.8 mV; Fig. 15d). Similarly, MNF and CV measured from the M-waves were both lower at 2H (MNF: 78.0 ± 24.4 Hz, $p < 0.05$ and CV: 3.0 ± 0.5 m/s, $p < 0.05$) post-exercise compared to the pre-exercise levels (MNF: 97.1 ± 7.2 Hz and CV: 4.1 ± 0.3 m/s). No changes were observed in MNF (2D: 88.0 ± 26.7 Hz and 4D: 90.0 ± 28.2 Hz) or CV (2D: 3.7 ± 0.6 m/s and 4D: 3.7 ± 0.7 m/s) during the recovery period (Fig. 15e and f).

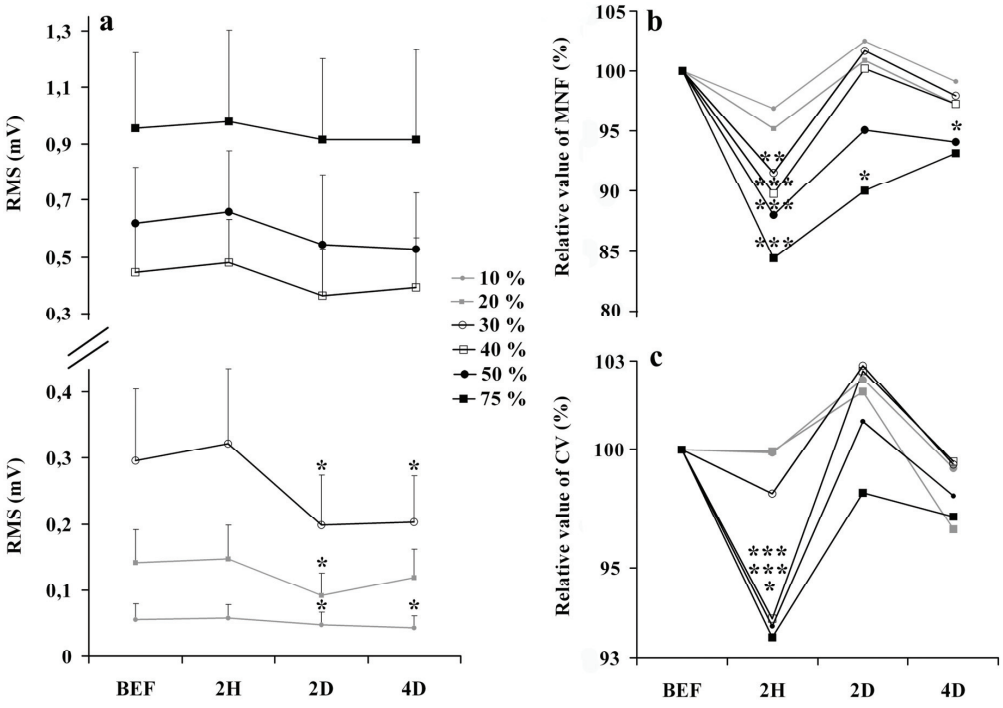

FIGURE 16 Mean (n=9) root mean square (RMS) values (a), relative values of mean power frequency (MNF) (b) and relative values of mean muscle fibre conduction velocity (CV) (c) over time in BBM muscle during submaximal isometric elbow flexion contractions. BEF = before, 2H = two hour, 2D = two days and 4D = four days post-exercise. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ with respect to the pre-exercise value. Note that time axis is not continuous, but categorical.

5.2.4 Motor unit characteristics

Number of identified MUs. In the exercise group, the total number of MUs with CoVISI ≤ 25%, identified in all the submaximal contractions of each session was as follows: BEF: 356 MUs, 2H: 265 MUs, 2D: 361 MUs and 4D: 373 MUs. Furthermore, the average number of MUs per contraction and subject across all contraction levels was: BEF: 6.6 ± 2.5 MUs, 2H: 4.9 ± 2.1 MUs, 2D: 6.7 ± 2.4 MUs and 4D: 6.9 ± 2.6 MUs. When compared to BEF values, the mean number of MUs identified at 2H was reduced at 30% (from 7.0 ± 2.2 MUs to 5.3 ± 2.9 MUs, $p < 0.01$), 40% (from 7.4 ± 3.5 MUs to 3.1 ± 1.1 MUs, $p < 0.01$), 50% (from 6.2 ± 2.2 MUs to 4.8 ± 1.9 MUs, $p < 0.05$) and 75% (from 6.2 ± 2.5 MUs to 4.0 ± 2.3 MUs, $p < 0.01$) of MVC. The number of identified MUs did not differ significantly between the different sessions with the exception of 2H.

Mean MU discharge rate. In the exercise group, MDR increased 2H post-exercise at the two highest contraction levels (50% and 75% of MVC) from 19.5 ± 4.4 pps (BEF) to 22.6 ± 5.0 pps ($p < 0.01$) and from 24.5 ± 5.6 pps (BEF) to 28.3 ± 3.0 pps ($p < 0.05$), respectively (Table 5 and Fig. 17d). However, MDR returned

to the pre-exercise level at 2D (50%: 19.0 ± 5.0 pps and 75%: 24.7 ± 5.2 pps) and 4D (50%: 17.7 ± 4.0 pps, and 75%: 24.5 ± 4.9 pps). CoVISI ranged from 13 ± 7% at 10% of MVC to 18 ± 6% at 75% of MVC, and did not change significantly between the sessions.

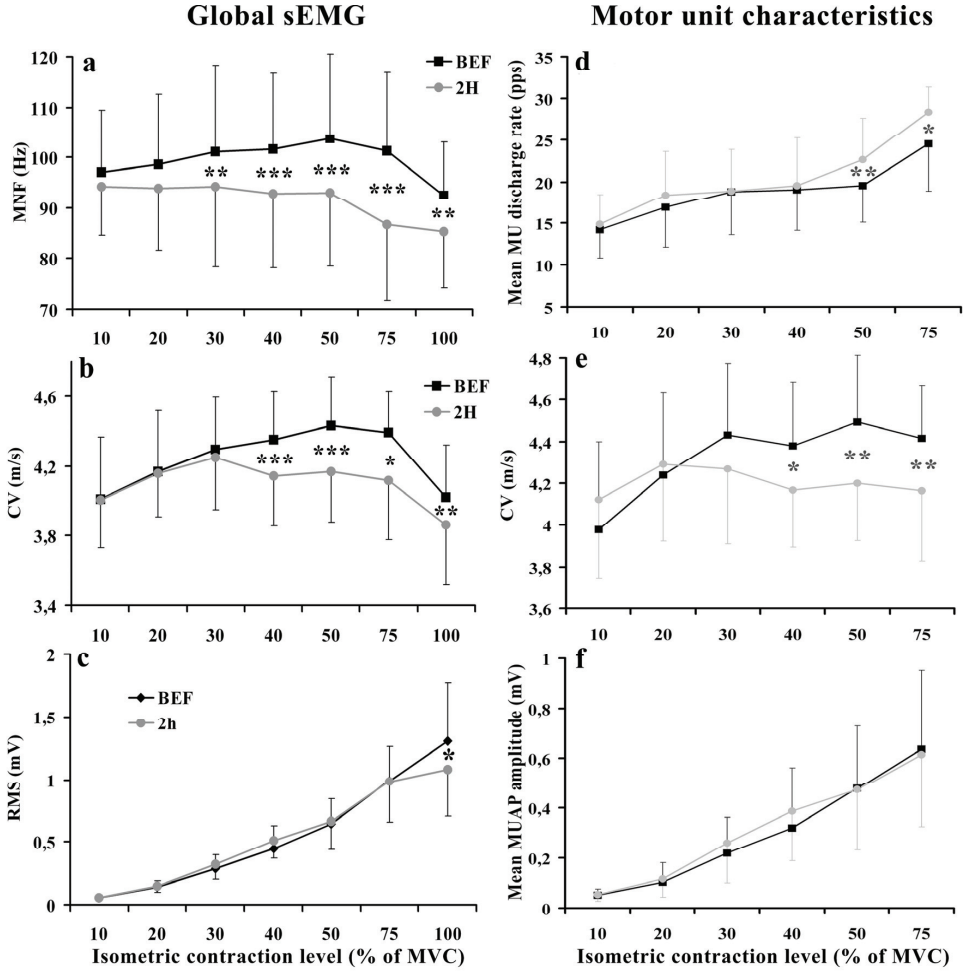

FIGURE 17 Global sEMG variables (n=9) (a, b and c, III) and mean MU characteristics (d, e, and f, IV) at different isometric contraction levels before (BEF) and two-hours post-exercise (2H). *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ between the 2H and BEF values.

Conduction velocity. In contrast to MDR, a reduction in mean CV was observed at 2H at 40% of MVC (from 4.4 ± 0.3 m/s (BEF) to 4.2 ± 0.3 m/s, $p < 0.05$), 50% (from 4.5 ± 0.3 m/s (BEF) to 4.2 ± 0.3 m/s, $p < 0.01$) and 75% of MVC (from 4.4 ± 0.3 m/s (BEF) to 4.2 ± 0.3 m/s, $p < 0.01$) (Table 5 and Fig. 17e).

Peak-to-peak MUAP amplitude. AMP did not show acute changes at 2H (Fig. 17f). However, a reduction from BEF values in AMP was observed at 20% of MVC (from 0.10 ± 0.08 mV to 0.07 ± 0.04 mV) and 30% of MVC (from 0.22 ±

0.14 mV to 0.18 ± 0.14 mV, $p < 0.05$) at 2D. Moreover, the three lowest contraction levels from 10% to 30% of MVC, showed a reduction in AMP at 4D: from 0.05 ± 0.02 mV (BEF) to 0.04 ± 0.02 mV ($p < 0.05$) at 10% of MVC, from 0.10 ± 0.08 mV (BEF) to 0.08 ± 0.04 mV ($p < 0.05$) at 20% of MVC and from 0.22 ± 0.14 mV to 0.18 ± 0.13 mV ($p < 0.01$) at 30% of MVC.

TABLE 5 Mean MU discharge rates (MDR) and mean MU conduction velocities (CV) in the exercise group (n = 9) at different submaximal isometric contraction levels relative to maximal voluntary contraction (MVC) before (BEF), two-hours (2H), two days (2D) and four days (4D) post-exercise.

	MDR (pps)				CV (m/s)			
MVC	BEF	2H	2D	4D	BEF	2H	2D	4D
10 %	14.1 ± 3.4	14.9 ± 3.6	13.8 ± 3.6	13.2 ± 3.5	4.0 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	4.0 ± 0.3
20 %	16.9 ± 4.8	18.4 ± 5.2	16.4 ± 4.2	16.4 ± 3.6	4.2 ± 0.4	4.3 ± 0.4	4.3 ± 0.5	4.2 ± 0.3
30 %	18.9 ± 5.2	18.9 ± 4.9	16.9 ± 3.7	16.3 ± 3.8	4.4 ± 0.3	4.3 ± 0.4	4.5 ± 0.5	4.4 ± 0.3
40 %	19.1 ± 4.9	19.5 ± 5.7	18.4 ± 4.4	17.3 ± 4.2	4.4 ± 0.3	**4.2 ± 0.3***	4.5 ± 0.5	4.4 ± 0.4
50 %	19.5 ± 4.4	**22.6 ± 5.0****	19.0 ± 5.0	17.7 ± 4.0	4.5 ± 0.3	**4.2 ± 0.3****	4.6 ± 0.4	4.5 ± 0.3
75 %	24.5 ± 5.6	**28.3 ± 3.0***	24.7 ± 5.2	24.5 ± 4.9	4.4 ± 0.3	**4.2 ± 0.3****	4.5 ± 0.4	4.4 ± 0.3

Mean ± SD, ** = $p < 0.01$ and * = $p < 0.05$, significant difference between the BEF and 2H values.

Regression slopes. When characteristics of the individual MUs of the exercise group were plotted against the relative force level, the slope of the regression line for MDR was significantly steeper at 2H than BEF ($p < 0.05$, Fig. 18a). The slope of the regression line for CV was significantly reduced at 2H with respect to BEF ($p < 0.05$, Fig. 18b). In the case of AMP, no significant changes in the slopes were observed (Fig. 18c). Furthermore, the validation group did not show significant changes in the regression slopes for MDR, CV or AMP between the different days (D1-1st and D2, Fig. 18d, e and f).

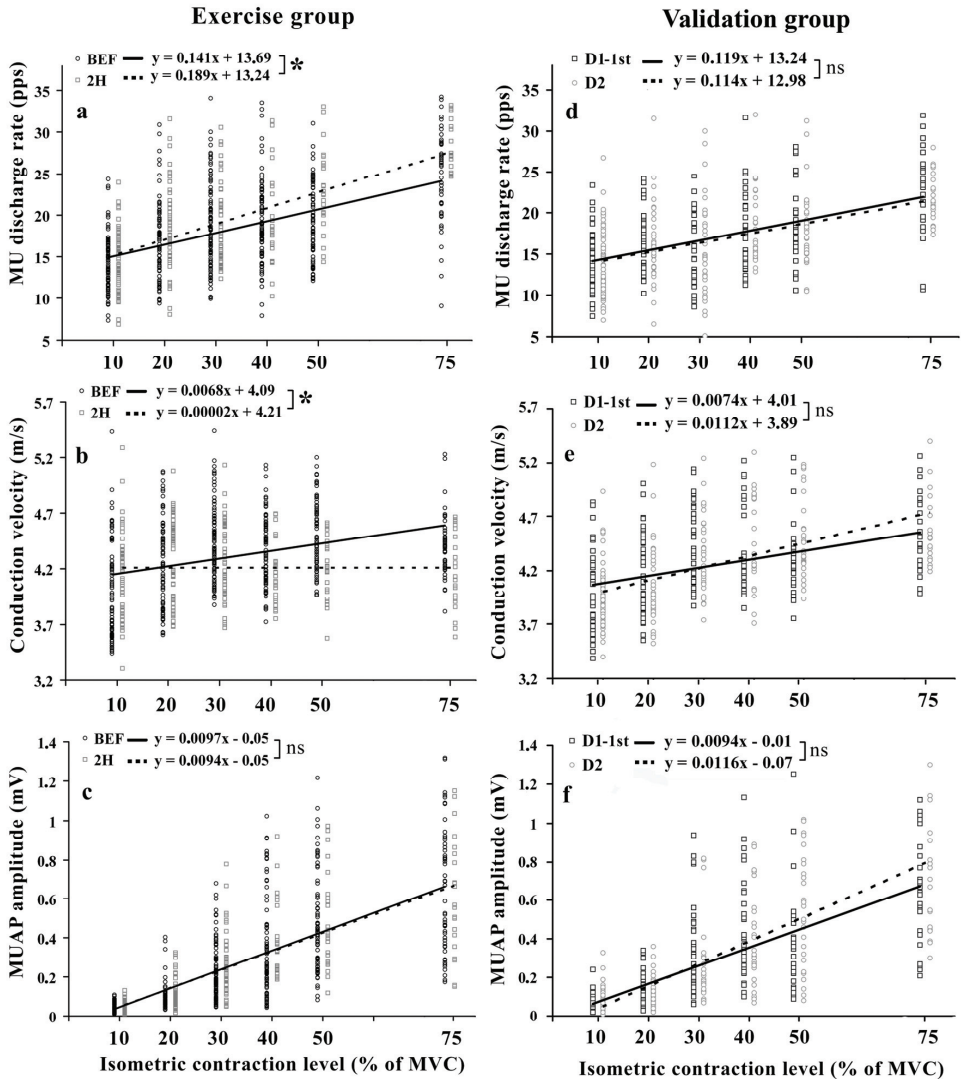

FIGURE 18 Discharge rate (a and d), muscle fibre conduction velocity (b and e) and peak-to-peak MUAP amplitude (c, f) of all identified MUs and their regression slopes against relative isometric contraction level in the exercise group (n=9) (left) before (BEF) and two-hours (2H) after the exercise, and in the validation group (n=7) (right) on day one (D1-1st) and day two (D2). * = $p < 0.05$, significant difference between the slopes of linear regression lines at BEF and 2H. ns = non-significant.

5.2.5 Antagonist muscle activity and skin temperature

Antagonist muscle activity was monitored and it remained at a constant low level throughout the experiment.

During the exercise, the skin temperature increased from an initial value of 32.9 ± 1.3 °C to 33.5 ± 1.4 °C ($p < 0.05$) at the mid-point of the exercise, and to

33.8 ± 1.4 °C ($p < 0.05$) at the end of the exercise, although it returned to the pre-exercise level prior to the 2H measurement session. Therefore, there were no statistically significant changes in skin temperature between the measurement sessions (BEF: 32.1 ± 1.2 °C, 2H: 32.6 ± 1.1 °C, 2D: 31.9 ± 1.2 °C and 4D: 31.8 ± 1.2 °C).

5.2.6 Muscle and subcutaneous tissue thickness and muscle soreness

Thickness of BBM muscle increased slightly from a pre-exercise value of 10.2 ± 2.2 mm to 11.7 ± 2.7 mm ($p < 0.05$) at 2H, and remained elevated up to 4D post-exercise (2D: 11.9 ± 3.4 mm, $p < 0.05$ and 4D: 12.8 ± 3.1 mm, $p < 0.05$). However, there were no changes in muscle thickness of brachialis muscle or subcutaneous tissue thickness after the exercise.

The subjects did not perceive soreness in the BB before the exercise. However, subjective perceived muscle soreness increased after the exercise (Fig. 19) and peaked in all subjects at 2D.

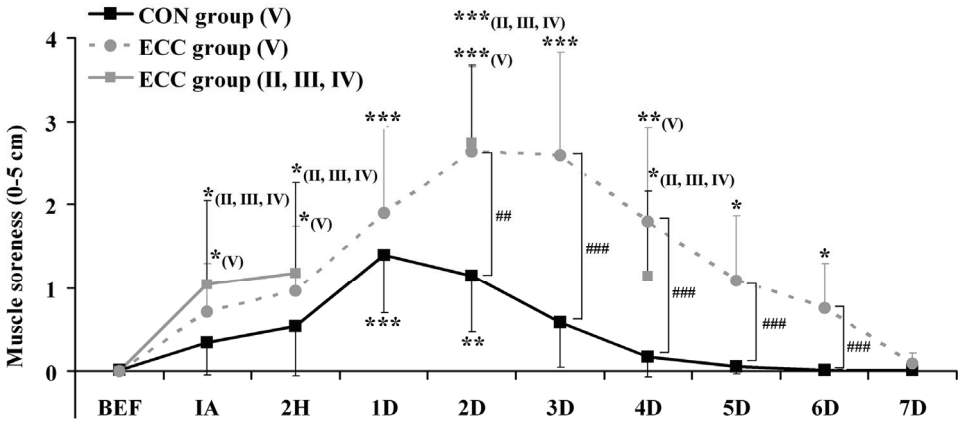

FIGURE 19 Subjective perceived muscle soreness before (BEF), immediately after (IA), two hours after (2H) and during the follow up period (1D-7D = day 1-day 7) in the concentric (CON) group (n=12), eccentric (ECC) group (n=12) of experiment V and ECC group of experiment II, III, IV. *** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$ with respect to pre-exercise values. ### = $p < 0.001$, ## = $p < 0.01$ and # = $p < 0.05$ difference between the groups (V). Note that time axis is not continuous, but categorical.

5.3 Effect of contraction type on acute responses (V)

5.3.1 Maximal force production capability

Isometric MVC test. Isometric MVC was reduced from BEF values (CON: 366 ± 74 N and ECC: 334 ± 108 N) in both groups at IA (CON: 280 ± 40 N, $p < 0.01$, and ECC: 234 ± 94 N, $p < 0.001$). More prolonged reduction in isometric MVC

was observed only in ECC group at 2H post-exercise (from BEF: 334 ± 108 N to 2H: 249 ± 93 N, $p < 0.01$). Furthermore, the reduction in isometric MVC was higher in ECC group at 2H post-exercise ($p < 0.01$) when compared to CON group (Fig. 14).

Passive twitch force. Maximal passive twitch force decreased from the BEF values in both groups at 2H post-exercise (CON: from 92.0 ± 17.9 N to 71.0 ± 17.3 N, $p < 0.01$ and ECC: from 78.2 ± 20.4 N to 42.0 ± 17.9 N, $p < 0.001$, Fig. 9). Furthermore, the reduction was significantly more prominent in the ECC group ($p < 0.01$, Fig. 14).

Voluntary activation level. VAL decreased in ECC group after the exercise (BEF: 84.7 ± 11.8 and 2H: 73.4 ± 16.1 N, $p < 0.05$, Fig. 14). However, this reduction in VAL was not significantly different from the CON group (BEF: 86.4 ± 10.1 N, 2H: 82.9 ± 10.3 N).

5.3.2 sEMG variables during maximal voluntary contraction

RMS. Both groups showed reduction in RMS both in BBM and BBL muscles both at IA and 2H after the exercises (Table 6). These reductions did not differ between the groups.

MNF. In BBM muscle, MNF showed reduction in ECC group ($p < 0.01$) at 2H post-exercise when compared to BEF values (Table 6 and Fig. 20b). Furthermore, this reduction was greater ($p < 0.05$) in ECC group when compared to CON group. In BBL muscle, only ECC group ($p< 0.01$) showed time dependent reduction at 2H post-exercise. However, this reduction was not significantly different from the CON group (Table 6).

CV. CON group showed increase in CV in BBM muscle at IA the exercise ($p < 0.001$, Table 6 and Fig. 20a). However, this change was not significantly different from the ECC group.

TABLE 6 Surface EMG during isometric MVC test.

		BBM muscle		BBL muscle	
		CON group	ECC group	CON group	ECC group
RMS (mV)	BEF	0.45 ± 0.17	0.37 ± 0.14	0.43 ± 0.20	0.28 ± 0.11
"	IA	**0.29 ± 0.14*****	**0.24 ± 0.10*****	**0.28 ± 0.15****	**0.20 ± 0.08****
"	2H	**0.30 ± 0.16***	**0.25 ± 0.14****	**0.32 ± 0.18***	**0.22 ± 0.11***
MNF (Hz)	BEF	105.4 ± 25.3	105.9 ± 27.4	85.6 ± 19.2	83.0 ± 25.4
"	IA	114.9 ± 32.6	104.7 ± 22.7	96.1 ± 28.8	80.4 ± 25.2
"	2H	**90.9 ± 14.9#**	**80.3 ± 12.7**#**	74.9 ± 15.6	**70.9 ± 18.5****
CV (m/s)	BEF	4.21 ± 0.54	4.16 ± 0.34	4.10 ± 0.58	4.29 ± 0.61
"	IA	**4.63 ± 0.47*****	4.24 ± 0.41	4.40 ± 0.62	4.25 ± 0.74
"	2H	**4.10 ± 0.49##**	**3.43 ± 0.44***##**	**3.69 ± 0.63***	**3.47 ± 0.79****

Mean ± SD, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$ with respect to before (BEF). # = $p < 0.05$, ## = $p < 0.01$ difference between concentric (CON) group (n=12) and eccentric (ECC) group (n=12).

At 2H time point, significant reductions were observed in CV of BBL muscle both in CON group ($p < 0.05$) and ECC group ($p < 0.01$). In BBM muscle,

reduction in CV was observed only in ECC group ($p < 0.001$) at 2H post-exercise (Table 6 and Fig. 20b). Moreover, this reduction in CV in the BBM muscle of the ECC group was significantly greater ($p < 0.01$) than in the CON group at 2H post-exercise (Table 6 and Fig. 20b).

5.3.3 Maximal M-wave properties

RMS. Initial value of RMS decreased in both CON group (BEF: 0.63 ± 0.28 mV, IA: 0.45 ± 0.24 mV, 30MIN: 0.43 ± 0.22 mV and 2H: 0.51 ± 0.26 mV, $p < 0.05$) and ECC group (BEF: 0.66 ± 0.35 mV, IA: 0.46 ± 0.23 mV, $p < 0.05$, 30MIN: 0.44 ± 0.25 mV, $p < 0.01$ and 2H: 0.36 ± 0.25 mV, $p < 0.01$) at IA, 30MIN and 2H after the exercises (Fig. 20c and d). In addition, the reduction in initial value of RMS was greater in ECC group when compared to CON group at 2H post-exercise ($p < 0.05$).

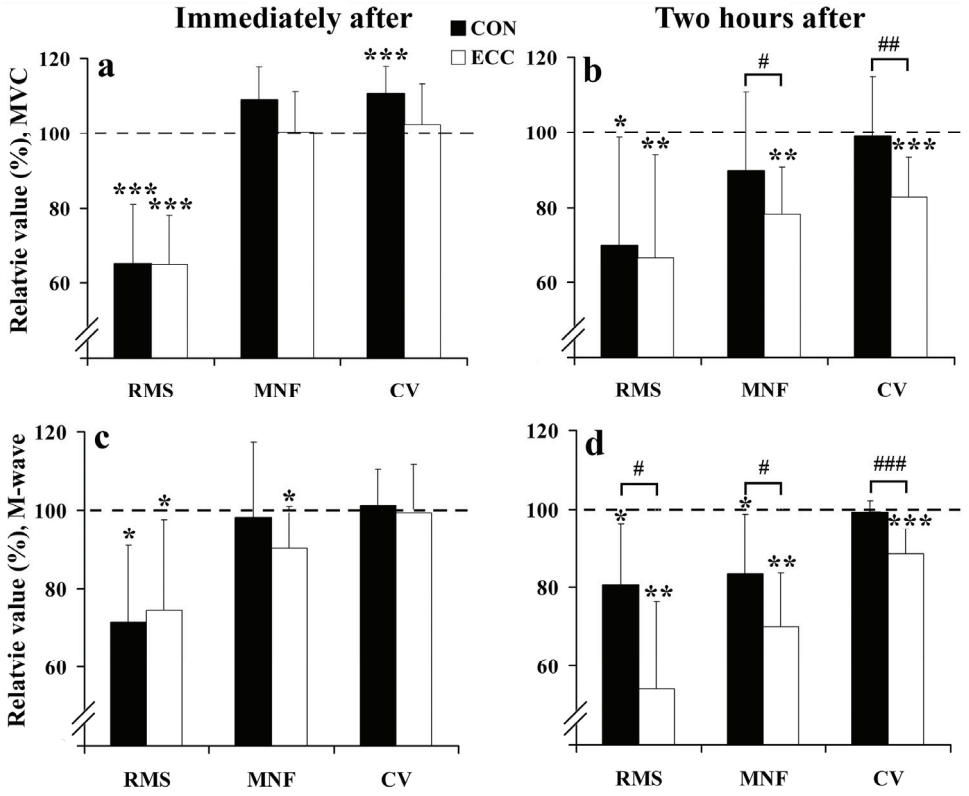

FIGURE 20 Relative values of sEMG variables measured from BBM muscle during isometric maximal voluntary contraction (MVC) test (a and b) and maximal M-wave (c and d) in concentric (CON) group (n=12) and eccentric (ECC) group (n=12). Dotted line corresponds to before exercise value. *** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$ with respect to the pre-exercise values. ### = $p < 0.001$, ## = $p < 0.01$ and # = $p < 0.05$ difference between the groups.

MNF. Initial value of MNF decreased in both CON and ECC groups at 2H post-exercise (BEF: 102 ± 24 Hz, 2H: 84.0 ± 20 Hz, $p < 0.05$) and ECC (BEF: 108 ± 30 Hz, 2H: 73.2 ± 17 Hz, $p < 0.01$), with greater reduction seen in the ECC group as compared to the CON group ($p < 0.05$, Fig. 20d). Moreover, significant reduction in initial value of MNF was observed in the ECC group also at IA (96.3 ± 25 Hz, $p < 0.05$) and 30MIN (88.0 ± 24 Hz, $p < 0.01$) time-points (Fig. 20c). The regression slope with absolute MNF values during the length of supramaximal monopolar electrical stimulation showed reduction only in ECC group (BEF: -2.64 ± 0.88 Hz/s and 2H: -1.86 ± 0.85 Hz/s, $p < 0.01$) at 2H post-exercise. This change was significantly different from the CON group ($p < 0.05$).

CV. Reduction of initial value of CV from BEF value of 4.33 ± 0.36 m/s was detected only in ECC group at 30MIN (3.91 ± 0.24 m/s, $p < 0.01$) and 2H (3.82 ± 0.3 m/s, $p < 0.001$) post-exercise (Fig. 20d). This reduction differed significantly from the CON group (30MIN, $p < 0.05$ and 2H, $p < 0.01$).

5.3.4 Antagonist muscle activity and skin temperature

There were no changes in the triceps brachii (antagonist) muscle activity. Brachialis (synergist) muscle did show a reduction in RMS in both groups, although only IA the exercise (CON: from 0.80 ± 0.37 mV to 0.51 ± 0.21 mV and ECC: from 0.80 ± 0.43 mV to 0.54 ± 0.28 mV, $p < 0.01$). This reduction in brachialis did not differ between the groups.

There were no statistically significant changes in skin temperature in CON (BEF: 33.4 ± 0.8 °C, IA: 33.7 ± 1.1 °C, 30MIN: 33.2 ± 0.6 °C and 2H: 33.2 ± 1.0 °C) or ECC group (BEF: 33.6 ± 0.7 °C, IA: 34.2 ± 0.8 °C, 30MIN: 33.5 ± 0.5 °C and 2H: 33.3 ± 0.8 °C) between the measurement sessions.

5.3.5 Muscle and subcutaneous tissue thickness and muscle soreness

Muscle thickness of BBM muscle did not change in CON (BEF: 11.6 ± 2.0 mm, 2H: 11.9 ± 2.1 mm) or ECC group (BEF: 13.3 ± 2.6 mm, 2H: 13.8 ± 2.7 mm). Similarly, the thickness of brachialis muscle did not change in CON (BEF: 29.4 ± 3.6 mm, 2H: 30.5 ± 3.2 mm) or ECC group (ECC: BEF: 27.7 ± 3.5 mm, 2H: 27.6 ± 3.1 mm).

The subjects did not perceive soreness in the BB muscle before the exercise. However, subjective perceived muscle soreness increased after the exercise in both groups (Fig. 19). Furthermore, the muscle soreness was greater in the ECC group as compared to CON group at 2D ($p < 0.01$), 3D, 4D, 5D and 6D ($p < 0.001$) post-exercise (Fig. 19).

5.3.6 Blood variables

Blood lactate concentrations showed slight, but non-significant increase in both groups IA the exercises with respect to the BEF values. The blood lactate concentration was thereafter on the pre-exercise level at 2H and 1D (Fig. 21a).

Blood myoglobin concentration increased in both groups at 2H when compared to pre-exercise level. In addition, ECC group showed acute increase in myoglobin concentration at IA the exercise and more prolonged elevation still at 1D post-exercise. Furthermore, the increase in myoglobin concentration was higher in the ECC group than in CON group at IA, 2H and 1D ($p < 0.05$, Fig. 21b).

Blood [K^+] showed increase in ECC group from BEF value of 4.17 ± 4.4 mmol/l to 4.42 ± 0.28 mmol/l at IA ($p < 0.05$). No change was observed in the CON group (BEF: 4.25 ± 0.35 mmol/l and IA: 4.33 ± 0.25 mmol/l). However, the change in [K^+] was not significantly different between the groups. Blood [Na^+] did not show significant changes.

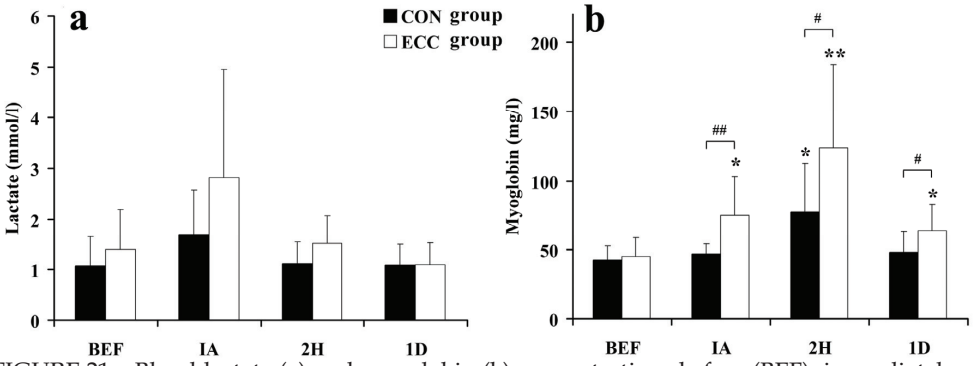

FIGURE 21 Blood lactate (a) and myoglobin (b) concentrations before (BEF), immediately after (IA) and two hours after (2H) the exercises in the concentric (CON) group (n=12) and eccentric (ECC) group (n=12). ** = $p < 0.01$ and * = $p < 0.05$ with respect to pre-exercise values. ## = $p < 0.01$ and # = $p < 0.05$ difference between the groups.

6 DISCUSSION

This study investigated the effects of intensive eccentric and concentric exercise on sarcolemmal function in global whole muscle and individual MU levels. Intensive eccentric exercise has previously been shown to induce prolonged loss of muscle force production and morphological changes in muscle fibres and its sarcolemma, which has been interpreted as damage. The current results indicated that sarcolemmal AP propagation can be impaired especially after eccentric exercise at relatively early phase of progression of EIMD, but is recovered prior to the recovery of the muscle force production capability.

6.1 Validation of methodology (I, II, IV)

Use of EMG to examine electrophysiological function of sarcolemma is less direct method when compared to experiments with isolated muscle fibres. However, it is the best method currently available for *in vivo* human studies, where traditionally standard single bipolar EMG electrodes have been applied. However, the standard EMG techniques have several methodological limitations that cannot be controlled, such as effect of anatomical structures and their shift due to physical activity on EMG signal and lack of reliable CV estimation (Farina et al. 2001, Merletti et al. 1999, Mesin et al. 2008, Rainoldi et al. 2004, Sadoyama et al. 1985). For this reason, development and validation of high-density sEMG methodology was performed in the current research to increase the accuracy of investigation of sarcolemmal function by overcoming some important limiting factors of sEMG methodology. This improved significantly the physiological relevance of the interpretations made from the current sEMG signals.

6.1.1 Innervation zone location

Shift of innervation zone with increasing isometric contraction level. The current results showed that IZ location can shift by an average of 0.6 ± 0.4 cm (10% vs. 100% MVC, $p < 0.001$, Table 2.) with increasing isometric contraction level in BBM muscle. Furthermore, this shift resulted in significant changes in RMS, MNF and CV of the nearby sEMG channels (Fig. 11). This is well in line with previous studies, since placement of sEMG electrodes over IZ has been shown to distort sEMG variables such as RMS, MDF and CV (Farina et al. 2001, Hogrel et al. 1998, Merletti et al. 1999, Rainoldi et al. 2004, Roy et al. 1986, Sadoyama et al. 1985). Therefore, it is important to pay attention to the distortion effect of IZ to sEMG signals. This was done in the current experiment by exclusion of the electrodes overlaying IZ from further analysis.

The current results are partly in line with observations of Masuda et al. (1985). They indicated a 0.2 cm IZ shift in a single individual with increasing isometric contraction level (30% and 40% of MVC) in BB muscle, but not at the other contraction levels (from 40% to 70% MVC). In the present experiment, a significant IZ shift was observed between wide ranges of isometric contraction levels (Table 2). Furthermore, Martin and MacIsaac (2006) have shown a significant IZ shift with changes in joint angle in BB, but not with increasing isometric contraction level (20%, 40% and 60% MVC). The reason for their contradictory findings could be a low number of subjects ($n = 5$), small relative difference in the isometric contraction levels (from 20% to 60% MVC) and spatially limited sEMG measurements (single array electrode with inter-electrode distance of 10 mm. In addition, spatial dependency of IZ shifts in the medial-lateral direction of the muscle cannot be taken into account if single array electrodes are used.

A shift in IZ location with increasing isometric contraction level could be explained by shortening of the muscle fibres, and thus a lengthening of the (distal) tendon due to increasing force. There seems to be some spatial dependency in this relationship, since the IZ shift was only significant in the four most medial electrode columns (Table 2). However, generalization of this issue must be made cautiously, because two subjects out of twelve exhibited a reversal in the spatial dependency of the IZ shift. The spatial dependency could be explained by uneven force transmission or load distribution in the muscle due to its anatomical features. Some evidence exists to support the notion of non-uniform spatial behaviour of muscles during contractions. Hodgson et al. (2006) observed a complex distribution of intramuscular velocities of muscle tissue during isometric contractions in the human soleus muscle with phase-contrast magnetic resonance imaging. Their results indicate that some parts of the muscle may move more than others, even during isometric contractions. In addition, the complexity of muscular force transmission, as reviewed by Huijing and Jaspers (2005), supports the possibility of uneven force transmission in the muscle tissue during contractions. Furthermore, Masuda and Sadoyama (1988) observed variation in the distribution of IZs in the medial-lateral direction of BB, and argued that these IZs may belong to different

MUs. We observed a similar dependency in many of our subjects. Such anatomical complexity combined with functional complexity in neural strategies, and mechanical variation in the intensity, speed and direction of the muscle contraction, may impair the quality of sEMG recordings, if these factors are not taken into account. Due to these factors, the current results cannot necessarily be generalized to other muscles than the studied BBM muscle.

In addition to the general anatomical and functional complexity of the muscle, there are individual differences in anatomy and function of the muscles. We observed that the location of the main IZ was highly variable along the BBM muscle belly between subjects. In addition, in many of our subjects, IZ was located in the region that has been suggested to be a suitable place for sEMG electrodes (Hermens et al. 1999). Furthermore, four of our subjects had two clearly different IZs separated by 3.3 ± 0.7 cm in the proximal-distal direction of the muscle. This is well in line with the findings of histochemical staining studies, where a 6 cm spread of motor end plates has been observed in the proximal-distal direction of human BB (Aquilonius et al. 1984a). Such complexity in IZ distribution is a significant confounding factor, for example in CV estimation (Nielsen et al. 2007). However, these limiting factors can be overcome with multi-channel sEMG recordings. This is possible because, the channels directly over IZ and at the end of the fibre regions of the muscle can be excluded from further analysis. In addition, a high number of sEMG channels allow the average to be calculated over all physiologically relevant channels. This improves the reliability of various sEMG variables by decreasing the variance due to electrode placement and changes in muscle geometry (Farina et al. 2001, Farina et al. 2004).

Reliability of innervation zone location measurement. IZ location showed very good trial-to-trial and day-to-day reliability, which indicates successful electrode repositioning. It should also be noted that the ICC of IZ location was lowest at the highest isometric contraction level (Table 3). This is probably due to the fact that maximal contraction can only be maintained for a few seconds, and is usually performed with higher fluctuations in the force level, leading inevitably to higher variation in the conditions for the determination of IZ location. It is also possible to reduce the influence of IZ region on sEMG variables by following the changes in muscle geometry (e.g. IZ shift in multi-channel sEMG recordings) simultaneously with sEMG recordings, and choosing the sEMG channels to be analysed accordingly. This could be particularly beneficial in dynamic muscle contractions involving large ranges of motion.

6.1.2 Spatially dependent changes

Current findings showed that changes in sEMG variables in the human BBM muscle in response to eccentric arm exercise are dependent on the location of the recording electrodes. Site-dependent changes in sEMG variables were evident in the transverse and longitudinal axes of the muscle. This finding is in line with those of a previous experiment by Hedayatpour et al. (2008). They

have shown site-dependent effects of eccentric exercise on the changes in sEMG amplitude and spectral parameters during sustained submaximal isometric contractions of quadriceps femoris muscle. However, they did not study these changes during maximal contractions as was the case in the current experiment.

The observed site-dependency could be explained by 1) uneven damage of muscle fibres or MUs (Jones et al. 1986a, Vijayan et al. 2001), 2) reduced neural drive in the MUs, due to possible central fatigue (Loscher & Nordlund 2002) and muscle pain (Le Pera et al. 2001, Madeleine et al. 2006), and 3) changes in muscle geometry, such as a change in muscle fibre length and orientation (Ishikawa et al. 2006). The current data emphasize the importance of geometrical properties of the muscle as an explanatory factor for the site-dependency, particularly the effect of IZ location. This is supported by the fact that the region where IZs were concentrated in the current subjects (between rows 6 to 10, Fig. 12 and 11) was less likely to exhibit significant changes in sEMG variables from pre- to post-exercise. The sEMG signals in the channels near IZ regions were of low quality, with a low amplitude and high frequency content (Fig. 13), as reported in many previous papers (Farina et al. 2001, Hogrel et al. 1998, Rainoldi et al. 2000, Rainoldi et al. 2004, Roy et al. 1986, Sadoyama et al. 1985). Therefore, RMS, MNF and CV values were biased in these channels, and thus systematic changes could not be clearly observed.

RMS values of the single bipolar electrodes were unaffected by the exercise in the current experiment, as has been the case in some previous studies (Bajaj et al. 2002, Chen 2003, Day et al. 1998, Michaut et al. 2001, Pearce et al. 1998, Semmler et al. 2007a). However, the global RMS, RMS of some of the transverse ROWS, and RMS of all the longitudinal COLUMNS decreased acutely. An acute decrease in RMS (< 1 day) has been shown previously (Piitulainen et al. 2008). Some studies have even shown a prolonged decrease in RMS after eccentric exercise (Hortobagyi et al. 1998, Kroon & Naeije 1991, Nie et al. 2007).). Furthermore, three of the 59 single bipolar channels showed a reverse trend in RMS changes compared to changes in global aRMS. These channels were near or at the edge of the average (n=9) IZ region (Fig. 13a). Similar variability was true also for spatial changes of MNF (Fig. 13b). Some previous studies have shown a decrease in MNF after exercise leading to EIMD (Chen 2003, Day et al. 1998, Jaskolski et al. 2007, Linnamo et al. 2000b, Warren et al. 2000). The current results showed that a small shift in IZ location due to methodological or exercise related reasons can dramatically change the RMS and MNF values in these channels. In such cases, the physiological conclusions would be invalid and should therefore be attributed to artifact and be neglected. Therefore, the use of multiple sEMG electrodes can improve the validity of sEMG measurements in muscle fatigue experiments, especially if the electrodes are arranged in arrays parallel to the muscle fibres. In such a system, the location of the recording electrode with respect to IZs and tendon regions can be detected (Sadoyama et al. 1985), and the focus can be on the physiologically relevant propagating part of the sEMG signal. In an ideal situation, a multichannel electrode-grid (Masuda & Sadoyama 1988) would be used to

obtain two-dimensional information about the electrophysiological events from the underlying muscle.

6.1.3 Decomposition of high-density sEMG signals

Performance of CKC method. At moderate contraction levels, the accuracy obtained by the CKC method is comparable to that obtained by decomposition of intramuscular recordings, as confirmed by the direct comparison of decomposition results from high-density sEMG and intramuscular recordings (Holobar et al. 2009, Holobar et al. 2010). In the current experiment, it was not possible to directly assess the decomposition accuracy, since the use of intramuscular recordings was not possible at the high contraction force levels. Therefore, extensive simulation tests (with simulated high-density sEMG signals) were performed in order to assess the capability of the CKC algorithm to accurately extract MDR of identified MUs at muscle contraction levels ranging from 20% to 75% of MVC. Although dependent on the excitation level, the average absolute error between the simulated and estimated MDR was ~ 0.2 pps, negligibly small when compared to ~ 4 pps differences in experimental MDR between pre-exercise and 2H post-exercise contractions (at 50% and 75% of MVC, Fig. 17d). Therefore, the performance and validity of the CKC decomposition algorithm was considered sufficient.

Consistency of CKC method. The consistency of CKC method was tested with experimental signals from separate group of subjects, where estimated MDR, CoVISI, and MUAP shapes were examined with a known physiological range of values (Table 4 and Fig. 18). The identified MDR, CV and AMP per contraction level remained constant throughout all sessions in the validation group (Table 4). Therefore, the CKC method was considered consistent.

Strict MU selection criteria were applied in the current experiment to minimize the risk for false identification of the MUs. All the MUs with CoVISI larger than 25% were excluded from further processing. The effect of strict MU selection was evident as lower number of identified MUs at high submaximal isometric contraction levels and at contractions performed 2H after the exercise, presumably due to the higher number of active MUs and their higher discharge rates. This resulted in more interference in the sEMG signal, and made its accurate decomposition more challenging. Nevertheless, the number of MUs analyzed for all subjects was in the order of hundreds. However, the proportion of MUs identified for single subject per contraction was still small (~ 6 MUs) when compared to the total number of 120 MUs in the BB muscle (Miller et al. 1993). This is still high number when compared to conventional intramuscular EMG recordings. Generalization of the CKC results to the whole muscle is only possible if MUs with different properties (size, type, etc.) are considered to have uniform distribution within the muscle cross-section. There is limited information available about this issue, however it has been shown that motor end plates are randomly distributed through out the cross section of BB muscle (Aquilonius et al. 1984b). It is also noteworthy that sEMG typically identifies large and superficial MUs, with the average depth up to ~ 1 cm in muscle tissue

(Holobar et al. 2009). Thus, although crosstalk from brachialis muscle beneath the BB muscle (thickness in the current subjects 10.2 ± 2.2 mm, III) is possible, it is highly unlikely that a significant number of MUs from brachialis muscle were detected in this study. The activity of the triceps brachii muscle was monitored during the experiment, and it did not change between the measurement sessions.

It is noteworthy that the recruitment thresholds of individual MUs were not examined, since this would have required an additional ramp prior to the desired contraction level, and would have resulted in muscle fatigue at the highest contraction levels. Due to this methodological limitation, only the MDR was investigated.

Finally, swelling of the muscle could be critical for sEMG decomposition, by changing the distance between the active MUs and pick-up electrodes. However, the swelling of BBM ranged from 1.5 mm to 2.6 mm at the 2H, 2D and 4D time points, and remained at the same level from 2H onwards (III). Furthermore, the subcutaneous tissue thickness remained unchanged throughout the experiment. Thus, the observed minor changes in the BBM muscle geometry are not considered a major limitation.

6.2 Responses to eccentric and concentric exercises (II, III, IV)

Eccentric exercise induced typical symptoms of EIMD, such as a prolonged reduction of maximal force production capability, a delayed increase in muscle soreness and an increase in the permeability of the muscle sarcolemma to myoplasmic proteins, indicated by an increase in blood myoglobin concentration. Similar, but significantly less evident symptoms were observed after concentric exercise. Furthermore, the eccentric exercise caused impairment in sarcolemmal AP propagation observed both in global muscle level and individual MU level and affected the MU control. In general, greater reductions were observed in MNF and CV after eccentric than concentric exercise, both during maximal voluntary and electrically elicited contractions. This difference was most evident at two hours post-exercise, where the CON group showed either a recovery or less significant reduction in sEMG variables than the ECC group. RMS values were reduced after both exercise modes, with a greater reduction after eccentric than concentric exercise, although this was only evident during electrically evoked contractions at two hours post-exercise.

6.2.1 Maximal force production capability

Previous investigations have observed a greater reduction of maximal voluntary force production capability after repeated eccentric contractions compared to repeated concentric contractions (Jones et al. 1989, Smith & Newham 2007). In addition, this reduction is often more prolonged after eccentric contractions (Jones et al. 1989, Smith & Newham 2007). The present

results are well in line with these findings, since the ECC group showed greater reductions than CON in isometric MVC test as well as in electrically evoked twitch force at 2H post-exercise (Fig. 14). Similar disparity in the reductions of twitch force and its evolution over time up to 4 hours after eccentric and concentric exercise has been shown explicitly by Smith and Newham (2007).

There are many possible explanations for these results. Most importantly, it appears that the eccentric exercise caused more "damage" to the muscle fibres than the concentric one, since the permeability of the sarcolemma increased more after the eccentric exercise indicated by a greater increase in blood myoglobin concentration (Fig. 21b) and the subjects reported higher muscle soreness levels after the eccentric exercise (Fig. 19), as is often the case (Jones et al. 1989). Therefore, the eccentric exercise caused greater symptoms of muscle damage and induced greater reduction in maximal voluntary force production capability, although it is not metabolically more demanding than the concentric exercise (Asmussen 1953). Although, all or most MUs are very likely recruited during maximal eccentric contraction, there is some evidence for lower neural drive and thus less MU activity during maximal eccentric contractions than concentric contractions (Westing et al. 1991), despite the fact that eccentric contractions often show higher maximal force values (Katz 1939, Westing et al. 1991). This disparity may be due to lower MU discharge rate during maximal eccentric contractions when compared to concentric ones, as has been observed in submaximal force levels (Søgaard et al. 1996). Eccentric MVC was also significantly higher than concentric MVC in the current research (average concentric MVC: 268 ± 64 N and eccentric MVC: 327 ± 64 N, $p < 0.001$). Therefore, it may be that especially FT fibres and their force transmitting sarcolemmas (Street & Ramsey 1965) are predominantly subjected to extremely high mechanical stress during sufficiently intensive or long lasting eccentric exercise. This is supported by morphological observations, where FT fibres show greater signs of damage than ST fibres after eccentric contractions (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b, Vijayan et al. 2001).

The current research focused on the role of sarcolemma in explaining the prolonged loss of force production associated to EIMD. The increased blood myoglobin concentration indicated that the current eccentric exercise model had a significant influence on the sarcolemma. This was reflected to its functional sEMG measures as well. Interestingly, the reduction in isometric MVC and electrically evoked twitch force was accompanied by a simultaneous reduction in sarcolemmal AP CV and other sEMG variables (Fig. 14 and 9). Most of the sEMG variables were recovered two days post-exercise, and all sEMG variables were completely recovered four days post-exercise, as was the maximal force production capability. For example, the recovery of MNF seemed to follow the recovery pattern of maximal isometric force production (Fig. 14 and 9b). A similar concurrent recovery pattern of sEMG power spectral variables and voluntary maximal force production has been observed previously by several investigators (Chen 2003, Day et al. 1998, Jaskolski et al. 2007). Nevertheless, it seems that intensive eccentric exercise may induce impairment in sarcolemmal function, which may at least partly explain the

reduction in maximal force production during the recovery period as possible site for E-C coupling failure.

MVC may be also reduced due to fatigue of spinal and/or suprasipnal origins. In the current research the central fatigue was monitored by measurement of voluntary activation level (VAL). The eccentric exercise did result in a reduction in VAL at 2H post-exercise, although this change was not different from the CON group (Fig. 14). However, the reduction in VAL cannot solely explain the reduction of isometric MVC, since clear between group differences were observed in electrically evoked passive twitch force, where central factor are bypassed by supramaximal electrical stimulation of the final common pathway (motor nerve).

6.2.2 High-density sEMG variables

In the current research, the function of sarcolemma was described with high-density sEMG variables in maximal isometric voluntary and electrically evoked contractions and in submaximal isometric voluntary contractions. In the latter case, the observations were done both in the global whole muscle level and for individual MU level. Sarcolemmal excitability was estimated with measurement of sEMG RMS amplitude and the sarcolemmal AP propagation properties were described with CV and MNF.

The focus of the current research was on BBM muscle (I, II, II, IV, V), although BBL muscle was also measured, but only in the last experiment (V). The results were similar in both heads of BB muscle (BBM and BBL, Table 6). Since most of the measurements were done only for the BBM muscle the focus of the following discussion will be on it.

Isometric MVC and M-wave. In general, the greatest reductions in sEMG variables were seen 2H post-exercise, with the exception that RMS was already significantly reduced IA the exercise in both ECC and CON groups (Fig. 16 and 13). Only MNF showed reduction still at 2D after eccentric exercise, while other variables had returned back to pre-exercise level (Fig. 16).

The acute (≤ 2 hours) reduction of RMS after both eccentric and concentric exercise indicates total loss of the excitability of the sarcolemma in some muscle fibres, or more general reduction in the AP amplitudes of many fibres. Furthermore, the reduction in RMS was significantly greater after eccentric than concentric exercise as M-wave RMS amplitude indicated (Fig. 20). These findings are in line with literature, since previous experiments have shown either an acute reduction (< 1 day) in RMS (Hortobagyi et al. 1998, Michaut et al. 2002, Piitulainen et al. 2008), a more prolonged reduction (≥ 1 day) in RMS (Hortobagyi et al. 1998, Kroon & Naeije 1991), or no reduction in RMS (Chen 2003) after eccentric exercise comparable to the current exercise model. There are some interesting findings available in the literature to explain the impaired sarcolemmal excitability.

Firstly, sarcolemmal AP amplitude may decrease due to a change in resting membrane potential because of modified ion concentration gradients over the sarcolemma, as a result of increased sarcolemmal permeability (McNeil

& Khakee 1992) caused by activation of stretch activated Na^+ and Ca^{2+} ion channels (McBride et al. 2000, Stauber 1989) and/or acute sarcolemmal damage (McNeil & Khakee 1992) as has been observed after eccentric loading. Secondly, it is known from morphological experiments that especially FT fibres are prone to EIMD (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b, Vijayan et al. 2001), and these fibres are the main contributors to RMS values due to their large MU size and thus their high MUAP amplitude. The current results showed that especially the MUs that dominate the sEMG signals at high contraction levels were most affected (see Fig. 17 and discussion below). Therefore, it is likely that some of the high threshold MUs may have lost their excitability or had significant reduction in their MUAP amplitude especially after the eccentric exercise. Thirdly, one possible factor that may explain the reduced RMS is the fact that CV was significantly reduced after eccentric exercise and with greater degree than after the concentric exercise. This together with possible increase of depolarization zone length (Merletti et al. 1999) will cause a change in muscle fibre AP shape and thus may decrease AP amplitude. Fourth possible explanation could be impaired neuromuscular transmission, although this is very improbable event, since McFadden and McComas (1996) have argued that impairment in the neuromuscular junction is unlikely, since the safety factor for proper function of neuromuscular junctions is high due to a high concentration of postsynaptic Na^+ channels (Flucher & Daniels 1989a), especially in FT fibres (Ruff & Whittlesey 1992).

Most of the aforementioned events are shown to occur after eccentric exercise, but not as clearly after concentric exercise. Therefore, it was surprising that no differences between the groups were observed in RMS during the isometric MVC (Fig. 20b). However, the initial value of RMS during the monopolar motorpoint electrical stimulation showed a greater reduction of RMS in the ECC group than the CON group at 2H post-exercise (Fig. 20d). The latter result from the electrically evoked contractions is probably a more valid indicator of gross sarcolemmal fatigue, since during the voluntary contractions the RMS values are affected by control strategies and possible fatigue of the central nervous system. The reduction in VAL in ECC at 2H could suggest central fatigue at the supraspinal level or at the spinal level, although the specific site of reduced voluntary activation related to EIMD has not been localized yet (Prasartwuth et al. 2005, Racinais et al. 2008).

In addition to the loss of sarcolemmal excitability, sarcolemmal AP propagation properties were also impaired at early phase of EIMD. This was evidenced by reduction of CV and MNF in both isometric MVC and electrically elicited conditions 2H after eccentric exercise, while no reduction or significantly less pronounced reduction was observed after concentric exercise (Fig. 15b, c, e, f and 13b, d). These results are in line with the literature, since acute (≤ 2 hours) reductions of MNF (Linnamo et al. 2000a, Sbriccoli et al. 2001) has been detected after eccentric exercise when measured during maximal voluntary and electrically evoked contractions. To our knowledge, the effect of maximal eccentric and concentric exercise on CV has not been studied during maximal conditions before this series of experiments. However, a reduction of

MNF has been observed during MVC after maximal concentric exercise (Linnamo et al. 2000a).

The possible explanation for the impairment of sarcolemmal AP propagation after eccentric exercise overlaps the ones discussed above for RMS. The greater increase in sarcolemmal permeability, as the increased blood myoglobin concentration indicated (Fig. 21b), may disturb the normal ion concentrations (e.g. $[Na^+]$ and $[K^+]$) over the sarcolemma, and possibly slow down or even block the sarcolemmal or transverse tubular system AP conduction. It has indeed been argued that sarcolemmal AP propagation velocity may be dependent on interstitial $[K^+]$ (Hodgkin & Horowicz 1959, Juel 1988). For this reason, the currently observed increase in plasma $[K^+]$ in the ECC group could partly explain the greater reductions of M-wave RMS, MNF and CV in the ECC group. There is evidence that extracellular $[K^+]$ may increase during muscular activity due to leakage of K^+ from ATP and $Ca2^+$-sensitive channels (Burton et al. 1988, Pallotta 1985). Nevertheless, there seems to be a large flow of K^+ into the interstitial space during muscle activity (Sjogaard 1990). Furthermore, it may be that the increase in sarcolemmal permeability due to its initial wounding (McNeil & Khakee 1992) and activation of different stretch activated Na^+ and Ca^{2+} channels (McBride et al. 2000, Stauber 1989) preferentially loads the Na^+-K^+ pumps in the eccentrically exercised FT fibres. Interestingly, the reduction of immediate energy stores, adenosine 5'-triphosphate (ATP) and phosphocreatine (PCr), supplying the ion pumps show a greater depression in FT fibres than slow twitch fibres shortly (< 1.5 min) after 25 s of maximal sprint cycling (Karatzaferi et al. 2001), and the depression in ATP and PCr is still evident 6 min after similar all out effort (Bogdanis et al. 1995). However, it seems that the loss of ATP and PCr are insignificant immediately after cessation of eccentric exercise (Bonde-Petersen et al. 1972) and the Na^+-K^+ pump seems to have a high safety factor, since only 2-6% of its capacity is in use under normal conditions (Clausen 1986). Furthermore, the activation of stretch activated Na^+ channels has been observed to cause a depolarization of resting membrane potential for more than 24 hours after eccentric contractions (McBride et al. 2000), which may cause a slow inactivation of voltage-gated TTX sensitive Na^+ channels that are largely responsible for AP propagation (Ruff 1999). Therefore, the inactivation of voltage-gated Na^+ channels could block or slow down the AP propagation in the affected muscle fibres and thus result in a depression of the M-wave amplitude and power spectral variables.

Submaximal voluntary isometric contractions. The current results showed that, sarcolemmal AP conduction was impaired only during high force levels (50 - 100% of MVC) of the sub- and maximal isometric contractions (Fig. 17), where MUs that comprise of FT fibres are likely to dominate the sEMG signals. These are the same group of fibres known to be the most sensitive to show morphological changes after eccentric contractions (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b, Vijayan et al. 2001).

RMS did not differ from the pre-exercise values at any of the submaximal force levels at 2H (Fig. 17c). This suggests that the same number of MUs were

active, although the absolute force levels differed due to muscle fatigue. However, it is possible that depolarization zones of the APs may have elongated due to the eccentric exercise. This could increase RMS of sEMG because of greater temporal overlapping of APs (Dimitrova & Dimitrov 2003). The recruitment of MUs comprising of FT fibres in the BB muscle begins extensively from at 40% of MVC onwards (Gydikov & Kosarov 1974) and continues up to 88% of MVC (Kukulka & Clamann 1981). In the present experiment, CV and MNF at the highest submaximal force levels (40-75% for CV and 30-75% for MNF) were clearly reduced at the 2H point (Fig. 17a, b). Before the eccentric exercise, MNF and CV showed an increasing trend with increasing isometric force level up to 50% of MVC, but this relationship was biased at the higher force levels two hours post-exercise (Fig. 17a, b). These findings suggest that the sarcolemmal AP propagation of higher threshold MUs could be predominantly affected after eccentric exercise. Based on Henneman's size principle (Henneman 1957), this result is well in line with previous findings of the susceptibility of FT fibres to disruption of their membrane systems after eccentric contractions (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b, Vijayan et al. 2001).

The susceptibility of FT fibres to disruption is usually explained by mechanical differences in the contractile properties of FT and ST fibre types (Lieber et al. 1991, Macpherson et al. 1996). This is especially true in eccentric muscle contractions, since maximal eccentric contractions usually involve higher forces than maximal isometric and concentric contractions, which was true also in the current research (average concentric MVC: 268 ± 64 N and eccentric MVC: 327 ± 64 N, $p < 0.001$). Moreover, based on voluntary activation level and sEMG measurements, it appears that there is less voluntary drive to muscle and thus less MU activity during maximal eccentric contractions than in maximal concentric or isometric contractions (Babault et al. 2001, Eloranta & Komi 1980, Westing et al. 1991). Furthermore, Søgaard et al. (1996) have shown, albeit only at very low submaximal force levels (10% of MVC), that the same number of MUs are firing but with lower firing rates during eccentric contractions than in concentric contractions. In any case, it seems that higher mechanical stress is applied over a smaller number of active muscle fibres during maximal eccentric contractions, and most importantly, over their force transmitting sarcolemma (Street & Ramsey 1965). In addition, there is some evidence that high threshold MUs could be selectively recruited before lower threshold MUs during voluntary eccentric contractions at low force levels (Howell et al. 1995, Nardone et al. 1989). In contradiction to those results, many authors have not found differences in the recruitment order between contraction types, but again only at low force levels (Pasquet et al. 2006, Søgaard et al. 1996, Stotz & Bawa 2001).

It has been shown that sEMG amplitude may increase in isometric submaximal constant-force tasks immediately after eccentric exercise, although the submaximal force level was expressed relative to the post-exercise MVC force (Semmler et al. 2007b). This phenomenon has been explained by an increase in MU synchronization and prolonged AP duration (Dartnall et al.

2008). In contrast to these findings, no increase in RMS was observed in any of the isometric submaximal contractions in any of the follow-up measurements in the present research (Fig. 18 and 11). However, the earliest follow-up measurements were not performed until 2H post-exercise. Therefore, the immediate increase in RMS in isometric submaximal contractions observed by Semmler et al. (2007b) may involve different mechanisms to the later events that occur in the presence of EIMD. These contradictory findings may also be explained by the differences in the applied exercise models. If the eccentric exercise is submaximal and conducted with short recovery periods between consecutive contractions, it may be that MUs with lower recruitment thresholds are more affected than with the currently used exercise protocol with relatively long recovery periods. Interestingly, RMS decreased at the three lowest isometric submaximal force levels (10%, 20% and 30% of MVC) at 2D (Fig. 16a). This reduction suggest that MUs with lower recruitment thresholds are 1) less affected than the higher threshold MUs by the current exercise model and/or 2) their recovery occurs more rapidly. This is supported by the fact that isometric MVC was still reduced at 2D and thus the absolute submaximal contraction levels were at lower force level as compared to BEF. Therefore, the task demand for the lower threshold and less affected MUs might have been lower.

Finally, since the comparisons between eccentric and concentric exercises were done only in during MVC and supramaximal nerve stimulation, involving contribution from most if not all MUs, it may be that eccentric exercise affects the gross sarcolemmal function of the FT fibres substantially more than concentric exercise. However, the effect of maximal concentric exercise on different threshold MUs should be studied in more detail to fully confirm this hypothesis.

6.2.3 Single motor unit characteristics (IV)

Such as was the case in global whole muscle sEMG variables, single MU characteristics were only altered 2H after the eccentric exercise in the following manner: 1) MDR increased at the two highest isometric contraction levels; 2) a reduction in CV was observed at the three highest isometric contraction levels; 3) the regression slope between individual MU discharge rates and relative force level was steeper than pre-exercise; and 4) the regression slope between CV and relative force level was lower than pre-exercise.

Mean discharge rate. Before the eccentric exercise, MDR, CV and AMP increased with increasing isometric contraction level (Fig. 17 and 12), as is normally the case with recruitment of additional faster and larger MUs in non-fatigued muscle (Andreassen & Arendt-Nielsen 1987, Freund et al. 1975, Moritani & Muro 1987, Sadoyama & Masuda 1987). However, 2H after the eccentric exercise, MDR was higher than BEF at the two highest isometric contraction levels (50% and 75% of MVC, Fig 11d). Furthermore, when individual MU discharge rates were plotted against relative contraction level, the regression slope was significantly steeper at 2H than BEF (Fig. 18a). At the same time, the largest decline in maximal muscle force production was

observed (Fig. 14). However, there were no changes in MDR at any of the lower contraction levels (Table 5. and Fig. 17d). These results indicate a greater increase in the rate coding of active MUs per unit of force at 2H than pre-exercise. This suggests that at high contraction levels the central nervous system attempts to compensate for impaired force production of the muscle by increasing the rate coding of the active MUs, and possibly also with the recruitment of additional MUs 2H post-exercise. Some supporting evidence for this hypothesis exists, since a reduction in recruitment thresholds of MUs and an increase in minimum MU discharge rate has been observed in BB muscle after submaximal eccentric exercise (Dartnall et al. 2009). However, it should be noted that these results were limited to low contraction levels (≤ 20% MVC) and the increase in discharge rate was only observed immediately after the exercise, but not at the 24 hour time-point.

Based on Henneman's size principle (Henneman 1957), the larger and faster MUs are recruited after the smaller and slower ones in isometric contractions. In the BB muscle, which contains a larger proportion of FT fibres (~ 60%) than slow twitch fibres (~40%) (Johnson et al. 1973), this recruitment continues up to approximately 88% of MVC (Kukulka & Clamann 1981), and the recruitment of larger and faster MUs appears to begin extensively at 40% of MVC onwards (Gydikov & Kosarov 1974). It is noteworthy that in the current experiment, statistically significant changes in both MDR and CV were only observed at or above the 40% MVC level. This observation is in line with previous morphological findings of the selective effect of eccentric contractions on FT fibres, while the slow twitch fibres of the lower threshold MUs remain intact (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b, Vijayan et al. 2001).

Mean MU conduction velocity. In normal situations, an increase in isometric contraction force is observed with a simultaneous increase in muscle fibre CV (Andreassen & Arendt-Nielsen 1987, Sadoyama & Masuda 1987). However, when individual MU CV values were plotted against relative contraction level, the regression slope was significantly lower at 2H than BEF (Fig. 18b). This was mainly due to a reduction in CV at the three highest isometric contraction levels (40%, 50% and 75% of MVC) at 2H when compared to pre-exercise values (Table 5 and Fig. 17e). Therefore, MUs with high CV seemed to be more affected by the eccentric exercise. This result emphasizes the possibility of predominant impairment of sarcolemmal AP conduction in MUs comprising of FT fibres 2H post-exercise. The explanation for this is related to sensitivity of FT fibres to disruption of their sarcolemma and transverse tubular system after eccentric contractions (Jones et al. 1986a, Lieber & Friden 1988, Takekura et al. 2001b), as discussed above at the global whole muscle level (see discussion in 6.2.2).

Several studies have observed direct morphological damage of muscle fibres in the early stages (< 6 hours) after eccentric contractions (McNeil & Khakee 1992, Newham et al. 1983a, Takekura et al. 2001b, Vijayan et al. 2001), although the damage has usually become more severe at the later stages (> 12 hours) of the progression of EIMD (Jones et al. 1986a, Newham et al. 1983a,

Takekura et al. 2001b, Vijayan et al. 2001). In general, it appears that only mild damage is usually observed in the time window (2H post-exercise) where the greatest reduction in CV was observed in the current research (Fig. 17b, e). More strikingly, CV was already recovered two days post-exercise, when morphological changes are usually the most severe. This contradiction may be explained by the findings of McNeil and Khakee (1992). They observed an acute increase in sarcolemmal permeability immediately after cessation of eccentric exercise, but these symptoms were already attenuated 24 hours post-exercise, probably due to the sealing of most of the membrane wounds. Therefore, it may be that the slowing of sarcolemmal AP conduction is mainly due to an early increase in sarcolemmal permeability caused by initial wounding (McNeil & Khakee 1992) and activation of stretch-sensitive ion channels (McBride et al. 2000, Yeung et al. 2003), and consequently an acute disturbance of intra and extracellular ion concentrations (Balnave & Allen 1995a, Yeung et al. 2003). Furthermore, it appears that the latter delayed morphological abnormalities are not necessarily related to a disturbance in ion concentrations over the sarcolemma at the latter stages of EIMD (> 2 days), but could instead be related to adaptive processes in the muscle fibres (Yu et al. 2004).

6.3 Possible mechanisms for impaired sarcolemmal function

Main objective for this research was to investigate *in vivo* the role of sarcolemma for prolonged loss of force production capability associated to EIMD. For this reason, the possible mechanisms for impaired sarcolemmal function after maximal eccentric exercise will be summarised in this chapter.

The mechanisms behind EIMD are complex and diverse involving contribution of different biological structures of the neuromuscular system (Fig. 4). In the literature, based on animal and/or human models, several hypotheses have been presented to explain the mechanisms behind EIMD and the accompanied prolonged loss of muscle force production (Balnave & Allen 1995b, Belcastro et al. 1998, Lieber & Friden 1988, Morgan & Allen 1999, Warren et al. 1999). The sarcolemma has been suggested to be involved (Fuglevand 1995), but is only one possible factor among others.

The possible impairment of sarcolemmal function remains unclear in human subjects due to methodological limitations. With high-density sEMG system the effect of some limitations can be suppressed and thus it is currently the best method to study electrophysiological function of sarcolemma. Based on the current high-density sEMG results it seems that the function of sarcolemma is affected at early the phase of EIMD. The principal reason for impaired sarcolemmal AP propagation seems to be disturbance of ion concentrations over the sarcolemma. In particular, interstitial accumulation of K^+ is of importance (Hodgkin & Horowicz 1959, Juel 1988). There is a large flow of K^+ into the interstitial space during muscle activity (Sjøgaard 1990). The leakage of K^+ can occur for example through ATP and Ca^{2+}-sensitive channels (Burton et al.

1988, Pallotta 1985) (Fig. 22). Furthermore, McNeil and Khakee (1992) showed that, eccentric exercise is associated with initial wounding of the sarcolemma, which may enhance the K^+ leakage. However, the initial wounding was attenuated 24 hours post-exercise, probably due to the sealing of most of the membrane wounds. Therefore, it may be that the slowing of sarcolemmal AP conduction is due to an early increase in sarcolemmal permeability caused by the initial wounding. It is also known that, the morphological changes often become more severe at the later stages (> 12 hours) of EIMD (Jones et al. 1986a, Newham et al. 1983a, Takekura et al. 2001b, Vijayan et al. 2001). Interestingly this is not the case for sarcolemmal function, since it showed partial recovery at 2D and full recovery at 4D (Fig. 15). Therefore, the delayed morphological abnormalities may be more related to adaptive processes in the muscle fibres (Yu et al. 2004), while the functional recovery of the sarcolemma occurs more rapidly.

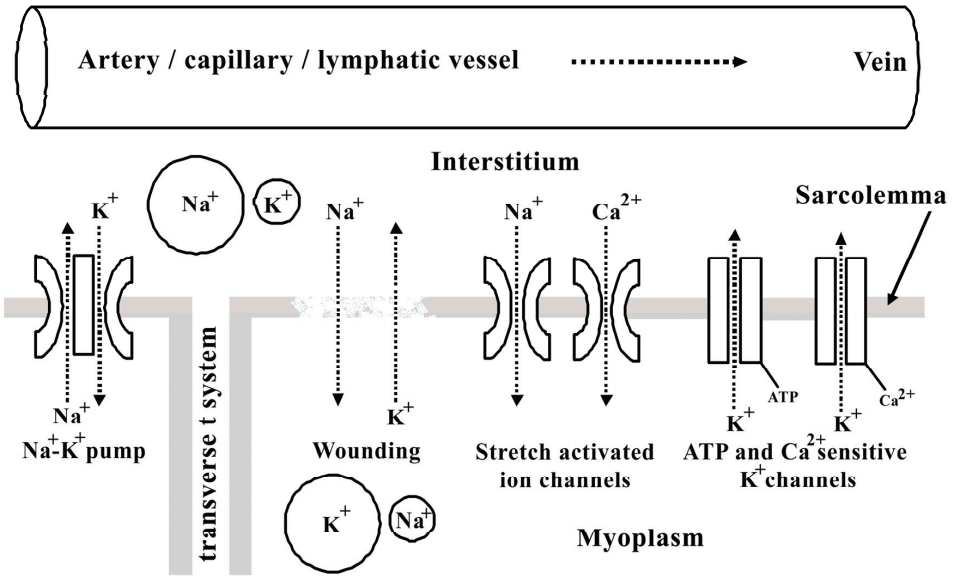

FIGURE 22 Eccentric exercise induced events that could enhance muscle activity related increase in interstitial $[K^+]$ and thus lead to sarcolemmal dysfunction, such as damage of sarcolemma and leakage from ATP and Ca^{2+} sensitive K^+ channels. The increase in interstitial $[K^+]$ is opposed by Na^+-K^+ pump and washout into blood and lymphatic vessels. Relative $[K^+]$ and $[Na^+]$ over the sarcolemma are presented with different sized circles in the figure. In normal situation, the $[K^+]$ is small in interstitium and high in myoplasm. $[Na^+]$ are in opposite directions.

In addition to K^+ leakage through membrane wounding and ATP and Ca^{2+}-sensitive channels, there are other mechanisms that may influence the sarcolemmal excitability. For example, there are various stretch sensitive ion channels in muscle fibres which may be activated due to eccentric exercise, such as stretch activated Na^+ and Ca^{2+} channels (McBride et al. 2000, Stauber 1989)

(Fig. 22). This is known to cause an acute depolarization of resting membrane potential lasting for more than 24 hours after eccentric contractions (McBride et al. 2000), which may cause a slow inactivation of voltage-gated TTX sensitive Na^+ channels that are largely responsible for AP propagation (Ruff 1999).

Interestingly, the most vulnerable muscle fibres for disruption after eccentric loading are the FT fibres (McNeil & Khakee 1992, Newham et al. 1983a, Takekura et al. 2001b, Vijayan et al. 2001). This is the same population of muscle fibres, of whose sarcolemmal AP propagation seemed to be the most affected in the current research. It is not clear what makes the FT fibres so sensitive to high force eccentric contractions. This is usually explained by mechanical differences in the contractile properties of the FT and ST fibre types (Lieber et al. 1991, Macpherson et al. 1996). The FT fibres are able to produce substantially more force with much higher rate than the ST fibres. Therefore, it may be that FT fibres and their force transmitting sarcolemma (Street & Ramsey 1965) are subjected to exceptionally high mechanical stress especially during maximal eccentric exercise, where MU activity seems to be lower (Babault et al. 2001, Eloranta & Komi 1980, Westing et al. 1991) and, thus stress to muscle fibres higher (Katz 1939, Westing et al. 1991) when compared to concentric one.

The sensitivity of the FT fibres to disruption after eccentric loading may explain partly the reduced CV at high isometric force levels in the current research. However, additional factors do exist. Firstly, the FT fibres have lower density of capillaries when compared to ST fibres (Sillau 1985). This will impair the removal or washout of K^+ from the interstitium and thus make the FT fibre more susceptible to lose its excitability during strenuous activity (Fig. 22). Furthermore, it has been shown that FT fibres are more vulnerable than ST fibres to decline of sarcolemmal AP amplitude during repetitive stimulation (Hanson 1974). Hicks and McComas (1989) observed that in contrast to FT fibres, repetitive stimulation of ST fibres actually causes membrane hyperpolarisation and increase in sarcolemmal AP amplitude possibly by protective enhancement of Na^+-K^+ pump activity in the ST fibres. Therefore, it appears that that the excitability of ST fibres is preserved, whereas it is more readily lost in the FT fibres. In normal situation, this would not be a serious problem, since the FT fibres are "designed" to function in phasic way – producing high force for brief periods of activity only. For this reason, it may be that the events leading to loss of excitability of the FT fibre, such as K^+ accumulation into the interstitial space, could be part of intrinsic safety mechanism of the muscle fibre to avoid excessive fatigue, which could otherwise lead to unrecoverable permanent damage and cell death of FT fibres. Therefore, especially in the case of maximal eccentric exercise, known to cause selective damage to FT fibres, it would not be surprising if some of the FT fibres belonging to the fastest and largest MUs would lose their excitability. Finally, it is very likely that prior to the total loss of excitability of a muscle fibre during the evolution of its fatigue, its AP propagation capability will be impaired or slowed down.

7 PRIMARY FINDINGS AND CONCLUSIONS

The present study showed that both maximal eccentric and concentric exercises caused a reduction of maximal force production capability, which was associated with a delayed increase in muscle soreness and an increase in the permeability of the muscle sarcolemma to myoplasmic proteins. These symptoms were significantly higher after the eccentric exercise. Furthermore, greater impairments were observed in global whole muscle excitability and sarcolemmal AP propagation after eccentric than concentric exercise. Moreover, distortion of motor control and AP propagation was observed also in individual MU level after eccentric exercise. These findings were especially evident at high contraction levels and relatively early (< two days) phase after the exercise(s). Specific findings and conclusions of the present research are as follows:

1) IZ can shift significantly, even during isometric contractions in BBM muscle, and this shift may bias RMS, MNF and CV if sEMG electrodes are located near IZ (I). The current findings underline the importance of individual investigation of IZ locations prior to the placement of conventional sEMG electrodes. With this procedure, it may be possible to avoid misleading results in sEMG experiments due to inter-individual differences in IZ location, and its shift due to muscle activity.

2) Site-dependent changes of sEMG variables after eccentric exercise can be detected in BBM muscle (II). These changes are not solely caused by physiological events occurring in the neuromuscular system, but are also influenced by anatomical factors, such as IZ and tendon regions. Therefore, if conventional single bipolar electrodes are used, placement of the electrode over or near these regions should be avoided. The multichannel sEMG system provides more valid and consistent recordings, and thus emphasizing the true physiological exercise-induced changes in sEMG variables.

3) The performance and consistency of CKC decomposition algorithm to indentify individual MUs from high-density sEMG signals proved to be sufficient even at relatively high isometric contraction forces (up to 75% of MVC) (IV). This is an encouraging result, since the novel technique extends observation of MU behaviour in muscle fatigue research closer to maximal contractions, while this has previously been limited to low contraction levels only. Therefore, the observations can be done over wide range of MUs and most importantly allows the observation of firing patterns and MUAP properties of the high threshold MUs.

4) Sarcolemmal function may be impaired at relatively early (≤ 2 days) phase after intensive eccentric exercise leading to EIMD in the BBM muscle (III). This was supported by an increase in the time of AP propagation along the sarcolemma, and a reduction in sEMG power spectral variables. It seems that the membrane systems of the muscle fibre population are not evenly affected by eccentric exercise. In particular, MUs with higher recruitment thresholds seem to be prone to sarcolemmal dysfunction after intensive eccentric exercise.

5) Control and electrophysiological properties of individual MUs may be disturbed in human BBM muscle at relatively early (≤ 2 days) phase after eccentric exercise (IV). The impairments seem to be related to slowing of sarcolemmal AP propagation. It appears that after maximal eccentric exercise, the central nervous system attempts to compensate for the loss of muscle force production by increasing the rate coding of active MUs.

6) Both eccentric and concentric exercise can induce reductions in maximal force production of elbow flexors and AP propagation over the sarcolemma together with increase in sarcolemmal permeability (V). However, these reductions were significantly greater after eccentric than concentric exercise. Therefore, the increased permeability of sarcolemma may partly explain the impairment of sarcolemmal AP propagation after eccentric exercise in the early stage (≤ 2 hours) of EIMD.

Based on the present findings and recent literature, it seems that the acute loss of force production in EIMD could be partially explained by failure of the E-C coupling process at the sarcolemmal level, but the prolonged loss of force production is caused by impairment in the E-C coupling processes beyond the sarcolemma. The early loss of muscle force production seems to be related to slowing of sarcolemmal AP conduction, and presumably a reduction in force production of the predominantly affected FT fibres and respective MUs. It appears that the central nervous system attempts to compensate for this force loss by increasing the rate coding of active MUs at submaximal contractions after the maximal eccentric exercise.

YHTEENVETO (FINNISH SUMMARY)

Lihassolukalvon toiminnallinen mukautuminen fyysiseen kuormitukseen

Yksittäinen raskas fyysinen kuormitus voi aiheuttaa lihasarkuutta ja voimantuottokyvyn heikentymisen useiksi päiviksi tai jopa viikoiksi. Tämä ilmiö vaikuttaa ihmisen fyysiseen toimintakykyyn ja koskee useimpia ihmisiä niin työssä kuin vapaa-ajallakin. Tämä ilmiö on havaittu ja kuvattu jo vuosisata sitten. Jo tällöin havaittiin, että lihasarkuus ja toimintakyvyn heikkeneminen syntyvät etenkin jos fyysinen aktiivisuus sisältää lihaksen aktiivisen venymisen, ns. eksentrisen lihastyön. Eksentrisen lihastyön vastakohta on konsentrinen lihastyö, jossa aktiivinen lihas lyhenee. Morfologiset tutkimukset mikroskopian avulla ovat osoittaneet muutoksia lihassolujen rakenteissa, kuten lihassolukalvossa, etenkin eksentrisen kuormituksen jälkeen. Nämä muutokset on tulkittu vaurioksi ja siksi tätä ilmiötä kutsutaan myös lihasvaurioksi. Lihasvaurion syntymekanismi ja syyt siitä aiheutuvalle voimantuottokyvyn pitkäaikaiselle heikkenemiselle eivät ole täysin selvillä. Ei myöskään tunneta, miten havaitut vauriot vaikuttavat lihassolun ja sen kalvorakenteiden toimintaan. Lihassolukalvolla on tärkeä tehtävä lihassolun supistumiskyvyn kannalta, sillä se välittää hermoston välittämän supistumiskäskyn koko lihassoluun ja sen voimaa tuottaville proteiinirakenteille. Tämä käsky, aktiopotentiaali, etenee aktiivisena prosessina hermolihasliitoksesta pitkin lihassolukalvoa kohti jännettä. Aktiopotentiaalin eteneminen aiheuttaa biosähköisiä jännitteen muutoksia lihaskudoksessa, jotka voidaan mitata elektromyografian (EMG) avulla. Tämän tutkimuksen tarkoitus oli selvittää eksentrisen ja konsentrisen kuormituksen vaikutuksia lihassolukalvon toimintaan ihmisillä. Lihassolukalvon aktiopotentiaalin johtumisominaisuudet mitattiin monikanavaisella pinta-EMG:llä koko lihaksen ja yksittäisten motoristen yksiköiden tasolla monilla eri isometrisillä (lihaspituus ei muutu) voimatasoilla. Motoriset yksiköt ovat lihaksen pienin toiminnallinen yksikkö, ts. joukko lihassoluja joita hermottaa yksittäinen liikehermo. Työ sisälsi uuden monikanavaisen EMG-metodologian kehitystyötä, validaatiota ja soveltamista tutkimuskäyttöön. Menetelmä osoittautui validiksi ja toistettavaksi. Tutkimuksen tulokset osoittivat sekä maksimaalisen eksentrisen että maksimaalisen konsentrisen kuormituksen aiheuttavan lihasarkuutta, voimantuottokyvyn alenemista ja lihassolukalvon läpäisevyyden kasvua. Nämä muutokset olivat kuitenkin merkittävästi suuremmat eksentrisen kuormituksen jälkeen. Eksentrisen kuormituksen jälkeen havaittiin suurempi hidastuminen koko lihaksen keskimääräisessä lihassolukalvon aktiopotentiaalin johtumisessa verrattaessa konsentriseen kuormitukseen. Tämän lisäksi yksittäisten motorisien yksiköiden hermostollinen kontrolli häiriintyi ja solukalvon aktiopotentiaalin johtuminen hidastui eksentrisen kuormituksen jälkeen. Muutokset olivat erityisen voimakkaita suurilla voimatasoilla ja suhteellisen aikaisessa vaiheessa (< kaksi päivää) kuormituksien jälkeen. Näin ollen lihassolukalvon toiminnan heikkeneminen selittää osin aikaisen voimantuottokyvyn laskun eksentrisen kuormituksen jälkeen. Tämän lisäksi vaikuttaa siltä, että lihassolukalvon toiminta häiriintyy ek-

sentrisen kuormituksen jälkeen etenkin lihassoluvauriolle herkimmillä suurilla ja nopeilla lihassoluilla ja niiden muodostamilla suurilla ja nopeilla motorisilla yksiköillä, jotka aktivoituvat vasta suuremmilla lihasvoimatasoilla. Lihassolukalvon toiminta palautui noin kaksi vuorokautta eksentrisen kuormituksen jälkeen, joten se ei ole merkittävä tekijä pitkäaikaisessa voimantuottokyvyn laskussa. Mahdolliset selittävät tekijät rajoittuvatkin lihassolukalvon aktiopotentiaalin johtumisen jälkeisiin tapahtumiin, kuten lihassolun sisään tunkeutuvien lihassolukalvosta muodostuvien poikittaisten putkirakenteiden aktiopotentiaalin johtumiseen. Näiden rakenteiden toiminnan mittaaminen ei ole tällä hetkellä mahdollista ihmiskoehenkilöillä, joten vastaus tähän kysymykseen jää vielä avoimeksi. Lihassolukalvon toiminnan heikkeneminen liittyi sen läpäisevyyden kasvuun, joka on merkki lihassolukalvon vauriosta. Näin ollen aikaisemmat havainnot vaurioituneista lihassoluista ovat merkittäviä lihassolun supistumiskyvyn kannalta. Keskushermosto näytti kompensoivan heikentynyttä motoristen yksiköiden voimantuottokykyä tihentämällä supistumiskäskyjä yksittäisille motorisille yksiköille eksentrisen kuormituksen jälkeen. Tämä on normaali tapa keskushermostolle lisätä voimantuottoa submaksimaalisilla voimantuottotasoilla. Kyseiset tulokset antavat merkittävää lisäinformaatiota lihassolukalvon toiminnasta ja sen adaptaatiosta, jota voidaan hyödyntää erilaisten lihassairauksien tutkimuksessa, sillä juuri lihassolukalvon toiminta on häiriintynyt monissa lihassairauksissa. Lisäksi tässäkin tutkimuksessa kehitetty monikanavainen EMG metodologia vaikuttaa lupaavalta menetelmältä, joka laajentaa motorisien yksiköiden toiminnan ja adaptaation tutkimuksen aiempaa korkeammille voimatasoille ja voi toimia tulevaisuudessa mahdollisena lihassairauksien diagnostisena menetelmänä.

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