SARCOLEMMAL EXCITABILITY AFTER ECCENTRIC EXERCISE IN MAN

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ABSTRACT

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Experiments were carried out to test the sarcolemmal excitability after intensive eccentric elbow flexor exercise (two sets of 20 repetitions) in humans. Electrically elicited surface compound muscle action potential (M-wave) properties from 30 s stimulation trains (20 Hz) were recorded from the biceps brachii muscle immediately after the exercise and during 48 h follow-up period. The results indicated that M-wave properties (area, amplitude, root mean square and duration) were reduced when measured immediately post-exercise. However, this was not the case two days after the exercise, although subjects had clear symptoms of delayed-onset muscle soreness and the maximal voluntary isometric and eccentric torques were still depressed by 12.2 ± 9 % ($P < 0.001$) and 17.7 ± 9 % ($P < 0.001$), respectively. Plasma concentrations of K^+ and Ca^{2+} showed an acute post-exercise increase of 5.4 ± 5.4 % and 1.7 ± 2.1 %, respectively, suggesting an acute loss of normal ion distribution across the sarcolemma. These findings suggest, that disturbance of sarcolemmal excitability is not the major factor on eccentric exercise induced prolonged loss of muscle strength. However, the eccentric exercise may decrease the sarcolemmal excitability acutely, which seems to be related to increased sarcolemmal permeability.

Keywords: M-wave, electromyogram, muscle fatigue, delayed-onset muscle soreness, sarcolemma

CONTENTS

1 BACKGROUD

1.1 Neuromuscular fatigue

1.1.1 Definition of fatigue and weakness

Neuromuscular fatigue can be observed as a decreased performance, usually determined by force generating capacity. During a sustained maximal contraction, the force will decline steadily and fatigue will be observed from the start of the exercise (Bigland-Ritchie et al. 1983). In contrast, during submaximal contractions the target force is maintained for longer time period. In this case, the fatigue will be defined as an inability to maintain the target force, even though the maximal force generating capacity is impaired earlier during the contraction. In order to clarify the definition of neuromuscular fatigue, it is usually defined as a loss of *maximal* force generating capacity (Bigland-Ritchie et al. 1986, Gandevia 1995).

Vollestad (1997) has further defined neuromuscular fatigue as any exercise-induced reduction in the maximal capacity to generate force or power output. This definition allows quantification of fatigue in different types of exercise and intensities. Furthermore, a muscle weakness is distinguished from neuromuscular fatigue as a chronic impairment in the force or power output generation, in contrast to the acute effect of the neuromuscular fatigue (Vollestad 1997).

1.1.2 Sites of neuromuscular fatigue

The sites of neuromuscular fatigue can be divided as central and peripheral. The possible central sites for neuromuscular fatigue include the central nervous system, motoneurones and neuromuscular junction (NMJ) (Edwards RHT 1978). Thus, motivational factors, integration of sensory information and motor signal transferring influence to the central fatigue (Vollestad 1997). Although, the motor signal transfer seems to be adequate with normal physiological excitation rate in healthy humans (Bigland-Richie et al. 1982,Gandevia et el. 1995, Wood & Slater 2001). Central fatigue

may be defined as any exercise-induced reduction in maximal voluntary contraction force which is not accompanied by the same reduction in maximal evocable force (Bigland-Richie et al. 1978). Focus of the present experiment is on the peripheral events in neuromuscular fatigue and, thus are discussed in more detail.

Peripheral factors of neuromuscular fatigue

Peripheral factors of neuromuscular fatigue include loss of excitation of muscle fibres, decreased tetanic release of Ca^{2+} into cytosol, impaired binding of Ca^{2+} to troponin, impaired crossbridge turnover, reduced rate of ATP utilization and regeneration (Nagesser et al. 1993, Warren et al. 1993, Warren et al. 2001, Vollestad 1997). In addition, impairment in muscle force transmission might also affect the force and power output during fatigue (Huijing 1999, Monti et al. 1999, Boriek et al. 2001).

Propagation of action potential (AP) on excitable membranes of muscle fibers, sarcolemma and t-tubule, may be impaired during exercise due to the imbalance of Na^{+} and K^{+} ions over the membranes (Nielsen et al. 2004, Ørtenblad&Stephenson 2003, Overgaard & Nielsen 2001, Sejersted 1992, Sejerstedt & Sjogaard 2000, Yensen et al. 2002). This could lead to decrement of sarcoplasmic reticulum (SR) Ca^{2+} release into the cytosol and attenuation of the Ca^{2+} binding to troponin C. Consequently, fewer cross-bridges would be formed between contractile proteins resulting in reduced force or power generation (Warren et al. 1993, Warren et al. 2001). The cross-bridge cycling depends also on formation of ATP molecules trough aerobic and anaerobic metabolic pathways (Vollestad 1997). Furthermore, affinity of Ca^{2+} binding to troponin might change because of fatigue related metabolites (Westerblad et al. 1991).

Vollestad (1997) states, that one of the most complicating factors in human fatigue studies with voluntary muscle actions, is the motor unit (MU) activation pattern. In voluntary actions the MUs are activated by size principle (Henneman et al. 1965) in order of activation threshold, from low to high threshold MUs (Milner-Brown et al. 1973). By supramaximal transcutaneous electrical stimulation the MU activation pattern is exceptional or abnormal, because all the MUs are recruited at the same instant of time allowing a simpler experiment protocol (Mandrile et al. 2003). By use of electrical stimulation method, central factors will be excluded and, thus the function of muscle in question can be directly obtained (Bigland-Richie et al. 1978). Although, caution must

be taken in such experiments, because excessive rate in electrical stimulation may lead to block of neuromuscular transmission (Jones 1996). This can be avoided by using adequate stimulation frequency.

A reduced release of Ca^{2+} from SR during stimulated contractions is demonstrated by a faster decline in twitch force than tetanic force and is called low-frequency fatigue (LFF). Furthermore, the tetanic force is recovered relatively fast as twitch force needs hours or days for complete recovery. For this reason, LFF provides an estimation of excitation-contraction (EC) coupling efficacy. (Edwards et al 1977.) LFF is usually estimated by the ratio between low (10 to 20 Hz) and high (50 HZ) frequency electrically evoked force (Vollestadt 1997). In contrast, high frequency stimulation will lead to electrical excitation failure in the sarcolemmal membrane during the stimulated contraction and results as decreased tetanic force (Bigland-Ritchie et al. 1979). This phenomenon is known as high frequency fatigue (HFF). Usually, HFF recovers within minutes or hours after exercise, whereas LFF may persist for days (Davies & White 1981).

1.1.3 Temporal aspects of neuromuscular fatigue

Early metabolic factors contributing to peripheral neuromuscular fatigue
Studies on isolated muscle fibers have shown that brief repetitive tetanic stimulation results as an early decrease in tension (~ 10 %) after 10-20 successive tetani. This is followed by relative stability in the tension, before a final rapid decline in the tetanic force (Westerblad & Allen 1991). The final stage of such fatigue is characterized by an increase of resting cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and a decrease of tetanic $[Ca^{2+}]_i$. These changes may explain much of the simultaneous fall in force. (Lee et al. 1991.) The underlying mechanism for the cellular $[Ca^{2+}]_i$ regulation by SR Ca^{2+} uptake or release is not fully understood, although its essential role in fatigue is generally accepted.

Neuromuscular fatigue is associated with metabolic changes as increased cytosolic concentrations of H^+ ($[H^+]$), Pi ([Pi]), ADP ([ADP]) and AMP ([AMP]), and simultaneous decrease of ATP ([ATP]) and ([PCr]) levels (Nagesser et al. 1993). Such changes might lead to decrease in tetanic $[Ca^{2+}]_i$ during final stage of fatigue because of

a failure of the SR Ca^{2+} release process, loss of Ca^{2+} from the SR and/or precipitation of calcium phosphate (Ca-Pi) within the SR (Westerblad & Allen 1991, Fryer et al. 1995, Duke & Steele 2000). The precipitation of Ca-Pi reduces the amount of Ca^{2+} available for release from the SR (Fryer et al. 1995) and, thus impairs SR function.

It has been demonstrated earlier, that intracellular acidosis evoked by increased $[H^+]$ concentration seems to have little effect on force production (Adams et al. 1991, Pate et al. 1995), thus the effect of pH and $[H^+]$ can be discarded. Interestingly, an increase in cytosolic [Pi] and [ADP] results as leakage of Ca^{2+} from SR, possibly via dysfunction of Ca^{2+} pump (Duke & Steele 2000). Naturally, increasing [Pi] is accompanied by PCr breakdown during the progression of neuromuscular fatigue. Moreover, it has been observed that a decrease of PCr level impairs SR Ca^{2+} uptake (Duke & Steele 1999). In addition, increased [ADP] has also an important inhibitory influence on the SR Ca^{2+} uptake (MacDonald & Stephenson 2001). Furthermore, the increase in cytosolic Mg^{2+} concentration during fatigue may emphasize the inhibitory effect of [Pi] on SR Ca^{2+} release (Steele & Duke 2003).

These kind of acute of metabolic changes during fatigue are recovered relatively fast, in minutes or hours. As this is the case, long-term reduction in force production must be caused by other factors than the immediate metabolic factors.

Later factors contributing to peripheral neuromuscular fatigue

The immediate energy stores are restored in minutes or hours after cessation of exercise. Nevertheless, impaired function of the muscle may persist several days, especially after eccentric exercise. Thus, factors other than immediate metabolic factors contribute to the delayed muscle weakness and soreness. This phenomenon is also known as delayed-onset muscle soreness (DOMS).

The reason for reduced muscle strength during DOMS remains unclear. Ultrastructural muscle damage has been observed after eccentric exercise, suggesting contractile machinery damage in the level of sarcomeres (Friden et al. 1981, Roth et al. 1999). Selective damage to possibly force bearing proteins such as dystrophin (Komulainen et al. 1998) may disturb lateral force transmission of the muscle cell and, thus impair muscle strength (Huijing 1999, Monti et al. 1999). The lateral force transmission seems

to be important especially in muscles which fasicles are longer than 35 mm, because such muscles are composed of overlapping arrays of short muscle fibers which are terminated intrafascicularly (Paul et al. 2002).

In mice, it is also noted that after eccentric exercise the sarcolemma itself may be damaged (Friden & Lieber 1998, Komulainen et al. 1998). This is important as sarcolemma is an active force transmitter of the muscle cell (Street & Ramsey 1965). However, in humans, Yu et al. (2002a) did not recognize sarcolemmal disturbance and muscle cell degeneration after eccentric muscle actions. Furthermore, any damage to the excitable membranes in muscle cell (sarcolemma, t-tubular system, SR) could lead to failure of AP spreading and, thus impair the EC coupling (Allen 2001, Warren et al. 2001). In addition, altered muscle energy metabolism may also explain the prolonged strength loss, because decrease in muscle glycogen content has been observed as late as two days after cessation of eccentric exercise (Asp et al. 1998).

Profound question of initiating factor in the muscle damage remains unanswered. Is the damage to the muscle tissue mechanical or chemical in nature? There is no evidence of extensive mechanical damage due intensive exercise in humans (Yu et al. 2002a), in contrast to the animal studies (Komulainen et al. 1998). The most likely explanation for the undeniable increase in protein catabolism (whole body and myofibrillar) (Rennie et al. 1981, Fielding et al. 1991) and ultrastructural damage of sarcomeres (Friden et al. 1981, Roth et al. 1999) are the exercise activated proteases. These enzymes are able to initiate proteolysis of various proteins in the muscle cell after exercise (Belcastro, 1993, Belcastro et al. 1998, Saido et al. 1994.)

Yu et al. (2002b and 2004) argued that the observed ultrastructural damage is due to adaptive remodeling process in the myofibrils. Calpain is a non-lysosomal protease, which is activated by increase in resting $[Ca^{2+}]_i$ (Belcastro et al. 1998), as is the case in $[Ca^{2+}]_i$ in the final stage of fatigue (Lee et al. 1991). Calpain is capable of immediate cleavage of receptor proteins, soluble and membrane associated enzymes, cytoskeletal proteins and myofibrillar proteins (Saido et al. 1994). Because of these properties, calpain may play a key role in the initiating of the disassembly and remodeling of the filamentous attachments to plasma membrane (Belcastro et al. 1998).

At later stage of the muscle damage (few days after the cessation of exercise) an inflammation process may follow. During the inflammation process a lysosomal proteases from monocytes and macrophages play a primary role in muscle cells protein turnover (Tidball 1995). Malm et al. (2000) and Yu et al (2002a) did not found inflammation in human muscle during DOMS. On contrary, Paulsen et al. (2005) did find infiltration of neutrophils and monocytes in muscle biopsies after eccentric exercise and argued that the eccentric exercise cause muscle damage and inflammation, which initiates delayed leukocytosis by stimulation of bone marrow.

1.2 Structure and function of membrane structures of the muscle cell

Skeletal muscle cells are surrounded by outer plasma membrane termed as sarcolemma, which is consisted of phospholipid bilayer, imbedded with protein complexes such as ion channels (Green 2004). Invaginations of the sarcolemma extend into cell interior, forming a extensive network of transversely (t-) running tubular system. T-tubular system allows the propagation of AP to the terminal cistarnae of the SR throughout the muscle cell. The internal spaces of these t-tubules are open to extracellular space, thus t-tubules are considered as part of the sarcolemma. (Clausen 2003.)

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 $[Na^+]$ and $[Cl^-]$ are low intracellularly and high extracellularly. For $[K^+]$ the opposite is true. During AP the ion concentrations are temporary reversed. The depolarization is initiated by voltage-gated Na^+ channels (NaChs) and is followed by repolarization by opening of the K^+ channels. With assistance of Cl^- channel opening and Na^+-K^+ pump activity the $[Na^+]$ and $[K^+]$ are restored. Simultaneously the resting membrane potential is achieved. It is worth mentioning, that Na^+-K^+-ATPase enzyme activity is essential for Na^+-K^+ pump function, and thus the excitability of the cell relies to it. (Green 2004.)

The signal for muscle contraction involves the propagation of AP along the sarcolemma and the t-tubular membrane. T-tubular system is important link in activation of voltage

sensor molecules, like skeletal dihydropyridine receptor (DHPR). As result of DHPR activation, Ca^{2+} are released from SR (Melzer et al. 1995). The actual activation mechanism in t-tubule and SR interspace leading to Ca^{2+} release from SR is not fully understood. The NaChs play a central role in initiation of AP on the sarcolemma (Denac et al. 2000). For this reason, NaChs are concentrated in the region of NMJ, but are expressed throughout the sarcolemma (Colledge & Froehner 1998). NaChs overal architecture is similar to Ca^{2+}-channels, with detailed differences. Also voltage-dependent K^+-channels are similar to Ca^{2+}-channels, but have simpler structure. (Marban et al. 1998.)

During strenuous exercise the sarcolemmal excitability may be reduced, because of altered ion concentrations across the sarcolemma. Sarcolemmal excitability is defined by inward current that is required to depolarize the sarcolemma enough to reach the threshold potential that triggers sufficient amount of Na^+ channels to elicit an AP. Intra- and extracellular [Na^+] and [K^+] contribute in great deal to the threshold potential of sarcolemma. During exercise, increase in extracellular [K^+] and intracellular [Na^+], may reduce sarcolemmal excitability. In addition, because the ratio between the area of excitable membrane and the extracellular volume it faces is much larger for the T-tubules than for the sarcolemma, the alterations in [K^+] are pronounced in the T-tubules. Consequently, the T-tubules may be more sensitive for excitation failure than sarcolemma. (Sejerstedt & Sjogaard 2000.)

It is demonstrated earlier, that the activity of Na^+-K^+-pumps increases during muscle activity (Nielsen & Clausen 1997). Such increment would oppose or relieve the alterations in [Na^+] and [K^+] and, thus force production during neuromuscular fatigue (Nielsen & Clausen 2000). Overgaard et al. (1999) showed with isolated muscle cells, a linear relation between decrease in M-wave area and tetanic force when [Na^+] and [K^+] were altered resembling fatigued situation or muscle fibers were fatigued with continuous electrical stimulation (120 s, 30 Hz). They also noticed that increase in Na^+-K^+-pump activity, induced by β_2-adrenoceptor agonist salbutamol, induces recovery of M-wave area and tetanic force. This was also related to simultaneous recovery of muscle fiber excitability. They concluded that loss of muscle fiber excitability is an important factor in fatigue induced by high frequency (30 Hz) stimulation. For this reason, M-wave area might be a good indicator for muscle fiber excitability.

Nielsen et al. (2004) studied the excitability of t-tubular system in isolated rat skeletal muscle and concluded that reduction in the chemical gradient for K^+, during intensive exercise, may depress t-tubular function. Although, simultaneous increase in the intracellular [Na^+] protects t-tubular function by stimulating the Na^+-K^+-pump.

1.3 Transcutaneous motor point electrical stimulation

The neural control (supraspinal, spinal and reflex activation) and the NMJ can be bypassed by transcutaneous electrical motor point stimulation, eliciting directly the sarcolemmal AP. This method allows the investigation of the sarcolemmal excitability in the muscle of interest, by simultaneously measured surface EMG. Furthermore, the time domain and frequency domain features of M-wave and fiber conduction velocity (CV) may be calculated. Usually a strain of impulses for certain time period (e.g. 15 to 30 s) are used with relatively low frequency (15 to 30 Hz). Low frequency is essential to avoid overlapping of successive M-waves with stimulus artifact. (see review Merletti et al. 1992.)

Merletti et al. (1998) studied the repeatability of electrically evoked EMG signals and founded, that the spectral variables, mean power (MPF) and median frequency (MDF), are more repeatable than those based on the amplitude variables, such as average rectified value (ARV) and root mean square (RMS) (Merletti et al. 1995, Merletti et al 1998). This is the case at least in human vastus lateralis and tibialis anterior muscles. In ARV, RMS and CV only changes in high magnitude may be detected (Knaflitz et al. 1996). It is worth to mention, that the repeatability of CV is improved if it is calculated from two double differential signals by cross correlation technique, as reported by McGill and Dorfman (1984).

The optimal position of the stimulation and detection electrodes is critical for repeatability of the measurements. Thus, careful repositioning before each measurement could lead to more repeatable results (Merletti et al. 1998). Linsen et al. (1993) have found the CV as the most repeatable variable during voluntary contractions in biceps

brachii muscle. Thus, the repeatability of EMG variables may be different in different muscles and conditions (Merletti et al. 1998).

Major problem related to the measurement of EMG during electrically evoked contractions is stimulation artifact. It may contaminate the measured M-wave if the nonlinear decay of the artifact overlaps with the M-wave. There are several artifact removal methods available, both hardware and software techniques (O'Keeffe et al. 2001). In biceps brachii the artifact amplitude seems to depend greatly by the distance of the detecting electrodes from stimulation point - the further the better. The desired location for detecting electrode is in the middle between the innervation zone and tendon (Mandrile et al. 2003). Increased distance between detecting and stimulating electrodes is beneficial, because the prolongation of latency between the stimulus and the M-wave. Thus, the risk for overlapping artifact with M-wave is reduced.

The measured EMG is sensitive for EMG electrode location (Roy et al. 1986) and movement with respect to the source (Merletti et al. 1992) during electrically elicitecd contraction. Furthermore, the shape of stimulation waveform, interelectrode distance, current intensity (which has a linear relationship with artifact amplitude) or spatial filter used for signal detection did not affect the artifact amplitude. As this is the case, the only option to remove the overlapping artifact from electrically elicited EMG signal is to use some of the blanking or removal methods available (O'Keeffe et al. 2001).

Since, in the electrical stimulation all MUs contributing to the muscle contraction are activated in the same instant of time, the detection of failures of neuromuscular transmission due to fatigue (Bigland-Ritchie & Woods 1984) or pathologies (Chisari et al. 2001, Chisari et al. 1998) are possible. The measured EMG signal (M-wave) gives information of fatigability properties of the active MUs during electrical stimulation (Mandrile et al. 2003).

Chissari et al. (2001) used the electrical motor point stimulation technique to investigate the sarcolemmal excitability in Steinert disease. They were able to separate the nature of the disease by the trend of ARV of surface EMG during the stimulation. The patients with sarcolemmal excitability alteration had decreasing pattern during the stimulation and the patients with no sarcolemmal excitability alteration had increasing trend as did

the control group. Furthermore, Chisari et al. (1998) observed in myotonic dystrophy patients alterations in the trends of CV and MDF during the stimulation when compared to the healthy controls. The lower absolute CV during the electrical stimulation in the patients might suggest a reduced fiber size. In addition, they observed an early increased rate of decrease in MDF during the stimulation, possibly because of abnormalities in inactivation of Na^+-channels, which is found in myotnic dystrophy patients (Franke et al. 1990). This could result as increased afterdepolarization of the AP and thus lengthening of depolarization zone (Chisari et al. 1998). In healthy subjects, the trend of ARV is usually a dome-shaped curve and CV and MDF are decreasing slightly during the progression of the stimulation. (Merletti et al. 1998, Chisari et al. 1998.)

1.4 The purpose of the experiment

The actual cause of strength loss after eccentric exercise remains unclear, but seems to be caused by disturbance in EC coupling (Warren et al. 2001). As the EC coupling could be affected by reduced sarcolemmal excitability, the purpose of the present study was to determine if the sarcolemmal excitability (determined as M-wave properties) is altered after eccentric exercise and how such alteration is recovered. Thus, the aim was to follow the temporal recovery of the sarcolemmal excitability and asses if the eccentric exercise and the consequent DOMS have contribution to the sarcolemmal excitability. Also the temporal relationships of functional capacity of elbow flexors among with other indices of muscle damage were investigated.

2 MANUSCRIPT FOR PUBLICATION

Sarcolemmal excitability after eccentric exercise in man

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Abstract

Experiments were carried out to test the sarcolemmal excitability after intensive eccentric elbow flexor exercise (two sets of 20 repetitions) in humans. Electrically elicited surface compound muscle action potential (M-wave) properties from 30 s stimulation trains (20 Hz) were recorded from the biceps brachii muscle immediately after the exercise and during 48 h follow-up period. The results indicated that M-wave properties (area, amplitude, root mean square and duration) were reduced when measured immediately post-exercise. However, this was not the case two days after the exercise, although subjects had clear symptoms of delayed-onset muscle soreness and the maximal voluntary isometric and eccentric torques were still depressed by 12.2 ± 9 % ($P < 0.001$) and 17.7 ± 9 % ($P < 0.001$), respectively. Plasma concentrations of K^+ and Ca^{2+} showed an acute post-exercise increase of 5.4 ± 5.4 % and 1.7 ± 2.1 %, respectively, suggesting an acute loss of normal ion distribution across the sarcolemma. These findings suggest, that disturbance of sarcolemmal excitability is not the major factor on eccentric exercise induced prolonged loss of muscle strength. However, the eccentric exercise may decrease the sarcolemmal excitability acutely, which seems to be related to increased sarcolemmal permeability.

Keywords: M-wave, electromyogram, muscle fatigue, delayed-onset muscle soreness, sarcolemma

2.1 Introduction

It is well known in the literature, that eccentric exercise is accompanied with a prolonged loss of muscle force, muscle pain, increased joint stiffness, muscle swelling, muscle protein degradation and efflux to circulation. All these effects are well known symptoms of delayed onset muscle soreness (DOMS) (e.g. Warren et al. 1999b). The cause for the loss of muscle strength is not fully understood, but has usually been attributed to disturbance of excitation-contraction (EC) coupling during the progression of muscle damage. Thus, this is related to defect of Ca^{2+} kinetics in the sarcoplasmic reticulum (SR) (Warren et al. 1993).

Ultrastructural muscle damage has also been observed after eccentric exercise, suggesting contractile machinery damage in the sarcomere level (Friden et al. 1981, Roth et al. 1999). Selective damage of force bearing proteins such as subsarcolemmal dystrophin (Komulainen et al. 1998) or intermediate filament desmin (Barash, et al. 2002) may disturb lateral force transmission in the sarcolemma (Street & Ramsey 1965) and, therefore impair muscle strength (Huijing 1999, Monti et al. 1999). Animal studies have noted that the sarcolemma itself may be damaged (Friden & Lieber 1998, Komulainen et al. 1998). In humans, however, Yu et al. (2002a) did not recognize sarcolemmal disturbance, loss of desmin or muscle cell degeneration after eccentric muscle actions. However, any damage in the excitable membranes in muscle cell (sarcolemma or t-tubular system) could lead to a failure in action potential (AP) propagation and, thus impair the EC coupling (Allen 2001).

It could therefore hypothesised that excitability of sarcolemma could be disturbed in DOMS. The sarcolemmal excitability is defined by the inward current that is required to depolarize the sarcolemma enough to reach the threshold potential that triggers sufficient amount of Na^{+} channels to elicit an AP (Sejerstedt & Sjogaard 2000). Although, eccentric exercise does not seem to initiate extensive damage in human sarcolemma (Yu et al. 2002a), permeability of sarcolemma may still be modified. This could possibly lead to ionic disturbance over the muscle cell membrane and affect the resting membrane potential and excitability. McBride et al. (2000) have observed in animals, that eccentric exercise induced prolonged membrane depolarization due to

increased cation conductance via stretch-activated ion channels in the sarcolemma. Thus, the eccentric exercise may affect sarcolemmal excitability due to increased sarcolemmal permeability and, therefore, decrease the force production capability of the muscle.

Sarcolemma itself is sensitive to changes in intra- and extracellular concentrations of Na^+ ($[Na^+]$) and K^+ ($[K^+]$) (Overgaard & Nielsen 2001, Nielsen et al. 2004, Yensen et al. 2002). These ion concentrations contribute naturally to the threshold potential of the sarcolemma. Especially the lateral invaginations of sarcolemma (t-tubules) are possible sites of the excitation failure during DOMS, because the ratio between the area of excitable membrane and the extracellular volume it faces is much larger for the t-tubules than for the sarcolemma. Therefore, the alterations in $[K^+]$ are pronounced in the t-tubules (Sejerstedt & Sjogaard 2000). As a result, individual AP propagation may be inadequate to reach all the terminal cistarnae of the SR. This could lead to a decrement of SR Ca^{2+} release into the cytosol and to an attenuation of the Ca^{2+} binding to troponin C. Thus, fewer cross-bridges would be formed between contractile proteins resulting in reduced force or power generation (Vollestad 1997).

The excitability of the muscle cell relies on Na^+-K^+-ATPase enzyme activity dependent Na^+-K^+ pump function (Green 2004). Therefore, the phosphorylation of the Na^+-K^+ pump relies on ATP stores, which is in turn depends on phosphocreatine (PCr) level. Muscle fatigue is associated with metabolic changes such as increased cytosolic levels of H^+, Pi, ADP and AMP, and simultaneous decrease of ATP and PCr levels (Nagesser et al. 1993). This may lead to dysfunction of muscle cell ion pumps, with simultaneously increased activation of stretch-activated ion channels. This could then alter the ion distribution across the membranes of muscle cell by increasing the resting cytosolic $[Na^+]$ (Yeung et al. 2003) and $[Ca^{2+}]$ (Balnave & Allen 1995, Duke & Steele 2000). Therefore, the metabolic changes together with mechanical stimuli could affect the sarcolemmal excitability during the progression of muscle fatigue in voluntary and electrically elicited actions.

In muscle fatigue experiments the sarcolemmal excitability has been studied with electrically elicited EMG signals. Electrical stimulation has the advantage of standardized and repeatable conditions as compared to voluntary muscle actions, since it

controls motor unit (MU) firing frequency, MU recruitment, eliminates cross-talk from nearby muscles and is independent of subjects motivation to perform muscular contraction (Merletti et al 1990). In electrical stimulation all the MUs are recruited at the same time. Thus, simultaneously measured EMG signal provides analysis of M-wave, which contains information about membrane properties of the active MUs (Merletti et al. 1992).

Even though disturbances at EC coupling level have been suggested to be responsible at least partly for the strength loss after eccentric actions (e.g. Warren et al. 2001), some uncertainty exists for the contribution of the different mechanisms involved. Therefore, the aim of the present study was to verify if sarcolemmal excitability is modified during two day post-exercise period after eccentric exercise and whether it could further explain the EC coupling dysfunction.

2.2 Methods

Subjects

One female and eleven male volunteers (age 25.4 ± 2.8 yr; height 179.7 ± 5.8 cm; weight 76.9 ± 11.8 kg) participated in the study. Nine male subjects carried out the whole experiment. One female and two males were included only for the repeatability measurements of the electrically elicited EMG parameters. No subject had known symptoms of neuromuscular disorders. The subjects were 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 whole study period. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of 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.

Study protocol

The measurements consisted of three different sessions on different days and five identical measurements (Fig. 1). During familiarisation session (1-2 day before exercise session) subjects practised submaximal eccentric actions, the electrode locations were determined and the measurements for repeatability analysis (PRE) were conducted. Baseline measurements were done before (BEF) the eccentric exercise and followed-up 48 hour post-exercise [immediately after (IA) and one hour (1h) after and two days after 2D the eccentric exercise]. The measurements were identical in each session.

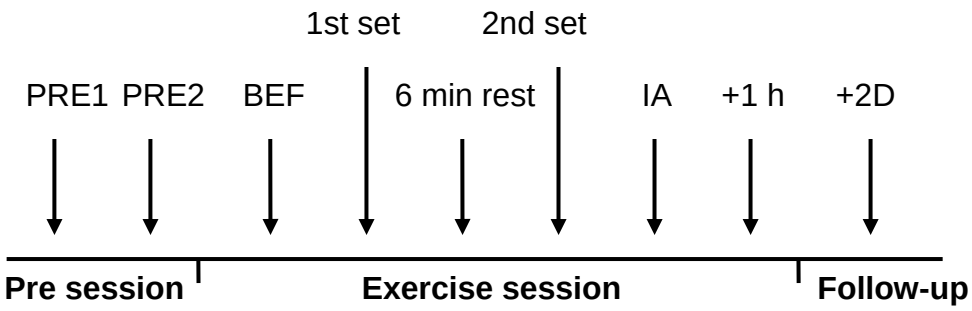

Figure 1. Schematic presentation of the study protocol. Repeatability measurements (PRE1, PRE2 and BEF), baseline measurement (BEF) and follow-up measurements [immediately after (IA), one hour after (1h) and two days after (2D) the eccentric exercise].

The tetanic electrical stimulation was repeated during the PRE measurement to analyze trial-to-trial repeatability. Day-to-day repeatability was acquired by comparing of PRE and BEF measurements. Warm-up protocol before each session consisted of three submaximal eccentric and isometric elbow flexor actions.

Exercise protocol

Subjects performed two sets of twenty maximal eccentric contractions (2 x 20) with elbow flexors of the right arm on a motorized isokinetic dynamometer (Komi et al. 2000) (Fig 2). The supinated right forearm was attached to the lever arm of the dynamometer and the force applied to the wrist by the elbow flexors was measured by a strain gauge transducer. The range of motion in the elbow joint was 120° (from 50° to 170°) during eccentric actions. Eccentric angular velocity was 1 $rad \cdot s^{-1}$ and there was one second brake in initial and end positions. Maximal eccentric actions were done with maximal preactivation. While the lever arm returned to the initial position (0.26 $rad \cdot s^{-1}$) subjects were instructed to relax their arm muscles.

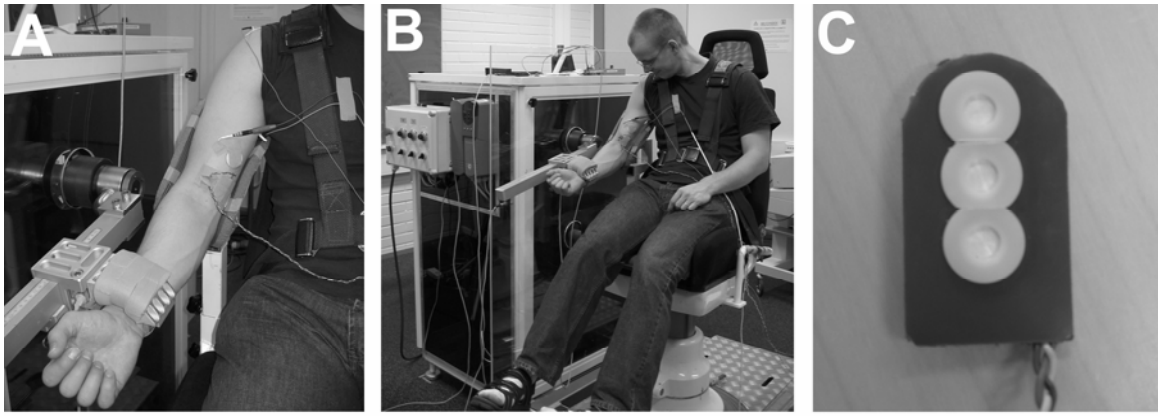

Figure 2. The motorized isokinetic dynamometer (A and B) and linear electrode (C) used in the present experiment.

One set lasted 4 minutes and there was 6 minutes brake between the sets. Total work time in the exercise was 1 min 40s. There was 10 s rest between consecutive eccentric actions, in order to minimize the metabolic fatigue.

EMG recordings

A dual single differential detection system was applied to detect surface EMG signals. In this system, a linear electrode was used and it consisted of three sintered Ag/AgCl

miniature skin electrodes (NT615T, Nippon Koden, Tokyo, Japan) (Fig. 2). These electrodes formed two pairs of bipolar electrodes with 10-mm interelectrode distance in each. EMG signals were pre amplified (100-fold) and high-pass filtered (3-dB bandwith, 10 Hz) with pre amplifier (NeuroLog 824, Digitimer Ltd.,Hertfordshire, England). EMG signal was further amplified (2- or 5-fold) with isolation amplifier (NeuroLog 820). All signals were sampled at 4000 samples/s per channel and converted to digital data by a 32-bit analogous to digital converter (Power 1401, CED Ltd., Cambridge, England), displayed real-time (Signal software, CED Ltd.) on computer screen and stored on computer hard disk. The EMG measuring circuit was isolated from electrical circuit by optical isolator between the isolation amplifier and A/D converter.

Impedance between EMG electrodes was always below 5 kΩ (1.67 kΩ ± 0.54 kΩ). EMG electrode was always located between the main motor point and the distal tendon of the biceps brachii muscle. One ground electrode (NI-4560, Ag/AgCl, Unomedical Ltd., Gloucestershire, Great Britain) was placed over ipsilateral acromion of scapula. Optimal electrode position was determined according to a large and clear shaped M-wave, and it had to be similar in shape in the both single differential signals. This procedure ascertained, that the electrodes were always oriented longitudinally to the muscle fibres. EMG electrode preparation was done during the familiarisation session before the PRE measurement and the optimal electrode position was marked to the skin to allow accurate repositioning later in the study.

Stimulation technique

The monopolar transcuteneous electrical stimulation technique was used in this study. The locations of the main motor points of biceps brachii were examined with a ball pointed (á 8 mm) pen electrode according to motor thresholds. After this determination one small round (á 1.25 cm) negative adhesive electrode (V-Trodes, Metler Electronics corp., Anaheim, CA, USA) was placed over the main motor points. A large oval (5.08 X 10.16 cm) positive adhesive electrode (V-Trode) was placed on the opposite side of the upper arm.

Supramaximal electrical stimulation was determined as a current level, which was 10 % above the level needed to initiate maximal M-wave. Maximal M-wave was verified before each measurement as a point where yielding of the M-wave occurred. Constant

current stimulator (DS7A, Digitimer Ltd.) was used with rectangular pulses of 100 μs. Electrical stimulation train of 20 Hz lasted for 30 s. Subjects were relaxed and seated with elbow angle adjusted to 140º on the isokinetic dynamometer during the stimulation. The electrical stimulation was always done first in each measurement. No stimulation artefact suppression was needed, because the detected M-waves did not overlap with the stimulation artefacts.

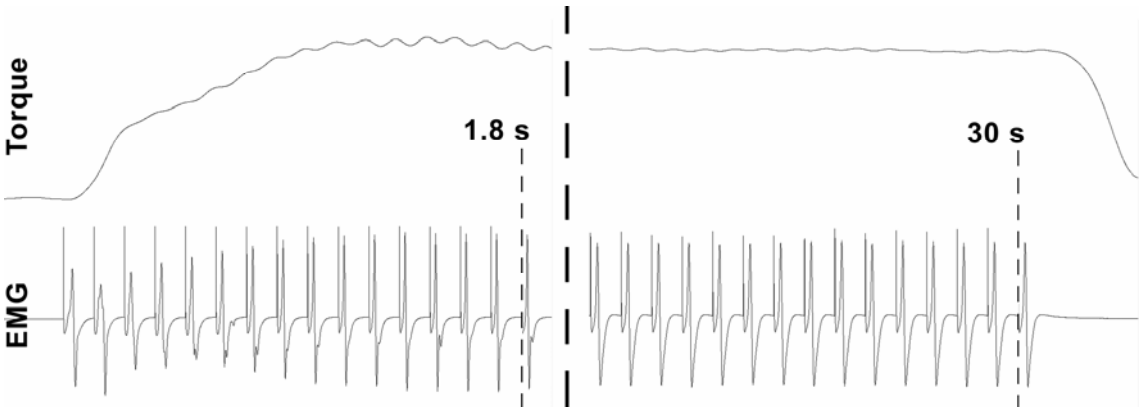

Figure 3. Initial and terminal part of a single 20 Hz tetanic supramaximal stimulation from one subject. Note that, the M-waves became stabled shortly after the torque reached the tetanic level.

Torque measurements

All maximal voluntary torque (MVC) measurements were done in the same motorized isokinetic dynamometer as in the eccentric exercise. The torque was obtained by multiplying the measured force with lever arm length of the subject. Isometric MVC was measured with an elbow joint angle of 140º during 4 s contraction. Eccentric MVC measurement was conducted the same way as was for the eccentric contraction during the exercise itself. Subjects performed three contractions to obtain the maximum torque, which was then used in the analysis.

Muscle soreness and range of motion

Subjective muscle soreness of the right arm elbow flexors was measured with visual scale of continuous line starting from 1 cm (no pain) and ending at 5 cm (worst possible pain) (Appendix 1). The assessment of the soreness level was done while investigator palpated the biceps brachii muscle.

Range of motion of the elbow joint was measured from the right arm with a goniometer, while subjects were standing on erect posture facing straight forward. Relaxed arm angle (RANG) was assessed while subject remained his/her right arm relaxed on the side of the body. During flexed arm angle (FANG) measurement subjects flexed elbow join as much as possible trying to touch ipsilateral shoulder.

Blood samples

Blood sample of 10 ml was drawn from the ulnar vein of non-exercised arm BEF, IA, 1h after and 2D after the exercise. Nova Biomedical STAT Profile pHOX Plus L-analyzer (Nova Biomedical, Waltham, MA USA) was used for analysing blood lactate and electrolyte (Na^+, K^+, and Cl^{2-}) concentrations, and blood pH. Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan) was used to extract total leukocyte count and three different sub-fractions (%) of leukocytes, lymphocytes, medium sized leukocytes (monocytes, eosinophils and basophils) and neutrophils. Plasma creatine kinase (CK) activity was analyzed by Vitros DT 60 (Ortho-Clinical Diagnostics, Rochester, NY USA).

Signal processing

The first 5 seconds of the electrically elicited contraction was disregarded because of EMG signal instability due to movement artefact in the beginning of the contraction (Fig. 3). After 5 seconds, signal remained stable in all subjects. M-waves from each 1 s epoch were then averaged, and thus a sequence of 25 averaged M-waves (ABS) were obtained. The beginning and end of each M-wave were determined by setting a threshold for each subject individually (Fig. 4), because the shape of the M-wave varied between different subjects. The threshold was usually lower than zero because of the slow recovery of the M-wave. After determining the “boundaries” of the M-wave the individual M-wave properties [(duration (DUR), area (ARE), peak to peak amplitude (AMP) and root mean square (RMS)] were calculated between the cursors (Fig. 4) and averaged for each 1 second epoch. Median frequency (MDF) and mean power frequency (MPF) were also calculated (see below).

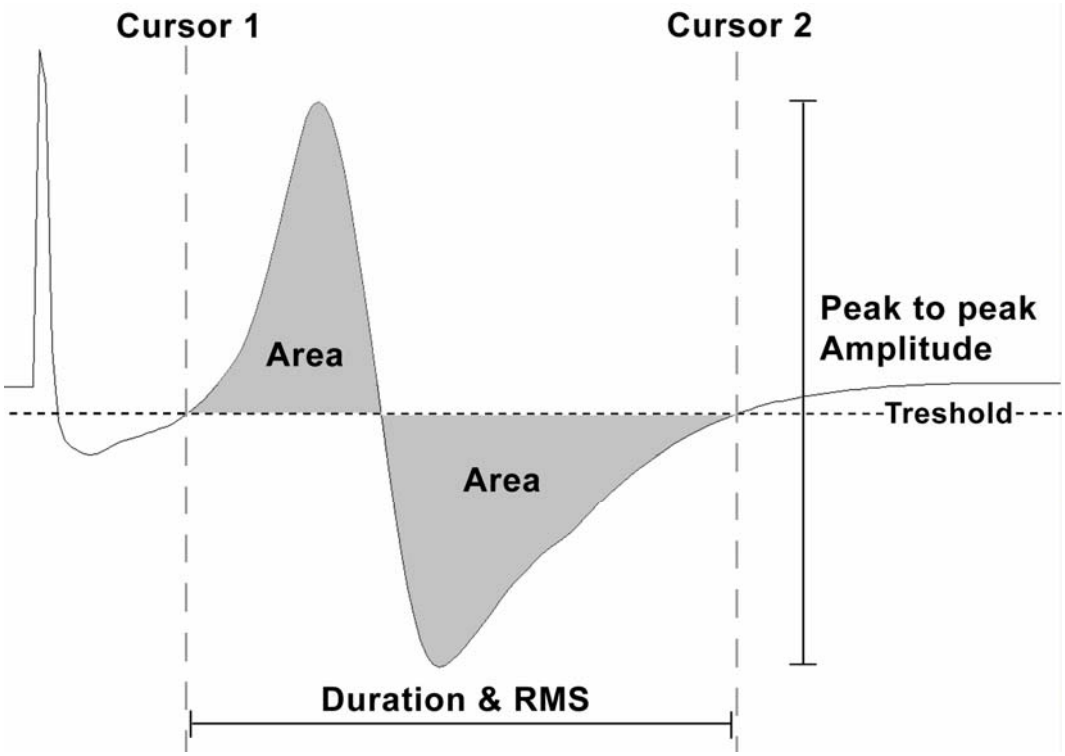

Figure 4. Schematic presentation of the setting of the threshold to calculate M-wave parameters for one subject.

For the MDF and MPF calculation, the first 10 M-waves from each 1 s epoch was first averaged, and then zero padded to reach 4096 data points, which was the size of the fast fourier transformation (FFT) window used to acquire power spectrum with frequency resolution of 1 Hz. From power spectrum MDF was determined as a frequency where the power was accumulated to 50 % and MPF was determined as a weighted mean of the power spectrum. The possible changes in the spectral shape due to different rate of change in MDF and MPF during the electrical stimulation (see Merletti et al. 1992) were estimated by using the two-tailed Pearson correlation coefficient.

To obtain the rate of change for each of the EMG parameter during the electrical stimulation, EMG parameters were normalized (NORM) with respect to their initial values (IV). IV was determined as the first epoch of the analyzed data. In addition, a regression-free area-ratio fatigue index (AR) (see Merletti et al. 1991) was calculated for each EMG parameter and was used as a fatigue index.

RMS and average EMG (aEMG) were obtained from the voluntary EMG signal with 0.5 s window. The middle of the window was placed at the maximal point of the torque signal of each isometric and eccentric MVC test individually.

Repeatability analysis

Trial-to-trial repeatability of electrically elicited EMG parameters were tested by comparing two repeated tetanic stimulations (PRE1 and PRE2) during familiarisation session. Similarly, day-to-day repeatability was tested by comparing two tetanic stimulations executed in different days before the exercise (PRE1 and BEF). The consistencies of ABS values, corresponding NORM values, the IV and the AR were tested (Table 1). The repeatability was assed by one-way random model of intraclass correlation coefficient (ICC) (Weir 2005).

Statistical analysis

All data were analysed by general linear model repeated measures analysis of variance to asses the effect of the eccentric exercise on measured parameters. Either the BEF was compared to the following measurements or successive measurements were compared to each other. Multiple comparisons were done with a significance level of $P < 0.05$. All values in the text are presented as means ± SD.

2.3 Results

Force production

Following the eccentric exercise of the elbow flexors the isometric and eccentric MVC decreased by 28.6 ± 8 % ($P < 0.001$) and 34.9 ± 5 % ($P < 0.001$) respectively, and remained below the initial level still two days after (2D) the eccentric exercise (Fig. 5).

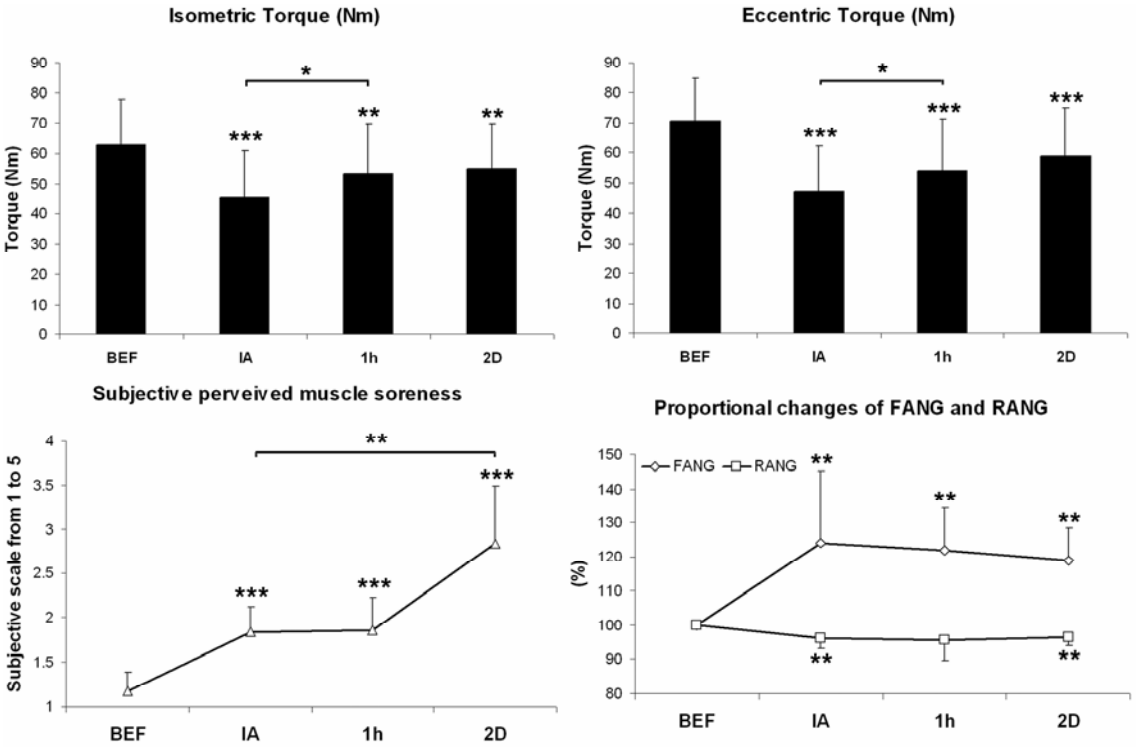

Figure 5. Maximal voluntary torques, subjective perceived muscle soreness and relaxed (RANG) and flexed (FANG) arm angle changes before (BEF), immediately after (IA), one hour after (1h) and two days after (2D) the eccentric exercise. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Muscle soreness and range of motion

Subjective muscle soreness of the right arm elbow flexors increased immediately after the eccentric exercise and increased even further on 2D. Flexed arm angle (FANG) showed an acute increase of 6.9 ± 6.7° ($P < 0.01$) due to eccentric exercise and it stayed above the initial level throughout the recovery period (Fig. 5). Relaxed arm angle (RANG) decreased immediately post-exercise by 5.9 ± 6.8° ($P < 0.01$) and was recovered 1h after the exercise. However, it was once again reduced on 2D (Fig. 5).

Blood measurements

Blood lactate concentration and pH did not change after the exercise. This was also the case for plasma creatine kinase (CK) activity. Significant changes were observed in the plasma electrolyte concentrations (Fig. 6). Plasma potassium ion and calcium ion concentrations increased immediately post-exercise by 5.4 ± 5.4 % ($P < 0.05$) and 1.7 ± 2.1 % ($P < 0.05$), respectively and returned to the initial level soon (1h) after the acute elevation. Sodium ion concentrations were unchanged (Fig 6). Moreover, blood total leukocyte count increased acutely, although its sub-fractions were unchanged (Fig 6).

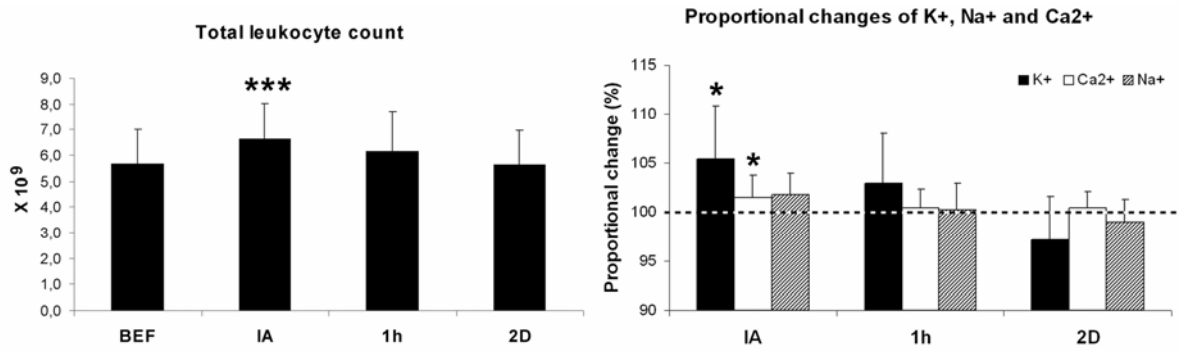

Figure 6. Total leukocyte count and proportional plasma ion changes before (BEF), immediately after (IA), one hour after (1h) and two days after (2D) the eccentric exercise. * = $P < 0.05$, *** = $P < 0.001$.

Voluntary and electrically elicited EMG parameters

The only significant difference in voluntary EMG variables was observed in the isometric MVC test, in which the RMS and aEMG decreased immediately post-exercise. No changes were observed in the EMG during the eccentric MVC test.

M-wave properties of area (ARE), duration (DUR), peak-to-peak amplitude (AMP) and root mean square (RMS) measured during electrical stimulation revealed a significant reduction immediately after the eccentric exercise. These changes were recovered 1h after the exercise. Interestingly, AMP was in higher level two days post-exercise as compared to baseline value (Fig 9).

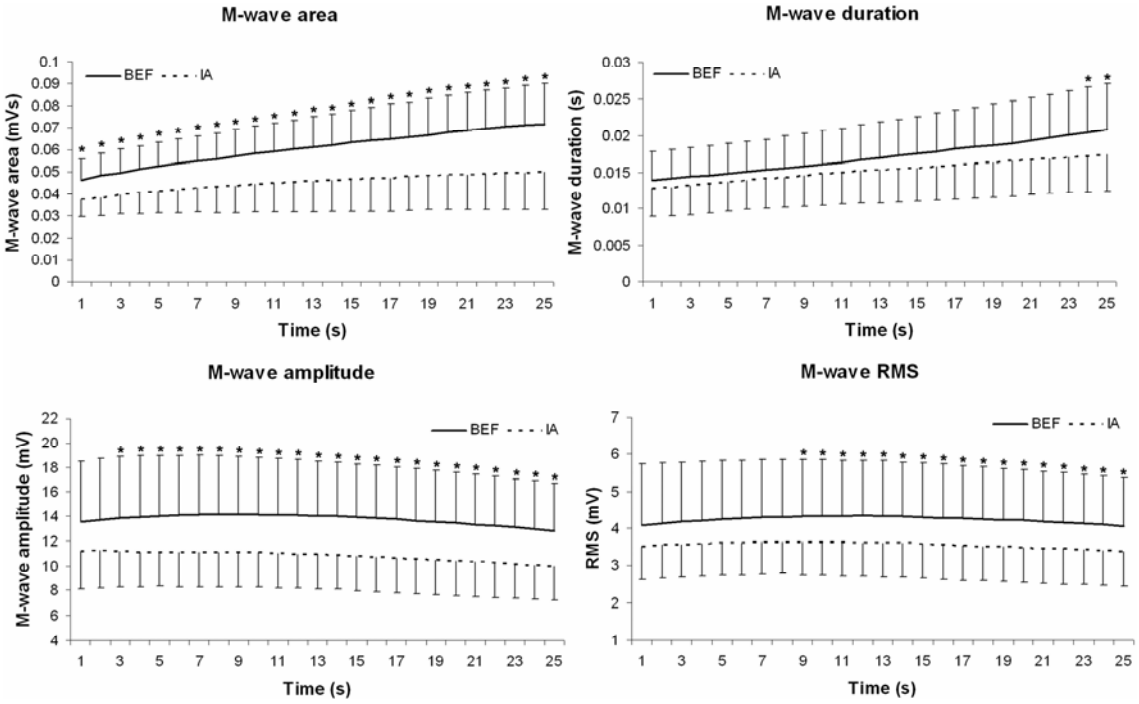

Figure 7. Time courses of M-wave area, duration, amplitude and RMS during the electrical stimulation before (BEF) and immediately after (IA) the eccentric exercise. * = $P < 0.05$.

The eccentric exercise affected the normalized (NORM) values for median frequency (MDF) and M-wave duration (DUR). Consequently, the rate of change in MDF was higher in the beginning of the electrical stimulation immediately after the eccentric exercise (Fig 8) and the rate of change in DUR was lower in the end of the electrical stimulation 1h after the eccentric exercise (Fig 8).

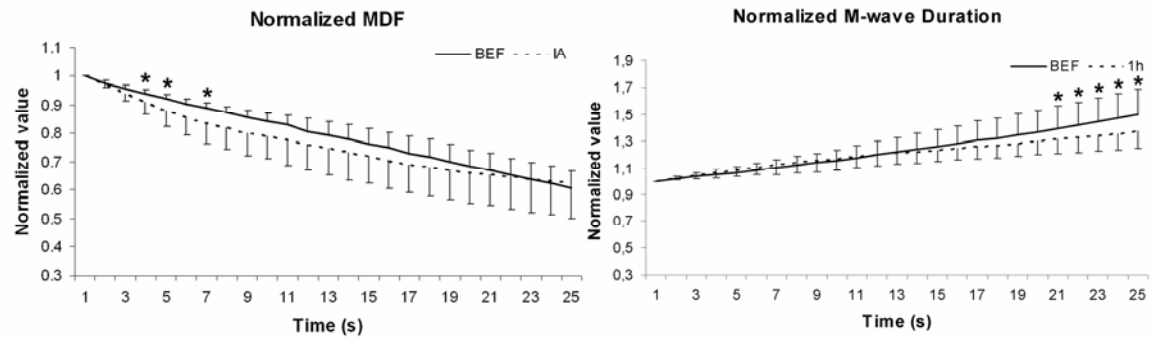

Figure 8. Time courses of electrically elicited M-waves normalized median frequency before (BEF) and immediately after (IA) the eccentric exercise and normalized M-wave duration BEF and one hour (1h) after the eccentric exercise. * = $P < 0.05$.

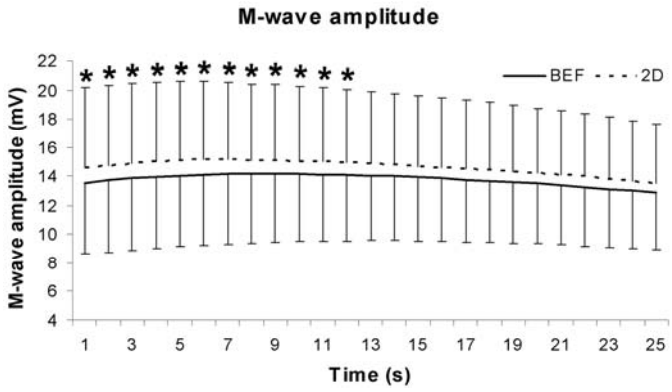

Fig 9. Time course of M-wave amplitude during electrical stimulation before (BEF) and two days (2D) after the eccentric exercise. * = P < 0.05.

The initial value (IV) of M-wave area decreased immediately after the eccentric exercise. It was recovered to the initial level there after (Fig 10). Interestingly, the IV of M-wave amplitude was increased on 2D (Fig 10).

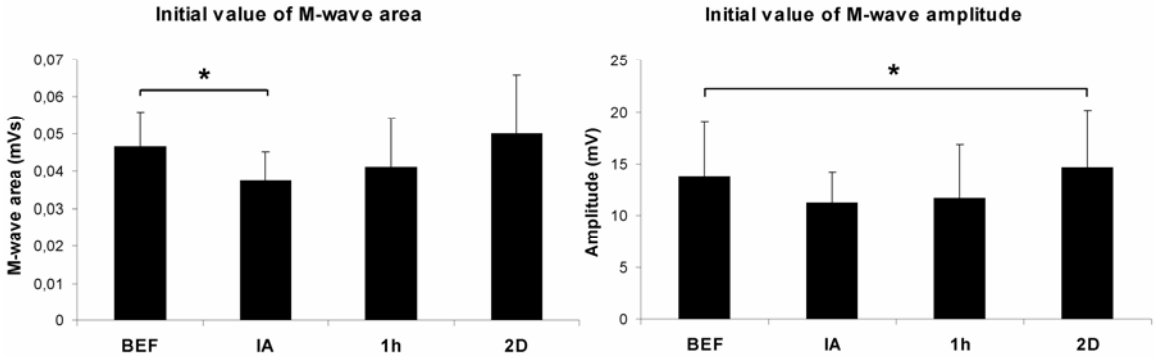

Figure 10. Initial values of M-wave area and amplitude before (BEF), immediately after (IA), one hour after (1h) and two days after (2D) the eccentric exercise. * = P < 0.05.

Repeatability analysis

The regression free area ratio fatigue index (AR) and IV calculated from electrically elicited EMG parameters showed excellent or good repeatability. Furthermore, the absolute (ABS) values of each 1 s epoch from the last 25 s of electrical stimulation expressed good or excellent repeatability. The normalized (NORM) values showed acceptable repeatability only in DUR and with lesser extent in ARE, MPF and MDF. Any data with poor repeatability was disregarded from further analysis. (Table 1).

Table 1. Intraclass correlation coefficient, ICC (%), for day to day and trial to trial. ICC values between 80 % and 100 % represent excellent repeatability and ICC values between 60 % and 80 % represent good repeatability. Bold values represent passed reliability test in the present study. AR = regression free area-ratio fatigue index, IV = initial value, ABS = average absolute values, NORM = normalized values, M-wave: area = ARE, duration = DUR, peak-to-peak amplitude = AMP, root mean square = RMS, mean power frequency = MPF, median frequency = MDF.

EMG parameter	Day to day ICC & Error Variance		Trial to Trial ICC & Error Variance	
ARE AR	**93**	**7**	**94**	**6**
ARE IV	**76**	**24**	**95**	**5**
DUR AR	**87**	**13**	**92**	**8**
DUR IV	**96**	**4**	**97**	**3**
AMP IV	**81**	**19**	**90**	**10**
RMS IV	**91**	**9**	**97**	**3**
MPF AR	**83**	**17**	**91**	**9**
MPF IV	**84**	**16**	**84**	**16**
MDF AR	**81**	**19**	**92**	**8**
MDF IV	**89**	**11**	**94**	**6**
ARE ABS	**> 83**	**< 17**	**> 95**	**< 5**
ARE NORM	**> 84**	**< 16**	**> 77**	**< 23**
DUR ABS	**> 92**	**< 8**	**> 96**	**< 4**
DUR NORM	**> 76**	**< 24**	**> 71**	**< 29**
AMP ABS	**> 83**	**< 17**	**> 89**	**< 11**
AMP NORM	> 2	< 98	> 28	< 72
RMS ABS	**> 87**	**< 13**	**> 93**	**< 17**
RMS NORM	> 40	< 60	> 54	< 46
MPF ABS	**> 67**	**< 33**	**> 85**	**< 15**
MPF NORM	**> 68**	**< 32**	**> 58**	**< 42**
MDF ABS	**> 83**	**< 17**	**> 93**	**< 7**
MDFNORM	**> 67**	**< 33**	**> 75**	**< 25**

2.4 Discussion

The eccentric exercise in the present study caused prolonged loss of muscle strength and clear symptoms of DOMS. The main findings were an acute decrease of M-wave ARE, DUR, AMP and RMS after the eccentric exercise. Furthermore, these M-wave properties did not show any prolonged impairment. Acute increase in plasma [K^+] and [Ca^{2+}] were also observed.

The exercise protocol included 10 s rest between eccentric contractions to minimize the metabolic fatigue. That was also the case, since blood lactate concentrations were unchanged throughout the experiment. This may be explained by considerably lower ATP turnover rate in eccentric actions as compared to concentric ones (Beltman et al. 2004). Thus, changes in muscle metabolites could also be lower in magnitude (Bonde-Petersen et al. 1972). Plasma CK was unchanged 2D after the exercise. This was probably due to a small muscle group used in the exercise and due to the possibility that the blood CK activity may have peaked later than two days after eccentric exercise, as has been observed earlier on elbow flexors (Lee & Clarkson 2003). Despite the small muscle mass involved in the exercise the total leukocyte count was elevated immediately after the eccentric exercise. Similar elevation has also been observed after eccentric exercise by other investigators (Malm et al. 1999, Paulsen et al. 2005, Pizza et al. 1996). In addition, they have suggested that the acute elevation may be due to increased circulatory stress. However, we did not observe delayed lekocytosis, again possibly due to small muscle group used in the exercise.

Reduction in the voluntary EMG activity immediately after the eccentric exercise during the isometric MVC test could suggest some alteration in the sarcolemmal excitability. Interestingly, that was not the case on 2D. However, the voluntary EMG activity does not provide reliable information from peripheral muscle fatigue, because of the possibility of central fatigue (Gandevia 2001) and the possibility of some inhibition at the spinal cord level (Gregory et al 2003, Proske & Morgan 2001, Proske 2005). Therefore, our later discussion is based mainly on electrically elicited EMG parameters.

The loss of muscle strength is usually emphasised immediately after heavy exercise, which is more or less dependent on the changes of crucial metabolites such as decrease of ATP and PCr levels (Nagesser et al. 1993). However, PCr/Cr ratio does not seem to decrease after eccentric exercise in humans if the frequency of the contractions is low (Nielsen et al. 2005) as was the case in the present study. Other possible contributors to the strength loss include a damage of force transmitting structures (Huijing 1999, Monti et al. 1999) or failure of EC coupling (Warren 2001).

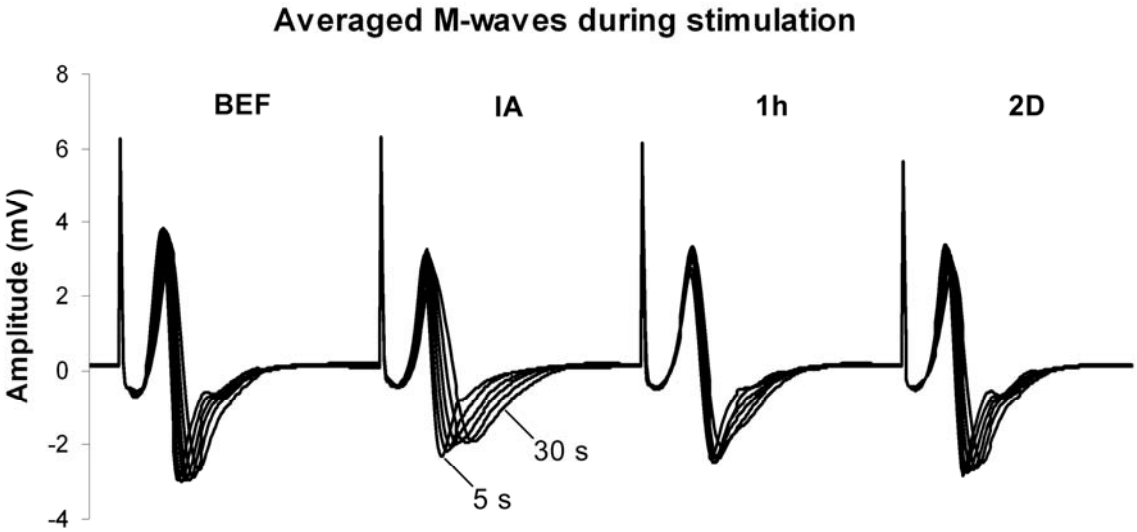

Figure 11. An example of six superimposed M-waves with 5 s intervals during tetanic stimulation from one subject.

EC coupling could be impaired by disturbances in the sarcolemmal excitability, which seems to be the case after the eccentric exercise in this experiment (Fig 7 and Fig 11). Sarcolemmal excitability may be affected due to many reasons, for e.g. because of direct damage to the sarcolemma itself or because of immediate energy source deficiency in the muscle cells. The common feature or outcome would be disturbed ion distribution and/or transport over the plasmalemma, either due to permeability changes in the sarcolemma or deficiency of the ion pump function. These changes would modify the shape of the M-wave (amplitude, duration, area, etc.), as was the case in the present experiment.

As mentioned above, M-wave properties of ARE, AMP and RMS in the M-wave trains were decreased immediately after the eccentric exercise (Fig 7 and Fig 11). This could be due to failed excitation of some MUs (Merletti et al. 1992). However, near unity

slopes, near zero intercept and high correlation coefficient of the normalized MDF and MPF BEF and immediately post-exercise observed in the present experiment invalidate this suggestion (Fig 12). This stationary in these parameters indicate no change in the shape of the power spectrum. Such change in the power spectrum shape would be expected if failed excitation of some MUs had been occurred (see Merletti et al. 1992). Other option for the decreased M-wave properties of ARE, AMP and RMS would be increased plasma [K^+]. It has been found earlier in animal studies, that depolarization of resting membrane potential due to increased extracellular K^+ may block muscle fibers excitability, increase the pulse strength necessary to initiate AP, decrease overshoot of action potential and reduce AP amplitude (Ovegaard & Nielsen 2001, Nielsen et al. 2004, Yensen et al. 2002). The possible mechanism for this could be a slow inactivation of TTX-sensitive Na^+ channels due to membrane depolarization (Ruff 1999), and it is the voltage-gated Na^+ channels that are the ones responsible for the propagation of AP in muscle cells.

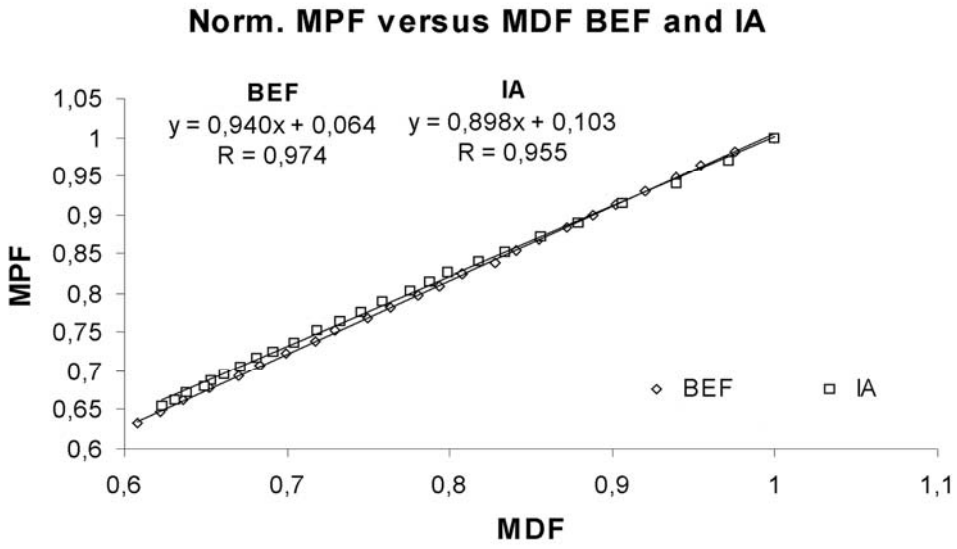

Figure 12. Correlation between normalized mean power frequency (MPF) and median frequency (MDF) before (BEF) and immediately after (IA) eccentric exercise.

A surprising finding was an acute decrease in the M-wave DUR after the eccentric exercise. This could be due to increased intramuscular temperature and, thus increased muscle fiber conduction velocity (CV) (Stålberg 1966). This possibility could also be supported by a slight, but non-significant acute increase in the MDF and MPF. It has been shown earlier that cooling of intramuscular temperature decreases median

frequency of EMG signal (Merletti et al. 1984) and, that intermittent muscular activity increases CV (Van der Hoeven & Lange 1994).

A very interesting increase in the rate of change in MDF was observed in the beginning of the electrical stimulation immediately after the eccentric exercise. Similar increase has been found in myotonic dystrophy patients (Chisari et al. 1998). Reduction in CV can compress the MDF to lower frequencies during electrical stimulation, however there are also some other factors which could contribute to the change in MDF (Merletti et al 1990, Naeije & Zorn 1982). In myotonic dystrophy, abnormal reopening of Na^+-channel is observed (Franke et al. 1990), which could cause a prolonged afterdepolarization and thus lengthening of AP depolarization zone and reduction in MDF (Chisari et al 1998). Similarly, eccentric exercise could result in prolonged intracellular AP and negative after-potential (Arabadzhiev et al. 2005, Dimitrova & Dimotrov 2003) due to disturbed ion distribution and/or transport over the sarcolemma. Consequently, it is tempting to propose that the increased activity of the stretch-activated ion channels would be partly responsible for our observation of acute increase in the rate of decline in MDF.

Sarcolemmal AP propagation does not seem to be impaired in animal models after eccentric contractions (Warren et al. 1993, 1999, Ingalls et al. 1998), even though McBride et al. (2000) have observed depolarization of the resting membrane potential in animals. Our finding of increase in plasma $[K^+]$ and $[Ca^{2+}]$ immediately post-exercise could suggest a loss of normal ion distribution across the sarcolemma and, thus a change in the resting membrane potential.

In the present study, the $[K^+]$ was measured from the sample drawn from the vein of the non-exercised arm and, therefore, the $[K^+]$ in interstitium of the exercised muscle must have been in a much higher level than our observation indicates. This idea is supported by prior demonstration of markedly higher elevation in interstitial $[K^+]$ as compared to the venous $[K^+]$ at similar work intensities (Juel et al. 2000). Furthermore, it is demonstrated earlier that, a reduction in the intracellular K^+ will dramatically reduce force production of a single skinned muscle fiber due to a loss of excitability of the t-tubular system (Ørtenblad&Stephenson 2003), which is likely the weakest part of the sarcolemmal AP propagation (Sejerstedt & Sjogaard 2000). All together, our observations of modified M-wave properties may suggest reduced sarcolemmal

excitability immediately after the eccentric exercise, although it has been reported that there is a marked safety margins for plasmalemmal depolarization (Cairns et al. 1995; Yensen et al. 2002).
Moreover, plasma ion concentrations were normalized soon after (1h) the acute increase of [K^+] and [Ca^{2+}]. This could imply an insignificant role of ion disturbances in the later stages of DOMS. However, we did not measure the ion concentrations on interstitium level. In the following experiments the use of microdialysis could offer more detailed analysis of ion concentration shifts during DOMS.

None of the electrically elicited EMG parameters were impaired 2D as compared to BEF values. On the contrary IV and the absolute AMP values during the electrical stimulation were enhanced on 2D. These findings support the idea of unaffected sarcolemmal excitability disturbance on the later stage of muscle damage.

Moderate eccentric exercise protocol was used in this study to avoid excessive swelling of the soft tissues. With more intensive exercise model alterations in the sarcolemmal excitability would probably be more evident, especially immediately post-exercise. The low frequency stimulation (20 Hz) used in this study is physiologically acceptable. However, with a slightly higher stimulation frequency (from 30 to 40 Hz), the time dependent alterations in the electrically elicited EMG signal could occur with higher rate, thus allowing a shorter stimulation period or greater amount of stimuli. This could be beneficial for more distinct detection of the alterations in the sarcolemmal excitability.

In conclusion, the results of the present experiment suggests that disturbance of sarcolemmal excitability is not the major factor on eccentric exercise induced prolonged loss of muscle strength. However, the eccentric exercise may alter the sarcolemmal excitability acutely, which seems to be related to the increased sarcolemmal permeability. This alteration is most likely due to increased activation of stretch-activated ion channels (McBride et al. 2000) and not so much due to extensive mechanical damage in the sarcolemma, since such damage has not been observed in humans (Yu et al. 2002a). Thus, the prolonged loss of muscle strength in DOMS relies most likely on impairment of EC coupling (Warren 1993, Warren et al. 2001) caused by other factor(s) than sarcolemmal excitability.

2.5 Acknowledgements

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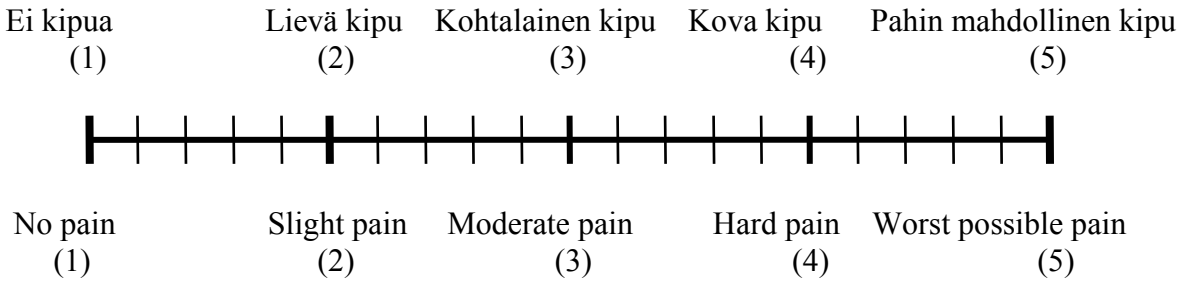
4 APPENDIXES

Apendix 1. Subjective continuous scale for assessment of perceived muscle soreness.



Appendix 2. Informed consent form.

Tutkittavan nimi_____
syntymäaika:_____._____._____

Yhteystiedot

_____

SUOSTUMUSASIAKIRJA INFORMED CONSENT FORM

SUOSTUMUS

Minua on pyydetty osallistumaan tutkimukseen ”LIHASSOLUKALVON SÄHKÖISEN TOIMINNAN ADAPTAATIO FYYSISESSÄ KUORMITUKSESSA”. Vakuutan, että luettuani koehenkilötiedotteen ja keskusteltuani vastuullisen tutkijan kanssa suostun tutkimukseen vapaaehtoisesti ja ilman painostusta. Minulla on ollut riittävästi aikaa harkita osallistumistani. En ole lääketieteellisestä tutkimuksesta annetun lain 488/1999 7-10 §:n tarkoittama henkilö (alaikäinen, raskaana/imettävä, vanki tai kehitysvammaisuuden/mielenterveyshäiriön/muun syyn vuoksi vajaakykyinen). Tiedän voivani milloin tahansa peruuttaa suostumukseni ja siten keskeyttää osallistumiseni tutkimukseen sen vaikuttamatta oikeuksiini. Ymmärrän tutkimuksen tarkoituksena olevan lihasväsymyksen ja lihasvaurion aiheuttamien lihassolukalvon sähköisen toiminnan muutoksien ja niiden suorituskykyyn aiheuttavien vaikutusten tutkimisen, ja ettei tutkimuksesta ole minulle terveydellistä hyötyä. Tiedän, että tutkittavat tutkimustoimenpiteet saattavat aiheuttaa haittoja. Mikäli haittavaikutuksia ilmenee, lupaan viipymättä ilmoittaa niistä tutkimushenkilökunnalle.

Tiedän, että henkilökohtaisia tietojani käsitellään luottamuksellisesti ja tutkimustulosten julkaisuissa tutkittavien tunnistaminen ei ole mahdollista.

Vakuutan, että terveydentilani on hyvä ja että en ole minkään lääkehoidon alaisena. Lupaan viipymättä ilmoittaa tutkimushenkilökunnalle mikäli terveydentilassani on tapahtunut muutos tai tutkimuksen aikana minulla ilmenee mahdollisesti lääkkeestä tai toimenpiteestä johtuvia sivuvaikutuksia. Ymmärrän, että annettujen ohjeiden ja rajoitusten, kuten tutkimusta edeltävän raskaan liikunnan välttämisen, tarkoituksena on varmistaa turvallisuutta, ja lupaan noudattaa kaikkia tutkijoiden ja tutkimuslääkäreiden antamia ohjeita. Hyväksyn sen, että tutkija voi keskeyttää osallistumiseni suostumuksestani riippumatta. Suomalaisella, ja tarvittaessa ulkomaisella, viranomaisella on lupa tutustua tutkimuksen koehenkilöasiakirjoihin ja tutkimusaineistoon.

_____ ___/___/2005 _____
tutkimushenkilön allekirjoitus

Tutkijan osuus:
Vakuutan, että olen antanut tutkittavalle ennen tämän asiakirjan allekirjoittamista riittävän selvityksen tutkittavan oikeuksista sekä tutkimukseen liittyvistä yksityiskohdista siten kuin lääketieteellisestä tutkimuksesta annetun lain 488/1999 6§:ssä edellytetään. Vakuutan, että kaikkea tutkimuksen aikana saatavaa tietoa käsitellään luottamuksellisesti ja että tutkimusryhmän ulkopuolisille annettavasta tiedosta (esim. julkaisut) tutkittavien henkilöllisyys ei ole tunnistettavissa. Tutkittavalla on oikeus milloin tahansa tutkimuksen kestäessä (myös syytä ilmoittamatta) peruuttaa suostumuksensa tutkimukseen, ilman että peruutus vaikuttaisi tutkittavan oikeuteen saada tarvitsemaansa hoitoa.

_____ ___/___/2005 _____
tutkijan allekirjoitus ja nimenselvennys

Appendix 3. Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles
for
Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52
nd
WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must 17.C 2 continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and

therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical

research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed

consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician’s judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.