The effect of short term lower limb ischaemia on human plantar-flexors muscle force and related neuromechanical mechanisms

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ABSTRACT

The effect of short term lower limb ischaemia on human plantar-flexors muscle force and related neuromechanical mechanisms

Ischemic lower limb is a syndrome that causes neuromuscular dysfunction in both acute and chronic periods. Only a short period of ischemia can produce intracellular metabolic disturbances affecting peripheral and spinal mechanism of human motor control. It affects mainly sensory and motor neuron excitability, neuromuscular transmission and skeletal muscle contractile mechanism.

The aim of this study was to assess the influence of short term ischaemia in lower limb on plantar-flexors (PF) muscle force production, eliciting by H-reflex and M wave recruitment curves and to determine the main site in the neuromuscular system affected by the pathogenic condition of ischaemia.

Seventeen healthy adult volunteers (11 male and 6 female) participated in the study with their Informed consent, experimental procedures were performed in accordance with the Declaration of Helsinki, and the study was the approved by the Ethics Committee. None of the subject had any history of vascular or other medical deficits known to affect neuromuscular function. The subjects lay prone on a table with both legs extended and the right foot attached (with respect to muscle tone) and secured to the force platform. The force platform was in vertical position. In order to induce ischemia blood pressure cuff was placed around the thigh 15 cm above the knee before the experiment started and inflated to a pressure of 200 mmHg for 10 min after the first series of measurements ended. Blood occlusion was repeatedly checked by an auscultation of the popliteal artery. During the experiment, the plantar-flexors (PF) force, H and M-responses of soleus muscle evoked by tibial nerve stimulation were measured at rest, during 10 minutes of ischaemia, 10 and 20 minutes after the occlusion was released. Obtained data were analyzed by means of one-way repeated measures ANOVA with Tukey post hoc analysis ($p<0.05$).

Background EMG activity of the soleus muscle was not significantly different between the four periods of experiment. However, threshold, recruitment

curves and transmission across the synapses of Ia afferent were significatnly altered during ischemia. At the post-ischeamic period the plantar flexors force fall significantly compare to pre-ischemic values and to ischaemia.
In conclusion our results show that ischaemia significantly reduce the mechanical performance (force) of plantar-flexors muscle for at least 20min and induce metabolic intracellular processes influencing excitability of both peripheral nerves and muscle fibers. These changes resulted in alteration of neuromechanical coupling.

Keywords: H-reflex, Ischaemia, Nerve excitability, Synapse transmission, Plantar-flexors muscle force, Neuromechanical coupling.

TABLE OF CONTENTS

ABBREVIATIONS

MU	Motor Unit
CNS	Central nervous system
ADP	Adenosine diphosphate
ATP	Adenosine-5'-triphosphate
EMG	Electromyography
H-reflex	Hoffmann reflex
M-wave	Motor response
PFs	Plantar flexors
MN	Motoneuron
H-reflex	Hoffmann reflex, response of afferent fibers
M-wave	Motor response of alpha motoneuron
Hmax	Maximal amplitude of Hoffmann reflex
Mmax	Maximal amplitude of motor response
EPSPs	Excitatory Postsynaptic Potentials
CMAP	Compound Muscle Fiber Action Potential
E-C coupling	Excitation Contraction Coupling
AP	Action Potential
PSI	Presynaptic inhibition

PREFACE

The initial baseline data of this work has been presented in the International congress of the Czech and Slovak Physiological Societies,
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Thank you

DECLARATION

The study described and presented in this thesis are the original work of the author, except where authors in the literature are cited.

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1. INTRODUCTION

The peripheral nerve is vascularized by a longitudinal network of vessels in the perineurium, epineurium and endoneurium, linked by many anastomoses. Significant reduction of blood flow in this network makes the nerve susceptible to ischemic vascular disease.

Ischaemia can play a central role in the pathophysiology and in the development of acute and chronic peripheral nerve dysfunction (Laghi Pasini, 1996, Teunissen, 2000, Hatzipantelis, 2001). For instance, ischemic lower limb is a syndrome that causes neuromuscular dysfunction in both acute (e.g. thrombosis) and chronic (e.g. diabetic peripheral neuropathy) periods.

To accurately introduce this topic, a comprehensive understanding of the neurophysiology of peripheral and spinal mechanism of human motor control system and a basic understanding of skeletal muscle biomechanics is required. Only a short period of ischemia can produce intracellular metabolic disturbances affecting peripheral and spinal mechanism of human motor control (Myogoros et al.1997, Grosskreutz 1999, Hogan, 1999, Zakutansky et al, 2005). This pathogenic condition affects mainly sensory and motor neuron excitability, neuromuscular transmission and skeletal muscle contractile mechanism and thus forces capacity.

1.1. Neurophysiology of human motor control system

The term motor system refers to the neural pathways that control the sequence and pattern of muscle contraction. The structures responsible for the neural control of skeletal muscle activity are distributed throughout central and peripheral control systems. As skeletal muscles can only contract in response to excitation of the motoneurons that supply them, all motor acts depend on neural circuits that eventually impinge on the alpha motoneurons that form the output of the motor system. Each motoneuron supplies a number of skeletal muscle fibers. An alpha motoneuron together with the skeletal muscle fibers it innervates constitutes a motor unit (MU) which is the basic element of motor control. For this

reason alpha motoneurons are referred to as the final common pathway of the motor system (Basmajian, 1985).

1.1.1. Excitability of sensory and motor neurons

The excitability of a neural tissue can be defined by the relationship between stimulation amplitude and stimulation duration, otherwise known as the strength–duration curve. There are two points on this curve that can be used to define the excitability of the tissue, the rheobase and the chronaxie (Fig. 1).
The rheobase of an excitable tissue is the minimum stimulus amplitude needed to elicit a response at infinitely long pulse durations; the chronaxie is the minimum pulse duration for a response when the stimulus amplitude is twice the rheobase (Ashley, 2005).

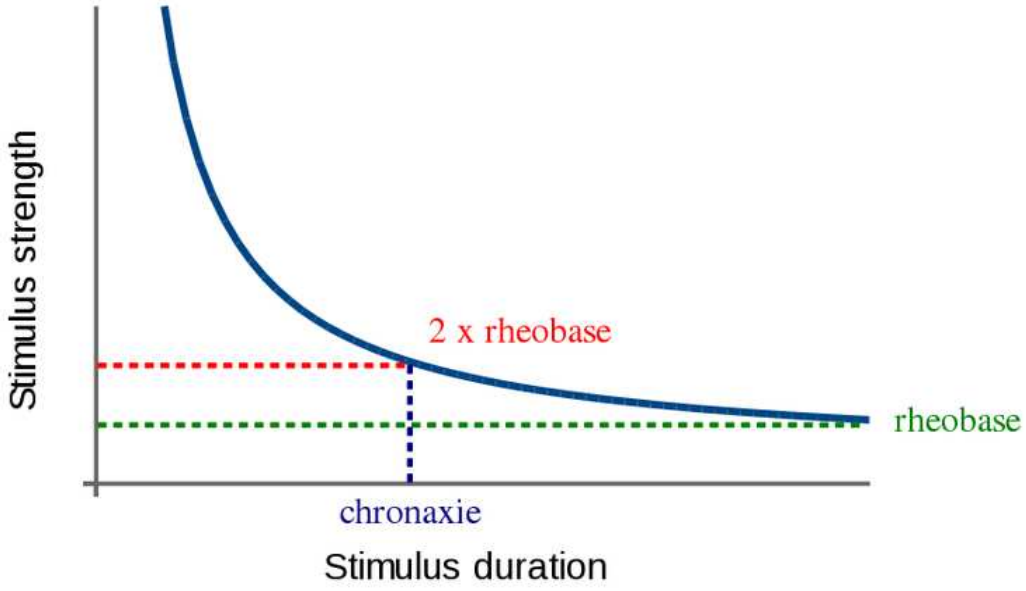

FIG. 1. Typical strength–duration curve obtained from skeletal muscle. The rheobase is the asymptote to the lower portion ofthe curve. The chronaxie is the pulse width required to produce a response at twice the rheobase (adapted from Ashley, 2005).

The excitability of the motoneurons is an intrinsic property which depends on the total membrane conductance, the membrane potential relative to threshold, and the presence of neuromodulators such as 5-HT (Capaday, 1997).

Excitability measures can provide evidence of altered axonal membrane properties and are complementary to conventional nerve conduction studies. A membrane potential is an important determinant of excitability and one part of the experimental protocol was design to determine the changes in a range of excitability properties related with alterations in membrane potential relative to threshold before, during and after ischaemia. Axonal membrane ion channels are voltage dependent and their function is related to the resting membrane potential of the axon and to changes in membrane potential. Excitability measurements are now being also used to provide functional information about neuromuscular disease (Kierman and Bostock, 2000). Membrane potential can vary indirectly by ischaemia

1.1.2. Excitability changes during and after ischaemia

Short term periods of ischaemia can paralyze the electrogenic Na^+-K^+ pump, increase $[K^+]_0$ and produce membrane depolarization. Previous electrophysiological studies performed in healthy subjects (Lin et al., 2002; Zakutansky et al., 2005) describes an increase in the excitability of the cutaneous afferent and motor axons by decreasing the threshold for both types of axons during ischaemia. This increase in peripheral excitability can be accompanied by a decrease in the efficiency of the Ia-fiber motoneuron synapse.

After ischaemia heightened activity of electrogenic Na^+-K^+ pump leads to axonal hyperpolarization. Grosskreutz (1999) reported that after ischaemia paradoxically, ectopic activity can be quite intense, particularly in sensory axons. This is probably due, in part, to activation of an inwardly rectifying conductance that is expressed more on sensory axons than on motor axons.

Decreases in motor and sensory thresholds indicate an acutely sensitive response; however, this response is limited to the axonal length, and does not uncover any information about alterations in synapse transmission with ischemia.

Synaptic transmission at the Ia-afferent terminals is controlled by presynaptic inhibitory mechanisms (Rudomin, 1990) and the terminals possess complex time, amplitude, and use dependent release properties (Capaday and Stein, 1986, Capaday, 2002).

1.1.3. Neuromuscular junction

The intrinsic excitability of the a-motoneurons is not the only factor involved in the net output of the motoneuron pool as a whole. A neuromuscular junction is the synapse or junction of the axon terminal of a motoneuron with the motor end plate, the highly – excitable region of muscle fiber plasma membrane responsible for initiation of action potentials across the muscle surface (Fig 2). Upon the arrival of an action potential at the axon terminal, voltage-dependent calcium channels open and Ca^{2+} ions flow from the extracellular fluid into the motor neuron's cytosol. The influx of Ca^{2+} triggers a biochemical cascade that causes neurotransmitter containing vesicles to fuse to the motor neurons cell membrane and release acetylcholine into synaptic cleft, a process known as exocytosis. Acetylcholine diffuses across the synaptic cleft and binds to the nicotinic acetylcholine receptors that are densily distributed at the motor end plate. These receptors are legand-gated ion channels, and when bound by acetylcholine, they open, allowing sodium to flow in and potassium ions to flow out the muscle cytosol. Because of the differences in electrochemical gradients across the plasma membrane, more sodium moves in than potassium out, producing a local depolarization of the motor end plate known as an end-plate potential. This depolarization spreads across the surface of the muscle fiber into transverse tubules eliciting the release of calcium from the sarcoplasmic reticulum, thus initiating muscle contraction. The action of acetylcholine is terminated when the enzyme acetylcholinestrase degrades the neurotransmitter and the unhydrolysed neurotransmitter diffuses away (Pocock G., Richards Ch., 1999).

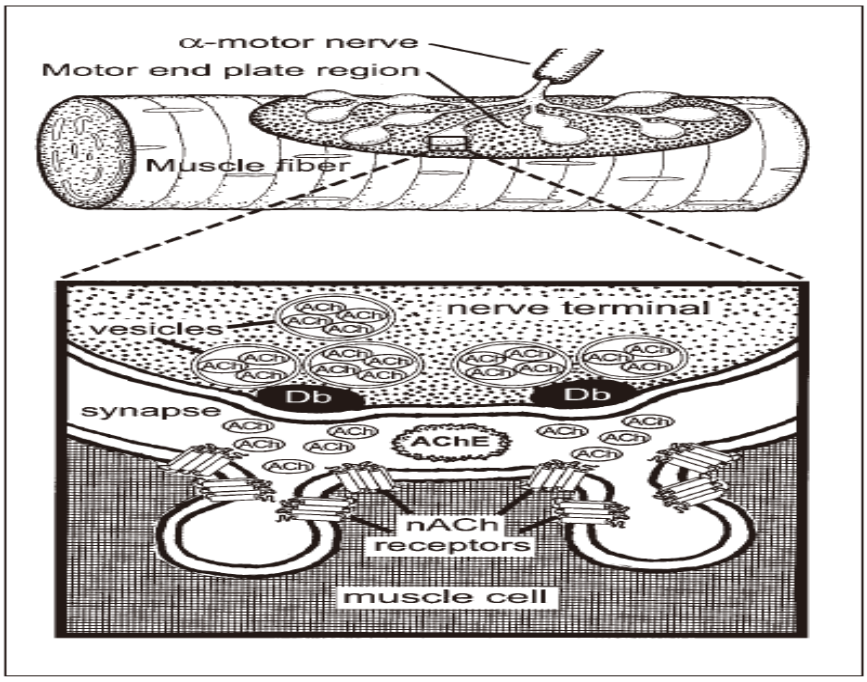

Figure 2. The motor end plate: proposed site of dysfunction during ischaemia

Top illustration: The junction between the motor neuron and the muscle fiber. The motor neuron terminates in multiple swellings termed synaptic boutons.

Bottom illustration: presynaptic boutons are separated from the postsynaptic muscle cell by the synaptic cleft. Within each bouton are many synaptic vesicles containing ACh, clustered around dense bars (Db). The Db is the site of ACh release into the synapse. Across the synapse from the Db, the postsynaptic muscle cell membrane forms junctional folds that are lined with nicotinic Ach receptors (nACh). ACh released into the synapse activates nACh receptors, and then is inactivated by the acetylcholinesterase enzyme (AChE). (adapted from MaCpartland, 2004)

1.1.4. Influence of ischaemia on the neuromuscular junction

Lundborg (1970) firstly noted that the neuromuscular junction is the most susceptible site of neuromuscular system to ischaemia. Also Hatzipantelis et al (2001) in recent years, design an experimental animal model using

electrophysiological method to evaluate the consequences of direct ischemic damage. The 80minutes arterial occlusion of the neuromuscular system of adult's rats causes a gradual reduction in twitch and titanic tension. After 50.7 (4.3) min of ischaemia, the muscle stops functioning under direct stimulation. For this duration of ischaemia the nerve function remained intact. This implicates significant alteration of function of the neuromuscular junction under acute ischaemia. From the above is obvious that the phenomenon of ischaemia interfere not only with neural circuits but also with contractility and endurance in skeletal muscle. The fact is that little is known about the influence of short term ischaemia on the human neuromuscular junction.

1.1.5. Neuromechanical properties of muscle fibers

Skeletal muscles contain three distinct types of motor units, slow-twitch (S), fast fatiguing (FF) and fatigue resistant (FR) (Enoka, 1988). These muscle fibers allow varied contraction force outputs and durations, which is important given the divers range of activities that skeletal muscles are required to achieve. They can be distinguished by force output, contraction duration and by color in histochemical studies. Also, the diameter of a motor nerve (axon of the motoneuron) is directly related to the type of muscle fibers recruited and number of muscle fibers it supplies.

The slow twitch (S or type I) are also called slow oxidative. These fibers split ATP at a slow rate and have a high capacity to generate ATP by oxidative metabolic processes. They are dense with capillaries and are rich in mitochondria and myoglobin giving the muscle fibers reddish colour. They produce relatively small contractile forces and are resistant to fatigue. These fibers are able to maintain a constant contractile force for very long periods. Type I fibers are oxygen dependant and as such are generally found in the deep parts of muscles where the blood supply is greatest. They are innervated by small motoneurons and are therefore the first to be recruited during voluntary

contraction due to the large input resistance in the motoneuron. (Henneman, 1965)

The fast, fatigue-resistance (FR or type IIA) or fast oxidative fibers contains a large amount of myoglobin, mitochondria and blood capillaries.They have a high capacity for generating ATP by oxidative metabolic processes and split ATP at a rapid rate. They are innervated by intermediately sized motoneurons and are recruited during moderately intensive tasks. Produce relatively fast and strong twitches and while they are fatigue resistant the contractile forces does decrease over time.

The fast fatigable (FF or type IIB) or also called fast glycolytic fibers. They have a low content of myoglobin, mitochondria and blood capillaries but large amounts of glycogen. They generate ATP by anaerobic metabolic processes and are generally found on the superficial layer of skeletal muscles. These fibers are innervated by the largest motoneurons and are only recruited during powerful voluntary contractions. They produce the fastest and strongest twitches (about 10 times the force produced by slow-twitch fibers) but fatigue quickly. The large motoneurons that innervate these muscle fibers, have a lower resistance than smaller motoneurons, and therefore have a faster action potential conduction time along the nerve axon (Enoka, 1988).

The sizes of the smallest motoneurons therefore also predict the stimulation threshold, the type of muscle fibers, and the number of the skeletal muscle fibers it innervates. This size principle dictates the order of recruitment of motoneurons as has been shown during investigations using the H-reflex (see below).

The total muscle output that results during specific tasks is result of both the number of motor units recruited and the firing rates of those motor units, which are activates to suit to the specific demand of task and have the appropriate mechanical characteristics . Such mechanism are functionally useful in providing smooth control of the muscle output via peripheral circuitry, and lessening the amount of control required by the central nervous system over the α-motoneuron pool during normal movement (Tucker, 2005).

1.1.6. Control of mechanical properties of skeletal muscle

The control mechanism of skeletal muscle relies on complex communication between the central and peripheral nervous system, including the integration of information from receptors that lay within many peripheral structures.

1.1.6.1. Central control system

The excitatory and inhibitory inputs that motoneuron pool receives from both peripheral and central origins establish the excitability of a motoneuron (Basmajian, 1985). The influence upon any motoneuronal pool is the tonic facilitatory influence which originates from the motor centers in the brain, the cortex, cerebellum and reticular formation (Kalezic, 2004). The supraspinal presynaptic inhibition, the influence of supraspinal centers on spinal-mediated presynaptic inhibiton, plays an important role in the regulation of normal muscle activity is particularly well described in studies detecting changes in muscle activity in patients with spinal cord injury. Also variable supra spinal influences on normal muscle activity are shown during different mental tasks or wakefulness (Burke, 1989,). The process of fatigue can also be partially regulated by central mechanism (Gandieva, 1992). Central mechanisms are not effective controllers of skeletal muscle activity alone, however, subsequent
proprioceptive input (e.g. muscle spindle) is constantly required to monitor kinesthetic sensations, and update the centrally generated program movement (Burke, 1989).

1.1.6.2. Peripheral control system

There are a variety of specialized receptors located in the muscles, tendons, fascia, and skin which provide information to appropriate parts of the central nervous system (CNS) concerning the state of the force and length characteristics of skeletal muscles.

The muscle spindle is the primary sensory structure within the muscle. The spindle system provides approximately one-third of the total peripheral input to postural muscle motoneurons, and is especially important in maintaining tonus and static posture in these muscles. They receive innervations from the fusimotor system- gamma motor system, which keeps the spindle in an active state, and therefore ready to fire at any particular length or tension.

Skeletal muscles contain a variety of sensory fibers wrapped around each of the intrafusal fibers. The larger group Ia and the smaller group II. The Ia afferent fibers connect with monosynaptic excitatory projection on the motoneuron pool of the same muscle and the group II afferent fibers with the disynaptic contact. The Ia and II group fibers modify their discharge rate as the mechanoreceptors endings of these fibers are elongated. They can be elongated, either by stretching the muscle which stretches the spindle capsule and thus the intrafusal fibers (discharge rate increases), or by contracting the intrafusal fibers via gamma fiber excitation (discharge rate decreases). Afferent fibers with free nerve endings and having wide range of diameters (groups II, III, and IV) have been identified. Pacinian corpuscles are specific skin sensors sensitive to touch and pressure, supplied by fibers of group I and II.

The Golgi organ is essentially a force sensor, therefore, it will respond in a fashion similar to an externally applied tension (during stretch) or an internally applied tension (during a voluntary contraction). The spindle, on the other hand, is sensitive to length and velocity, thus it will respond differently, depending on whether it is being elongated during a stretch or shortening during a voluntary contraction (Basmajian, 1985).

All information from central and peripheral influences converges at the spinal cord level, and it is the net effect of all the inhibitory and excitatory inputs to the motoneuron that determines its ability to fire.

The resulting mechanical activity that occurs in the firing muscle depends also on the Renshawn cells, cutaneus receptors, temperature and pain receptors, fatigue, hyperpolarisation of neurons and interneurones in the spinal

cord. They are all important peripheral modulators of skeletal muscle activity (Hagbarth, 1952, McMullan et al., 2004, Tucker 2005).

1.2. Muscle contraction

Skeletal muscles contract according to the generally accepted concept of sliding filament theory (Huxley A. and Niedergerke (1954), Huxley H. and Hanson (1954). That states that the exertion of a force output by skeletal muscle is accompanied by the sliding of thick and thin filaments past one another (Fig. 3).

The action potential originating in the CNS propagates by activating sodium dependent channels along the axon toward synaptic cleft. The action potential reaches the motor neuron terminal and causes a calcium ion influx through the calcium- dependent channels. The Ca^{+} influx causes vesicles containing the neurotransmitter acetylcholine to fuse with the plasma membrane, releasing acetylcholine out into the extracellular space between the motor neuron terminal and the motor and plate of the skeletal muscle fiber. The acetylcholine diffuses across the synapse and binds to and activates nicotinic acetylcholine receptors on the neuromuscular junction of the muscle cell. Activation of the nicotinic receptor opens its intrinsic sodium/potassium channel, causing sodium to rush in and potassium to trickle out. Because the channel is more permeable to sodium, the muscle fiber membrane becomes more positively charged, triggering an action potential.

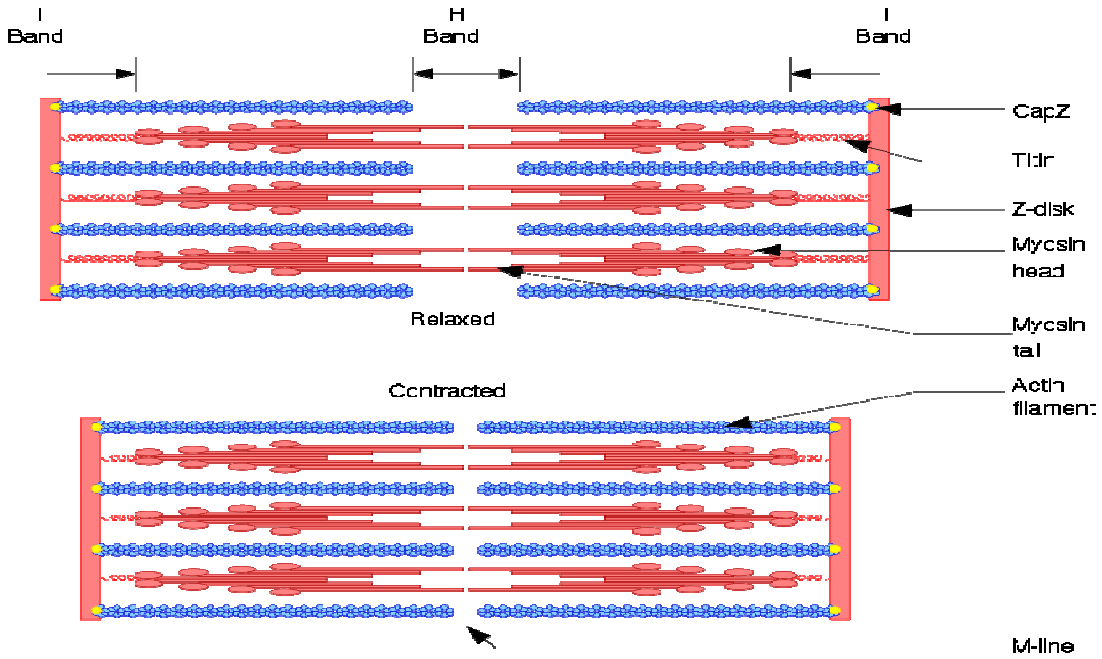

Figure 3. Sliding filament model of muscle contraction (adapted from Paulev, 2000).

The action potential spreads through the muscle fibers network of T-tubules, depolarizing the inner portion of the muscle fiber. The depolarization activates L-types voltage-dependent calcium channels (dihydropyridine receptors) in the T-tubule membrane, which are in closeproximity to calcium-release channels (ryanodine receptors) in the adjacent sarcoplasmic reticulum. Activated voltage-gated calcium channels physically interact with calcium-release channels to activate them, causing the sarcplasmic reticulum to release calcium. The calcium binds to the troponin C present on the actin-containing thin filaments of the myofibrils. The troponin then allosterically modulates the tropomyosin.

Myosin (which has ADP and inorganic phosphate bound to its nucleotide binding pocket and is in ready state) binds the newly uncovered binding sites on the thin filament. Myosin is now bound to actin in the strong binding state. The release of ADP and inorganic phosphate are tightly coupled to the power stroke. This will pull the Z-bands towards each other, thus shortening the sarkomere and the I band. ATP binds myosin, allowing it to release actin and be in the weak binding state (a lack of ATP makes this step impossible, resulting in the rigor state). The myosin then hydrolyzes the ATP and uses the energy to move into

the “cocked back” conformation. The previous two steps repeat as long as atp is available and calcium is present on thin filament. While the above steps are occurring, calcium is actively pumped back into the sarcoplasmic reticulum.

When calcium is no longer present on the thin filament, the tropomyosin changes conformation back to its previous state so as to block the binding sites again. The myosin ceases binning to the thin filament, and the contraction cease (Trojan et al, 1996, Paulev, 2000)

1.2.1. The mechanical output of the activated muscle – the force

In addition to the excitation from the nervous system, the force that a skeletal muscle exerts depends on two contraction factors.

The first factor is muscle mechanics. Muscle mechanics, represents a group of effect that depend on the characteristics of the force-generating units in muscle. An action potential in a skeletal muscle fiber is an all or none event, but the force exerted by a muscle fiber is not always the same. It depends on internal factors, such as the history of previous action potentials, and on external factors such as muscle fiber length and the speed of moment. The interdependence of external mechanical variables (muscle length, force velocity, power) gives the internal contractile state (e.g. availability of Ca^{++}, rate of occurrence of action potentials) of skeletal muscle (Enoka, 1988).

According to the sliding-filament theory (described above) the output of a force by muscle is accompanied by the sliding of thick and thin filaments past one another. An explanation to this theory can be given by crossbridge theory. The crossbridge theory suggests that crossbridges extending from thick filaments are able to attach to the thin filaments and then undergo a structural-chemical transition that exerts a tensile force. As the length of the muscle changes the number of thin filaments binding sites available for the crossbridges will change. That means that the tension varies as the amount of overlap between thick and thin filaments within sarcomere varies (Enoka, 1988). The rate of change of muscle length (velocity) play also major role in mechanical muscle force output.

The second factor is muscle architecture and refers to effects due to different arrangements of these force generating units within muscle.

The sarcomere is the basic functional unit of muscle. The effect that muscle exerts on rigid link depends on how the sarcomeres are arranged within the muscle (arrangement of force generating units). The intrinsic properties of the force-generating units within myofilaments, are further modified by the organization of the muscle fibers within the muscle that is the architectural or design features of muscle. The major three architectural influences are the average number of sarcomeres per muscle fiber (i.e., the in-series effect), the number of fibers in parallel (i.e., the in-parallel effect) and the angle at which the fibers are orientated relative to the line of pull of the muscle (i.e., the degree of pinnation), (Enoka, 1988).

The activation of muscle fiber is accompanied by change in the electric potential at the membrane. Electromyography represents an extracellular view of force generating muscle fibers that associated with the propagation of action potential. These electromyography techniques are well established and are reliable for measuring the positive or negative components of evoked potentials (DeLisa, 1994).

1.2.2. Thermo-mechanical aspect of muscle contraction

Muscular contractions can be classified according to either length changes or force levels to isotonic and isometric. In an isotonic contraction, tension remains unchanged and the muscle's length changes. There are two types of isotonic contractions: a) concentric and b) eccentric. In a concentric contraction, the force generated is sufficient to overcome the resistance, and the muscle shortens as it contracts. The force generated is insufficient to overcome the external load on the muscle and the muscle fibers lengthen as they contract.

The rate of positive work or work done by the muscle during contractions is indicated as power production. Power is determined as the product of the force and velocity of the skeletal muscle contraction. Power production is limited by the

rate at which energy is supplied for the muscle contraction e.g. ATP production (Fig.4.) and rate at which the myofilaments can convert chemical energy into mechanical work (power).

The chemical energy stored at ATP, i.e. creatine phosphate, is transformed by bioenergetics processes into mechanical and electrical work. During the processes of which conformation protein changes also occur in the field of these forces, representing spatial relocations of atoms and groups of atoms comprising the macromolecular complexes involved. This process can be assumed to represent one of the direct transformations of chemical energy into mechanical energy proceeding in interaction with a source of free energy generated during the process of fermentation reaction. This conclusion also seems to be confirmed by the fact that execution of mechanical work in living organism takes place under isobaric and isothermal conditions, whereby any assumption of thermal energy as the source of mechanical work is excluded. (Otahal in Valenta, 1993).

The production of contraction work and heat is related with the effectiveness of muscle contraction. Both forms of energy (heat and work of contraction represent the predominant consequences of the chemical reactions governing muscle contraction during the period of contraction. By experimental findings it was confirmed that: a) the release rate of all forms of energy encountered in muscle contraction are constant for a given load, b)Energy release rates change with the load in proportion to Hills factor F_0-F, c) During contraction the rate of energy release is constant for a given value of the relative load F/F_0, i.e. the release of energy in contraction is a linear function of the time of contraction. (Otahal in Valenta, 1993).

1.2.3. Energy sources for muscle contraction

ATP recycled form ADP in mitochondria is the immediate source of energy for muscle contraction (Fig.4). Although a muscle fiber contains only enough ATP

to power a few twitches, its ATP "pool" is replenished as needed. There are three sources of high-energy phosphate to keep the ATP pool filled.

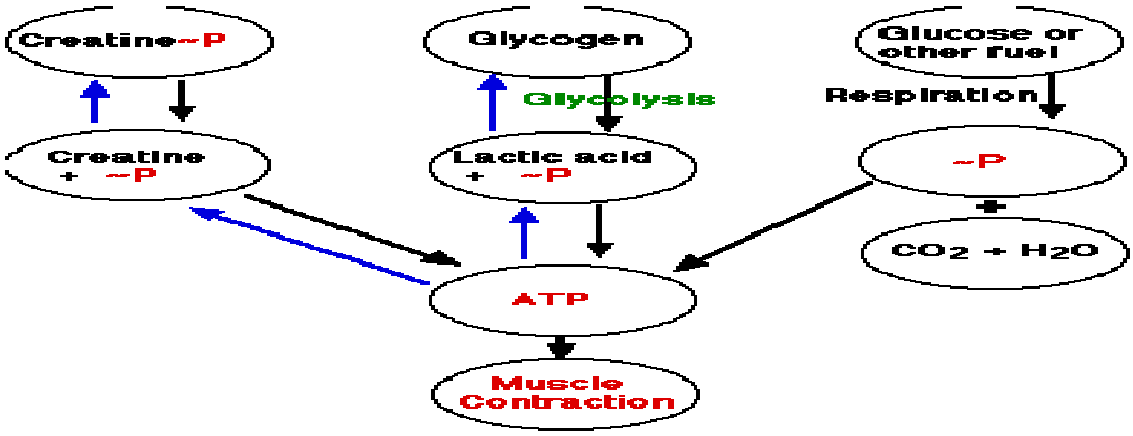

Figure 4. Three sources of high-energy phosphate of ATP (adapted from Gastin, 2001).

The phosphate group in *creatine phosphate* is attached by a "high-energy" bond like that in ATP. Creatine phosphate derives its high-energy phosphate from ATP and can donate it back to ADP to form ATP.

Creatine phosphate + ADP ↔ creatine + ATP

The pool of creatine phosphate in the fiber is about 10 times larger than that of ATP and thus serves as a modest reservoir of ATP.

Skeletal muscle fibers contain about 1% *glycogen*. The muscle fiber can degrade this glycogen by glycogenolysis producing glucose-1-phosphate. This enters the glycolytic pathway to yield two molecules of ATP for each pair of lactic acid molecules produced. Not much, but enough to keep the muscle functioning if it fails to receive sufficient oxygen to meet its ATP needs by respiration. However, this source is limited and eventually the muscle must depend on cellular respiration.

Cellular respiration not only is required to meet the ATP needs of a muscle engaged in prolonged activity (thus causing more rapid and deeper breathing), but is also required afterwards to enable the body to resynthesize glycogen from

the lactic acid produced earlier (deep breathing continues for a time after exercise is stopped). The body must repay its oxygen debt. (Gastin, 2001)

1.2.4. Alteration of muscle force and the role of ischaemia

The mechanisms accounting for an alteration in strength could arise from a variety of factors, which can be deviated into two categories; neurological and skeletal muscle dysfunction, as it is well known that the output from these two sources controls force production (Enoka, 1988).

Within the neuromuscular system, there are several potential sites that could affect voluntary force output, such as excitatory drive to the lower motoneurons, motoneuron excitability, neuromuscular transmission, sarcolemma excitability, excitation-contraction (E-C) coupling, and contractile mechanisms.

Reduction or fatigue in force output will generally occur when the demand of the ATPases exceeds the ability of the muscle to maintain ATP production through oxidative phosphorylation and substrate level phosphorylation e.g. during relatively high-intensity exercise. When working muscle is made ischemic, O_2 availability to the mitochondria can become compromised, and ATP generation by oxidative phosphorylation will be inadequate for the demand of the ATPases, resulting in attenuation of developed tension. The extent of the contractile dysfunction will depend on the duration and severity of the ischemic episode. During conditions of partial ischemia, the reduction in force development is generally proportional to the reduction in O_2 availability, and force generation will achieve a new steady state at which demand of the ATPases can be met by oxidative metabolism (Gladden et al., 1978, Hogan et al., 1998).

The recovery of force production after a fatiguing bout of high-intensity exercise follows a time course that is partially dependent on the restoration of homeostatic conditions in the intracellular environment. There is some evidence that, if a brief ischemic period is imposed in the midst of steady-state skeletal muscle contractions, subsequent blood flow reperfusion during the contractile

period can result in some degree of recovery in force development, demonstrating that the ‘‘fatigue’’ process under these conditions may be rapidly reversible. The factors that allow recovery of muscle function on reperfusion after an episode of brief blood flow cessation are not well understood (Hogan et al., 1998). Whereas recovery of force development after a period of brief ischemia will certainly be dependent on restoration of blood flow, it remains unclear whether it is the reinstatement of O_2 availability or some other factor related to blood flow [associated with either delivery of a substrate(s) other than O_2 or removal of metabolic waste products trapped in the tissue] that may result in a potentially rapid recovery of force development (Westerblad et al, 1991, Hogan et al, 1996).

Recently, Clark et al. (2006) studied a skeletal muscle contractile properties applying periodic cessation of blood occlusion in humans. The muscle cross-sectional area assesses by serial axial plane MRI scans and plantar flexors muscle measured by custom-modified dynamometer did not significantly change when the knee was in the flexed position.

1.3. H-reflex and M wave evoked potentials

The Hoffman reflex (Hoffmann, 1918) and motor M wave recruitment curves are induced in a variety of muscles by passing the percutaneous electrical stimuli through a mixed peripheral nerve within these muscles (Fig. 5).

The direct M-wave generally has higher activation threshold that the H-reflex because of the relatively thinner size of the motor axons compared to the muscle spindle Ia afferents (Fig.6). The M-wave occurs at a shorter latency (i.e. in the soleus muscle approximately at 8-10ms) to the H-reflex (i.e. in the m. soleus approximately at (30-45ms) (DeLisa et al, 1994)

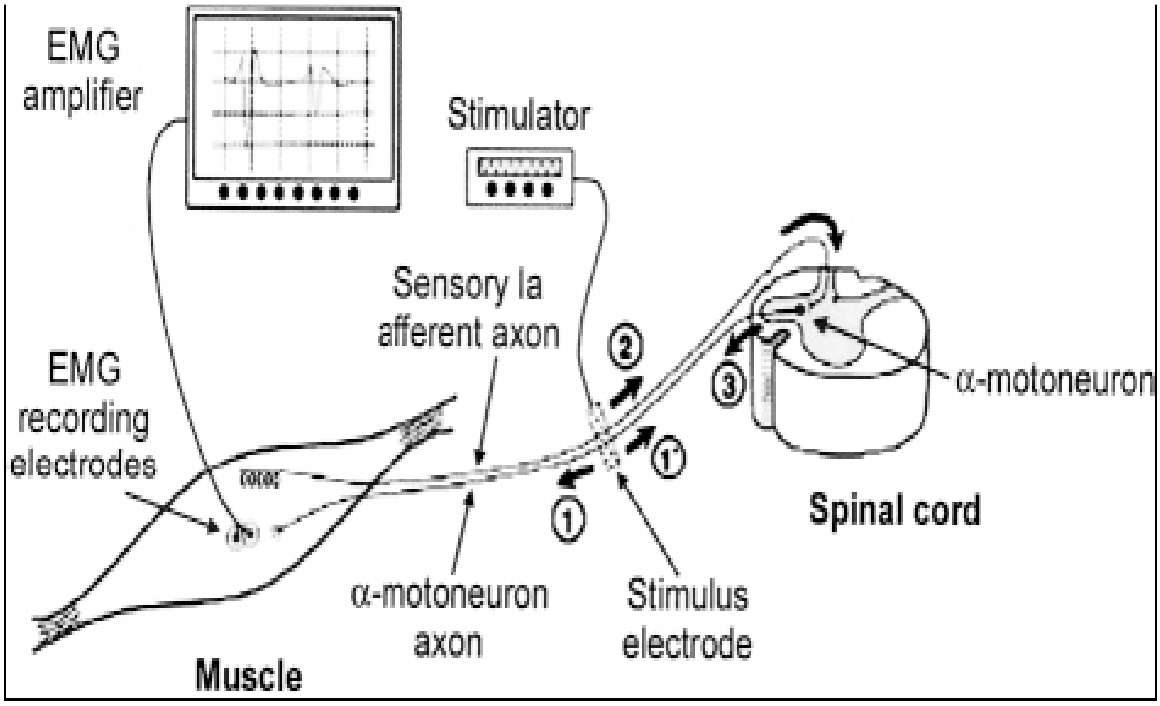

Figure 5. Eliciting Hoffmann reflex (H-reflex) and muscle response (M-wave) pathways. When a short-duration, low-intensity electric stimulus is delivered to the mixed peripheral nerve, action potentials are elicited selectively in sensory Ia afferents due to their large axon diameter (response 2). These action potentials travel to the spinal cord, where they give rise to excitatory postsynaptic potentials, in turn eliciting action potentials, which travel down the alpha motor neuron (aMN) axons toward the muscle (response 3). Subsequently, the volley of efferent action potentials is recorded in the muscle as an H-reflex. Gradually increasing the stimulus intensity causes action potentials to occur in the thinner axons of the aMNs (response 1), traveling directly toward the muscle and recorded as the M-wave. At the same time, action potentials propagate antidromically (backward) in the aMN toward the spinal cord (response 1) to collide with action potentials of the evoked reflex response (response 3), thereby resulting in partial cancellation of the reflex response. At supramaximal stimulus intensities, orthodromic (toward the muscle) and antidromic (toward the spinal cord) action potentials occur in all MN axons; the former gives rise to a Mmax, whereas the latter results in complete cancellation of the H-reflex (adapted from Palmieri et al., 2004).

. This is because the M-wave volley has to travel only along the motor axon while the H-reflex volley begins in the afferent fiber, synapses into the motoneuron in the spinal cord, then travels along the efferent fiber before eliciting a response in the target muscle (Capaday, 1997).

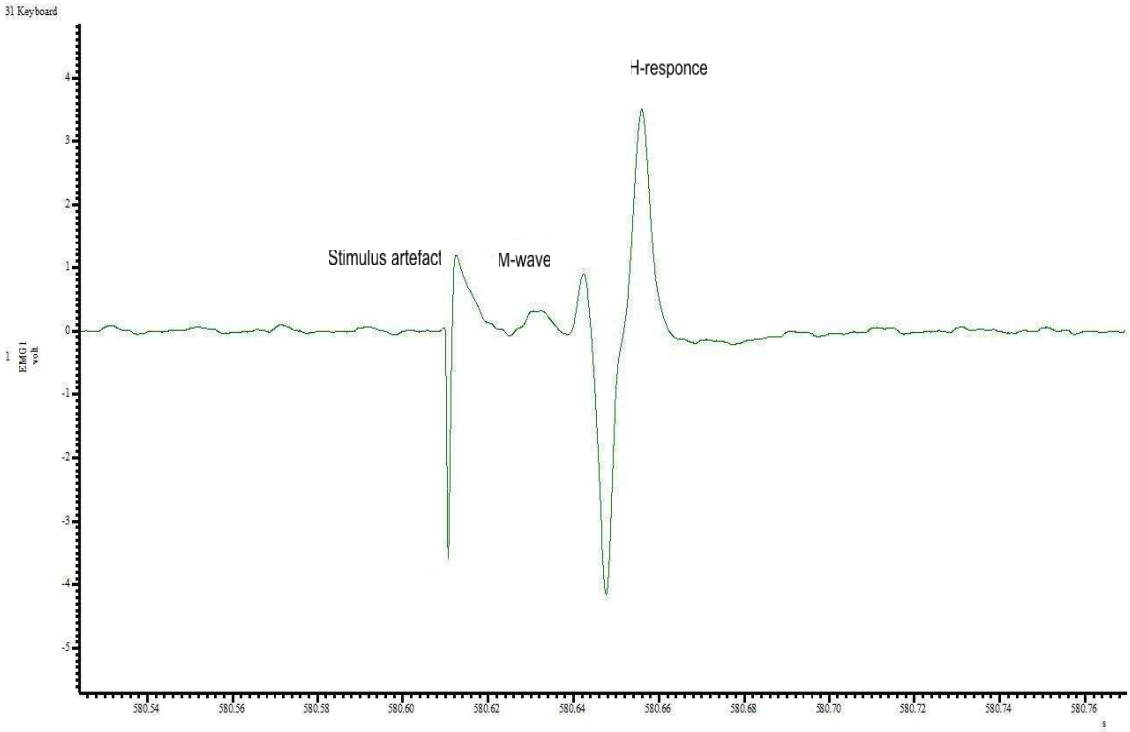

Fig. 6. Response of the H-reflex and M-wave to the electrical stimulation (Spike 2)

As the neural circuitry of the H-reflex is largely the same as the monosynaptic stretch reflex (tendon-tap reflex), the H-reflex has often been described as the electrical analog of the stretch reflex. However, since the H-reflex is evoked by the direct activation of the afferents, the sensory endings of the muscle spindle sense organs are bypassed along with the influences of gamma motoneuron activity on spindle sensitivity (Fig.7).

Fig.7. H-reflex and M-wave recruitment curves obtained with stimulus duration of 0.5ms. Note the clear separation of the threshold of group I afferent fibres and that of the a-motor fibers. Note also the obvious sigmoidal shape of the early portion of the H-reflex recruitment curve (adapted from Capaday, 1997).

1.4. Eliciting the H-reflex in the human plantar- flexors muscles

The H-reflex is most commonly studied in the triceps surae, with greatest focus on the soleus muscle because of the larger separation between the stimulus thresholds of its H-reflex and M-wave (Capaday, 1997). Electrical stimulation of the posterior tibial nerve in the popliteal fossa at various intensities evokes a direct (M-wave) and a reflex response (H-reflex) of the soleus muscle. The mechanical response induced by the tibial nerve stimulation at the popliteal fossa is generated by the whole plantar-flexors group, and possibly diminished by concurrent pretibial muscle activation. It has been established that the maximal

Hoffmann reflex (Hmax) of the soleus muscle produced by a sub-maximal electrical stimulation of the posterior tibial nerve may essentially be related to the activation of the slowest-twitch units in the examined motor pool, which are easily

excited by an Ia afferent volley (Pierrot-Deseilligny and Mazevet, 2000, Maffiuletti et al., 2000, Zehr, 2002). Instead the compound muscle action potential (Mmax), due to the direct depolarization of the a-motoneurons, is the electrical response to the activation of the entire motor pool, including fast-twitch units. Despite these reports, very little is known on the force output capacity of those motor units contributing to the maximal H-reflex.

1.5. Force platform

Force platform or force plate are measuring instruments that measure the ground reaction forces generated by a body standing on or moving across them, to quantify balance, gait, muscle force output and other parameters of biomechanics. The simplest force plates measure only the vertical component of the force in the geometric center of the platform. More advanced models (Kistler) measure the three-dimensional components of the single equivalent force applied to the surface and its point of application, usually called the centre of pressure, as well as the vertical moment of force (Robertson, 2004).

Force platforms may be classified as a single-pedestal or multi-pedestal and by the transducer (force and moment transducer) type e.g. piezoelectric sensors, piezoresistive. Single pedestal models, sometimes called load cells, are suitable for forces that are applied over a small area. For studies of movements, such as gait analysis, force platforms with at least three pedestals and usually four are used to permit forces that migrate across the plate. They should be distinguished from pressure measuring systems that, although they too quantify center of pressure, do not directly measure the applied force vector. Pressure measuring plates are useful for quantifying the pressure patterns under a foot over time but cannot quantify horizontal or shear components of the applied forces (Robertson, 2004).

2. AIMS

In conclusion, current knowledge about pathphysiological mechanisms and biomechanical consequences of short term ischemia of the lower limb is limited. Thus our aims were:

1) To assess the influence of short term ischaemia on the excitability parameters of sensory and motor neurons.

2) To evaluate the affection of neuromuscular transmission on this pathogenic condition of ischaemia.

3) To assess the plantar-flexors muscle force production, eliciting by H-reflex and M wave recruitment curves under ischemic and post-ischaemic conditions.

4) To determine the main site in the neuromuscular system affected by the pathogenic condition of short term ischaemia.

3. METHODS

This chapter describes the application of research methodology for the analysis of study design, the population studied and sampling criteria. Further, comment on issues concerning the reproducibility of the study and the validity/reliability of the experimental methods. Ethical issues that were considered, the equipment used in the research and the experimental procedures for collecting the data. Also describes the statistical test that was used in order to examine each research question addressed in this study.

3.1. Subjects selection

Seventeen healthy adult volunteers (11 male and 6 female) participated in the study during the period between February 2007 and October 2007.

In order to be recruited in to the study all volunteers were interviewed by the investigator to confirm the following inclusion criteria:

i) No previous injury or history of lower limbs or spine surgery;

ii) No functional limitations of the knee or ankle during plantar and dorsal flexion;

iii) No medical history of vascular, pulmonary or other medical deficits known to affect neuromuscular function;

Exclusion criteria were as follows:

i) The presence of severe low back pain and/ or limb disability affecting the function of triceps surae;

ii) Recent consumption of drugs or caffeine;

iii) Undergoing any training tasks before the measurement;

iv) Age over 45;

v) Any symptoms associated with muscle or any other pain sensation;

vi) Subject was excluded from the study, as the H-reflex could not be obtained before the M-wave in the soleus muscle.

3.2. Ethical approval

All experimental procedures were performed in accordance with the Declaration of Helsinki (Appendix 1), and the study had the approval of the Charles University Ethics Committee. The nature and purpose of the study was explained to the volunteers and written information about the research was provided before the subjects signed a consent form (Appendix 2).

3.3. Study design - experimental procedures

All subjects underwent identical experimental protocol with constant internal environment, temperature and body position. Before recording the outcome measures, the whole procedure was explained in detail to each of them.

3.3.1. Position of the subject

Subject positioning is crucial during H-reflex testing, because factors such as eye closure, head position, joint position or angle, remote muscle contractions, and muscle length, affect the H-reflex amplitude. In previous work, was found that comfort positioning subjects supine or prone and maintaining the same hand and head position throughout testing allows for reliable H-reflex measures (Hugon, 1973, Zehr and Stein, 1999, Hwang, 2002, Knikou and Rymer, 2002, Palmieri et al 2002).

The subject lay prone with both legs extended, on a standard physiotherapy table, with the face hole that allowed regular respiration. That position does not alter (from the influence of vestibular system) the strength of background muscle and reflex activity (Knikou and Rymer, 2002). The foot was outside the table and the ankle was positioned with respect to muscle tone. The right foot (barefoot) was attached and secured to the force platform. The foot was slightly attached from the distal points of first till the fifth metatarsal. The force platform was secured in vertical position (Fig. 8). The relaxation, comfort and

body adequate support of subject was maintained throughout the experiment to reduce the potential of any depressive influence on the soleus muscle and motoneuron pool (Hugon, 1973).

3.3.2. Recording H-reflex from the plantar flexors muscles

The superficial group of muscles – gastrocnemius, soleus, and plantaris- forms a powerful muscular mass in the calf of the leg that plantarflexes the foot. The other muscles (flexor hallucis longus, flexor digitorum longus and tibialis posterior) involved with plantar flexion just weakly assist. All these muscles are supplied by the tibial nerve and posterior tibial vessels (Moore and Dalley, 1999).

The large size and constitution of the two-head gastrocnemius and soleus support their different functional characteristic during standing and during movement of the lower limb (postural function). The soleus is most active when the foot is in dorsiflexion (lengthening contraction), while the gastrocnemii are most active when the foot is plantar flexed, during strong contraction or rapid development of tension. The soleus also de-recruits differently to the gastrocnemius during plantar flexion in humans and their motor neuron pools function quite separately (Herman, 1967, Nardone and Schieppati, 1988).

The H-reflex is most commonly studied in the soleus muscles, because of the larger separation between the stimulus thresholds of its H-reflex and M-wave (Capaday, 1997).

There are however limitations to extrapolating complete and comparative results from many investigations, due to the close proximity of these muscles and their tendency to co activate during certain movements, which can result in cross talk particularly when recording by surface electromyography and due to the large variability that exist between the experimental protocols off different authors.

3.3.3. Placement of the electrodes

Before placing the EMG electrodes, the skin was shaved, if necessary (in male), over the site of application, and gently cleaned with methylated spirit in order to reduce the electrical impedance between the skin and the electrodes (below 10KΩ). It was then left to dry.

To record the H-reflex and M-response, unipolar surface electrode was placed along the muscle fiber of the right soleus. The reference placed just above the external malleolus and the ground placed between the stimulating and the active electrodes (Fig. 8). To elicit the H-reflex and M-wave recruitment curves, an anode covered with gauze and wetted in saline was placed just above the patella and a point metal cathode (0.5cm in diameter) was fixed over the posterior tibial nerve in the popliteal fossa. This arrangement reduces the artifact of electrical stimulus and allows more selective stimulation of the tibial nerve when compared to the longitudinal stimulating electrode arrangement (Delisa et al., 1994, Capaday, 1997,).

It is well understood that electrode size, and type, will affect the recorded signal. This is because the surface signal is determined by the contributions of many active motor units and their interference pattern and because the recording electrode records the average potentials under its area. For this reason, the same types of electrodes were used in the current investigation.

3.3.4. Stimulation arrangement

The H-reflex size is known to depend on the stimulating frequency and intensity. The impedance of the tissues across the leg is relatively constant and the stimulating current would also be relatively constant (Hugon, 1973). The stimulus duration is an important parameter since it allows for a separation of a threshold of Ia-fibers from that of α-motor fibers. Hugon suggested that it should be 1ms, with shorter durations while Capaday suggest that 0.5ms duration is a good compromise for eliciting the response in the appropriate nerve fibres and

minimizing unpleasant sensation. Briefer stimuli would further separate the thresholds, but the necessarily greater stimulus intensities required makes such stimuli unpleasant, because nociceptive nerve terminals in the skin will be stimulated (Capaday, 1997).

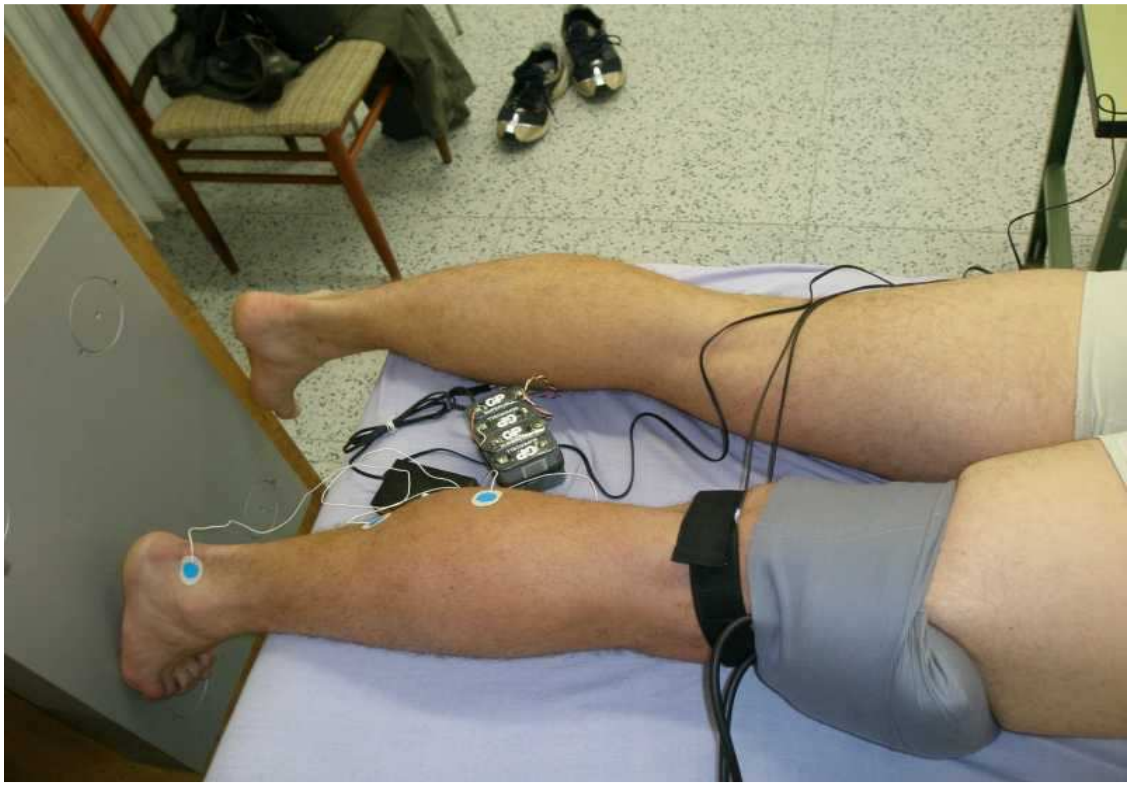

Figure 8. Subject position during experiment

Hugon suggest that period between stimuli should be no less than 5 seconds. However recent studies indicate that stimuli given 3 seconds apart will avoid any post activation depression when the test muscle is at rest, and that stimuli may be given up to 4Hz without loss of reflexes amplitude if the test muscle is voluntarily contracting (Burke et al., 1989, Capaday, 1997, Zehr, 2002).

To overcome trial-to- trial variability of the H-reflex, it has been suggested that the reflex response to 10 stimuli are averaged for each experimental condition (Hugon, 1973), however it is common for up to 20 (Knikou and Rymer, 2002).

3.3.5. Monitoring stimulus intensity

Monitoring the stimulating current alone does not guarantee that the same number of nerve fibres is stimulated at different times in the same task, at different contraction levels of the same task, or in different tasks. The use of constant current stimulator will promote the use of stable stimulus intensity throughout the study, as it will reduce the effect that changes in the skin-electrode impedance may have on the size of the responses obtained (Hugon, 1973, Zehr, 2002).

At present, the best measure of stimulus intensity is the M-wave amplitude (response of the a-motor fibres to direct stimulation of the nerve) that was also followed in this study. The assumption is that stimulation of a constant number of a-motor fibres also ensures stimulation of a constant number of afferent fibres (Capaday and Stein, 1986). It is commonly agreed that for each task investigated the possible discharge rates of afferent and efferent fibres relative to their refractory period should be considered (Capaday and Stein, 1986; Capaday et al., 1995). The relative refractory period of human nerves is between 4 and 5 ms, whereas the highest discharge rates of soleus motor units is between 10 and 15 spikes/s (i.e., one spike every 60–100 ms). Therefore, only a small fraction of motor axons will be refractory at any time. However, since the objective is to maintain the M-wave strength, or to normalize (explained below) the size of the reflex response to the maximal M-wave strength with little regard to the actual stimulus intensity required, the source of the current may be in practical terms irrelevant (Capaday, 1997).

3.3.6. Monopolar surface electrodes

Monopolar surface electrodes were used for recording surface muscle activity from the soleus. Monopolar recordings have been described to yield higher peak-to-peak reflex amplitudes when the potentials are measured just above the soleus fibers (Gerilovsky et al. 1989), while at increased stimulus intensities the H-reflex regardless of the change in its amplitude is characterized

by a shorter duration and especially by a smaller interval between the beginning and the end of the potential. Most authors preferred monopolar recordings (rather than bipolar recording) of leg muscles due to better reproducibility of the shape, and easier distinction between the M-wave and H-reflex (Gydikov et al., 1976, Garland et al., 1994, Knikou and Taglianeti, 2006).

3.3.7. EMG - data collection and storage

To record the H-reflex and M-wave evoked potentials, monopolar surface electrode (Ag/AgCl ,Ambu Blue Sensor NF) were taped over the right soleus (motor point) and reference placed just above the external malleolus. The ground was placed between the stimulating and the active electrodes. To elicit the H-reflex and M-wave recruitment curves, an anode covered with gauze and wetted in saline was placed just above the patella and a point metal cathode (0.5cm in diameter) was fixed over the posterior tibial nerve in the popliteal fossa.
Constant voltage stimulation was provided by single rectangular pulses of 0.5ms duration at a minimum interpulse interval of 10sec. The stimulating impulses were gradually increased, from H-reflex threshold to above the saturated state of the maximal M-wave. The EMG signal was amplified (GrassTelefactor) and were sampled by the input-output interface device CED Power 1401 (Cambridge Electronic Design, UK), while the Spike 2 software (Cambridge Electronics Department, UK) was used for data acquisition and further processing. Input signals were digitalized at 16bit (A/D card) resolution at the rates of 10 kHz and continuously stored on PC hard disk for further analysis. Further analysis was performed off-line.

3.3.8. Force- data collection and storage

Force platform (Kistler Instruments, Switzerland) with sampling frequency 5 kHz was used to record the plantar-flexors force output produced during the determination of H and M wave recruitment curves. The force platform was in

vertical position and when the foot touches the force plate the reaction forces were converted into electrical signals. An obtained signal was continuously stored (BioWare software) on PC hard disk for further offline analysis.

All data were obtained from subject's touches the force plate barefoot to eliminate variations caused by shoe design.

3.3.9. Amplitudes analysis

The amplitudes of the H-reflex, M-wave and Nmax were measured as peak-to-peak values in our study. Peak to peak amplitude analysis involve measuring the difference between the maximum and minimum (positive and negative values) peak of the response from the raw traces (Fig. 9).

The soleus H-reflex and M-wave measured as peak-to-peak amplitude are significantly larger from reflex values representing their size as area under the full-wave rectified waveform. In addition, the reflex measured as area appears to detect or to be more prone to homosynaptic depression (Palmieri et al 2002, Knikou and Taglianeti, 2006).

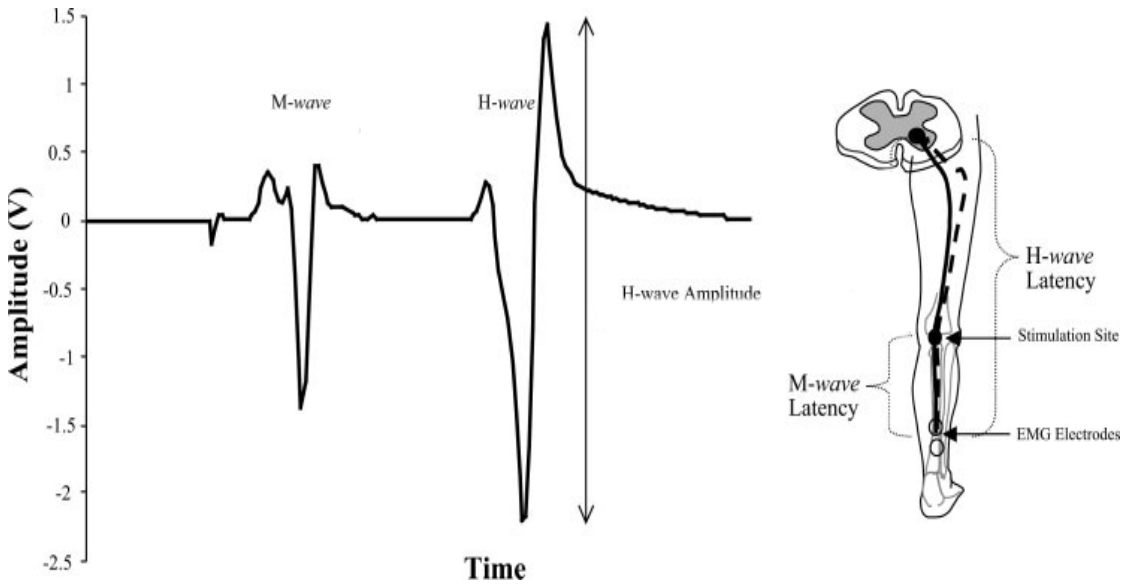

Fig. 9. Example of the soleus H-reflex evoked potential (peak to peak) and pathway (adapted from Clark et al., 2006).

3.4. Background level of motor activity

The background level of motor activity is a very important variable. During the course of the experiment at the time the H-reflex was measured was also followed. For a stimulus of fixed strength the amplitude of the H-reflex depends on the level of activity of the motor pool. H-reflexes of small or large amplitude relative to Mmax, a measure of maximum recruitment of the motor pool, increase approximately linearly and relatively slowly with the background level of motor activity (Capaday, 1997).

3.5. Normalisation factors in H-reflex studies

The amplitude of the H-reflex varies among subjects; therefore, it was necessary to normalize this value so between-subject comparisons can be made. These amplitude variations can result from variations in skin resistance, different amounts of subcutaneous fat, and locations of the nerve relative to the stimulus, among others.

3.5.1. Maximal M-wave amplitude

The most advocated method of H-reflex normalization is eliciting the H-reflex at a percentage of the maximal M-wave amplitude (Mmax). This method entails finding the amplitude of the Mmax and then adjusting the stimulation intensity to produce an H-reflex with amplitude equal to some percentage of the Mmax amplitude.

To elicit an H-reflex that is 10% of the Mmax, the first step was to measure Mmax. Next, the stimulation intensity was adjusted to produce H-reflex amplitude that was10% of the Mmax amplitude. Theoretically, this method allows for evaluation of the same proportion of the motoneuron (MN) pool for every subject. Eliciting soleus H-reflex amplitude that is 10% of the soleus Mmax means that 10% of the soleus MNs is being recruited from the entire soleus motoneuron pool (Palmieri et al 2002).

Another advantage of this method is it allows the stimulating and recording conditions to be monitored by examining the M-wave. If a constant stimulus is being delivered, then the M-wave amplitude should stay stable. Movement of the cathode relative to the nerve is the main technical factor limiting trans-cutaneous stimulation at constant intensity over the course of an experiment. During tasks such as walking and running the large angular displacement of the knee produces large displacements of the cathode relative to the underlying nerve. However, even during tasks involving no apparent movement of the knee, such as during quiet standing or isometric activation while seated, contraction of the underlying muscles may displace the stimulating electrode away from the nerve. Therefore, changes in M-wave amplitude can alert that the stimulating electrode may have shifted from its original position (decreased M-wave amplitude if the electrode was shifted away from the nerve and increased amplitude if it was moved closer, can be observed) or that the recording electrodes have moved relative to the muscle (Capaday, 1997).

3.5.2. Ratio of maximal H-reflex (Hmax) and M-wave amplitudes

When comparing across different conditions, it is a common method to obtain the full H/M-wave maximal amplitude of each of the conditions for H-reflex normalization. It is preferred over the H-reflex at a percentage of M-wave under these circumstances because of movement of the stimulating or recording electrodes, which makes it more difficult to assume the same portion of the MN pool is being stimulated (Zehr 2002).

The Hmax is an indirect estimate of the number of MNs being recruited and the Mmax represents the entire MN pool, the Hmax/Mmax ratio can be interpreted as the proportion of the entire MN pool capable of being recruited. Similar to expressing H-reflex as a percentage of Mmax, normalization of Hmax to Mmax is based on the assumption that the M-wave amplitude is a stable value. If the Mmax amplitude changes, this method of normalization is not

effective. Therefore, it is useful the raw Hmax and Mmax values to be reported under these circumstances. In cases when Mmax is stable and the ratio is used as the dependent measure, it should be reported that Mmax was analyzed and no differences were detected, so it can be assured that a change in the ratio is due to a change in H-reflex amplitude rather than M-wave amplitude.

A disadvantage of this method is that the H-reflex is less susceptible to facilitation and inhibition at higher amplitudes. Therefore, changes in Hmax may underestimate the amount of facilitation or inhibition under a given condition (Crone et al., 1990).

3.6. Arterial occlusion intervention – Ischaemia

In order to induce ischemia by mechanical pressure, we used a conventional sphygmomanometer. While the subject lied prone on an examination table and before we start any assessment a blood pressure thigh cuff was wrapped around the thigh of the investigated right leg. The cuff was fixed at mid-thigh level 15cm above the knee.

Ischaemia was achieving by using manual inflation of the cuff to an occlusion pressure of 200mmHg and maintained for 10 minutes after the first series of measurements ended. Using occlusive pressure at 200mmHg we avoid causing direct muscle and neural damage (Nitz et al, 1986, Schulte et al, 2008). Blood occlusion was repeatedly checked by an auscultation of the popliteal artery. Pneumatic tourniquet cuff inflation and deflation took approximately 8-10 second and 10 seconds, respectively. During all experiment and assessment procedures the cuff remains in the same position. All 17 subjects received the arterial occlusion intervention.

3.7. Experimental procedures

The experimental procedure of the study was divided in four periods:

i) The H-reflex and M-wave recruitment curve was obtained, before the cuff was inflated and ischaemia was induced (at rest). During these excitation of sensory and motor axons, the skeletal muscle contractile mechanism and thus forces capacity on plantar-flexors muscle force production was recorded. These values were used as control values.

ii) After completion of first, resting series measurement, ischaemia was induced to the right leg by inflated the blood pressure thigh cuff to an occlusion pressure of 200mmHg. Blood occlusion was checked via auscultation of popliteal artery (Korotkoff sounds) under the cuff. After the 5 minutes of ischaemia, a second recruitment curve of H-reflex, M-wave and plantar-flexors force output was obtained (second series). All these values were obtained during ischaemia (with inflated cuff).

iii) The cuff occlusion pressure was immediately released on completion of the above mention recruitment curves (the deflated thigh cuff remains in place). After 10 minutes from releasing the occlusion a third series of H and M-responses of soleus muscle and plantar-flexors muscle force output evoked by tibial nerve stimulation were measured (post-ischaemic values).

iv) A final recruitment curves of H-reflex, M-wave and plantar-flexors force were obtained 20 minutes after occlusion was released and reperfusion restored.

3.8. Analysis of outcome measures

Electromyographic and force evoked potential changes analysis allows the study of different aspects of the neuromuscular system function under ischaemia. The parameters chosen for measurement in the electromyographic and force

capacity changes in people with no known neuromuscular or vascular diseases were:

3.8.1. H-reflex and M-wave recruitment curves

The direct identification of neuronal circuitry in the human is limited by the obvious restrictions placed upon human experimentation. The H-reflex allows an indirect look into the organization and connectivity within the sensory-motor systems of the human.

H-reflex recruitment curve was obtained by gradually increasing the stimulus intensity from zero to an intensity that would elicit the maximum amplitude of the M-wave. Beginning with a low-intensity stimulus and gradually increasing its intensity until the depolarization of primary afferent fibers (Ia afferents) arising from the muscle spindle. The muscle spindle itself is not being stimulated, because we were activating the nerves electrically, thereby effectively bypassing the spindle. Activation of the Ia afferents results in action potentials being propagated toward the spinal cord.

If the activity in the Ia afferents is sufficient to cause depolarization of the presynaptic terminal, neurotransmitters are released into the synaptic cleft at the Ia-aMN synapse, eliciting excitatory postsynaptic potentials (EPSPs) in the MNs. If the EPSPs are able to depolarize the MNs (this depends on MN membrane potential and the size of the EPSPs), action potentials are generated, causing acetylcholine release at the neuromuscular junction, contraction of the muscle, and appearance of an H-reflex tracing on the EMG. At low levels of stimulation, the afferent fibers are preferentially stimulated due to their intrinsic properties and their larger diameter. As the stimulus intensity continues to increase, more Ia afferent fibers are recruited as they begin to reach their threshold, resulting in activation of more MNs and increasing the amplitude of the H-reflex (Capaday & Stein, 1986, Lin et al., 2002).

When the H-reflex measurements was performed, it was an advantage to use a stimulation intensity that produces M-wave responses corresponding to a

constant percentage of the maximal M-wave to ensure that the same number of motor axons are recruited in each trial, which indicates that stimulus intensity to the efferent nerve is also kept constant (Capaday, 1997).

Thus, in the present study, the electrical stimulation was first increased to obtain a maximal compound muscle fiber action potential (Mmax), and the stimulation intensity was adjusted and continuously monitored to evoke a muscle action potential (M-wave) with peak-to-peak amplitude equal to 2.5% of the CMAP. With incremental application of stimulation from zero, the following notable parts, as describe further below, of the recruitment curve will be observed: H-reflex threshold, M-wave threshold, H-reflex and M-wave latencies, maximal evoked amplitudes of H-reflex (Hmax) and M-wave (Mmax).

3.8.2. Excitability measurements of sensory and motor axons

One part of the experimental protocol was design to determine the changes in a range of excitability properties related with alterations in membrane potential relative to threshold before, during and after ischaemia.

To assess the influence of short term ischaemia on the excitability parameters of sensory and motor neurons and to determine the strength–duration properties (see chapter 1.1.1) of the soleus H-reflex and of soleus motor axons, the thresholds required to produce an H-reflex and an M-wave were measured.

The excitability of the motoneurons is an intrinsic property which depends on the total membrane conductance, the membrane potential relative to threshold, and the presence of neuromodulators such as 5-HT(serotonin); as mentioned previously in the introduction. However, the intrinsic excitability of the a-motoneurons is not the only factor involved in the net output of the motoneuron pool as a whole. Synaptic transmission at the Ia-afferent terminals is controlled

by presynaptic inhibitory mechanisms and the terminals possess complex time, amplitude, and use dependent release properties (Capaday, 1997).

Cadapay and Stein (1986) on the basis of neural modeling studies and animal experiments that for a fixed level of a-motoneuron pool activity and stimulus intensity, the H-reflex output depends on the level of presynaptic inhibition of Ia-afferent terminals in the spinal cord. That means the H-reflex is a measure of the efficacy of synaptic transmission when measurements are made at matched levels of motor activity and also that, the intrinsic excitability of motoneurons and the recruitment gain of the motor pool may be related factors (Devanne et al., 1995).

Changes in the excitability of the H-reflex can indicate the existence of more complex interactions, which may provide insight into the integrative processes of some neuronal circuitries. Knikou and Conway (2001) showed that application of pressure to the sole of the foot significantly depressed the soleus H-reflex. It was demonstrated that electrical stimulation of the plantar skin also produced inhibition of the soleus H-reflex if the hip was in a flexed position. However, changing the position of the hip to extension resulted in the same electrical stimulation of the plantar skin to produce facilitation of the H-reflex. This study indicates that stimulation of the foot sole accesses an inhibitory and facilitatory pathway onto the soleus H-reflex pathway, and that the afferent feedback associated with hip joint angle is used as a switch between them.

In order to assess whether the excitability of afferent and efferent axons change during and after ischaemia, both threshold intensity measurements of H-reflex and M-wave were performed during all four periods. Threshold for H-reflex and M-wave was fixed at 2,5% of H-reflex and M-wave maximal amplitudes (Hilgerwoord et al., 1994). That means changes in excitability of sensory and motor fibers were measured by the difference in stimulus intensity required to evoke wave amplitudes of H-reflex and M-wave seen at threshold level during the resting period. As a result of this procedure, a decrease in stimulus intensity

represented an increase in excitability; conversely, an increase in stimulus intensity represented a decrease in excitability of afferent and efferent fibers (Zakutansky et al, 2005).

3.8.3. Latency

The length of the H-reflex pathway, which takes into account limb length, was important to keep in mind when examining the amount of time it takes for the H-reflex to appear on the EMG. Before becoming visible on the EMG, the action potentials making up the H-reflex have to travel up the afferent fibers to MNs and then down the motor axons to the muscle. The time it takes for the H-reflex to appear on the EMG relative to the introduction of the stimulus is referred to as its latency. The closer the muscle is to the spinal cord, the shorter the latency of the H-reflex. For example, the soleus H-reflex tracing appears on the EMG at a latency of approximately 35 milliseconds after stimulus delivery, whereas the vastus medialis H-reflex appears after only approximately 20 milliseconds

The M-wave is a muscle response to the direct stimulation of motor axons with high threshold electrical stimulus. Due to the relatively short path the action potentials must travel for a muscle response to occur, the M-wave tracing appears on the EMG at a shorter latency than the H-reflex (ie, shows up first in the tracing). In the soleus, for example, the M-wave appears at approximately 8 to 10 milliseconds; as mentioned previously, the H-reflex appears at approximately 35 milliseconds (Pierrot-Deseilligny & Mazevet, 2000, Falco et al., 1994).

3.8.4. Maximal amplitudes (Hmax & Mmax)

The next parameter that was measured and analyze was the H-reflex maximal amplitude (Hmax). This is a measure of maximal reflex activation or, stated differently, is an estimate of the number of MNs one is capable of activating in a given state. For example, if an athlete has his peroneal Hmax

measured preseason and then again immediately after sustaining a lateral ankle sprain, we could compare the measurements to determine if the muscle was affected after the injury. We would expect that the peroneal H-reflex would decrease from the preseason measurement and infer that the peroneal muscles are inhibited, preventing the athlete from recruiting MNs during a contraction (Palmieri et al., 2004).

To compare H-reflexes between all subjects and ischaemia conditions, reflex amplitude was normalized to the maximum evoked motor response (Mmax). That is, the amplitudes of both the H-reflex and the M-wave were normalized to the largest M wave that was evoked during stimulation (see chapter 3.5.1.).

The maximal amplitude of M-wave (Mmax) represents activation of the total motoneurone pool and, therefore, maximum muscle activation. It has been generally assumed to be of constant amplitude as long as the recording conditions are unchanged and when muscular fatique is absent. When the Mmax is reached, every MN that supplies the muscle of interest is thought to be activated and thus the value should be stable. (Crone et al.,1999, Pierrot-Deseilligny & Mazevet, 2000, Zehr, 2002).

3.8.5. Synapse transmission - Hmax/Mmax

In our study the sensory transmission across the Ia-alpha motoneuron synapse was assessed with the ratio of H-reflex maximal amplitude and M-wave maximal amplitude. With this ratio was evaluated the affection of neuromuscular transmission on this pathogenic condition of ischaemia (Zakutansky et al., 2005).

The Hmax/Mmax ratio also removes much of the variation in absolute H-response size due to variation in local anatomy and the location of recording electrodes. The H/M ratio is a standard tool to diminish the effects of peripheral conditions on response sizes (Hilgerwoord et al., 1994, Zakutansky et al., 2005). The ratio of maximal H-reflex amplitude and maximal amplitude of M-wave (Hmax/Mmax) was used for H-reflex normalization (see chapter 3.5.2.).

3.8.6. The Stimulation-Force curve of the plantar muscles

One of the aims of this study was to comprehensively investigate and to assess the plantar-flexors muscle force production, eliciting by H-reflex and M-wave recruitment curves under ischemic and post-ischaemic conditions. In this study we report the efficacy of interventions (ischaemia) designed to selectively target the plantar flexors muscle force output. The changes in skeletal muscles contractile properties as a consequence of blood flow occlusion were reported.

The contraction of the plantar flexors was induced by a rectangular electrical pulse (0,5ms in duration) delivered by a custom-made stimulator (Dissa, Denmark). The stimulus was delivered to the tibial nerve (during evocation of H-reflex and M-wave recruitment curve) through electrode. The electrical stimulus used to elicit the Hoffmann reflex was progressively increased, until a maximum H-reflex (Hmax) was obtained. The muscle compound action potential (M-wave) was recorded by increasing the electrical stimulus until the M-wave and the corresponding output reached a plateau. The level of stimulation was then set 10-15% above this point (supramaximal stimulation) to ensure maximal muscle activation (Klass et al., 2004). The M-wave and the mechanical twitch in response to single and paired supramaximal stimuli were recorded.

The degree of muscle activation during contraction was assessed and recorded by force platform (Kistler Industries, Switzerland) with sampling frequency 5 kHz. An obtained signal was continuously stored (BioWare software) on PC hard disk for further offline analysis. The assessment of plantar flexors force capacity ended when the H-reflex and M wave recruitment curves were obtained and supramaximal stimulation was applied. Peak twitch tension, i.e. the highest value of the maximal muscle twitch response were measured. Data were expressed in absolute terms (N/ms).

Very little is known on the force output capacity of those motor units contributing to the H-reflex.

3.8.7. Latency

The latency of plantar flexors muscle maximal force output was measured to detect possible differences in the contractile characteristics of the muscles to the electrical stimulus. Latency time was measured from the stimulus artifact to the point where the maximal response of plantar flexors muscle force twitch (the action potential) was elicited by the supramaximal transcutaneus stimulation of the tibial nerve.

3.8.8 Neuro-mechanical coupling

Another parameter that was measured and compare was the changes in a range of neuro-mechanical coupling properties of skeletal muscle relative to threshold before, during and after ischaemia.

To assess the influence of short term ischaemia on the neuro-mechanical parameters of excitation-contraction coupling and to determine the difference in stimulus strength properties of contractile machinery, the thresholds intensity required to produce a plantar flexors muscle twitch were measured in all four periods. The stimulus intensity for threshold measurement was delivered at the same time with the thresholds required to produce an H-reflex and an M-wave. That means changes in neuromechanical coupling were measured through the evaluation of the evoked electromyographic and force output potentials.

Those were assessed by the difference in stimulus intensity required to evoke peak wave of plantar flexors muscle twitch seen at threshold level during all periods of experiment. As a result of this procedure, a decrease in stimulus intensity represented an increase in strength-duration properties of neuromechanical coupling

The single pulse stimulus intensity required to elicit plantar flexors muscle twitch evoked potential seen at threshold level, were followed to compare axonal and skeletal muscle neuromechanical mechanisms.

3.8.9. Mmax - Force relation

Pre-ischaemic, ischaemic and post-ischaemic measures of plantar-flexors muscle force output related to coumpaound muscle fiber action potential (Mmax) were measured. A single, supramaximal electrical stimulation was delivered to the tibial nerve with stimulation intensity set at 15% above the level of maximal amplitude of M-wave to ensure maximal muscle activation (Klass et al., 2004). The M-wave and the mechanical twitch in response to single and paired supramaximal stimuli were recorded. The peak-to-peak amplitude of Mmax was assessed, as well as the maximal peak twitch force of PFs muscle.

Caution must be taken in such experiments, because excessive rate in electrical stimulation may lead to block of neuromuscular transmission (Jones, 1996). This was avoided by using adequate stimulation frequency (see chapter 3.3.4 and 3.3.5.). Thus, simultaneously measured EMG evoked potential provides analysis of M-wave, which contains information about membrane properties of the active MUs (Merletti et al., 1992).

The recovery of force production after ischaemia follows a time course that is partially dependent on the restoration of homeostatic conditions in the intracellular environment (Hogan et al., 1999). There are a number of sites that can contribute to the force- capacity aspect during and after ischaemia.

The potential force-failure mechanisms known to exist at this level include all the above mention parameters but also contractile machinery and energy metabolism. Very little is also known whereas recovery of force development after period of short ischaemia will be dependent on restoration factor of blood flow.

3.9. Statistical analysis

To compare the data of the measurements those were done before, during ischaemia and two times in post-ischaemic period, conventional statistical methods were used to calculate means, standard deviations (SD), and standard errors of the mean (SE). When not specified, data were reported as means ± SE.

Obtained data were analyzed by means of a one-way ANOVA with repeated measures. When significant main effects were observed, Tukey test was used for post hoc analysis. A probability of $p<0.05$ was chosen as the significant level in all analyses.

4. RESULTS

4.1 H-reflex and M-wave native traces

All participants in the present study were found to be within normal limits for each of the individual variable measures (e.g. the H-reflex could be obtained before the M-wave in the soleus muscle).

H-reflex and M-wave native traces were recorded from soleus muscle with constant stimulus intensity and duration of 0.5ms during all four phases of the experiment. Increasing the stimulus intensity was accompanied by a progressive increase in the amplitude of both H and M responses and a subsequent decline in the H-reflex amplitude while the M-wave continued to grow.

This allowed comparison of the properties of sensory and motor axons evoked potential to stimuli delivered on the tibial nerve and its effectiveness.

4.1.1. Evoked potential

Characteristic evoked potential of H-reflex and M-wave amplitudes using constant stimulus intensity are depicted in Fig. 10. In this figure it can be seen the effectiveness of the same stimulus intensity on H-reflex and M-wave amplitudes before, during and after ischaemia. From these evoked potentials can be observed that the effectiveness of stimulus strength differ during the course of experiment. The H-reflex amplitude that was elicited 10min after ischaemia significantly differs in comparison with the amplitude evoked with identical (54V) stimulus strength during ischaemia. All post-ischaemic values of H reflex amplitudes increase significantly compare to values during ischaemia. To describe this phenomenon more accurately H-reflex and M-waves recruitment curves were obtained.

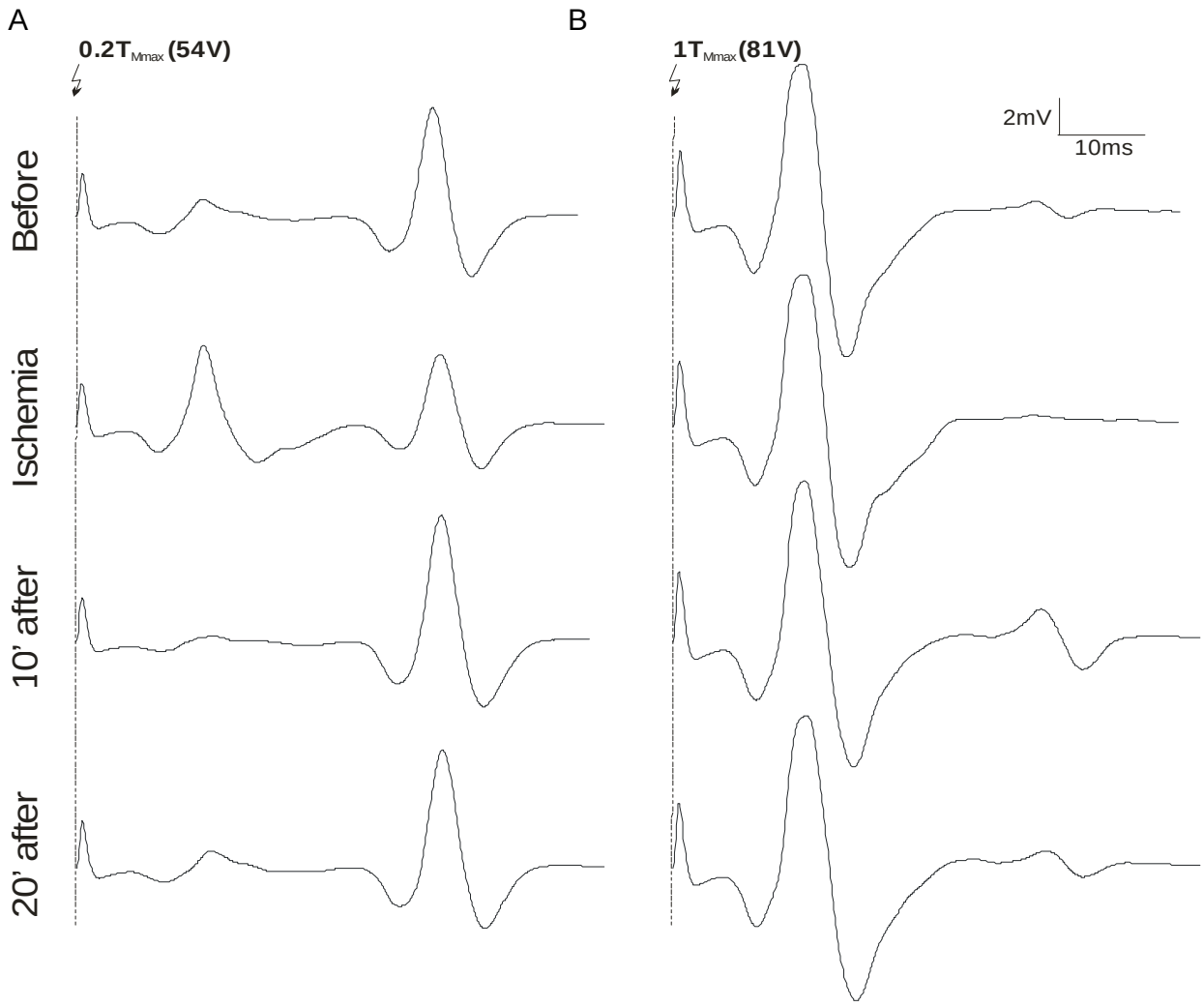

Fig.10. Evoked potentials of the H-reflex (A) and M-wave (B) to the same stimulus strength before, during ischaemia, 10 and 20 minutes after reperfusion. Note the significant changes between H-response before vs during ischaemia and 10min after ischaemia ($P < 0.05$).). Identical stimulus elicited smaller H-reflex amplitude during ischaemia but after 10min from occlusion release the amplitude significantly increased. The post-ischaemic values of H reflex amplitude increase significantly also compare to values during ischaemia.

4.1.2. Recruitment curves

H-reflex and M-wave recruitment curves were analyzed to explore the above mention changes (Fig. 13a, 13b). In this figure we can observe decreases in amplitude of H-reflex to the same single pulse intensity and a shift to the left of both recruitment curves. This indicates an increase in excitability of sensory and motor axons. To evaluate these excitability findings threshold values of H-reflex and M-wave amplitudes were measured.

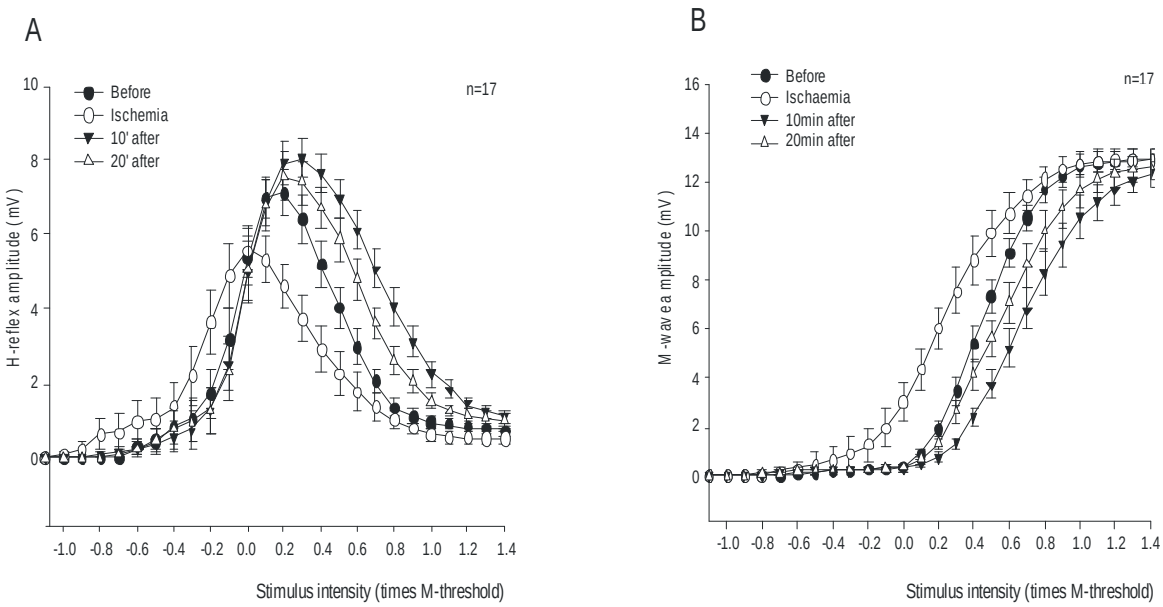

Fig.13. Mean changes in the H-reflex **(A)** and M-wave **(B)** recruitment curves. H-reflex recruitment curve during ischaemia is smaller than those before and after reperfusion and is shifted spatially to the left. Similarly, both post-ischaemic recruitment curve of H-response was significantly greater as compared with those during ischaemia ((P <0.001). The M-wave recruitment curve is significantly shifted to the left during ischaemia as compared with those obtained before ischaemia and 10 and 20min after reperfusion.

4.1.3. Thresholds

In order to compare the difference in stimulus strength, threshold intensity measurements for H-reflex and M-wave were analyzed in all four periods (Fig. 14a, 14b). The stimulus intensity for threshold measurement was fixed at 2.5% of the maximal H-reflex and M-wave amplitude (Hilgervoord et al., 1994). Before ischaemia, the H-reflex threshold occurred at 45.000 ± 2.627% (mean ± standard error) while during ischaemia the H-reflex threshold decrease significantly ($P<0.05$) and occurred at 39.000 ± 1.897%.

This reduction on H reflex threshold was associated with significant differences in the M wave threshold (58.313 ± 3.297% versus 48.188 ± 2.266%) during ischaemia. It means that short term ischaemia can increase the excitability of the sensory and motor fibers. After reperfusion, H-reflex (46.938 ± 2.798% for 10min after and 47.438 ± 2.672% for 20min after reperfusion) and M-wave threshold (63.938 ± 3.362 for 10min after and 61.313 ± 3.376% for 20min after reperfusion) increase significantly and overpass the values during ischaemia ($P<0.05$).

The motor axons became hyperexcitable 10 minutes after ischaemia and overpass the initial values significantly. The excitability of sensory fibers after reperfusion restored to the level before ischaemia. The level of post-ischaemic excitability for H-reflex and M-wave was significantly greater than during ischaemia.

4.1.4. Latency

Two measures of nerve conduction were made. First, the time interval between stimulus onset and the initial trace of the H-wave was calculated and represents the time required for signal propagation through the reflex arc [including the Ia afferent, synaptic delay at the motorneuron, and the smaller diameter (lower threshold Type I) efferent motor units back across the neuromuscular junction] (chapter 3.3.9., Fig. 9).

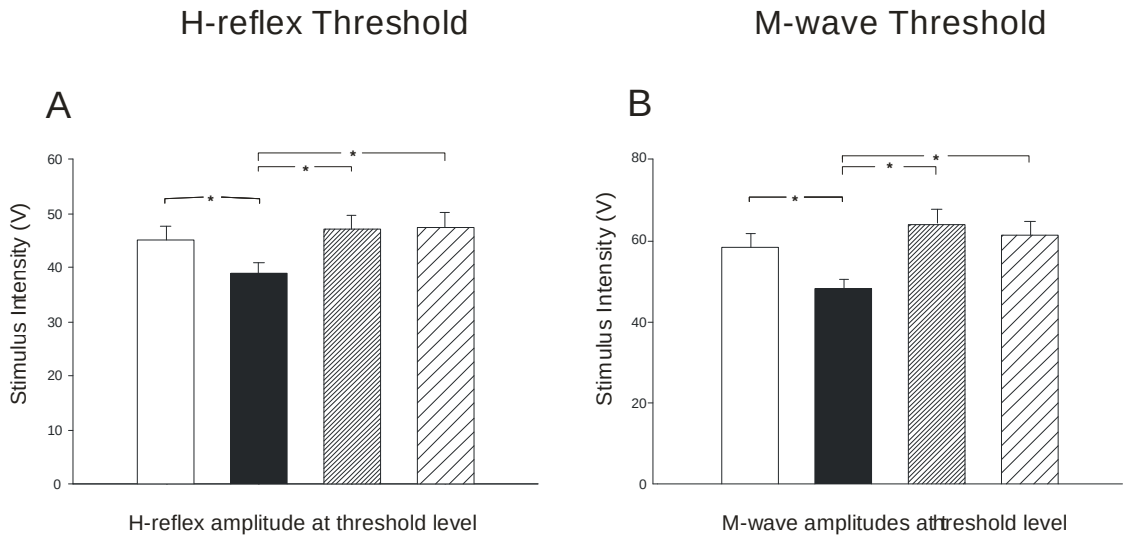

Fig.14. A threshold changes was measured using 0.5ms stimulus duration. Note, that ischaemia produced significant hyperexcitability of sensory **(A)** and motor **(B)** axons in both post-ischaemic conditions. The decrease of threshold of sensory fibers between initial data and ischaemia was associated also with a change of threshold intensity for motor fibers. Measurements after reperfusion (after 10 and 20min) showed that M-wave threshold significantly increase compare to the data during ischaemia ($P < 0.05$).

Second the time difference between stimulus onset and the initial trace of the M-wave was calculated and represents the time required for conduction down the larger diameter (higher threshold Type II) motor units to the terminal branches and propagation across the neuromuscular junction.

In addition, the H-reflex latency results from multiple comparison analysis shows significantly longer latency during ischaemia and post-ischaemic period compared to the values before ischaemia ($P<0.05$).The post-ischaemic latency values slightly recovered compared to ischaemic condition.

For the M-wave latency there was a significant delay in the values 10 and 20min after reperfusion compared to initial values. Even 20min after occlusion

was removed the M-latency was still significantly longer from the latency during ischaemia. It is important to note, that the M-wave latency didn't show any significant difference during ischaemia.

4.2. Characteristics of the H-reflex and M-wave maximal responses and ratio

Background EMG activity of the soleus muscle was not significantly different between the four periods of experiment (Fig. 15f). The same level of background motor activity at the time the H-reflex was measured is a very important finding; because we can exclude interferences with spontaneous background activity of the skeletal muscle. This activity can occur also from some painful stimulus or unpleasant feelings (stress) during the experiment. There is evidence that pain and stress can significantly altered the H-reflex amplitude (Svensson et al 2000).

Devanne and colleagues (1995, 1997) provide the evidence that even the recruitment gain of the human corticospinal pathway depends on the background level of motor activity. This is probably also true for other motor pathways (Cappaday, 1997).

Background EMG was assessed in terms of amplitude analysis.

4.2.1. Maximal EMG amplitudes (Hmax and Mmax)

The peak-to-peak amplitude of soleus Hmax and the Hmax/Mmax ratio (Fig. 15e) during ischaemia was significantly decreased ($P<0.01$) compared to the initial data. The values relative to the H-reflex and M-wave maximum amplitudes parameters are summarized in Figure 15a, b.

On the other hand, the Hmax and the Hmax/Mmax ratio significantly increased in both post-ischaemic periods as compared with the values during ischaemia ($P<0.001$). A significant Hmax increase was also found between the ischaemic period and 10min after reperfusion.

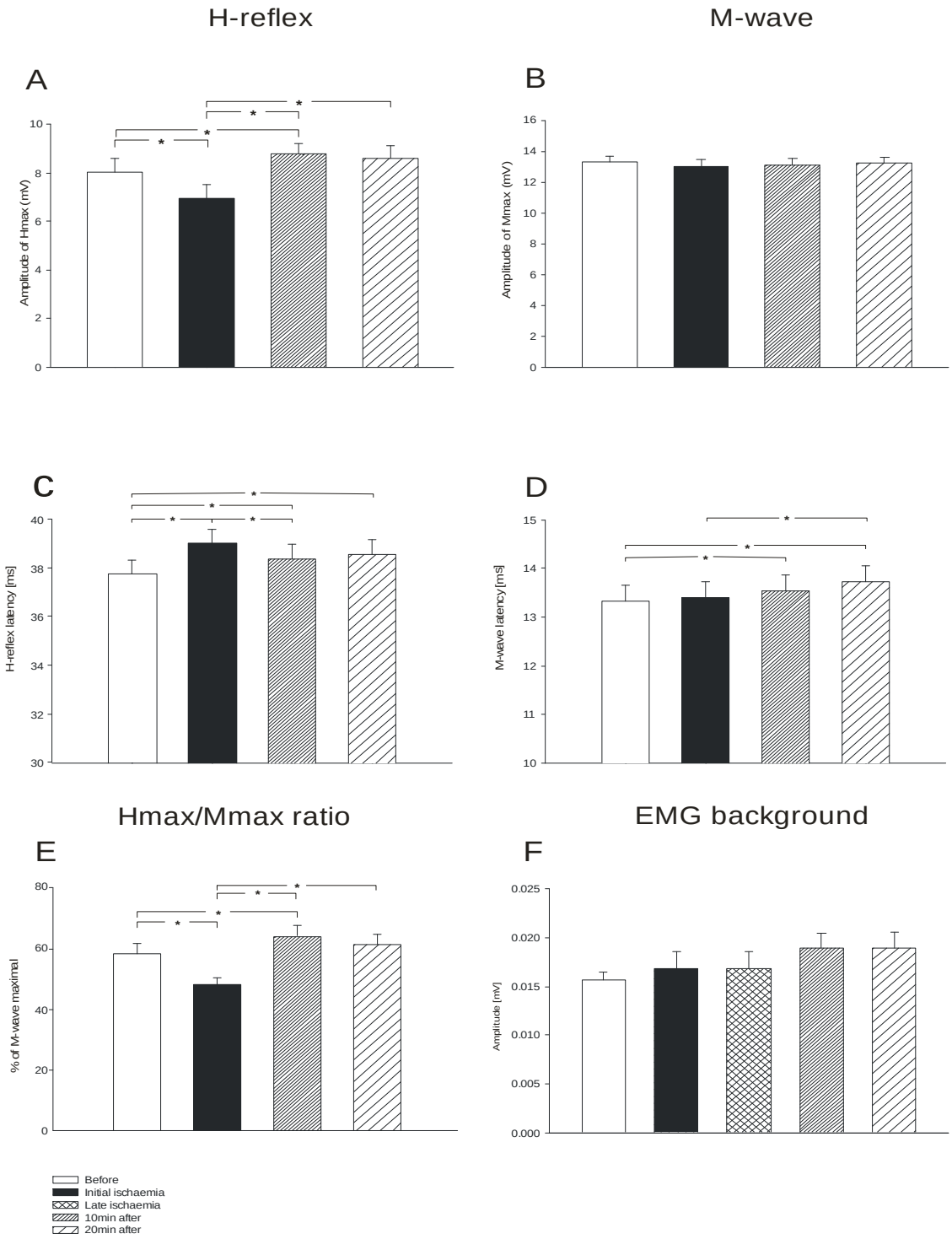

Fig.15. Comparison of the effect of ischaemia to the maximal amplitude of H-reflex (A) and M-wave (B), to the latency (C), (D), to the Hmax/Mmax ratio(E) and to the EMG background changes(F) for all 17 subjects.

4.2.2. Hmax/Mmax- sensory transmission across the Ia-alpha motoneuron

The effectivity of the sensory to motoneuron transmission expressed as Hmax/Mmax was affected significantly by acute ischemia of the lower limb. However, no significant change of the Mmax was observed. That means no significant differences or alteration of NM junction function were recorded during the course of the experiment. From the above, it would be safe to conclude that all significant changes in Hmax/Mmax ratio are due to a change in Hmax amplitude (Fig. 15e).

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4.3. The plantar-flexors muscle force output factors and evoked potential

One of the overall goals of this study was to determine the main site in the neuromuscular system affected by the pathogenic condition of short term ischaemia. By design, the subjects received interventions (artery occlusion) designed to alter the normal function of the nervous and muscular system in an attempt to gain a substantial amount of various measured physiological variables. We identified the following variables that we felt may theoretically be predictive of neuromuscular disturbances.

Neural property variables as analyse above was: H-reflex and M-wave excitability, H-reflex and M-wave latency, H-reflex and M-wave maximal amplitudes, ratio of H-reflex and M-wave maximal amplitudes (Hmax/Mmax).

In the following chapters muscle property variables will be presented: maximal amplitude of muscle force twitch (Nmax), Latency of Nmax, induction of PFs evoked force, the ratio of force and M-wave maximal responses.

A comparable series of recording made before, during and after ischaemia is illustrated in Fig.16. Note that the plantar-flexors muscle force (N) output activated by the same stimulus intensity changes during ischaemia and after reperfusion. The maximal force of muscle twitch (Nmax) was significantly lower in both reperfusion intervals in compare to values during ischaemia.

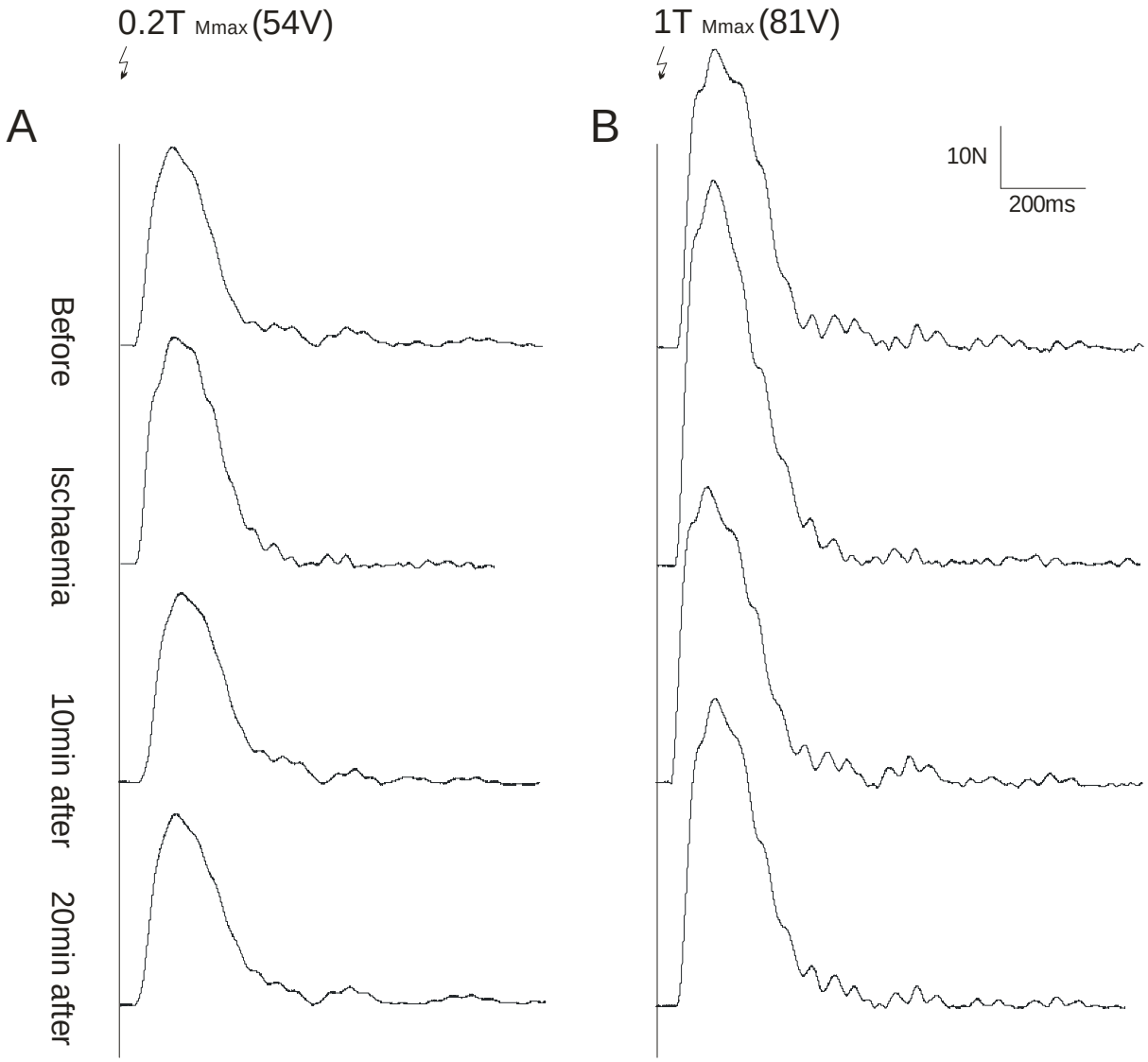

Fig.16. Characteristic mechanical responses of the plantar flexors muscle (A) and (B) to the same intensity stimulus during the course of the experiment. It can be seen the difference between the traces recorded before, during occlusion and after reperfusion.

4.3.1. The mechanical output of PFs muscle

Changes in developed force of plantar-flexors (PF) were analyzed during each experimental period (Fig 17a). There was no significant difference in force development between control period and during ischaemia. At the post-ischeamic phase the force fall significantly (69.065 ± 4.678 for 10min after and 70.765 ± 4.794 for 20min after reperfusion) compare to control (75.389 ± 5.054) and to ischaemia (79.292 ± 5.374) phase ($P<0.001$).

It means that short term ischaemia can produced marked changes in post-ischaemic measures of plantar-flexors muscle force output (Nmax*)* and not even the reperfusion and restoration of blood flow allowed the muscle to reach the control values.

4.3.2. Latency of evoked PFs muscle twitch

Figure 17b presents the latency of maximal plantar flexors muscle twitch. The latency responses were evaluated by the measurement of the time difference between stimulus onset and maximal evoked muscle twitch force. The time required for conduction (signal propagation across the neuromuscular junction) down to the skeletal muscle fiber and their maximal contraction.

Therefore, to detect possible differences in the contractile characteristics of the muscles to the electrical stimulus the latency of evoked PFs muscle twitch was measured. The values show significant slowing of the muscle mechanical response induced by the tibial nerve stimulation during ischaemic and post-ischaemic periods ($P<0.001$). These results indicate that total blood flow occlusion produce a delay in force development and the muscle force cannot recover even within 20min.

4.3.3. Description of the force threshold

The full sequence of threshold measurements, as described in the methods (see chapter 3.8.8), was obtained to define threshold intensity required

to produce a plantar flexors muscle twitch. The influence of short term ischaemia on the excitability values of excitation-contraction coupling was measured using 0.5ms stimulus duration. The thresholds stimulus intensity (V) was related with alterations in membrane potential, during and after ischaemia. Initial (control) values were obtained at rest (48.188 ± 2.590) before the effect of short term ischaemia on different neuromechanical parameters was compared.

The ischaemia produced a decreased of the threshold significantly ($P<0.05$), reducing the stimulus intensity required to evoke a particular response (42.375 ± 2.240), whereas reperfusion returned the threshold near to the initial values. Results 10 and 20min after the occlusion of thigh cuff was released (49.875 ± 2.805 and 50.625± 2.668) showed that flexors muscle twitch evoked potential seen at threshold level significantly increase compare to the data before ischaemia ($P < 0.05$).

According to these results the threshold of PFs muscle correlates with the excitability results of H-reflex and M-wave amplitudes. Both results show significant decrease in threshold for the sensory and motor fibers and skeletal muscle neuromechanical coupling during ischaemia.

Measurements after reperfusion showed that stimulus intensity required to evoke peak wave of plantar flexors muscle twitch seen at threshold level and M-wave and H-reflex threshold significantly increase compare to the data before ischaemia ($P < 0.05$). These show that the excitability of sensory and motor axons and force evoked potentials returned to the baseline (control values).

Those were assessed (see chapter 3.8.8) by the difference in stimulus intensity during all periods of experiment. The single pulse stimulus intensity required to elicit plantar flexors muscle twitch evoked potential seen at threshold level, were followed to compare axonal and skeletal muscle neuromechanical coupling.

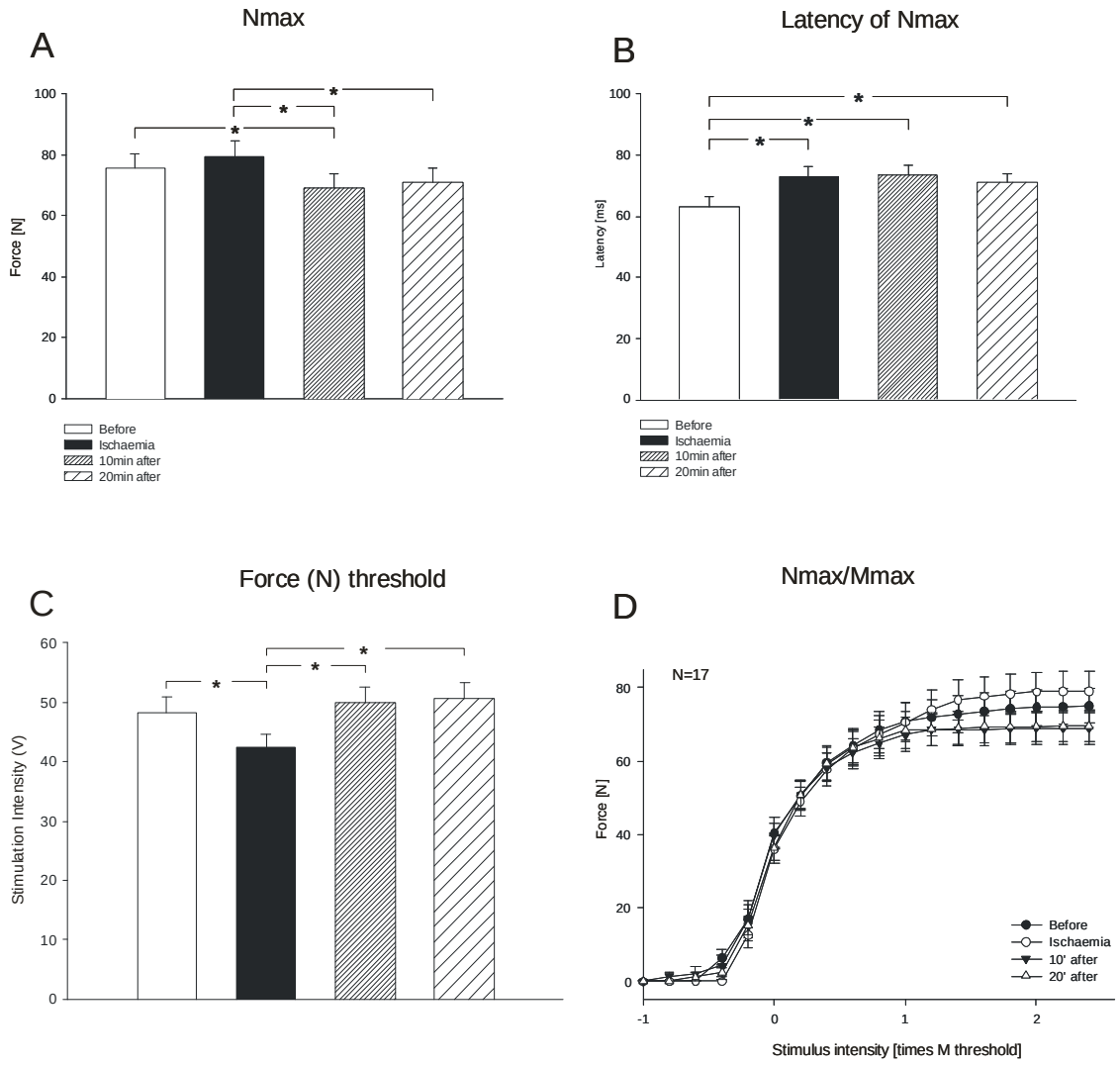

Fig.17. Comparison of Force evoked potential measurements during experiment.

A. Maximal response of evoked twitch peak of PFs muscle mechanical reaction to electrical stimulation of tibial nerve at popliteal area

B. Changes in maximal muscle twitch reaction latency

C. Relation between stimulus intensity and PFs muscle twitch response

D. Recruitment curve of PFs Force (N) displaying the increase of maximal amplitudes with increase of the stimulus intensity. The mean Nmax/Mmax values in the subjects rises at lower stimulus intensities relative to M-threshold and reaches a higher maximum.

4.3.4. Plantar-flexors muscle force output related to Mmax

From the plantar-flexors force recruitment curve is obvious that the muscle twitch was evoked before the M-wave was elicited and when only H response was present (Fig. 17d). This implicates that activation of muscles with monosynaptic reflex loop produces mechanical response.

These results indicate that peak twitch force evoked by stimulus lower than those evoking M-wave (M-threshold) and that the first motor units activated at low stimulus strength by the reflex pathway.

The total activation of the soleus motorneuron pool produces maximal amplitude of M-wave and also maximal amplitude of plantar flexors force twitch, during supramaximal stimulation of tibial nerve. That was verified by these results.

The maximal neural activation of the PFs muscle was clearly correlated with the Mmax response (CMUP) which represents the activation of the total motor neurone pool. The constant maximal amplitude of the M-wave in all four phases of measurement (pre-ischaemic, ischaemic and post-ischaemic conditions) means that the recording conditions were unchanged during the course of the experiment.

From the above results (Mmax was analyzed and no differences were detected) it can be save to conclude that during the course of experiment plantar-flexors muscle fatigue was absent and the intra-individual variability of EMG and force evoked potential responses was decrease.

5. DISCUSSION

The present study here reveals different structures within neuromuscular system that can be affected by lower limb short-term ischaemia. The study of these structures may provide important insights employed into the performance of neuromuscular periphery.

The hypothesis tested in the present study was to determine the main site in the neuromuscular system affected by the pathogenic condition of lower limb short term ischaemia.

Our results suggest that, there are more vulnerable structures involved in neuromuscular function to short term ischaemia. These important findings can be divided in three main categories:

5.1. Effect of short-term ischaemia on peripheral nerve function

The results presented in previous chapter (chapter 4) shows that there is experimental evidence of alterations induced by short-term ischaemia on peripheral nerve function. The neural structures were significantly affected by short term ischaemia on their excitability parameters, efficiency of the transmission across the synapses of Ia afferent terminals and latency. The mechanisms located beyond the sensory and motor neurons alterations, indicating:

5.1.1. Alteration of excitability

Short-term ischemia of lower limb significantly increases excitability of afferent and motor neuron axons by decreasing significantly H-reflex and M-wave thresholds elicited from soleus muscle. The differences in excitability and threshold were also evaluated by the H-reflex and M-wave recruitment curves (fig. 13a,b).

The finding of increased excitability of the afferent and motor motor fibers during ischaemia is partly in agreement with that of Lin et al. (2002) and Zakutansky et al (2005).

Lin and colleagues (2002) observed the differences in behavior of sensory and motor axons of median nerve to ischaemic disturbances. Their experiments were performed using a computerized threshold tracking program (describe by Bostock et al., 1998) and the results determined that sensory axons have more significant threshold decrease during ischaemia compare to motor axons.

Zakutansky and colleagues (2005) found, that during acute (5min) ischemia the H-reflex threshold decreased to 82,7% of pre-ischemic values and M wave threshold to 82,6% respectively.

They results also show that, H-wave threshold failed to return to the control level of excitability, whereas the motor axons were fully recovered after 5 minutes of recovery. However, this finding is in contrary to our evidence report, when the H-reflex threshold was measured 10min after reperfusion without any significant differences form the values obtained before ischaemia. One possible explanation for the discrepant finding is the post-ischaemic conditions under which the H-reflexes were measured. In the present study, they were obtained 10min after the occlusion was released and the ischaemia of lower limb was induced for 10min. Whereas Zakutansky et al obtained their post-ischaemic results 5min after release of occlusion with ischaemic time set at 5min.

This means that afferent fibers at early stage of lower limb ischaemia can be more affected by ischemia. These results indicate that sensory Ia fibers are more vulnerable to altered metabolic processes (extracellular K+ accumulation) than motor axons (described below).

However, other possibility explaining the discrepant findings could be differences in the electrical stimulus duration that was delivered to the tibial nerve.

In our experiment, H-reflex and M-wave recruitment curves were obtained with stimulus duration of 0.5ms. In contrary to the stimulus duration of 1ms that was used from Zakutansky and colleagues.

The stimulus duration is an important parameter (see chapter 3.3.4.) since it allows for a separation of the threshold of Ia-fibres from that of a-motor fibres. Cappaday (1997) suggest a stimulus duration of 0.5 ms, because it is a good compromise between the need to separate thresholds and the need to minimize unpleasant sensations. According to his experience, this stimulus duration gives a better separation of the threshold of Ia-afferent fibres from that of a-motor fibres compared to a stimulus of 1 ms duration. Shorter stimuli would further separate the thresholds, but the necessarily stronger stimulus intensities make such stimuli unpleasant, because nociceptive nerve terminals in the skin will be stimulated.

From the threshold results in Fig. 14 can be conclude that excitability changes in afferent sensory and efferent motor axons occur during ischaemia. These changes were evaluated with differences in thresholds required to produce an H-reflex and M-wave evoked potential.

Mogyoros et al (1997) in their study presents evidence that ion channels are likely candidates to be involved in these excitability changes: Na+ channels adn K+ channels. The consequent increase in extracellular K^+ ions, especially in the restricted space under the myelin sheath can alter the function of Na^+/K^+ pump. This interpretation is supported by the observation that depolarization by applied currents produces similar changes in excitability and accommodation to those occurring in the first 10 min of ischaemia (Baker and Bostock, 1989; Bostock et al.,1991b).

Other studies have also shown that during brief period of ischaemia, axonal excitability increase due to membrane depolarization, caused by altered function of the electrogenic sodium pump and extracellular K+ accumulation (Bostock et al., 1991a and 1994, Mogyoros et al., 1997, Grosskreutz et al., 1999, Lin et al., 2002).

Modulation of the H-reflex threshold during ischaemia can probably result from altered function of different ion channels and from changes in metabolic processes (influenced by pH) due to dysfunction of the electrogenic Na^+ pump and subsequent K^+ accumulation.

However, it doesn't mean that threshold reflect membrane potential. Grosskreutz and colleagues (1999) provide evidence that threshold varies appropriately with membrane potential: threshold rises and its reciprocal (excitability) decreases when axons are hyperpolarized; threshold decreases and excitability increases when they are depolarized. Their data suggest a non-linearity in the relationship between threshold and membrane potential during ischaemia. The near-linear relationship between the excitability indices and the reciprocal of threshold does not imply a similar relationship to membrane potential. Further, at their conclusions support the view that the changes in axonal excitability during ischaemia and after its release are not due solely to changes in membrane potential.

The present findings are therefore consistent with the theory that, ischaemia changes the accommodative properties of axons and thus change the excitability of human nerve (Kugelberg, 1944; Bostock et al., 1991a).

In this part of the study we have analysed further the excitability changes in afferent and motor axons in peripheral nerve during short-term lower limb ischaemia. The results of peripheral nerve excitability changes in the current experiment correspond with the findings of previous mentioned studies performed by different techniques and methods (e.g. long-lasting depolarizing and hyperpolarizing currents, threshold tracking).

This shows that the amplitude component of H-reflex can be one of the valuable techniques to assess sensory and motor neurons excitability during ischaemia.

5.1.2. Affection of synapse transmission

The increased excitability values of the sensory and motor fibers produced by short term ischemia were followed by a decrease of maximal amplitude of H-reflex and Hmax/Mmax ratio. These results are also consistent with the findings of Zakutanksy and colleagues (2005).

The decrease of Hmax/Mmax ratio was affected mainly by the alteration of Hmax amplitude, because the maximal amplitude of M-wave (CMAP) remains unchanged during all phases of experiment.

The strength of the H-reflex maximal amplitude can be modulated by primary afferent depolarization which reduces the amount of neurotransmitter released from the Ia afferent presynaptic terminals. Primary afferent depolarization acts as an inhibitor to the circuit by reducing the potential for an action potential to be elicited in the target motoneurons (Sabbahi and DeLuca, 1981). The majority of the changes in H-reflex strength are attributed to changes in the amount of presynaptic inhibition (Schieppati, 1987, Zehr, 2002)

There is extensive modulation of transmission in the H-reflex pathway. Peripheral feedback from muscle spindle receptors, Golgi tendon organs, cutaneous afferents, joint afferents, and vestibular inputs can all affect the amplitude of the H-reflex (Schieppati, 1987). Thus, careful control over these peripheral inputs was maintained by monitoring the posture of the volunteers and the contraction of other muscles.

Modulation of the maximal H-reflex amplitude was followed by decrease of the Hmax/Mmax ratio and that can resulted from a number of factors, including:
a) changes in membrane potential arising from excitatory or inhibitory inputs or intrinsic properties changes in Ia fibers and alpha motoneurons,
b) presynaptic inhibition of the Ia fibers,
c) variation in the amount of Ia neurotransmitter release,

d) changes in neuromuscular junction (seems unlikely in our experiment to affect transmission across the synapses of Ia afferent terminals, because the maximal amplitude of M-wave remain unchanged),and
e) biomechanical properties of muscle tissue (will be analyzed separately further bellow in chapter 5.2).

With respect to the results mention above our findings are driven primarily by the Ia afferents. The decrease of Hmax/Mmax ratio during ischaemia seems to be affected by the altered efficiency of the transmission across the synapses of Ia afferent terminals. It is, however, not clear whether the efficiency of the synaptic transmission is dependent on the degree of the neurochemical transmitter depletion or on a change in the Ca^{+} permeability. It is worth noting that a decrease in the amount of neurochemical transmitter by the activation of a N-type Ca^{2+} channel in the presynaptic terminal was found to be a cause of the long term adaptation of the monosynaptic gill withdrawal reflex (Komiyama et al., 1999).

The decreased synapse transmission (evaluated by decreased value of Hmax/Mmax ratio) can be due to increases in presynaptic inhibition of group Ia-afferent terminals projecting directly to the motoneurons (with matched level of background level of motor activity).

Presynaptic inhibition (PSI) of the H-reflex is a major factor that must be considered when interpreting H-reflex data, but other factors (which may also relate to or interact with PSI) must be considered when evoking and interpreting the H-reflex (Capaday et al., 1995). Presynaptic inhibition (PSI) is mediated by the action of an inhibitory interneuron (using gamma aminobutyric acid as the neurotransmitter; Rudomin and Schmidt, 1999) acting on the Ia afferent terminals, leading to a reduction in neurotransmitter release and a concomitant reduction in motoneuron depolarization induced by Ia activity. Thus, afferent transmission can be altered without a corresponding effect on the postsynaptic (e.g., motoneuron) membrane. Frank and Fourtes (1957) demonstrated that in

the presence of PSI there was no change in the postsynaptic membrane potential, despite activity in the Ia afferents. Furthermore, the motoneurons remained receptive to other inputs that were unaffected by PSI. This provided conclusive evidence that PSI could selectively alter transmission in a monosynaptic reflex pathway, and it has recently been demonstrated that this mechanism is selective enough to affect different collaterals from the same muscle spindle afferent (Rudomin and Schmidt, 1999).

That means the short term ischaemia can cause alteration in sensory transmission across the Ia-alpha motoneuron synapse and decrease the effect of the Ia fiber onto the motor pool. This may be viewed as a mechanism whereby the central nervous system attempts to offset the increased excitability of the incoming sensory fiber activity with a concomitant increase in presynaptic inhibition to these fibers (Zakutansky et al., 2005)

Further research is needed using other techniques and protocols to determine how ischemia may act to influence presynaptic interneurons and thus give more neurophysiologic explanation of our observed response.

One particularly interesting finding from the present study is the observation of an increased latency time in the H- wave (during ischaemia and both post-ischaemic periods) and M-wave (significant delay in the post-ischaemic values) indicates a slowing in nerve conduction time required for signal propagation through the reflex arc following 10min of lower limb ischaemia. These findings suggest that slowing in axonal nerve conduction velocity occurred only after ischaemia for larger diameter (higher threshold Type II) motor units. In addition to longer transmission time through the spinal reflex loop during ischaemia for Ia afferent and the smaller diameter (lower threshold type I) efferent motor units.

The H-reflex and M-wave latency gives only an indication of a global change of sensory and motor fibers conduction velocity; therefore this measure is probably not sensitive enough to detect such small localized alterations.

5.2. Effect of short-term ischaemia on skeletal muscle function

We assess the plantar-flexors muscle force production under ischemic and post-ischaemic conditions by eliciting the H-reflex and M-wave recruitment curves. The results of the muscle property variables demonstrate that short term ischaemia can produce marked changes in: a) plantar-flexors muscle force output (Nmax*)*, b) latency of the muscle mechanical response induced by the tibial nerve stimulation, c) neuromechanical parameters of PFs muscle.

Interestingly, the maximal activation of the PFs muscle was clearly correlated with the Mmax response (CMUP). Mmax represents the activation of the total motor neurone pool. The constant maximal amplitude of the M-wave in all four phases of measurement means that the recording conditions were unchanged during the course of the experiment.

The mechanical responses were not altered from the displacement (e.g. contraction of underlying muscles) of the stimulating electrode away from the tibial nerve during the experiment. Thus, it seems that, the maximal muscle twitch was not functionally affected with fatigue-induced reaction, on the other hand ischaemia create intracellular metabolic disruption (severity depends on the time duration of ischaemia). Another parameter that supports these conclusions is that the supramaximal transcutaneus electrical stimuli that were delivered to tibial nerve were given 3 second apart to avoid any postactivation depression of the PFs muscle (Capaday, 1997).

5.2.1. The force output adaptation to short term ischaemia

One of the most complicating factors in human neuromuscular control studies 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 mechanisms 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 was avoided by using adequate stimulation frequency (see chapter 3.3.4 and 3.3.5.). Thus, simultaneously measured EMG evoked potential provides analysis of M-wave, which contains information about membrane properties of the active MUs (Merletti et al., 1992).

The recovery of force production after ischaemia follows a time course that is partially dependent on the restoration of homeostatic conditions in the intracellular environment (Hogan et al., 1999). There are a number of sites that can contribute to the force- capacity aspect during and after ischaemia.

The potential force-failure mechanisms known to exist at this level include all the above mention parameters (motoneuron excitability, neuromuscular transmission, sarcolema excitability, excitation-contraction coupling), but also contractile machinery and energy metabolism. Very little is also known whereas recovery of force development after period of short ischaemia will be dependent on restoration factor of blood flow.

The present study demonstrated that after 10min of ischaemia mechanical performance of plantar-flexors muscle force output (Nmax*)* was significantly reduced for at least 20min. Even restoration of blood flow with oxygenated blood allowed the muscles to reach the initial values.

The analysis of the mechanical responses induced by supramaximal electrical stimulation of tibial nerve allows one to indirectly investigate the possible muscle intracellular changes responsible for the reduced plantar flexors mechanical performance (as assessed from the peak evoked forces)after reperfusion. The decrease in the force twitch observed at both post-ischaemic results, without any change in the M-wave, can be explained by an alteration of the excitation-contraction (E-C) coupling (Duchateau et al., 1985, Bigland-Ritchie, 1986).

When the total reduction in blood flow is sudden and severe (as in our experiment) the working skeletal (Stainsby et al., 1990, Hogan et al., 1994) or cardiac muscle (Allen, 1987) result in a fall in force production that can occur within seconds of the ischemic initiation. The complete elimination of blood flow in lower limb certainly resulted in intracellular metabolic disruption (because of the rapid reduction in oxidative phosphorylation) that contributed to the fall in force. The total ischemia produces acidosis, because of increased lactate production. As the ischaemic time increases, acidosis causes dysfunction of the calcium pump, which is a Ca–ATPase, and reduces the time of release of calcium from troponin C because the amount of troponin C that binds up calcium is low. Half relaxation time therefore remains stable, because this depends on the velocity of sequestration of calcium by the sarcoplasmic reticulum (Hatzipantelis, 2001).

The metabolic instability that was developed during this period (of 10min ischaemia) was severe enough to significantly change the PFs muscle evoked twitch force for at least 20min.

The restoration of plantar flexors muscle force depends on the degree of metabolic disturbance that occurred and the time allowed for recovery. However, even when the metabolic disturbances are corrected during the recovery period, there can remain substantial contractile dysfunction (Baker et al., 1993, Nagesser, 1992), and it has been recently suggested (Bruton et al., 1998) that the sustained impairment may be related to prolonged elevated (slow

reabsorption from sarcoplasmic reticulum) intracellular Ca^{2+}. In addition, recovery of muscle function after fatiguing contractions in whole muscle may depend in part on keeping blood flow elevated and washing out metabolic waste products (Bogdanis et al., 1996).

Hogan and colleagues (1999) have shown that whole muscle contractility is affected within seconds on initiation of ischemia and that this process is dependent on O_2 availability and not some other factor related to the cessation of blood flow. The time course involved with recovery of force production on restoration of normal perfusion after a period of ischemia, and whether this process may be related to O_2 availability or the washout of various metabolites that may inhibit contractility, is unknown.

In the present study we found that after reperfusion PFs muscle force was decreased even 20min after occlusion was removed.

Another interesting finding of the present study was the observation of a significant slowing of the time between stimulus onset and maximal evoked twitch force during ischaemia and post-ischaemic phases of experiment. This increased latency time of maximal force (Nmax) correlates absolutely with H-relfex latency alteration caused by ischaemia.

The physiological mechanisms of force-time properties of evoked contractions are driven by a number of different factors and in general are thought to provide information on intracellular Ca^{2+} transients, muscle fiber type, and cross-bridge function (Westerblad et al., 1997, Ortenblad et al., 2000).

These results indicate that total blood flow occlusion produce a delay in force development and the muscle force cannot recover within 20min. The present latency analysis has shown prolonged time changes in the evoked twitch of the PFs muscle compound action potential. These modifications may, to some extent, be attributable to peripheral myopathic and neurogenic adaptations such as a slower electrical propagation related to impaired muscle membrane excitability due to amount and rate of sarcoplasmic reticulum Ca^{2+} release (Dutka et al., 2005, Ortenblad et al., 2000).

5.2.2. Efficacy of ischaemia on neuromechanical threshold

Several factors accounting for a change in neuromechanichal threshold of muscle fibers evoked by direct electrical stimulation (e.g disturbance in E-C coupling, as the E-C coupling could be affected by reduced sarcolemmal excitability, or reduced rate of ATP utilization and regeneration). The propagation of action potential (AP) on excitable membranes of muscle fibers, sarcolemma and t-tubule, may be impaired during ischaemia due to the imbalance of Na^+ and K^+ ions over the membranes. This could lead to decrement of sarcoplasmic reticulum 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 of skeletal muscle (Yensen et al. 2002, Nielsen et al. 2004).

The cross-bridge cycling depends on formation of ATP molecules trough aerobic and anaerobic metabolic pathways. Furthermore, affinity of Ca^{2+} binding to troponin might change because of ischaemia related metabolites. (Vollestad, 1997, Warren et al.,2001).

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 firing frequency, MU recruitment, eliminates cross-talk from nearby muscles and is independent of subject's motivation to perform muscular contraction. In electrical stimulation (determined as M-wave properties) all the MUs are recruited at the same time (Merletti et al 1992, Avela et al., 1999).

Excitation-contraction coupling is one of the potential force-reducing mechanisms. The results of PFs muscle twitch threshold correspond absolutely with the result of H-reflex and M-wave threshold.

In this study, the amplitude of the M-wave recorded in the soleus muscle did not change significantly during and after ischaemia. This observation, which

is in agreement with previous studies using periodic bouts of Ischaemia and supramaximal stimulus intensity (Duchateau et al., 2002, Clark et al., 2006) suggests that short rest periods between each muscle activation are sufficient to maintain the neuromuscular excitability at a normal level.

According to these results the neuromechanical threshold of PFs muscle correlates with the excitability changes of H-reflex and M-wave amplitudes. Both results show significant decrease in threshold of sensory and motor axons related to decreased threshold of PFs muscle and excitation of neuromechanical coupling during ischaemia. These increased excitability values associated with the decrease of threshold.

Both post-occlusive measurements showed that stimulus intensity required to evoke peak wave of PFs muscle and M-wave and H-reflex response significantly increase compare to the data during ischaemia ($P < 0.05$). These show that the excitability of EMG and force evoked potentials returned to the baseline (control values before ischaemia).

There is another one parameter that must be kept in mind when PFs muscle twitch force is analyzed. Ischaemia produces acidosis, because of increase lactate production. It is well known that acidosis decreases excitability in an excitable tissue and vice versa. Muscles, however, can balance effect of intracelular acidosis because of lactate and hydrogen production by efflux of potassium which can reach up to 8mM which helps to preserve excitability of the muscle fibre during fatigue. (Hatzipantelis et al, 2001, Sostaric et al, 2006).

It is suspected that the reduced excitability contributes to a reduction in calcium release by the sarcoplasmic reticulum and a consequent decrease in the force of muscle contraction (Lindinger, 2006). That finding is in agreement with our post-ischaemic results of PFs muscle force and neuromechanical threshold. The post-ischaemic mechanical performance of PFs was significantly reduced and in opposite the neuromechanical threshold was increased compare to the values during ischaemia.

5.3. Effect of short-term ischaemia on neuromuscular junction

Except the above mention neuromuscular mechanisms that can be altered by tourniquet short-term ischaemia also neuromuscular junction can be affected.

According to Lundborg (1970) and Hatzipantelis et al (2001) the neuromuscular junction is the most susceptible site of neuromuscular system to ischaemia. Hatzipantelis and colleagues (2001) design an experimental animal model applying 80minutes blood flow occlusion on peripheral nerve of the rats. Their results indicates that under ischaemic conditions the neuromuscular function probably stops because acidosis causes a reduction in the time that calcium channels remain open, a reduction in the number of synaptic vesicles and of their acetylcholine contents and reduction in the permeability of muscle membrane to sodium and potassium.

Contrary to their findings, we did not observe from our measurements any effect of short-term ischaemia on the human neuromuscular junction. Our assertion is based on the results of maximal amplitude of M-wave (CMAP) and the values form M-wave latency before and during ischaemia. Both responses remain unchanged. The present results indicate that neuromuscular transmission during this experiment was unaffected.

The fact is that little is known about the influence of short term ischaemia on the human neuromuscular junction and further research is needed.

One particularly interesting findings from the current study was the observation of PFs muscle twitch response before M-wave action potential was evoked. This is obvious from the plantar flexors muscle recruitment curves (Fig. 17d) elicited during all four phases of our experiment. This mechanical response was recorded by force platform and was elicited by tibial nerve stimulation when only H-reflex was present. Relatively few studies have attempted to evaluate the relation between mechanical responses of PFs activated by low intensity stimulation of sensory fibers. In agreement with our finding are only the studies of Maffiuletti and colleagues (2000) and Scaglioni and others (2003), presenting investigation, showed the relative contribution to the plantar-flexors torque of the

soleus motor units activated by H and M waves, even if the testing position was at 90^0 of knee flexion. That was evaluated because it markedly reduces the mechanical contribution to the evoked twitch of the gastrocnemii. That means they results interpreting only the twitch evoked by soleus muscle and it doesn't correspond to all PFs muscle group. The studies were done in healthy human subjects but without any intervention.

6. GENERAL CONCLUSION

The primary purpose of this study was to reveals different structures within neuromuscular system that can be altered by short-term ischaemia. It has been demonstrated that during and after ischaemia numerous alterations in the electrical and mechanical properties of human peripheral nerves and muscle occurs. To this end, our major findings were that:

a) excitability of both peripheral nerves and muscle fibers are highly influenced by metabolic intracellular processes (affected by pH). However, current extent of this effect remains unknown. It is well known that acidosis decreases excitability in an excitable tissue and vice versa. Indeed, the results of the present study appear to confirm the concept that the accumulation of K^+ accompanies ischaemia and has effect on excitability of nervous and muscle tissues.

b) there is extensive modulation of transmission across the synapses of Ia afferent in the H-reflex pathway due to ischaemia. The alterded synapse transmission can be due to increases in presynaptic inhibition of group Ia-afferent terminals projecting directly to the motoneurons.

c) during the experiment the human neuromuscular junction was not affected by short-term ischemia. Assertion based on M-wave values.

d) short-term ischaemia (10min) induce intracellular metabolic disruption that result to significantly reduced mechanical performance (force) of plantar-flexors muscle for at least 20min. This reduction of maximal evoked twitch force was accompanied with increased latency of Nmax.

e) the mechanical muscle twitch evoked by tibial nerve stimulation at the early stage of H-reflex was recorded and observed during all four phases of experiment. This action potential appears before the M-wave was elicited. The mechanisms responsible for these results remain to be explored.

6.1. SUGGESTIONS FOR FUTURE RESEARCH

Although the equipment used in this research was satisfactory, there ways in which researcher undertaking similar work in the future could benefit from additional equipment if available. In my opinion the stimulator that was used for electrical stimulation of tibial nerve proved adequate for the purposes of the study. However, it would have been helpful to use stimulator with more technical options e.g. with automatic repetitive stimulation function to selectively evaluate the action potential transmission across neuromuscular junction. Also the assessment during natural motor task e.g. walking can help to understand better the basic principles of human motor control. During this evaluation the different biomechanical and neurophysiological properties of soleus and gastrocnemius muscles must be taken into consideration (Taborikova, 1968). The soleus is most active when the foot is in dorsiflexion (lengthening contraction), while the gastrocnemii are most active when the foot is plantar flexed, during strong contraction or rapid development of tension. The soleus also de-recruits differently to the gastrocnemius during plantar flexion in humans and their motor neuron pools function quite separately. However, soleus contains mainly fatigue-resistant fibers and plays a dominant role in the mechanical time course of the triceps surae (Herman, 1967, Nardone and Schieppati, 1988).

Apart from considerations about the equipment and methods used, there are other ways in which research of this kind can be improved. This study involves only few healthy young subjects. Future studies could involve also patients with radicular symptomatology to identify the most susceptible site of neuromuscular system affected by peripheral nerve compression.

Further research might ideally employ a prospective randomized control trial using different types of physiotherapy methods. A successful trial could be difficult but expensive, requiring the cooperation and collaboration of many physiotherapist. The value of physiotherapy for improving outcomes of patients with radicular symptomatology (Vele et al, 2005) also requires controlled prospective evaluation.

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APPENDIX I.

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.

APPENDIX II

Žádost
o vyjádření Etické komise UK FTVS k výzkumnému projektu

Název: Vliv krátkodobé ischemie na funkci plantárních flexorů a neuromuskulární ploténky
Forma projektu: doktorandská práce
Autor/ hlavní řešitel/ Mgr.Paris Charalampidis
Školitel: Prof.Ing. Stanislav Otáhal, DrSc., MUDr. Jakub Otáhal, PhD.

Zajištění bezpečnosti pro posouzení odborníky:
Řešitel bude provádět samostatné EMG a dynamonetrické měření pod dohledem školitele.

Etické aspekty výzkumu
Experimentů se zůčastní zdravé dospělé osoby ve věkovém rozmezí 20-40 let. Účast v experimentu je dobrovolná a není spojena s žádnou finanční odměnou.

Datum: …… ……
Podpis autora (řešitele)

Příloha:

1. Základní informace pro účastníka výzkumu
Experimenty budou prováděny na zdravých dospělých osobách ve věkovém rozmezí 20-40 let. Cílem je zjistit, vliv kratkodobé ischemie dolní končetiny vyvolanou pomocí komprese stehenní mažety tonometru na funkci plantárních flexorů a neuromuskulární ploténky při nebolestivé elektrické stimulaci n. tibialis v oblasti podkolenní jamky. Pomocí této stimulace bude vyvolán a zaznamenán i monosynaptický spinální reflex, vybavitelný z m. soleus. Jeho komponentamy jsou H-reflex a M-vlna. H-reflex je ekvivalent fázického svalového napínacího reflexu, kdy místo použití neurologického kladívka pro vyvolání reflexu se používá elektrického stimulu aplikovaného přímo na nerv zásobující příslušný sval. Při stimulaci n. tibialis se díky podráždění motorických vláken (M vlna) objeví kontrakce svalů a následná motorická odpověď‘, která bude měřena pomocí kistlerovy desky. Elektrická aktivita svalů bude snímána EMG přístrojem GrassTelefactor s galvanickým oddělením splňující normy EU. Stimulace se bude provádět povrchovou elektrodou monopolární technikou. Tato technika je neinvazivní, působí minimální nepříjemnosti spojené s aplikací elektrod (odstranění ochlupení, očistění pokožky lihobenzinem na malé ploše v oblasti aplikace elektrod). Jedná se o jednorázové měření, nevyžadujeme dlouhodobý účastnický závazek. Další zpracování a prezentace výsledků bude probíhat

anonymně s ohledem na zákon utajování osobních údajů. Účastník může účast kdykoliv beztrestně odmítnout či odstoupit z výzkumu.

2. Informovaný souhlas

INFORMACE A INFORMOVANÝ SOUHLAS PACIENTA S VYŠETŘENÍM V RÁMCI DOKTORANDSKÉ PRÁCE: VLIV KRÁTKODOBÉ ISCHÉMIE NA FUKNCI PLANTÁRNÍCH FLEXORŮ A NEUROMUSKULÁRNÍ PLOTÉNKY

Jméno pacienta:

Úvod: Tážeme se Vás, zda souhlasíte se zařazením do této studie zkoumající charakteristiky EMG biosignálu a motorické odpovědi platárních flexorů během a po ischémii, pomocí povrchových elektrod a Kistlerovy desky.

Účel studie: Účelem této studie je zkoumání charakteristik elektrické aktivity svalů a jejich síly v závislosti na aplikaci ischemie, vyvolanou pomoci komprese stehenní mažety tonometru. Analýza funkce plantárních flexorů a neuromuskulární ploténky při nebolestivé elektrické stimulaci n. tibialis v oblasti podkolenní jamky může přinést nové informace, které by mohli přispět k hlubšímu poznání biomechanické a neurofyziologické podstaty u akutních (thrombóza) nebo chronických (periferni diabetická neropatie) onemocnění.

Průběh studie: Povrchovými elektrodami nalepenými na kůži nad svalem bude snímána elektrická aktivita svalů během nebolestivé elektrické stimulaci n. tibialis v oblasti podkolenní jamky. Svalová odpověd vyvolaná touto stimulací bude snínána pomocí kistlerovy desky na které bude přitisknuté Vaše chodidlo . Měření proběhne ve čtyřech fázích a to před ischémii, během ischémie, 10min po ischémii a 20min po ischémii. Tato data budou dále počítačově zpracována a analyzována.
Rizika: Ze zařazení do studie pro Vás nevyplývají žádná rizika.
Dobrovolná účast: Vaše účast v této studii je zcela dobrovolná.
Důvěrnost informací: Zpracování dat proběhne v souladu s platnými předpisy o využití informací týkajících se zdravotního stavu pacientů v lékařském výzkumu, včetně anonymní prezentace výsledků vyšetření na lékařských kongresech a v odborném tisku.
Informovaný souhlas: Měl jsem dostatek času na rozhodnutí a příležitost informovat se na podrobnosti studie. Všechny moje otázky týkající se studie byly odpovězeny k mé spokojenosti. Rozumím, že moje účast v této studii je dobrovolná a že mohu účast odmítnout. Jsem informován/a, že se všemi údaji, které by mohly odhalit moji totožnost, se bude zacházet důvěrně. Obdržím kopii mnou podepsaného, datem opatřeného písemného informovaného souhlasu. Souhlasím s účastí v této studii.

Jméno pacienta, datum narození, datum, podpis.

APPENDIX III

Vyjádření a Souhlas Etické komise UK FTVS k výzkumnému projektu