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

Comprehensive Abstract of Doctoral Dissertation

by

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Summary

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 ischaemia can produce disturbances affecting peripheral and spinal mechanism of human motor control. The aim of this study was to assess the influence of short term ischaemia in lower limb on plantar-flexors 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 participated in the study with their Informed consent. The subjects lay prone on a physiotherapy table with both legs extended and the right foot attached and secured to the force platform. Ischemia was induced by blood pressure cuff placed around the right thigh 15 cm above the knee and inflated to a pressure of 200 mmHg for 10 min. During the experiment, the plantar-flexors 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, thresholds, recruitment curves and transmission across the synapses of Ia afferent were significantly 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 10min 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.

Introduction

Ischemic lower limb is a syndrome that causes neuromuscular dysfunction in both acute (thrombosis, embolism, injury to large arteries) and chronic (diabetic peripheral neuropathy) periods. Arterial occlusion caused by mechanical compression induces ischemic conditions beneath and distal to the tourniquet cuff. Only a short period of ischemia can produce intracellular metabolic disturbances affecting peripheral and spinal mechanism of human motor control (Mogyoros et al., 1997; Grosskreutz et.al., 1999; Hogan et al., 1999; Zakutansky et al., 2005). By this pathogenic condition sensory and motor neuron excitability, neuromuscular transmission and skeletal muscle contractile mechanism and thus forces capacity can be affected.

Previous electrophysiological H-reflex 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 was accompanied by a decrease in the efficiency of the Ia-fiber motoneuron synapse.

It is experimentally proved that arterial occlusion lasting up to 80minutes in adult rat's causes a gradual reduction in muscle 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 (Hatzipantelis et al., 2001). This implicates significant alteration of function of the neuromuscular junction under acute ischaemia. Recently, Clark et al. (2006a,b) 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 flexor muscle measured by custom-modified dynamometer did not significantly change when the knee was in the flexed position.

To date, reports using excitation of sensory and motor axons to investigate plantar-flexors (PFs) muscle force output changes after short term ischaemia and the post-ischaemic reactions have not been published.

Aims

In conclusion, current knowledge about the consequences of pathophysiological mechanisms induced by ischaemia on biomechanical properties of the lower limb skeletal muscles 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.

Methods

Seventeen healthy adult volunteers (11 male and 6 female; mean age 27.41yr ±1.06) participated in the study with their Informed consent to the nature and purpose of the experiment. All experimental procedures were performed in accordance with the Declaration of Helsinki, and the study had the approval of the Charles University Ethics Committee. None of the subject had any history of vascular or other medical deficits known to affect neuromuscular function.

Experiment procedures

All subjects underwent identical experimental protocol with constant internal environment, temperature and body position. The subjects lay prone on a physiotherapy table with both legs extended and the right foot (barefoot) attached (with respect to muscle tone) and secured to the force platform. The position of the right ankle was continually controlled by goniometer during the experiment (Knikou and Conway, 2001).

The force platform was calibrated in vertical position. During the experiment, the plantar-flexor (PF) force, H and M-responses of soleus muscle evoked by tibial nerve stimulation were measured at rest, during 10 minutes of ischaemia (starting after 6 minutes), 10 and 20 minutes after the occlusion was released.

Ischaemia

In order to induce ischemia by mechanical pressure, we used a conventional sphygmomanometer. Before we start any assessment a blood pressure thigh cuff was wrapped around the thigh, 15cm above the knee (mid-thigh level) of the investigated right leg. Ischaemia was achieving, after the first series of measurements ended by using manual inflation of the cuff to an occlusion pressure of 200mmHg (Krishnan et al., 2006) and maintained for 10 minutes. 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 (Korotkoff sounds) of the popliteal artery under the tourniquets cuff. During post-ischaemic assessment procedures the cuff remains (deflate) in the same position.

EMG recordings

To record the H-reflex and M-response, unipolar surface electrode (Ag/AgCl) were taped over the right soleus and reference placed just above the external malleolus and the ground 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 (Capaday, 1997). 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. In all four periods of measurements, changes in single pulse stimulus intensity required to elicit H-reflex, M-wave and PF force amplitudes seen at threshold level,

were followed to compare axonal excitability. A decrease in single pulse intensity means an increase in excitability and vice versa.

The EMG signal was amplified (GrassTelefactor) and digitalized at 16bit and 10kHz (Power1401 + Spike2 CED, UK) and continuously stored on PC hard disk for further offline analysis.

Muscle force experimental data collection

Force platform (Kistler Instruments, Switzerland) with sampling frequency 5 kHz was used to record the plantar-flexor force output produced during the determination of H and M wave recruitment curves. Obtained mechanical responses were continuously stored (BioWare software) on PC hard disk for further offline analysis.

Neuromechanical coupling

Another parameter that was measured and compare was the changes in a range of neuromechanical coupling properties of skeletal muscle relative to threshold before, during and after ischaemia. To assess the influence of short term ischaemia on the neuromechanical 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. 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.

Mmax - Force relation

Pre-ischaemic, ischaemic and post-ischaemic reactions of plantar-flexors muscle force output related to the compound 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 and were given more than 3 sec apart to avoid any postactivation depression of the PFs muscle (Capaday, 1997; Klass et al., 2004). During the measurement caution was taken also about the rate of stimulation, because excessive rate in electrical stimulation may lead to block of neuromuscular transmission (Jones, 1996). This was avoided by using adequate stimulation frequency. 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. Simultaneously measured EMG evoked potential provides analysis of M-wave, which contains information about membrane properties of the active MUs (Merletti et al., 1992).

Statistical analysis

To compare the experimental 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 and standard errors of the mean (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.

Results

Electromyographic and force evoked potential changes analysis allows the study of different aspects of the neuromuscular system function under ischaemia. The parameters chosen for further analysis were:

H-reflex and M-wave native traces

Characteristic evoked potential of H-reflex and M-wave amplitudes using constant stimulus intensity are depicted in Fig. 1. It can be seen that the effectiveness of stimulus strength differ during the course of experiment. 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. To describe this phenomenon more accurately H-reflex and M-waves recruitment curves were obtained.

Recruitment curves

H-reflex and M-wave recruitment curves were analyzed to explore the above mention changes (Fig. 2a, 2b). In this figures 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 both evoked amplitudes were measured.

Thresholds

In order to compare the difference in stimulus strength, threshold intensity measurements for H-reflex and M-wave were obtained in all four periods (Fig. 3a, 3b). 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%.

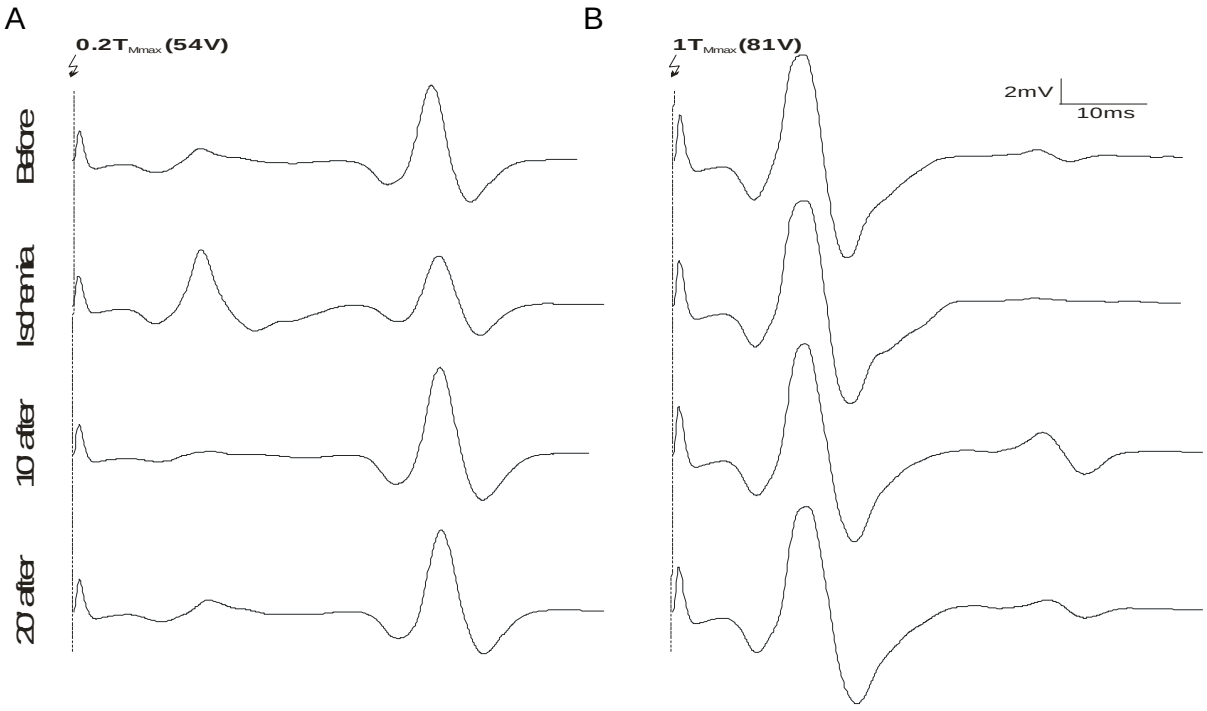

Fig.1. Evoked potentials of the H-reflex **(A)** and M-wave **(B)** to the same stimulus strength before, during ischaemia, 10 and 20 minutes after reperfusion. Note the significant changes between H-response before vs during ischaemia and 10min after ischaemia ($P < 0.05$). Identical stimulus elicited 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. M-wave remain unchanged.

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.

The level of post-ischaemic threshold for H-reflex and M-wave was significantly greater than during ischaemia. After occlusion release, 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 and sensory axons became less excitable 10 minutes after ischaemia and reach the control values. That means the excitability of sensory and motor fibers 10min after reperfusion restored to the level before ischaemia.

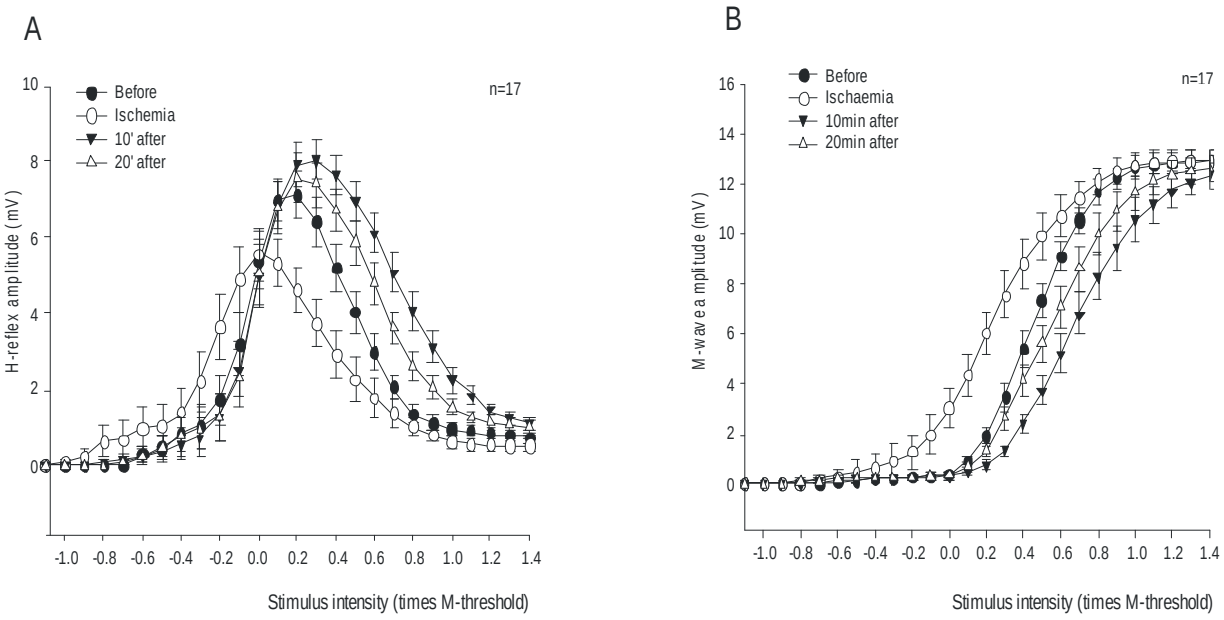

Fig.2. Mean changes in the H-reflex **(A)** and M-wave **(B)** recruitment curves. H-reflex recruitment curve during ischaemia is smaller than those before and after reperfusion and is shifted spatially to the left. Similarly, both post-ischaemic recruitment curve of H-response was significantly greater 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.

Latency

The time interval between stimulus onset and the initial trace of the H-response was calculated. The results from multiple comparison analysis shows significantly longer latency during ischaemia and post-ischaemic periods compared to the values before ischaemia (P<0.05). The post-ischaemic latency values (20min after) slightly recovered compared to ischaemic condition (Fig. 4c).

The M-wave latency represents the time required for conduction velocity of efferent fibers down to the terminal branches and propagation across the neuromuscular junction. There was a significant delay in the values 10 and 20min after reperfusion compared to initial values (Fig. 4d). 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.

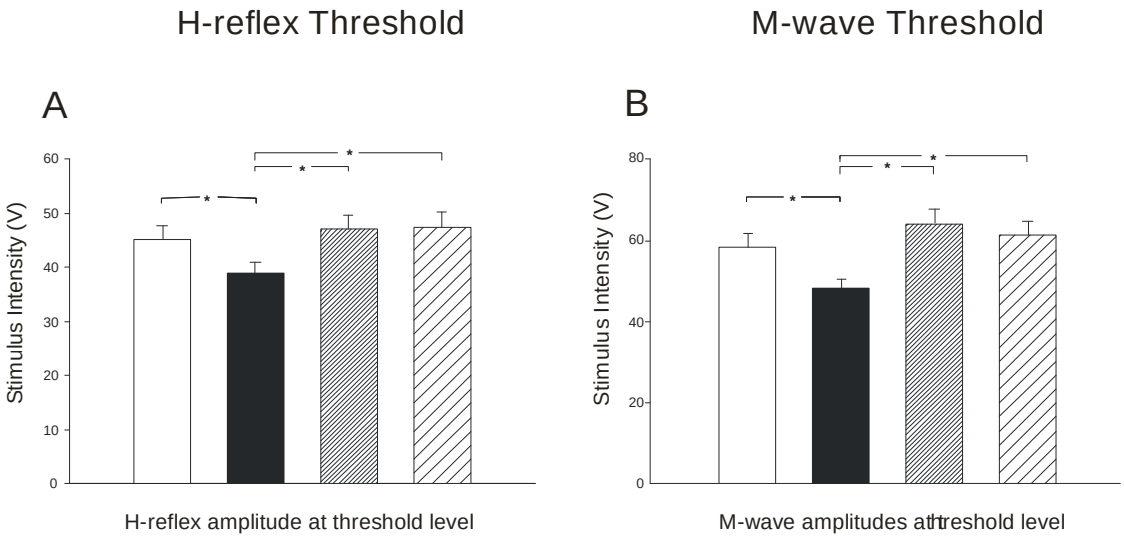

Fig. 3. Threshold changes were measured using 0.5ms stimulus duration. Note that ischaemia produced significant hyperexcitability of sensory **(A)** and motor **(B)** axons. The decrease of threshold of sensory fibers between initial data and ischaemia was associated also with a change of threshold intensity for motor fibers. Measurements after reperfusion (after 10 and 20min) showed that H-reflex and M-wave thresholds significantly increase compare to the data during ischaemia ($P < 0.05$).

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

Background EMG activity of the soleus muscle was assessed in terms of amplitude analysis and was not significantly different between the four periods of experiment (Fig. 4f).

The maximal peak-to-peak amplitude of soleus (Hmax) and the Hmax/Mmax ratio (Fig. 4e) 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 4a, 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 ($P<0.001$).

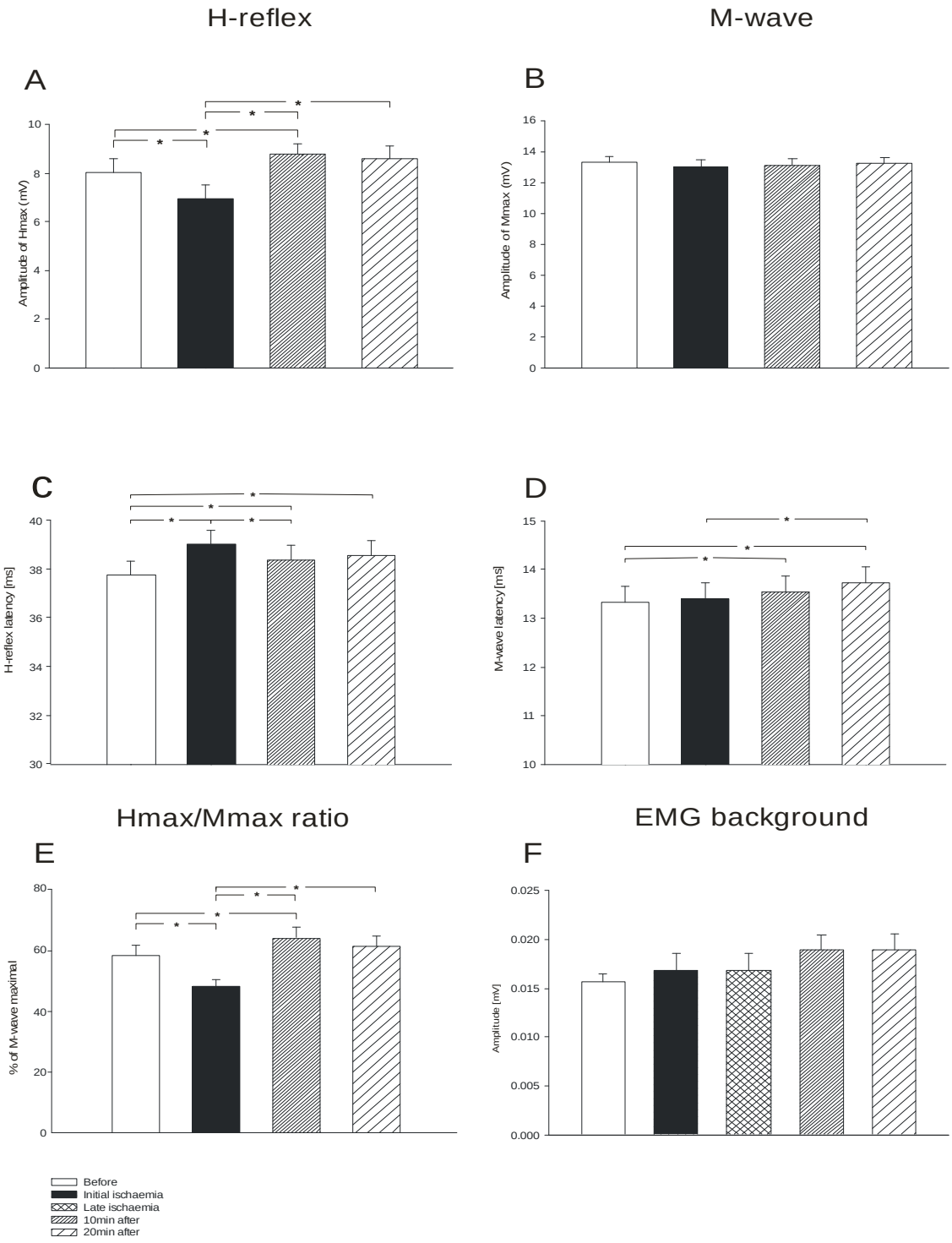

Fig.4. Comparison of the effect of ischaemia to the maximal amplitude of H-reflex **(A)** and M-wave **(B),** to the latency **(C), (D)**, to the Hmax/Mmax ratio **(E)** and to the EMG background changes **(F)** for all 17 subjects.

A significant Hmax increase was also found between the period before ischaemia and 10min after reperfusion. 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 and the mechanical responses were not altered from the displacement (e.g. from contraction of underlying muscles) of the stimulating electrode away from the tibial nerve during the experiment. Mmax represents the total activation of the soleus motoneurone pool.

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. 4e).

The plantar-flexors muscle mechanical output

A comparable series of recording made before, during and after ischaemia is illustrated in Fig.5. Note that the plantar-flexor 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. Changes in developed force of plantar-flexor (PF) were analyzed during each experimental period (Fig 6a). There was no significant difference in force development between control period and during ischaemia.

At the first post-ischeamic measurements (10min after reperfusion) the force fall significantly (69.065 ± 4.678 for) compare to control (75.389 ± 5.054) and to ischaemia (79.292 ± 5.374) values ($P<0.001$). The PFs force remained significantly reduced 20min after reperfusion (70.765 ± 4.794) compare to the force values during ischaemia. It means that short term Ischaemia can produced marked changes in post-ischaemic measures of plantar-flexor muscle force output (Nmax*)* and not even the reintroduction and restoration of blood flow allowed the muscle to reach the control values.

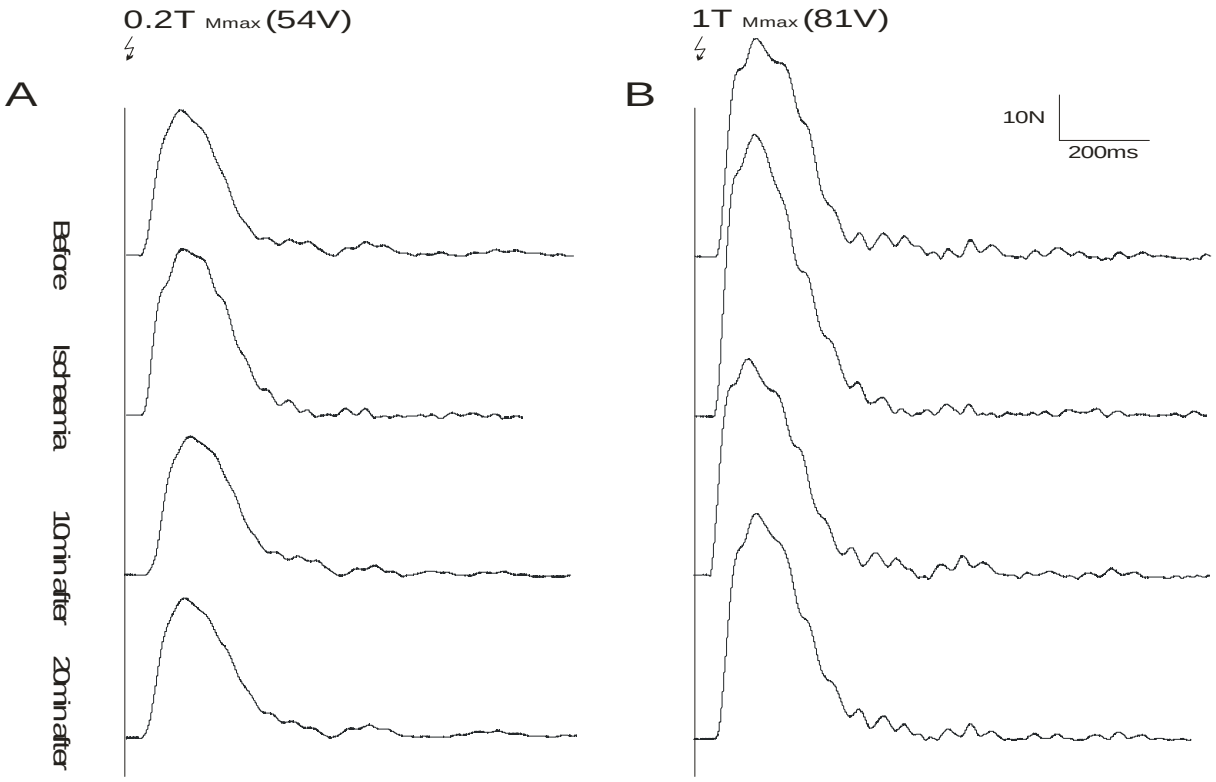

Fig.5. Characteristic mechanical responses of the plantar flexor muscles **(A)** and **(B)** to the same intensity stimulus during the course of the experiment. It can be seen the difference between the traces recorded before, during occlusion and after reperfusion.

Latency of evoked PFs muscle twitch

Figure 6b 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 twitch latency cannot recover even within 20min.

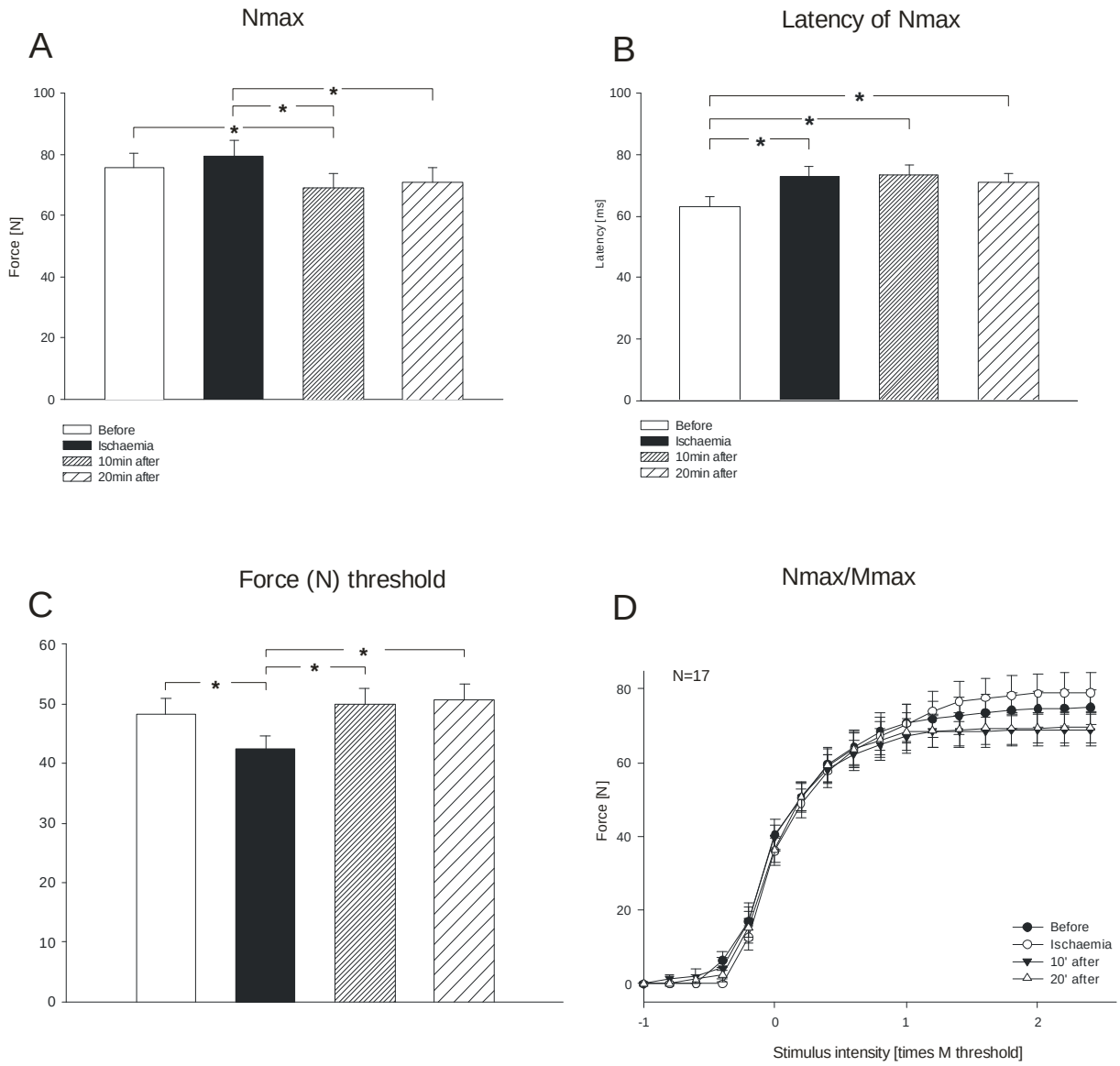

Fig.6. Comparison of Force evoked potential measurements during experiment.
A. Maximal response of evoked twitch peak of PFs muscle mechanical reaction to electrical stimulation of tibial nerve at popliteal area.
B. Changes in maximal muscle twitch reaction latency.
C. Relation between stimulus intensity 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.

The muscle twitch strength-duration properties

The full sequence of threshold measurements was obtained to define the differences in 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 initial (control) thresholds stimulus intensity (V) values were obtained at rest (48.188 ± 2.590) before the effect of short term ischaemia on different neuromechanical parameters were 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 plantar-flexors muscle twitch evoked potential seen at threshold level significantly increase compare to the data during 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 evoking peak wave of plantar-flexors muscle twitch seen at threshold level and M-wave and H-reflex threshold significantly increase compare to the data during ischaemia ($P < 0.05$). These show that the excitability of sensory and motor axons and the excitability of plantar-flexors muscle fibers returned to the baseline (control values).

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. 15d). This implicates that activation of monosynaptic reflex loop produces mechanical response of PFs muscles. These results indicate that peak twitch force was evoked by stimulus lower than those evoking M-wave (M-threshold) and that the first motor response 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 isometric activation of the PFs muscle was clearly correlated with the Mmax response (CMUP) which represents the total activation of the soleus motoneurone pool. The constant maximal amplitude of the M-wave in all four phases of experiment ensures that the recording conditions were unchanged.

Discussion

Our results suggest that, there are more vulnerable to short-term lower limb ischaemia structures involved in neuromuscular function. The main findings of the present study are significant alterations of peripheral nerve excitability, affection of synapse transmission and force-failure mechanisms known to exist at this level.

Pathophysiological changes on peripheral nerve excitability

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.

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. These differences in excitability and threshold were evaluated by the H-reflex and M-wave recruitment curves (Fig. 2a,b). The finding of increased excitability of the afferent and motor fibers during ischaemia is partly in agreement with that of Lin et al. (2002) and Zakutansky et al. (2005). Zakutansky and colleagues 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 reperfusion. 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. The discrepant findings could be due to differences in the electrical stimulus duration (Capaday, 1997) that was delivered to the tibial nerve (in our study 0,5ms instead of 1ms) and due to different post-

ischaemic conditions under which the H-reflexes were measured. In the present study the experimental data were obtained 10min after the occlusion was released and the ischaemia of lower limb was induced for 10min (instead of 5min). These results indicate that sensory Ia fibers are not more vulnerable to altered metabolic processes (extracellular K+ accumulation) than motor axons.

The mechanisms located beyond the sensory and motor neurons alterations is supported by the observation that membrane depolarization by applied currents produces similar changes in excitability 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 ion channels and from changes in metabolic processes (influenced by pH) due to dysfunction of the electrogenic Na^+ pump and subsequent K^+ accumulation. The present findings of peripheral nerve excitability changes correspond with the findings of previous mentioned studies performed by different techniques and methods (e.g. long-lasting depolarizing and hyperpolarizing currents, threshold tracking).They are also supported 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). 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 (Burke et al., 1989).

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

seems unlikely to be due to changes in neuromuscular junction because the maximal amplitude and latency of M-wave remains unchanged. The majority of the changes in H-reflex strength are attributed to changes in the amount of presynaptic inhibition (Schieppati, 1987; Zehr, 2002). With respect to the results 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. The decreased synapse transmission (evaluated by decreased value of Hmax/Mmax ratio) can be due to increases in presynaptic inhibition (Avela et al., 2001; Zakutansky et al., 2005) of group Ia-afferent terminals projecting directly to the motoneurons (with matched level of background level of motor activity). It is, however, not clear whether changes in Ca^{++} permeability or the degree of the neuromechanical transmitter depletion can affect the efficiency of the synaptic transmission (Komiyama et al., 1999).

One particularly interesting finding from the present study is the observation of an increased latency in the H-wave (during ischaemia and at 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.

Alteration of 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 plantar-flexors muscle force output (Nmax*)*, latency of the muscle mechanical response and to neuromechanical parameters of PFs muscle.

Force output and latency during ischaemia

The maximal activation of the PFs muscle (Nmax) was clearly correlated with the Mmax response which remains unchanged during the course of 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.

The analysis of this 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 after reperfusion. 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 (alteration of pH), 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. (Hatzipantelis et al., 2001; Clausen, 2003). The metabolic instability that was developed during 10min of ischaemia was severe enough to significantly change the PFs muscle evoked twitch force for at least 10min. Even restoration of blood flow with oxygenated blood allowed the muscles to reach the initial values.

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). These dysfunction can be related to prolonged elevated (slow reabsorption from sarcoplasmic reticulum) intracellular Ca^{2+} (Bruton et al., 1998). 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).

Interestingly, the PFs muscle force was not significantly altered during ischaemia. That finding is in contrary to the reports of Stainsby et al., 1990 and Hogan et al., 1999 that the sudden and total reduction in blood flow result in a fall in force production within seconds of the ischemic initiation. One possible explanation for the discrepant findings could be difference in the conditions under which the muscle was assessed and the type of muscle fibers.

The decrease in the muscle twitch force observed at both post-ischaemic results, without any change in the M-wave, can be also explained by an alteration of the excitation-contraction coupling and due to intracellular metabolic processes (Duchateau and Hainaut, 1985; Bigland-Ritchie et al., 1986, Avela et al., 2001).

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 in post-ischaemic phases. This increased latency of maximal force (Nmax) correlates absolutely with H-reflex latency alteration caused by ischaemia. These changes can be due to some peripheral neurogenic or myopathic adaptations resulted in slower electrical impulse propagation and related to impaired muscle membrane excitability caused by the amount and rate of Ca^{2+} release from sarcoplasmic reticulum (Dutka et al., 2005, Ortenblad et al., 2000). All physiological mechanisms of strength-duration properties are driven by several factors but in general can provide information on intracellular Ca^{2+} transients, muscle fiber type, and cross-bridge changes (Westerblad et al., 1997; Ortenblad et al., 2000, Clark et al., 2006a).

Efficacy of ischaemia on neuromechanical threshold

The result of PFs neuromechanical threshold 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, 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 muscle force evoked potentials returned to the baseline (before ischaemia).

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 action potential propagation on excitable membranes of muscle fibers (sarcolemma and t-tubule) may be altered during ischaemia because of the membrane Na^{+} pump dysfunction (Yensen et al. 2002; Duchateau et al., 2002; Nielsen et al. 2004; Piitulainen et al., 2007).

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 known that acidosis decreases excitability in an excitable tissue and vice versa. Muscles, however, can balance effect of intracellular 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 fiber 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.

Effects of ischaemia on neuromuscular junction

Except the above mention neuromuscular mechanisms that can be impaired by 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 by 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 results 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.

The observation of PFs muscle twitch response before M-wave action potential was evoked is another one interesting findings from the current study. This is obvious from the PFs muscle recruitment curves (Fig. 6d) elicited during all four phases of our experiment. This mechanical response was recorded by the force platform and was elicited by low intensity stimulation of the sensory fibers (only H-reflex was present). In agreement with our finding are only the studies of Maffiuletti and colleagues (2000) and Scaglioni and others (2003). Their studies were done in healthy human subjects but without any intervention. They presenting investigations, showed the relative contribution to the plantar-flexors torque of the soleus motor units activated by H and M waves. The testing position of lower limb was at 90^0 of knee flexion. That position was evaluated because it markedly reduces the mechanical contribution of gastrocnemii muscles to the evoked twitch. That means they results interpreting only the twitch evoked by soleus muscle and it doesn't correspond to all PFs muscle group.

Epilogue

The aim of the present study was to determine the main site in the neuromuscular system affected by the pathogenic condition of lower limb short-term ischaemia. We identified the following variables that we felt may theoretically be predictive of neuromuscular disturbances.

Excitability of peripheral nerves and muscle fibers are highly influenced by metabolic intracellular processes (affected by pH). It is 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. However, current extent of this effect remains unknown.

There was extensive modulation of transmission across the synapses of Ia afferent in the H-reflex pathway due to ischaemia. These altered synapse transmission can be due to increases in presynaptic inhibition of group Ia-afferent terminals

projecting directly to the motoneurons. Also, during the experiment the human neuromuscular junction was not affected by short-term ischemia. Assertion based on M-wave values.

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

The further study of the above mention structures may provide important insights employed into the performance of neuromuscular periphery in disease states.

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