

POST-ACTIVATION POTENTIATION AND FATIGUE OF QUADRICEPS MUSCLE AFTER CONTINUOUS ISOMETRIC CONTRACTIONS AT MAXIMAL AND SUBMAXIMAL INTENSITIES

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ABSTRACT

The dominance of fatigue or post-activation potentiation (PAP) depends on the type, intensity, and duration of exercise and duration of the recovery before contractility is tested. Although the decrease in PAP magnitude with decreased exercise intensity is well documented (Vandervoort et al., 1983; Behm et al., 2004), it is not clear how PAP and fatigue influences the contractile properties of skeletal muscle when exercise is of different intensity but with the same amount of work performed. Thus it is important to understand the manifestation of PAP and fatigue of skeletal muscle after continuous maximal and submaximal contractions but with the same amount of work performed.

Eight healthy untrained men (age 23–27 years, mass 83.5 ± 5.4 kg) performed maximal sustained isometric knee extension for 30 s (MVC-30 s) and on the other occasion the same subject performed sustained isometric knee extension for 60 s at 50% of maximal (50% MVC-60 s). We assumed that the amount of performed work was the same during both MVC-30 s and 50% MVC-60 s exercises. The experimental order was randomized. The contractile properties of quadriceps muscle evoked by electrical stimulation at 1 Hz (P 1), 10 Hz (P 10), 20 Hz (P 20), and 50 Hz (P 50) as well as contraction time (CT) and relaxation time (RT) of single twitch (P 1) and EMG_{rms} of v. lateralis muscle were recorded before and immediately after the exercises (0 min) and 1, 2, and 3 min following the exercises.

A significantly greater potentiation ($p < 0.05$) of P1 was observed after 30-s MVC (MVC-30 s) compared with the 60-s MVC (50% MVC-60 s) immediately after exercise and at 1 min of recovery. No changes in P 1 contraction time (CT) were observed during 3 min recovery period, however half relaxation of P 1 ($\frac{1}{2}$ RT) was more prolonged ($p < 0.05$) immediately after 50% MVC-60 s exercises. Moreover, immediately and 1 min post exercise the P 10 force after MVC-30 s exercise was higher ($p < 0.05$) compared to 50% MVC-60 s exercise. No differences between MVC-30 s and 50% MVC-60 s exercises were observed at high stimulation frequencies, maximal voluntary contraction force (MVC) as well as for EMG_{rms} values during 3 min recovery period.

The main finding of the present study was that PAP was observed after both maximal and submaximal intensity exercises when the same amount of work was performed. The more intensively exercise is performed, the more PAP offsets fatigue straight after exercise (maximal intensity); while after submaximal exercise PAP becomes more evident only during the recovery period.

Keywords: skeletal muscle, isometric exercise, maximum voluntary contraction, recovery.

INTRODUCTION

The performance of skeletal muscle is affected by its contractility history. While fatigue will impair performance, post-activation potentiation (PAP) acts to improve performance (Vandervoort et al., 1983; Baudry, Duchateau, 2004; Masiulis et al., 2006). The dominance of fatigue or PAP depends on the type, intensity, and duration of exercise and duration of the recovery before contractility is tested. It has been shown

that intense and very short (about 5–10 s) isometric contractions induce PAP, i. e., an increase in contractile force evoked by a single twitch and/or low-frequency stimulation, lasting for 5–10 min (Houston, Grange, 1990). Temporal characteristics of contraction are also affected in a way that force development and relaxation occur at a faster rate (O'Leary et al., 1997; Hamada et al., 2000). The magnitude of PAP is greater in muscles

with highest proportion of type II muscle fibers (Vandervoort et al., 1983; Hamada et al., 2000). Phosphorylation of myosin regulatory light chains has been implicated as the underlying mechanism of PAP in human skeletal muscles (Houston, Grange, 1990).

It was shown that voluntary contractions at < 75% MVC produced little or no potentiation. It was concluded that maximal (vs. submaximal) voluntary contractions lasting approximately 10 s cause the greatest twitch potentiation (Vandervoort et al., 1983). The fact that potentiation can occur during submaximal exercise with leg extensors in humans is supported by P. D. Gollnick et al. (1974), who observed that fast-twitch fibres are used at lower force levels than generally believed, and by the previous work by O. M. Rutherford et al. (1986), who also observed potentiation early in repetitive exercise performed at 30 and 45% MVC.

Although the decrease in PAP magnitude with decreased exercise intensity is well documented (Vandervoort et al., 1983; Behm et al., 2004), it is not clear how PAP and fatigue influences the contractile properties of skeletal muscle when exercise is of different intensity but with the same amount of work performed. Thus it is important to understand the manifestation of PAP and fatigue of skeletal muscle after continuous maximal and submaximal contractions with the same amount of work performed.

METHODS

Subjects. Eight healthy untrained men (aged 23–27 years, mass 83.5 ± 5.4 kg) gave their informed consent to participate in this study. The subjects were physically active but did not take part in any formal physical exercise or sport program. Each subject read and signed written informed consent form consistent with the principles outlined in the Declaration of Helsinki.

Force Measurements. The equipment and technique used for measuring force were the same as used in the previous studies (Skurvydas, Zachovajevs, 1998; Masiulis et al., 2006). Briefly, before performing explosive strength training session the subjects were seated in a steel framed straight-backed adjustable chair and appropriate adjustments were made to ensure an optimal riding position. A seatbelt attached to the side of the chair passed around the subject's waist and chest to firmly secure the pelvis and upper

body for minimizing uncontrolled movements. The right leg was clamped in a force-measuring device with the knee kept at an angle of 90° (full extension being 180°) during the whole experiment. A 6-cm-wide plastic cuff, placed around the right leg just proximal to the malleoli, was tightly attached to a linear variable differential transducer. The output of the transducer, proportional to isometric knee extension force, was amplified and digitized at a sampling rate of 1 kHz by a 12-bit analogue-to-digital converter incorporated in a personal computer. The digitized signal was stored on a hard disk for subsequent analysis. The output from the force transducer was also displayed on a voltmeter in front of the subject. Maximal voluntary contraction (MVC) force was determined.

Electrical Stimulation. A high-voltage stimulator (MG 440, Medicor, Budapest, Hungary) was used to deliver electrical stimuli to the quadriceps muscle through surface electrodes (9×18 cm) padded with cotton cloth and soaked in saline solution. One stimulation electrode was placed just above the patella, while another one covered a large portion of the muscle belly in the proximal third of the thigh. The electrical stimulation was always delivered in trains of square wave pulses of 1-ms duration (voltage 150 V, which induced approximately 60–80 percent of MVC). To maximize recruitment of fibres, the highest possible stimulation voltage was employed. The subjects were familiarized with electrical stimulation during the introductory visit before the onset of experiments. We measured the contractile force of the quadriceps muscle, evoked by electrical stimulation at 1 (P 1), 10 (P 10), 20 (P 20), and 50 (P 50) Hz (the duration of each electrical stimulation series was 1 s). The rest interval between muscle electrical stimulation was 3 s. Contraction time (CT) and relaxation time (RT) of single twitch (P1) of quadriceps muscle was also recorded.

Electromyography. The electromyogram (EMG) was recorded only during voluntary contraction. Two Ag-AgCl electrodes (FE1 surface electrode, Medicor, Budapest, Hungary) were used. The electrodes were placed over the vastus lateralis muscle approximately 15-cm proximal to the superior border of the patella, and approximately 3-cm proximal to the distal stimulation electrode. The electrodes were of rectangular shape (5×10 mm) and were glued to a plastic plate to maintain a distance of 10 mm between

them. The skin was shaved and cleaned with ethanol, and the electrodes, covered with electrode jelly, were placed over the muscle. The EMG signals (amplified $\times 2500$ and bandpass filtered between 10 and 1000 Hz; MG 440, Medicor, Budapest, Hungary) were analogue-to-digital converted (12 bit) at a sampling rate of 2500 Hz and stored on hard disk for subsequent analysis. Sample duration of 2 s was always used. During calculations of electromyogram root mean square (EMG_{rms}), adjustments for the resting signal level were made (Mathiassen, Winkel, 1990; Ratkevicius et al., 1995). The resting EMG_{rms} was evaluated at the beginning of each experiment after the electrodes were positioned on the vastus lateralis muscle.

Experimental Protocol. Two experiments were carried out with 1 week interval in between them. Before the experiments the subject performed a 5 min warm-up and was seated in the experimental chair right afterwards. After 5 min, the initial contractile properties of muscle (Ini) were recorded in the following sequence: P 1, P 10, P 20, P 50 and MVC (MVC was reached twice with 1 min rest in between) at knee angle of 90° . During MVC, the 2 s EMG_{rms} of v. lateralis muscle was recorded when the contraction force reached plateau. To determine the impact of the exercise intensity on neuromuscular system, we chose two exercises of different intensity but with the same amount of work performed. The subject performed maximal sustained isometric knee extension for 30 s (MVC-30 s) and on the other occasion the same subject performed sustained isometric knee extension for 60 s at 50% of maximal (50% MVC-60 s). We assumed that the amount of performed work was the same during both MVC-30 s and 50% MVC-60 s exercises. The

experimental order was randomized. The contractile properties of quadriceps muscle and EMG of v. lateralis muscle were recorded immediately after the exercise (0 min) and 1, 2, and 3 min following the exercise.

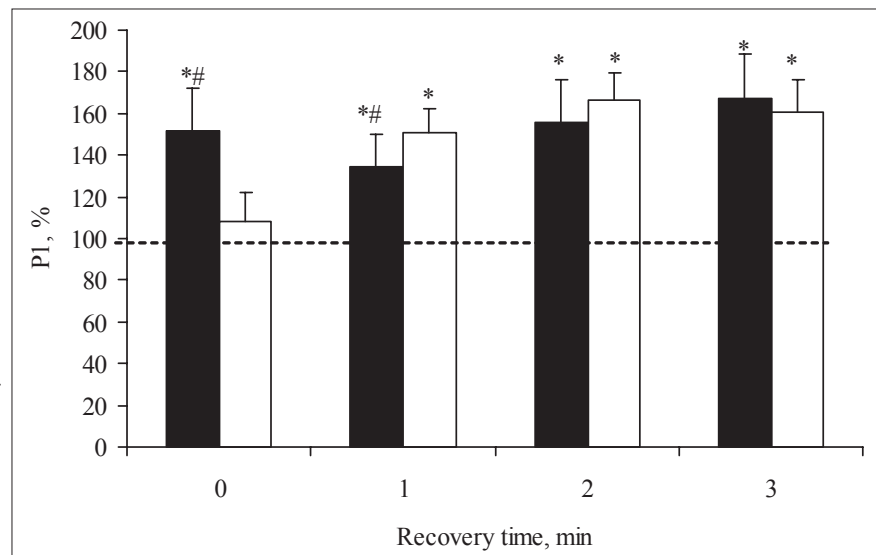
Statistics. The results obtained have been processed using the methods of mathematical statistics by calculating the means (\bar{x}) and standard deviations of mean ($\pm SD$). The differences for repeated measures in mean values were evaluated by using Student's t-test for paired data. The level of significance of the difference between arithmetic means was considered relevant when the p value was less than 0.05. The analyses were done making use of the Microsoft Excel 2000.

RESULTS

The twitch force (P 1) was significantly elevated above pre-exercise value immediately (0 min) after 30-s MVC (MVC-30 s) exercise ($p < 0.05$), but it remained unchanged after 60-s MVC (50% MVC-60 s) exercise when compared with an initial value (Fig. 1). Already at 1 min, P 1 was potentiated after both exercises and remained elevated until 3 min of recovery period ($p < 0.05$) (Fig. 1). Statistically significant differences ($p < 0.05$) were observed for P 1 immediately (0 min) and 1 min following the exercises after MVC-30 s and 50% MVC-60 s were compared (Fig. 1).

No changes in twitch CT were observed during 3 min recovery period after MVC-30 s and 50% MVC-60 s exercises ($p > 0.05$). Twitch $\frac{1}{2}$ RT was prolonged ($p < 0.05$) immediately after MVC-30 s and 50% MVC-60 s exercises, but no changes were observed at 1 min after exercises

Fig. 1. The time-course of changes in P 1 after sustained MVC for 30 s at maximal (MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery



Note. The dashed line indicates the initial mean level (100%). P1 — muscle contraction force at a stimulation rate of 1 Hz; Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$; # — significant ($p < 0.05$) difference between MVC-30 s and 50% MVC-60 s ($n = 8$).

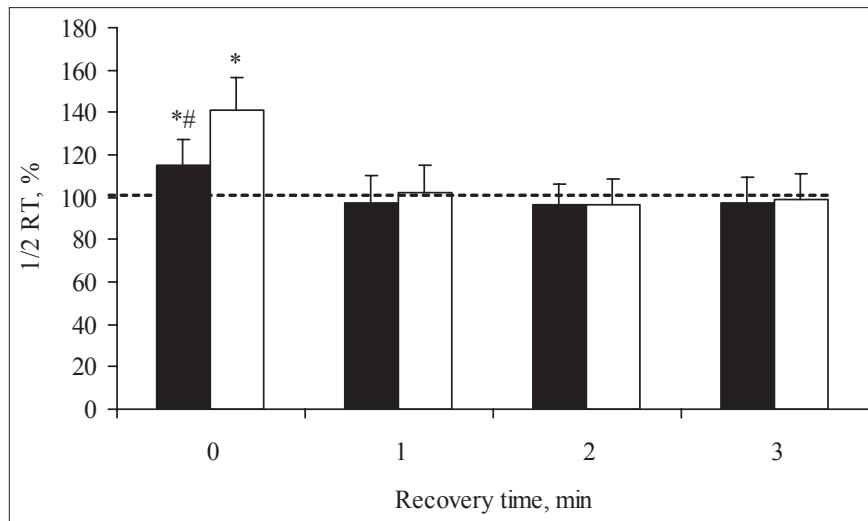


Fig. 2. Half relaxation time ($\frac{1}{2}$ RT) of single twitch (P 1) after MVC for 30 s at maximal (MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery

Note. The dashed line indicates the initial mean level (100%). Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$; # — significant ($p < 0.05$) difference between MVC-30 s and 50% MVC-60 s ($n = 8$).

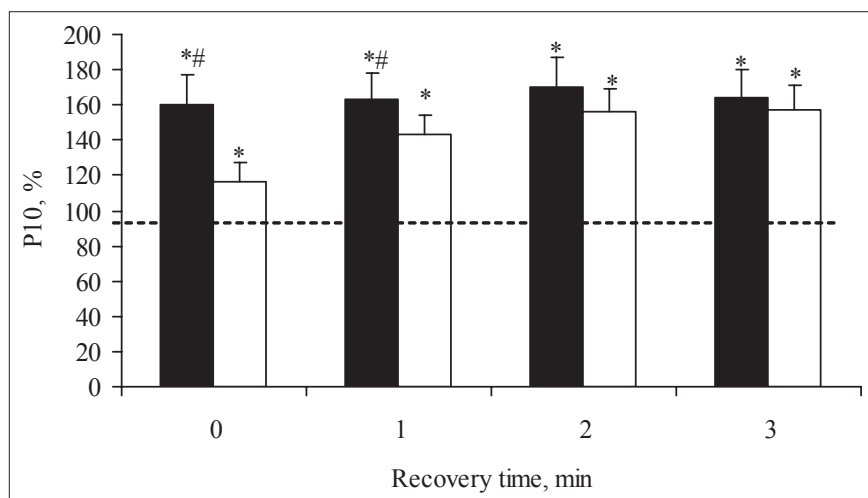


Fig. 3. The time-course of changes in P 10 after sustained MVC for 30 s at maximal MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery

Note. The dashed line indicates the initial mean level (100%). P 10 — muscle contraction force at a stimulation rate of 10 Hz; Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$, # — significant ($p < 0.05$) difference between MVC-30 s and 50% MVC-60 s ($n = 8$).

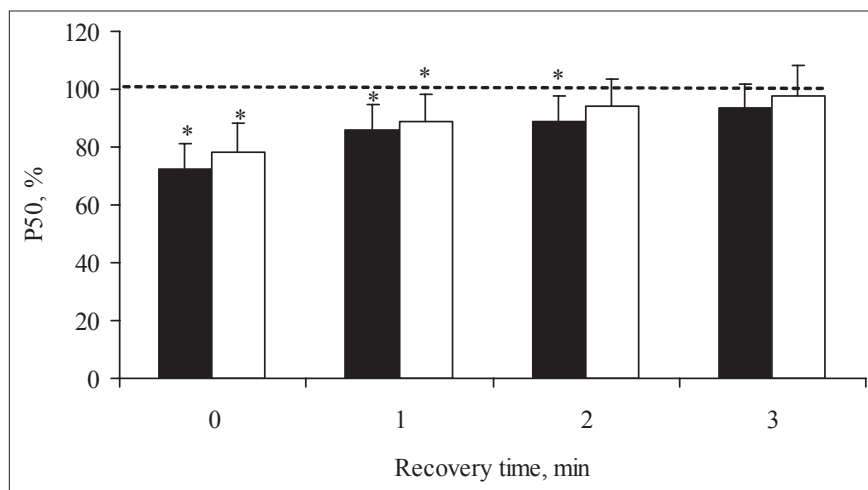


Fig. 4. The time-course of changes in P 50 after sustained MVC for 30 s at maximal (MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery

Note. The dashed line indicates the initial mean level (100%). P 50 — muscle contraction force at a stimulation rate of 50 Hz; Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$ ($n = 8$).

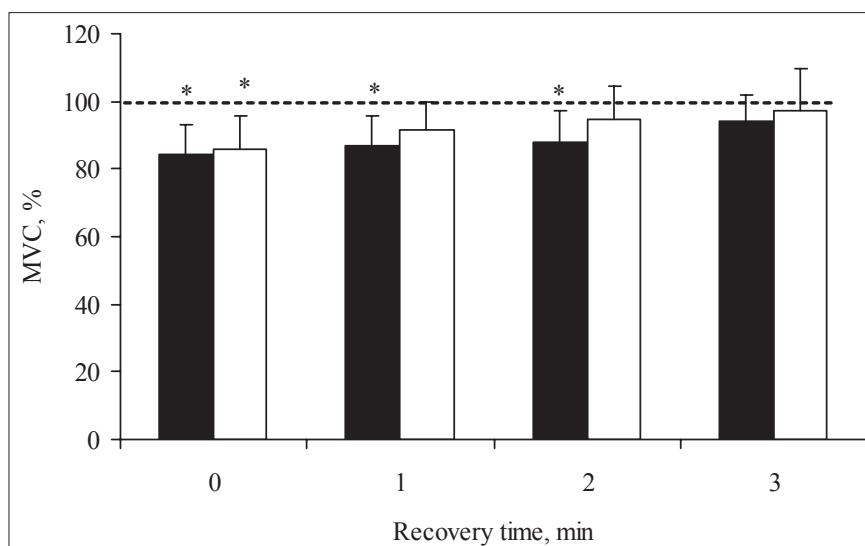
compared to the control value (Fig. 2). Right after 50% MVC-60 s exercise (at 0 min), $\frac{1}{2}$ RT was significantly prolonged ($p < 0.05$) when compared to the MVC-30 s exercise (Fig. 2).

Following both fatiguing tasks the response evoked by 10 Hz (Fig. 3) was significantly affected ($p < 0.05$) at every time point during 3-min recovery. Furthermore, immediately and 1 min post exercise the P 10 force after MVC-30 s exer-

cise was significantly higher ($p < 0.05$) compared to 50% MVC-60 s exercise (Fig. 3). It should be emphasized that P 1 and P 10 values at 3 min after 50% MVC-60 s were significantly higher ($p < 0.05$) than the values immediately (0 min) after 50% MVC-60 s exercise (Fig. 1, 3).

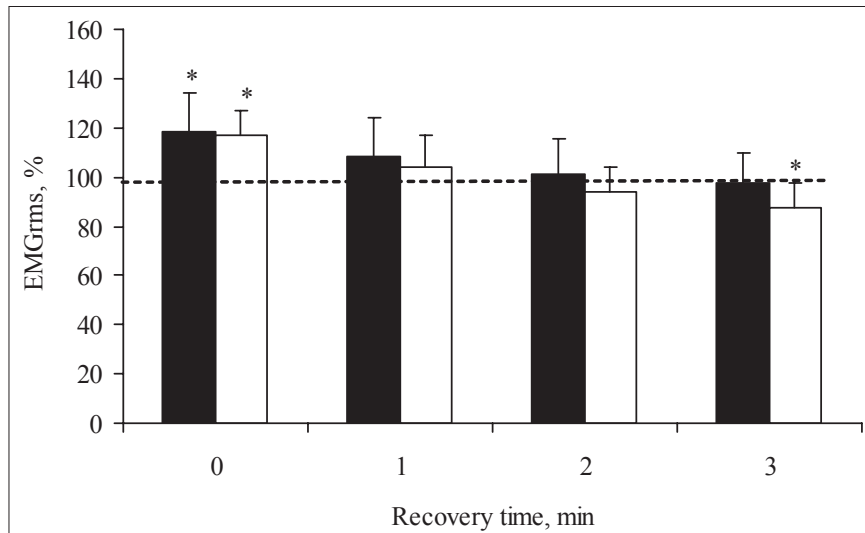
The P 50 response (Fig. 4) was depressed immediately after MVC-30 s exercise and did not recover to its initial level by 2 min ($p < 0.05$). A similar

Fig. 5. The time-course of changes in MVC after sustained MVC for 30 s at maximal (MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery



Note. The dashed line indicates the initial mean level (100%). Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$ ($n = 8$).

Fig. 6. The time-course of changes in EMG_{rms} after sustained MVC for 30 s at maximal (MVC-30 s, solid bars) and for 60 s at submaximal (50% MVC-60 s, open bars) intensities, right after (0 min) and following 1, 2 and 3 min of recovery



Note. The dashed line indicates the initial mean level (100%). EMG_{rms} — electromyogram root mean square. Values are means \pm SD. Statistically significant differences from initial are indicated as: * — $p < 0.05$ ($n = 8$).

observation was after 50% MVC-60 s exercise, but force at P 50 recovered within 2 min (Fig. 4).

Both exercise protocols resulted in a decreased MVC force (Fig. 5). The depression in MVC persisted throughout the 2-min recovery period after MVC-30 s ($p < 0.05$) and for 1 min after 50% MVC-60 s ($p < 0.05$). No differences between MVC-30 s and 50% MVC-60 s exercises were observed at high stimulation frequencies as well as in MVC (Fig. 4, 5). Values for EMG_{rms} recorded immediately after both exercises were significantly augmented, while at 3 min after 50% MVC-60 s exercise were significantly reduced ($p < 0.05$) (Fig. 6).

DISCUSSION

The main finding of this study was that PAP was observed after both maximal and submaximal intensity exercises when the same amount of work performed. Furthermore, the more intensively ex-

ercise is performed, the more PAP offsets fatigue straight after exercise (maximal intensity); while after submaximal exercise PAP becomes more evident only during the recovery period.

Greater prolongation of relaxation time and force depression after the 50% MVC-60 s exercise at 0 min could be induced by various metabolic products. Prolongation of relaxation would logically be an effective mechanism to combat the effects of fatigue by increasing the time for Ca^{2+} release to optimize cross-bridge kinetics. Twitch properties have been reported to be affected by H^+ accumulation and increases in ADP (Cady et al., 1989). It was pointed out, that the slowing of relaxation increasing the degree of fusion of low stimulation tetanus. This process, which was thought to minimize fatigue was called “muscle wisdom” (Marsden et al., 1983). Following 50% MVC-60 s fatiguing task, $\frac{1}{2}$ RT of P 1 was more prolonged compared to MVC-30 s (Fig. 2), therefore after 50% MVC-60 s greater “muscle

wisdom” occurred. Also the fact that after 50% MVC-60 s P 10 was higher than P 1 immediately after exercise, can prove that the “muscle wisdom” occurred (Fig. 1, 3). Muscle wisdom is a description for the process by which the activation rates of motor units are modulated by the CNS to optimize force during sustained contractions (Marsden et al., 1983).

Specifically, C. D. Marsden and colleagues (1983) argued that during a prolonged contraction the contractile rate of the muscle slows, allowing lower activation rates to produce the maximal force from the muscle during fatigue. It has been shown that during MVC held for at least 60 s, there was a gradual decline in force, a progressive decline in means firing rates of motor units (Bigland-Ritchie et al., 1983; Marsden et al., 1983), and a decline in contractile speed (Bigland-Ritchie, 1993). It has been suggested that as the muscle fatigues, motor units decrease their firing rates to match the decreasing contractile speed so that lower frequencies, which are less fatiguing, could still generate relatively higher forces (Marsden et al., 1983; Enoka, Stuart, 1992; Bigland-Ritchie, 1993).

Concurrently with PAP and “muscle wisdom” muscular fatigue occurs after both MVC-30 s and 50% MVC-60 s. A prolonged duration of intense contraction (10–60 s) induces a substantial disturbance of metabolic profile causing the metabolic fatigue (Green, 1997; Sahlin et al., 1998). An increase in ADP and P_i (Westerblad et al., 2002) occurs with a concomitant decrease in concentration of ATP and PCr (Houston, Grange, 1990). The consequence of these metabolic alterations is a reduction of free Ca^{2+} concentration in response to action potential (Westerblad et al., 1998) and impaired function at the level of cross-bridges (Westerblad et al., 2002), which in turn results in a decrease of contraction force for very low, low and high stimulation frequencies (Fig. 1, 3–4). Restoration of metabolic homeostasis following the exercise occurs within the range of minutes and is concomitant with rapid recovery of contractility (Houston, Grange, 1990). Therefore, P 1 and P 10 increases even more compared to post-exercise value, since PAP may persist for longer than 3 min (O’Leary et al., 1997; Baudry, Duchateau, 2004) (Fig. 1, 3).

The inactivation of MVC (Fig. 5) could originate from the inhibition of spinal motoneurons (Behm et al., 2004). Metabolites reduce the mechanical thresholds of group III and IV afferents

(Loring, Hershenson, 1992). Since they have little background discharge and their presence is both widespread and dense, group III and IV afferents can have massive increases in their input to the central nervous system (Gandevia, 1998). Thus, the perception by the central nervous system of fatigue-induced metabolic disruptions can be effectively transmitted to the motoneuron. Moreover, indirect evidence indicates that Ia afferents from intrafusal stretch receptors can contribute up to 30% of the motoneuron excitation with sustained fatiguing isometric contractions (Gandevia, 1998). Yet if the contraction is sustained for more than 1–2 s with the development of fatigue, discharge frequency diminishes (Gandevia, 1998). The resulting disfacilitation of motoneurons, while not providing direct inhibition, may decrease motoneuron excitability. Hence, it is possible to have muscle potentiating effects and voluntary force decrements concurrently. In addition, Linnamo et al. (1998) showed that central fatigue was increased with higher exercise intensity; therefore MVC force recovery after MVC-30 s can be slower (Fig. 5).

During 50% MVC-60 s exercise, the task could be performed without activating all motor units. This suggests that some motor units during 50% MVC-60 s were not activated or had time to recover, which could affect the faster recovery of P 50 (Fig. 4) and MVC (Fig. 5) force compared with MVC-30 s, when presumably all motor units were activated.

There were no significant changes in EMG_{rms} amplitude at 1 min after both exercises when P 1 and P 10 were potentiated (Fig. 6). These divergent changes in EMG_{rms} and potentiation of P 1 and P 10 (Figs. 1, 3) suggest that different processes control increases in EMG_{rms} , potentiation of force for very low and low stimulation frequencies, and fatigue of tetanic force (Fig. 4). This observation indicates that the mechanisms for potentiation is located beyond the muscle cell membrane and do not involve changes in the ECC. The finding, that EMG_{rms} amplitude after 50% MVC-60 s at 3 min was significantly reduced indicates the more efficient changes in muscle contractile characteristics after 50% MVC-60 s (Fig. 6).

Summing up, simultaneous PAP, “muscle wisdom” and fatigue in quadriceps after both MVC-30 s and 50% MVC-60 s were observed. The primary observations which present evidence for coexistence of fatigue and potentiation are the

depressed MVC and high-frequency responses while the P 1 is enhanced. Furthermore, the more intensively exercise is performed, the more PAP offsets fatigue straight after exercise. Thus, if elements associated with fatigue such as increased P_i were attenuating force output, their effects were easily masked by mechanisms of force potentiation at least during the first 3 min after MVC-30 s and 50% MVC-60 s exercises.

CONCLUSION

Coexistence of post-activation potentiation and fatigue when performing exercises with the same amount of work depends on exercise intensity. The more intensive exercise is, the more potentiation counteracts fatigue immediately after exercise; while after submaximal exercise post-activation potentiation becomes more evident only during the recovery period.

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KETURGALVIO ŠLAUNIES RAUMENS POSTAKTYVACINĖ POTENCIACIJA IR NUOVARGIS ATLIKUS NENUTRŪKSTAMĄ MAKSIMALAUS IR SUBMAKSIMALAUS INTENSYVUMO KRŪVĮ

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SANTRAUKA

Kuris iš fenomenų — raumenų nuovargis ar postaktyvacinė potenciacija (PAP) — bus vyraujantis, priklauso nuo fizinio krūvio tipo, intensyvumo, trukmės ir poilsio laiko tarp stimulų trukmės. Žinoma, kad PAP silpnėja mažėjant fizinio krūvio intensyvumui, tačiau kaip PAP ir nuovargis veikia griaučių raumenų savybes atliekant skirtingo intensyvumo, bet tokios pačios apimties darbą, nėra aišku. Todėl svarbu nustatyti PAP ir nuovargio poreiškį atlikus 30 s maksimalųjį ir 60 s submaksimalųjį, bet tokios pačios apimties nenutrūkstamą krūvį.

Buvo tiriama sveiki aktyviai nesportuojantys vyrai (amžius 23—27 m., svoris — $83,5 \pm 5,4$ kg) ($n = 11$). Tiriamieji atliko 30 s maksimalųjį (MVC-30 s) ir 60 s submaksimalųjį krūvį, t. y. naudodami 50% pastangų (50% MVC-60 s) koją tiesė per kelio sąnarį. Darytina prielaida, kad abiejų krūvių metu atlikto darbo apimtis buvo vienoda. Krūviai atlikti atsitiktine tvarka. Keturgalvio šlaunies raumens susitraukimo jėga, sukelta 1 (P 1), 10 (P 10), 20 (P 20) ir 50 (P 50) Hz stimuliavimo dažniu, raumens susitraukimo (CT) ir atsipalaidavimo iki pusės jėgos P 1 ($\frac{1}{2}$ RT) trukmė ir EMG_{rms} buvo registruojami iš karto atlikus krūvį (0 min) ir praėjus 1, 2 ir 3 min po jo.

P 1 jėga užregistruota iš karto po (0 min) ir praėjus 1 min po MVC-30 s krūvio buvo didesnė, lyginant su reikšmėmis po 50% MVC-60 s krūvio ($p < 0,05$). P 1 susitraukimo laikas (CT) nepakito per 3 min atsigavimo laikotarpį, tačiau P 1 atsipalaidavimo iki pusės jėgos ($\frac{1}{2}$ RT) trukmė labiau pailgėjo iš karto po 50% MVC-60 s nei po MVC-30 s krūvio ($p < 0,05$). Iškart po MVC-30 s krūvio (0 min) ir praėjus 1 min P 10 jėga po buvo reikšmingai didesnė ($p < 0,05$), palyginti su 50% MVC-60 s. Jokio skirtumo tarp MVC-30 s ir 50% MVC-60 s krūvių nenustatyta stimuliuojant raumenis dideliais stimuliavimo dažniais, testuojant MVJ ir EMG_{rms} atsigavimo metu.

Postaktyvacinės potenciacijos ir nuovargio sąveika atliekant tokios pačios apimties fizinį krūvį, priklauso nuo krūvių intensyvumo. Kuo intensyviau atliekamas fizinis krūvis, tuo labiau potenciacija paslepia nuovargį iškart atlikus krūvį. Atlikus submaksimalų fizinį krūvį potenciacija labiau išryškėja per atsigavimo laikotarpį.

Raktažodžiai: griaučių raumenys, izometrinis krūvis, maksimalioji valinga jėga, atsigavimas.

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