

## EKSPERIMENTINIAI TYRIMAI

### Low frequency fatigue of quadriceps muscle after sustained maximum voluntary contractions

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**Key words:** skeletal muscle, low frequency fatigue, recovery.

**Summary.** Immediately after sustained maximum voluntary contractions for 60 s, greater quadriceps muscle fatigue and, especially, low frequency fatigue is observed. The results of our study have shown that immediately after the exercise there was a significant ( $P < 0.05$ ) decrease in muscle force induced by low (20 Hz) and high (50 Hz) stimulation frequencies and maximum voluntary contractions (it is not muscle length-dependent) and it did not recover to its initial (pre-exercise) level 15 min after the end of exercise. These observations suggest the observed low frequency fatigue may consist of 2 phases: an early, rapid recovery phase (to 3 min), likely related to muscle potentiation and metabolite build-up, and a slow recovery phase, which is not dependent on metabolite levels, but is especially dependent on muscle mechanical damage. There is an increase in low frequency fatigue during a slow recovery phase and it is more pronounced when low frequency fatigue is registered at short muscle length.

#### Introduction

In humans, low frequency fatigue (LFF) occurs in concentric, isometric and eccentric exercise (1–3). Although the mechanism for production of LFF is unknown, both metabolite build-up and elevation in intracellular  $Ca^{2+}$  concentration as well as muscle mechanical damage have been suggested to play a role in its development (4–7). It has been shown in mouse skeletal muscle that the decrease in sarcoplasmic reticulum  $Ca^{2+}$  release associated with fatigue (particularly with LFF) has at least two components: 1) a metabolic dependent component, which recovers within 1 h and 2) a component dependent on the elevation of the  $[Ca^{2+}]_i$ –time integral, which recovers more slowly (7). The theory proposed by E. R. Chin et al. (7), however, does not evaluate the influence of muscle post-tetanic potentiation and muscle mechanical damage on LFF. Post-tetanic potentiation influences the development of muscle LFF (8, 9).

In a series of experiments designed to investigate the effects of length, isometric maximal voluntary contractions were performed at both short and long muscle length and the muscles were subsequently tested at an intermediate length (4). The contractions at long length resulted in greater LFF and pain, despite the

fact that they generated less force than those at the short length. Furthermore, there is a length-dependent component in the generation of LFF and muscle pain (4). D. L. Morgan (10) has showed that isometric contractions at long length will lead to overextension and damage of some sarcomeres. In this situation the active sarcomeres would be working at a shorter length than predicted from overall fiber length and the force-frequency curve will be shifted to the right (10, 11). To test this theory of muscle damage it would be necessary to verify whether there had been a change in force-length relationship.

We hypothesize that muscle LFF is dependent on the coexistence of four components: a) a fast-developing and rapidly recovering component that depends on muscle potentiation and metabolite build-up; b) a fast-developing, slow-recovering, metabolite-dependent component; c) a slow-developing, slow-recovering,  $[Ca^{2+}]_i$ –time integral dependent component; d) a fast-developing but slow-recovering component that depends on muscle mechanical damage. To verify this hypothesis, a research protocol was chosen during which isometric exercise was performed to induce muscle fatigue caused both by metabolic and non-metabolic changes as well as muscle damage.

## **Materials and methods**

### *Subjects*

Healthy untrained men (mean age  $22.9 \pm 1.8$  years,  $n=12$ ) (mean body weight  $77.5 \pm 5.2$  kg) gave their informed consent to take part in the experiments. The subjects were physically active but did not take part in any formal physical exercise or sport program.

### *General protocol*

Experiment was designed to examine the time-course of recovery of muscle contraction and relaxation properties after isometric sustained maximum voluntary contractions (MVC) for 60 s performed at knee angles of  $90^\circ$  (full extension  $180^\circ$ ).

### *Force measurements*

The equipment and technique for measuring force were the same as used in a previous study (3, 8, 9, 12). Subjects sat upright in a chair with a vertical back support. A strap secured the hips and thighs to minimize uncontrolled movements. The right leg was clamped in a force-measuring device with the knee at an angle of  $90^\circ$  or  $135^\circ$ . 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 installed in an IBM-compatible personal computer. The digitized signal was stored on the hard disk for subsequent analysis. The output from the force transducer was also displayed on a voltmeter in front of the subject. Therefore the subjects had visual feedback of the muscle force output.

### *Electrical stimulation*

Equipment and procedure for electrical stimulation were essentially the same as described previously (3, 8, 9, 12). A high-voltage stimulator (MG 440, Medicor, Budapest, Hungary) was used. Electrical stimuli to the quadriceps muscle were delivered through surface electrodes ( $9 \times 18$  cm) padded with cotton cloth and soaked in saline. One stimulation electrode was placed just above the patella, while the other 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 induces 65–85% of maximal voluntary contraction force; MVC). With an aim of recruiting the greatest number of fibers the highest stimulation voltage possible was chosen. The subjects were introduced to electrical stimulation before the experiments began.

The following data were measured: the force of the quadriceps muscle, elicited by electrical stimulation

for 1 s. at 20 Hz (P20) and 50 Hz (P50), MVC and muscle relaxation time from 100% to 50% of P50 (RTP50). Rest interval between muscle stimulations was 3 s. and between MVC was 1 min. The ratio of P20/P50 was calculated for the evaluation of LFF (1, 9). The contractile force was measured at knee joint angles of  $135^\circ$  (short length; SL) and  $90^\circ$  (long length; LL) in a randomized order.

### *Experimental protocol*

The subject was seated in the chair and after 5 min, muscle contractile properties were recorded at both SL and LL in the following sequence: P20, P50 and MVC (MVC was reached twice). During the period of recovery, MVC was evaluated once. Immediately after the exercise (A0) and 3 (A3), 7 (A7) and 15 (A15) min following the exercise the contractile properties of skeletal muscle were tested at both SL and LL. MVC at A0 was force size at the end of MVC-60 s (58–60 s).

### *Data and statistical analysis*

The two-way analysis of variance (two way ANOVA) for repeated measures was used to determine differences between the groups. When the ANOVA was significant, a paired Student's (t) test was used to determine differences between the groups. One-way ANOVA for repeated measures was used to test the statistical differences within each group (pre - vs. post-fatigue). When the ANOVA was significant, a paired Student's (t) test was used to determine differences between separate measurements. Statistical significance was set at  $P < 0.05$ .

## **Results**

Are muscle contraction and relaxation properties dependent on muscle length?

Both the force evoked by electrostimulation and the relaxation time are muscle length-dependent: 1) force-frequency curve registered at  $135^\circ$  of knee angle (SL) at frequencies between 20 Hz and 50 Hz is steeper than the one at  $90^\circ$  (LL) since P20/P50 is significantly smaller ( $P < 0.05$ ) whereas 2) RTP50 is significantly ( $P < 0.05$ ) shorter at SL than at LL (Table).

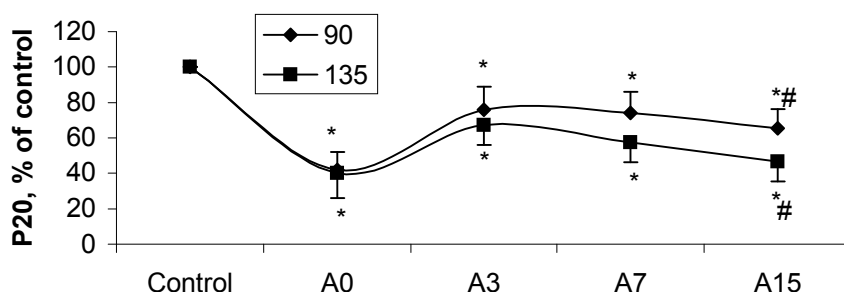
Is time-course of recovery of muscle contractile properties length-dependent?

The results of our study have shown that immediately after the exercise (A0) there was a significant ( $P < 0.05$ ) decrease in muscle force induced by low (20 Hz) and high (50 Hz) stimulation frequencies and MVC (it is not muscle length-dependent) and it did not recover to its initial (pre-exercise) level 15 min after the end of exercise (Fig. 1–3). During recovery period there was a significant ( $P < 0.05$ ) decrease in P20/P50 that is

**Table. Control values of indices of men's electrostimulation-induced contractions of quadriceps muscle, MVC and RTP50 (mean±SD)**

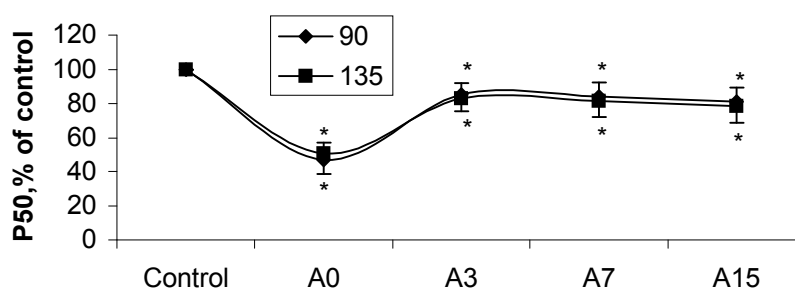
Angle	P20, N	P50, N	MVC, N	P20/P50	RTP50, ms
90°	348.4 51.4	442.7 68.4	719.8 79.2	0.77 0.06	84.3 10.1
135°	422.7 60.1	634.8 79.2	747.1 89.5	0.66 0.06	73.5 8.2
Difference between 90° and 135° angles	<0.05	<0.05	ns.	<0.05	<0.05

P20 and P50 – muscle contraction force evoked by stimulating of quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximum voluntary contraction force. RTP50 – muscle relaxation time from 100% until 50% of P50.



**Fig. 1. The changes in P20 % compared to pre-exercise values of electrostimulation-induced muscle contractions for 60 s at long muscle length (knee-joint angle of 90°)**

A0, A3, A7 and A15 – immediately, 3 min, 7 min and 15 min after exercise. P20 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz. \* – significantly ( $P<0.05$ ) different from the initial mean level. # –  $P<0.05$  between 90° and 135° knee angles mean level.

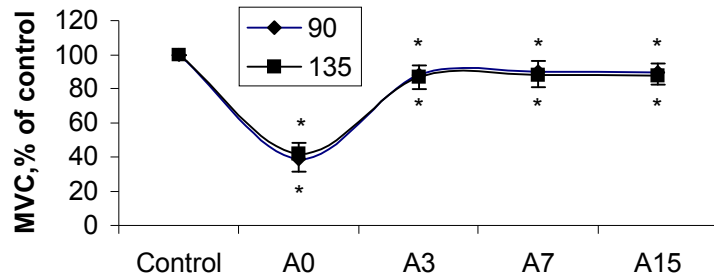


**Fig. 2. The changes in P50 percentage compared to pre-exercise values of electrostimulation-induced muscle contractions for 60 s. at long muscle length (knee-joint angle of 90°)**

A0, A3, A7 and A15 – immediately, 3 min, 7 min and 15 min after exercise. P50 – muscle contraction force evoked by stimulating quadriceps muscle at 50 Hz frequencies. \* – significantly ( $P<0.05$ ) different from the initial mean level.

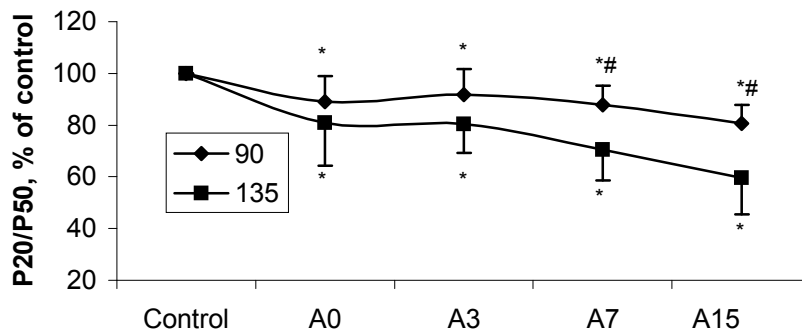
indicative of LFF (Fig. 4). From 3 min to 15 min during recovery LFF increased significantly ( $P<0.05$ ) and it is muscle length-dependent, because LFF is more pronounced ( $P<0.05$ ) in SL than LL (Fig. 4). Immediately after the exercise there was significant ( $P<0.05$ ) increase in muscle half relaxation time (RTP50), which was

not length-dependent. Three minutes after the end of exercise the muscle RTP50 recovered to its initial level while 7 min and 15 min after the end of exercise the half relaxation time was significantly ( $P<0.05$ ) shorter than pre-exercise value (Fig. 5). During the recovery period, only changes in P20 and P20/P50 were length-dependent.



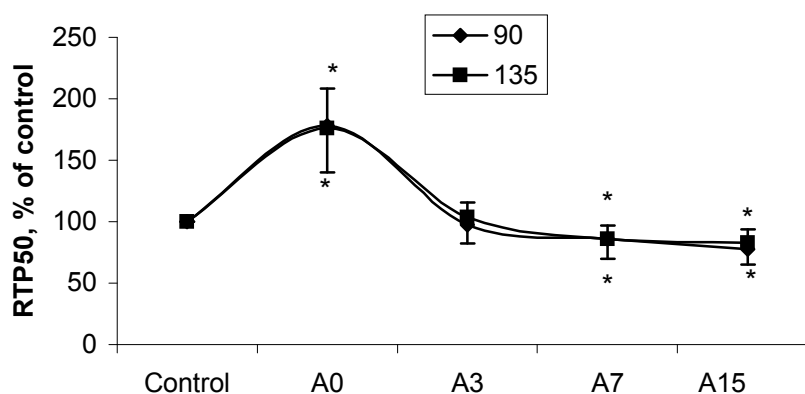
**Fig. 3. The changes in MVC % compared to pre-exercise values for 60 s at long muscle length (knee-joint angle of 90°)**

A0, A3, A7 and A15 – immediately, 3 min, 7 min and 15 min after exercise. MVC – maximum voluntary contraction force. \* – significant ( $P < 0.05$ ) different from the initial mean level.



**Fig. 4. The changes in P20/P50 after sustained maximum voluntary contractions for 60 s at long muscle length (knee-joint angle of 90°)**

A0, A3, A7 and A15 – immediately, 3 min, 7 min and 15 min after exercise. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. \* – significant ( $P < 0.05$ ) different from the initial mean level. # –  $P < 0.05$  between 90 and 135 knee degree angles mean level.



**Fig. 5. The changes in muscle relaxation time from 100% until 50% of P50 (RTP50) after sustained maximum voluntary contractions for 60 s at long muscle length (knee-joint angle of 90°)**

P50 – muscle contraction force induced by electrostimulation at 50 Hz. A0, A3, A7 and A15 – immediately, 3 min, 7 min and 15 min after exercise. \* – significant ( $p < 0.05$ ) different from the initial mean level.

## Discussion

Our main findings are as follows: 1) there are two periods recovery of muscle fatigue: rapid recovering (to 3 min) and slow recovering; 2) there is an increase in LFF during muscle slow recovery period and it is more pronounced when LFF is registered at short muscle length.

The isometric exercise chosen by us was analogous to the one performed by M. E. Houston and R. W. Grange (13), i. e. maintaining MVC for 60 s. There is no doubt, therefore, that muscle fatigue arising after MVC-60 s is dependent on metabolic factors. It has been shown that 10 min after the MVC-60 s, twitch tension was similar to control values and phosphorylation of myosin regulatory light chain was similar to the one observed at rest, ATP and PCr concentration returned to control level while lactate concentration remained elevated (13). Therefore rapid recovery of muscle contraction force and half relaxation time within 3 min after the exercise in our case could also be dependent on metabolic factors (Table), (Fig. 1–3).

It has been established that one of the mechanisms of LFF depends on the decrease in sarcoplasmic reticulum  $Ca^{2+}$  release (5). A. A. Kabbara and D. G. Allen (14) provide strong evidence that the reduction in releasable  $Ca^{2+}$  is in some way related to the metabolic changes associated with fatigue and it is consistent with the hypothesis that  $Ca^{2+}$  precipitates with phosphate in the sarcoplasmic reticulum. It has been shown that contraction and relaxation times depend on the myoplasmic  $Ca^{2+}$  transient (15). Therefore our finding that the half relaxation times were decreased during recovery might be an indirect proof of a decreased myoplasmic  $Ca^{2+}$  transient.

Within 7–15 min after the exercise the metabolic component may have fully recovered (16), but the  $[Ca^{2+}]_i$ –

time-integral dependent component may be active enough to counteract recovery of the metabolic-dependent component (7). It may be that this  $Ca^{2+}$  dependent long lasting component of LFF has a longer onset time than the metabolic component. This would explain the rapid initial decrease in LFF at 3 min of recovery and a subsequent increase in LFF at 15 min of recovery (Fig. 4). Previous studies have typically examined LFF at 10, 30 and/or 60 min of recovery (7, 17) and therefore would not have detected the changes in low frequency force response between 3 and 15 min seen here although a similar decrease and subsequent increase in LFF have previously been reported (3, 18).

Slow recovery of muscle contraction force after exercise (Fig. 1–3) indicates that fatigue is of non-metabolic origin and that it might be associated with muscle damage which is a fast developing but slow recovering muscle fatigue component. J. M. Saxton and A. E. Donnelly (19) have shown that the force loss in human biceps, damaged by lengthening contractions, was the greatest when the muscle was tested at short lengths and the least when the muscle was extended, indicating a shift to the right of the length-tension relationship.

## Conclusion

In conclusion, immediately after sustained maximum voluntary contractions for 60 s a greater quadriceps muscle fatigue and, especially, LFF is observed. These observations suggest the LFF observed here may consist of 2 phases: an early, rapid recovery phase (to 3 min), likely related to muscle potentiation and metabolite build-up, and a slow recovery phase, which is not dependent on metabolite levels but is especially dependent on muscle mechanical damage. There is an increase in LFF during a slow recovery phase and it is more pronounced when LFF is registered at short length.

## Keturgalvio šlaunies raumens mažų dažnių nuovargis atliekant izometrinį fizinį krūvį

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**Raktažodžiai:** griaučių raumenys, mažų dažnių nuovargis, atsigavimas.

**Santrauka.** Po izometrinio fizinio krūvio, atliekamo maksimaliu intensyvumu, žmogaus raumenyse pasireiškia mažų dažnių nuovargis. Tyrimo duomenimis, iš karto po krūvio statistiškai patikimai ( $p < 0,05$ ) sumažėjo raumens jėga, sukelta mažų (20 Hz) ir didelių (50 Hz) stimuliavimo dažnių, bei maksimali valingoji jėga (nepriklausoma nuo raumens ilgio). Šių jėgų reikšmės nesinormalizuoja praėjus 15 min. po krūvio. Yra dvi raumenų mažų dažnių nuovargio išnykimo fazės: greitoji raumens atsigavimo fazė, kuri trunka iki 3 min., priklauso nuo raumens potenciacijos bei „metabolinio“ nuovargio sąveikos, ir lėtoji raumens atsigavimo po krūvio fazė, kuri nepriklauso nuo metabolitų kiekio, bet labiausiai ją veikia mechaninis raumeninių skaidulų sužalojimas. Mažų dažnių

nuovargis padidėja lėtosios atsigavimo fazės metu ir tai dar labiau išryškėja, kai mažo dažnio nuovargis registruojamas esant trumpam raumens ilgiui.

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*Received 7 February 2003, accepted 25 September 2003*

*Straipsnis gautas 2003 02 07, priimtas 2003 09 25*