THE INFLUENCE OF REPEATED BOUTS OF HIGH-INTENSITY VELOERGOMETRIC EXERCISE ON LOW FREQUENCY FATIGUE AND RECOVERY OF QUADRICEPS MUSCLE AT DIFFERENT MUSCLE LENGTH

by

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The aim of this study was to establish the effect of one day training exercise on the time-course of muscle recovery after high-intensity veloergometric exercise in untrained subjects (males, age 25.4 ± 1.7 years, n=12) what causes muscle fatigue, especially low frequency fatigue (LFF). Subjects performed three, 1 min exercise bout at approximately 130% VO_2max with a 4-min rest period between each work bout. The same experiment (second experiment) was repeated 48 h after the first experiment. After first and second experiment exercises muscle contraction force induced by stimulating the muscle at 20 Hz and 50 Hz frequencies and changes in the maximal voluntary contraction force were established. The contractile force was measured at knee-joint angles of 135^o and 75^o. The results of the experiments carried out have shown that 1) the exercise repeated 48 h after applying the first experiment brought about no changes either in muscle resistance to LFF (irrespective of muscle length at which muscle contraction force was registered) or in the time-course of changes in LFF after the exercise; 2) following exercises of both experiments LFF was significantly greater at a shorter muscle length though the time-course of muscle recovery after exercise did not depend on muscle length.

Key words: electrical stimulation, low frequency fatigue, recovery, repeated bout effect, veloergometric exercise

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Unfamiliar eccentric exercise frequently results in muscle damage, the symptoms of which include strength loss, pain and elevated creatine kinase activity (Newham et al. 1987, Balnave and Thompson 1993, Nosaka and Clarkson 1995). It has been shown that following recovery, a repeated bout of the same exercise results in minimal symptoms of muscle damage and has been referred to as the "repeated bout effect" (Nosaka and Clarkson 1995, Brown et al. 1997). The repeated bout effect has subsequently been demonstrated in human and animal models, and with various types of activities using different muscle group (Balnave and Thompson 1993, Nosaka and Clarkson 1995, Brown et al. 1997). It has been concluded that when a bout of eccentric exercises is repeated 48 h after the initial bout, there is no change in the characteristic of time-course of creatine kinase and muscle strength (Smith et al. 1994). The data of these experiments therefore do not point to the presence of repeated bout effect.

In humans, low frequency fatigue (LFF) occurs in concentric and isometric exercise (Edwards et al. 1977, Ratkevicius et al. 1995, Ratkevicius et al. 1998, Skurvydas et al. 1999) but it is mostly observable in eccentric exercise (Newham et al. 1987). Although the mechanism for production of LFF is unknown, both metabolite build-up and elevation in intracellular Ca^{2+} concentration as well as muscle damage have been suggested to play a role in the development of LFF (Westerblad et al. 1993, Lamb et al. 1995, Chin and Allen 1997, Chin et al. 1997).

What are the changes taking place in muscle resistance to LFF during repeated bout effect? It has been shown that due to repeated bout effect recovery of LFF accelerations (Newham et al. 1987) and LFF diminishes (Sacco and Jones 1992). Therefore it has been suggested that when performing repeated loads the function of the muscle can be impaired (with LFF manifesting itself) without apparent muscle damage (Balnave and Thompson 1993, Brown et al. 1997). In all these cases repeated bout effect manifested itself when performing exercises after training with eccentric exercise. It is not clear therefore if prior concentric exercise will cause repeated bout effect and

how it will influence the time-course of muscle recovery after LFF registered at different muscle length.

Our working hypothesis is if time-course of recovery of LFF after performing high-intensity veloergometric exercise does not depend on muscle damage, but on metabolic factors, then prior exercise of the same type should not have essential influence on LFF changes at both short and long muscle length. The aim of the study was to establish the impact of repeated bouts of high-intensity veloergometric exercise on time-course of low frequency fatigue of quadriceps muscle at different muscle length. Besides we wanted to find out how soon contractile properties of the skeletal muscle are restored after high-intensity veloergometric exercise, which causes very strong metabolic disturbance (Choi et al. 1994).

Methods

Subjects

Healthy untrained men (age 24.4 ± 1.9 years, n=12) (weight 74.8 ± 6.3 kg) gave their informed consent to take part in all experiments within the study. The untrained subjects were physically active but none took part in any formal physical exercise or sport program.

Force measurements

The equipment and technique of measuring force was the same as has been used in a previous study (Ratkevicius et al. 1998, Skurvydas and Zahovajevas 1998). Subjects were placed in the experimental chair. They sat upright in the experimental chair with a vertical back support provided. A strap secured the hips and thighs to minimise uncontrolled movements. The right leg was clamped in the force-measuring device with the knee semi-flexed. 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 digitised at a sampling rate of 1 kHz by a 12-bit analogue-to-digital converter installed in an IBM-compatible personal computer. The digitised signal was

stored on hard disk for subsequent analysis. The output from the force transducer was also displayed on a voltmeter in front of the subject.

Electrical stimulation

Details of equipment and procedure for electrical stimulation were essentially the same as has been described previously (Ratkevicius et al. 1998, Skurvydas and Zahovajevas 1998). A high voltage stimulator (MG 440, Medicor, Budapest, Hungary) was used. Electrical stimuli to the quadriceps muscle were delivered through surface electrodes (9x18 cm) padded with cotton cloth and soaked in saline solution. 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 part of the thigh. The electrical stimulation was always delivered in trains of square wave pulses of 1-ms duration (voltage 150 V, which induce 65-70 per cent of MVC). With an aim of recruiting the greatest number of fibres the highest stimulation voltage possible was chosen. The subjects were introduced to electrical stimulation.

The following data were measured: the force of the quadriceps muscle, aroused by electrical stimulation under 20 Hz (P20) and 50 Hz (P50) frequencies (the duration of each electrical stimulation series was 1 s) and maximal voluntary contraction force (MVC) (top of the MVC was reached, held some 2 seconds and relaxation). Rest interval between muscle electrostimulation pattern was 10 s and between MVC was 1 min. The ratio of P20/P50 was calculated for the evaluation of LFF (Edwards et al. 1977). The contractile force was measured at knee-joint angles of 135^{o} and 75^{o} in randomised way.

Experimental protocol

Two experiments with an interval of 48 h were performed. Experiments 1 and 2 were designed to examine changes in muscle contraction properties after performing high-intensity veloergometric exercise (three, 1 min exercise bout at approximately 130% of maximal oxygen consumption (VO_2max) with a 4-min rest period between each work bout). The rate of pedalling was 70 times per min.

A week prior to experiment 1 the VO_2max was established. VO_2max was established indirectly using the methods proposed by Conconi et al. (1996). The

work was performed by the subjects on the veloergometer (Ergometer EX1) produced by the "Kettler" company. The rate of pedalling was 70 times per min. The initial load was to 50 W. This load was used for warm-up during the period of 4 min. Then the load was being increased by 25 W every 1 min. The subjects continued exercising until they could maintain the working intensity required. Throughout testing electrocardiogram was registered. On the basis of electrocardiogram according to the number of R cogs during the last 10 s of each minute the pulse of contraction of the heart was calculated.

First experiment. The subject was seated in the experimental chair and after 5 min muscle contractile properties were recorded in the following sequence: P20, P50 and MVC (MVC was reached 3 times). Then the subjects undertook 5 min of light exercise as warm-up: they pedalled for 5 min on e veloergometer with the rate of pedalling 70 times per min and the work load amounting to 50 W. Then the subjects performed three 1 min veloergometric exercise bout at approximately 130% VO_2max with a 4-min rest period between each work bout. Then the subjects were seated in the experimental chair once again and both voluntary and electrostimulation-induced muscle contraction properties were registered (they were registered 2-3 min following the end of the veloergometric exercise). Besides, MVC was registered but twice. Following this, the subjects remained seated in the chair for 60 min during which the muscle contractile properties were tested again (muscle contractile properties were tested 30 min and 60 min after the exercise). In addition, blood samples were obtained before and 2, and 30 min after the end of exercises and lactate concentration was (Kulis et al. 1988). The following day (24 h after the exercise) all voluntary and electrostimulation-induced muscle contraction properties were tested once again likewise as before the veloergometric exercise. In addition, during the following day the subjects subjectively evaluated their muscle pain (during walking) according to 10-point scale.

Experiment 2: 48 h after the start of the first experiment the second experiment was performed. During experiment 2 the same muscle contraction properties and lactate concentration were tested. Testing was carried out in the same sequence as in the case of experiment 1.

Data and statistical analysis

The two-way analysis of variance (two way ANOVA) for repeated measures was used to determine differences between the experiments. When the ANOVA was significant, a paired Student's t test was used to determine differences between the experiments 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

There was no difference between control values of first and second day experiments (Table 1). There was no difference between MVC values registered in 90^o and 135^o angle of knee but P20/P50 in 90^o angle was bigger than in 135^o ($P<0.05$).

Table 1. Control values of indices of electrostimulation-induced contractions of quadriceps muscle and MVC (mean ± SD)

Experi-ments		P20		P50		P20/P50		MVC	
		N		N				N	
		90^o	135^o	90^o	135^o	90^o	135^o	90^o	135^o
First		22,4	28,4	29,6	44,7	0,75	0,64	69,1	72,3
	SD	6,1	7,9	7,1	10,9	0,09	0,08	14,5	15,3
Second		22,7	27,4	30,2	44,1	0,74	0,63	69,7	74,2
	SD	7,1	8,9	8,9	14,1	0,08	0,08	13,7	14,9
		ns.		ns.		ns.		ns.	

P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximal voluntary contracion force. ns. – between first and second experiments. P20, P50 and MVC registered at knee-joint angles of 90^o and 135^o.

After the loads experienced both during experiment 1 and experiment 2 there was a significantly ($P<0.001$) decrease in P20, P50 and MVC and 1 h after

the end of exercise they did not recover to the initial level. In both cases, however, the force induced by low stimulation frequencies (20 Hz) decreased significantly (P<0.05) more than in the case of stimulation at high frequencies (50 Hz). In addition, in both cases LFF was significantly greater at a shorter muscle length though the time-course of recovery of LFF did not depend on muscle length (Fig. 1, 2 and 3). The decrease in force induced at high stimulation frequencies (50 Hz), however, did not differ in respect to registration angles.

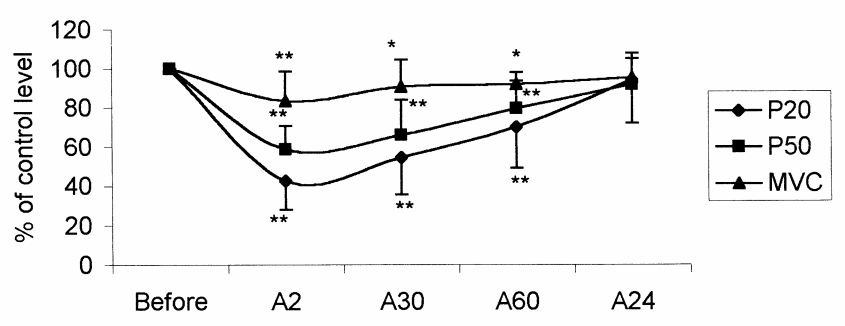

Fig. 1. Changes in the P20, P50 and MVC (registered at knee-joint angle of 90°) of persons after exercises of the first experiment. A2, A30, A60 and A24 – P20, P50 and MVC registered 2 min, 30 min, 60 min and 24 h after the end of the exercise. * - ** P<0,05 and P<0,001 to initial level. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximal voluntary contraction force.

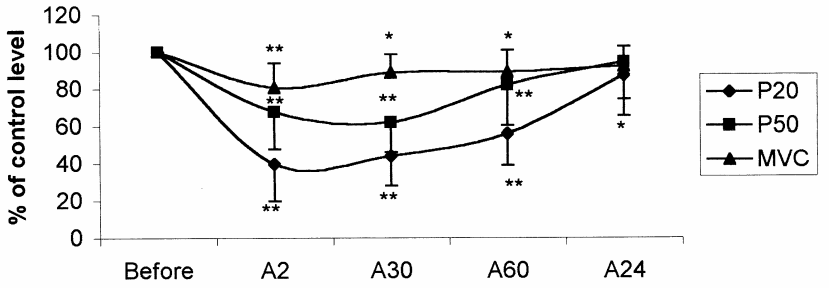

Fig. 2. Changes in the P20, P50 and MVC (registered at knee-joint angle of 135°) of persons after exercises of the first experiment. A2, A30, A60 and A24 – P20, P50 and MVC registered 2 min, 30 min, 60 min and 24 h after the end of the exercise. * - ** P<0,05 and P<0,001 to initial level. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximal voluntary contraction force.

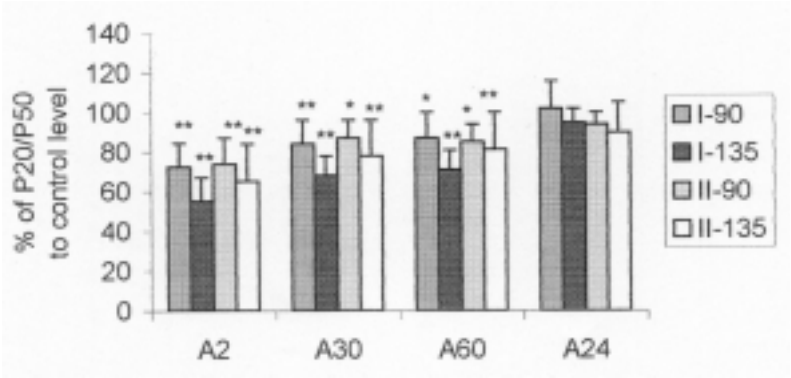

Fig. 3. Changes in the P20/P50 of persons after first (I) and second (II) exercise exercises. A2, A30, A60 and A24 – P20/P50 registered 2 min, 30 min, 60 min and 24 h after the end of the exercise. * - ** P<0,05 and P<0,001 to initial level. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. P20 and P50 registered at the knee-joint angle of 90^{o} (I-90 and II-90) and the knee-joint angle of 135^{o} (I-135 and II-135).

Besides, 2 min and 30 min after the first experimental exercise lactate concentration in blood was 9.2±1.6 mmol/l (in rest 1.1±0.3 mmol/l) and 4.5±1.7 mmol/l respectively, after the second experimental exercise 6.7±1.4 mmol/l (in rest 0.8±0.2 mmol/l) and 3.3±0.3 mmol/l respectively. There was a significant ($P<0.05$) difference between lactate concentration in blood 2 min after first and 2 min after the second experiment.

Following 24 h after the load of the first and second experiments the subjects experienced a similar muscle pain, i.e. 1.1±0.6 and 0.8±0.5 points respectively.

Discussion

The first finding of our study is that repeated load applied 48 h after the first load did not bring about any change either in muscle resistance to LFF (irrespective of muscle length at which muscle contraction force was registered) or in time-course of changes in LFF after the exercise, though lactate concentration in blood 2 min after the first experiment was significantly bigger than after the second experiment.

The second finding of our study is that following the load of both days LFF was significantly greater at a smaller length of the muscle though the time-course of LFF recovery was not dependent on the muscle length.

The main causes of low frequency fatigue

It has been established that manifestation of muscle pain 24-48 h after the load (Newham et al. 1987, Jones et al. 1989, Jones 1996, Brown et al. 1997), greater decrease in muscle force at a smaller muscle length (Wood et al. 1993, Saxton and Donnelly 1996) and slow recovery of muscle contraction force taking several days (Newham et al. 1987, Jones et al. 1989) point to the origin of muscle damage. In our case however the main reason of LFF origin cannot be associated with muscle damage since 24 h following the load (in the case of experiments 1 and 2) muscle pain was not significant and the force induced by high stimulation frequencies decreased to a similar extent at both muscle length. Also 24 h following the load P50 and MVC were no different from their control values (Fig.1, 2 and 3). The results of our research (first and second experiments) however do not allow us to deny entirely that after the load applied there could be no manifestation of muscle damage whatever since the subjects still experienced muscle pain and 24 h following the load the force induced by low stimulation frequencies (20 Hz) registered at a shorter muscle length was significantly smaller if compared to the control one ($P<0.05$).

It has been established that one of the mechanisms of LFF depends on the decrease in sarcoplasmic reticulum Ca^{2+} release (Westerblad et al. 1993). It has been shown recently that decrease in sarcoplasmic reticulum Ca^{2+} release associated with fatigue (particularly with LFF) has at least two components: 1) a metabolic 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 (Chin et al. 1997). Kabbara and Allen (1999) provide strong evidence that the reduction in releasable Ca^{2+} is in some way related to the metabolic changes associated with fatigue. The results obtained recently by Binder-MacLeod and Russ (1999) support the theory that there are two components to LFF: a rapidly recovering, metabolite-dependent component and slow-developing, slow-recovering component that is not a result of metabolite build-up. The results of our research (Fig. 3), however, contrary to the results

obtained by Binder-MacLeod and Russ (1999) show that there is even recovery of LFF after the load while the results of the authors mentioned above testify that during the period between 2 min and 20 min after the load there is even an increase in LFF. We think, however, that in our case as well, similarly to the results obtained by Binder-MacLeod and Russ (1999), immediately after the exercise this metabolic component has an influence on muscle fatigue and a decrease in muscle contraction force 1 h and more after the load apparently points to ever more reliable non-metabolic origin of LFF. There is no doubt that after the load applied by us there would be a higher accumulation of Pi, ADP and H^+ in the muscles which is known to decrease muscle contraction force (Green 1997, Saugen et al. 1997, Sahlin et al. 1998). It has been shown recently that at higher work intensities (in this case force of single fibres was reduced to 30 % in 42±7 s) the reduction in Ca^{2+} sensitivity of the contractile proteins is largely due to the accompanying acidosis and takes on increasing role in fatigue (Chin and Allen 1998).

It has been shown that the reduction in force (especially in stimulation at low frequencies), Ca^{2+} release and contractile protein inhibition observed during fatigue are closely associated with reduced muscle glycogen concentration (Chin and Allen 1997). These findings also suggest that the changes in Ca^{2+} release associated with fatigue and recovery have two components-one which is glycogen dependent and another which is independent of glycogen but depends on previous activity. Choi et al. (1994) shown that 1 h after research protocol similar to ours muscle glycogen was not restored to its initial level. We think therefore that in our case as well the manifestation of LFF 1 h following the end of the load may be glycogen dependent.

It has been shown, that if fatigue is induced with intermittent tetanic contractions, recovery is far slower and requires several hours to be complete (Lännergren et al. 1996). During recovery in this case there is often a secondary reduction in the force response to stimulation and in extreme cases force may be abolished for 20-30 min. The extreme effect on the force during recovery has been called “post-contractile depression” (PCD) (Lännergren et al. 1996) and the underlying mechanisms seem in many respects to resemble LFF in mammalian muscle. We think that PCD may have also had influence on time-course of muscle force recovery.

Why was LFF more markedly expressed at a shorter muscle length?

There is a possibility that in the fatiguing exercise, the end sarcomeres of the fibre overextend and damage those in the middle section of the fibre. In this situation the active sarcomas would be working at a shorter length than predicted from the overall fibre length and the force-frequency curve will be shifted to the right (Jones 1996). Measurements of the length-tension relationship of muscles damaged by stretching are consistent with this happening (Jones 1996, Saxton and Donnelly 1996). In addition, since at a shorter muscle length P20/P50 is also smaller, consequently, after a decrease in Ca^{2+} release at a shorter muscle length a greater decrease in P20 also takes place.

Why did not preliminary load experienced cause either LFF disappearance or decrease in LFF?

It has been shown that due to repeated bout effect there was acceleration in the recovery of low frequency fatigue (LFF) (Newham et al. 1987) and diminished LFF (Sacco and Jones 1992). This contradicts to the results of our research which indicate that due to preliminary load no increase in muscle resistance to LFF took place. This contradiction might be explained by the fact during the experiment conducted by Sacco and Jones (1992) they observed muscle contractions of eccentric type while in our case we mainly observed muscles contractions of concentric type. The results of our research, however, coincide with the data of other authors indicating that the function of the muscle can be impaired (LFF manifesting itself) without apparent muscle damage (Balnave and Thompson 1993, Brown et al. 1997). During the experiment carried out by these authors though exercises of eccentric type were performed. We conclude therefore that in our case repeated bout effect did not manifest itself since in our case there were hardly any muscle contraction of the eccentric type.

Therefore many theories have been proposed to explain the repeated bout effect but a specific mechanism has not been identified (McHugh et al. 1999). Therefore another alternative explanation of the results of our research is also possible. The results of our research do not allow us to deny the fact that there may have been increase in muscle resistance to LFF called fort by one

mechanism but LFF may also have arisen due another mechanism since causes of origin of LFF are numerous (Westerblad et al. 1993, Jones 1996). Possibly, in the case of the second load there may have been a better distribution of the work load among the fibers and, consequently, a smaller load may have fallen on fast-twitch muscle fibers that are less resistant to fatigue which in its turn may have decreased muscle fatigue. Since in that case greater load may have fallen on slow-twitch muscle fibers thus muscle energetic may have shifted more to aerobic (this is also evident from the results of our research that after the second load there was smaller concentration of lactate in the blood). In addition, it is not clear at all how repeated bout effect is influenced by metabolic factors. For example, it has been shown recently (Blomstrand and Saltin 1999) that following a decrease in the concentration of muscle glycogen fat and amino acid oxidation increases. It is not clear whether after a load of this kind muscle glycogen was fully restored or not. Has been shown recently that endurance time and VO_2 during cycling were unaffected by the prior (two days) eccentric exercises which caused muscle damage, but venous blood lactate concentration was higher (Gleeson et al. 1998). It has been concluded that the higher blood lactate concentration during cycling exercise after prior eccentric exercise may be attributable to an increased rate of glycogenolysis possibly arising from an increased recruitment of Type II muscle fibres. It has been shown that the apparent reduction in glycogenolysis and glycolysis in response to short-term training occurs during the adjustment phase to steady-state exercise (Green et al. 1995).

In conclusion, there is no changes in time-course of recovery of low frequency fatigue of quadriceps muscle at different muscle length after performing high-intensity veloergometric exercise following a prior exercise of the some intensity, though lactate concentration in blood was significantly smaller after the second experiment. Therefore there is no protective effect of high-intensity veloergometric exercise against low frequency fatigue induced by the same type of exercise in the human quadriceps muscle.

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