EFFECT OF FOUR JUMPING ENDURANCE TRAININGS ON METABOLIC FATIGUE AND ON INDIRECT SYMPTOMS OF SKELETAL MUSCLE DAMAGE

AUTHORS: Skurvydas A., Kamandulis S., Stanislovaitis A., Mamkus G., Mickevičienė D.

Laboratory of Human Motorics, Lithuanian Academy of Physical Education, Kaunas, Lithuania

ABSTRACT: The aim was to analyze the effect of four trainings on the neuromuscular adaptation of the knee extensors muscles, and particularly the connection with neuromuscular fatigue and exercise-induced muscle damage (EIMD). The subjects were healthy untrained men (age 20.8 ± 1.2 years, n=11). The four jumping endurance trainings (JETs) were repeated every 3 days and each consisted of five series of 20 jumps performed with maximal intensity with 10 s intervals between the series. The maximal voluntary contraction force (MVCF) and electrically evoked muscle contraction force at high and low frequencies, jump height (JH), muscle pain, creatine kinase (CK) activity and lactate concentration were measured before and after first and fourth JET.

The main findings in this study are that four JETs, caused: 1) no changes in decrease both of JH and MVCF 3 min after JET and no changes in their recovery rate (up to 60 min) either; 2) smaller low frequency fatigue (LFF) 3 min after JET; 3) smaller secondary decrease in electrically induced muscle force at high stimulation frequencies from 3 min until 60 min after JET; 4) smaller manifestation of indirect symptoms of EIMD 24 h after JET.

Our study showed that four JETs caused no changes in jumping performance but increased resistance of skeletal muscle to LFF and EIMD. It is evident that brief training effect manifest itself rather in electrically induced muscle performance than in voluntary muscle performance.

INTRODUCTION

The evidence of energy deficiency being a determinant of fatigue during high-intensity exercise is strong [8,9]. This type of fatigue is often referred to as metabolic [8,9]. Another type of fatigue, however, known as non-metabolic fatigue, or fatigue that occurs in the absence of significant metabolic disturbance has also been documented [5,27,28]. This type of fatigue is most readily apparent after eccentric exercise [4,6]. Then, most frequently, muscle function decreases due to skeletal muscle damage [4,6]. The well documented symptoms of exercise-induced muscle damage (EIMD) include disruption of intracellular muscle structure, sarcolema and extracellular matrix [17], prolonged impairment of muscle function measured during both voluntary and electrically stimulated contractions [6,20], protein leakage from the injured muscle fibres, acute inflammation reaction and delayed-onset muscle soreness, stiffness and swelling [2,7]. Non-metabolic fatigue is frequently accompanied by the so-called low-frequency fatigue (LFF), that is associated with excitation-contraction coupling failure [5,19,28].

It has been observed that in several days (or even in several weeks) after repeating the previous load muscle fatigue and exercise–induced muscle damage turns out to be less than after the first load applied [16,18]. This is the so-called repeated bout effect (RBE) the origin of which is associated with changes in the muscle [16,18] and/or in neural mechanisms [3,26]. Neuromuscular adaptation is usually studied on the basis of applying loads for several weeks but little is known about changes in neuromuscular adaptation after performing three-four intensive trainings that cause a brief training effect. It is not clear, however, how such a cycle of four intensive trainings aimed at developing jumping endurance will affect neuromuscular system resistance to both metabolic fatigue and EIMD. Besides, it is not clear if muscle resistance to fatigue and damage will manifest itself in the same when voluntary muscle force and force evoked by electrical stimulation at different (high and low) frequencies will be tested.

The purpose of the study was to analyze the effects of four trainings aimed at developing jumping endurance on the neuromuscular adaptation of the knee extensors muscles, and particularly the connection with neuromuscular fatigue and exercise-induced muscle damage.
MATERIALS AND METHODS

Subjects. Healthy untrained men (age 20.8±1.2 years, body mass 78.2±6.7 kg, height 181.7±6.9 cm, body mass index 21.8±2.4 kg/m²) (n=11) gave their informed consent to take part in the experiment within the study. The untrained subjects were physically active but did not take part in any formal physical exercise or sport programme. Each subject read and signed written informed consent form consistent with the principles outlined in the Declaration of Helsinki. This study was approved by the Ethics Committee of Kaunas Medical University.

Jumping endurance training. We have chosen a cycle of four jumping endurance trainings (JET I, JET II, JET III, JET IV) conducted every 72 h, during which the subjects performed stretch-shortening exercise: 5 series of 20 jumps with counter-movement to an angle of 90 degrees at the knee joint executed with maximal intensity with 10 s intervals between series. Jumps were performed from the multicomponent Kistler force plate (type 9286A, USA). During the jumps hands of the subjects were on the waist. The subjects were motivated to perform each jump as high as possible. During JET each jump was registered and the first three and last three jump height (JH) of each series were counted and averaged. A similar research protocol was applied in previous researches [21,22].

Measurements. Muscle force. The equipment and technique for measuring muscle force were the same as has been used in a previous studies [20,21]. Subjects sat upright in the experimental 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 kept at an angle of 90 degrees. 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 a personal computer. The digitised 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.

Equipment and procedures for electrical stimulation were essentially the same as has been described previously [20,21]. 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 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 in duration (voltage 150 V). With an aim of recruiting the greatest number of fibres the highest stimulation voltage possible was chosen. Prior to stimulating the muscle at 150 V the subjects were acquainted with electrical stimulation (the muscle was stimulated 2-3 times by a single stimulus at 70-90 V).

The following data were obtained: the force of the quadriceps muscle, aroused by electrical stimulation at 20 Hz (P20) and 100 Hz (P100) frequencies (the duration of each electrical stimulation series was 1 s) and maximal voluntary contraction force (MVCF) (the peak of MVCF was reached and maintained some 2 seconds before relaxation). MVCF was reached 3 times. The rest interval between muscle electrostimulations was 10 s and between voluntary contractions was 1 min. The ratio of P20/P100 kinetics after the exercise was used for the evaluation of LFF [15,19].

Counter-movement jump (CMJ). Each subject performed maximal voluntary jumps on the multicomponent Kistler force plate (type 9286A, USA) (after 2-3 trial jumps 3 jumping attempts every 20 s were allowed per person and the best attempt was counted) 1 min before, as well as 1 and 60 min after the first and fourth JET. The force signal was registered and analysed with the help of a personal computer. Techniques for CMJ were the same as described previously [22]. The participants started from an erect standing position with knees fully extended (knee = 180°). After the verbal command “Go”, they performed a downward countermovement to the 90° of knee angle and then jumped vertically for maximum height in one continuous movement. Subjects were instructed to hold their hands on the waist in all cases.

Blood lactate. Capillary blood samples (50 µl) from the fingertip were taken before and 5, 10, 20, 30 and 60 min after first and fourth training for blood lactate (La) level determination. An enzymatic (using the membrane with lactateoxidase ferment) method making use of an EXAN-G Universal (Lithuania) analyzer was applied [11]. Prior to each test the analyser was calibrated with standard 5 mmol/l lactate solution.

Plasma CK activity. Approximately 5 ml of blood was drawn from the arm vein and immediately centrifuged for 10 min to obtain plasma for CK activity determination. Plasma samples were pipetted into microcentrifuge tubes and stored in a -20°C freezer until analysed. Plasma CK (IU/L) activity was determined by using a “Monarch” (Instrumentation Laboratory SpA, USA-Italy) automatic biochemical analyser 1 h before, as well as 24 h after first and fourth JET.

Muscle soreness. Muscle soreness was reported subjectively using a visual analogue scale of 0 to 10, where 0 represented “no pain” and 10 represented “intolerably intense pain”. This evaluating method of muscle soreness has also been used in our previous researches [20,21]. The participants were required to indicate the severity of soreness in their quadriceps in response to muscle compression, as well as when standing up and walking at the start of each daily session.

Experimental protocol. After the JET I and JET IV neuromuscular fatigue as well as indirect symptoms of exercise muscle damage was being established. Neuromuscular fatigue was established in respect to the impairment in voluntary muscle performance (JH and MVCF), and force evoked by electrostimulation at different frequencies within 60 min after exercising. The indirect symptoms of EIMD chosen were: increase in CK activity in the blood and muscle soreness at 24 h after exercising.
Effect of four jumping endurance trainings on metabolic fatigue and on indirect symptoms of skeletal muscle damage

The experimental design is shown in Fig. 1. After measuring La and CK activity in the blood the subject was seated in the experimental chair and after 5 min, muscle contractile properties were recorded in the following sequence P20, P100 and MVCF (MVCF was reached 3 times and the best value was taken for evaluation). Then the subjects undertook warm up which consisted of 5 min running on the spot with an intensity that corresponded to heart rate (HR) 130-150 beats per minute (it comprised about 70 percent of maximum HR); then the subject performed 10 squat-stands. HR was measured with a Polar HR recorder (Polar Electro). Afterwards the height of CMJ was established. Then the subjects attempted to perform 3-5 continuous jumps serving as model of JET. About 1 min later JET (5 series of 20 jumps with counter-movement to 90 degrees angle in the knee executed with maximal intensity with 10 s intervals between series) was undertaken. Height of CMJ was repeatedly established 1 min after JET. After the jumps 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 after the end of the jumping exercise, MVCF was reached twice). The subjects remained seated and at 10, 30 and 60 min after JET the testing procedure was repeated in the sequence, as prior to the load. Before JET and at 5, 10, 20, 30 and 60 min after JET lactate (La) concentration in the blood was measured. Besides, at 24 h after JET muscle soreness and CK activity was determined.

**Statistical Analyses.** The two-way analysis of variance (two way ANOVA) for repeated measurements was used to determine differences between the groups. When the ANOVA was significant, an unpaired Student’s t test was used to determine differences between separate measurements. Statistical significance was set at P<0.05.

**RESULTS**

There were no significant differences in pre-exercise values of quadriceps muscle contraction force induced by different electrical stimulation frequencies, MVCF as well as JH before the first and the fourth JET (table 1). The JH decrease was significant during JET I and IV (P>0.05, JET I and IV compared) (Fig. 2). It was 84.5±9.8 % and 85.4±12.6 % of pre-exercise value after JET I and IV respectively at 60 min after the exercise (P<0.05, compared to pre-exercise value).

There was a significant decrease in MVCF during first and fourth JET and it had not recovered to its initial value within 60 min after the exercise (P<0.05) (Fig. 3). No significant difference in the dynamics of MVCF between JET I and IV has been found.

**TABLE 1. PRE-EXERCISE VALUES OF INDICES OF ELECTROSTIMULATION-INDUCED CONTRACTION FORCE AT DIFFERENT STIMULATION FREQUENCIES OF QUADRICEPS MUSCLE, MVCF AND HEIGHT OF CMJ (MEAN ± SD)**

<table>
<thead>
<tr>
<th>Jumping endurance training</th>
<th>Height of CMJ [cm]</th>
<th>P20 [N]</th>
<th>P100 [N]</th>
<th>P20/P100 [%]</th>
<th>MVCF [N]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>39.8 ± 4.7</td>
<td>166.5 ± 58.2</td>
<td>221.7 ± 72.7</td>
<td>79.9 ± 6.1</td>
<td>485.8 ± 80.2</td>
</tr>
<tr>
<td>IV</td>
<td>40.2 ± 3.8</td>
<td>178.8 ± 51.5</td>
<td>248.8 ± 73.7</td>
<td>77.1 ± 6.2</td>
<td>477.1 ± 101.5</td>
</tr>
</tbody>
</table>

Legend: MVCF – maximal voluntary contraction force. CMJ – counter-movement jump. P20 and P100 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 100 Hz frequencies.

**FIG. 2.** CHANGES IN THE HEIGHT OF JUMPS DURING JUMPING ENDURANCE TRAINING I (JET I) AND IV (JET IV) (MEAN ± SD). JET I and IV each consisted of 5 series of 20 continuous maximal jumps with counter-movement to 90 degrees in the knee and with a 10 s interval between the series. 1a, 2a, 3a, 4a and 5a - height average of three first jumps of each series and 1b, 2b, 3b, 4b and 5b - height average of the three last jumps of each series.

Legend: * – P<0.05, compared to height average of three first jumps.
The quadriceps muscle contraction force induced by different electrical stimulation frequencies significantly decreased at 3 min after JET I and IV (Fig. 4). Besides there was a significantly greater decrease in muscle force induced by low (P20) than high (P100) electrostimulation frequencies. There was less decrease in P20 (Fig. 4A) and P20/P100 force ratio (Fig. 5) after JET IV than JET I (P<0.05). Between 3 and 60 min after JET I and IV there was a significant secondary decrease in P20, P100 and P20/P100 force ratio. However secondary decrease in P100 was less after JET IV than JET I (P<0.05). No statistically significant difference in the secondary decrease of P20, as well as P20/P100 force ratio between JET I and IV has been found.

There was a significant raise in La concentration in the blood after JET I and IV and 60 min after the exercise it was statistically significantly higher than prior to the exercise (Fig. 6). No statistically significant difference in the dynamics of La concentration in the blood between JET I and IV has been found. At 24 h after JET I and IV muscle pain in the legs was 3.6±1.9 points and 1.1±0.6 points (P<0.05, JET I and IV compared) respectively, whereas CK concentration at 24 h after JET IV was significantly less than after JET I (Fig. 7).

**DISCUSSION**

The main findings in this study are that four JETs, caused:

1) no changes in decrease both of JH and MVCF 3 min after JET and no changes in their recovery rate (up to 60 min) either; 2) smaller LFF 3 min after JET; 3) smaller secondary decrease in electrically induced muscle force at high stimulation frequencies from 3 min until 60 min after JET; 4) smaller manifestation of indirect symptoms of EIMD (CK and muscle soreness) 24 h after JET.

Main causes of fatigue and exercise-induced muscle damage during and after JET. In performing jumping exercises with maximum intensity both types of fatigue, metabolic and non-metabolic, i.e. the one associated with mechanical muscle damage, can arise in the muscles. There is no doubt that after such a load that has been undergone by our subjects accumulation of Pi, ADP and $H^+$ in the muscles, that is known to decrease muscle contraction force [8], occurred. We did not register these metabolites after exercise but a significant increase in La concentration shows that neuromuscular fatigue might have arisen due to metabolic factors as well. It has been established that when performing dynamic high intensity exercise of a similar duration as in our case energy is produced in both anaerobic and aerobic way [8].

Exercise-induced skeletal muscle damage after performing JET in the case of our experiment causes indirect symptoms of muscle damage. This is evident from the increased muscle soreness and increased CK activity (Fig. 7). Besides, the manifestation of LFF (Fig. 5) points to the origin of non-metabolic fatigue. Exercise-induced muscle damage in our case is supported by the findings of other authors showing that well documented symptoms of muscle damage include prolonged impairment of muscle function measured during
both voluntary and electrically stimulated contractions, protein leakage from injured muscle fibres, acute inflammation reaction and delayed-onset muscle soreness [2,6].

Notwithstanding the obvious fact that 100 jumps performed with maximum intensity bring about muscle damage, not only the damage mechanism itself but also its effect on the time-course of muscle function and neuromuscular performance remains to be cleared. The clarity of this phenomenon is complicated by the fact that decrease in muscle and neuromuscular function during and after JET is a complex process that depends on a great number of interrelated factors that often are very difficult to be considered separately. There is no doubt that not only muscles but also mechanisms of central motor control participate in this process. It has been shown that the fatigue of the neuromuscular system induced by JET may not be attributed to muscle damage alone since it could also be caused by differences in the modulation of reflex and stiffness interaction as well as compensation by central motor command [10].

There is a delay in muscle force development after eccentric exercise, and this delay is suggested to be due to secondary degradation processes affecting excitation-contraction coupling [13]. The progressive nature of structural damage after eccentric exercise is poorly understood. The mechanism is thought to initially involve mechanical insult followed by an accumulation of intracellular calcium, which triggers calcium-mediated processes [5,27]. During recovery in this case there is often a secondary reduction in the force response to stimulation and force may be abolished for 20-30 min. This is in agreement with our findings since there was significant decrease ($P<0.05$, Fig. 4) in P20 and P100 from 3 min after JET till 60 min after exercise.

LFF is characterized by a relative loss of force at low frequencies of stimulation and it is important to mention that the force is not impaired or there is but relatively low impairment at high frequencies [15,19,21]. In our case there was a decrease in the force evoked not only by low stimulation frequencies (20 Hz) but by high stimulation frequencies (100 Hz) as well (Fig. 4). It is but rarely that one can find physical load capable of bringing about a decrease in the force evoked by low stimulation frequencies since in nearly all of the cases there is a greater or smaller decrease in the force evoked by high stimulation frequencies as well [15,19,21]. Although the underlying mechanism is unknown, both metabolite build-up and elevation of intracellular $Ca^{2+}$ concentration, as well as mechanical damage to the muscle, have been suggested to play a role in the development of LFF [5,27].

It has been shown that recovery of force and $[Ca^{2+}]$ after fatigue follows a complex time course. It has been shown recently that the decrease in $Ca^{2+}$ release from sarcoplasmic reticulum 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 $[Ca^{2+}]$, time integral, which recovers more slowly [5]. We think that in our case at 3 min after the exercise this metabolic component has an influence on muscle fatigue. The elevated $[Ca^{2+}]$-time integral induces prolonged reduction in $Ca^{2+}$ release in the absence of any metabolic alterations associated with fatigue, since it is believed to activate some process that results in the disruption of proteins involved in excitation-contraction coupling [12]. As it has been shown by Smith et al. [23], after concentric exercise all subjects show a progressive decrease in twitch response, with a minimum after 10-30 min and a subsequent complete recovery within 1-2 h. They have suggested that the development of LFF occurs during the period of metabolic recovery and at a rate apparently dependent on the metabolic work done. In our case changes in the muscle force after exercise also indicate the presence of a secondary increase in LFF during recovery period.

Warren et al. [25] suggested that during and after eccentric exercise most of the early strength loss results from a failure of excitation-contraction coupling and a slow loss of contractile proteins during the days after injury prolongs the recovery time. It remains for the scientists to clear up if there was a greater decrease in muscle force because of the failure of excitation-contraction coupling or due to muscle sarcomeres damage. It might be speculated that in our
case the LFF manifestation is indicative of the excitation-contraction coupling failure since it has been established that one of the mechanisms of LFF depends on the decreased release of Ca$^{2+}$ from the sarcoplasmic reticulum [28].

**What is the effect of four JETs on muscle fatigue and EIMD?**

The results of our research have revealed that four JETs are not enough to improve jump height and jumping endurance (Fig. 2) but they are enough to increase muscle resistance to fatigue, particularly, fatigue associated with EIMD. The training applied during our experiment was not of specific character aimed at developing height of jump but it was rather directed towards developing jumping endurance. It has been established that there occurs a significant improvement in speed endurance, but after 10 – 20 trainings [24]. It has been observed, however, that even two exercise bouts can increase the rate of developing power [1]. Besides, as found by Green [9], even a single intensive training can bring about changes in Na$^+$, K$^+$ - ATPase what, in its turn, can improve the efficiency of muscle activation.

We speculate that a decrease in muscle mechanical damage due to four training aimed at developing jumping endurance can be accounted for by the adaptation of muscular and /or neural system. Due to the adaptation of the neural system there may have occurred changes in the motor control. Thus, for example, in conditions of repeated bouts ever greater number of muscle fibres of the slow-twitch type may have been recruited and these fibres are more resistant to fatigue than muscle fibres of the fast twitch type [19]. Besides, in repeated bouts muscle tension may have been distributed among a greater number of muscle fibres thus avoiding the enormous mechanical stress on the muscle fibres likely to experience the greatest damage. This hypothesis of the RBE has been substantiated by several scholars [3,26]. There is no doubt that the results of our research can be interpreted on the basis of the neural hypothesis of the origin of the RBE. In other words, it can be asserted that during JET IV the muscles experienced a mechanical damage of a more moderate character since they performed their work more economically during physical bout applied.

If the neural hypothesis as to the origin of RBE were not true to fact, i.e. after JET IV the muscles experienced the same stress (tension), as after the JET I, then more moderate muscle damage might be explained by muscle adaptation. It has been established that even after a single physical bout undergone muscles became more resistant to damage when repeated bout was applied in several days or even in several weeks [16]. Several explanations, as to why the muscle becomes more resistant to mechanical damage, are available. One of the most wide–spread hypotheses maintains that the manifestation of the RBE is due to the fact that there occurs an increase in the number of sarcomeres in the muscle fibre and strengthening of the weak sarcomeres, most sensitive to mechanical damage, takes place [14,17]. It has been found that 5 trainings consisting of eccentric exercise were enough for the number of sarcomeres to be increased [14]. The results of our research do not allow us to say for sure which of the two hypotheses, the neural or muscular one, is chiefly confirmed by our experiment. More research in this field is necessary.

**CONCLUSIONS**

Four jumping endurance trainings caused no increase in jumping performance but increased resistance of skeletal muscle to low frequency fatigue and exercise–induced muscle damage. It is evident that brief training effect manifest itself rather in electrically induced muscle performance than in voluntary muscle performance.

**REFERENCES**

Effect of four jumping endurance trainings on metabolic fatigue and on indirect symptoms of skeletal muscle damage


