DE GRUYTER OPEN
Pol. J. Sport Tourism **2018**, *25, 3-9*
DOI: 10.2478/pjst-2018-0001

Original research papers

LOW-LEVEL LASER THERAPY AND THE RECOVERY OF MUSCLE FUNCTION AFTER A SINGLE SESSION OF NEUROMUSCULAR ELECTRICAL STIMULATION: A CROSSOVER TRIAL

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Abstract
Introduction. Neuromuscular electrical stimulation is applied in muscle atrophy and in muscle strength and endurance training in athletes. Muscle soreness and temporary reduction in muscle strength may occur as adverse effects. Laser therapy has been used as a method of counteracting delayed onset muscle soreness following volitional exercise, but not following electrical stimulation. The aim of the study was to determine whether low-level laser therapy applied prior to electrical stimulation accelerates the recovery of muscle strength and decreases the duration and intensity of muscle soreness at rest after intensive isometric neuromuscular electrical stimulation of the quadriceps femoris muscle. ***Material and methods.*** A randomised crossover trial was carried out on 24 healthy, recreationally active men aged 22-24 years. Low-level laser therapy or sham laser therapy was applied prior to a single session of neuromuscular electrical stimulation of the quadriceps femoris muscle with typical technical and training-related parameters. Irradiations were performed immediately prior to and shortly after electrical stimulation as well as 24, 48, 72, and 96 hours after this procedure. Muscle soreness was examined using the VAS scale in the same time periods. Quadriceps moments of force were recorded with the use of a Biodex 4 Pro device during maximum voluntary static contraction and during electrical stimulation that triggered a tetanic contraction of the quadriceps femoris muscle reaching the level of maximum tolerance. ***Results.*** No significant differences were noted in the severity of quadriceps soreness and in the magnitude of the decrease in the moments of force of maximum voluntary contractions after stimulation preceded by laser therapy and that preceded by sham irradiations. ***Conclusions.*** In the group studied, laser therapy applied before single electrical stimulation with typical parameters did not bring about a faster recovery of muscle strength or a more rapid decrease in soreness than sham laser therapy used prior to electrical stimulation. Further research on larger groups of subjects with the application of various procedures as well as research on training programmes is needed.

Key words: low-level laser therapy, neuromuscular electrical stimulation, delayed onset muscle soreness, exercise-induced muscle damage

Introduction

Physical exercise is defined as the activity of the skeletal muscles with accompanying functional changes and, in the case of repeatability, with morphological changes in the whole body [1]. Skeletal muscle activity naturally leads to muscle fatigue, whose scope depends on such factors as exercise intensity, duration, and frequency as well as environmental conditions [2]. Skeletal muscle fatigue results in biochemical, biomechanical, and subjective changes. They may be determined by testing changes in the levels of enzymes in blood plasma and the typical activity of creatine kinase (CK) and lactate dehydrogenase (LDH), as well as by defining morphological changes with medical imaging methods, conducting dynamometric tests, and determining the intensity of muscle soreness [3-5]. Muscle fatigue is manifested through a decrease in muscle strength at different times after exercise as compared to the pre-exercise value [6, 7]. Moreover, qualitative tests such as muscle soreness tests are carried out before and after exercise. Soreness, especially delayed, is one of the main symptoms of myocyte damage [8].

The long-lasting consequences of muscle microdamage in athletes, such as muscle pain and weakening, may lead to earlier exhaustion during training, a lower ability to tolerate training loads and, as a consequence, diminished sports performance [9]. This is the reason why researchers seek effective methods of limiting these effects [2-4, 6, 8].

The application of various physical modalities in different periods of sports training as a way of preventing or treating the effects of delayed onset muscle soreness has been perceived as significant for years [3, 8, 10]. There are works describing the advantages of using sauna and baths [11, 12], infrared therapy, and massage in accelerating recovery through improving peripheral circulation, circulatory and respiratory parameters, as well as metabolite removal, leading to alleviated muscle soreness and reduced fatigue [8, 11-12].

Therefore, recent years have seen an increased interest in the application of low-level laser therapy (LLLT) and light-emitting diode therapy (LEDT). There are numerous studies describing the effectiveness of LLLT and LEDT in wound healing [13], inflammation reduction [14], or bone healing [15]. It has been reported that LLLT may prove beneficial in preventing and reducing the intensity of delayed onset muscle soreness and may accelerate post-exercise recovery when applied shortly before training [16]. It is also reported that when applied after training, it may produce beneficial effects such as increased muscle power and reduced muscle soreness [17]. However, the most recent systematic review concerning the application of LLLT with the aim of reducing muscle soreness and damage and improving its post-exercise function pointed to some evidence that implied the doubtful effectiveness of applying LLLT prior to volitional exercise. Still, this evidence came either from methodologically flawed studies or from small sample trials [18].

In terms of technical and training-related parameters, various forms of neuromuscular electrical stimulation (NMES) are used to prevent muscle atrophy and strength loss, particularly in the case of limb immobilisation. It is also applied as a method of muscle strength and endurance training in athletes [19] or even perceived as a method which, in certain cases, is more effective than volitional training [20].

However, NMES may lead to fatigue-related muscle dysfunctions, which tend to be more intensive and to last longer than in the case of volitional exercise [21]. Apart from the promising reports on animals [22], there is still a scarcity of data on the application of LLLT in accelerating muscle strength recovery and decreasing muscle soreness after NMES. Therefore, the aim of this study was to examine whether LLLT applied prior to a single NMES session prevents or diminishes muscle soreness and if it reduces muscle fatigue and accelerates the recovery of muscle strength after NMES.

Material and methods

A randomised, double blind, crossover trial was performed. Blinding involved both study participants and investigators, who calculated moments of force (T.S.), assessed pain intensity (E.J.), and analysed the data (I.C.). The subjects were not informed which intervention (LLLT or sham LLLT) they were exposed to. The investigators did not know if the NMES-related measurements, assessments, and calculations they were performing had been preceded by LLLT or sham LLLT.

The study included 24 healthy males (physiotherapy students from a university of physical education) aged 20-23 (mean = 20.9, SD = 0.82) years who were physically active but did not do sports professionally and did not perform strenuous exercise. The exclusion criteria were as follows: lower limb injuries experienced within a year before the study, an inflammatory state, anti-inflammatory drug use, and contraindications to NMES and LLLT. The candidates were informed about the planned procedure, were instructed not to do intensive physical exercise during the study, and were told to inform the researchers should any of the exclusion factors occur (e.g., a cold/illness or medicine use).

All the study participants underwent two interventions, that is NMES preceded by LLLT and NMES preceded by sham LLLT. In order to have an interval between these two interventions, we randomly assigned the participants to one of the following two groups: (1) LLLT and NMES (n = 12) and (2) sham LLLT and NMES (n = 12). In the first stage of the study, LLLT was applied in group 1, while sham LLLT was used in group 2. In the second stage, after an 8-day interval, a reverse procedure was applied. All the participants (n = 24) underwent NMES preceded or not preceded by LLLT. The 8-day wash-out period, which was assumed as sufficient based on the literature [23], helped the muscles to recover their function fully after the first NMES and made it possible to avoid the overlapping of intervention effects. Prior to LLLT or sham LLLT, pain intensity was determined, and the moments of force of the quadriceps examined during maximum voluntary contraction (MVC) in isometric conditions were calculated.

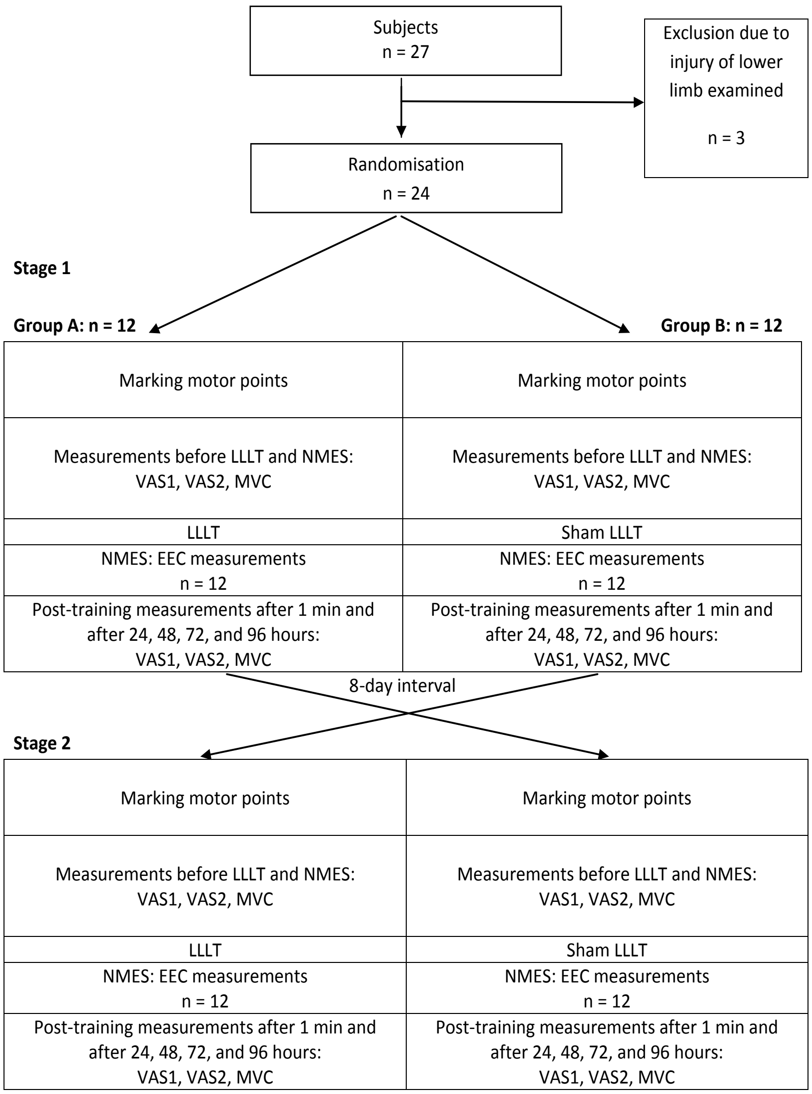
During NMES, the moments of force of electrically-evoked contractions (EEC) were measured. Immediately after NMES as well as 24, 48, 72, and 96 hours after NMES, further MVC measurements were made, and pain severity in the quadriceps treated with NMES was assessed. The procedure is illustrated in Figure 1.

Position and research device

The moments of force of the isometric muscle contractions were investigated using a Biodex System 4 Pro measuring device (Biodex Medical System, USA). The measuring station consisted of a chair, a dynamometer, and a computer with the necessary software. There are different ways of investigating moments of force in isometric conditions. In the current study, the procedures were performed according to a protocol similar to the one applied in the most comparable and reliable research, that is the study conducted by Jubeau et al. [24]; however, the specificity and requirements of the device applied were taken into account. The study participants were examined in a sitting position. The pelvis, thighs, and trunk were stabilised with the use of transverse straps, which were stretched across the abdomen and thighs, and two oblique straps were crossed on the chest. The participants used stabilisation handles as extra support. The knees were bent at an angle of 100° measured from 0° extension. The dynamometer shaft axis was aligned with the joint rotational axis. The non-dominant lower limb was examined.

NMES. Electrically-evoked isometric contractions of the quadriceps femoris muscle were induced through electrical stimulation with the use of the Sonicator Plus 940 (Mettler Electronics Corporation, USA). It generated pulsed, two-phase, square-waved, symmetrical currents with a frequency of 80 Hz and a phase duration of 300 μs. An impulse wave was applied with the 5-s "on" time and 15-s "off" time, a 1.5-s increase in intensity, and a 0.5-s decrease in intensity. Two electric circuits were used. Round, self-adhesive electrodes with a 5-cm diameter were placed on the skin, which had been rinsed with alcohol and had dried. They were placed over the motor points of the quadriceps femoris muscle, according to the typical rules of locating electrodes, in a way similar to that in the study by Aldayel et al. [25], and with the use of a typical electrical stimulation technique [26]. The location of the electrodes is illustrated in Figure 2.

Motor points, defined as points on the skin overlying the muscles where the lowest possible electrical stimulus causes muscle contraction, were found by inducing single muscle con-



EEC = electrically-evoked contractions; LLLT = low-level laser therapy; NMES = neuromuscular electrical stimulation; MVC = maximum voluntary contraction; VAS1 = pain severity in pressure test; VAS2 = pain severity in squat test.

Figure 1. Research protocol

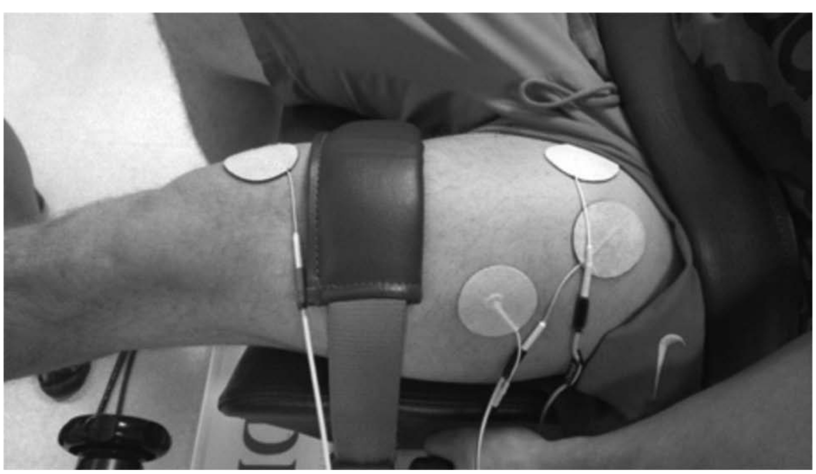


Figure 2. The location of the electrodes

tractions with a point electrode with a 5-cm diameter using interrupted current (frequency = 1 Hz, duration of impulse = 300 µs) generated by a TRIO STIM device (Mettler Electronics Corporation, USA). Detailed data on the technical parameters of NMES are presented in Table 1. After the contractions occurred, electrical stimulation was stopped in order to avoid muscle fatigue, and a motor point was marked with a sterile marker pen.

LLLT. Laser irradiations were performed with a BTL 5000 device (BTL Industries Limited, London), with a cluster probe consisting of four semiconducting lasers emitting laser radiation with a wave length of 830 nm and a power of 200 mW each (Table 1). The therapy was applied in six areas (25 cm^2 each), that is 5 cm above the middle of the line connecting the anterior superior iliac spine and the base of the patella and 5 cm below that spot, in two skin areas above the vastus medialis belly, as well as in two areas above the vastus lateralis belly. A dose of 30 J was applied in each area. The therapy was administered immediately before NMES. The procedure of sham therapy was the same; however, the applicator remained switched off, which the study participants were not informed about.

Muscle soreness examinations. In order to obtain a subjective assessment of the intensity of muscle soreness, we applied a standard visual analogue scale (VAS). The participants were asked to mark the severity of the pain they experienced on a 100-mm scale, where 0 meant "no pain" and 100 meant "worst imaginable pain". The investigator checked pain severity by pressing the fingers for 3 seconds in four areas, located (1) 5 cm proximally and (2) 5 cm distally to the central area between the base of the patella and the anterior superior iliac spine as well as in the motor points of (3) the vastus medialis and (4) the vastus lateralis, which were determined for the purposes of NMES. The pressure was applied by the same researcher (M.C.), with the same repeatability of force and time as well as constancy of pressure, to the greatest possible extent. Afterwards, standing with their feet spread shoulder-width apart, the subjects performed a squat slowly to 90° knee flexion and came back to the starting position. Pain severity was assessed twice, that is after applying pressure (VAS1) and after performing the squat (VAS2). The assessment was made using separate forms.

MVC. The investigations were performed with the use of the same device that registered the moments of force, with the same settings, and with the same body positions adopted by the subjects as during NMES. To determine MVC, we asked the study participants to extend the lower limb at the knee joint, with the maximum strength possible, for 3 seconds and with a 30-s interval. They performed the exercise three times, and the results of the best trials were included in the analysis.

EEC. During NMES, the moments of force of each electrically-evoked muscle contraction (EEC) were measured. The measurement, which commenced with the sixth contraction, was performed in an uninterrupted manner. The first five contractions were excluded from the analysis. This procedure was similar to that adopted by Snyder-Mackler [27], who noted that the force generated by the first five contractions was weak (less than 10% MVC). The highest EEC value observed in the first phase of the present study was 31.53% MVC, while in the second phase, it was 36.77% MVC.

The project was approved by the Research Ethics Committee of the University of Physical Education in Warsaw (SKE 01 – 14/2014).

Statistical analysis

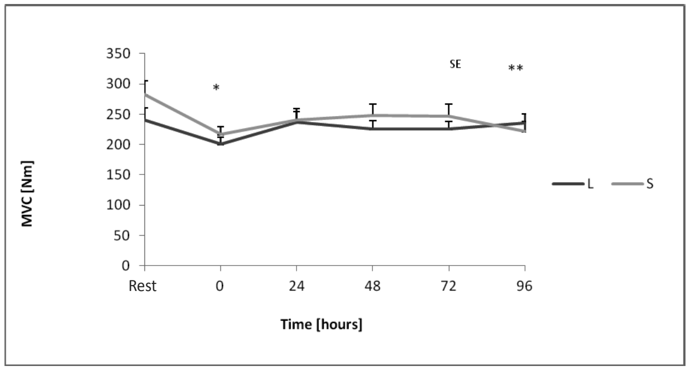
A corrected pairwise t-test for multiple hypotheses was used to analyse muscle strength changes, while the Kruskal-Wallis test was employed to measure pain intensity. EEC values were presented in the form of %MVC. The findings regarding pain intensity were shown on a T scale. The sham LLLT was normalised to the actual intervention (LLLT). The Anderson-Darling normality test was used to test the distribution of data. The level of significance was set at $p < 0.01$. The analyses were conducted with Statistica 12.0 software.

Table 1. Neuromuscular electrical stimulation and low-level laser therapy parameters

Neuromuscular Electrical Stimulation, NMES		Low-Level Laser Therapy, LLLT	
Technical parameters:			
Pulse frequency	80 Hz	Number of laser diodes	4
Pulse duration	300 µs	Wavelength	830 nm (infrared)
Waveform	Symmetrical biphasic	Frequency	Continuous output
Stimulus intensity:		Optical output	200 mW
Stimulus on time	5 s	Irradiation characteristics:	
Stimulus off time	15 s	Energy	30 J on each point
Contraction intensity	Maximal tolerance	Total energy delivered per muscle	180 J
Number of repetitions	45	Application mode	Cluster probe held stationary in skin contact at 90° angle and slight pressure

Results

Significant changes in the moments of force of the knee extensors were noted immediately after NMES ($p < 0.05$) and 96 hours after the intervention ($p < 0.01$). The results were compared with MVC values obtained before the intervention. Compared to the sham therapy, a smaller decrease in moments of force was observed after LLLT (Table 2); however, it was not significant. In both cases, a full recovery of muscle strength did not occur after 96 hours (Figure 3).



L = LLLT irradiation, S = sham LLLT irradiation; MVC = torque of maximum voluntary contraction; SE = standard error; * and ** = statistically significant changes of MVC after NMES in successive measurements at $p < 0.05$ and $p < 0.01$, respectively.

Figure 3. Curves representing changes in muscle torque before, immediately after (0), and 24, 48, 72, and 96 hours after neuromuscular electrical stimulation

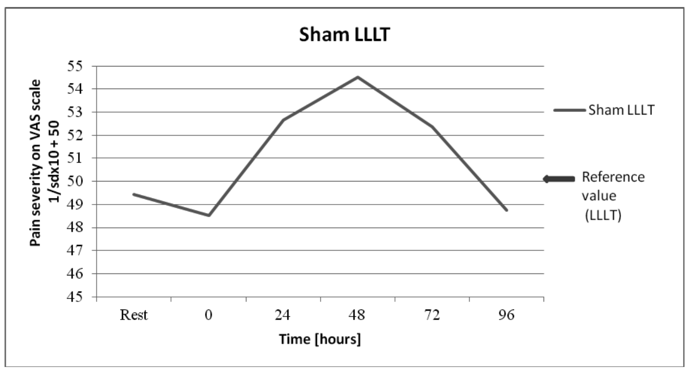


Figure 4. Changes in pain severity after sham LLLT in regard to LLLT in the pressure test (VASI) (T scale)

Table 2. Percentage changes in muscle torque (MVC) before, immediately after (0), and 24, 48, 72, and 96 hours after neuromuscular electrical stimulation in regard to MVC measured before LLLT and sham LLLT

Intervention	MVC rest	MVC0	% change	MVC24	% change	MVC48	% change	MVC72	% change	MVC96	% change
LLLT	240.29	200.975	16.36*	236.333	1.65	225.583	6.12	225.548	6.14	236.03	1.77
Sham LLLT	282.95	216.717	23.41*	240.658	14.95	247.883	12.39	246.55	12.86	221.728	21.64**

MVC = torque of maximum voluntary contraction; MVC rest, MVC0, MVC24, MVC48, MVC72, and MVC96 = MVC measurements after, immediately after, and 24, 48, 72, and 96 hours after NMES, respectively; % change = percentage of MVC change in respect to resting value; * = $p < 0.05$; ** = $p < 0.01$.

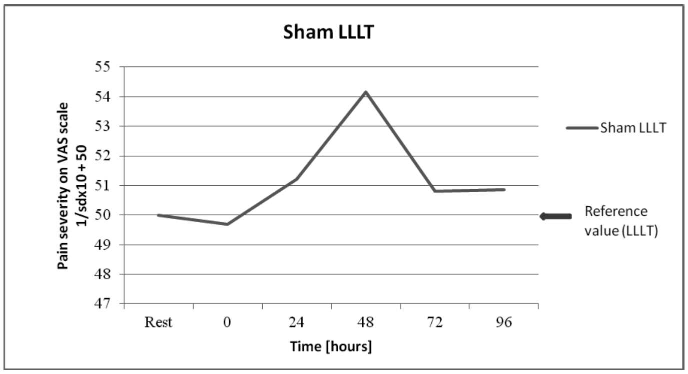


Figure 5. Changes in pain severity after sham LLLT in regard to LLLT in the squat test (VAS2) (T scale)

Moreover, no significant differences in pain severity were found between the groups at further stages during the pressure test ($p > 0.05$) or the squat test ($p > 0.05$) (Figures 4 and 5).

Discussion

LLLT is applied to reduce pain [28, 29] and inflammatory conditions [14, 30] and to accelerate bone growth [15, 31] as well as wound and ulceration healing [13, 32]. Research on LLLT as a method of preventing or alleviating delayed onset muscle soreness has started to be carried out only recently [16-18]. Alves et al. [33] imply that LLLT effectively enhances muscle recovery shortly after damage. Baroni et al. [34] confirmed that LLLT applied before eccentric exercise increases MVC immediately after exercise and for the next two days. De Souza [35] showed that LLLT used prior to exercising ankle plantar flexors (performance of 100 muscle contractions at a speed of 90°/s) considerably reduced muscle fatigue.

The findings of Douris et al. [36] indicate that LLLT reduces muscle soreness after eccentric training of the elbow flexors (study participants had to hold maximum weight in the range of 45° to 135° of elbow flexion until they were no longer capable of controlling it).

Other researchers have not confirmed the findings regarding LLLT benefits that stem from reducing the effects of muscle fatigue after volitional exercise. Having conducted an experiment on healthy females subjected to eccentric exercise (elbow flexion), Macedo et al. [37] did not note an LLLT-related acceleration of post-exercise muscle recovery. However, the authors suggested that similar research should be replicated using a larger sample size and with a longer period of intervention. Kobordo's study on 27 volunteers who were asked to perform three 3-second isometric contractions of the elbow flexors (at an angle of 90°) revealed that LLLT delivered pre- and post-exercise was ineffective for muscle soreness reduction, nor did it increase muscle strength or function [38].

The present study sought to find out whether or not a single application of LLLT affected muscle fatigue brought about by a single NMES intervention. Changes in quadriceps femoris strength and soreness were observed. Previous studies on animals (no research has been carried out on humans) indicate that LLLT may reduce the effects of muscle fatigue after NMES [39]. The findings of the study did not confirm that LLLT applied in healthy and active male individuals prior to a single NMES intervention involving inducing 45 quadriceps femoris muscle contractions reaching pain tolerance levels significantly changed the course of objective and subjective post-exercise reactions. There was also no confirmation that LLLT administered before a single NMES session reduced the effects of electrically-evoked quadriceps femoris fatigue. It is worth bearing in mind that a randomised crossover trial was performed, which made it possible to avoid a number of interfering factors. The study group was homogeneous, and all the subjects underwent both interventions. What is more, there was an interval between the interventions so as to avoid the overlapping of intervention effects. Therefore, the results seem to be reliable; whether there is a causal relationship between the ineffectiveness of the irradiations and the parameters of LLLT or those of electrical stimulation remains a matter of debate and is worth verifying in future studies. Inadequate parameters, such as an improper dose or wavelength of the laser radiation, a too small exposure area, and other factors that are hard to define may be some of the main causes of the ineffectiveness of LLLT. There is no set agreement and there is wide variability among researchers and practitioners as to LLLT parameters in general practice, and the use of that modality in studies such as this one has the same limitation. It cannot be excluded that the parameters of LLLT applied were not the most efficient ones. Therefore, future studies should seek to identify optimal laser therapy doses. Moreover, other and larger muscle areas need to be treated with laser therapy [16]. On the other hand, a single session of NMES might have been insufficient to evoke a degree of muscle fatigue and damage that would make it possible to prove the effectiveness of LLLT irradiations compared to passive recovery. The stimulation was conducted with the use of parameters and techniques appropriate for obtaining heavy workloads. However, the limitation of this technique – relying on local phenomena, with no central command processes from the central nervous system and with spatial limitations related to stimulating superficial muscle fibres – might have led to insufficient muscle fatigue. The authors of the present study are currently implementing a project in which laser therapies are applied for 3 weeks prior to each of the six NMES training sessions. The findings of this study will be presented in future papers.

Conclusions

In the study presented in the current article, low-level laser therapy irradiations administered prior to a single session of neuromuscular electrical stimulation of the quadriceps muscle did not prove to be effective in reducing signs and symptoms of delayed onset muscle soreness. Infrared laser irradiations of the skin above quadriceps femoris muscle bellies in young, recreationally active men seem to neither alleviate muscle pain nor accelerate muscle strength recovery. Nonetheless, it seems necessary to continue research on the application of low-level therapy in reducing the symptoms of exercise-induced skeletal muscle fatigue. Further research should also focus on other methods of irradiation as well as LLLT doses, particularly in NMES training programmes.

Acknowledgements

This study was carried out under the research project DS. 204 implemented by the Józef Piłsudski University of Physical Education in Warsaw, Faculty of Physical Education and Sport in Biała Podlaska, which was financed by the Polish Ministry of Science and Higher Education. All the tests were conducted in the Regional Center for Research Development in Biała Podlaska co-financed under the European Regional Development

Fund, Operational Programme Development of Eastern Poland 2007-2013: Laboratory of Molecular Diagnostics, Laboratory of Modern Technologies, Rehabilitation of the Musculoskeletal System, Laboratory of Biomechanics and Kinesiology, Laboratory of Body Posture Diagnostics.

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Submitted: July 24, 2017
Accepted: September 29, 2017