The Effect of Low Level Laser Irradiation (Ga-Al-As - 655nm) On Skeletal Muscle Fatigue induced by Electrical Stimulation in Rats

Rodrigo Álvaro B. Lopes-Martins¹, Rodrigo Labat Marcos¹, Patrícia Sardinha Leonardo¹, Antônio Carlos Prianti Jr.¹, Marcelo Nicolas Muscará², Flavio Aimbire³, Lúcio Frigo⁴, Vegard I. Iversen⁵, Jan Magnus Bjordal*⁶,⁷

¹ - Laboratory of Pharmacology and Phototherapy of Inflammation, Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo. Av. Prof. Lineu Prestes, 1524, Butantan, São Paulo – SP, Brazil; 05508-900

² – Laboratory of Biochemical Pharmacology of Free Radicals, Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo. Av. Prof. Lineu Prestes, 1524, Butantan, São Paulo – SP, Brazil; 05508-900

³ - Laboratory of Animal Experimentation, IP&D UNIVAP R. Shishima Hifumi, 2911, 12240-000. São José dos Campos, SP, Brazil

⁴ – Universidade Cruzeiro do Sul, Centro de Ciências Biológicas e Saúde. Av. Dr. Ussiel Cirilo, 225 São Miguel Paulista, 08060-070 - São Paulo, SP - Brasil

⁵ - Department of Physiology, Jonas Lies vei 91, N-5009 Bergen, Norway.

⁶ - Section of Physiotherapy Science, Institute of Public Health and Primary Health Care, University of Bergen, Kalfarveien 31, 5018 Bergen, Norway

⁷ – Institute of Physical Therapy, Bergen University College, Møllendalsv.6, 5009 Bergen, Norway

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* Author for Correspondence
Jan M. Bjordal
Inst. Physical Therapy
Bergen University College
Møllendalsv. 6
5009 Bergen
Norway
Fone: +47 55585663
FAX: +47 55298364
Email: jmb@hib.no
Abstract

We investigated if low level laser irradiation (LLLI) can reduce muscular fatigue during tetanic contractions in rats. 32 male Wistar rats were divided in 4 groups receiving LLLT doses of 0 (control group), 0.5, 1.0 and 2.5 J/cm². Irradiation lasted 32, 80 and 160 seconds respectively with a fixed power density of 31.25 mW/cm². The total energy doses were 0.08, 0.2 and 0.4 Joules respectively. Electrical stimulation induced 6 tetanic muscle contractions in the tibial anterior muscle. Contractions were stopped when the muscle force fell to 50% of the initial value for each contraction (T50%). There was no significant difference between the 2.5 J/cm² laser-irradiated group and the control group in mean T50%-values. Laser-irradiated groups 0.5 J/cm² and 1.0 J/cm² had significantly longer T50% values than the control group. The relative peak force for the 6th contraction in the laser irradiated groups were significantly higher at 92.2 % (SD +/- 12.6%) for 0.5 J/cm², 83.2 % (SD +/- 20.5%) for 1.0 J/cm² and 82.9 % (SD +/- 18.3%) for 2.5 J/cm² respectively, than for the control group, 50% (SD +/- 15%). Laser groups receiving 0.5 J/cm² and 1.0 J/cm², showed significant increases in mean performed work compared both to the control group and their 1st contraction values. Groups receiving laser irradiation with doses of 1 and 2.5 J/cm² also showed significantly lower levels of Creatine Kinase (CK) in plasma than the non-irradiated control group. We conclude that these doses of LLLT inhibit development of muscular fatigue in rats.

INTRODUCTION
Fatigue is a common experience in daily life, although the cellular and physiological mechanisms involved are not fully understood. The main features of muscular fatigue are decreased muscle strength and impaired motor control, and subsequently muscular pain. Several studies have investigated how fatigue develops during different types of exercise in order to elucidate the process and mechanisms involved. Decreased muscle strength has been reported after fatigue in different parts of the human body (7, 24, 40). Physical fitness is a major factor in preventing fatigue, but within common physiological limits anyone may experience a state of neuromuscular fatigue if the time period is long enough (16). Whether age-dependent changes in physical performance result from alterations in muscle fibers, neuromuscular junction, peripheral nerve and/or central motor neurons have been difficult to ascertain in vivo (19). In patients with chronic neck pain of muscular origin, both early onset of increased electromyographic activity and a lack of post exercise vasodilatation have been observed compared to healthy controls (28). Exercise-induced muscle injury in humans may occur after strenuous exercises, particularly if the exercises involve a large amount of eccentric contractions (9).

There are several definitions for muscular fatigue and one of them is the incapacity to maintain force by muscular contraction for a period of time. From an athlete’s perspective, Vollestad (52) definition of fatigue consist as any exercise-induced reduction in the maximal capacity to generate muscle force might be appropriate. Verburg et al. (51) also states that fatigue develops during maximal or high-intensity exercise and during prolonged sub-maximal exercises, as evidenced by a decline in the force-generating capacity during maximal (test) contractions. It has been shown that a considerable component of fatigue is due
to processes on the surroundings of the muscular junction (4, 31, 42) i.e., inhibition of neural excitation-muscular contraction coupling (1, 18, 44). Most hypotheses for the mechanism of this type of fatigue are based on changes in muscle function and local biochemical changes which occur during high-intensity exercise or tetanic stimulation (51). It is also well established that altering oxygen \((O_2)\) delivery to the contracting skeletal muscle affects human performance. In this respect, reducing \(O_2\) supply (hypoxia) will increase the rate of muscle fatigue, whereas increasing \(O_2\) supply (hyperoxia) will reduce the rate of fatigue (23).

Numerous studies have used experimental models involving maximal voluntary contractions, tetanic stimulation and ischemic conditions (37, 53, 5) to study skeletal muscle fatigue. In experimental models, it has been demonstrated that a reduced force generation is following intense, sustained voluntary contractions and tetanic electrical stimulation of animal muscles in vitro (4, 13, 15). Delayed onset muscle soreness (DOMS) is often observed after eccentric exercises. DOMS is manifested clinically through transient muscle pain and impaired maximal muscle strength. Physiological manifestation may, for example, be increased levels of creatine kinase (CK), which is considered to be an indirect sign of widespread microtraumas inside the muscle. It has been shown that the anti-inflammatory drug diclofenac reduces minor muscle damage and CK-levels in DOMS. Several tools has been used in order to prevent muscle fatigue in healthy athletic subjects as well as in pathological conditions such as nutritional supplements (14, 47, 48) fluid reposition (10, 27), Creatine (33) and Arginine (32, 38).

Low level laser irradiation (LLLI) is a therapy form which has been used to treat muscle damage and muscular pain, but the biological mechanisms behind
observed beneficial results in clinical trials, remain unclear (8) LLLI may be
administered with different wavelengths of the visible and near-infrared spectra
including HeNe (632.8nm), Ga-Al-As (805 or 650 nm) and Ga-As (904 nm) (34).
LLLI has been shown to reduce the duration of acute inflammation (36), and
accelerate tissue repair in tendon and muscle injuries (3). Positive effects from
LLLI may be related to modulation of the oxidative chain, and a recent review
concluded that LLLI increased cellular redox activity (29). Stimulatory effects from
LLLI have been observed for mitochondrial activity in cell cultures (53), and in
hypoxic animal tissue (54) of diabetic mice. Marked increases in arteriolar and
collateral blood flow have also been observed in injured tissue after LLLI (25,30).
In clinical settings, painful fibromyalgia is associated with impaired local circulation
and can be attenuated by an increase in muscle blood flow (26), and this disorder
also seems to benefit from LLLI (21).
In this perspective it is a clear need to investigate if LLLI can prevent hypoxia and
muscular fatigue. We decided to use the experimental model of electrical nerve
stimulation to induce fatigue by tetanic tibial muscle contraction in rats, and test if
LLLI could prevent the anticipated reduction of muscle force or the anticipated
increase in CK levels. This model with electrically stimulated tetanic contraction
has limitations, but we considered it suitable for initial experimental studies with
laser irradiation in muscular fatigue.
METHODOLOGY

All experiments were carried out in accordance with the guidelines of Vale do Paraíba University for human and animal care, Protocol Number 045/2003/CEP.

Material

The experiments were carried out on Male Wistar rats weighing between 150 and 200 g, with food and water “ad libitum” provided by Central Animal House of the Research and Development Department of Vale do Paraíba University (UNIVAP). All rats were placed in a box and 32 animals were randomly divided into four groups of eight animals as follows:

A) Control group – non-irradiated

B) Treatment group receiving LLLI administered with 1.0 J/cm²

C) Treatment group receiving LLLI administered with 2.5 J/cm².

D) Treatment group receiving LLLI administered with 5.0 J/cm².

Experimental procedure

Animal preparation

Rats were anaesthetized intraperitoneally with Sodium Pentobarbitone (40 mg.kg⁻¹) and the former tibial muscle and tibial nerve were both isolated and immediately fixed to a surgical table. In the insertion region next to the plantar region from metatarsus, the muscle and tendon was connected to an isometrical transducer...
(Ugo Basile; Vareze, Italy) and the nerve was connected to a bipolar electrode. The muscle was submitted to a constant tension of 0.1 Newton, which was achieved by titrating electrical stimulation intensity with a pulse frequency 0.2 Hz. (Figure 1)

<< Figure1>>

**Electrical stimulation for induction of muscular fatigue**

The electrical stimulation was administered with an intensity 6-7 mA, and biphasic pulses of 2 ms duration (53). To induce a tetanic contraction, the frequency was raised from 0.2 up to 60 Hz and kept there until the muscular force fell to 50% of its value at the onset of that particular contraction (Figure 2).

<< Figure2>>

This procedure was repeated 6 times for every 10 minutes during the following 60 minutes. The muscle force was measured by a dynamometer in Newtons (UGO BASILE GEMINI 7070 Physiograph). In order to avoid tissue death, electrical stimulation was kept constant only until the tension from muscular contraction declined to 50% under the maximum recording. The muscle was then rested until the next contraction commenced 10 minutes after the start of the previously induced contraction.

In order to prevent muscle desiccation a continuous dropping of warmed saline solution was used over the tibial muscle.
**Laser Irradiation**

A diode laser of the InGaAlP type (model: Dermolaser AD2040) with a continuous output power of 2.5 mW and wavelength of 655 nm (visible red) was used. The optical power was calibrated using a Newport Multifunction Optical Meter model 1835C. The stability of laser during the laser irradiation was measured collecting light with a partial reflect (4%). The spot size was 0.08 cm², and the laser illumination spot was placed in contact with the central part of dissected tibial muscle belly. Irradiated animals received irradiation in this single point at the beginning of the muscle contraction. Irradiation lasted 32, 80 and 160 seconds respectively with a fixed power density of 31.25 mW/ cm². The total delivered energy for irradiated groups were 0.08, 0.2 and 0.4 Joules respectively.

**Outcomes**

Muscular fatigue was defined by three parameters:

1) The maximal force elicited at the onset of each of the 6 electrically induced tetanic contractions

2) The time taken for an electrically induced tetanic contraction to have its force reduced to 50% of the value at the onset of that particular contraction.

3) The work performed by each electrically induced titanic contraction (Power in Watts = force (Newton) multiplied by time in seconds)

Muscle damage was indirectly measured by the levels of Creatine Kinase (CK). CK levels were measured from blood samples collected in vivo during the experiment between the electrical stimulation sessions. The blood samples were collected from each animal, and then analysed.
Statistical Analysis

The obtained data were first plotted for analysis of normal distribution, and statistical analysis was then performed with parametric tests if the data were normally distributed. The statistical level of significance was set at $p<0.05$. After confirmation obtained data were tested statistically by an analysis of variance (ANOVA). The mean value and its standard error (SEM) were calculated and differences between control group data and the irradiated groups data were tested statistically with t-Student test.
Results

In all the contractions a typical non-linear decrease of force could be seen over time (Figure 3).

In the control group, the mean peak contraction force decreased in a successive, but non-linear manner to 50\% (SD +/- 15\%) of the first mean peak contraction force in the 6th contraction. For the laser-irradiated groups the mean peak contraction force was significantly higher at 92.2 \% (SD +/- 12.6\%) for 0.5 J/cm^2, 83.2 \% (SD +/- 20.5\%) for 1.0 J/cm^2 and 82.9 \% (SD +/- 18.3\%) for 2.5 J/cm^2 respectively for the 6th contraction (Figure 3).

In the control group, the mean time to 50\% decrease of the maximal force of each particular contraction did not change significantly over time and remained between 92\% (SD +/- 40.4\%) and 110\% (SD +/- 12.5\%) of the first contraction. For the laser-irradiated groups the mean time to 50\% decrease of the maximal force showed different patterns for different doses. There was no statistically significant difference between the change in time to 50\% decrease of maximal force in the laser group receiving a dose of 2.5 J/cm^2 and the control group for any of the six contractions. The laser group receiving 0.5 J/cm^2 had a significantly longer time to 50\% decrease in maximal force of the 2nd, 3rd and 4th contractions than the control group, while the laser group receiving 1.0 J/cm^2 had significantly longer times to 50\% decrease in maximal force for all contractions than did the control group (Figure 4).
In the control group, the mean work elicited before a 50% decrease of the maximal force of each contraction dropped sharply from the first to the second contraction (67%, SD +/-6.4%), and decreased less in a linear manner from the 3rd to the 6th contraction. For all laser-irradiated groups the mean work elicited was significantly higher than the control group for every single contraction. However, this was significantly more pronounced in the 0.5 J/cm² and the 1.0 J/cm² groups, than in the 2.5 J/cm² (Figure 5).

We measured CK levels by spectrophotometry before the 1st tetanic contraction and after the 3rd and 6th contraction. In the control group we found a linear reduction of that CK levels were reduced by 15% after the 3rd contraction and 30% after the 6th contraction. For the laser groups group we found a significantly higher reduction of CK levels at all time points except after the 6th contraction for the 0.5 J/cm² group. The laser dos-response patterns for CK level reductions differed form the other outcomes in the sense that the lowest laser dosage also induced the smallest reductions of CK levels. The largest reduction by 80% of CK levels were seen with a dose of 1,0 J/cm2 after the 6th contraction, while the high laser dose (2.5 J/cm²) induced significantly better CK level reductions than the low dose laser group (0.5 J/cm² ) (Figure 6).
DISCUSSION

In this trial we have demonstrated that LLLT can postpone the fatigue response to repeated tetanic contractions in skeletal muscles. Our results also show some differences in effects between the different doses used. Within the common therapeutic dose range between 0.5 and 2.5 J/cm², LLLI provided superior muscular performance by generating consistently higher maximal force for all three doses involved. However, the highest LLLI dose was not superior to controls when only the times to half values of maximal force were considered. This indicates that some of the beneficial effects on cellular metabolism may be lost if LLLI doses are too high. Unpublished results of our group using the experimental model of intra-vital microscopy demonstrated that high energy densities of LLLI could induce arteriolar constriction, which may eventually cause a worsening of fatigue. Optimal LLLI dosage seems to be a critical success factor, but it also needs to be tailored to the specific biological mechanism we want to modulate. For instance, the highest dose group was not significantly different from controls for fatigue, but it was clearly superior to controls in reducing post-exercise CK-levels. A preventive LLLI-effect on inflammatory reactions to the strenuous exercises induced in the experiment is suggested from the two highest doses of 1 and 2.5 J/cm². The observed dose-response pattern for LLLI in muscular fatigue is familiar, and has been observed previously in reviews of connective tissue metabolism and inflammation. Indeed, fibroblast cell metabolism is stimulated and collagen production increased after LLLI with a dose-range which is slightly lower than what is required to suppress inflammatory reactions (6, 38, 41, 50). The discrepancy between dose-response patterns for LLLI in inhibition of CK release and fatigue prevention in our study, suggests that LLLI induce both
inflammatory modulation and reduction of oxidative stress reduction. The ability to enhance cellular survival in hypoxic tissue has previously been demonstrated in LLLI-treated dogs with myocardial infarcts (34).

Some considerations are necessary before initiating and optimizing clinical use. Firstly, the doses we used here are “in situ” doses, because the muscles were dissected and the animal skin was removed. This model may not reflect the complex pain processes involved in development of muscular pain and fatigue in humans. Secondly, the irradiated muscle in our experiment was only one millimeter thick, and thus, most of the rat tibial muscle received an adequate LLLI dose. In a clinical setting, increased doses are needed to penetrate human skin (45). Although LLLI can exhibit positive effects on hemoglobin and blood cells (43), particularly hemoglobin absorbs laser light and may hamper an even distribution of the LLLI dose (47). In larger human skeletal muscles it may also be difficult to distribute adequate LLLI doses throughout the muscle belly.

Few clinical studies have investigated the effects of LLLI in DOMS, but two studies found no significant effects from doses of combined laser and LED therapy by red 660nm and infrared 820 and 950 nm wavelengths with 11 J/cm² and 31 J/cm² respectively (11,12). There are several possibilities for the poor results in these studies. Either the LED and laser combination, the combination of different wavelengths or doses above 10 J/cm² may have caused the poor results. In addition, they only assessed pain and range of motion and did not measure the ability to elicit force or perform work. In sports, quick and adequate restoration of physiological processes after strenuous training or competition is important and several options exist to achieve this. To our knowledge, LLLI has not been used
as an adjunct in controlled trials which investigate if LLLI can restore bodily processes after sports.

In other areas such as chronic muscle pain syndromes, clinical studies have shown that doses of 2 J/cm² with monochromatic 904nm pulsed laser have achieved good results (21,22). Several treatment options are available for the treatment of chronic muscle pain, but currently none seem to provide the ultimate balance between benefits and harms (35). In addition, some disorders like chronic neck muscle pain and fibromyalgia may be recalcitrant and no single treatment has provided convincing results. Common treatments for muscle pain are often associated with transient painful perceptions like massage and acupuncture and pharmaceutical muscle relaxants may cause addiction and inflict poorer prognosis in low back pain (17). Fibromyalgia is a chronic condition where fatigue and hypoxia seem to play an important part (26). Although exercise therapy has been used with some long-term success in fibromyalgia, the level of intensity must be increased slowly in order to avoid episodes of exercise-induced fatigue and increased pain (20). The demonstrated reduction in fatigue development by LLLI in our study, may be part of the explanation why LLLI seem to reduce pain in fibromyalgia patients (22).

In this perspective, there is clearly room for a risk-free and effective alternative, and LLLI seems a likely contender. Future clinical studies are needed to systematically elucidate the possible mechanisms behind LLLI in muscle fatigue and muscle pain and the optimal administration and dosage of LLLI.

**Conclusion**
This animal study showed that LLLI could reduce the debilitating influence of local fatigue on muscle force, and possibly reduce muscle damage after strenuous exercises in a dose-dependent manner. Further studies in clinical settings are warranted.

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Figure 2

Baseline Contraction

Tetanic Contraction

A

B
Figure 3

- Control
- 0.5 J/cm²
- 1 J/cm²
- 2.5 J/cm²
Figure 4

Time of decay up to 50% of peak force contraction

- Control
- 0.5 J/cm²
- 1 J/cm²
- 2.5 J/cm²

Time (min)

60 10 20 30 40 50 60

60 80 100 120 140 160
Figure 5

![Graph showing work (% of 1st Contraction) over time (min) for different light intensities: Control, 0.5 J/cm², 1 J/cm², and 2.5 J/cm². The graph indicates a decrease in work over time.](image-url)
Figure 6

Creatine Kinase
% of Activity

Contraction

Control

0.5 J/cm²

1 J/cm²

2.5 J/cm²

*
Figure 1: Laser irradiation (red – 655 nm wavelength) of the rat tibial anterior muscle belly. The distal tendon end is connected by wire to the dynamometer.

Figure 2: Typical Record of Rat Tibial Skeletal Muscle Tetanic Contraction induced by Electrical Stimulation. A – Peak Force of contraction in Newton; B – time to decrease do 50% of maximal contraction. The area under the curve represents our parameter for performed workload.

Figure 3: % Muscular force in Newton over time for a tetanic contraction induced by electrical nerve stimulation. The initial peak force was taken as 100%. Each contraction was induced every 10 minutes, in a total of 6 tetanical contractions both for control and irradiated animals. (*p<0.05)

Figure 4: Graph showing group mean times with SEM for contraction force to be reduced to 50% of the maximal force achieved from the onset of each of 6 maximal muscular contractions. The initial time was taken as 100%. The treatment group receiving doses of 1.0 J/cm² (triangles) were less fatigued and able to maintain more than 50% muscle force significantly longer than the control group at contractions 2 through 6. (*p<0.05)

Figure 5: Graphic showing the area under the curve of muscular fatigue after the tetanical contraction. The initial area was taken as 100%. Muscular contraction intensity relation with the time for this tension decreased 50% in maximum amplitude. (*p<0.05).
**Figure 6:** Figure showing CK activity reduction during the electrical stimulations and tetenical contractions. The first dosage was in rest, before the titanic stimulation and taken as basal activity or 0%. (*p<0.05).


