Lasers in Medical Science (2019) 34:749–758
https://doi.org/10.1007/s10103-018-2655-x

ORIGINAL ARTICLE

Modulation of exercise-induced muscular damage and hyperalgesia by different 630 nm doses of light-emitting diode therapy (LEDT) in rats

Alan B. Vasconcelos[1] · Fernando K. Nampo[2] · Júlio C. Molina[3] · Miriam B. Silva[1] · Alan S. Oliveira[1] · Tarlyson R. de Angelis[3] · Amanda L. Hasuda[3] · Enilton A. Camargo[1] · Solange P. Ramos[3]

Received: 19 March 2018 / Accepted: 30 September 2018 / Published online: 17 October 2018

Abstract

We compared the acute effects of different doses of 630 nm light-emitting diode therapy (LEDT) on skeletal muscle inflammation and hyperalgesia in rats submitted to exercise-induced muscle damage (EIMD). Wistar rats were divided into five experimental groups ($n = 5$–8/group): sedentary control (CON); exercise + passive recovery (PR); and exercise + LEDT (1.2 J/cm^2, 1.8 J; 4.2 J/cm^2, 6.3 J; 10.0 J/cm^2, 15 J). After 100 min of swimming, the rats in the LEDT groups were exposed to phototherapy on the triceps surae muscle. For mechanical hyperalgesia evaluation, paw withdrawal threshold was assessed before and 24 h after swimming. Immediately after hyperalgesia tests, blood samples were collected to analyze creatine kinase (CK) activity and the soleus muscle was removed for histological and tumor necrosis factor (TNF)-α immunohistological analyses. In all LEDT groups, plasma CK activity was reduced to levels similar to those measured in the CON group. Paw withdrawal threshold decreased in the PR group (-11.9 ± 1.9 g) when compared to the CON group (2.2 ± 1.5 g; $p < 0.01$) and it was attenuated in the group LEDT 4.2 J/cm^2 (-3.3 ± 2.4 g, $p < 0.05$). Less leukocyte infiltration and edema and fewer necrotic areas were found in histological sections of soleus muscle in LEDT (4.2 J/cm^2) and LEDT (10.0 J/cm^2) groups compared to the PR group. Also, LEDT (4.2 J/cm^2) and LEDT (10.0 J/cm^2) groups showed less immunostaining for TNF-α in macrophages or areas with necrosis of muscle fibers compared to the PR group. LEDT (4.2 J/cm^2, 6.3 J)-reduced muscle inflammation and nociception in animals submitted to EIMD.

Keywords Inflammation · Pain · Skeletal muscle · Physical exercise · Light-emitting diode · Phototherapy

Introduction

Exercise-induced muscle damage (EIMD) can occur after a single bout of unaccustomed exercise or after an intense effort involving eccentric contraction. Mechanical overload and metabolically damaged muscle fibers can lead to a local inflammatory process, mainly characterized by cytokine production and consequent leukocyte infiltration, muscle fiber necrosis, increased levels of circulating creatine kinase (CK), and delayed onset muscular soreness, resulting in a temporary loss of performance [1–4]. The CK circulating levels (a biochemical marker of muscle injury) and the inflammatory process symptoms peak between 24 and 72 h following the physical effort and are accompanied by reduced muscle function and loss of physical performance for a period up 5 to 7 days [1, 3, 5–8]. Thus, a key issue in avoiding EIMD may be the prevention of cell necrosis and consequent inflammation, preventing loss of function, and inflammatory symptoms.

The photobiomodulation induced by low-level light sources ranging from red to near-infrared wavelengths has been suggested to be useful in prevention of EIMD [9–12]. Animal studies have demonstrated that light-emitting diode therapy (LEDT) can prevent muscle necrosis and inflammatory infiltration at energy densities ranging from 1 to 133 J/cm^2, with total energy delivered from approximately 1 to 10 J per muscle [13–17]. However, experimental and clinical studies have shown that energy dosimetry has an important

✉ Enilton A. Camargo
enilton.camargo@pq.cnpq.br

[1] Department of Physiology, Federal University of Sergipe, Marechal Rondon Av., São Cristóvão, SE CEP 49100-000, Brazil

[2] Latin American Institute of Life and Natural Sciences, Federal University of Latin American Integration, Foz do Iguaçu, PR, Brazil

[3] Department of Histology, State University of Londrina, Londrina, PR, Brazil

influence on different parameters of EIMD, since some doses are more efficient in decreasing CK release, while other doses prevent delayed onset muscular soreness and associated loss of performance [16, 18, 19]. It is not clear which doses can avoid both muscle necrosis and pain. It is also important to point out that an in vitro study demonstrated that phototherapy at low energy density (1 to 2 J/cm^2) induced the expression of inflammatory cytokines by monocytes, including tumor necrosis factor-α (TNF-α), whereas energy density above 3 J/cm^2 inhibited this effect [16]. This result suggests that low doses of energy may stimulate the polarization of proinflammatory macrophages (TNF-α-positive) in damaged muscles and it may increase pain and tissue disorganization. Total energy delivered and energy density may also influence pain relief. Previous works have demonstrated that irradiation above 2.0 J/cm^2 on chemically or surgically inflamed hind paws of rats could inhibit mechanical pain threshold, whereas no effect was observed for application of 1 J/cm^2 [20, 21]. One study demonstrated that irradiation of inflamed tissues could decrease mechanical pain threshold, but no effect was observed in TNF-α production and other signs of inflammation [22]. Therefore, it is not clear which parameter is better for preventing inflammation and pain associated with EIMD.

Another concern is about inhibition of production of proinflammatory cytokines, since these can delay inflammatory cell infiltration [13, 14, 22, 23], and tissue repair [24]. Considering that inner layers of human tissues might not receive adequate doses of energy to avoid EIMD, it is necessary to evaluate the effects of different energy doses of LEDT on the muscle damage markers and signs and symptoms of inflammation. Thus, clinical and experimental studies show differences regarding the parameters used and the prophylactic versus therapeutic application, which shows that better parameterization for LEDT treatment is necessary [11, 12, 22, 24–29].

Based on this situation, several animal models of physical exercise can be useful to study inflammation and acute pain associated to EIMD [14, 30–33] and can help evaluate the effect of varying phototherapy parameters. Thus, in order to mimic a strenuous session of physical exercise, our group previously used the protocol of swimming for 100 min in rats [13], which induce acute inflammatory parameters such as injury to the skeletal muscle accompanied by cell infiltration, necrosis, and edema after 24 h of exercise, but no study has considered the variation of LEDT energy doses in this model and its impact on inflammation and hyperalgesia in this condition.

In this study, we compared the effect of different doses of LEDT (630 nm) on the inflammation of the skeletal striated muscle and the hyperalgesic response in experimental animals submitted to exercise, in order to contribute to the discussion about varying energy doses of LEDT for the treatment of EIMD.

Materials and methods

Experimental design

Male Wistar rats (250–300 g) from the animal breeding facility of State University of Londrina (UEL) were used. Animals were maintained at 21 ± 2 °C with free access to water and rodent chow (Nuvilab® CR1, Nuvital, Colombo, Brazil) in a light-dark cycle of 12 h. The experimental procedures were carried out according to the standards of the Brazilian Society of Laboratory Animal Care and were approved by the Ethics Committee on the Use of Animals under protocol number 124/2014.

The animals were randomly divided into five groups (n = 5–8): sedentary control group (CON), passive recovery exercised group (PR), and three groups of exercised animals subjected to different applications of LEDT (LEDT 1.2 J/cm^2, LEDT 4.2 J/cm^2, and LEDT 10 J/cm^2). The experiments were carried out during the animals' light cycle, between 8 a.m. and 16 p.m.

A sample size of five animals per group was calculated considering a frequency of 0.8% of fields containing necrotic or inflammatory cells 24 h after an EIDM protocol [13] with a statistical power of 80% (β = 20%) and statistical significance of 5% (α). We also estimated 30% sample loss during EIDM protocol and sample handling, so eight animals were included in each group.

EIMD protocol

The animals were familiarized with the aquatic environment for 5 min per day for 5 consecutive days before the EIMD protocol. The exercised groups (PR, LEDT 1.2 J/cm^2, LEDT 4.2 J/cm^2, and LEDT 10 J/cm^2) swam for 100 min in a plastic container (40-cm depth, 52-cm diameter) under continuous supervision with water temperature of approximately 30 °C [13].

Light-emitting diode therapy application

The LEDT application was performed immediately after the exercise protocol by irradiating both hind legs at a 630-nm wavelength with spectral bandwidth of 20 nm. The LEDT was administrated using a Bios Therapy II device (Bios Equipamentos Medicos, São José dos Campos, Brazil), with power output of 300 mW, using an optic fiber with 1.35-cm spot diameter, spot area of 1.43 cm^2, and power density of 200 mW/cm^2. The irradiation was applied during 6 s, 21 s, and 50 s to deliver 1.8 J, 6.3 J, and 15 J at energy densities of 1.2 J/cm^2, 4.2 J/cm^2, and 10.0 J/cm^2, respectively. These doses and the therapeutic application of LEDT were chosen based on previous studies in our laboratory using the EIMD model [13, 14] and others from the literature [16, 22, 27, 34–36]. The radiation source was kept stationary in contact with the skin surface at a single point located in the middle of the triceps surae muscle belly.

Mechanical hyperalgesia evaluation

To test the mechanical hyperalgesia, we used an electronic von Frey instrument (Insight, Ribeirão Preto, Brazil). The animals were transferred and kept in individual cages (Insight, Ribeirão Preto, Brazil) for 30 min. After this period, a hand-held force transducer was used to apply mechanical stimuli to both paws, in triplicate, before and 24 h after the swimming protocol. The intensity of stimulus required to evoke withdrawal behavior was recorded as the withdrawal threshold (g). For the analysis, we considered the mean of the three consecutive measurements performed on each paw of the animal, with minimum and maximum intervals of 1 and 5 min respectively. The data were expressed as the difference between the initial and final stimulus intensity values (Δ). The evaluator was blinded to the experimental groups.

Hematological and creatine kinase analysis

The animals were anesthetized intraperitoneally with xylazine hydrochloride (0.02 g/kg, Virbaxyl® 2%, Virbac do Brasil, São Paulo, Brazil) and ketamine hydrochloride (0.1 g/kg, Francotar® 10%, Virbac do Brasil, São Paulo, Brazil) 24 h post-exercise. Blood samples were collected by heart puncture. Creatine kinase (CK) analysis was performed using the Dimension Xpand Plus Integrated Chemistry System (Siemens, Erlagen, Germany) and hematological analysis was performed with an automated hematology analyzer (BC 2800 veterinary, Mindray Medical, Nanshan, China).

Histological procedures

After blood sampling, animals were euthanized by exsanguination under anesthesia and the right soleus muscle was removed and fixed in Bouin's solution for 24 h and embedded in histological paraffin. Five longitudinal sections (5 μm) of the muscle were serially obtained, stained with hematoxylin and eosin (H&E), and analyzed with an optical microscope. Ten images were randomly captured from each slice at tenfold magnification using a Moticam imaging device (Motic Group, Xiamen, China) and analyzed with the Motic Image Plus 2.0 software (Motic, Xiamen, China). Each image was divided into 20 fields (50 × 50 μm/field). A trained evaluator unaware of the group's identity counted any field with muscle fibers and recorded the fields containing damaged muscle fibers, inflammatory cell infiltrate, and edema.

Immunohistochemistry procedure

The left soleus muscle was removed and fixed in buffered formalin solution for 24 h and embedded in histological paraffin. Transversal sections (5 μm) of the muscle were submitted to immunohistochemical protocol for detection of cells expressing tumor necrosis factor (TNF)-α. For antigen retrieval, the slides were submerged in sodium citrate buffer (pH 6.0, 100 °C) for 5 min. For the reduction of non-specific binding, the sections were treated with 5% skimmed milk diluted in distilled water for 1 h. The sections were topped using TNF-α primary antibody (#555212, Santa Cruz Biotechnology, Dallas, USA) at 1:50 dilution in phosphate-buffered saline (pH 7.2). The sections were incubated overnight in a humid chamber at 4 °C and were subsequently treated with secondary antibody (rabbit anti-mouse IgG, #A9044, Sigma-Aldrich, St. Louis, USA) and incubated for 2 h at room temperature. These sections were incubated with diaminobenzidine (DAB) and counter-stained with Mayer's hematoxylin (Sigma-Aldrich, St. Louis, USA) for 10 min.

Five random slides per group were analyzed to identify areas of necrosis in muscle tissue and TNF-α immunostaining in inflammatory cells by an evaluator unaware of group identity.

Statistical analysis

The Kolmogorov–Smirnov test was applied to verify the dependent variables' distribution. One-way ANOVA with Tukey's post hoc test were used to compare mean differences between different treatment groups. Data are expressed as means and standard derivations. The histological data were analyzed by the chi-square test with Yates's correction. Differences were considered statistically significant if $p < 0.05$.

Results

The initial body weight of animals was not different among the groups: CON (313.5 ± 45.3 g), PR (305.3 ± 34.9 g), LEDT 1.2 J/cm^2 (301.9 ± 32.1 g), LEDT 4.2 J/cm^2 (297.8 ± 57.78 g), and LEDT 10.0 J/cm^2 (285.3 ± 24.8 g) ($p = 0.7129$).

In order to evaluate mechanical hyperalgesia, the paw withdrawal threshold was measured. No difference was observed for baseline values of intensity of stimulus needed to elicit withdrawal behavior among the groups studied: CON (35.0 ± 2.2 g), PR (37.4 ± 2.3 g), LEDT 1.2 J/cm^2 (37.2 ± 1.8 g), LEDT 4.2 J/cm^2 (36.0 ± 1.5 g), and LEDT 10.0 J/cm^2 (36.3 ± 2.0 g). Twenty-four hours after the swimming protocol, we found a reduction ($p < 0.01$) in the paw withdrawal threshold to the mechanical stimulus in the PR group compared to the control group (Fig. 1). The LEDT 4.2 J/cm^2 group showed a significant reversion of mechanical hyperalgesia ($p < 0.05$) in comparison to the PR group. This group did not differ from the CON group at this time point. On the other hand, in the groups LEDT 1.2 and LEDT 10.0 J/cm^2, no alteration of the paw withdrawal threshold was observed in comparison to the

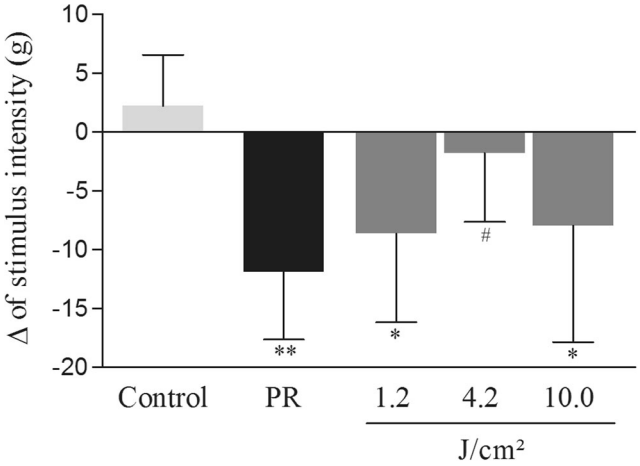

Fig. 1 Mechanical hyperalgesia (g) in non-exercised animals (control), animals subjected to 100 min swimming exercise and passive recovery (PR), and animals exercised and submitted to LED therapy (1.2 J/cm^2, 4.2 J/cm^2, or 10.0 J/cm^2). Data are shown as variation (Δ) of paw withdrawal threshold intensity measured after 24 h of exercise subtracted from basal values. $^{**}p < 0.001$ or $^{*}p < 0.05$ compared to control group. $\#p < 0.05$ compared to PR group, one-way ANOVA and Tukey's post hoc test

PR group and these groups still differed from the CON group ($p < 0.05$ each) (Fig. 1).

Twenty-four hours after the exercise, CK levels were higher ($p < 0.001$) in the PR group compared to the CON group (Fig. 2). The groups LEDT 1.2 J/cm^2, LEDT 4.2 J/cm^2, and LEDT 10.0 J/cm^2 had lower ($p < 0.001$ each) serum CK release in comparison to the PR group at levels that did not differ from the CON group (Fig. 2).

The PR and LEDT 1.2 J/cm^2 groups presented increased ($p < 0.05$) frequency of histological fields with necrotic muscle fibers, leukocyte infiltration, and edema in comparison to the CON group (Table 1; Fig. 3). However, the LEDT 1.2 J/cm^2 group presented reduced ($p < 0.05$) frequency of fields with leukocyte infiltration in comparison to the PR group, which was not observed for the frequency of necrotic muscle fibers ($p = 0.35$) and edema ($p = 0.94$). Muscle damage and inflammatory areas were scarce in the LEDT 4.2 J/cm^2 and LEDT 10 J/cm^2 groups in comparison to PR and LEDT 1.2 J/cm^2 groups. The LEDT 4.2 J/cm^2 group presented the lowest frequency of necrosis, edema, and leukocyte infiltration fields in comparison to PR and other LEDT groups ($p < 0.05$). Furthermore, the LEDT 4.2 J/cm^2 group had a lower frequency of edema ($p < 0.05$) and leukocyte infiltration ($p < 0.05$) but not necrosis ($p = 0.09$) when compared to the CON group.

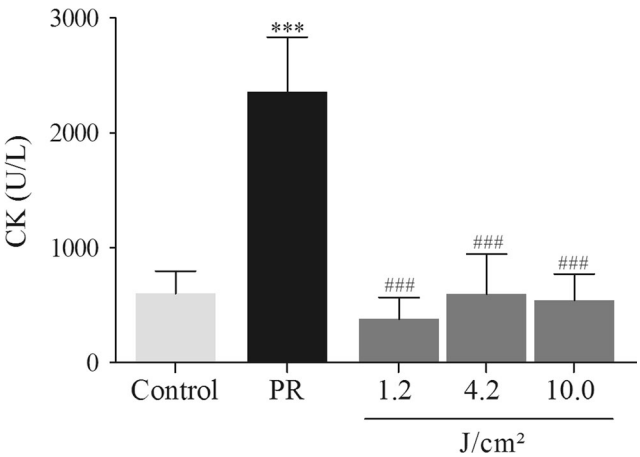

Fig. 2 Levels of blood creatine kinase in Wistar rats at rest (control), submitted to 100 min of swimming exercise and passive recovery (PR), or LED therapy (1.2 J/cm^2, 4.2 J/cm^2, or 10.0 J/cm^2). Data are expressed as mean ± SD $^{***}p < 0.0001$ in comparison to control group, $\#\#\#p < 0.0001$ in comparison to PR group. One-way ANOVA and Tukey's post hoc test

Table 2 shows that no difference was observed in total leukocyte, polymorphonuclear, or mononuclear counts in the peripheral blood of animals from all groups 24 h after induction of muscle inflammation.

Tumor necrosis factor-α immunostaining was observed in several muscle cells in the PR, LEDT 1.2 J/cm^2, and 10.0 J/cm^2 groups (Fig. 4). Moreover, the PR group presented necrotic muscle fibers infiltrated with a high number of TNF-α-positive leukocytes (Fig. 4). On the other hand, few TNF-α-positive leukocytes were observed in necrotic fibers in the LEDT 1.2 J/cm^2 group (Fig. 5). In LEDT 4.2 J/cm^2 and 10 J/cm^2 groups, TNF-α-positive leukocytes were scarcely found.

Discussion

The data in the present study confirm previous demonstrations that LEDT reduces some conditions associated with clinical signs of EIMD [13, 14], while adding important information not only about the dose needed to affect muscular inflammatory damage but also nociceptive alterations induced by exercise. These effects seem to depend on the dose applied, and the inhibition of TNF-α production may be linked to the observed effects.

The swimming protocol induced an increase of the activity of CK in the bloodstream of the animals subjected to passive recovery. It is well known that CK extravasation is caused by disorganization of the cytoskeleton and sarcolemma, a phenomenon associated with calcium regulation and ATP depletion [37, 38]. Post-exercise treatment with LEDT attenuated the increase in CK activity independently of the dose applied, suggesting that this treatment may have contributed to preserving skeletal muscle structure. This result agrees with the demonstration that LEDT-reduced CK activity in animal models of continuous or intermittent exercise [13, 14]. Indeed, human studies have also confirmed this finding, since in healthy subjects undergoing a protocol of eccentric extensor isokinetic contractions (five sets of 15 replicates), the combination of LASER (904 nm) and LEDs (604 nm and 875 nm) at energy densities between 0.83–5.83 J/cm^2 (each diode) with total energy delivered of 39.37 J per site attenuated the increase in CK activity from 24 to 96 h after intervention [11].

Table 1 Number (*n*) and frequency (%) of fields containing necrosis, edema, and leukocyte infiltration

	Control ($n = 5962$)	PR ($n = 10{,}173$)	LEDT 1.2 J/cm^2 ($n = 10{,}097$)	LEDT 4.2 J/cm^2 ($n = 7403$)	LEDT 10.0 J/cm^2 ($n = 8019$)
Necrosis	02 (0.03%)	95 (0.93%)[a]	81 (0.80%)[a]	10 (0.13%)[b, c]	50 (0.62%)[a, d]
Edema	415 (6.96%)	936 (9.20%)[a]	925 (9.16%)[a]	356 (4.80%)[a, b, c]	635 (8.59%)[a, b, c, d]
Leukocyte infiltrate	83 (1.39%)	357 (3.50%)[a]	278 (2.75%)[a, b]	41 (0.55%)[a, b, c]	133 (1.65%)[b, c, d]

[a] $p < 0.05$ compared to the control group. [b] $p < 0.05$ compared to the PR group. [c] $p < 0.05$ compared to LEDT 1.2 J/cm^2 . [d] Group $p < 0.05$ compared to LEDT 4.2 J/cm^2 group; chi-square test with Yates's correction. *PR*, passive recovery; *LEDT*, light-emitting diode therapy

The protective effect of LEDT, evidenced by the reduction of CK activity, might be partly related to lower tissue inflammatory response. Indeed, in the present study, the groups LEDT 4.2 J/cm^2 and LEDT 10.0 J/cm^2 showed a decrease in inflammatory parameters in muscle, such as edema, necrosis, and infiltration of cells, indicating that these treatments are more effective in maintaining cellular viability and attenuating inflammation. Interestingly, in the group LEDT 4.2 J/cm^2, we found reduced frequency of inflammatory parameters to values lower than those of the control group. Animal studies have demonstrated that phototherapy with energy densities above 2 J/cm^2 (1.2 J) can inhibit the expression of chemokines (monocyte chemotaxis protein-1), inflammatory mediators, and cytokines, reducing stimulus to inflammatory cell migration into tissues [21, 39, 40]. Interestingly, in spite of the reduction of CK activity in animals of the group LEDT 1.2 J/cm^2, these animals presented several areas of muscular necrosis. This phenomenon was also reported by de Almeida et al. [34], who showed that the lowest total energy used (0.1 J) reduced CK activity, but did not reduce the expression of cyclooxygenase-2. The mechanisms involved in prevention of the inflammatory process may be related to blunting of oxidative stress (associated with increased cytochrome c activity and ATP production) and inhibition of redox-sensitive activation of the nuclear factor-κB pathway in the injured tissues [41].

Besides the inflammatory alterations detected by histological analysis, the exercise protocol applied increased the number of cells expressing TNF-α, an effect not observed in animals submitted to post-treatment with LEDT at 4.2 J/cm^2 (6.3 J) and 10.0 J/cm^2 (15 J). However, once again, the treatment with LEDT at 1.2 J/cm^2 (1.8 J) did not affect TNF-α immunostaining in injured muscle, reinforcing that the LEDT

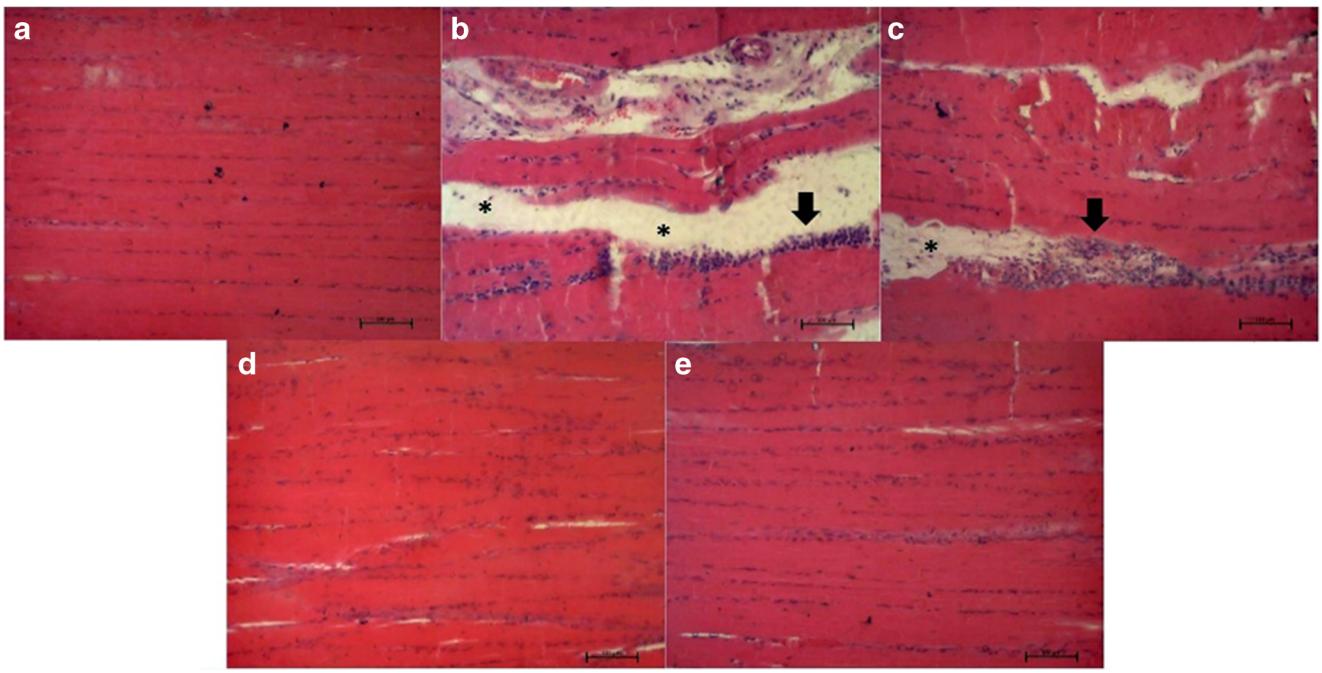

Fig. 3 Photomicrography of the soleus muscle of Wistar rats. **a** Control animal illustrating a healthy area of skeletal muscle, with absence of necrosis, edema, and inflammation. **b** Animals submitted to 100 min of swimming exercise (PR) presented several areas with infiltrated inflammatory cells and necrosis signals (arrow) and dissociation of muscle fibers due to edema (*). **c** Animals from the LEDT (1.2 J/cm^2) group presented areas with inflammatory cell infiltration in necrotic fibers (arrow) and edema (*). **d** and **e** Animals from the LEDT 4.2 10.0 J/cm^2 and LEDT 10.0 J/cm^2 groups. Most of analyzed fields did not present signs of muscle damage. 10 × magnification; hematoxylin and eosin staining

Table 2 Leukocyte counts

	Control ($n = 8$)	PR ($n = 7$)	LEDT 1.2 J/cm^2 ($n = 5$)	LEDT 4.2 J/cm^2 ($n = 5$)	LEDT 10.0 J/cm^2 ($n = 7$)
Total leukocytes (10^6 cell/mm^3)	5.75 ± 0.37	7.97 ± 0.11	8.86 ± 0.11	7.36 ± 0.18	8.75 ± 0.86
Polymorphonuclear cells (cell/mm^3)	0.90 ± 0.15	1.16 ± 0.28	1.33 ± 0.40	1.24 ± 0.45	1.78 ± 0.28
Mononuclear cells (cell/mm^3)	4.48 ± 0.20	6.70 ± 0.98	7.08 ± 0.10	5.99 ± 0.13	6.86 ± 0.69

PR, passive recovery; *LEDT*, light-emitting diode therapy

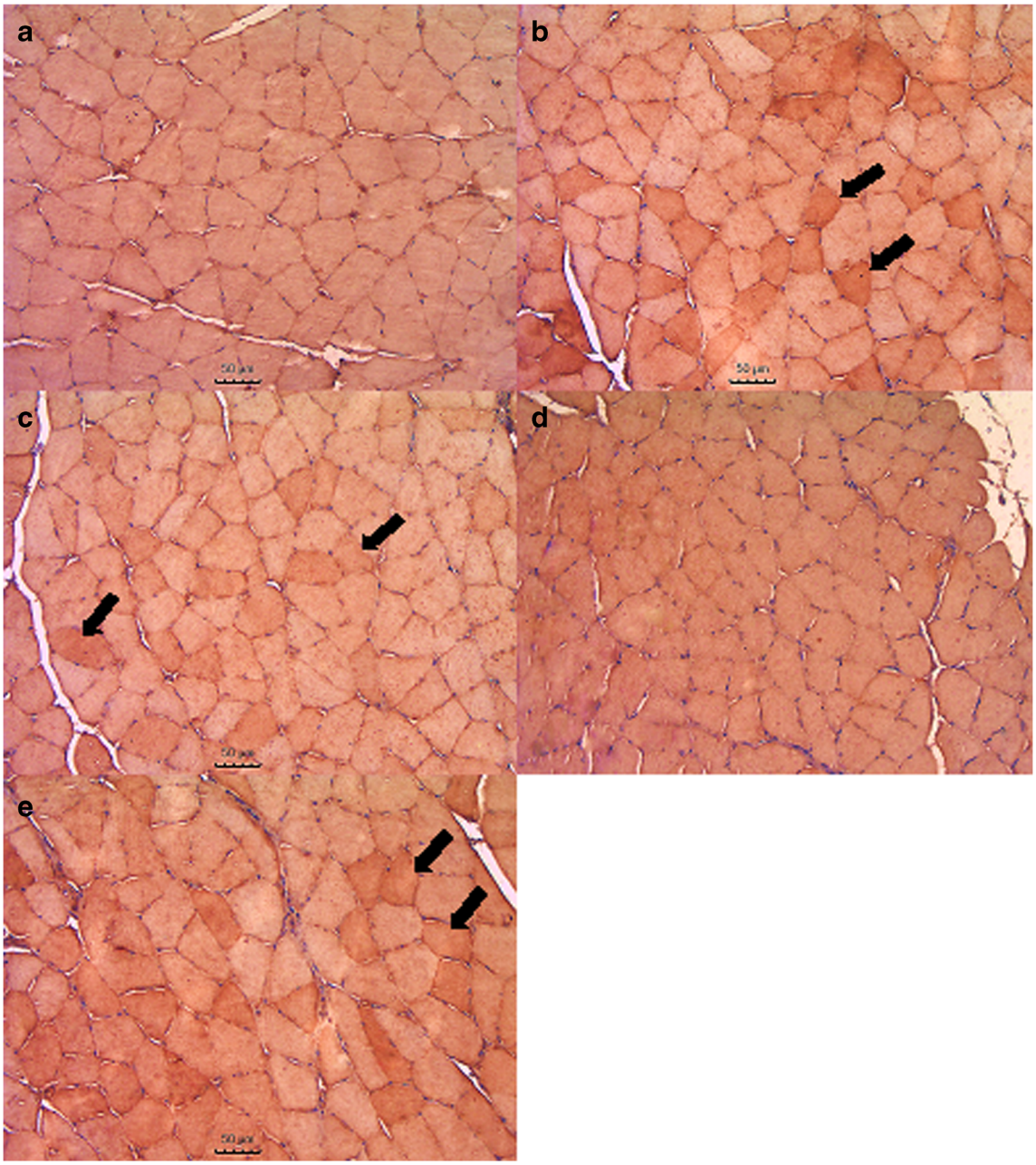

Fig. 4 Immunohistochemistry for TNF-α detection in Wistar rat soleus muscle. **a** Control animals presented no muscular fibers with immunostaining for TNF-α. **b** Animals submitted to 100 min of swimming exercise (PR). **c**–**e** Animals were submitted to exercise and treated with LEDT 1.2 J/cm^2 (1.8 J), 4.2 J/cm^2 (6.3 J), and 10.0 J/cm^2 (15 J) respectively. Black arrows indicate muscular fibers with immunostaining for TNF-α; tenfold magnification (bars = 50 μm)

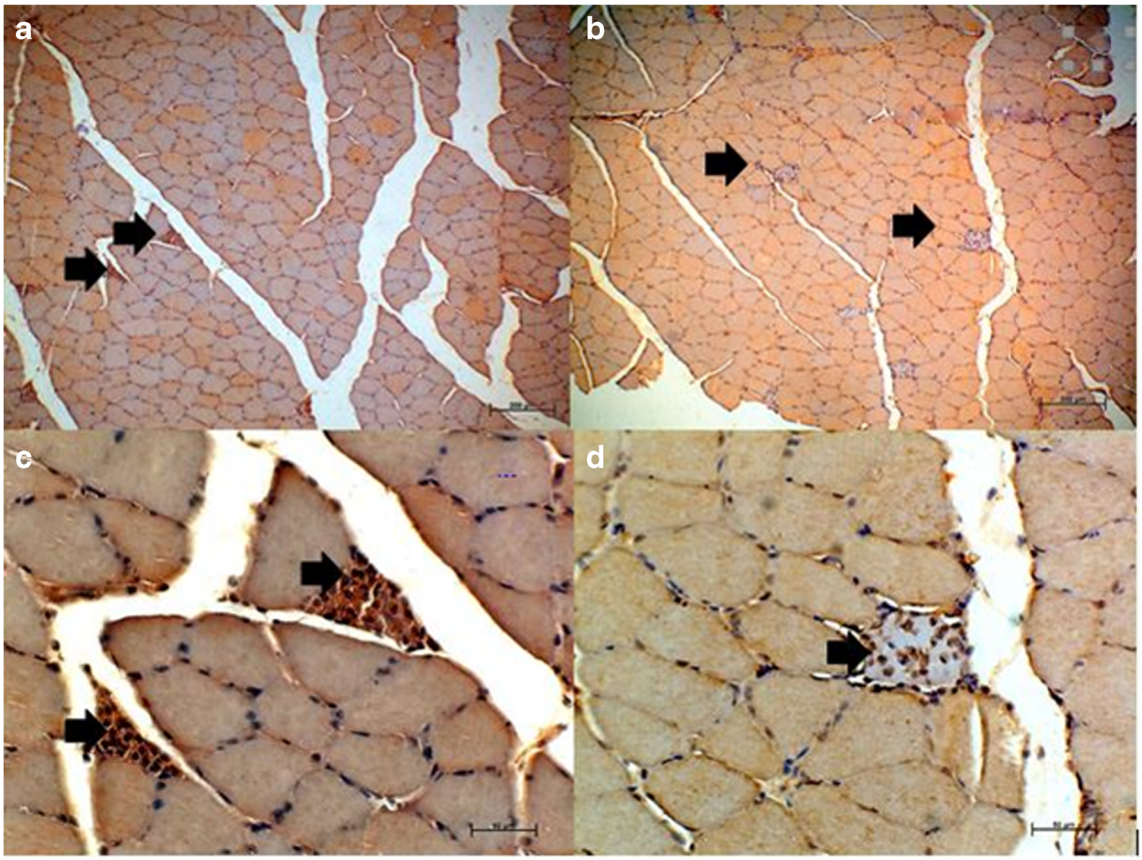

Fig. 5 TNF-α-positive leukocytes in Wistar rat soleus muscle. **a** and **c** Animals submitted to 100 min of swimming exercise (PR; 5 and 400-fold magnification, respectively). **b** and **d** Animals submitted to exercise and treated with LEDT 1.2 J/cm^2 (1.8 J); 5 and 400-fold magnification, respectively. Black arrows indicate necrotic muscular fibers infiltrated with TNF-α-positive leukocytes. **a** and **b** bars = 200 μm. **c** and **d** bars = 50 μm

dose is important. Tomazoni et al. [42] demonstrated that LASER therapy at 830 nm and 1 J (35.7 J/cm^2), 3 J (107.1 J/cm^2), and 9 J (321.4 J/cm^2) was effective in reducing TNF-α concentration in anterior tibialis muscle of rats with skeletal muscle inflammation induced by contusion.

In the present study, there was no change in the cell count after exercise. Previous studies have shown that 24 h after physical exercise the number of leukocytes in the bloodstream may be increased [14], decreased [13], or return to baseline values [43, 44]. Thus, although one method to attempt to evaluate the consequences of exercise on the organism is the measurement of hematological parameters such as leukocyte counts [45, 46], the conclusions from this parameter may be limited by experimental variabilities, which makes it hard to infer about the immune system function in this condition.

Considering the reduction of tissue inflammation by LEDT, we supposed it could be related to modulation of nociception in rats. We observed that the swimming exercise protocol resulted in reduction of the paw withdrawal threshold in rats, indicating the development of hyperalgesia. Interestingly, this response was less pronounced in the animals that received the intermediate dose of 4.2 J/cm^2 (6.3 J). The reduction of the histological outcomes is not the only factor that determined the reduction of hyperalgesia, because the application of the dose of 10.0 J/cm^2 (15 J) decreased histological alterations but it was not accompanied by alteration of hyperalgesia. Hough (1902) first proposed that pain appears in the early hours (12–24 h) and is related to rupture of muscle fibers or connective tissue. However, it is currently known that delayed onset muscular soreness may occur after exercise independently of the markers of EIMD [38, 47]. It has been suggested in this condition that neurotrophic factors such as the B_2 receptors/nerve growth factor pathway as well as glial cell line-derived neurotrophic factor pathway via cyclooxigenase-2 activation may contribute to this antihyperalgesic effect [48, 49]. In this respect, previous studies have demonstrated that phototherapy inhibited depolarization of afferent nerve fibers and inhibited the action of bradykinin, as well as the reducing cyclooxygenase-2 expression and consequently the production of prostaglandin E_2 [50–52].

More recently, a study demonstrated that LEDT (890 nm, 390 mW, and 20.8 J/cm^2) attenuated the nociception induced by endogenous mediators (glutamate, bradykinin, and prostaglandin E_2), by the activation of nociceptive channels (TRPV1, TRPA1, TRPM8, and ASIC) and by the increase

of PKA and PKC concentrations. These enzymes are responsible for the phosphorylation of nociceptive channels, which probably decreased their activation thresholds, thus potentiating the nociceptive response. This study also demonstrated the participation of C fibers in the reduction of these responses [24]. In addition, pre-administration of opioid antagonist (naloxone) or inhibitor of L and P selectins (fucoidin) inhibited the antinociceptive effect of LEDT (950 nm, 2 J/cm^2) [22]. Thus, it is possible that both local and central mechanisms can mediate the effects of LEDT found in our study.

Altogether, our data show that LEDT at 4.2 J/cm^2 (6.3 J) and 630 nm produced superior effects than other doses used for investigation of inflammation and hyperalgesia following a swimming protocol, which has not been demonstrated by others. Previous studies have investigated the effects of photobiomodulation using red laser (655 and 660 nm) and infrared phototherapy (830, 904, 904, and 940 nm) for the treatment of inflammation in the model of induction of skeletal muscle fatigue by electrical stimulation in rats. These studies have shown reduced CK activity, improved muscle performance by decreasing fatigue, reduced histological parameters indicating muscle damage, and decreased expression of mRNA for COX 2 in animals treated with laser phototherapy [16, 34–36]. The total energy used in these studies varied between 0.3 and 10 J, with optimal effects between 1 and 3 J for most outcomes. However, since these authors used different sources of photobiomodulation and experimental models, it is hard to compare their results with ours.

In summary, the present study indicates that LEDT at a dose of 4.2 J/cm^2 (6.3 J) and wavelength of 630 nm produced better effects, since it not only reduced the CK activity, inflammatory cell infiltration, muscle necrosis, and TNF-α immunostaining, but also mechanical hyperalgesia in rats. Furthermore, among the three doses tested that of 1.2 J/cm^2 (1.8 J) was insufficient to treat muscular inflammation and nociception; whereas, 10 J/cm^2 (15 J) produced anti-inflammatory actions not related to antinociceptive effects. In this way, the data obtained in this study shed new light on the effect of different doses of LEDT, a problem discussed in an incipient way in the clinical use of this therapy.

Acknowledgements The authors would like to thank the "Laboratorio de Óptica e Optoeletronica do Departamento de Física da Universidade Estadual de Londrina" for technical support; the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES); "Conselho Nacional de Pesquisa e Desenvolvimento Científico" (CNPq); and "Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná". ABSV was a recipient of scholarship from CAPES.

Compliance with ethical standards

The experimental procedures were carried out according to the standards of the Brazilian Society of Laboratory Animal Care and were approved by the Ethics Committee on the Use of Animals under protocol number 124/2014.

References

1. Ely MR, Romero SA, Sieck DC et al (2016) A single dose of histamine-receptor antagonists prior to downhill running alters markers of muscle damage and delayed onset muscle soreness. J Appl Physiol. https://doi.org/10.1152/japplphysiol.00518.2016
2. Enoka RM, Duchateau J (2008) Muscle fatigue: what, why and how it influences muscle function. J Physiol 586:11–23. https://doi.org/10.1113/jphysiol.2007.139477
3. Morgado JP, Monteiro CP, Teles J et al (2016) Immune cell changes in response to a swimming training session during a 24-h recovery period. Appl Physiol Nutr Metab 41:476–483. https://doi.org/10.1139/apnm-2015-0488
4. Silveira Coswig V, Hideyoshi Fukuda D, de Paula Ramos S, Boscolo Del Vecchio F (2016) Biochemical differences between official and simulated mixed martial arts (MMA) matches. Asian J Sports Med In press. https://doi.org/10.5812/asjsm.30950
5. Abdelmagid SM, Barr AE, Rico M et al (2012) Performance of repetitive tasks induces decreased grip strength and increased fibrogenic proteins in skeletal muscle: role of force and inflammation. PLoS One 7:e38359. https://doi.org/10.1371/journal.pone.0038359
6. Ascensão A, Rebelo A, Oliveira E et al (2008) Biochemical impact of a soccer match - analysis of oxidative stress and muscle damage markers throughout recovery. Clin Biochem 41:841–851. https://doi.org/10.1016/j.clinbiochem.2008.04.008
7. Chatzinikolaou A, Fatouros IG, Gourgoulis V et al (2010) Time course of changes in performance and inflammatory responses after acute plyometric exercise. J Strength Cond Res 24:1389–1398. https://doi.org/10.1519/JSC.0b013e3181d1d318
8. Paulsen G, Crameri R, Benestad HB et al (2010) Time course of leukocyte accumulation in human muscle after eccentric exercise. Med Sci Sports Exerc 42:75–85. https://doi.org/10.1249/MSS.0b013e3181ac7adb
9. Borsa PA, Larkin KA, True JM (2013) Does phototherapy enhance skeletal muscle contractile function and postexercise recovery? A systematic review. J Athl Train 48:57–67. https://doi.org/10.4085/1062-6050-48.1.12
10. Fritsch CG, Dornelles MP, Severo-Silveira L et al (2016) Effects of low-level laser therapy applied before or after plyometric exercise on muscle damage markers: randomized, double-blind, placebo-controlled trial. Lasers Med Sci 31:1935–1942. https://doi.org/10.1007/s10103-016-2072-y
11. de Paiva PRV, Tomazoni SS, Johnson DS et al (2016) Photobiomodulation therapy (PBMT) and/or cryotherapy in skeletal muscle restitution, what is better? A randomized, double-blinded, placebo-controlled clinical trial. Lasers Med Sci. https://doi.org/10.1007/s10103-016-2071-z
12. Vanin AA, Verhagen E, Barboza SD et al (2017) Photobiomodulation therapy for the improvement of muscular performance and reduction of muscular fatigue associated with exercise in healthy people: a systematic review and meta-analysis. Lasers Med Sci. https://doi.org/10.1007/s10103-017-2368-6
13. Camargo MZ, Siqueira CPCM, Preti MCP et al (2012) Effects of light emitting diode (LED) therapy and cold water immersion therapy on exercise-induced muscle damage in rats. Lasers Med Sci 27:1051–1058. https://doi.org/10.1007/s10103-011-1039-2
14. da Costa Santos VB, de Paula Ramos S, Milanez VF et al (2014) LED therapy or cryotherapy between exercise intervals in Wistar rats: anti-inflammatory and ergogenic effects. Lasers Med Sci 29:599–605. https://doi.org/10.1007/s10103-013-1371-9
15. Liu X-G, Zhou Y-J, Liu TC-Y, Yuan J-Q (2009) Effects of low-level laser irradiation on rat skeletal muscle injury after eccentric

exercise. Photomed Laser Surg 27:863–869. https://doi.org/10.1089/pho.2008.2443
16. Santos LA, Marcos RL, Tomazoni SS et al (2014) Effects of pre-irradiation of low-level laser therapy with different doses and wavelengths in skeletal muscle performance, fatigue, and skeletal muscle damage induced by tetanic contractions in rats. Lasers Med Sci 29:1617–1626. https://doi.org/10.1007/s10103-014-1560-1
17. Sussai DA, Carvalho PTC, Dourado DM et al (2010) Low-level laser therapy attenuates creatine kinase levels and apoptosis during forced swimming in rats. Lasers Med Sci 25:115–120. https://doi.org/10.1007/s10103-009-0697-9
18. Nampo FK, Cavalheri V, Ramos S de P, Camargo EA (2016) Effect of low-level phototherapy on delayed onset muscle soreness: a systematic review and meta-analysis. Lasers Med Sci 31:165–177. https://doi.org/10.1007/s10103-015-1832-4
19. Antonialli FC, De Marchi T, Tomazoni SS et al (2014) Phototherapy in skeletal muscle performance and recovery after exercise: effect of combination of super-pulsed laser and light-emitting diodes. Lasers Med Sci 29:1967–1976. https://doi.org/10.1007/s10103-014-1611-7
20. Laakso E-L, Cabot PJ (2005) Nociceptive scores and endorphin-containing cells reduced by low-level laser therapy (LLLT) in inflamed paws of Wistar rat. Photomed Laser Surg 23:32–35. https://doi.org/10.1089/pho.2005.23.32
21. Pereira FC, Parisi JR, Maglioni CB et al (2017) Antinociceptive effects of low-level laser therapy at 3 and 8 j/cm ^{2} in a rat model of postoperative pain: possible role of endogenous opioids: antinociceptive effects of low-level laser therapy. Lasers Surg Med 49:844–851. https://doi.org/10.1002/lsm.22696
22. Martins DF, Turnes BL, Cidral-Filho FJ et al (2016) Light-emitting diode therapy reduces persistent inflammatory pain: role of interleukin 10 and antioxidant enzymes. Neuroscience 324:485–495. https://doi.org/10.1016/j.neuroscience.2016.03.035
23. Belém MO, de Andrade GMM, Carlos TM et al (2016) Light-emitting diodes at 940nm attenuate colitis-induced inflammatory process in mice. J Photochem Photobiol B 162:367–373. https://doi.org/10.1016/j.jphotobiol.2016.07.008
24. Pigatto GR, Coelho IS, Aquino RS et al (2016) Light-emitting diode phototherapy reduces nocifensive behavior induced by thermal and chemical noxious stimuli in mice: evidence for the involvement of capsaicin-sensitive central afferent fibers. Mol Neurobiol. https://doi.org/10.1007/s12035-016-9887-1
25. Baroni BM, Rodrigues R, Freire BB et al (2015) Effect of low-level laser therapy on muscle adaptation to knee extensor eccentric training. Eur J Appl Physiol 115:639–647. https://doi.org/10.1007/s00421-014-3055-y
26. Cidral-Filho FJ, Martins DF, Moré AOO et al (2013) Light-emitting diode therapy induces analgesia and decreases spinal cord and sciatic nerve tumour necrosis factor-α levels after sciatic nerve crush in mice: light-emitting diode therapy analgesic effect. Eur J Pain 17:1193–1204. https://doi.org/10.1002/j.1532-2149.2012.00280.x
27. Cidral-Filho FJ, Mazzardo-Martins L, Martins DF, Santos ARS (2014) Light-emitting diode therapy induces analgesia in a mouse model of postoperative pain through activation of peripheral opioid receptors and the l-arginine/nitric oxide pathway. Lasers Med Sci 29:695–702. https://doi.org/10.1007/s10103-013-1385-3
28. de Almeida P, Lopes-Martins RAB, De Marchi T et al (2012) Red (660 nm) and infrared (830 nm) low-level laser therapy in skeletal muscle fatigue in humans: what is better? Lasers Med Sci 27:453–458. https://doi.org/10.1007/s10103-011-0957-3
29. Vanin AA, Miranda EF, Machado CSM et al (2016) What is the best moment to apply phototherapy when associated to a strength training program? A randomized, double-blinded, placebo-controlled trial: phototherapy in association to strength training. Lasers Med Sci. https://doi.org/10.1007/s10103-016-2015-7
30. Beck W, Gobatto CA (2013) Effects of maximum intensity aerobic swimming exercise until exhaustion at different times of day on the hematological parameters in rats. Acta Physiol Hung 100:427–434. https://doi.org/10.1556/APhysiol.100.2013.013
31. Balci SS, Pepe H (2012) Effects of gender, endurance training and acute exhaustive exercise on oxidative stress in the heart and skeletal muscle of the rat. Chin J Physiol. https://doi.org/10.4077/CJP.2012.BAA021
32. Isanejad A, Saraf ZH, Mahdavi M et al (2015) The effect of endurance training and downhill running on the expression of IL-1β, IL-6, and TNF-α and HSP72 in rat skeletal muscle. Cytokine 73:302–308. https://doi.org/10.1016/j.cyto.2015.03.013
33. Lamou B, Taiwe GS, Hamadou A et al (2016) Antioxidant and antifatigue properties of the aqueous extract of *Moringa oleifera* in rats subjected to forced swimming endurance test. Oxidative Med Cell Longev 2016:1–9. https://doi.org/10.1155/2016/3517824
34. de Almeida P, Lopes-Martins RÁB, Tomazoni SS et al (2011) Low-level laser therapy improves skeletal muscle performance, decreases skeletal muscle damage and modulates mRNA expression of COX-1 and COX-2 in a dose-dependent manner. Photochem Photobiol 87:1159–1163. https://doi.org/10.1111/j.1751-1097.2011.00968.x
35. Leal Junior ECP, Lopes-Martins RÁB, de Almeida P et al (2010) Effect of low-level laser therapy (GaAs 904 nm) in skeletal muscle fatigue and biochemical markers of muscle damage in rats. Eur J Appl Physiol 108:1083–1088. https://doi.org/10.1007/s00421-009-1321-1
36. Lopes-Martins RÁB, Marcos RL, Leonardo PS et al (2006) Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. J Appl Physiol 101:283–288. https://doi.org/10.1152/japplphysiol.01318.2005
37. Baumert P, Lake MJ, Stewart CE et al (2016) Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. Eur J Appl Physiol 116:1595–1625. https://doi.org/10.1007/s00421-016-3411-1
38. Douglas J, Pearson S, Ross A, McGuigan M (2016) Eccentric exercise: physiological characteristics and acute responses. Sports Med. https://doi.org/10.1007/s40279-016-0624-8
39. de Oliveira HA, Antonio EL, Silva FA et al (2018) Protective effects of photobiomodulation against resistance exercise-induced muscle damage and inflammation in rats. J Sports Sci 36:2349–2357. https://doi.org/10.1080/02640414.2018.1457419
40. Mantineo M, Pinheiro JP, Morgado AM (2014) Low-level laser therapy on skeletal muscle inflammation: evaluation of irradiation parameters. J Biomed Opt 19:98002. https://doi.org/10.1117/1.JBO.19.9.098002
41. Assis L, Moretti AIS, Abrahão TB et al (2012) Low-level laser therapy (808 nm) reduces inflammatory response and oxidative stress in rat tibialis anterior muscle after cryolesion. Lasers Surg Med 44:726–735. https://doi.org/10.1002/lsm.22077
42. Tomazoni SS, Frigo L, Dos Reis Ferreira TC et al (2017) Effects of photobiomodulation therapy and topical non-steroidal anti-inflammatory drug on skeletal muscle injury induced by contusion in rats-part 2: biochemical aspects. Lasers Med Sci. https://doi.org/10.1007/s10103-017-2299-2
43. McCarthy DA, Dale MM (1988) The leucocytosis of exercise. A review and model. Sports Med Auckl NZ 6:333–363
44. Kakanis MW, Peake J, Brenu EW et al (2010) The open window of susceptibility to infection after acute exercise in healthy young male elite athletes. Exerc Immunol Rev 16:119–137
45. Banfi G, Roi GS, Dolci A, Susta D (2004) Behaviour of haematological parameters in athletes performing marathons and ultramarathons in altitude ("skyrunners"). Clin Lab Haematol 26:373–377. https://doi.org/10.1111/j.1365-2257.2004.00642.x

46. Lippi G, Banfi G, Montagnana M et al (2010) Acute variation of leucocytes counts following a half-marathon run. Int J Lab Hematol 32:117–121. https://doi.org/10.1111/j.1751-553X.2008.01133.x
47. Mizumura K, Taguchi T (2016) Delayed onset muscle soreness: involvement of neurotrophic factors. J Physiol Sci 66:43–52. https://doi.org/10.1007/s12576-015-0397-0
48. Murase S, Terazawa E, Hirate K et al (2013) Upregulated glial cell line-derived neurotrophic factor through cyclooxygenase-2 activation in the muscle is required for mechanical hyperalgesia after exercise in rats. J Physiol 591:3035–3048. https://doi.org/10.1113/jphysiol.2012.249235
49. Murase S, Terazawa E, Queme F et al (2010) Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayed-onset muscle soreness). J Neurosci 30:3752–3761. https://doi.org/10.1523/JNEUROSCI.3803-09.2010
50. Yan W, Chow R, Armati PJ (2011) Inhibitory effects of visible 650-nm and infrared 808-nm laser irradiation on somatosensory and compound muscle action potentials in rat sciatic nerve: implications for laser-induced analgesia. J Peripher Nerv Syst JPNS 16:130–135. https://doi.org/10.1111/j.1529-8027.2011.00337.x
51. Lim W, Lee S, Kim I et al (2007) The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors. Lasers Surg Med 39:614–621. https://doi.org/10.1002/lsm.20533
52. Rennó ACM, Toma RL, Feitosa SM et al (2011) Comparative effects of low-intensity pulsed ultrasound and low-level laser therapy on injured skeletal muscle. Photomed Laser Surg 29:5–10. https://doi.org/10.1089/pho.2009.2715