Free Radical Research

ISSN: 1071-5762 (Print) 1029-2470 (Online) Journal homepage: http://www.tandfonline.com/loi/ifra20

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To cite this article: Paulo Cesar Lock Silveira, Debora da Luz Scheffer, Viviane Glaser, Aline Pertile Remor, Ricardo Aurino Pinho, Aderbal Silva Aguiar Junior & Alexandra Latini (2016): Low-level laser therapy attenuates the acute inflammatory response induced by muscle traumatic injury, Free Radical Research, DOI: 10.3109/10715762.2016.1147649

To link to this article: http://dx.doi.org/10.3109/10715762.2016.1147649

FREE RADICAL RESEARCH, 2016
http://dx.doi.org/10.3109/10715762.2016.1147649

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ORIGINAL ARTICLE

Low-level laser therapy attenuates the acute inflammatory response induced by muscle traumatic injury

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ABSTRACT

The purpose of this work was to investigate the effect of early and long-term low-level laser therapy (LLLT) on oxidative stress and inflammatory biomarkers after acute-traumatic muscle injury in Wistar rats. Animals were randomly divided into the following four groups: control group (CG), muscle injury group (IG), CG + LLLT, and IG + LLLT: laser treatment with doses of 3 and 5 J/cm^2. Muscle traumatic injury was induced by a single-impact blunt trauma in the rat gastrocnemius. Irradiation for 3 or 5 J/cm^2 was initiated 2, 12, and 24 h after muscle trauma induction, and the treatment was continued for five consecutive days. All the oxidant markers investigated. namely thiobarbituric acid-reactive substance, carbonyl, superoxide dismutase, glutathione peroxidase, and catalase, were increased as soon as 2 h after muscle injury and remained increased up to 24 h. These alterations were prevented by LLLT at a 3 J/cm^2 dose given 2 h after the trauma. Similarly, LLLT prevented the trauma-induced proinflammatory state characterized by IL-6 and IL-10. In parallel, trauma-induced reduction in BDNF and VEGF, vascular remodeling and fiber-proliferating markers, was prevented by laser irradiation. In order to test whether the preventive effect of LLLT was also reflected in muscle functionality, we tested the locomotor activity, by measuring distance traveled and the number of rearings in the open field test. LLLT was effective in recovering the normal locomotion, indicating that the irradiation induced biostimulatory effects that accelerated or resolved the acute inflammatory response as well as the oxidant state elicited by the muscle trauma.

ARTICLE HISTORY
Received 4 August 2015
Revised 19 January 2016
Accepted 25 January 2016
Published online
26 February 2016

KEYWORDS
Growth factors; inflammation; low-level laser therapy; muscle trauma; oxidative stress

Introduction

Since the mid-1960s [1], light energy has been used as a therapy for inflammatory processes and cell tropism, opening a new field in the interaction of electromagnetic energy with biological tissue. Photobiostimulation is the mechanism by which low-level laser therapy (LLLT) activates cellular function without causing significant tissue-level heating. LLLT can stimulate or inhibit certain biological process depending on the tissue type and the dose of the irradiation used. LLLT has been widely used to accelerate the resolution of inflammatory processes, skin wound repairing, rheumatic diseases, muscle injury, neuromuscular, and sports disorders, among others [2–6].

Regarding muscle damage, LLLT has shown to improve the process of muscular regeneration by promoting the generation of new myofibers [7,8] and by activating cellular processes linked to the stimulation of the antioxidant defenses and higher mitochondrial efficiency [9,10]. In this context Karu et al. [11] demonstrated that laser radiation is absorbed through mitochondrial chromophores, including complexes I and IV of the respiratory chain. Currently, it is understood that the photoactivation of mitochondrial components that are sensitive to the light laser can cause a transient activation in the respiratory chain components [12]. This enhanced mitochondrial capacity would provoke beneficial changes in the cellular redox state of both the cytoplasm and mitochondria, which may include increasing Krebs cycle turnover, with further production of ATP, and therefore protein synthesis and cell proliferation [13,14].

In addition, there is evidence demonstrating that LLLT reduces the production of reactive oxygen species (ROS) that occurs during the acute phase of skin and muscle injury [15–18]. Moreover, the reduction of ROS appears

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to be also involved in the anti-inflammatory effect of LLLT by reducing the activation of the transcription factor nuclear factor kappa B (NF-B) and the downstream interleukins, IL-1β and IL-6 [19,20]. These molecular mechanisms would accelerate the capillary hydrostatic pressure edema resorption and elimination of chemical mediator of inflammation [21].

After the initial muscle injury, oxidative stress could be increased due to a number of potential sites for ROS generation within the traumatized muscle [22]. Primary sources for free radicals may include mitochondria, xanthine oxidase enzymes, prostanoid metabolism, and NAD(P)H oxidases [23]. Additionally, the inflammatory status-associated cytokines will further promote the migration, proliferation, and survival of various cell types at the injury site, whereas the inflammatory cells will be responsible for the phagocytosis of cell debris [24]. Indeed, macrophage releases cytokines, which play a major role in muscle repair mechanism [25]. In line with this, the degree of the inflammatory response may determine the efficacy of muscle repair therapies. Therefore, it is feasible that earlier interventions during the inflammation might attenuate the deleterious effects induced by the muscular lesion.

Thus, the purpose of this work was to investigate the effect of early and long-term LLL irradiation on oxidative stress and inflammatory parameters after acute traumatic muscle injury. Furthermore, recovery of muscular functionality, vascular remodeling and fiber proliferative activity were also studied.

Methods

Animals

Male Wistar rats (250–300 g) obtained from the Central Animal House of the Universidade Federal de Santa Catarina (UFSC), Santa Catarina, Brazil, were caged in groups of six. offered commercial rat chow and water *ad libitum* and maintained on a 12-h light/12-h dark cycle. The animals were randomly divided into four groups ($n = 6$): control group (CG), muscle injury group (IG), CG + LLLT, and IG + LLLT: laser treatment with doses of 3 and 5 J/cm^2.

Induction of muscle injury

Muscle traumatic injury was induced as described by Rizzi [18]. Briefly, animals were submitted to muscle lesion after receiving an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (15 mg/kg). Gastrocnemius injury was induced by a single-impact blunt trauma in a press developed by the Industrial Center of Education and Research Equipment (CIDEP, Porto Alegre, RS, Brazil). Injury was elicited by a metal mass (0.459 kg) falling through a metal guide from a height of 18 cm. The impact kinetic energy delivered was 0.811 J. Sham rats were also anesthetized to ensure standardization, but without muscle trauma.

Laser irradiation

Animals received laser irradiation starting at three different time periods, 2, 12, and 24 h and lasting up to 5 days after injury. Irradiation for 3 or 5 J/cm^2 was initiated 2, 12, and 24 h after inducing muscle trauma, and the treatment was continued for five consecutive days. In all cases, LLL applications were done every 24 h after the first irradiation. Laser irradiation was performed over five distinct regions around the impact area (total area 2 cm^2) with the laser pen being kept perpendicular to the injury at a distance of 0.5 cm per point, as described by Pessoa [26]. Each treated point received 3 and 5 J/cm^2 or 0.3 and 0.5 J of energy density (9 and 12 s) using a previously calibrated semiconductor diode laser GaAs (Laserpulse. Ibramed) with pulsed emission (wavelength 904 nm, peak power 70 W, frequency 9.500 Hz, pulse time 60 ns, mean power output 40 mW, power density 400 mW/cm^2, spot size 0.10 cm^2) [15]. Equipment characteristics such as power and transmission time were measured by means of a laser power/energy monitor apparatus calibrated by National Institute Standards and Technology according to ISO 10012-1 to confirm the parameters of the device.

Induction of euthanasia and sample preparation

Two hours after the last irradiation, animals were killed by decapitation. The injured region of the gastrocnemius was surgically removed, immediately processed, and stored at −70 °C for subsequent analysis and homogenized in the appropriate buffer used for each biochemical technique. The homogenates were centrifuged at 1000 × *g* for 10 min at 4 °C, and the supernatants were kept at −70 °C until further used in the experiments. The maximal period between homogenate preparation and biochemical analysis was always <5 days.

Biochemical measurements

Griess nitrite assay

NO$^{\bullet}$ produced was estimated spectrophotometrically as nitrite formed (NO_2^-). To measure the NO_2^- content, the samples were incubated with the Griess reagent (1% sulfanilamide in 0.1 mol/l HCl and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride) at room temperature

(32 °C) for 10 min. Then, absorbance was measured at 540 nm using a microplate reader. Nitrite content was calculated based on a standard curve constructed with $NaNO_2$ [27]. Since no reducing agent or enzyme (nitrate reductase) was used to transform NO_3^- into NO_2^-, the positive reaction with the Griess reagent corresponds only to the NO_2^- species.

Lipid peroxidation

The formation of thiobarbituric acid-reactive substances (TBARS) during TBA-heating reaction was used as an index of lipid peroxidation, as previously described by Draper and Hadley [28]. Briefly, the samples were mixed with 1 ml of 10% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid. Subsequently, they were heated in a boiling water bath for 30 min. TBARS level was determined through absorbance at 532 nm using 1,1,3,3-tetramethoxypropane as an external standard. Results were expressed as TBARS level (nmol/mg protein).

Dityrosine autofluorescence determination

Dityrosine content, a measure of protein oxidation, was estimated by the fluorescence emitted at 410 nm. Briefly, 50 µl of plasma were added to 950 µl 6 M urea in 20 mM sodium phosphate buffer pH 7.4. After 30 min, samples were read using a fluorometer (excitation 315 nm; emission 410 nm). Results were expressed as fluorescence units per mg protein [29].

Protein carbonylation

Oxidative damage to proteins was measured by determining carbonyl groups based on the reaction with 2,4-dinitrophenylhydrazine (DNPH) [30]. Proteins were precipitated by adding 20% trichloroacetic acid and reacted with DNPH. The samples were then redissolved in 6 M guanidine hydrochloride; carbonyl contents were determined through absorbance at 370 nm using a extinction coefficient of $22{,}000 \times 10^6$ nM/ml for aliphatic hydrazones, and total protein contents were determined by absorbance of 270 nm in the same sample. Results were expressed as carbonyl level (nmol/mg protein).

Advanced oxidation protein products assay

The method for evaluating the contents of advanced oxidation protein products (AOPPs) was performed by spectrophotometry with a wavelength of 340 nm according to Witko-Sarsat [31]. Results are expressed in nmol of equivalents of chloramine T/mg protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm, as previously described [32]. Results were expressed in U SOD/mg protein.

Glutathione peroxidase assay

Glutathione peroxidase (GPX) activity was measured by using tert-butyl-hydroperoxide as substrate [33]. Enzyme activity was measured by monitoring the rate of disappearance of NADPH at 340 nm in 50 mM potassium phosphate buffer, pH 7.0, containing 1.0 mM EDTA, 2.0 mM glutathione (GSH), 0.2 U/ml GSH reductase, 1.0 mM azide, 0.2 mM tert-butyl-hydroperoxide, 0.2 mM NADPH, and supernatant containing 0.2–0.3 mg protein/ml. GPX activity was expressed as nmol of NADPH oxidized per minute per milligram of protein, using an extinction coefficient of 6.22 mM^{-1} cm^{-1} for NADPH.

Catalase activity

Catalase (CAT) activity was measured by the rate of decrease in hydrogen peroxide as absorbance at 240 nm [34], and results were expressed in U CAT/mg protein.

Quantitative reverse transcription PCR

Total RNA was extracted from muscle tissue using Trizol (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Single-stranded cDNAs were synthesized with random primers (Invitrogen) in 20 µl of reaction. Briefly, 1 µg of total RNA was incubated with these primers (1.25 ng/ml), and reverse transcriptase reaction was performed as previously described [35]. For real-time PCR, cDNA was mixed with 16SYBR Green.

PCR Master Mix (Applied Biosystems, Waltham, MA) and the forward and reverse primers were added to a final volume of 15 ml. Primer concentration was 300 nm, and the sequences used are listed in Table 1. Real-time PCR was carried out on an ABI 7500. Applied Biosystems with Sequence Detection Software v1.4. The cycling conditions included a hot start at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Specificity was verified by melting curve analysis and agarose gel electrophoresis. Each sample was analyzed in triplicate. Transcript levels were normalized to those of cyclophilin A, and relative expression levels were calculated using the 2–DDCt method [35]. Amplification efficiency for each set of primers was near 98%. RNA samples incubated without reverse transcriptase during cDNA synthesis, as well as PCR

Table 1. Sequences of primers employed for the determinations.

Originator	Sequence "Forward"	Sequence "Reverse"
VEGF	ATCATGCGGATCAAACCTCACC	GGTCTGCATTCACATCTGCTAT
BDNF	AAGGCTGCAGGGGCATAGAC	TGAACCGCCAGCCAATTCTC
IL-6	AAGAGACTTCCAGCCAGTTGCC	ACTGGTCTGTTGTGGGTGGTA
IL-10	GCCAAGCCTTGTCAGAAATGA	TTTCTGGGCCATGGTTCTCT

using water instead of template showed no amplification.

Behavioral test

Open field

We evaluated the effects of the laser treatment on behavior after 5 days from the muscle traumatic injury like motor activity marker according to open field arena. The apparatus, made of wood covered with impermeable formica, had a white floor of 60 cm × 50 cm (divided by black lines into 25 squares of 20 cm × 20 cm) and white walls 40 cm high. Each rat was placed in the center of the open field, and the distance (Meters) and rearing were registered in 5 min [36].

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test when p values were significant ($p < 0.05$). All analyses were performed using the Statistical Package for the Social Science (SPSS. v 17.0; IBM Corp., Armonk, NY) software.

Results

Effect of traumatic muscle lesion on oxidative stress parameters 2, 12, and 24 h after injury

Figure 1(A and B) shows that TBA-RS [$F_{(3,16)}$=6.82, $p < 0.01$] and protein carbonyl levels [$F_{(3,18)} = 5.81$, $p < 0.01$] were significantly increased 2, 12, and 24 h after muscle injury induction. Figure 1(C and D) also shows that the activity of the antioxidant enzymes SOD [$F_{(3,20)}$=5.88, $p < 0.01$], GPX [$F_{(3,18)}$=8.34, $p < 0.01$], and CAT [$F_{(3,18)}$=7.54, $p < 0.01$] was also significantly increased after the muscle lesion.

Effect of 5-day 3 J/cm^2 GaAs LLL irradiation on oxidative stress parameters after the induction of traumatic muscle lesion

Table 2 shows the effect of 3 J/cm^2 GaAs LLLT starting the irradiation 2, 12, and 24 h after the muscle injury with treatment lasting for up to 5 days on TBA-RS and protein carbonyl levels and antioxidant enzymes activities in injured skeletal muscle. It can be observed that TBARS [$F_{(3,20)}$=8.37, $p < 0.05$] and protein carbonyl levels [$F_{(3,19)}$=12.75, $p < 0.05$], and the activity of the antioxidant enzymes SOD [$F_{(3,20)}$=5.42, $p < 0.05$], GPX [$F_{(3,20)}$=5.43, $p < 0.05$], and CAT [$F_{(3,20)}$=8.45, $p < 0.05$] were significantly increased 5 days after the trauma and that these parameters were significantly normalized by 3 J/cm^2 GaAs LLLT, when starting the irradiation 2 h after the traumatic injury. Similar trauma-induced profiles were observed when the irradiation started 12 and 24 h after the muscle injury (12 h after injury: TBARS levels [$F_{(3,20)}$=10.67, $p < 0.05$], protein carbonyl levels [$F_{(3,19)}$=3.54, $p < 0.05$], GPX activity [$F_{(3,20)}$=3.04, $p < 0.05$], CAT activity [$F_{(3,18)}$=12.8, $p < 0.05$]; 24 h after injury: TBARS levels [$F_{(3,19)}$=11.25, $p < 0.05$], SOD activity [$F_{(3,20)}$=6.99, $p < 0.05$], CAT activity [$F_{(3,18)}$=10.04, $p < 0.05$]). However, only TBARS and protein carbonyl levels and CAT activity parameters were reverted by laser irradiation starting at 12 h after muscle lesion. In addition, only TBARS levels and CAT activity alterations were prevented by 3 J/cm^2 GaAs LLLT starting 24 h after muscle trauma. This first set of results shows that 3 J/cm^2 GaAs irradiation is more effective in preventing the induction of the oxidative stress status in the traumatic muscle if applied early (2 h after injury).

Effect of 5-day 5 J/cm^2 GaAs LLL irradiation on oxidative stress parameters after the induction of traumatic muscle lesion

Table 3 shows the effect of 5 J/cm^2 GaAs LLLT starting the irradiation 2, 12, and 24 h after the muscle injury with treatment lasting for up to 5 days on TBA-RS and protein carbonyl levels and antioxidant enzyme activities in injured skeletal muscle. It can be observed in the Table 2 that the lesion induced similar alterations in the oxidative stress parameters assessed in the injured muscle, as those observed before in Table 1 (LLLT initiated 2 h after injury: TBARS levels [$F_{(3,18)}$=24.08, $p < 0.05$], protein carbonyl levels [$F_{(3,20)}$=11.22, $p < 0.05$]. SOD activity [$F_{(3,19)}$=9.01, $p < 0.05$], GPX activity [$F_{(3,20)}$=6.94, $p < 0.05$], CAT activity [$F_{(3,18)}$=7.16, $p < 0.05$]; LLLT initiated 12 h after injury: protein carbonyl levels [$F_{(3,20)}$=4.71, $p < 0.05$], SOD activity [$F_{(3,20)}$=3.21, $p < 0.05$], GPX activity [$F_{(3,19)}$=8.24, $p < 0.05$], CAT activity [$F_{(3,18)}$=6.35, $p < 0.05$]; LLLT initiated 24 h after injury: TBARS levels [$F_{(3,19)}$=4.27, $p < 0.05$]. protein carbonyl levels [$F_{(3,18)}$=7.64, $p < 0.05$], SOD activity [$F_{(3,20)}$=8.78, $p < 0.05$], GPX activity [$F_{(3,19)}$=5.46, $p < 0.05$], CAT activity [$F_{(3,18)}$=10.04, $p < 0.05$]). These parameters were all significantly normalized by 5 J/cm^2 GaAs LLLT, starting the irradiation 2 h after injury. However, laser irradiation starting at 12 h after muscle lesion had no effect on the TBARS levels and SOD activity. Moreover, laser irradiation

starting at 24 h after muscle lesion only normalized the protein carbonyl levels.

Considering that 3 and 5 J/cm^2 GaAs irradiation induced similar positive effects in the injured muscle, the following experiments were performed only at the lower GaAs dose and starting the laser application 2 h after the trauma, with the application lasting for up to 5 days.

Effect of 5-day 3 J/cm^2 GaAs LLL irradiation, starting 2 h after the traumatic muscle lesion on plasmatic and/or muscle oxidative stress biomarkers

Figure 2(A and B) shows the effect of 5-day 3 J/cm^2 GaAs LLL irradiation, starting 2 h after the trauma, on nitrite concentrations in plasma and injured muscle. It could be observed in the figure that the trauma-

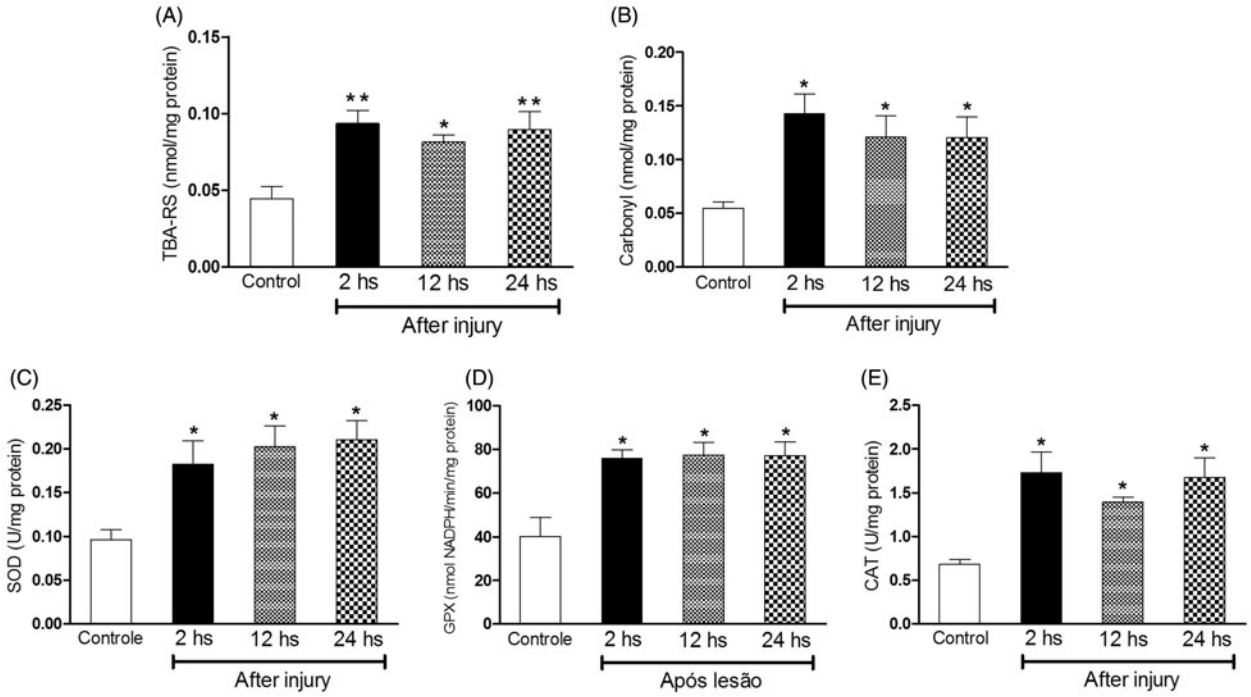

Figure 1. Effect induced by traumatic muscle injury on the content of TBA-RS (A), protein carbonyls (B), activity of antioxidant enzymes SOD (C), GPx (D), and CAT (E) 2, 12, and 24 h after injury. The bars represent the mean ± SEM for six animals. *$p < 0.05$. **$p < 0.01$ versus control (one-way ANOVA followed by *post hoc* Tukey test).

Table 2. Effect of low-power laser (GaAs at 3 J/cm^2 dose) irradiation 2, 12, and 24 h after muscle injury on the content of TBA-RS, protein carbonylation, and activity of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in homogenate of skeletal muscle.

Outset of irradiation 2 h after injury	Biochemical assays				
	TBA-RS	Carbonylation	SOD	GPX	CAT
Control group (CG)	0.05 ± 0.003	0.21 ± 0.01	0.09 ± 0.01	2.07 ± 0.20	2.19 ± 0.19
Injury group (IG)	0.08 ± 0.006*	0.34 ± 0.02*	0.18 ± 0.02*	3.00 ± 0.31*	3.55 ± 0.44*
CG + LLLT (3 J/cm^2)	0.06 ± 0.003	0.28 ± 0.01	0.14 ± 0.01	2.21 ± 0.17	2.77 ± 0.30
IG + LLLT (3 J/cm^2)	0.05 ± 0.003#	0.23 ± 0.01#	0.08 ± 0.01#	1.72 ± 0.20#	1.39 ± 0.25#
12 h after injury					
Control group (CG)	0.02 ± 0.002	0.15 ± 0.007	0.23 ± 0.02	2.38 ± 0.32	2.02 ± 0.12
Injury group (IG)	0.06 ± 0.001*	0.28 ± 0.03*	0.27 ± 0.02	3.91 ± 0.56*	3.00 ± 0.34*
CG + LLLT (3 J/cm^2)	0.02 ± 0.002	0.21 ± 0.05	0.30 ± 0.04	1.81 ± 0.37	3.16 ± 0.39
IG + LLLT (3 J/cm^2)	0.01 ± 0.001#	0.14 ± 0.01#	0.34 ± 0.03	3.25 ± 0.33	0.92 ± 0.20#
24 h after injury					
Control group (CG)	0.12 ± 0.01	0.17 ± 0.01	7.2 ± 0.45	2.77 ± 0.27	
Injury group (IG)	0.20 ± 0.03*	0.24 ± 0.01*	6.82 ± 0.44	4.40 ± 0.49*	
CG + LLLT (3 J/cm^2)	0.09 ± 0.006#	0.17 ± 0.006#	6.84 ± 0.51	3.35 ± 0.36	
IG + LLLT (3 J/cm^2)	0.09 ± 0.004#	0.19 ± 0.01	7.55 ± 0.43	1.78 ± 0.21#	

Irradiation started 2 h after inducing the trauma and lasted during five consecutive days. The bars represent the mean ± SEM for six animals. *$p < 0.05$ versus control. #$p < 0.05$ versus IG (ANOVA one-way followed by *post hoc* Tukey test).

Table 3. Effect of low-power laser (GaAs at 5 J/cm^2 dose) irradiation 2, 12, and 24 h after muscle injury on the content of TBA-RS, protein carbonylation, and activity of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in homogenate of skeletal muscle.

	Biochemical assays				
Outset of irradiation	TBA-RS	Carbonylation	SOD	GPX	CAT
2 h after injury					
Control group (CG)	0.11 ± 0.01	0.19 ± 0.02	0.09 ± 0.01	3.76 ± 0.25	3.66 ± 0.23
Injury group (IG)	0.21 ± 0.02*	0.39 ± 0.06*	0.24 ± 0.03*	5.34 ± 0.32*	5.67 ± 0.55*
CG + LLLT (5 J/cm^2)	0.14 ± 0.04	0.29 ± 0.02	0.17 ± 0.03	4.84 ± 0.42	4.88 ± 0.22
IG + LLLT (5 J/cm^2)	0.07 ± 0.01#	0.21 ± 0.04#	0.13 ± 0.02#	3.30 ± 0.20#	3.97 ± 0.54#
12 h after injury					
Control group (CG)	0.03 ± 0.006	0.04 ± 0.004	0.11 ± 0.014	4.25 ± 0.11	2.17 ± 0.11
Injury group (IG)	0.035 ± 0.003	0.09 ± 0.006*	0.19 ± 0.018*	5.95 ± 0.52*	3.64 ± 0.41*
CG + LLLT (5 J/cm^2)	0.02 ± 0.004	0.06 ± 0.004#	0.14 ± 0.02	4.13 ± 0.32#	2.95 ± 0.46
IG + LLLT (5 J/cm^2)	0.02 ± 0.003	0.06 ± 0.008#	0.13 ± 0.003	4.20 ± 0.40#	1.62 ± 0.19#
24 h after injury					
Control group (CG)	0.79 ± 0.02	0.06 ± 0.008	0.07 ± 0.011	6.92 ± 0.64	3.79 ± 0.15
Injury group (IG)	1.10 ± 0.20*	0.13 ± 0.03*	0.15 ± 0.005*	9.98 ± 1.83*	7.30 ± 0.56*
CG + LLLT (5 J/cm^2)	0.69 ± 0.08	0.07 ± 0.005#	0.08 ± 0.007	7.81 ± 1.05	6.23 ± 0.65*
IG + LLLT (5 J/cm^2)	1.00 ± 0.07	0.06 ± 0.009#	0.09 ± 0.008	9.30 ± 0.82	5.34 ± 0.55

Irradiation started 2 h after inducing the trauma and lasted during five consecutive days. The bars represent the mean ± SEM for six animals. *$p < 0.05$ versus control. #$p < 0.05$ versus IG (ANOVA one-way followed by *post hoc* Tukey test).

induced increased levels of nitrite were significantly reduced by the LLL treatment in plasma [$F_{(3,18)}$=9.21, $p < 0.05$] and also in the rat muscle [$F_{(3,19)}$=4.88, $p < 0.05$]. Similarly, the plasma protein oxidation biomarkers, including the trauma-induced increased levels of AOPP [$F_{(3,16)}$=48.45, $p < 0.05$] and dityrosine [$F_{(3,17)}$=27.11, $p < 0.05$] levels, were significantly reduced by the LLLT (Figure 2C and D).

Effect of 5-day 3 J/cm^2 GaAs LLL irradiation, starting 2 h after the traumatic muscle lesion on muscular inflammatory markers

Figure 3 shows the triggering effect of 5-day 3 J/cm^2 GaAs LLL irradiation, starting 2 h after the trauma, on the expression of interleukins IL-6 and IL-10 and VEGF and BDNF, factors that promote vascular endothelial growth and myosatellite cell proliferation. It is shown in the figure that trauma provoked a significant increase in the mRNA content of the proinflammatory cytokine IL-6 [$F_{(3,8)}$=35.01, $p < 0.05$], VEGF [$F_{(3,9)}$=19.31, $p < 0.05$], and BDNF [$F_{(3,8)}$=14.80, $p < 0.05$], while the gene expression for IL-10 was significantly reduced [$F_{(3,6)}$=7.10, $p < 0.05$]. This effect was completely abolished by the 5-day 3 J/cm^2 GaAs LLL treatment. Additionally, the LLLT *per se* elicited higher and significant expression of IL-10.

Effect of 5-day 3 J/cm^2 GaAs LLL irradiation, starting 2 h after the traumatic muscle lesion on motor and functional muscular measurements

In order to assess whether the preventive effect of LLLT shown above has a significant impact in improving the functionality of the traumatized muscle, the locomotor activity, and the number of rearings were measured in the behavioral test apparatus, open field. Figure 4(A and B) shows a significant reduction in the distance traveled [$F_{(2,20)}$=11.42, $p < 0.05$] and in the number of rearings [$F_{(2,21)}$=14.19, $p < 0.05$] in traumatized animals. The figures also show that the laser irradiation treatment significantly reverted the negative effect of trauma on muscular functionality or locomotor activity.

Discussion

We used a model of traumatic muscle injury in rats to evaluate the influence of early LLLT on oxidative stress parameters and inflammatory mediators in the injured area. as well as the functional recovery of the targeted muscle. First, we assessed whether the injury model induced changes in oxidative stress parameters 2, 12, and 24 h after injury. As seen in Figure 1, the parameters had a significant increase in all the investigated time points. This model reproduced with efficiency a traumatic muscle injury, where an inflammatory response characterized by a elevation in oxidant stress biomarkers is generated [37,38].

LLLT has been shown to be effective in reducing the inflammatory response, promoting tissue repair, decreasing pain, and reducing fatigue in several studies, even in animal models or in clinical studies [39–42]. The GaAs laser irradiation (904 nm) with high peak power is demonstrated to be more efficient in muscular injuries than low-power infrared wavelengths (780–860 nm) whose outputs are continuous [43–45]. According to the World Association of Laser Therapy (2006), the GaAs

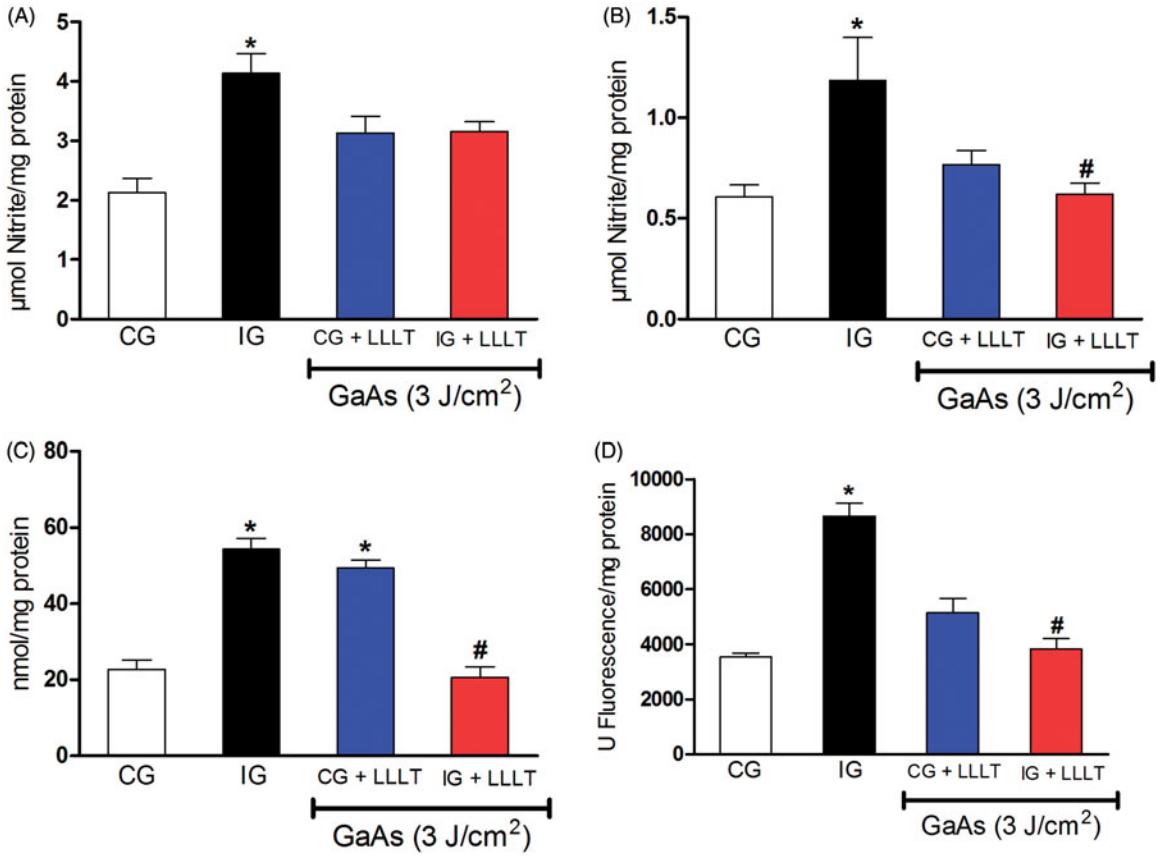

Figure 2. Effect of low-power laser (GaAs at 3 J/cm^2 dose) irradiation (five consecutive days) after muscle injury on the concentration of nitrite in plasma (A) and muscle (B), and the content of AOPPs (C) and dityrosine (D) in plasma. Irradiation started 2 h after inducing the trauma. Nitrites and AOPPs levels were assessed spectrophotometrically and dityrosine was estimated by following the fluorescence emission at 410 nm. The bars represent the mean ± SEM for six animals. *$p < 0.05$ versus CG. #$p < 0.05$ versus IG (one-way ANOVA followed by *post hoc* Tukey test).

laser (904 nm) is recommended for musculoskeletal injuries [46].

Because of this, we decided to investigate the effect of the treatment with LLLT starting 2, 12, and 24 h after induction of muscle injury with two doses 3 and 5 J/cm^2 with 5 days of duration on oxidative stress parameters. As we can see (Tables 2 and 3), the groups that started treatment 2 h after injury in a dose of 3 and 5 J/cm^2 had a significant decrease compared to animals with muscle injury in all oxidative stress parameters analyzed, showing that the reduction in nonenzymatic oxidative stress can be induced earlier. Furthermore, the antioxidant status, namely GPX, SOD, and CAT activities, was recovered after LLLT. Even, when expression or content of these proteins were not measured in this study, the final biological result of an activated pathway will be the activity of the enzyme/protein. Therefore, it is feasible to propose that LLLT reset up the antioxidant status in the muscle, which probably will result in better recovery of the injured tissue. We decided to use a dose of 3 J/cm^2 with the outset of the irradiation 2 h after injury in the following experiments.

As shown in Figure 2, it is possible to suggest that the muscle injury induced an increase in markers of protein oxidation (AOPP and dityrosine) and the content of nitrite in plasma and in the injury muscle, and that the LLLT group decrease all parameters except the plasma levels of nitrite. LLLT may have induced a reduction in the oxidation of plasma proteins probably due to decreased production of ROS and RNS at the site of the injury. It is widely described that during inflammation there is an increase in NO generation and its downstream products (as shown in Figure 2A), favoring the establishment of oxidative stress, which may amplify the damage and alter the structure and function of proteins and lipids in cell membranes [47,48]. Such events may lead to increasing the signaling of cellular pathways that exacerbate the inflammatory response [48,49]. The LLLT caused a reduction in the oxidant and inflammatory biomarkers, indicating that LLLT has a positive biological effect by modulating the redox state and accelerating the recovery of injured tissue. In line with this, LLLT also reduced or resolved the acute inflammatory response, by diminishing the gene

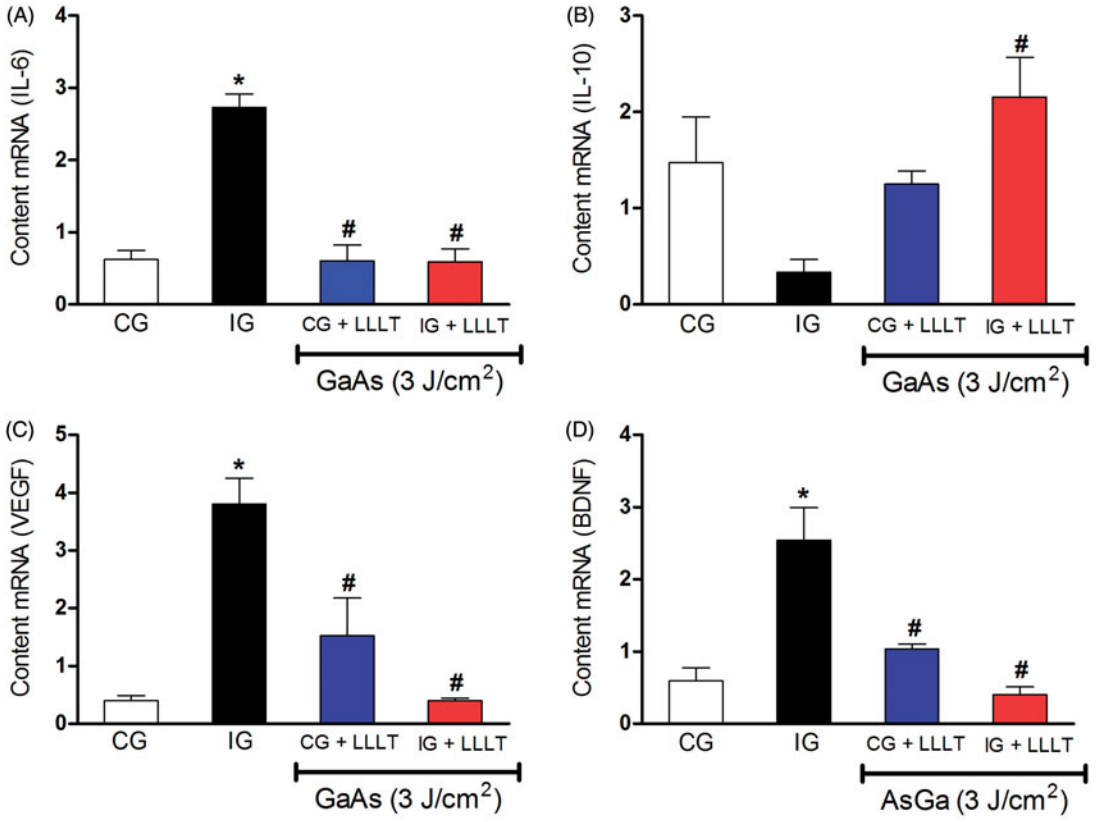

Figure 3. Effect of low-power laser (GaAs at 3 J/cm^2 dose) irradiation (five consecutive days) after muscle injury on the gene expression of IL-6 (A), IL-10 (B), vascular endothelial growth factor (VEGF; C) and brain derived neurotrophic factor (BDNF; D) in the injured skeletal muscle. Irradiation started 2 h after inducing the trauma. The bars represent the mean ± SEM for six animals. *$p < 0.05$ versus CG #$p < 0.05$ versus IG (one-way ANOVA followed by *post hoc* Tukey test).

expression of IL-6 with concomitant increase in IL-10 expression (Figure 3A and B). Some studies have shown that LLLT accelerates the resolution of the inflammatory acute phase, decreasing its deleterious effects, due to stimulation of the respiratory chain and, consequently, more efficient ATP production, or by reducing RNO and ROS production at site of the trauma [41,50–52].

Five days after the traumatic injury, the muscle tissue is still at an early stage of the acute inflammatory process, characterized by swelling as a result of macrophages and neutrophil infiltration [53]. At this point, there is also a parallel increase in VEGF expression due to disruption of the sarcomeres and vascular structure in the muscle fibers, which in turn will induce extravasation of blood and local hypoxia [54]. The promoter region of the VEGF gene contains a hypoxia response element (HRE) which, once activated, induces phosphorylation of HIF-1α, and when that happens, the transcription of VEGF is increased [55]. Moreover, some other studies also suggest VEGF expression could be activated by inflammatory molecules, ROS generation, and/or low energy state [56–60].

In adult skeletal muscle, BDNF expression is found in myogenic progenitors known as satellite cells [61]. In healthy adult skeletal muscle, satellite cells are mitotically quiescent, sequestered between the basement membrane and sarcolemma of myofibers [62,63]. In response to injury, however, they become activated, triggering proliferation and differentiation to repair damaged fibers [64,65]. Muscle injury also results in upregulation of BDNF expression at a time when satellite cell activation and proliferation occur, suggesting that BDNF may play a role in mediating the satellite cell response to injury, particularly in the acute response [66,67]. According to Tidball [53], BDNF has a similar role to other inflammatory cytokines (IL-6 and TGF-β) in the regulation of muscle, which showed that BDNF expression was proportional to the extent of invasion of T cells and macrophages [68,69]. As shown in Figure 3(C and D), the laser application induced a decrease in gene expression of these proteins, VEGF and BDNF, demonstrating an anti-inflammatory effect that can be justified, hypothetically due to the stimulus of the local metabolism and more efficient resolution of the acute inflammatory response.

To confirm this hypothesis, we analyzed behavioral parameters of motor activity, such as traveled distance and number of rearing in the open field test. The Figure 4(A and B) suggests that the group that received GaAs laser with a dose of 3 J/cm^2 starting the irradiation 2 h

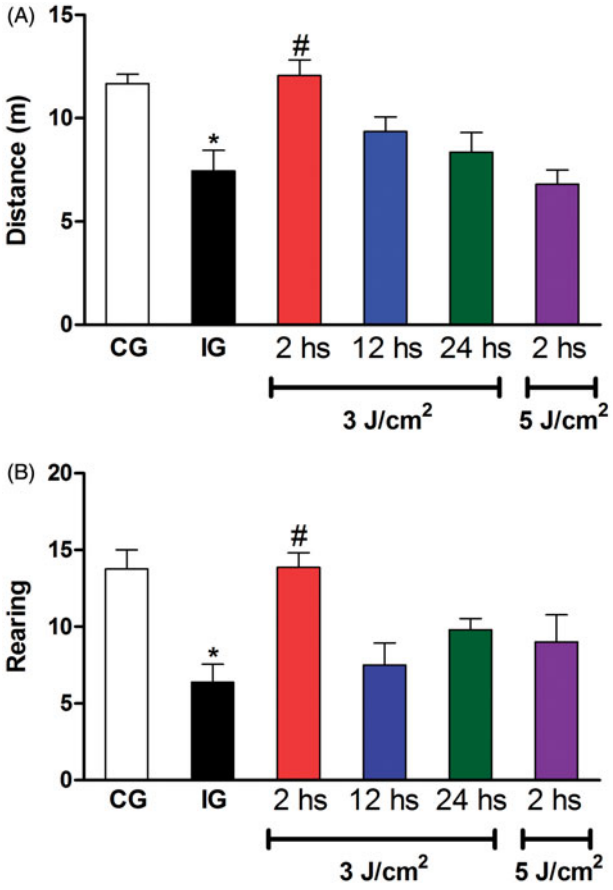

Figure 4. Effect of low-power laser (GaAs at 3 and 5 J/cm^2 dose) irradiation (five consecutive days) after muscle injury on the traveled distance (A) and the number of rearings (B) in the open field apparatus. Irradiation started 2, 12, and 24 h after inducing the trauma. The bars represent the mean ± SEM for six animals. *$p < 0.05$ versus CG. #$p < 0.05$ versus IG. and $p < 0.05$ versus IG with GaAs and 3 J/cm^2 dose (one-way ANOVA followed by *post hoc* Tukey test).

after injury, showed an improvement in locomotor and exploratory activity in relation to the IG corroborating earlier results. However, when irradiation was started 12 or 24 h after the trauma induction, or even when higher irradiation dose was used, the recovery effect of LLLT was lost. This suggests that the sooner the inflammation and oxidant status is resolved, the faster is the functional recovery of the traumatic muscle.

Taken together, these findings strongly suggest that laser irradiation induced biostimulatory processes that accelerated or resolved the acute inflammatory response, as well as, the oxidant state elicited by the muscle trauma and this effect was dose and time dependent.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Funding information

This work was supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil, 479222/2013-4), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil, PVE: 004_2013 and 88881.062164/2014-01, INCT 189405 16/2014), FAPESC/CNPq (Programa de Apoio a Núcleos de Excelência PRONEX; NENASC Project). Latini A is a CNPq fellow (311451/2013-6).

References

[1] Mester E. The use of the laser beam in therapy. Orv Hetil 1966;107:1012–1016.
[2] Ribeiro MS, Da Silva Dde F, De Araujo CE, De Oliveira SF, Pelegrini CM, Zorn TM, Zezell DM. Effects of low-intensity polarized visible laser radiation on skin burns: a light microscopy study. J Clin Laser Med Surg 2004;22:59–66.
[3] Giuliani A, Fernandez M, Farinelli M, Baratto L, Capra R, Rovetta G, et al. Very low level laser therapy attenuates edema and pain in experimental models. Int J Tissue React 2004;26:29–37.
[4] Rochkind S, Drory V, Alon M, Nissan M, Ouaknine GE. Laser phototherapy (780 nm), a new modality in treatment of long-term incomplete peripheral nerve injury: a randomized double-blind placebo-controlled study. Photomed Laser Surg 2007;25:436–442.
[5] Rubio CR, Cremonezzi D, Moya M, Soriano F, Palma J, Campana V. Helium-neon laser reduces the inflammatory process of arthritis. Photomed Laser Surg 2010;28:125–129.
[6] Shefer G, Barash I, Oron U, Halevy O. Low-energy laser irradiation enhances de novo protein synthesis via its effects on translation-regulatory proteins in skeletal muscle myoblasts. Biochim Biophys Acta 2003;1593:131–139.
[7] Baptista J, Martins MD, Pavesi VC, Bussadori SK, Fernandes KP, Pinto Junior Ddos S, Ferrari RA. Influence of laser photobiomodulation on collagen IV during skeletal muscle tissue remodeling after injury in rats. Photomed Laser Surg 2011;29:11–17.
[8] Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. J Biomed Sci 2009;16:1–16.
[9] Lakyova L, Toporcer T, Tomeckova V, Sabo J, Radonak J. Low-level laser therapy for protection against skeletal muscle damage after ischemia-reperfusion injury in rat hindlimbs. Lasers Surg Med 2010;42:665–672.
[10] Yasukawa A, Hrui H, Koyama Y, Nagai M, Takakuda K. The effect of low reactive-level laser therapy (LLLT) with helium-neon laser on operative wound healing in a rat model. J Vet Med Sci 2007;69:799–806.
[11] Karu TI. Multiple roles of cytochrome c oxidase in mammalian cells under action of red and IR-A radiation. IUBMB Life 2010;62:607–610.
[12] Manteifel VM, Karu TI. Structure of mitochondria and activity of their respiratory chain in subsequent generations of yeast cells exposed to He-Ne laser light. Izv Akad Nauk Ser Biol 2005;6:672–683.
[13] Lubart R, Eichler M, Lavi R, Friedman H, Shainberg A. Low-energy laser irradiation promotes cellular redox activity. Photomed Laser Surg 2005;23:3–9.

[14] Karu TI. Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. Photochem Photobiol 2008;84:1091–1099.
[15] Silveira PC, Silva LA, Freitas TP, Latini A, Pinho RA. Effects of low-power laser irradiation (LPLI) at different wavelengths and doses on oxidative stress and fibrogenesis parameters in an animal model of wound healing. Lasers Med Sci 2011;26:125–131.
[16] Silveira PC, da Silva LA, Pinho CA, De Souza PS, Ronsani MM, Scheffer Dda L, Pinho RA. Effects of low-level laser therapy (GaAs) in an animal model of muscular damage induced by trauma. Lasers Med Sci 2013;28:431–436.
[17] Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. Am J Physiol Regul Integr Comp Physiol 2010;298:1173–1187.
[18] Rizzi CF, Mauriz JL, Freitas Correa DS, Moreira AJ, Zettler CG, Filippin LI, et al. Effects of low-level laser therapy (LLLT) on the nuclear factor (NF)-kappaB signaling pathway in traumatized muscle. Lasers Surg Med 2006;38:704–713.
[19] Pires D, Xavier M, Araujo T, Silva JA Jr, Aimbire F, Albertini R. Low-level laser therapy (LLLT; 780 nm) acts differently on mRNA expression of anti- and pro-inflammatory mediators in an experimental model of collagenase-induced tendinitis in rat. Lasers Med Sci 2011;26:85–94.
[20] Basso FG, Pansani TN, Turrioni AP, Bagnato VS, Hebling J, de Souza Costa CA. In vitro wound healing improvement by low-level laser therapy application in cultured gingival fibroblasts. Int J Dent 2012;2012:1–6.
[21] da Silva JP, da Silva MA, Almeida AP, Lombardi Junior I, Matos AP. Laser therapy in the tissue repair process: a literature review. Photomed Laser Surg 2010;28:17–21.
[22] Bar-Shai M, Carmeli E, Ljubuncic P, Reznick AZ. Exercise and immobilization in aging animals: the involvement of oxidative stress and NF-kappaB activation. Free Radic Biol Med 2008;44:202–214.
[23] Halliwell B. Biochemistry of oxidative stress. Biochem Soc Trans 2007;35:1147–1150.
[24] Li YP. TNF-alpha is a mitogen in skeletal muscle. Am J Physiol Cell Physiol 2003;285:370–376.
[25] Serrano AL, Baeza-Raja B, Perdiguero E, Jardi M, Munoz-Canoves P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. Cell Metab 2008;7:33–44.
[26] Pessoa ES, Melhado RM, Theodoro LH, Garcia VG. A histologic assessment of the influence of low-intensity laser therapy on wound healing in steroid-treated animals. Photomed Laser Surg 2004;22:199–204.
[27] Hevel JM, Marletta MA. Nitric oxide synthase assays. Methods Enzymol 1994;233:250–258.
[28] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–431.
[29] Kirschbaum B. Correlative studies of urine fluorescence and free radical indicators. Clin Nephrol 2002;58:344–349.
[30] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–478.
[31] Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996;49:1304–1313.
[32] Bannister JV, Calabrese L. Assays for superoxide dismutase. Methods Biochem Anal 1987;32:279–312.
[33] Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114–121.
[34] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–126.
[35] Jiang L, Saetre P, Radomska KJ, Jazin E, Lindholm Carlstrom E. QKI-7 regulates expression of interferon-related genes in human astrocyte glioma cells. PLoS One 2010;5:1–16.
[36] Aguiar AS Jr, Araujo AL, da-Cunha TR, Speck AE, Ignacio ZM, De-Mello N, Prediger RD. Physical exercise improves motor and short-term social memory deficits in reserpinized rats. Brain Res Bull 2009;79:452–457.
[37] Huard J, Cao B, Qu-Petersen Z. Muscle-derived stem cells: potential for muscle regeneration. Birth Defects Res C Embryo Today 2003;69:230–237.
[38] Powers SK, Talbert EE, Adhihetty PJ. Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle. J Physiol (Lond) 2011;589:2129–2138.
[39] Douris P, Southard V, Ferrigi R, Grauer J, Katz D, Nascimento C, Podbielski P. Effect of phototherapy on delayed onset muscle soreness. Photomed Laser Surg 2006;24:377–382.
[40] Leal Junior EC, Lopes-Martins RA, Frigo L, De Marchi T, Rossi RP, de Godoi V, et al. Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. J Orthop Sports Phys Ther 2010;40:524–532.
[41] Servetto N, Cremonezzi D, Simes JC, Moya M, Soriano F, Palma JA, Campana VR. Evaluation of inflammatory biomarkers associated with oxidative stress and histological assessment of low-level laser therapy in experimental myopathy. Lasers Surg Med 2010;42:577–583.
[42] Chen AC, Arany PR, Huang YY, Tomkinson EM, Sharma SK, Kharkwal GB, et al. Low-level laser therapy activates NF-kB via generation of reactive oxygen species in mouse embryonic fibroblasts. PLoS One 2011;6:1–8.
[43] Bjordal JM, Couppe C, Ljunggren AE. Low level laser therapy for tendinopathy. Evidence of a dose–response pattern. Phys Ther Rev 2001;6:91–99.
[44] Bjordal JM, Couppe C, Chow RT, Tuner J, Ljunggren EA. A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. Aust J Physiother 2003;49:107–116.
[45] Enwemeka CS. Attenuation and penetration of visible 632.8 nm and invisible infra-red 904nm light in soft tissues. Laser Ther 2001;13:95–101.
[46] World Association of Laser T. Consensus agreement on the design and conduct of clinical studies with low-level laser therapy and light therapy for musculoskeletal pain and disorders. Photomed Laser Surg 2006;24:761–762.
[47] Filippin LI, Moreira AJ, Marroni NP, Xavier RM. Nitric oxide and repair of skeletal muscle injury. Nitric Oxide 2009;21:157–163.
[48] Filippin LI, Cuevas MJ, Lima E, Marroni NP, Gonzalez-Gallego J, Xavier RM. The role of nitric oxide during healing of trauma to the skeletal muscle. Inflamm Res 2011;60:347–356.

[49] Shi X, Garry DJ. Muscle stem cells in development, regeneration, and disease. Genes Dev 2006;20:1692–1708.
[50] Gomes LEA, Dalmarco EM, André ES. The brain-derived neurotrophic factor, nerve growth factor, neurotrophin-3, and induced nitric oxide synthase expressions after low-level laser therapy in an axonotmesis experimental model. Photomed Laser Surg 2012;30:642–647.
[51] Wu J-Y, Chen C-H, Wang C-Z, Ho M-L, Yeh M-L, Wang Y-H. Low-power laser irradiation suppresses inflammatory response of human adipose-derived stem cells by modulating intracellular cyclic AMP level and NF-κB activity. PLoS One 2013;8:1–9.
[52] Laraia EM, Silva IS, Pereira DM, dos Reis FA, Albertini R, de Almeida P, et al. Effect of low-level laser therapy (660 nm) on acute inflammation induced by tenotomy of Achilles tendon in rats. Photochem Photobiol 2012;88:1546–1550.
[53] Tidball JG. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005;288:345–353.
[54] Tang K, Breen EC, Gerber HP, Ferrara NM, Wagner PD. Capillary regression in vascular endothelial growth factor-deficient skeletal muscle. Physiol Genomics 2004;18:63–69.
[55] Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16:4604–4613.
[56] Cohen T, Nahari D, Cerem LW, Neufeld G, Levi BZ. Interleukin 6 induces the expression of vascular endothelial growth factor. J Biol Chem 1996;271:736–741.
[57] Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J, Jelkmann W. Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 1999;94:1561–1567.
[58] Kosmidou I, Xagorari A, Roussos C, Papapetropoulos A. Reactive oxygen species stimulate VEGF production from C2C12 skeletal myotubes through a PI3K/Akt pathway. Am J Physiol Lung Cell Mol Physiol 2001;280:585–592.
[59] Schäfer G, Cramer T, Suske G, Kemmner W, Wiedenmann B, Höcker M. Oxidative stress regulates vascular endothelial growth factor – a gene transcription through Sp1- and Sp3-dependent activation of two proximal GC-rich promoter elements. J Biol Chem 2003;278:8190–8198.
[60] Clark AN, Youkey R, Liu X, Jia L, Blatt R, Day YJ, et al. A1 adenosine receptor activation promotes angiogenesis and release of VEGF from monocytes. Circ Res 2007;101:1130–1138.
[61] Mousavi K, Jasmin BJ. BDNF is expressed in skeletal muscle satellite cells and inhibits myogenic differentiation. J Neurosci 2006;26:5739–5749.
[62] Schultz E. Changes in the satellite cells of growing muscle following denervation. Anat Rec 1978;190:299–311.
[63] Schultz E, Lipton BH. The effect of Marcaine on muscle and non-muscle cells in vitro. Anat Rec 1978;191:351–369.
[64] Snow TR. Substrate effects on myocardial performance during normoxia and hypoxia. Am J Physiol 1978;235:144–149.
[65] Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev 2004;84:209–238.
[66] Griesbeck O, Parsadanian AS, Sendtner M, Thoenen H. Expression of neurotrophins in skeletal muscle: quantitative comparison and significance for motoneuron survival and maintenance of function. J Neurosci Res 1995;42:21–33.
[67] Omura T, Sano M, Omura K, Hasegawa T, Sawada T, Nagano A. Different expressions of BDNF, NT3, and NT4 in muscle and nerve after various types of peripheral nerve injuries. J Peripher Nerv Syst 2005;10:293–300.
[68] Sobue G, Yamamoto M, Doyu M, Li M, Yasuda T, Mitsuma T. Expression of mRNAs for neurotrophins (NGF, BDNF, and NT-3) and their receptors (p75NGFR, trk, trkB, and trkC) in human peripheral neuropathies. Neurochem Res 1998;23:821–829.
[69] Clow C, Jasmin BJ. Brain-derived neurotrophic factor regulates satellite cell differentiation and skeltal muscle regeneration. Mol Biol Cell 2010;21:2182–2190.