Post-resistance exercise photobiomodulation therapy has a more effective antioxidant effect than pre-application on muscle oxidative stress

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Abstract

This study evaluated the effect of photobiomodulation therapy (PBMt) before or after a high-intensity resistance exercise (RE) session on muscle oxidative stress. Female Wistar rats were assigned to one of the following groups: Sham (non-exercised, undergoing placebo-PBMt); NLRE (exercised, undergoing placebo-PBMt); PBMt + RE (pre-exercise PBMt); RE + PBMt (post-exercise PBMt). The RE comprised four climbs bearing the maximum load with a 2 min rest between each climb. An 830-nm aluminum gallium arsenide diode laser (100 mW; 0.028 cm²; 3.57 mW/cm²; 142.8 J/cm²; 4 J; Photon Laser III, DMC, São Paulo, Brazil) was applied 60 s before or after RE in gastrocnemius muscles. Analyses were performed at 24 h after RE: lipoperoxidation using malondialdehyde (MDA) and protein oxidation (OP) on Western blot. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activity were spectrophotometrically assessed. Nitric oxide (NO) level was determined by the Griess reaction. The MDA and OP levels were significantly higher in the NLRE group. Increased OP was prevented in all PBMt groups; however, increased MDA was prevented only in the RE + PBMt group. The RE + PBMt group had higher SOD activity compared to all other groups. A higher GPx activity was observed only in the PBMt + RE compared to Sham group, and CAT activity was reduced by RE, without PBMt effect. NO levels were unchanged with RE or PBMt. Therefore, PBMt application after a RE session has a more potent antioxidant effect than previous PBMt. Rats submitted to post-RE PBMt illustrated prevention of increased lipoperoxidation and protein oxidation as well as increased SOD activity.

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Graphic abstract

The photobiomodulation can attenuate oxidative stress induced by resistance exercise. A more evident benefit shows to be obtained with the application after exercise, in which it has increased the activity of superoxide dismutase.

Keywords Exercise · Laser irradiation · Low-light-level therapy · Reactive oxygen species · Photobiomodulation

1 Introduction

Previous studies have shown the impact of acute aerobic exercise on the production of oxidizing agents [1–3]. This would be a result of increased O_2 uptake and a pro-inflammatory response analogous to stress following tissue ischemia [4]. The higher O_2 consumption induces an increased electron flow in the skeletal muscles active, as well as favoring greater “deviation” of these electrons, from the mitochondrial respiratory chain, resulting in the synthesis of reactive oxygen species (ROS) [4–6]. These deviations result in a univalent reduction in the total electron flow of the O_2 consumed, which increases the synthesis of ROS and a large spectrum of radical species, such as superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($OH^{\cdot}$) and non-radicals such as hydrogen peroxide (H_2O_2) [7]. The decomposition of dihydrogen peroxide occurs in the presence of ferrous ions (Fe^{2+}) and $O_2^{\cdot-}$ (Fenton’s reaction). This reaction forms H_2O_2 in an acid environment and is catalyzed by the superoxide dismutase (SOD) enzyme. Hydroxyl radical ($OH^{\cdot}$) is the end product of Fenton’s reaction. $OH^{\cdot}$ is a very reactive and there is no specific antioxidant against this free radical (FR). This FR causes lipid peroxidation and protein oxidation [8].

Nevertheless, it has also been suggested oxidative stress specific to anaerobic exercise [e.g., resistance exercise (RE)] mediated through ischemia/reperfusion and pathways such as the increase in xanthine (XO) and NADPH (NOXs) oxidases [9–11]. NADPH oxidases (NOXs) catalyze the O_2 to superoxide ($O_2^{\cdot-}$) or H_2O_2 [12]. It was shown that the NOX

members contribute to cytosolic superoxide production both at rest and during contractile muscle activity to a larger extent than in the mitochondria [13, 14]. The XO reduces hypoxanthine to xanthine and xanthine to uric acid [15]. Upon contraction, XO activity is considerably increased and further impacts lipid peroxidation, protein oxidation, muscle damage and edema [16]. During high-intensity activity, a lot of ATP is consumed, and hypoxanthine and xanthine concentrations rise. Then, XO induces $O_2^{\cdot-}$ formation by utilizing molecular oxygen, thereby exacerbating reactive oxygen species (ROS) [10].

ROS play a key role in exercise-induced adaptation by a phenomenon similar to hormesis [17]. Hormesis is a physiological strategy for best resource allocation that ensures homeostasis [18]. On the other hand, high levels of ROS during rigorous muscular work does interfere with redox signaling that mediates increased force production, heat shock response, muscle growth and metabolic adaptations [19, 20]. Highly oxidative environment is inductive to catabolic pathways such as inflammation, proteolysis, and apoptosis [21]. Moreover, it has shown that the production of ROS after strenuous exercise results in muscle damage and delayed onset of muscle soreness and inflammation [22, 23]. This condition is evident during periods of intensified exercise training when ROS release may exceed the protective action of the antioxidant systems [24].

Recently, photobiomodulation therapy (PBMt) with low-level laser has appeared as a means to modulate the excessive oxidative stress induced by acute exercise [25]

and improve muscle recovery in both application before [26] and after [27, 28] in different exercises or fatigue protocols. An experimental study applied treadmill running in decline (downhill running) in rats to evaluate the PBMt role in the inhibition of inflammation, reduction of CK activity and oxidative stress. Authors found increases in defense against oxidative stress (increased activity of SOD) 24 h and 48 h after exercise [29].

There are several mechanisms to explain the effects of PBMt on muscle tissue, including higher energy metabolism and ATP synthesis; stimulation of defenses against oxidative stress; prevention and repair of muscle damage; modulation of gene expression by activation of transcription factors; possible increase in the excitability of muscle fibers [30].

To interact with the living cell, light must be absorbed by intracellular chromophores. Suggested candidates for endogenous chromophores include porphyrins, flavins, mitochondrial cytochromes, and the plasma membrane NADPH oxidase system, which contains flavoproteins and cytochrome *b* [31, 32]. These chromophores are photosensitizers; they absorb visible light and transfer it to nearby O_2 molecules, thus producing ROS [33]. Previous experiments have shown that ROS stimulate signal transduction processes for transcription factor activation, gene expression, muscle contraction and cell growth [34, 35]. The isolated plasma membranes also generate ROS upon illumination. The plasma membranes possess an electron transfer chain complex called the “NADPH oxidase complex,” which consists of photosensitizer molecules. This transmembrane electron chain transfers electrons from NADPH to molecular O_2 through flavocytochrome *b* containing the electron carriers cytochrome *b* and FAD [36], all known as photosensitizers [37–39].

The oxidative status is controlled by a wide spectrum of dietary exogenous antioxidants such as tocopherols, ascorbate, carotenoids, and phenolic compounds, and by endogenous antioxidants such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and catalase [40]. These enzymes are responsible for removing $O_2^{\cdot-}$, hydroperoxides and H_2O_2 , respectively [41]. The ROS production by cellular photosensitizers competes with the antioxidant action of photobiostimulation. The enhanced antioxidant activity can arise either by light-induced antioxidant activity or by light-induced increase in the cellular electron transfer process [33]. There is evidence in the literature showing that light-induced ROS increases the activity of various antioxidants such as SOD and catalase (CAT) or stimulates their synthesis in the cell [42, 43]. This cell defense mechanism eliminates damaging oxidative metabolites. In addition, it is worth highlighting the role of nitric oxide (NO) in the regulation of oxidative stress. NO may simultaneously have both pro- and anti-oxidant effects depending on the relative concentration of individual

reactive species present [44]. NO production in response to illumination may proceed by two different types of mechanisms. NO may be produced enzymatically following an increase in NO synthase (NOS) activity, resulting from physiological changes brought about by illumination. In such a case, the increase in NO may occur long after illumination. However, if the NO is released directly by a photochemical mechanism, the increase in NO will occur during illumination or shortly thereafter, and the chemical properties of NO are highly relevant to its production [33]. For a photochemical mechanism to exist, there must be a molecule that serves as a store capable of releasing NO [33]. Moreover, illumination produces superoxide [45], which reacts quickly with NO to produce peroxynitrite. Thus, the amount of NO produced/released must exceed that of the superoxide to have an effect. In many cases, illumination will lower NO concentrations by producing superoxide.

Lower protein oxidation and lipoperoxidation levels were found in the gastrocnemius muscle of rats when PBMt was used before high-intensity RE [46]. Likewise, for practical purposes of strength and conditioning, it is important to determine whether the use of pre- or post-RE PBMt has a similar impact on oxidative stress. Prior or posterior PBMt application have been studied in humans and animals related to the muscular and oxidative damage generated by an aerobic exercise [47, 48], endurance [49] and strength training [50] and fatigue protocol [51, 52]. However, there is little information from studies that determined the repercussion of PBMt application, before versus after an only maximum load RE session, in the muscle oxidative stress. A previous study has shown that post-exercise PBMt decreased the serum creatine kinase levels in rats, without any effect on PBMt when applied before exercise [53]. Therefore, we may speculate that the moment of PBMt application may be a key factor for the modulation of exercise-induced oxidative stress. This study aimed to compare the effectiveness pre-irradiation of PBMt to irradiation after a high-intensity RE session, while evaluating markers of muscle oxidative stress in rats, to examine the isolated effects on the muscle.

2 Methods

2.1 Animals

A total of 37 female Wistar rats (weight: 200–250 g; 12 weeks old) were allocated into one of the four experimental groups: Sham (non-exercised, undergoing placebo-PBMt); NLRE (exercised, undergoing placebo-PBMt); PBMt + RE (pre-exercise PBMt); RE + PBMt (post-exercise PBMt). Experimental protocol was carried out in accordance with the ARRIVE guidelines and National Institutes of Health guide for the care and use of Laboratory animals

(NIH Publications No. 8023, revised 1978). The study protocol was approved by the Institutional Research Ethics Committee at the Uninove University, São Paulo—SP, Brazil (process: 8868250615). Based on rats' availability in our animal's facility, several protocols were performed using female rats that have been published [54, 55]. Moreover, the landscape of female athletics has changed considerably in the last decades, in which female athletic participation in competitions has significantly increased worldwide [56].

2.2 Load test and RE session

Maximum load test and RE protocol were carried out on a ladder adapted for rodents as previously reported [46, 53, 57, 58]. The RE session was performed after 72 h of rest by using the maximum load. The rats completed four climbs bearing the maximum load (i.e. 100% load) with a 2-min time rest between climbs. Sham group underwent exercise adaptation without the maximum load test, or a RE. Rats were euthanized 24 h after RE with sodium pentobarbital (180 mg/kg; i.p.) to separate the left gastrocnemius muscle. It has previously been reported that there is increased lipoperoxidation 24 h post-RE in a rat model [46]. Figure 1 illustrates the experimental timeline for each set.

2.3 PBMt protocol

DMC Laser Photon III[®] aluminum gallium arsenide diode laser system (São Carlos, SP, Brazil) was used for irradiation as illustrated in Table 1. We used 4 J of radiant energy,

following a recent study showing that such energy is suitable to modulate oxidative stress induced by RE [46]. The laser was used in direct contact with the shaved skin on both legs to central, proximal, and distal sites on the gastrocnemius muscle. Irradiation was delivered 60 s before or after RE, as designed in the experimental follow-up (Fig. 1; supplemental data). Sham and NLRE groups were manipulated in the same way as those in the PBMt groups, but with the equipment turned off.

2.4 Muscle collection

Animals were euthanized with a urethane overdose (4.8 g kg⁻¹ i.p.) and gastrocnemius muscles of the left hind paw were quickly removed dipped in liquid nitrogen and stored at −80 °C until further processing.

2.5 Lipid peroxidation

Lipoperoxidation was determined using malondialdehyde (MDA) with thiobarbituric acid reactive substances (TBARS) assay. For this, 50 mg gastrocnemius was homogenized in 500 µL SDS lysis buffer (0.05 M Tris-HCl; 0.5% SDS; 1 mM DTT; pH 8.0) following the 20-cycle schedule with a 5-s pause between each cycle and at a speed of 4 m/s on Bead Ruptor 12 homogenizer (OMNI International, USA). An aliquot of 100 µL was centrifuged for 10 min at 800×g at 4 °C, and a 10 µL of supernatant was used for the Bradford assay. A mixture containing 250 µL of non-centrifuged homogenate, 50 µL

Fig. 1 Schematic timeline of the study design

Table 1 Laser irradiation parameters

Experimental groups	Wave length (nm)	Laser beam (cm ²)	Radiant power (mW)	Irradiance (W/cm ²)	Radiant exposure (J/cm ²)	Radiant energy (J)	Irradiation time per site (s)
Sham	—	—	—	—	—	—	40
NLRE	—	—	—	—	—	—	40
L + RE	830	0.028	100	3.57	142.8	4	40
RE + L	830	0.028	100	3.57	142.8	4	40

of 4% BHT, 300 μL of 20% acetic acid, pH 3.5 and 300 μL 0.78% of the thiobarbituric acid aqueous solution was prepared. The mixture was heated at 95 °C for 60 min and centrifuged at 12,000g for 15 min. Then, a 100 μL of the supernatant was pipetted in duplicate into a 96-well plate to determine the absorbance at 532 nm with Spectra Max M5 spectrophotometer (Molecular Devices, CA, USA). All assays were performed in triplicate.

2.6 Protein oxidation

Measurement of carbonyl groups added into proteins by oxidative reactions was evaluated with ABCAM kit AB178020 (Abcam, Cambridge, MA, USA) for an equal protein load (20 μg) [58, 59]. Samples were loaded onto SDS-PAGE gels and DNP conjugated proteins were detected by western blotting using primary DNP antibody and HRP conjugated secondary antibody. Bound antibody was detected by using chemiluminescence with a Amersham Imager 600 system (GE Health Care, Little Chalfont, UK, USA).

2.7 Antioxidant enzymatic activity

Muscles (~50 mg) were homogenized in phosphate buffer (0.1 M; pH 7.4), and samples were centrifuged twice for 15 min each (800 and 13,400 $\times g$). Catalase (CAT) activity was assessed by mixing the homogenates with 10 mM H_2O_2 (10% v/v) and 50 mM phosphate buffer (90% v/v); the decrease in H_2O_2 over 5 min at 30 °C was measured in 240 nm. Superoxide dismutase (SOD) activity was evaluated by adding nitro blue tetrazolium, βNADH , and phenylmethanesulfate, and absorbance was measured at 560 nm for 5 min at 30 °C. GPx catalyzes the reaction of reduced glutathione hydroperoxides (GSH) to form oxidized glutathione (GSSG) and the hydroperoxide reduction product, enzyme activity was determined by measuring NADPH consumption in the reduction reaction coupled to the GPx. Approximately 30 mg of gastrocnemius was homogenized in 200 μL of 50 mM Tris–HCL buffer; 1 mM EDTA; pH 7.6 and then centrifuged for 15 min at 400 $\times g$ and 4 °C. The supernatant was then transferred to a 1.5 mL tube and centrifuged for 15 min at 13,400 $\times g$ and 4 °C. The resulting supernatant was used in the assessment of GPx activity. The decrease in NADPH absorbance at 340 nm was determined on a 96-well plate spectrophotometer containing 10 μL centrifuged homogenate, 180 μL reaction medium (50 mM Tris–HCL; 1 mM EDTA; 0.2 mM NADPH; GSH Glutathione reductase 0.1 U/mL (G3664 SIGMA); pH 7.6) and 10 μL 0.5 mM Terc-Butyl Hydroperoxide. Absorbance was monitored for 5 min, with an interval of 30 s between each reading. All assays were performed in triplicate.

2.8 Nitrate, nitrite, and NOx

A total of 50 mg gastrocnemius was homogenized in 700 μL PBS pH 7.4 and centrifuged at 800 $\times g$ for 20 min at 4 °C. Then, 100 μL of the supernatant was mixed with a 100 μL of 10% Perchloric Acid, incubated for 5 min at room temperature and centrifuged for 10 min at 800 $\times g$ for deproteinization. In a 96-well plate, 50 μL Griess reagent (1% sulfanilamide, 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride and 2.5% H_3PO_4) in 50 μL of the deproteinized sample was added and after 5 min incubation at room temperature, nitrite absorbance was read at 540 nm. This was followed by the addition of 50 μL of vanadium solution (0.8% vanadium chloride III and 9 mM HCl) to each well and after 5 min incubation at room temperature; the nitrate absorbance was read at 540 nm. All assays were performed in triplicate.

2.9 Statistical analysis

Data were analyzed using GraphPad Prism 6.01 (GraphPad Software, La Jolla, CA, USA) and are shown as mean $\pm$ 1 SD. Shapiro Wilks' *W* test was applied to exam the normality of data. Levine's test for equality was used to determine whether group variances were equal in the population. One-way ANOVA (Tukey post-hoc) was performed with multiple comparison tests, and Kruskal–Wallis test followed by Dunn's multiple comparison tests were used to assess non-normal data. Effect size (η^2) was calculated using GPOWER software (version 3.1.9.2, Franz Faul, Universität Kiel, Germany). Effect size was judged as small ($\eta^2=0.1$), medium ($\eta^2=0.25$) or large ($\eta^2=0.40$) (Cohen 1992). Statistical significance was considered as $p \leq 0.05$.

3 Results

As illustrated in Fig. 2, MDA and OP levels, were significantly higher in the NLRE group compared to the Sham group. Post-ER PBMT has attenuated the increase in MDA level and prevented high OP content.

Regarding the antioxidant enzymes, the RE + PBMT group showed greater SOD activity when compared to the other experimental groups (Fig. 3a). The CAT activity was significantly lower in the exercised animals, and no PBMT effect was observed (Fig. 3b). Significant differences in GPx activity were observed only between PBMT + RE and Sham groups (Fig. 3c).

There were no differences in muscle expression of nitrate, nitrite and NOx index between experimental groups, regardless of the moment of PBMT application (Fig. 4).

Fig. 2 Lipoperoxidation (**a**) and oxidized protein (**b**) levels in gastrocnemius muscle in rats not exercised without PBMT application (Sham), rats that only underwent a resistance exercise session (NLRE), and rats that were submitted to PBMT irradiations before (PBMT+RE) or after (RE+PBMT) to a resistance exercise session (**a** effect size=0.22; **b** effect size=0.19). Same letters above bars

into graphs indicate values not different. Different letters above bars into graphs indicate a significant difference between means. Number of animals for each experiment is shown inside the bars. One-way ANOVA (Tukey post-hoc) was applied in the comparisons. * $p < 0.05$ vs. Sham; # $p < 0.05$ vs. NLRE; @ $p < 0.05$ vs. PBMT + RE

Fig. 3 Enzymatic activity of superoxide dismutase (SOD, **a**), catalase (CAT, **b**) and glutathione peroxidase (GPx, **c**) in gastrocnemius muscle in rats not exercised without PBMT application (Sham), rats that only underwent a resistance exercise session (NLRE), and rats that were submitted to PBMT irradiations before (PBMT+RE) or after (RE+PBMT) to a resistance exercise session (**a** effect size=0.15; **b**

effect size=0.20; **c** effect size=0.12). Same letters above bars into graphs indicate values not different. Different letters above bars into graphs indicate a significant difference between means. Number of animals for each experiment is shown inside the bars. One-way ANOVA (Tukey post-hoc) was applied in the comparisons. * $p < 0.05$ vs. Sham; # $p < 0.05$ vs. NLRE; @ $p < 0.05$ vs. PBMT + RE

4 Discussion

Several studies have documented a positive effect of PBMT on exercise-induced muscle fatigue, injury, inflammation, and oxidative stress [46, 53, 60, 61]. However, the best time for PBMT (pre-RE or post-RE) remains unclear. PBMT irradiation before or after physical exercise has distinct features but also has common mechanisms of action [30]. Clinical trials using LLLT and LEDT before exercise

reported a preventive effect against mitochondrial dysfunction and muscle damage mediated by ROS and reactive nitrogen species (RNS), as well as modulation of energetic metabolism [2, 61–65]. LLLT and LEDT radiation after the physical exercise aims not only to alleviate the mitochondrial and metabolic dysfunction, but also the repair microlesions produced from mechanical and metabolic stress resulting from muscle contraction and ROS/RNS [29, 30, 66–69].

Fig. 4 Expression of nitrate (a), nitrite (b) and NOX index (c) in gastrocnemius muscle in rats not exercised without PBMT application (Sham), rats that only underwent a resistance exercise session (NLRE), and rats that were submitted to PBMT irradiations before (PBMT+RE) or after (RE+PBMT) to a resistance exercise session (a effect size=0.09; b effect size=0.08; c effect size=0.05). Same

letters above bars into graphs indicate values not different. Different letters above bars into graphs indicate a significant difference between means. Number of animals for each experiment is shown inside the bars. One-way ANOVA (Tukey post-hoc) was applied in the analysis of nitrate and NOX. Kruskal–Wallis test (Dunn's post-hoc) was applied in the analysis of nitrite

For example, a comparison of the effect of PBMT in irradiated rats before or/and after a strength training program showed that post-irradiation was more effective in reducing concentrations of protein carbonyls compared to the other groups irradiated before or before plus after exercise [50]. For other conditions, a systematic review illustrated that phototherapy improves muscular performance and accelerates recovery mainly when applied before exercise [70].

In this study, all measurements were performed in the same period (24 h after exercise or application of PBMT). Only the time of application of PBMT was changed in the experimental groups (before or after exercise). As shown in Fig. 2a, lipoperoxidation was prevented only in animals irradiated after RE, while pre-irradiation had a null effect. Despite the lack of information to establish PBMT time-dependent mechanisms of action, the maintenance of MDA levels in the RE+PBMT group to values close to the Sham group has important cellular implications. The prevention of increased protein oxidation in the RE+PBMT group may be secondary to the inhibition of increased MDA levels. In addition, proteins may be directly oxidized by the action of ROS. Thus, a higher SOD expression in the RE+PBMT group suggests that the prevention of protein oxidation may result from the activity/impact of PBMT on ROS since SOD forms the first line of defense against superoxide radicals as SOD radicals to form H_2O_2 and O_2 [7].

Possibly, PBMT improves mitochondrial function and dismutation of $O_2^{\cdot-}$ via SOD and decreases the formation of $ONOO^-$ [71]. Alterations in the turnover of proteins and the activation of specific genes can result in specific adaptations, increasing the activity of this enzyme [72]. It is known that physical exercise may decrease intracellular pH [73]. An accumulation of H^+ can inactivate the Cu–Zn-SOD (SOD-1 and SOD-3) through a protonation of the residue of histidine 61 in the active center of these enzymes. However, LLLT can reverse this process and re-activate SOD-1 and SOD-3 [71]. Liu et al. [29] trained rats on a treadmill in the declined plane at speed of 16 m/min until the animal's exhaustion.

Authors applied LLLT on a single point on gastrocnemius muscle after exercise and the results showed reduced muscle MDA levels 24 h and 48 h after exercise and increased activity of SOD.

Ogonovszky et al. [74] suggested that the decrease in ROS production induced by exercise training could increase the level of antioxidants, repairing the carbonylation process, and this may be mediated by the complex of proteasome enzymes. The candidate mechanisms underlying the prevention of protein oxidation in the RE+PBMT group do not seem to apply to the PBMT+RE group since PO levels in the latter group were preserved without a positive effect of PBMT on lipoperoxidation and SOD content. These findings suggest that the effect of pre-RE PBMT irradiation on protein oxidation is mediated by mechanisms different from those observed for post-RE irradiation.

The null effect of PBMT on MDA levels in the PBMT+RE group is an intriguing finding. In a previous study, we have analyzed the content of 4-hydroxynonenal as a marker of lipoperoxidation, in which the application of PBMT before ER has prevented an increase in aldehyde. It is difficult to understand the basis of this finding, but it is possible that the effects of PBMT can be linked to the metabolism of MDA and not only in production as an aldehyde derived from lipoperoxidation. Importantly, this mechanism can be moment-dependent, occurring only with the application of PBMT after RE. It is well known that light promotes changes in mitochondrial metabolism, which could accelerate the metabolism of MDA in the PBMT+RE group. A probable route for MDA metabolism involves its oxidation by mitochondrial aldehyde dehydrogenase followed by decarboxylation to produce acetaldehyde, which is oxidized by aldehyde dehydrogenase to acetate and further to CO_2 and H_2O [75, 76].

Catalase activity was reduced after RE only in group NLRE. Similar results were found by Eftting et al. [77] to analyze the effects of RE training on oxidative stress and inflammatory parameters on mice with obesity. The authors

suggested that hydrogen peroxide can be catalyzed under these conditions by other cell detoxification systems, such as glutathione and peroxins.

Contrary to the findings of these studies, no change in muscle catalase activity in rats after 24 h of RE has been reported [78]. These conflicting findings may be due to methodological details. Our RE protocol was implemented after 72 h of the adaptation, in which effort intensity was based on a maximal load test, while in the other study [78] the animals were exercised 24 h after adaptation, with subjective loads ranging from 85 to 95% of body weight. Furthermore, different findings for catalase activity may be related to measurement techniques. For example, catalase assays typically require the addition of a substrate (i.e., H_2O_2) followed by degradation assessment. It turns out that the V_{max} catalase increases as a function of H_2O_2 concentration [7]. Thereby, measurements might have been affected not only by the amount of active catalase but also by the concentration of H_2O_2 during the reaction period [79]. Ultimately, the lack of effect of PBMt on catalase activity corroborates previous research findings [2, 46].

There is little information about the acute effect of RE on GPx activity. In this study, the animals undergoing RE did not show higher GPx activity in the gastrocnemius muscle when compared to animal Sham. The lack of RE effect on GPx activity may be related to the type of muscle fiber. For example, Loureiro et al. have found lower GPx activity in the gastrocnemius muscle when compared to the soleus muscle in rats undergoing aerobic training [80]. Thus, it may be speculated that the low concentration of oxidative fibers in the gastrocnemius results in the lower adaptive response of GPx to RE [81].

Some antioxidant enzyme results of this study were different from our previous study [46]. It is believed that the reason for these conflicting results is differences in the way the variables were measured. Although the assessment of antioxidant levels in tissues has merit as a biomarker of oxidative stress, this approach is not without its weaknesses. For example, a concern associated with the measurement of tissue antioxidants is the potential for auto-oxidation during sample handling, resulting in antioxidants depletion in the tissue [82].

The increase in NO during exercise training has important implications for metabolic control through the blood supply, glucose uptake, oxidative phosphorylation and muscle contractility [83]. In this study, NO muscle was not changed after either RE or PBMt. In general, NO exerts biological effects by forming bonds with iron atoms and thiols. Thus, photolysis of these bonds could weaken the effects of NO, even though the concentration of free NO is increased [33]. This phenomenon was demonstrated by Borutaite et al. [84].

Güzel et al. have demonstrated an increase in plasma NO between 6 and 48 h after high-intensity RE in sedentary

men [85]. The fact that systemic NO concentrations do not necessarily reflect their local content makes it difficult to compare the muscle assessments undertaken in the present study with plasma NO dosing [86]. Thus, under the experimental conditions of this research, it may be possible that NO levels may be altered in the blood, as was not documented in the gastrocnemius. Finally, no effect of PBMt on NO concentration was observed. One explanation for this is that the wavelength here adopted (i.e. 830 nm) is not suitable for changes in the NO. This is based on preliminary observations that serum NO levels are elevated with irradiations below 740 nm [87, 88].

5 Conclusion

This study illustrates that the application of PBMt after a section of high-intensity RE has a more potent antioxidant effect than previous PBMt in rats. Rats submitted to post-ER PBMt illustrated prevention of increased lipoperoxidation and protein oxidation as well as increased activity of the SOD enzyme.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Bachur, J. A., Garcia, S. B., Vannucchi, H., Jordao, A. A., Chiarello, P. G., & Zucoloto, S. (2007). Anti-oxidative systems in rat skeletal muscle after acute physical exercise. *Applied Physiology, Nutrition, and Metabolism*, 32(2), 190–196.
2. De Marchi, T., Junior, E. C. P. L., Bortoli, C., Tomazoni, S. S., Lopes-Martins, R. Á. B., & Salvador, M. (2012). Low-level laser therapy (LLLT) in human progressive-intensity running: Effects on exercise performance, skeletal muscle status, and oxidative stress. *Lasers in Medical Science*, 27(1), 231–236.
3. Schneider, C. D., & Oliveira, A. R. D. (2004). Oxygen free radicals and exercise: Mechanisms of synthesis and adaptation to the physical training. *Revista Brasileira de Medicina do Esporte*, 10(4), 308–313.
4. Mastaloudis, A., Morrow, J. D., Hopkins, D. W., Devaraj, S., & Traber, M. G. (2004). Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. *Free Radical Biology and Medicine*, 36(10), 1329–1341.

5. Clarkson, P. M., & Thompson, H. S. (2000). Antioxidants: What role do they play in physical activity and health? *The American Journal of Clinical Nutrition*, 72(2), 637S–S646
6. Ji, L. L. (1999). Antioxidants and oxidative stress in exercise. *Proceedings of the Society for experimental Biology and Medicine*, 222(3), 283–292
7. Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiological Reviews*, 88(4), 1243–1276
8. Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative stress. *Sports Medicine*, 36(4), 327–358
9. Bloomer, R. J., & Goldfarb, A. H. (2004). Anaerobic exercise and oxidative stress: A review. *Canadian Journal of Applied Physiology*, 29(3), 245–263
10. Radak, Z., Zhao, Z., Koltai, E., Ohno, H., & Atalay, M. (2013). Oxygen consumption and usage during physical exercise: The balance between oxidative stress and ROS-dependent adaptive signaling. *Antioxidants & Redox Signaling*, 18(10), 1208–1246
11. Wiecek, M., Szymura, J., Maciejczyk, M., Kantorowicz, M., & Szygula, Z. (2018). Anaerobic exercise-induced activation of antioxidant enzymes in the blood of women and men. *Frontiers in Physiology*, 9, 1006
12. Brandes, R. P., Weissmann, N., & Schröder, K. (2014). Nox family NADPH oxidases: Molecular mechanisms of activation. *Free Radical Biology and Medicine*, 76, 208–226
13. Xia, R., Webb, J. A., Gnall, L. L., Cutler, K., & Abramson, J. J. (2003). Skeletal muscle sarcoplasmic reticulum contains a NADH-dependent oxidase that generates superoxide. *American Journal of Physiology-Cell Physiology*, 285(1), C215–C221
14. Sakellariou, G. K., Vasilaki, A., Palomero, J., Kayani, A., Zibrik, L., McArdle, A., et al. (2013). Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase (s) in the increased skeletal muscle superoxide generation that occurs during contractile activity. *Antioxidants & Redox Signaling*, 18(6), 603–621
15. Harrison, R. (2002). Structure and function of xanthine oxidoreductase: Where are we now? *Free Radical Biology and Medicine*, 33(6), 774–797
16. Judge, A., & Dodd, S. (2004). Xanthine oxidase and activated neutrophils cause oxidative damage to skeletal muscle after contractile claudication. *American Journal of Physiology-Heart and Circulatory Physiology*, 286(1), H252–H256
17. Kozakowska, M., Pietraszek-Gremplewicz, K., Jozkowicz, A., & Dulak, J. (2015). The role of oxidative stress in skeletal muscle injury and regeneration: Focus on antioxidant enzymes. *Journal of Muscle Research and Cell Motility*, 36(6), 377–393
18. Calabrese E, Baldwin LA (2002) The dose determines the stimulation (and poison): Development of a Chemical Hormesis Database
19. Peake, J. M., Markworth, J. F., Nosaka, K., Raastad, T., Wadley, G. D., & Coffey, V. G. (2015). Modulating exercise-induced hormesis: Does less equal more? *Journal of Applied Physiology*, 119(3), 172–189
20. Alleman, R. J., Katunga, L. A., Nelson, M. A., Brown, D. A., & Anderson, E. J. (2014). The “Goldilocks Zone” from a redox perspective—adaptive vs. deleterious responses to oxidative stress in striated muscle. *Frontiers in Physiology*, 5, 358
21. Ristow, M., & Schmeisser, S. (2011). Extending life span by increasing oxidative stress. *Free Radical Biology and Medicine*, 51(2), 327–336
22. Nikolaidis, M. G., Jamurtas, A. Z., Paschalis, V., Fatouros, I. G., Koutedakis, Y., & Kourtas, D. (2008). The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress. *Sports Medicine*, 38(7), 579–606
23. Retamoso, L. T., Junior, M. E. S., Lima, F. D., Busanello, G. L., Bresciani, G., Ribeiro, L. R., et al. (2016). Increased xanthine oxidase-related ROS production and TRPV1 synthesis preceding DOMS post-eccentric exercise in rats. *Life Sciences*, 152, 52–59
24. Slattery, K., Bentley, D., & Coutts, A. J. (2015). The role of oxidative, inflammatory and neuroendocrinological systems during exercise stress in athletes: Implications of antioxidant supplementation on physiological adaptation during intensified physical training. *Sports Medicine*, 45(4), 453–471
25. Sunemi, S. M., Silva, F. A., Antonio, E. L., Tucci, P. J. F., & Serra, A. J. (2019). Photobiomodulation: Newly discovered actions in resistance exercise. *Reactive Oxygen Species*, 7(21), 148–53
26. De Marchi, T., Leal-Junior, E. C. P., Lando, K. C., Cimadon, F., Vanin, A. A., da Rosa, D. P., et al. (2019). Photobiomodulation therapy before futsal matches improves the staying time of athletes in the court and accelerates post-exercise recovery. *Lasers in Medical Science*, 34(1), 139–148
27. De Marchi, T., Schmitt, V. M., Machado, G. P., de Sene, J. S., de Col, C. D., Tairova, O., et al. (2017). Does photobiomodulation therapy is better than cryotherapy in muscle recovery after a high-intensity exercise? A randomized, double-blind, placebo-controlled clinical trial. *Lasers in Medical Science*, 32(2), 429–437
28. de Paiva, P. R. V., Tomazoni, S. S., Johnson, D. S., Vanin, A. A., Albuquerque-Pontes, G. M., Machado, C. D. S. M., 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 in Medical Science*, 31(9), 1925–33
29. Liu, X.-G., Zhou, Y.-J., Liu, T.C.-Y., & Yuan, J.-Q. (2009). Effects of low-level laser irradiation on rat skeletal muscle injury after eccentric exercise. *Photomedicine and Laser Surgery*, 27(6), 863–9
30. Ferraresi, C., Hamblin, M. R., & Parizotto, N. A. (2012). Low-level laser (light) therapy (LLLT) on muscle tissue: Performance, fatigue and repair benefited by the power of light: Low-level-laser (Licht)-Therapie an Muskelgewebe-Möglichkeiten zur Verbesserung der Leistungsfähigkeit und zur Behandlung von Muskelermüdung und Muskelverletzungen. *Photonics & Lasers in Medicine*, 1(4), 267–286
31. Fraikin, G. Y., Strakhovskaya, M., & Rubin, A. (1996). The role of membrane-bound porphyrin-type compound as endogenous sensitizer in photodynamic damage to yeast plasma membranes. *Journal of Photochemistry and Photobiology B: Biology*, 34(2–3), 129–135
32. Edwards, A. M., & Silva, E. (2001). Effect of visible light on selected enzymes, vitamins and amino acids. *Journal of Photochemistry and Photobiology B: Biology*, 63(1–3), 126–131
33. Lubart, R., Eichler, M., Lavi, R., Friedman, H., & Shainberg, A. (2005). Low-energy laser irradiation promotes cellular redox activity. *Photomedicine and Laser Therapy*, 23(1), 3–9
34. Suzuki, Y. J., & Ford, G. D. (1999). Redox regulation of signal transduction in cardiac and smooth muscle. *Journal of Molecular and Cellular Cardiology*, 31(2), 345–353
35. Rhee, S. G. (1999). Redox signaling: Hydrogen peroxide as intracellular messenger. *Experimental & Molecular Medicine*, 31(2), 53–59
36. Vignais, P. (2002). The superoxide-generating NADPH oxidase: Structural aspects and activation mechanism. *Cellular and Molecular Life Sciences CMLS*, 59(9), 1428–1459
37. Pottier, R., & Truscott, T. (1986). The photochemistry of haemoporphyrin and related systems. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine*, 50(3), 421–452
38. Nisimoto, Y., Otsuka-Murakami, H., & Lambeth, D. J. (1995). Reconstitution of flavin-depleted neutrophil flavocytochrome b558 with 8-mercapto-FAD and characterization of the flavin-reconstituted enzyme. *Journal of Biological Chemistry*, 270(27), 16428–16434

39. Kale, H., Harikumar, P., Kulkarni, S., Nair, P., & Netrawali, M. (1992). Assessment of the genotoxic potential of riboflavin and lumiflavin: B. Effect of light. *Mutation Research/Genetic Toxicology*, 298(1), 17–23
40. Elosua, R., Molina, L., Fito, M., Arquer, A., Sanchez-Quesada, J., Covas, M., et al. (2003). Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis*, 167(2), 327–334
41. Powers, S. K., Ji, L. L., Kavazis, A. N., & Jackson, M. J. (2011). Reactive oxygen species: Impact on skeletal muscle. *Comprehensive Physiology*, 1(2), 941–969
42. Stadler, I., Evans, R., Kolb, B., Naim, J. O., Narayan, V., Buehner, N., et al. (2000). In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, 27(3), 255–261
43. Zhu, Q., Yu, W., Yang, X., Hicks, G. L., Lanzafame, R. J., & Wang, T. (1997). Photo-irradiation improved functional preservation of the isolated rat heart. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, 20(3), 332–339
44. Goss, S. P., Hogg, N., & Kalyanaraman, B. (1997). The effect of nitric oxide release rates on the oxidation of human low density lipoprotein. *Journal of Biological Chemistry*, 272(34), 21647–21653
45. Callaghan, G. A., Riordan, C., Gilmore, W. S., McIntyre, I. A., Allen, J. M., & Hannigan, B. M. (1996). Reactive oxygen species inducible by low-intensity laser irradiation alter DNA synthesis in the haemopoietic cell line U937. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, 19(2), 201–206
46. de Oliveira, H. A., Antonio, E. L., Arsa, G., Santana, E. T., Silva, F. A., Junior, D. A., et al. (2018). Photobiomodulation leads to reduced oxidative stress in rats submitted to high-intensity resistive exercise. *Oxidative Medicine and Cellular Longevity*, 2018, 5763256
47. Frigero, M., dos Santos, S. A., Serra, A. J., Machado, C. D. S. M., Portes, L. A., Tucci, P. J. F., et al. (2018). Effect of photobiomodulation therapy on oxidative stress markers of gastrocnemius muscle of diabetic rats subjected to high-intensity exercise. *Lasers in Medical Science*, 33(8), 1781–90
48. Guaraldo, S. A., Serra, A. J., Amadio, E. M., Antônio, E. L., Silva, F., Portes, L. A., et al. (2016). The effect of low-level laser therapy on oxidative stress and functional fitness in aged rats subjected to swimming: An aerobic exercise. *Lasers in Medical Science*, 31(5), 833–840
49. Miranda, E. F., Tomazoni, S. S., de Paiva, P. R. V., Pinto, H. D., Smith, D., Santos, L. A., et al. (2018). When is the best moment to apply photobiomodulation therapy (PBMT) when associated to a treadmill endurance-training program? A randomized, triple-blinded, placebo-controlled clinical trial. *Lasers in Medical Science*, 33(4), 719–727
50. Ferraresi, C., Parizotto, N. A., de Sousa, M. V. P., Kaippert, B., Huang, Y.-Y., Koiso, T., et al. (2016). Light-emitting diode therapy in exercise-trained mice increases muscle performance, cytochrome c oxidase activity, ATP and cell proliferation. *Journal of Biophotonics*, 9(9), 976
51. de Oliveira Silva, A. A., Leal-Junior, E. C. P., D'Avila, K. D. A. L., Serra, A. J., Albertini, R., França, C. M., et al. (2015). Pre-exercise low-level laser therapy improves performance and levels of oxidative stress markers in mdx mice subjected to muscle fatigue by high-intensity exercise. *Lasers in Medical Science*, 30(6), 1719–27
52. Ribeiro, B. G., Alves, A. N., dos Santos, L. A. D., Cantero, T. M., Fernandes, K. P. S., Dias, D. D. S., et al. (2016). Red and infrared low-level laser therapy prior to injury with or without administration after injury modulate oxidative stress during the muscle repair process. *PLoS ONE*, 11(4), e0153618
53. de Oliveira, H. A., Antonio, E. L., Silva, F. A., de Carvalho, P. T. C., Feliciano, R., Yoshizaki, A., et al. (2018). Protective effects of photobiomodulation against resistance exercise-induced muscle damage and inflammation in rats. *Journal of Sports Sciences*, 36(20), 2349–2357
54. Grandinetti, V., Carlos, F. P., Antonio, E. L., de Oliveira, H. A., Dos Santos, L. F. N., Yoshizaki, A., et al. (2019). Photobiomodulation therapy combined with carvedilol attenuates post-infarction heart failure by suppressing excessive inflammation and oxidative stress in rats. *Scientific Reports*, 9(1), 1–7
55. Manchini, M. T., Serra, A. J., dos Santos, F. R., Santana, E. T., Antonio, E. L., de Carvalho, P. D. T. C., et al. (2014). Amelioration of cardiac function and activation of anti-inflammatory vasoactive peptides expression in the rat myocardium by low level laser therapy. *PLoS ONE*, 9(7), e0101270
56. Nunes RA. Women athletes in the Olympic Games. 2019.
57. de Souza Oliveira, C., de Oliveira, H. A., Teixeira, I. L. A., Antonio, E. L., Silva, F. A., Sunemi, S., et al. (2020). Low-level laser therapy prevents muscle apoptosis induced by a high-intensity resistance exercise in a dose-dependent manner. *Lasers in Medical Science*, 2020, 1–4
58. Dos Santos, S. S., de Oliveira, H. A., Antonio, E. L., Teixeira, I. L. A., Mansano, B., Silva, F. A., et al. (2020). Low-level laser therapy prevents muscle oxidative stress in rats subjected to high-intensity resistance exercise in a dose-dependent manner. *Lasers in Medical Science*, 2020, 1–6
59. Grandinetti, V., Carlos, F. P., Antonio, E. L., de Oliveira, H. A., Dos Santos, L. F. N., Yoshizaki, A., et al. (2019). Photobiomodulation therapy combined with carvedilol attenuates post-infarction heart failure by suppressing excessive inflammation and oxidative stress in rats. *Science and Reports*, 9(1), 9425
60. Junior, E. C. P. L., Lopes-Martins, R. Á. B., Baroni, B. M., De Marchi, T., Taufer, D., Manfro, D. S., et al. (2009). Effect of 830 nm low-level laser therapy applied before high-intensity exercises on skeletal muscle recovery in athletes. *Lasers in Medical Science*, 24(6), 857
61. Junior, E. C. P. L., Lopes-Martins, R. Á. B., de Almeida, P., Ramos, L., Iversen, V. V., & Bjordal, J. M. (2010). Effect of low-level laser therapy (GaAs 904 nm) in skeletal muscle fatigue and biochemical markers of muscle damage in rats. *European Journal of Applied Physiology*, 108(6), 1083–1088
62. Lopes-Martins, R. Á. B., Marcos, R. L., Leonardo, P. S., Prianti, A. C., Jr., Muscará, M. N., Aimbire, F., et al. (2006). Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. *Journal of Applied Physiology*, 101(1), 283–288
63. de Almeida, P., Lopes-Martins, R. Á. B., De Marchi, T., Tomazoni, S. S., Albertini, R., Corrêa, J. C. F., et al. (2012). Red (660 nm) and infrared (830 nm) low-level laser therapy in skeletal muscle fatigue in humans: What is better? *Lasers in Medical Science*, 27(2), 453–458
64. Leal Junior, E. C. P., Lopes-Martins, R. A. B., Baroni, B. M., De Marchi, T., Rossi, R. P., Grosselli, D., et al. (2009). Comparison between single-diode low-level laser therapy (LLLT) and LED multi-diode (cluster) therapy (LEDT) applications before high-intensity exercise. *Photomedicine and Laser Surgery*, 27(4), 617–623
65. Baroni, B. M., Leal Junior, E. C. P., Geremia, J. M., Diefenthaler, F., & Vaz, M. A. (2010). Effect of light-emitting diodes therapy (LEDT) on knee extensor muscle fatigue. *Photomedicine and Laser Surgery*, 28(5), 653–658
66. Vieira, W., Goes, R., Costa, F., Parizotto, N., Perez, S., Baldissera, V., et al. (2006). Adaptation of LDH enzyme in rats undergoing

- aerobic treadmill training and low intensity laser therapy. *Rev Bras Fisioter*, 10(2), 205–211
67. Sussai, D. A., de Carvalho, P. D. T. C., Dourado, D. M., Belchior, A. C. G., dos Reis, F. A., & Pereira, D. M. (2010). Low-level laser therapy attenuates creatine kinase levels and apoptosis during forced swimming in rats. *Lasers in Medical Science*, 25(1), 115–20
 68. Ferraresi, C., de Brito, O. T., de Oliveira, Z. L., de Menezes Reiff, R. B., Baldissera, V., de Andrade Perez, S. E., et al. (2011). Effects of low level laser therapy (808 nm) on physical strength training in humans. *Lasers in Medical Science*, 26(3), 349–358
 69. Junior, E. C. L., de Godoi, V., Mancalossi, J. L., Rossi, R. P., De Marchi, T., Parente, M., et al. (2011). Comparison between cold water immersion therapy (CWIT) and light emitting diode therapy (LEDT) in short-term skeletal muscle recovery after high-intensity exercise in athletes—preliminary results. *Lasers in Medical Science*, 26(4), 493–501
 70. Leal-Junior, E. C. P., Vanin, A. A., Miranda, E. F., de Carvalho, P. D. T. C., Dal Corso, S., & Bjordal, J. M. (2015). Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: A systematic review with meta-analysis. *Lasers in Medical Science*, 30(2), 925–39
 71. Vladimirov, Y. A., Osipov, A., & Klebanov, G. (2004). Photobiological principles of therapeutic applications of laser radiation. *Biochemistry (Moscow)*, 69(1), 81–90
 72. Reid, M. B. (2008). Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radical Biology and Medicine*, 44(2), 169–179
 73. Allen, D. G., Lamb, G. D., & Westerblad, H. (2008). Skeletal muscle fatigue: Cellular mechanisms. *Physiological Reviews*, 88, 287–332.
 74. Ogonovszky, H., Sasvári, M., Dosek, A., Berkes, I., Kaneko, T., Tahara, S., et al. (2005). The effects of moderate, strenuous, and overtraining on oxidative stress markers and DNA repair in rat liver. *Canadian Journal of Applied Physiology*, 30(2), 186–195
 75. Esterbauer, H., Gebicki, J., Puhl, H., & Jürgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine*, 13(4), 341–390
 76. Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, 2014, 1–31.
 77. Effting, P. S., Brescianini, S., Sorato, H. R., Fernandes, B. B., Fidelis, G. D. S. P., Silva, P. R. L. D., et al. (2019). Resistance exercise modulates oxidative stress parameters and TNF- α content in the heart of mice with diet-induced obesity. *Arquivos brasileiros de Cardiologia*, 112(5), 545–52
 78. Rech, A., Radaelli, R., De Assis, A. M., Fernandes, J. R., Longoni, A., Vozari-Hampe, M. M., et al. (2014). The effects of strength, aerobic, and concurrent exercise on skeletal muscle damage in rats. *Muscle & Nerve*, 50(1), 79–86
 79. Ji, L. L. (1995). Exercise and oxidative stress: Role of the cellular antioxidant systems. *Exercise and Sport Sciences Reviews*, 23(1), 135–166
 80. Loureiro, A. C. C., Rêgo-Monteiro, I. C. D., Louzada, R. A., Ortenzi, V. H., Aguiar, A. P. D., Abreu, E. S. D., et al. (2016). Differential expression of NADPH oxidases depends on skeletal muscle fiber type in rats. *Oxidative Medicine and Cellular Longevity*, 2016, 1–10.
 81. Baldwin, K. M., & Haddad, F. (2001). Invited review: Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. *Journal of Applied Physiology*, 90(1), 345–357
 82. Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford: Oxford University Press.
 83. Galdino, G. S., Xavier, C. H., Almeida, R., Silva, G., Fontes, M. A., Menezes, G., et al. (2015). The nitric oxide/CGMP/KATP pathway mediates systemic and central antinociception induced by resistance exercise in rats. *International Journal of Neuroscience*, 125(10), 765–773
 84. Borutaite, V., Budriunaite, A., & Brown, G. C. (2000). Reversal of nitric oxide-, peroxynitrite- and S-nitrosothiol-induced inhibition of mitochondrial respiration or complex I activity by light and thiols. *Biochimica et Biophysica Acta (BBA) Bioenergetics*, 1459(2–3), 405–12
 85. Güzel, N. A., Hazar, S., & Erbas, D. (2007). Effects of different resistance exercise protocols on nitric oxide, lipid peroxidation and creatine kinase activity in sedentary males. *Journal of Sports Science & Medicine*, 6(4), 417
 86. Krause, M., Rodrigues-Krause, J., O'Hagan, C., De Vito, G., Boreham, C., Susta, D., et al. (2012). Differential nitric oxide levels in the blood and skeletal muscle of type 2 diabetic subjects may be consequence of adiposity: A preliminary study. *Metabolism*, 61(11), 1528–1537
 87. Keszler, A., Lindemer, B., Hogg, N., Weihrauch, D., & Lohr, N. L. (2018). Wavelength-dependence of vasodilation and NO release from S-nitrosothiols and dinitrosyl iron complexes by far red/near infrared light. *Archives of Biochemistry and Biophysics*, 649, 47–52
 88. Lohr, N. L., Ninomiya, J. T., Warltier, D. C., & Weihrauch, D. (2013). Far red/near infrared light treatment promotes femoral artery collateralization in the ischemic hindlimb. *Journal of Molecular and Cellular Cardiology*, 62, 36–42