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Low-level Laser Therapy Associated With High Intensity Resistance Training on Cardiac Autonomic Control of Heart Rate and Skeletal Muscle Remodeling in Wistar Rats

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Background and Objective: Phototherapy plus dynamic exercise can enhance physical performance and improve health. The aim of our study was to evaluate the effect of low-level laser therapy (LLLT) associated with high intensity resistance training (HIT) on cardiac autonomic and muscle metabolic responses in rats.

Study Design/Materials and Methods: Forty Wistar rats were randomized into 4 groups: sedentary control (CG), HIT, LLLT and HIT + LLLT. HIT was performed 3 times/week for 8 weeks with loads attached to the tail of the animal. The load was gradually increased by 10% of body mass until reaching a maximal overload. For LLLT, irradiation parameters applied to the tibialis anterior (TA) muscle were as follows: infrared laser (780 nm), power of 15 mW for 10 seconds, leading to an irradiance of 37.5 mW/cm^2, energy of 0.15 J per point and fluency of 3.8 J/cm^2. Blood lactate (BL), matrix metalloproteinase gelatinase A (MMP$_{-2}$) gene expression and heart rate variability (HRV) indices were performed.

Results: BL significantly increased after 8-weeks for HIT, LLLT and HIT + LLLT groups. However, peak lactate when normalized by maximal load was significantly reduced for both HIT and HIT + LLLT groups ($P<0.05$). MMP$_{-2}$ in the active form was significantly increased after HIT, LLLT and HIT + LLLT compared tom the CG ($P<0.05$). There was a significant reduction in low frequency [LF (ms^2)] and increase in high frequency [HF (un)] and HF (ms^2)] for the HIT, LLLT and HIT + LLLT groups compared with the CG ($P<0.05$). However, the LF/HF ratio was further reduced in the LLLT and HIT + LLLT groups compared to the CG and HIT group ($P<0.05$).

Conclusion: These results provide evidence for the positive benefits of LLLT and HIT with respect to enhanced muscle metabolic and cardiac autonomic function in Wistar rats. Lasers Surg. Med. 46:796–803, 2014. © 2014 Wiley Periodicals, Inc.

Key words: resistance training; LLLT; lactate; MMP$_{-2}$; HRV

INTRODUCTION

Resistance training elicits numerous health-related benefits [1]. Resistance exercise has been shown to improve lipid profile [2], glucose tolerance and insulin sensitivity,

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increase basal metabolic rate, heart rate variability (HRV) [3] and muscle and bone mass [1,4], and reduces body fat, blood pressure, and fatigue. In addition, phototherapy has been combined with dynamic exercise to enhance physical performance and improve health in experimental models [5,6] and clinical trials [7–9].

Phototherapy improves cellular activation via absorption of photons by chromophores (e.g., nicotinamide adenine dinucleotide (NADH) dehydrogenases and cytochrome C oxidase) present in the mitochondria [10]. Biophysics and biochemistry effects of phototherapy are believed to include increased electron transport in the mitochondrial respiratory chain, higher production of adenosine triphosphate (ATP) [11,12], gene modulation and tissue regeneration associated with both anti-inflammatory [5] and analgesic effects [13]. In this context, phototherapy associated with high intensity exercise can lead to pain relief, improve muscle performance, greater fatigue resistance, accelerate recovery after dynamic intense exercises and muscle healing after injuries [14].

The extracellular matrix (ECM) surrounding muscle fibers provides a protective effect and maintains functional integrity in these fibers. Recent evidence suggests resistance training, such as ladder climbing in an animal model, elicits ECM remodeling [15]. Among the enzymes involved in ECM remodeling is a matrix metalloproteinases (MMPs) [15,16]. Two isoforms of MMPs are gelatinase A (MMP_{-2}) and gelatinase B (MMP_{-9}), which play an important role during myogenesis and regeneration [17–19]. In this context, low-level laser therapy (LLLT) has been used to accelerate muscle healing. Alves et al. [20] showed that infrared LLLT has a positive effect on the inflammatory process, $MMP\text{-}_2$ activity as well as collagen organization and distribution in the repair process of the tibialis anterior (TA) muscle in rats following cryoinjury. In a study by Dias et al. [21], infrared LLLT promoted the expression of MMPs and stimulated oxidative metabolism of the masseter muscle in rats. Lastly, when infrared LLLT was applied after climbing training, there was reduced resting lactate levels, decreased muscle glycogen depletion and increased cross-section area of TA muscle fibers [22].

The effects of phototherapy on HRV responses have not been broadly investigated. He et al. [23] investigated the effects intravascular laser irradiation of blood (ILIB) therapy with a laser inserted and fixed into the femoral vein or inserted about 3 mm in the Neiguan (PC6) acupoint (laser acupuncture) in rats. The results showed that laser acupuncture reduced HR and ILIB therapy increased HRV modulation [23]. The improvement in HRV is related to cardiac sympathovagal balance, which leads to improvement in exercise tolerance and a reduction of cardiovascular disease risk [24].

A review of the literature [1–4,15,24] demonstrates the benefits of the physical training on cardiovascular, muscular and metabolic function are well-known. However, the LLLT is still rather controversial in the biomedical optics community. There are a variety of skin types, wavelengths, optical powers, device geometry and others parameters to consider when trying to identify the optimal dose for photobiomodulation-promoting stimulation and inhibition of biochemical and biophysical responses in biological tissue [11,12].

Previous studies from our group observed the potential effects of high intensity resistance training on collagen remodeling, aerobic capacity and cardiac autonomic function in rats [3,15]. However, the potential effects of LLLT associated with intense resistance training on these outcomes, to our knowledge, have not been investigated. Thus, the aim of this study was to evaluate the effect of LLLT associated with high intensity resistance training on functional performance in Wistar rats. Our hypothesis was that cardiac autonomic control and muscle metabolic remodeling in animals would be enhanced through the use of LLLT during resistance exercise training.

MATERIALS AND METHODS

Animals

Forty 2–4 month albino male *Wistar* rats weighing 250–300 g were used and cared for according to the European Communities Council Directive of November 24th, 1986. The procedure adopted in this study was approved by the Ethics and Research Committee of the Federal University of São Carlos (N.021/2006). Food (Nuvilab CR1, Nuvital Nutrientes S/A, Brazil) and water were available ad libitum. The animals were kept in polypropylene cages under controlled temperature (22 ± 2°C) and humidity (70%) with a 12/12-hour light-dark photoperiod. The animals were randomly distributed into four groups (10 rats/group): sedentary control (CG), high intensity resistance training (HIT), LLLT and, HIT plus LLLT.

Maximal Resistance Test

All the animals were submitted to a maximal resistant test (MRT) as previously described [3,15]. The rats climbed the first step load-free and subsequent climbs with the load progressively increasing by 10% of body mass (BM) on the first MRT (pre-training) and by 30% of BM on the subsequent MRT (post-training). Loading intervals were every 2 minutes until maximal overload and exhaustion. The criterion for interrupting the test was determined by the incapacity of the animal to perform a complete climb [3,15].

Blood Lactate

Blood lactate (BL) concentration was determinate as previously described [3,15]. The capillary tubes were previously calibrated with 25 μL heparin for blood collection. Blood samples were taken from the animal's tail at the beginning of the MRT and 1 minute after each climb. To prevent glycolysis, the blood samples collected were transferred to 2 mL tubes containing 50 μL 1% sodium fluoride and stored at −10°C. Blood lactate concentration was determined by an electroenzymatic method (YSI 1500 ® – Sport Lactate Analyzer, Yellow Springs, OH).

High Resistance Training

During the resistance training program, the rats climbed the ladder with the external load attached to the animal's tail as previously described [3,15]. After a one week load-free adaptation period, the HIT and HIT + LLLT groups performed 24 training sessions, three times per week for 8 weeks, in the afternoon. Each training session lasted between 6 and 10 seconds and consisted of 8 to 12 limb movements per climb, totaling 58 climbs. In all sessions, the repetition began with a load of 75% of BM and was increased (by 10% of BM) until reaching a maximal overload [3,15].

Low-Level Laser Therapy (LLLT)

Animals, in the LLLT and LLLT + HIT groups were treated with Lasertherapy (Twin laser, MMOptics, São Carlos, SP, Brazil). The irradiation parameters were as follows: infrared laser (780 nm) with a spot area of $0.04\,cm^2$ and an average optical power of 15 mW operated in a continuous mode during 10 seconds, leading to an irradiance of $37.5\,mW/cm^2$, energy of 0.15 J per point and fluence of $3.8\,J/cm^2$ [25]. The LLLT was applied to the center of both the right and left TA muscle (1 point at each limb) in contact mode at a 90° angle with the skin using slight pressure. For the HIT + LLLT group, therapy was applied immediately after HIT.

Heart Rate Variability

The animals were instrumented with femoral venous and arterial catheters, under tribromoethanol anesthesia (250 mg/kg, I.P.) as previously described [3,26]. Data were recorded (Powerlab 8/30, AD Instruments, Oxford, United Kingdom) at 500–1,000 samples per second, controlled via LabChart acquisition software (Lab Chart, Version 7, ADI, Oxford, United Kingdom). The heart rate (HR) was calculated from the pulse of the arterial pressure. The oscillating components were demarcated as very-low frequency (VLF: 0.01–0.20 Hz), low frequency (LF: 0.20–0.75 Hz), or high frequency (HF: 0.75–2.50 Hz) in absolute units (milliseconds squared per hertz), and the predominance of sympathetic and parasympathetic modulation was determined. The LF and HF components of HRV were also expressed in normalized units obtained from the calculation of the percentage of HF and LF, with their respective total power, after subtracting the VLF component [3,26].

MMP_{-2} Gene Expression

Animals were killed by decapitation after study completion. The TA muscle was removed from both posterior hindlimbs and the mass of muscles were measured. The muscle samples were frozen in liquid nitrogen and stored at −80°C. Zymography, performed as previously described [15,27]. Frozen tissue (25 mg) was incubated in 2 ml of extraction buffer (10 mM cacodylic acid, pH 5.0; 0.15 M NaCl; 1 M ZnCl 2; 20 mM CaCl 2 ; 1.5 mM NaN 3 ; 0.01% Triton X-100 [v/v]), at 48°C for 24 hours. After this period, the solution was centrifuged for 10 minutes (13 000*g* at 4°C). Samples were dried and resuspended in the same extraction buffer to apply 20 μg of total protein in each lane of sodium dodecylsulfate (SDS)-10% polyacrylamide gels prepared with 1 mg/ml gelatin. After electrophoresis, the gels were washed twice in 2.5% Triton X-100 to remove the SDS. Gels were incubated in buffer substrate (50 mM Tris-HCl, pH 8.0; 5 mM CaCl 2 ; 0.02% NaN 3) at 37°C for 20 hours. Gels were stained with Coomassie brilliant blue for 1.5 hours and detrained with acetic acid: methanol: water (1:4:5) for visualization of the activity bands [15,27]. All samples were evaluated in triplicate, to guarantee the precision and linearity of the analysis and each sample was normalized for the total amount of protein included. The gels were photographed with a Canon G6 Power Shot 7.1 mega pixels camera (Newport News, Virginia, USA). The averages of band intensity were measured using Gene Tools software (Syngene, Cambridge, United Kingdom). We identified bands that characterize the domains of MMP_{-2} according to molecular mass (72 kDa: pro-MMP_{-2}; and 64 kDa: active-MMP_{-2}), as previously described [15,27].

Statistical Analysis

The results are expressed as mean ± standard deviation (SD). Kolmogorov–Smirnov and Levene's tests were used to analyze the normality and homogeneity of variance. Two-way analysis of variance (ANOVA) was used for intragroup analysis. One-way ANOVA was used for intergroup analysis. When a significant difference was detected, the Tukey's post-hoc test was applied to identify the difference. Statistical Package for the Social Sciences^{TM} (SPSS – IBM, version 10.0.1, 1999) was used for all tests and a level of significance was set at $\alpha \leq 0.05$.

RESULTS

The results of BM and peak load during MRT are listed in Table 1. All the animals participating in HIT groups completed the training protocol. As expected, all the groups increased in BM; however, only HIT induced to significant increase in BM gain compared with controls (Table 1, $P < 0.05$). In addition, the maximal load was higher only in HIT and HIT + LLLT when contrasted with controls (Table 1). In this context, the animals in the HIT and HIT + LLLT groups were able to support higher loads compared to the animals in the control group. The results of workload indicate greater muscle performance, mainly the HIT + LLLT group demonstrate a 300% increase in workload [BM/maximal load ratio (%/g BM)] during the post-training MRT (Fig.1).

Blood lactate concentration showed a significant increase at post-training compared to peak of the baseline test with increasing load for the HIT, LLLT and, HIT + LLLT groups (Fig. 1). However, when LLLT was applied alone or together with HIT, the lactate levels increased only during the last workload of the post-training MRT. In contrast, when peak lactate was normalized by maximal load (peak lactate/maximal load), there were significantly lower levels for both the HIT and HIT + LLLT groups (Fig. 2), illustrating the positive effects of resistance training only or associated with lasertherapy. We emphasized the potential reduction of lactate levels when LLLT was applied, especially after HIT.

Figure 3 illustrates the analysis of MMP_{-2} activity in the TA extracts. MMP_{-2} expression in the active form was significantly increased for the HIT, LLLT and

TABLE 1. Results of Body Mass (BM) and Load Peak Before and After Training

	CG		HIT		LLLT		HIT + LLLT	
	BM (g)	Load Peak (g)	BM (g)	Load Peak (g)	BM (g)	Load Peak (g)	BM (g)	Load Peak (g)
Before	286 ± 19	303 ± 66	292 ± 27	262 ± 83	297 ± 11	309 ± 69	298 ± 18	288 ± 55
After	381 ± 33*	756 ± 102***	413 ± 38*	1021 ± 231***	403 ± 29*	786 ± 151**	401 ± 27*	1166 ± 140***
Change	95 ± 32	453 ± 121	121 ± 28	739 ± 207$^{†\#}$	107 ± 32	476 ± 172	103 ± 20	878 ± 161$^{†\#}$

Data showed as mean and standard deviation. Intragroup differences (*$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$); Intergroup differences: † between CG and # between LLLT.

HIT + LLLT groups compared with the CG ($P<0.05$), indicating positive effects of resistance training and/or lasertherapy on muscle remodeling. Then, the results indicated that HIT only, LLLT only or HIT associated with LLLT may induce upregulation of collagen synthesis in skeletal muscle.

HRV analysis results are illustrated in Fig. 4. There was a significant reduction of LF (ms^2) and a significant increase of HF (un) for the HIT, LLLT and HIT + LLLT groups compared with the CG ($P < 0.05$), showing the positive effects of resistance training and LLLT on cardiac autonomic control. HIT showed lower values of LF/HF ratio when contrasted with the CG; however, HIT + LLLT and LLLT showed lower values of LF/HF ratio compared with CG and HIT ($P < 0.05$), which can elucidate the potential effect of LLLT on sympathovagal balance. In this context, the TA (hindlimb) of rats was irradiated with LLLT, which resulted in modulation of HRV (greater HF and lower LF). This was a novel finding and similar to outcomes with exercise training. These adaptations indicate a reduction of sympathetic tone and a concomitant increase in parasympathetic tone.

DISCUSSION

This is the first study showing increased MMP$_{-2}$ expression and improvement of HRV when infrared laser was applied after resistance training in rats TA muscle. In addition, our study found increased maximal load and a reduction of BL concentration. These findings corroborate

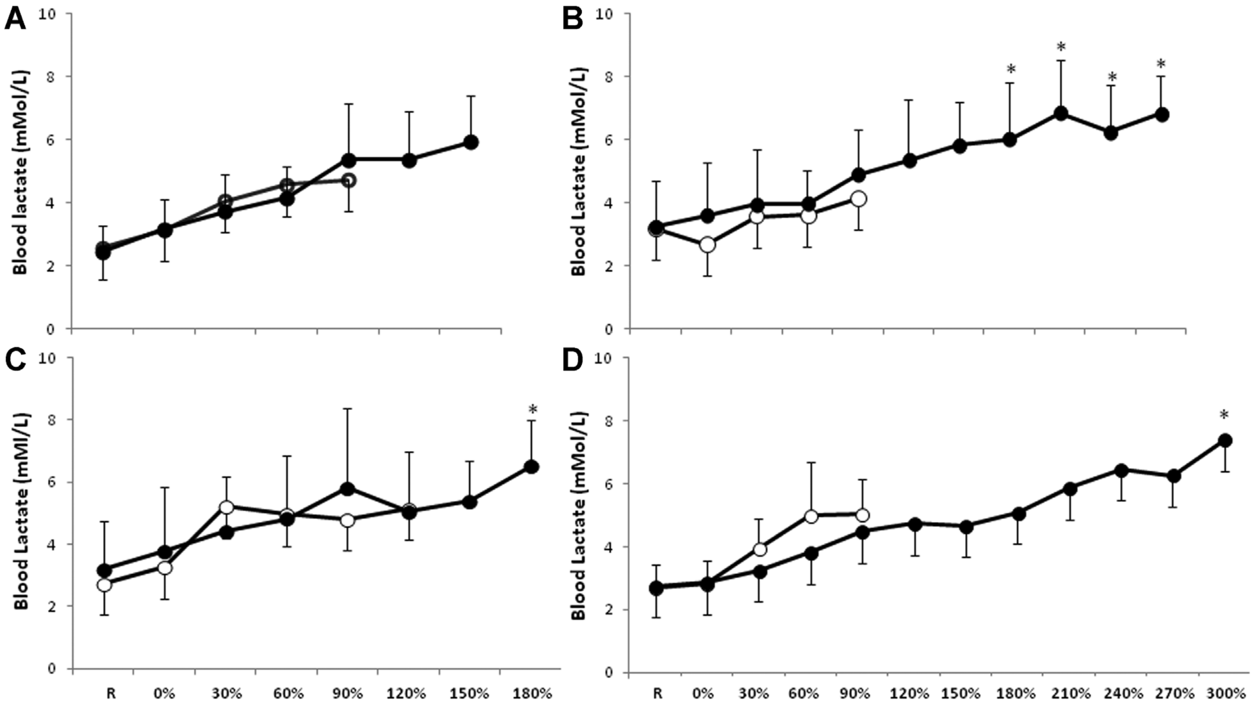

Fig. 1. Blood lactate concentration during maximal resistance test in pre-training (°) and post-training (•). The peak was obtained with load of 150% BM for the CG (A), 270% BM for HIT (B), 180% BM for the LLLT (C) and 300% BM for the HIT + LLLT (D). *$P < 0.05$, Two-way ANOVA.

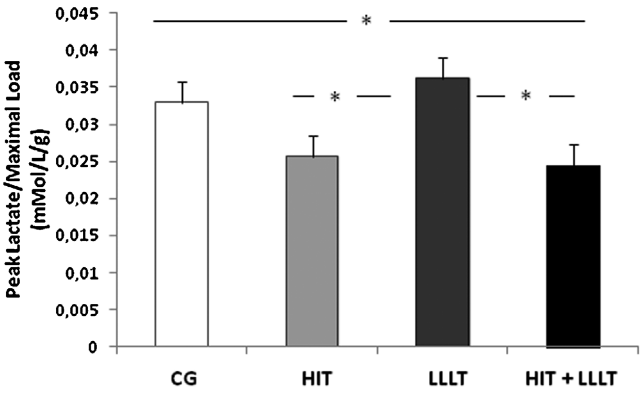

Fig. 2. Peak lactate normalized by maximal load (Peak lactate/maximal load). There were significant differences for the HIT and HIT + LLLT compared with LLLT as well as for the HIT + LLLT compared with CG. $^{*}P < 0.05$, one-way ANOVA.

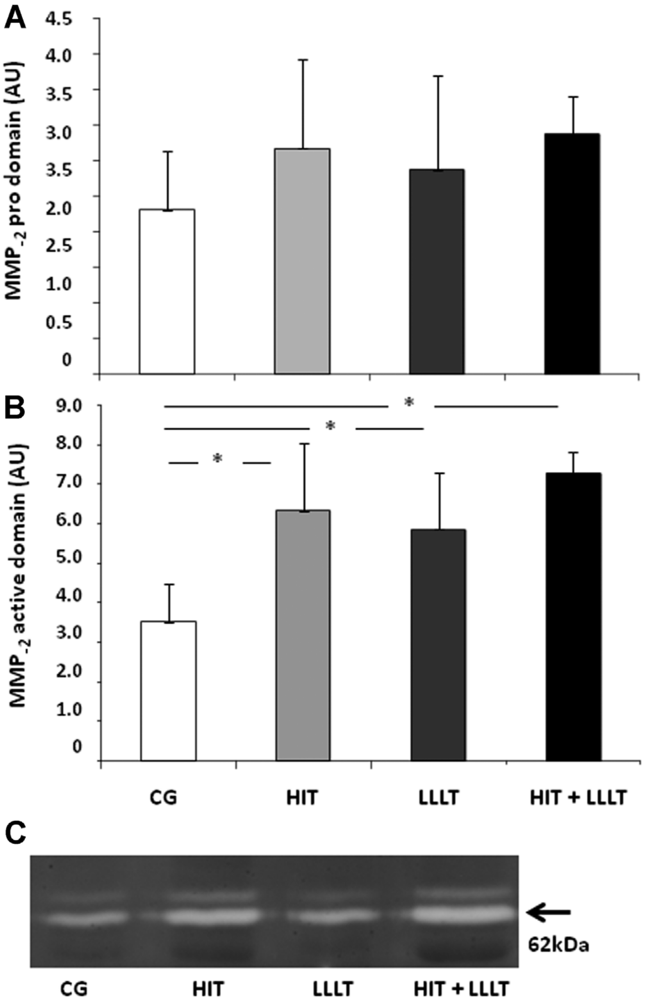

Fig. 3. MMp$_{-2}$ gene expression in TA muscles. In A MMP$_{-2}$ pro domain and in B MMP$_{-2}$ active domain. $^{*}P < 0.05$, one-way ANOVA.

other studies examining the potential benefits of phototherapy associated with physical exercise, demonstrating this approach produced lower BL levels and improved exercise performance in both human [28–30] and animal [22,31] models.

We used infrared laser for phototherapy, because this spectrum has significantly greater depth of penetration. In addition, lasers produce a monochromatic, coherent, and collimated beam. Optical properties of the biological tissue are related by varying rates of absorption, scattering, transmission, and reflection. Laser beam coherence is not lost when entering living biological tissue, but the coherence length is reduced with formation of speckles. There was a higher power density (intensity) within the speckle spot and lower intensity around it. These intensity differences in a speckle field are important, because the therapeutic effects depend on the radiation intensity threshold in deep tissue and the photon absorption cross section of the target molecule (porphyrins, cytochrome-c-oxidase, and others). It depends on wavelength, redox state, polarization, and temperature of the chromophore leading changes in mitochondrial metabolism [32,33].

High-intensity exercise leads to a deficiency in oxygen delivery to skeletal muscle; leading to the oxidative pathway (mitochondrial) being supplemented by anaerobic glycolysis with formation of lactic acid and changes in the muscle contraction process. It is associated with an increase of hydrogen ions that may compete with calcium ions in skeletal muscle troponin C (TnC), stopping the contractile process and causing abrupt cessation of exercise due to muscle fatigue [34]. However, phototherapy has ability to reduce BL levels and increase fatigue resistance [14]. It is known that HIT generates an increase in BL levels because use anaerobic metabolism, but phototherapy can increase local microcirculation [33] as well as improve both oxygen supply and removal of lactic acid [22,31,35] acutely. In addition, phototherapy can improve aerobic metabolism due to stimulation of mitochondrial fusion and formation of giant mitochondria [36,37] chronically.

In our study, we observed that HIT stimulated the remodeling of the collagen matrix when contrasted to the CG. It is well established that HIT may active MMP$_{-2}$ expression. Deus et al. [15] showed that TA muscle MMP$_{-2}$ activity improved after HIT. In addition, they found a positive correlation between BL concentration and MMP$_{-2}$ activity in TA muscle when Wistar rats performed high intensity climbing training. According to Carmeli et al. [16] HIT promotes muscle injury and protein turnover in skeletal muscle fibers with changes involving modulation between degradation and synthesis of ECM. Moreover, theses authors observed that the expression of MMP$_{-2}$ was dominant in muscles containing a high percentage of fast-twitch fibers in response to high intensity exercise [16]. MMPs are known to be up-regulated by muscle, where MMP$_{-2}$ is activated by nitric oxide synthesis and hepatocyte growth factor release for satellite cell activation, promoting both muscle repair and hypertrophy [38]. These data are important for several benefiting from improvement in muscle function (e.g., those with inflammatory

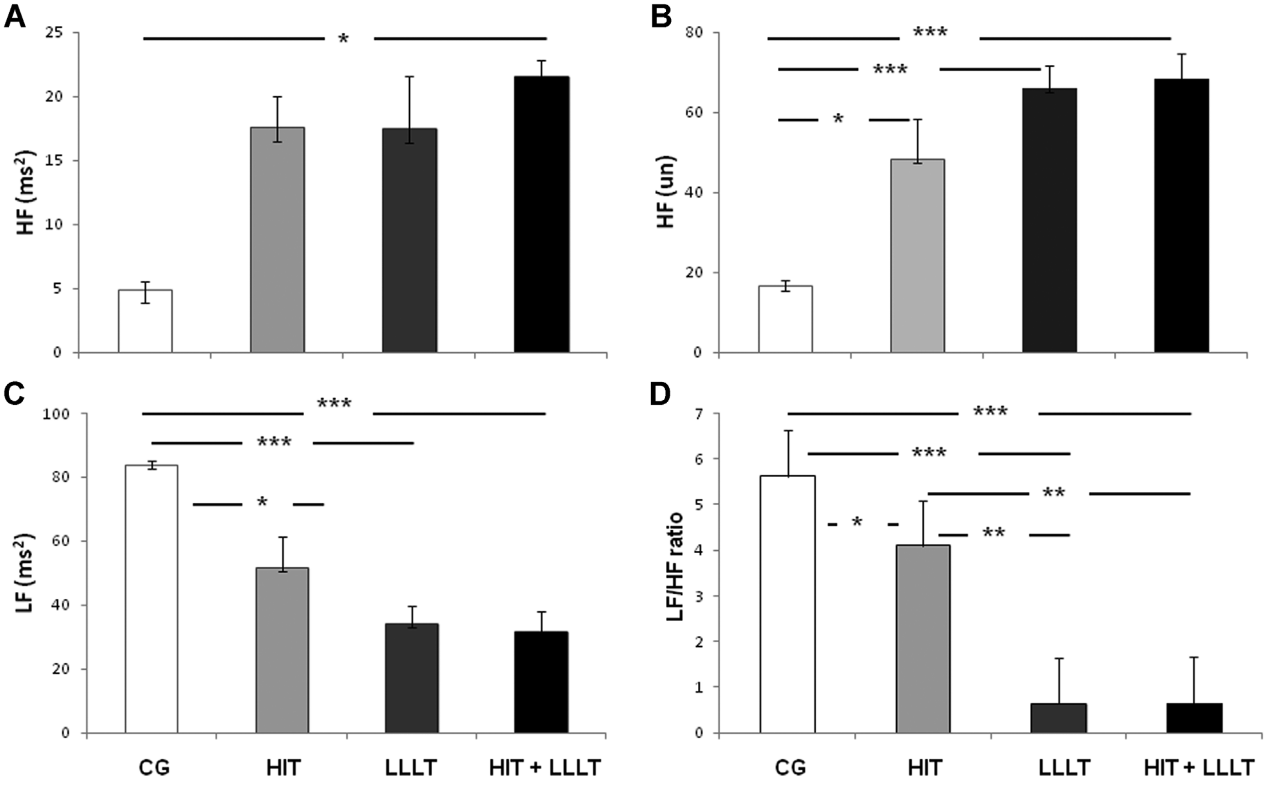

Fig. 4. Heart rate variability (HRV) response. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, one-way ANOVA.

conditions, neural disorders; myopathies as Duchenne muscular dystrophy, those who are aging or obese; people with cardiovascular and respiratory diseases; or in high performance athletes) [16,21,39].

However, in the present study, LLLT without physical exercise and their association promoted higher MMP$_{-2}$ gene expression. Other studies have found positive results of laser alone on MMP$_{-2}$ remodeling. For fatigue resistance and orofacial rehabilitation, Dias et al. [21] performed LLLT on rats masseter muscle. These authors found that LLLT increased the expression of MMP$_{-2}$ and MMP$_{-9}$ as well as stimulated oxidative metabolism with higher activity of NADH diaphorase [21]. Similar results related to MMP$_{-2}$ and increased collagen synthesis was obtained after injury to TA muscle [20] and the Achilles tendon [40] of rats when LLLT was applied to accelerate the tissue repair process.

MMPs can be influenced through the stimulation of cytokines and cell surface ECM receptors [41]. Corazza et al. [5] showed that resistance training and phototherapy regulated tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) as well as stimulated production of insulin growth factor-1 (IGF-1), enhancing anabolic activity and preventing sarcopenia in ovariectomized rats.

Surprisingly, we thought that the association of LLLT with HIT could potentiate MMP$_{-2}$ remodeling of TA muscle, however, we did not observed any difference when comparing LLLT and HIT groups, with the only difference apparent when compared to the CG. However, MMP$_{-2}$ in our study did not permit analysis of a dose response effect since we applied only lasertherapy. In this context, studies [42] have showed that MMP$_{-2}$ expression remodeling can be influenced in a dose-dependent manner to physical exercise.

Finally, our study showed that LLLT applied to the TA muscle improved some indices of the HRV frequency domain when compared to HIT training alone. This finding indicates potentially important systemic effects of phototherapy. We observed that HIT, HIT + LLLT and, LLLT alone promoted higher HF (un) and lower LF (ms^2) when compared to the CG. However, only LLLT and HIT + LLLT improved sympathovagal balance (LF/HF ratio) when compared to both the CG and HIT. These results can demonstrate the potential effects of LLLT, with or without resistance exercise training on sympathovagal balance, reflecting the absolute and relative interactions between the sympathetic and parasympathetic components of the system [43]. This is a novel result and should be explored further. In a recent study, LLLT was applied on the gastrocnemius muscle for 10 consecutive days, producing a significant reduction in the inflammatory profile of rats with heart failure [44]. In addition, LLLT exerted a cardioprotective effect as observed by regulation of expression of cardiac cytokines and contributed to the reversal of ventricular remodeling after myocardium infarction in rats [45]. These results together can explain the positive effects of autonomic nervous function, since the LLLT alone and associated to HIT showed systemic effects that leaded to an addition improvement of sympathetic and parasympathetic modulation in parallel to reduction of blood lactate release in higher intensities of resistance exercise training.

However, HIT and laser alone could elicit some positive effects of cardiac autonomic function in rats, as observed by the improvement of HF (un) and reduction of BF (ms^2). We previously demonstrated that HIT increased some indices of HRV when Wistar rats performed high intensity of climbing training [46]. In fact, physical training programs have been associated with significant benefits to the autonomic nervous system in humans and experimental models [47]. However, to our knowledge, this is the first study to show the effects of HIT with or without LLLT in this type of experimental model. Moreover, future studies needs to be focused on the potential effects of the association of LLLT with HIT in healthy and in systemic diseases.

CONCLUSION

The main results of the present study found that LLLT in conjunction with HIT increased MMP_{-2} gene expression and some HRV indices. It also appears LLLT has independent positive effects on HRV. These results provide evidence for the positive benefits of HIT associated with LLLT, which may prove to be an effective treatment combination in the future.

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