



Surface electromyography after lower level laser therapy application on skeletal muscles in individuals with heart failure

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Abstract

The purpose of this study was to investigate the effects of low-level laser therapy (LLLT) applied before a fatigue protocol through the effects on the electrical activation in the quadriceps muscle in patients with HF. Fourteen patients with the diagnosis of heart failure (HF) were selected for this double-blind, crossover type clinical trial. These participants have attended to a familiarization, LLLT, and placebo sessions, totaling three visits. The LLLT was applied in the quadriceps muscle (850 nm, 5 J per diode). The fatigue protocol consisted of concentric and eccentric isokinetic contractions (cc/ec) until exhaustion or up to 50 cc/ec. The muscular fatigue was evaluated with surface electromyography, by the analysis of integral, median frequency, and entropy. Only one application of LLLT is not able to decrease skeletal muscle activation in patients with HF. There was no reduction of muscle fatigue among the proposed protocols. Single LLLT session has no effect on the reduction of skeletal muscle fatigue in patients with HF.

Keywords Electromyography · Entropy · Low-level laser therapy · Heart failure

Introduction

Heart failure (HF) is a clinical syndrome that causes significant global morbidity and mortality [1]. Over the next two decades, the prevalence of HF will increase 46%, adding eight million newly diagnosed patients over 18 years old and affecting primarily white males [1, 2]. Structural and/or functional cardiac abnormality causes this condition, resulting in an insufficient cardiac output and/or elevated intracardiac pressures at rest or during stress [1]. The symptoms (e.g., breathlessness, ankle swelling, fatigue) are related to the low exercise tolerance and difficulties to perform daily life activities, as well as the decrease in the quality of life of these patients [1]. It is suggested that peripheral factors are the main determinants of

low exercise tolerance, like atrophy, decreased blood flow to muscle tissue, and intrinsic alterations of cellular metabolism, which trigger muscle fatigue [3, 4].

Recent studies show that low-level laser therapy (LLLT) decreases peripheral fatigue of skeletal muscles, increases muscle performance in healthy people, and improves tissue regeneration, as well as modulation of inflammation and pain [5, 6]. A systematic review [6] indicates that, besides improving muscle performance, LLLT applied before exercise may protect the skeletal muscle tissue, avoiding muscle fatigue injuries and improving recovery after exercise. Although the positive effects are documented in the literature for health subjects, there is still little evidence on the effects of LLLT in patients with HF.

Considering that muscle fatigue is a common symptom in patients with HF, one way to evaluate the effect of LLLT on muscle strength and fatigue is by the use of surface electromyography (EMG); indeed, to quantify the electrical activity during contraction in order to measure muscle action during movement is widely used for fatigue analysis [7, 8]. Traditional measures of signal analyses are limited to examining the root mean square (RMS) and median frequency (MF) [9]. However, dynamical systems approach has been used as well [8, 10, 11]. Entropy-based analyses describes the irregularity of time series and depends on several

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neuromuscular system parameters such as firing rate, muscle fiber types, motor unit types, and volumes conductor effects [8, 10]. Therefore, the multiscale entropy (MSE) analysis is applied to evaluate the signal in different time scales and to provide details about the dynamics of the signal [12].

The objective of this study was to evaluate the effects of LLLT on muscle performance and myoelectric activation of the quadriceps muscle in patients with HF during a fatigue protocol. In order to test in implications of LLLT within the temporal complexity of EMG during muscle fatigue at dynamic contractions, the hypothesis of this study is that a single LLLT intervention decreases muscle fatigue.

Methods

Study design and participants

This study is a crossover, double-blind clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier: NCT02508792). All data was collected at the Functional and Nutritional Evaluation Laboratory of the Federal University of Health Sciences of Porto Alegre (UFCSPA, Porto Alegre, RS, Brazil), between July 2015 and May 2016, approved by the human research ethics committee of UFCSPA and Cardiology Institute, University Foundation of Cardiology of Rio Grande do Sul (ICFUC) (approval numbers 995361/2015 and 1.112.281/2015).

Fifteen adults were recruited aging between 35 and 75 years old (61.2 ± 7.2 years old) to join the experiment. Most of them were recruited through the ICFUC in Porto Alegre, RS, Brazil, and others were recruited from a sample of convenience. Inclusion criteria were patients with a clinical diagnosis of heart failure and left ventricular ejection fraction (LVEF) less or around 45%, secondary to ischemic or idiopathic dilated cardiomyopathy and functional class II or III according to the *New York Heart Association (NYHA)* [13]. The participants should be in the stable stage of the disease and without any changes in drug therapy or symptoms exacerbation at least 4 weeks before participating in this study. They should not have participated or initiated a cardiac rehabilitation program. Exclusion criteria were to have any type of lower limbs musculoskeletal injury, orthopedic or rheumatic diseases, and uncontrolled hypertension. As well, those with other pulmonary and psychiatric diseases, without sufficient understanding of the tests application, presence of any LLLT therapy contraindication, and those who did not complete all steps of data collection were excluded from the study. Participants were briefed on the purpose and procedures of the study and signed an informed consent form prior to involvement in the study.

One participant was excluded from the sample for not completing all steps of data collection. Thus, the final sample size was 14 individuals (4 female) (61.2 ± 7.2 years old). Every

participant was submitted to LLLT and/or placebo therapy and was randomly assigned to one of the two groups by drawing lots (A or B). The randomization was performed by an assistant who was not involved in the experiment.

Sample size calculation

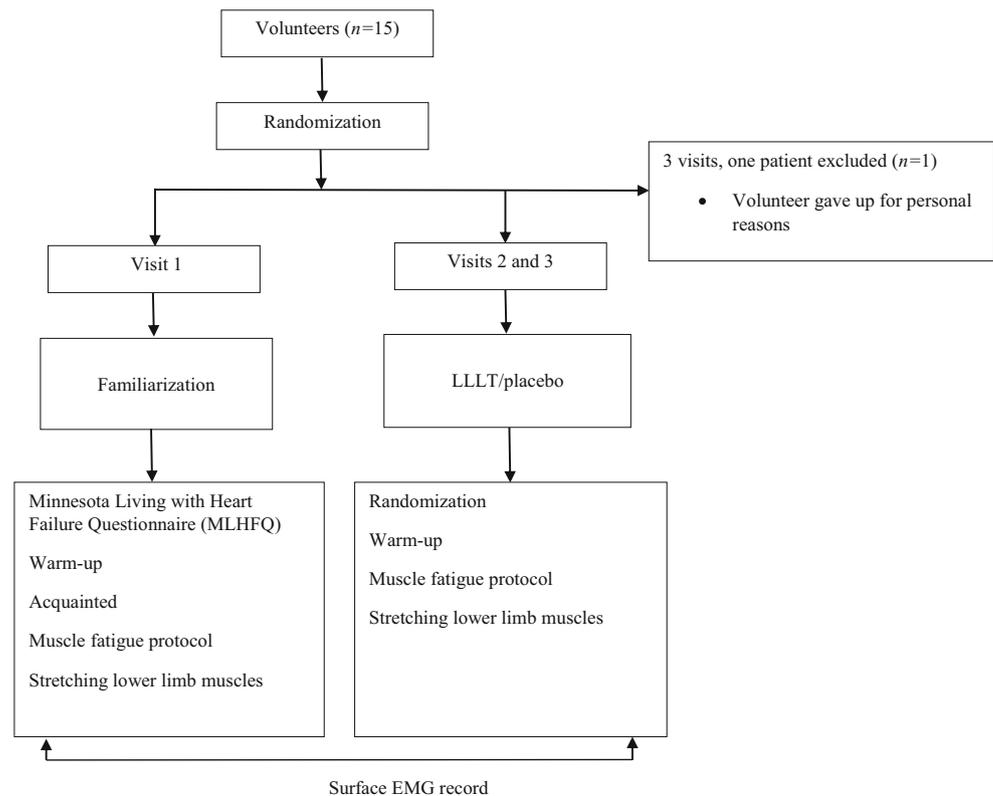
The sample size calculation was based on Wilson et al. [14], who has used as reference the last mean of integrated root-mean-square voltage/contraction (RMSV) in HF patients during maximal workload achieved and maximal-20 immediately before highest workload. Considering the 5% as significance level and 80% as statistical power, the minimum sample size recommended was 14 individuals. The calculation was done using the software OpenEpi, Version 3.0 (Rollins School of Public Health, USA).

Randomization

The randomization was performed with simple drawing lots, using two opaque envelopes corresponding to groups A or B. After randomization, the main researcher left the room and the assistant investigator, without informing the participants and the main researcher (double-blinded), applied the placebo or active laser form. Thus, those involved were blinded to treatment allocation and kept the integrity of the study design.

Exercise protocol

Participants were at the laboratory three times for data collection, with, at least, 2-day interval (Fig. 1). In the first visit, participants were informed about the consent form to join the study, their body size and mass were measured and they answered the *Minnesota Living with Heart Failure Questionnaire (MLHFQ)* [15] to evaluate the quality of life and the limitation in the ability to perform activities of daily living in patients with HF. As soon as the participants were introduced and familiar to the equipment, the fatigue protocol was engaged. At the end of each visit, they stretched their lower limbs (quadriceps, tibial, and gastrocnemius). In the other two visits, the LLLT or placebo was applied, followed by warm-up and fatigue exercise execution. The time for each stage of this study was about 60 min at each meeting. The fatigue protocol was performed using an isokinetic dynamometer (Biodex Advantage 4, Biodex Medical Systems, USA). Participants sat and stabilized on the isokinetic chair according to the instruction manual, keeping 90° of hip and knee flexion, allowing only the knee flexion and extension in the dominant leg. Before the muscle fatigue protocol, subjects performed a warm-up of the dominant lower limb with the isokinetic dynamometer, which consisted of five repetitions of quadriceps concentric and eccentric contractions (cc/ec) with voluntary submaximal force, at 180° s⁻¹ and continuous mode. Verbal encouragement was given during the

Fig. 1 Flow chart of data collection

execution of the protocol. The muscle fatigue protocol [16, 17] consisted of cc/ec to exhaustion or to 50 cc/ec, at 180° s^{-1} and continuous mode.

The muscle fatigue exercise protocol was performed at approximately the same time each visit to control the circadian rhythm, keeping at least 48-h interval on each test to minimize the effects of muscle fatigue. Before and in the end of the test, heart rate, blood pressure, and perceived effort (dyspnea and fatigue in the lower limbs) using the modified Borg scale [18] were measured.

Experimental protocol of LLLT

The LLLT was applied by a cluster-type applicator with infrared laser clusters (ISO: 13485, Model 2779, Chattanooga Group-Intelect® Mobile Laser, Austin, TX, USA), consisting of five-diode GaAlAs laser (850 nm), each one with $0,06 \text{ cm}^2$ area, $0,046 \text{ W/cm}^2$ power density, and 200 mW output power, operated in continuous mode. The cluster was applied for 30 s at each point, with 30 J per point (6 J each diode) and 180 J for the whole quadriceps muscle [6]. Before starting the experiments, the laser equipment was calibrated using a power meter (ILX Lightwave OMN-6810B Optical Multimeter; ILX Lightwave Corporation, Bozeman, MT, USA). The LLLT or placebo (laser turned off) was applied to the subject before each isokinetic exercise session in six areas of the quadriceps muscle (medial distal region, lateral distal region, medial

middle region, lateral middle region, proximal medial region, and lateral proximal region). The infrared wave length (850 nm) is invisible to the human eye, thus the laser was only activated after the application of the cluster to the skin, and it was not removed before irradiation had ended and the cluster was deactivated. This procedure blocked the participants' view of the laser beam. Participants and the responsible for the application of laser wore sunglasses with opaque lenses for eye protection.

Surface electromyography

The electromyographic signal was collected through a six channel wireless surface electrodes electromyography with 1 kHz sampling frequency and 16 bit resolution (BTS TMJOINT®, BTS SpA, BTS Bioengineering, Padova, Italy). The EMG signal visualization was performed by BTS EMG Analyzer Software (B&L, Engineering, Santa Ana, CA).

Before placing the electrodes, trichotomy was done. The skin was abraded with gauze and cleaned with 70% volume of isopropyl alcohol to reduce the skin impedance. The sensors were fixed to the skin by surface electrodes. For impedance control, a digital multimeter DT830D Hyx 54035 was used. Surface EMG was measured in two muscles of the dominant leg: *vastus medialis* and *rectus femoris*. The electrodes positioning followed the Surface Electromyography for the Non-

Invasive Assessment of Muscles (SENIAN) [19] recommendations, for thigh muscles. Electrodes were placed over the *vastus medialis* 80% of the distance from the anterior superior iliac spine to the joint space in front of the anterior border of the medial ligament; for the *rectus femoris*, it was measured halfway (50%) between the anterior superior iliac spine and the superior border of the patella. To confirm the positioning, the volunteer has extended the knee, without hip rotation, against ankle joint manual resistance. The electromyographic signal of the *vastus medialis* and *rectus femoris* muscles were obtained during the muscle fatigue exercise protocol in the isokinetic dynamometer equipment.

Data processing

Raw EMG signals were demeaned to remove DC noise. Then, zero mean raw EMG signals were filtered with low-pass fourth-order Butterworth digital filter, with 500 Hz cutoff frequency. Notch filters (fourth-order Butterworth digital filter) for 60 Hz and its multiple frequencies up to 500 Hz were applied to the raw EMG signal as well. Moving window (2-s long without overlap) along all data series was applied to calculate the median frequency (MDF) of filtered EMG, integral of EMG, and multiscale entropy (MSE) of filtered EMG.

Data analysis

MDF, integral, and MSE were calculated along the filtered EMG time series using a 2-s-moving-window without overlap. The linear trend of these variables during the fatigue protocol was depicted using single linear regression analysis. The slopes of those linear trends were compared between muscles (*m. vastus medialis* and *m. rectus femoris*) and between placebo and LLLT interventions. Two-way analysis of variance was run to make such comparisons. The level of statistical significance was set at $p < 0.05$.

Statistical analysis

Data was presented by descriptive measures of central tendency (mean and median) and variability (amplitude, standard deviation, and quartiles), as well as by absolute (n) and relative (%) distributions. For data normality distribution, the Shapiro–Wilk test was performed. For parametric data, two-way analysis of variance (ANOVA) with Tukey LSD post hoc test was performed. For comparison of non-parametric data, Kruskal–Wallis test or Wilcoxon test were used. Statistical Package for Social Sciences for Windows (SPSS Inc., Chicago, IL, USA, 2008) was used and the level of significance (α) was 5%. For other analysis, GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA) for Windows and scripts written in MATLAB (MathWorks Inc., USA) were used.

Results

The participants' characteristics are summarized in Table 1. Most of them were male (71.4%). The mean age was 61.2 (SD 7.2) years old, with 39.9 (SD 5.2) LVEF and 29.4 (SD 5.9) BMI. Participants were mostly persons with mild heart failure, class II (71.4%) or III (28.6%) according to the NYHA criteria. The ischemic cardiomyopathy type (64.3%) was the most common between the participants and they had a high quality of life score—MLHFQ (34.4 SD 17.4).

The muscular fatigue was evaluated with surface electromyography and the linear regression coefficients for integrals of *vastus medialis* and *rectus femoris* muscles along the fatigue protocol results are presented in Table 2. Placebo and LLLT interventions ($F = 0.8$ $p = 0.36$), and *vastus medialis* and *rectus femoris* muscles ($F = 0.04$ $p = 0.82$) presented similar linear relation. Those results show that integrals of *vastus medialis* and *rectus femoris* muscles increased during fatigue exercise protocol, and such an increase was not different between muscles nor compared placebo laser session.

For MDF of EMG spectra during fatigue protocol (Table 2), placebo and LLLT interventions ($F = 0.04$ $p = 0.83$), and *vastus medialis* and *rectus femoris* muscles ($F = 0.05$ $p = 0.82$) presented similar linear relation. Those results show that MDF of *vastus medialis* and *rectus femoris* muscles increased during fatigue exercise protocol, and such an increase was not different between muscles nor placebo and LLLT protocols.

For MSE of EMG signals, placebo and LLLT interventions ($F = 0.03$ $p = 0.84$), and *vastus medialis* and *rectus femoris* muscles ($F = 0.3$ $p = 0.55$) presented similar linear relation

Table 1 General characteristics of the study population

Age (years)	61.2 (SD 7.2)
Gender	
Male	10 (71.4%)
Female	4 (28.5%)
BMI (kg/m ²)	29.4 (SD 5.8)
Etiology of heart failure	
Ischemic	9 (64.2%)
Valve disease	2 (14.2%)
Congestive heart failure	1 (7.1%)
Dilated cardiomyopathy	1 (7.1%)
Myocardial hypertrophy	1 (7.1%)
Functional class (NYHA)	
II	10 (71.4%)
III	4 (28.5%)
Minnesota quality of life (score)	34.4 (SD 17.3)
LVEF (%)	39.9 (SD 5.1)

BMI body mass index, NYHA New York Heart Association, LVEF left ventricular ejection fraction (echocardiogram—Simpson's method)

Table 2 Linear regression model applied to integrals, median frequency (MDF), and multiscale entropy (MSE) of integrals of EMG signals during fatigue protocol

Variable	Intervention	Muscle	Mean of linear regression coefficient	SE	95% confidence interval	
					Lower bound	Upper bound
Integral	A	VM	1238.8	259.9	711.1	1766.4
		RF	1131.0	259.9	603.4	1658.7
	B	VM	946.9	273.9	390.7	1503.1
		RF	939.8	259.9	412.1	1467.4
MDF	A	VM	5.99	0.09	5.80	6.18
		RF	5.97	0.09	5.78	6.15
	B	VM	5.97	0.09	5.78	6.15
		RF	5.95	0.09	5.76	6.14
MSE	A	VM	0.05	0.09	-0.14	0.24
		RF	0.04	0.09	-0.14	0.23
	B	VM	0.11	0.09	-0.08	0.30
		RF	0.08	0.09	-0.10	0.28

Mean and standard deviation are presented for placebo (A) and low-level laser therapy (B) interventions and *m. vastus medialis* (VM) and *m. rectus femoris* (RF)

(Table 2). Those results show that MSE of vastus medialis and rectus femoris muscles increased during fatigue exercise protocol, and such an increase was not different between muscles nor placebo and LLLT protocols.

In relation to the fatigue in the lower limbs through the perceived effort, groups showed similar dyspnea-perceived effort. Both groups showed augmented fatigue sensation for lower limbs (2 [0–3] vs. 4 [1–7], $p = 0.001$ for the placebo group and $p = 0.002$ for the LLLT group) after the fatigue test (Fig. 2a, b).

Discussion

This study investigates lower limbs muscle activation through EMG signal complexity during dynamic contractions pre and post a fatigue protocol after LLLT application in individuals with HF. The hypothesis of this study was that a single LLLT intervention is able to decrease muscle fatigue levels. The results showed that a single LLLT application has not decreased muscle activation and has not improved muscle performance in subjects with HF as well.

It is known that muscle fatigue is a neuromuscular condition that disables the muscle to maintain the required level of contraction and strength, and is associated with the decrease in physical performance and perceived exertion to perform a daily life activity or to exercise [21, 22]. The neuromuscular system is complex dynamical system in which the EMG signal provides preliminary evidence for a better understanding of muscle activity and muscle fatigue [23, 24]. Muscle activity and fatigue can be detected in different time domains and the time-frequency analysis of the EMG signal can provide such evaluation, suggesting the irregularity of time series [8].

Patients with HF have a reduction in electromyographic activity that is related to the morphological disorders of the musculoskeletal system [25]. Within the exercise protocol of muscle

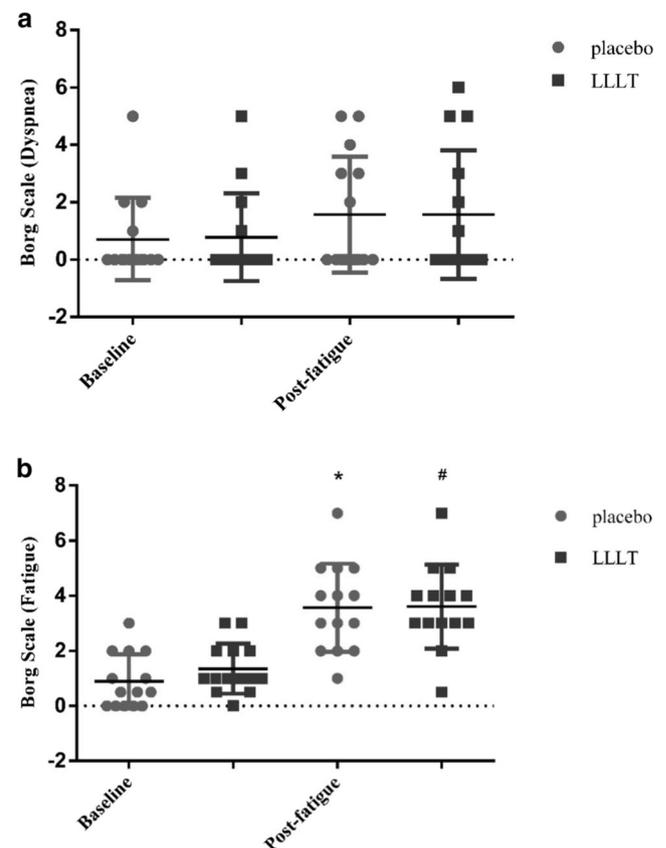


Fig. 2 **a** Dyspnea, pre and post fatigue protocol (BORG scale). **b** Fatigue in the lower limbs, pre and post fatigue protocol. (BORG scale) Values are median and minimum/maximum (Kruskal–Wallis, * $p < 0.001$ placebo group baseline vs. post fatigue, # $p < 0.002$, LLLT group baseline vs. post-fatigue)

fatigue, it was possible to observe the increase in muscle activity in the *rectus femoris* and *vastus medialis* muscles, as expected, since the maximal muscle contraction was encouraged. Wilson et al. [14] applied a fatigue bicycle protocol in HF patients and healthy individuals evaluated through EMG; and both groups reported leg fatigue and showed more muscle activation. However, patients with HF had increased muscle activations at much lower workloads. Our results showed that a single LLLT session (acute effect) was not sufficient to improve muscle performance in subjects with HF, since there was no difference in muscle fatigue reduction between LLLT intervention and placebo protocol. However, different laser therapeutic windows and studied populations must be considered. Antonialli et al. [20], with healthy and untrained individuals, showed that a single phototherapy intervention (low-intensity laser combined with LED) with different therapeutic doses increased the performance of those individuals, decreased late muscle pain, and showed improvement in the creatine kinase biochemical marker. The different populations and the chosen laser parameters may be related to such results. Vanin et al. [26] determined the optimal moment to apply phototherapy irradiation when used in association with strength training. They suggest that the increase in strength without muscle mass gain may indicate that pre-muscle training phototherapy may improve the overall muscle quality and efficiency. Moreover, the phototherapy applied (a) after strength training sessions (and before placebo) and (b) before and after training sessions, did not increase strength compared with the placebo group. The authors suggest that this may be related to the overall dose delivered to the muscle tissue. The need for further investigations on the effect of LLLT on individuals with HF at different doses of phototherapy and number of training sessions is important to optimize the therapeutic window. Another study [27], with healthy rats, showed that the combined use of different wavelengths may increase the effects of LLLT and may improve performance and protect skeletal muscle against the effects of fatigue.

Although some studies have showed the beneficial effects of LLLT on reducing muscle fatigue levels and improving muscular performance in rats with heart failure, healthy individuals, professional athletes, and elderly women, those effects are still controversial [6, 28–31]. Specific responses from LLLT intervention in individuals with HF remain unexplored. A systematic review with meta-analysis, included 39 randomized controlled trials to identify the effectiveness of phototherapy, the best application time in exercise protocols, and the effective parameters for improving muscular performance and for reducing muscle fatigue, showed that further investigation is required due the studies' methodological quality, small sample size, variability of exercise protocols, and parameters of phototherapy [31]. In this sense, to optimize therapeutic windows in different populations is a challenge, notwithstanding, studies including subjects with HF and LLLT are valuable for the health condition improvement of these subjects.

A study with elderly women under a fatigue exercise protocol [30] shows that photobiomodulation has reduced muscle fatigue with the increase of electromyographic fatigue index (EFI) compared to a placebo session. On the other hand, Toma et al. [29] did not observe any difference in EMG signal after fatigue in elderly women, despite the increase in the number of repetitions when LLLT was actively applied before the fatigue protocol. Similarly, our study showed that the values of the integral and MDF of EMG signal for the measured muscles increased during fatigue exercise protocol, as expected. Nevertheless, such increase was not different between muscles nor placebo and LLLT protocols, even considering that one of the effects of LLLT improves cellular activation at the mitochondrial level and ATP synthesis, an effect that could explain the decrease of muscular fatigue and consequent improvement in muscular performance [6, 32, 33]. Recently, other study suggests that LLLT when applied at a dose of 135 J, at the quadriceps muscle, was able to increase the frequency of pedaling at the final stage of the cycling test compared to the placebo, contributing to the maintenance of maximum effort for a longer period. One possible speculation about the increase of the frequency activation using LLLT may be related to the increased microcirculation and tissue oxygenation [34]. Still, the optimal dosage on patients HF remains unclear, as well as, other phototherapy parameters.

To the best of our knowledge, this is the first study to evaluate the entropy and other EMG variables during a lower leg muscle fatigue protocol after LLLT intervention in patients with HF. Traditional EMG analysis is limited to assess the physiological variability of the biological signal and it is usually based on the EMG signal amplitude parameters [10, 23]. Entropy-based analysis provides information on the rate of information production of dynamical systems, facilitating the detection of the onset of muscle activity without noise interference [35]. Therefore, Cashaback et al. [10] investigated the complexity of the surface EMG during muscle fatigue and the intensity of contraction using an isometric muscle fatigue protocol. They found that the complexity of the EMG signal in the brachial biceps of healthy individuals decreased near to exhaustion; as well, the complexity of surface EMG is dependent on contraction intensity. We found that MSE increased to a critical time scale for *vastus medialis* and *rectus femoris* muscles recorded from a HF subjects and the signal complexity showed similar patterns, possibly because a single intervention was not able to produce the desirable results.

MSE of *vastus medialis* and *rectus femoris* muscles increased during fatigue exercise protocol. Entropy is a measure of complexity in physiologic signals [36]. MSE refers to the organization of the signal across different time scales. Higher entropy suggests higher level of complexity within the signal [37]. In some pathologic conditions, physiologic signals might increase [37]. In terms of EMG signal, it suggests that more signal patterns appear within the signal when data is

evaluated in different time scales [36]. More EMG patterns is an indication that more motor units have been recruited, which is feasible under the need to maintain muscle torque during the task. As more long cycles of knee flexion and extension were developed, other motor units have been recruited to avoid the task failure [36]. Entropy similarity between muscles that have similar functions suggests that a common central nervous input might be responsible for this behavior [37]. Such similarity has also appeared when we compared the experimental protocols. Therefore, we can suggest that the effect of LLLT onto the muscle is not directly related to the central nervous system, but it is not clear either.

Regarding the perceived exertion for lower limbs, there was a difference between the baseline and fatigue condition, which shows that the fatigue protocol was effective. Notwithstanding, the acute use of LLLT was not able to reduce the fatigue sensation in lower limbs and dyspnea. Miranda et al. [38] showed that the combined use of phototherapy before exercise decreased the dyspnea and fatigue in the lower limbs in patients with COPD. Most patients with HF are limited in their physical activity and their ability to exercise is diminished. Bona et al. [39] have showed more coactivation of the antagonist muscles in walking in patients with chronic heart failure, which would justify the higher energy expenditure and the self-selected speed of the slower walking, limiting the functional capacity of these individuals. Thus, it is important to consider novel interventions, as LLLT, which can improve walking speed and exercise tolerance in HF patients in combination to the traditional approaches as the pharmacological, aerobic, and resistance training programs [28].

One limitation of our study is the single application of LLLT. Therefore, it is still necessary to understand the long-term and chronic effects of LLLT in persons/patients with HF. Besides, most of the participants were HF class II and their peripheral limitation may not be enough for LLLT to produce effects. Other LLLT parameters may be used based on the recent literature for trying to reach the therapeutic window for this population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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