Lasers in Medical Science (2019) 34:991–999
https://doi.org/10.1007/s10103-018-2689-0

ORIGINAL ARTICLE

Cold water immersion or LED therapy after training sessions: effects on exercise-induced muscle damage and performance in rats

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Received: 2 January 2018 / Accepted: 8 November 2018 / Published online: 19 November 2018

Abstract
Cryotherapy and phototherapy have been suggested as recovery methods due to their anti-inflammatory effects. They may also induce mitochondrial biogenesis, thus favoring endurance training adaptation. The aim of this study was to evaluate the anti-inflammatory and ergogenic effects of phototherapy or cold water immersion (CWI) applied daily after exercise in rats. Thirty-five rats were divided into five groups: control (CO), non-exercised (CE), passive recovery (PR), cold water immersion (CWI), and LED therapy (LED). The CO and CE groups were not submitted to training; however, the CE were submitted to an exhaustion test after the training period. Low-intensity swimming training (21 sessions, 45 min) was performed followed by passive recovery (PR), CWI (10 °C, 5 min), or infrared irradiation (940 nm, 4 J/cm^2). Forty-eight hours after the final training session, the CE, PR, CWI, and LED animals were submitted to an exhaustion test. The animals were euthanized 24 h later and submitted to hematological, creatine kinase (CK), and C-reactive protein (PCR) analysis. Gastrocnemius and soleus muscles were submitted to histological analysis. No differences in blood cell counts, CK, and PCR were detected between groups. The CE group presented an increased number of areas with necrosis in the gastrocnemius and soleus muscles. The PR group presented the highest frequency of areas with edema and inflammation followed by CWI and LED groups. None of the recovery methods improved the performance in the exhaustion test. Successive applications of recovery methods do not improve exercise performance, but downmodulate the inflammation and prevent muscle necrosis.

Keywords Cryotherapy · Phototherapy · Phagocyte · Performance

Introduction

Recovery methods have been used in physical training to prevent exercise-induced muscle damage (EIMD) and inflammatory reactions; however, it is not clear if they could improve muscle adaptation. Moderate- to high-intensity training loads can induce muscle adaptation, increasing exercise performance and aerobic adaptation [1]. However, a single bout of moderate to intense exercise may cause some degree of tissue damage, highlighted by temporary decrements in performance, EIMD, and delayed-onset muscle soreness (DOMS) [2–4]. A high-intensity load or an eccentric exercise cause disruption of the myofibrillar cytoskeleton, Ca^{2+} release from the sarcolemma, and adenosine triphosphate (ATP) depletion [3, 5], inducing an inflammatory reaction that causes further oxidative damage and the chemotaxis of phagocytic cells [3, 5, 6]. Thus, a stressful stimulus, such as high-intensity or prolonged exercise, should be followed by a recovery period for tissue repair and to achieve tissue adaptation [7, 8]. This may require increased time to recovery and any method that could prevent EIMD might be useful for individuals to have a prompt return to training sessions.

Cryotherapy in cold water immersion (CWI) is one of the most popular recovery methods and seems to be beneficial in physical recovery after endurance exercises, allowing faster

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recovery from training sessions or preserving performance after an acute bout of exercise [9, 10]. Reduced EIDM and improved performance have been reported after acute bouts of exercise in experimental [6, 10] and human studies [9, 11]. On the other hand, some authors demonstrated that the acute effects of CWI were associated with a reduced perception of fatigue but not a reduction in muscle damage markers [12–14]. Regarding chronic use, a study in overweight patients suggested that the association of cryotherapy with moderate-intensity aerobic exercise could improve the enzymatic antioxidant system and reduce oxidative damage markers [15]. This suggests that chronic use of CWI may contribute to muscle adaptation and blunt the effects of EIMD. However, in relation to muscle performance and adaptation, recent reports present controversial findings. Some suggest cryotherapy may stimulate mitochondrial biogenesis and improve adaptation to endurance exercise when applied after acute bouts of exercise or chronically after exercise sessions with high training loads [16, 17]. However, other studies did not find improvements in endurance performance or signaling for mitochondrial biogenesis after 6 weeks of sprint interval training [18] or after 21 days of high-intensity cycling training [19]. Another study did not find improvements in performance but upregulation of signaling cascades of mitochondrial biogenesis after chronic use of CWI following high-intensity interval training sessions [20]. It is not clear if cryotherapy could improve muscle performance when associated with moderate loads intended not to cause major muscle damage. The effects of chronic use of CWI on protection against EIMD are also not clear.

Phototherapy is a therapeutic modality that employs red to near-infrared irradiation, emitted by laser diodes or light-emitting diodes (LED), to decrease pain, inflammatory reactions, oxidative stress, and improve tissue repair [21–23]. Phototherapy has been suggested as a recovery method to prevent EIMD and DOMS, besides improving tissue repair and functional recovery [23–26]. Phototherapy can increase the mitochondrial electron transport rate and ATP synthesis, promote mitochondrial biogenesis, downmodulate proinflammatory intracellular signaling pathways, inhibit inflammatory cell infiltration, increase antioxidant status, and stimulate angiogenesis [6, 23, 27, 28].The acute effects of phototherapy have demonstrated that it can prevent EIMD and inflammatory reactions in animal studies [6, 10] and improve muscle performance recovery in human studies [24]. Taken together, these biological effects may favor endurance training adaptations and protect against EIMD. However, the effects of chronic application of phototherapy on endurance performance and EIMD are still unclear.

The aim of the present work was to evaluate and compare the effects of cryotherapy and phototherapy applied after endurance training sessions on physical performance adaptation and EIMD of Wistar rats submitted to swimming training. Considering that CWI and phototherapy have biological effects that may improve endurance adaptation, the use of these methods during physical training program could promote tissue adaptation under lower loads and with shorter periods of recovery. The methods also could improve anti-inflammatory pathways that may protect against EIDM induced by a fatiguing bout of exercise. The hypothesis was that even at low-intensity loads, both recovery methods may improve muscle performance and protect against EIMD.

Methods

Animals

Thirty-five male Wistar rats, 4 months old, weighing 300 ± 10 g, were housed at 20–22 °C, 12 h light/dark cycle, with free access to normocaloric rodent chow (NUVILAB®CR1, Nuvital, Colombo, Brasil) and tap water. The animals were randomly distributed into five groups:

- Control (CO, $n = 6$)
- Control submitted to exhaustion test (CE, $n = 7$)
- Trained submitted to passive recovery (PR, $n = 8$)
- Trained submitted to cold water immersion (CWI, $n = 7$)
- Trained submitted to LED therapy (LED, $n = 7$)

All experiments were previously approved by the Ethics Committee on the Use of Animals of the Universidade Estadual de Londrina (protocol 077/2013).

Exercise protocol

The PR, CWI, and LED groups were submitted to 4 days of familiarization with the water environment, resting for 5 min in the water, and increasing the water level by 10 cm per day. The exercise protocol was performed for 21 days according to a swimming protocol adapted from Da Costa Santos et al. [29]. The exercises were carried out 5 days per week, in plastic containers (45-cm diameter and 60-cm depth), filled with 40 cm of water, at 30 °C (± 2 °C). In the first and second training sessions, the animals swam for 7 min without additional load. From the third session onwards, individual loads were attached to their chest and weekly adjusted to their body weight. Training loads and time of exercise were progressively adjusted until day 21 (Table 1).

Recovery methods

Immediately after each training session, the animals were sedated (1 g/kg ketamine hydrochloride, Francotar® 10%, Virbac do Brasil, São Paulo, Brazil) and submitted to a

Table 1 Days of exercise protocol (time and load) and application of passive recovery (PR), cold water immersion (CWI), or phototherapy (LED) post-exercise sessions

Days	1–2°	3–5°	6–7°	8°	9–12°
Time (min)	7	10	Rest	15	20
Load (%body weight)	0	3	–	4	4
Treatment	–	–	–	PR, CWI, and LED	PR, CWI, and LED
Days	13–14°	15–16°	17–19°	20–21°	22°
Time (min)	Rest	25	30	Rest	Exhaustion test
Load (%body weight)	–	4	4	–	4
Treatment	–	PR, CWI, and LED	PR, CWI, and LED	–	–

recovery method (Table 1). The PR animals were allocated in their cages under supervision until they recovered from sedation.

The entire hind paws (from iliac crest and tail) of the CWI group were immersed in cold water, at 10 °C (± 2 °C) for 5 min. The water temperature was constantly monitored with a thermometer. After immersion in cold water, they were housed in their cages and supervised until conscious recovery.

The LED group was irradiated in both hind paws (surae triceps muscles) at 940-nm wavelength and 45-nm bandwidth. The LED apparatus contains 6 LEDs (Everlight electronics Co, Taipei, Taiwan) with a 2.7-mW power output, power density of 16.2 mW/cm^2, and irradiated area of 1 cm^2. The muscles were irradiated during 250 s, to achieve a total dose of 4 J with an energy density of 4 J/cm^2. The light source was attached to a support 1 cm above the belly of the triceps surae, with the rats lying in a supine position. The optical output of the LED apparatus was measured with a power meter (PD 300 Sensor Fotodiodo; Ophir Optronics, Jerusalem, Israel). The LED equipment was developed by the laboratory of Optics and Opticoeletronic of the Physical Department of the Universidade Estadual de Londrina. After receiving LED irradiation, the animals were housed in their cages and supervised until recovery from sedation.

Exhaustion test

On the 22nd day, the CE, PR, CWI, and LED animals were submitted to an exhaustion test to evaluate their performance, performed 72 h after the final swimming session. The CE animals were familiarized with the water environment as previously described for the trained groups. The rats had a load of 4% body weight attached to their chest and swam until exhaustion, i.e., until the animals could not remain on the surface of the water and sank for more than 5 s [6, 30]. Time to exhaustion was recorded by two calibrated observers blinded to the allocation of experimental groups.

Biochemical analysis and hematology

Twenty-four hours after the exhaustion test, the animals were anesthetized with an aqueous solution of xylazine hydrochloride (0.02 g/kg, Virbaxyl® 2%, Virbac do Brasil, São Paulo, Brasil) and ketamine hydrochloride (1 g/kg Francotar® 10%, Virbac do Brasil, São Paulo, Brasil) and blood samples were collected by heart puncture. The creatine kinase (CK) and C-reactive protein (CRP) levels were determined using an automated biochemical analyzer (Dimension EXL™, Siemens, Munich, Germany). The hematological analysis was carried out in an automated hematological analyzer (BC 2800 Veterinary, Mindray Medical, Nanshan, China). After blood sampling, the animals were euthanized with an overdose of anesthetic solution.

Histological analysis

The right soleus and gastrocnemius muscles were harvested, weighed, and immediately fixed in Bouin's solution. The epididymal fat depots were also removed and weighed. The muscles were embedded in histological paraffin and 10 sections of 7-μm semi-seriated slides (100-μm distance apart) were stained in hematoxylin and eosin.

Ten images of each slide (1-mm distance from each other) were captured at × 10 magnification using a digital camera (Moticam, Motic, Xiamen, China) coupled to an optical microscope. The images were analyzed by two blinded and calibrated histologists, using Motic Image Plus 2.0software (Motic, Xiamen, China). The images were divided into 100 fields of 10,000 μm^2, and the number of fields containing damaged muscle fibers and inflammatory infiltrate was recorded [31]. The muscle cells were considered damaged when they presented signs of necrosis (acidophilic cytoplasm, loss of striation, loss of nuclei, pyknotic nuclei, irregular contour, and degradation by phagocytic cells). The number of fields presenting inflammatory cells (macrophage and neutrophils) in connective tissue or in contact with muscle fibers was recorded, in addition to the number of inflammatory cells per field. Areas of edema were counted when the presence of areas containing acidophilic amorphous material and inflammatory cells interspersed between muscle fibers were observed.

Statistical analysis

The Kolmogorov-Smirnov test was applied to ascertain normal distribution of data. The ANOVA two-way and post hoc Tukey tests were applied to parametric data to compare mean differences between groups. The Kruskal-Wallis and post hoc Dunn tests were used to compare non-parametric data. The differences in frequency distribution between groups were tested with the chi-squared test with Yates correction or the Fisher exact test. Data are expressed as mean and standard deviation (parametric data) or median and interquartile intervals. Differences were considered significant if $P < 0.05$. All tests were carried out with the statistical program GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

There were no differences between groups regarding body weight, epididymal fat depot, or soleus and gastrocnemius muscle weights (Table 2).

Inflammation and muscle damage

There were no differences in hematological parameters, CK, or CRP protein levels between groups (Table 3).

The microscopic analyses of the soleus muscle demonstrated that the CE group presented an increased frequency of microscopic fields presenting necrotic muscle cells in relation to the CO (not submitted to exhaustion test), PR, CWI, and LED groups (Table 4, Fig. 1). Areas of necrosis presented similar frequency in the PR, CWI, and LED groups (Table 4). All swimming groups presented increased areas of edema in relation to the CO animals (Table 4). The edema areas were increased in the CE and PR in relation to the CWI and LED groups (Table 4). The frequency of edema areas was not significantly different in CWI and LED groups (Table 4). Inflammatory cell infiltration was increased in all groups in relation to the CO; however, the PR group presented an increased frequency of fields containing inflammatory cells compared to the CWI and LED groups (Table 4). The LED group presented a lower number of fields containing inflammatory cells than other trained groups (Table 4). The CWI and LED groups had a lower count of inflammatory cells per area compared to the PR group (Table 4).

In the gastrocnemius muscle, all groups presented an increased frequency of areas containing necrosis, edema, and inflammation in relation to the CO animals (Table 5). The CE group presented the greatest frequency of areas of necrosis, followed by the PR, LED, and CWI groups (Table 5). The CWI group presented reduced a frequency of necrotic areas in relation to LED animals (Table 5). The PR presented a large number of fields containing edema and inflammatory cells, whereas the LED group presented few edema and inflammatory areas (Table 5). In the gastrocnemius muscle, the LED group presented a low frequency of edema and inflammatory areas in comparison to CWI group (Table 5).

Swimming performance

The exhaustion test did not reveal differences in swimming time between the CE (151.1 ± 71.9 min), PR (156.6 ± 48.4 min), CWI (156.4 ± 34.2 min), and LED (151.1 ± 109.5 min) groups ($p > 0.05$, one-way ANOVA).

Discussion

The main finding of the present work is that the chronic use of CWI or LED therapy could decrease exercise-induced muscle damage and inflammatory reactions. However, the association of recovery methods with low training loads did not evoke improvements in exercise performance. A large variation in LED group performance was observed because some animals

Table 2 Body weight and weight of epididymal fat depot, soleous muscle, and gastrocnemius muscle

	CO	CE	PR	CWI	LED
Initial body weight (g)	328.3 ± 33.1	346.7 ± 39.8	375.1 ± 39.4	372.7 ± 8.4	363 ± 16.4
Final body weight (g)	363.8 ± 19.6	369.3 ± 40.2	388.9 ± 37.1	388.6 ± 25.9	404.6 ± 26.5
[1]Epididymal fat depot (g)	3.87 (3.58–5.10)	4.58 (4.04–5.75)	5.06 (4.16–6.67)	4.97 (4.45–5.41)	4.17 (3.23–6.15)
[1]Soleous muscle (g)	0.16 (0.16–0.20)	0.18 (0.16–0.21)	0.20 (0.16–0.21)	0.18 (0.17–0.18)	0.20 (0.18–0.24)
[1]Gastrocnemius muscle (g)	2.33 (2.31–2.38)	2.26 (2.16–2.26)	2.23 (2.17–2.38)	2.41 (2.22–2.61)	2.46 (2.35–2.62)

CO control group, *CE* animals submitted to training and no recovery method, *PR* animals submitted to training and passive recovery method, *CWI* animals submitted to training and cold water immersion, *LED* animals submitted to training and LED therapy; [1] data expressed as median (interquartile interval)

Table 3 Leukocytes, CK, and CRP levels of Wistar rats not trained (CO), not trained and submitted to exhaustion test (CE), submitted to a training program without use of recovery methods (PR), or submitted to cold water immersion (CWI) or phototherapy (LED) recovery methods

	CO ($n = 6$)	CE ($n = 7$)	PR ($n = 8$)	CWI ($n = 7$)	LED ($n = 7$)
[1]Leukocytes (cells/mm^3)	6950 (5600–8375)	6850 (5525–8800)	6100 (5200–8150)	7600 (5100–8275)	7300 (7300–8300)
Lymphocytes (cells/mm^3)	5211 ± 1927	4466 ± 1495	4604 ± 1339	5284 ± 1457	4482 ± 1035
Neutrophils (cells/mm^3)	1520 ± 384	2334 ± 1376	2076 ± 750	1749 ± 794	2715 ± 700
[1]CK (units/L)	492 (237–1168)	590 (223–1119)	465 (269–1256)	354 (247–756)	792 (320–1596)
[1]CRP (mg/ml)	4.4 (1–20)	9.0 (7–21)	3.5 (2–7)	4.0 (3–20)	4.0 (2–10)

[1] Data expressed as median (interquartile interval), Kruskal-Wallis test

improved physical performance whereas two rats decreased. This suggests that the use of phototherapy associated with low training loads may impair physical performance or may not improve physical adaptation in some subjects.

Cold water immersion is the most popular cryotherapy method used in the recovery of athletes. The acute effects of cryotherapy may decrease local blood influx and muscle temperature, and blunt the inflammatory process, attenuating the migration of inflammatory cells in EIMD [6, 9, 32]. Our results suggest that chronic application of CWI may induce anti-inflammatory adaptations, since the CWI group developed less inflammatory areas and did not improve circulating CRP levels when exposed to an exhaustion test. One adaptation induced by chronic application of cryotherapy in endurance-trained men is the mitochondrial biogenesis through the expression of peroxisome proliferator–activated receptor gamma coactivator-1α (PGC-1α) [16, 20]. In vitro and rodent studies have demonstrated that overexpression of PGC-1α can downmodulate the production of proinflammatory mediators, such as tumor necrosis factor-α (TNF-α), cyclooxygenase, and interleukin-12 (IL-12) [33, 34]. PGC-1α expression is also associated with aerobic adaptation; however, we did not observe any improvement in swimming performance in the CWI group. The results demonstrate that neither the training nor the recovery method stimulated significant improvements in performance.

Aguiar et al. [20] assessed the effects of post-exercise CWI on signaling molecules related to mitochondrial biogenesis and exercise performance in high-intensity interval–trained healthy individuals. The authors observed improved performance due to the training program but with no differences between the CWI and control groups [20]. No differences were found in mitochondrial biogenesis markers such as PGC-1α [20]. Another study applied CWI during a short training period in volleyball players and found no effects on muscle performance or inflammatory markers [35]. Whether cryotherapy could improve physical performance is not clear, unless our results demonstrate that chronic CWI produces an anti-inflammatory milieu in cooled muscles. Future studies addressing the impact of CWI on inflammatory pathways are necessary.

Some studies have demonstrated that phototherapy can prevent EIMD, blunting CK release into the bloodstream (a clinical marker of muscle necrosis), decreasing tissue necrosis and inflammation, and preventing loss of performance after an acute bout of exercise [6, 10, 24]. Camargo et al. [6] studied

Table 4 Necrosis, edema, and inflammatory areas in the soleous muscle of Wistar rats not trained (CO), not trained and submitted to exhaustion test (CE), submitted to a training program without use of recovery methods (PR), or submitted to cold water immersion (CWI) or phototherapy (LED) recovery methods

Groups	CO	CE	PR	CWI	LED
Number of animals	$n = 6$	$n = 7$	$n = 8$	$n = 7$	$n = 7$
Number of fields	$n = 60{,}000$	$n = 70{,}000$	$n = 80{,}000$	$n = 70{,}000$	$n = 70{,}000$
Necrosis (%)	06 (0.01)	2541 (3.63)**	224 (0.28)**¥¥	119 (0.17)*¥¥	138 (0.19)**¥¥
Edema (%)	3426 (5.7)	14,812 (21.1)**	21,008 (26.2)**¥¥	10,234 (14.6)**¥¥‡‡	9009 (13.9)**¥¥‡‡
Inflammation (%)	2052 (3.42)	6517 (9.31)**	12,528 (15.66)**¥¥	7147 (10.21)**‡‡	5397 (7.71)**¥¥‡‡††
[1]Inflammatory cells (cells/mm^2)	10.0 (6–25)	40.,0 (20–84)**	51.5 (27–83)**	29.0 (18–49)**‡	33.5 (17–61)** ‡

*$p < 0.05$; ** $p < 0.005$ in comparison to CO; chi-squared test

¥ $p < 0.05$; ¥¥ $p < 0.005$ in comparison to CE; chi-squared test

‡ $p < 0.05$; ‡‡ $p < 0.005$ in comparison to PR; chi-squared test

† $p < 0.05$; †† $p < 0.005$ in comparison to CRYO; chi-squared test

[1] Data expressed as median (interquartile intervals); Kruskal-Wallis and Dunn tests

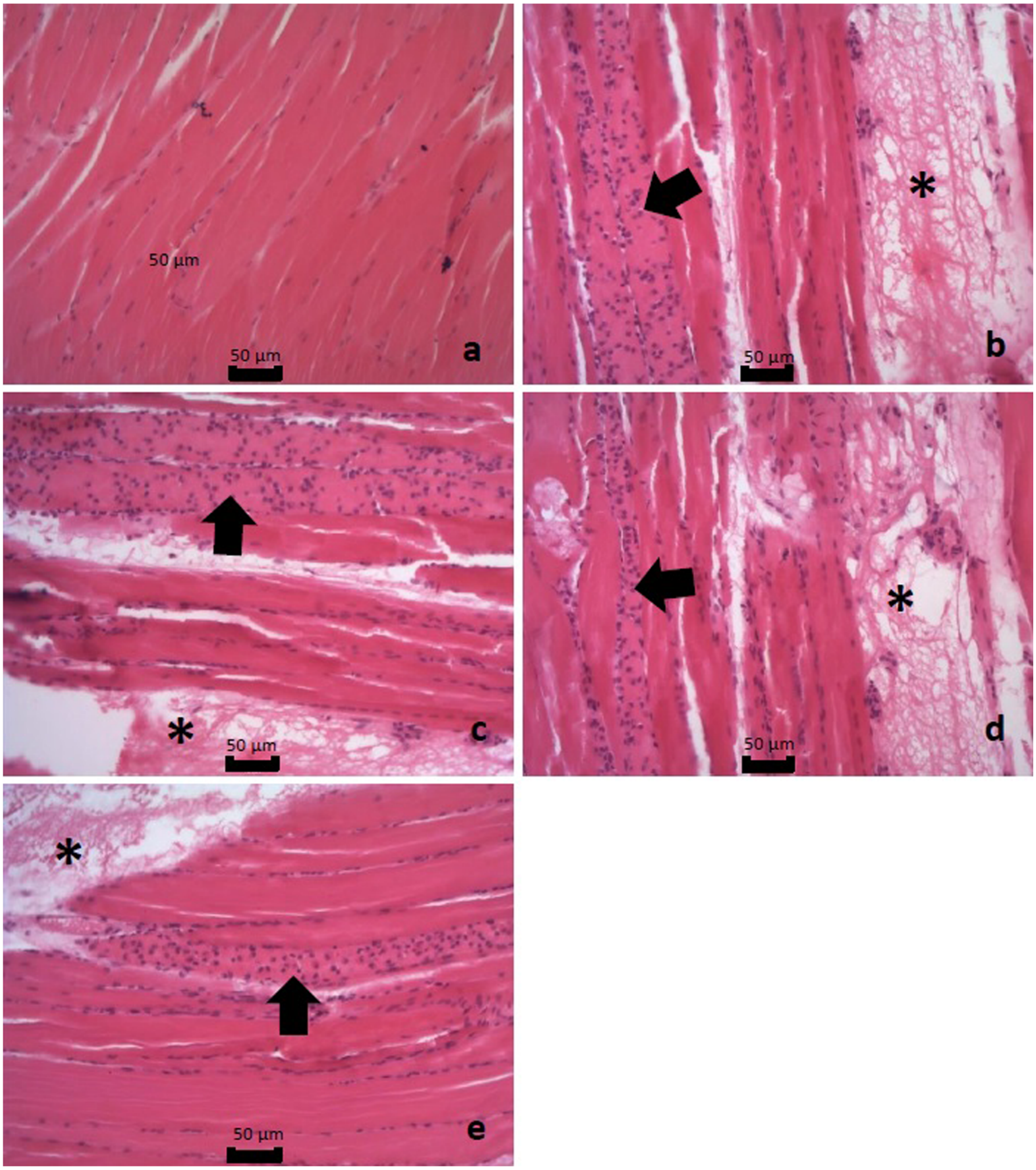

Fig. 1 Microscopic images of the soleous muscle of **a** control animals—CO, **b** control animals submitted to exhaustion test—CE, **c** passive recovery group—PR, **d** cold water immersion group—CWI, and **e** LED therapy group—LED. Arrows indicate necrotic muscle fibers infiltrated by phagocytic cells. The asterisks indicate areas of intramuscular edema. × 40 magnification, Hematoxylin & Eosin

the effects of CWI and LED therapy at 940 nm on inflammatory markers, muscle damage, and edema, after an exhaustion test in Wistar rats. The authors showed that LED therapy was more effective than CWI in preventing EIMD [6]. Another study demonstrated that irradiation at 808 nm after muscle injury also causes a reduction in oxidative and nitrative stress, reducing lipid peroxidation, nitrotyrosine formation, and nitric oxide production, besides reducing the inflammatory response induced by nuclear factor kB pathway, cyclooxygenase-2, TNF-α, and interleukin-1β [36]. Taken together, these studies suggest that LED therapy may prevent EIMD and presents anti-inflammatory properties.

Phototherapy acts on the mitochondrial enzyme cytochrome C oxidase, a key regulator of the mitochondrial respiratory chain. Phototherapy can increase the expression of cytochrome C oxidase and disrupt nitric oxide (a negative regulator) from the enzyme, increasing oxygen consumption and ATP synthesis [37, 38]. Phototherapy stimulates muscle repair by increasing expression of myogenic genes and growth factors, the proliferation of

Table 5 Necrosis, edema, and inflammatory areas in gastrocnemius muscle of Wistar rats not submitted to training (CO), not trained and submitted to exhaustion test (CE), and trained and submitted to passive recovery (PR), cold water immersion (CWI), or phototherapy (LED) recovery methods

Groups	CO	CE	PR	CWI	LED
Number of animals	$n = 6$	$n = 7$	$n = 8$	$n = 7$	$n = 7$
Number of microscopic fields	$n = 60{,}000$	$n = 70{,}000$	$n = 80{,}000$	$n = 70{,}000$	$n = 70{,}000$
Necrosis (%)	–	1169 (1.67)**	872 (1.09)**¥¥	315 (0.45)**¥¥‡‡	693 (0.99)**¥¥††
Edema (%)	1566 (2.61)	6055 (8.65)**	17,408 (21.76)**¥¥	4795 (6.85)**¥¥‡‡	4032 (5.76)**¥¥‡‡†
Inflammation (%)	577 (0.87)	2352 (3.36)**	7008 (8.76)**¥¥	2723 (3.89)**‡‡	1988 (2.84)**‡‡††
[1]inflammatory cells (cells/mm^2)	6.0 (4–14)	14.0 (8–25)**	28.0 (16–49)**¥	12.0 (6–18)‡‡	14.5 (7–29)‡‡

*$p < 0.05$; **$p < 0.005$ in comparison to CO;
¥ $p < 0.05$; ¥¥ $p < 0.005$ in comparison to CE;
‡ $p < 0.05$; ‡‡ $p < 0.005$ in comparison to PR;
† $p < 0.05$; †† $p < 0.005$ in comparison to CWI; chi-squared test
[1] Data expressed as median (interquartile intervals); Kruskal-Wallis and Dunn tests

satellite cells, angiogenesis, and collagen synthesis [39, 40]. Phototherapy also has anti-inflammatory effects and reduces pain and oxidative stress [6, 39, 40]. These effects may be useful to prevent or repair EIDM. Phototherapy also induces the expression of the mitochondrial electron transport chain [23], suggesting it may have beneficial effects on aerobic adaptation.

Phototherapy using LED and low-level laser irradiation has been demonstrated to prevent EIMD and blunt the inflammatory process in animal studies [6, 10]. Another study applied laser therapy during 6 weeks of aerobic training in aged rats and demonstrated a significant reduction in the expression of inflammatory cytokines IL-6 and TNF-α in gastrocnemius muscles [41]. Other experimental studies also demonstrated that a single or multiple irradiation could decrease migration of inflammatory cells into damaged tissues, decrease the expression of TNF-α in macrophages and skeletal muscle, and blunt oxidative stress [6, 36, 40, 42–44]. Chronic application of phototherapy may induce anti-inflammatory effects for longer periods of time. A reduction in inflammatory infiltration could be seen 60 h after muscle injury [42], 7 days after connective tissue damage [44], and 21 days after nerve crush injury [43].

Irradiation of cultured fibroblasts in vitro demonstrated that red irradiation upmodulated genes associated with complexes I and IV of the mitochondrial respiratory chain [23]. An experimental study demonstrated that phototherapy can increase the expression of cytochrome C oxidase in red and intermediate muscle fibers of rat temporalis muscle [45]. In addition, phototherapy also induces angiogenesis and upregulation of enzymatic antioxidative systems [36]. These effects suggest that phototherapy may favor aerobic adaptation. However, our study did not find any improvement in physical performance. Indeed, it is not clear if chronic application of low-level irradiation could improve physical adaptation, despite it may blunt inflammatory reactions.

Our study showed that both CWI and LED therapy can reduce inflammation without generating performance results. However, the training protocol used was low to moderate intensity, which may not have provided sufficient stimulus to improve swimming performance. Thus, high-intensity training models should be tested to verify the existence of other effects of recovery methods on fitness and exercise performance.

Conclusion

The studied training did not improve swimming performance in Wistar rats, but decreased EIMD after an acute bout of exhausting exercise. LED therapy and cryotherapy, in addition to the training, did not alter performance in the swimming exercise when applied daily after the training sessions, in relation to the passive recovery group. However, LED therapy and CWI were effective in preventing muscle injury and inflammatory reactions. Further studies are needed to evaluate the effects of the successive application of CWI and phototherapy at other exercise intensities, and in an experimental model that allows observation of performance changes resulting from the training.

Funding information This study was financially supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, for the purchase of laboratory reagents and for the post-graduation grant for VBCS (grant no.1085489/2013).

Compliance with ethical standards

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

Ethical approval All experiments were previously approved by the Ethics Committee on the Use of Animals of the Universidade Estadual de Londrina (protocol 077/2013).

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