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Article

Effects of low-level laser therapy (LLLT) on skeletal muscle fatigue and damage

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Abstract: The present study aimed to evaluate the effects of low-level laser therapy (LLLT) on exercise performance and skeletal muscle damage. A randomized, double-blind, placebo-controlled study involved 24 male college swimming athletes. LLLT was administered prior to exercise using a He-Ne laser at 632.8 nm, with a power output of 5 mW, a total irradiation duration of 300 s, and an energy density of 0.3 J/cm^2 per diode or placebo, applied to two points on the rectus femoris muscles bilaterally. The performance in a 200-m breaststroke swim, as well as thigh and leg girth, blood lactate levels, creatine kinase (CK), and lactate dehydrogenase (LDH) levels were assessed before and immediately after the swimming protocol. The LLLT group demonstrated a significant improvement in 200-m breaststroke performance ($p < 0.05$) and a significant reduction in thigh circumference, blood lactate, CK, and LDH levels ($p < 0.05$) when compared to the placebo group. Pre-exercise photobiomodulation by LLLT improved the 200-m breaststroke swimming performance, and reduced muscle fatigue and damage.

Keywords: LLLT; fatigue; muscle damage; photobiomodulation

1. Introduction

Skeletal muscle fatigue is an inevitable phenomenon in routine athlete training and competition. Muscle damage, a frequent consequence of intense physical activities, diminishes muscular power and upsurges the risk of injury and discomfort [1]. Such damage materializes during the progression of muscle fatigue, manifesting as structural disruptions like sarcomeric disarray and membrane tears, which lead to the leakage of enzymes like creatine kinase [2]. Additionally, it triggers inflammatory responses, marked by the release of cytokines and the infiltration of phagocytic cells, thereby impacting athletes' performance and their recovery post-exercise [3].

Creatine kinase (CK), also known as creatine phosphokinase, is more abundant in skeletal and smooth muscle, mainly in the cytoplasm and mitochondria of cells, and is directly related to intracellular energy operation, muscle contraction, and ATP regeneration [4]. Lactate dehydrogenase (LDH) is present in almost all tissues, and is most abundant in skeletal muscle, with five isoenzymes [5]. It has been shown that the levels of CK and LDH in serum and skeletal muscle increase when the body undergoes heavy exercise [6]. This may be due to the increase of free radicals in tissues and organs when the organism undergoes heavy exercise. The lipid bilayer of the cell membrane undergoes peroxidation, which damages the integrity of the cell membrane, making the blocking effect of the cell membrane on CK and LDH weakened, and the flow of CK and LDH from the cell membrane to the outside of the membrane, which leads to the increase of the serum levels [7]. Therefore, elevated serum CK and LDH levels are important markers of skeletal muscle damage.

LLLT involves the use of laser light with a power output of 1–500 mW and a specific wavelength within the red to near-infrared spectrum (600–1000 nm) [8]. Numerous studies, both on animals and humans, have demonstrated the potential of LLLT to reduce muscle fatigue and expedite muscle recovery following physical activity [9,10]. When applied prior to exercise, LLLT has been found to lower blood lactate levels and decrease the concentration of muscle damage indicators, such as CK and LDH [11,12]. This indicates that LLLT may help to reduce the likelihood of muscle fatigue and injury, potentially improving exercise performance. However, since many of these studies have focused on single muscle groups and have been conducted in controlled settings, the impact of LLLT in actual competitive environments and across various sports disciplines requires further investigation.

While the majority of randomized clinical trials have shown the efficacy of PBMT in enhancing exercise performance and accelerating recovery in controlled settings like laboratories or semi-controlled environments such as field tests, the true test of its practical application lies in its application in real-world competitive scenarios and across diverse sports [13]. This study aimed to explore the impact of pre-exercise LLLT (He-Ne laser, 632.8 nm) on the performance of a 200-m breaststroke swim, muscle fatigue, and muscle damage, considering the need for further research in real competition settings and different sports modalities to validate previous laboratory findings.

2. Methods

2.1. Subjects

The study was conducted in accordance with the Helsinki Declaration and received approval from the Ethics Committee at Zhaoqing College. Participants were healthy male college swimmers, aged between 18 to 23, with a mean age of 22.4 ± 0.6 years. Each participant provided their informed consent and committed to actively engage in the study procedures.

2.2. Randomized, double-blind and placebo-controlled trial

The study consisted of two phases spaced approximately 2 weeks apart. 16 Subjects were randomized to Group A and Group B. Individuals in Group A were administered active and placebo LLLT during Phases I and II, respectively, while those in Group B underwent the reverse procedure, receiving placebo and then active LLLT in Phases I and II, respectively (as depicted in **Figure 1**). The allocation to groups was masked for both the participants and the observers.

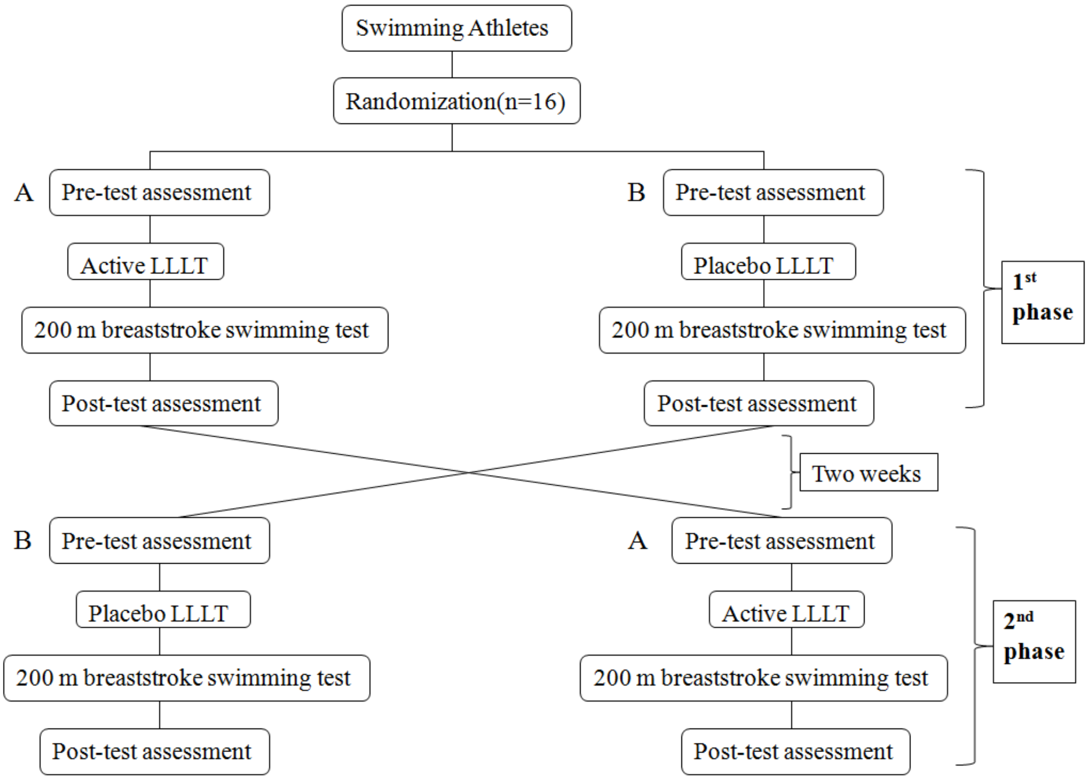

Figure 1. The flow chart of the study procedures.

2.3. Exercise protocol

Participants underwent a 200-m breaststroke swimming test, which was timed with a stopwatch following a 15-min warm-up. They were instructed to begin and swim the 200 m with maximum effort, sprinting as quickly as possible throughout the event.

2.4. LLLT protocol

Participants received either active or placebo LLLT both prior to and following the 200-m breaststroke swim. The LLLT was administered using a low-level helium-neon laser (kx-350-1b, Guilin Kangxing Medical Instrument Co., Ltd., Guilin, China) irradiating the abdominal location of the rectus femoris muscle (laser parameters are shown in **Table 1**), with two points irradiated on each side (**Figure 1**). The control group was operated in the same way as the active LLLT group but without the light source. In order to guarantee the experiment's double-blind nature, every participant was required to wear black eye masks while being irradiated.

Table 1. Low-level laser therapy (LLLT) parameters.

Laser source	**Wavelength**	**Output power**	**Power density**	**Spot size**	**Power density**	**The energy density per point**	**Irradiation time**	**Number of points**	**Total energy delivered**
Helium-neon	632.8 nm	50 mW	10 mW/cm^2	4.9 cm^2	10 mW/cm^2	3 J/cm^2	300 s on each point	4	60 J

2.5. Outcome measures

2.5.1. 200 m breaststroke swimming performance

The performance of the 200 m breaststroke was timed using a stopwatch.

2.5.2. Thigh and calf circumference

Thigh and calf circumference were measured by tape measure.

2.5.3. Blood lactate level

Blood was obtained from the fingertip of the ring finger both immediately before and after the exercise. To prevent any sample contamination, the initial drop of blood was deliberately not collected, and the subsequent blood was then analyzed using a portable lactate Scout+ analyzer (SensLab GmbH, Leipzig, Germany).

2.5.4. CK and LDH levels

Blood samples were collected by a nurse who was unaware of the group assignments, obtained from an antecubital vein prior to exercise and right at the conclusion of the exercise routine. Post-collection, the blood was centrifuged at 2700× *g* for 10 min at 4 ℃. Subsequently, the serum was promptly transferred into Eppendorf tubes and preserved at −80 ℃ for future analysis. Serum CK and LDH levels were determined with CK and LDH kits, respectively.

2.6. Statistical analysis

The analysis of the data was conducted utilizing SPSS software, with results presented as the mean ± standard deviation for each cohort. Independent samples *t*-tests were employed to assess significant disparities between the groups receiving LLLT and those receiving placebo LLLT.

3. Results

3.1. Effect of LLLT on 200 m breaststroke performance

As shown in **Table 2**, there was a significant difference between LLLT group and the placebo group in 200 m breaststroke performance ($p < 0.05$).

Table 2. 200 m breaststroke performance.

	Placebo	**LLLT**
Total time (s)	272.5 ± 1.1	264.7 ± 0.8*

* $p < 0.05$.

3.2. Effect of LLLT on thigh and calf circumference

The results in **Table 3** showed that before exercise, there was no significant difference in thigh circumference between the placebo group and the LLLT group ($p > 0.05$); after exercise, thigh circumference was significantly lower in the LLLT group compared to the placebo group ($p < 0.05$). Before and after exercise, there was no significant difference in calf circumference between the placebo and LLLT groups ($p > 0.05$).

Table 3. Changes in thigh and calf circumference before and after exercise.

	Thigh circumference (cm)		Calf circumference (cm)	
	Before exercise	**After exercise**	**Before exercise**	**After exercise**
Placebo	53.6 ± 2.6	55.1 ± 1.9	37.0 ± 1.5	37.1 ± 1.4
LLLT	53.2 ± 2.6	54.0 ± 2.5*	35.9 ± 1.2	36.1 ± 1.1

* $p < 0.05$.

3.3. Effect of LLLT on blood lactate level

Prior to the exercise, no notable differences in blood lactate concentrations were observed between the placebo and LLLT groups ($p > 0.05$). However, post-exercise, a significant reduction in blood lactate concentrations was noted in the LLLT group in contrast to the placebo group ($p < 0.05$) (as detailed in **Table 4**).

Table 4. Changes in Blood lactate levels before and after exercise.

	Blood lactate (mmol/L)	
	Before exercise	**After exercise**
Placebo	2.21 ± 0.3	13.4 ± 0.93
LLLT	2.16 ± 0.42	11.6 ± 1.1*

* $p < 0.05$.

3.4. Effect of LLLT on CK and LDH levels

Prior to the exercise, no significant disparities were found in CK and LDH levels between the placebo and LLLT groups ($p > 0.05$). Following the exercise, however, the levels of CK and LDH were considerably reduced in the LLLT group when compared to the placebo group ($p < 0.05$), as illustrated in **Table 5**.

Table 5. Changes in CK and LDH levels before and after exercise.

	Before exercise		After exercise	
	Placebo	**LLLT**	**Placebo**	**LLLT**
CK (U/mL)	0.39 ± 0.18	0.38 ± 0.19	0.36 ± 0.2	0.31 ± 0.16*
LDH (U/L)	2120.0 ± 230.0	2043.8 ± 415.7	2310.6 ± 192.5	2157.7 ± 246.7*

* $p < 0.05$.

4. Discussion

Skeletal muscle fatigue and damage can seriously affect athletic ability and performance [14]. In the field of sports medicine, LLLT has attracted wide attention because of its simple, safe, effective, and green characteristics. The beneficial outcomes of LLLT stem from its interaction with biological tissues, which can stimulate bioenergetic and proliferative effects at the cellular and molecular levels [15,16]. In recent years, LLLT has emerged as a novel strategy to postpone the onset of skeletal muscle fatigue, enhance human motor performance, and boost athletic capabilities. Studies have shown that laser irradiation (904 nm, 15 mW average power, four laser doses 0.1, 0.3, 1.0, and 3.0 J) was administered immediately before the initial contraction in the treatment groups [17]. Studies have found that LLLT treatment before centrifugal exercise can effectively reduce the increase of muscle protein in serum and the decrease of muscle strength [18]. Tomazoni et al. [19] found that LLLT before exercise has an important antioxidant effect, which can reduce exercise-induced oxidative stress, thereby improving exercise performance and improving post-exercise recovery. Consequently, it has been demonstrated that infrared laser irradiation at a dosage of 1.0 J prior to exercise can improve skeletal muscle performance and reduce muscle damage and inflammation following exercise. According to the results of the 200 m breaststroke test in this experiment, it can be seen that subjects in the placebo control group had deeper fatigue and needed a longer time to complete the 200 m breaststroke test, while subjects in the LLLT group had stronger body working ability and needed shorter time to complete the 200 m breaststroke test. It can be seen that LLLT pretreatment before exercise is beneficial for improving athletic performance.

A meta-analysis has recognized that a variety of wavelengths (655, 660, 800, 808, 810, 830, 850, and 970 nm) have been utilized in research examining the effects of LLLT on exercise capacity and muscle performance [20,21]. It has been noted that red laser light penetrates human skin less effectively compared to infrared laser light [22]. In the present study, a red laser at a wavelength of 632.8 nm was employed. Additionally, the dosage and therapeutic protocols are deemed crucial for the successful application of LLLT in muscle tissue [22,23]. With this in mind, the power output was increased and the duration of irradiation was extended, enhancing the dose applied to each of the four targeted sites to ensure adequate exposure of the human rectus femoris muscle. In related research, Zhao et al. [24] explored the impact of red light (630 nm) on the sleep quality and endurance of female basketball players from the Chinese national team. The athletes were treated with red light therapy for 14 days, with 30 min of irradiation each time, and the athletes’ sleep quality, 12-min endurance run, and serum melphalan were measured after the treatment. After 14 days of whole-body red light irradiation, the athletes’ sleep quality, serum melphalan levels, and endurance capacity were significantly improved. It was shown that LLLT pre-adaptive irradiation also increased the efficiency of the flexor muscle groups. In addition, according to the results of the swimming test in this experiment, it can be known that the subjects in the placebo control group had deeper fatigue and took longer time to complete the 200 m breaststroke test, while the subjects in the LLLT irradiation group had stronger organism working ability and took a shorter time to complete the 200 m breaststroke test, so we believe that the LLLT could be a means to improve the human body’s exercise capacity, improve the exercise performance as well as promote the means of recovery from body fatigue.

Muscle hardness is closely related to the contraction strength of muscles in the non-fatigued state, so the muscle condition test system used to observe muscle hardness can accurately reflect the muscle tone and fatigue degree. Under the premise of consistent exercise load, the greater the change occurred in muscle circumference, the deeper the fatigue is proved to be. Muscle stimulation will cause contraction, and muscle contraction can cause bone displacement. Due to the characteristics of the swimming program, the muscles have to resist the resistance from the water under the premise of driving the displacement of bone-excluded muscles. Generally, nerve impulses are transmitted to the muscles, which then contract. When the nerve impulses are lost, the muscle relaxes. Within a reasonable range, a decrease in muscle stiffness is a sign that the muscle is in good condition. In conjunction with the observation of the data of thigh circumference and calf circumference of the subjects after completing the 200 m breaststroke test in this experiment, it can be known that the data of thigh circumference and calf circumference of the subjects in the LLLT irradiation group stabilized after completing the 200 m breaststroke test. On the contrary, the data on thigh circumference and calf circumference of the subjects in the placebo control group changed significantly ($p < 0.05$). Thus, while the calf muscle tone increased, the faster the muscle reached fatigue. In other words, the less resistant it is to fatigue. The reason for this is most likely because the subjects in the LLLT irradiation group were illuminated while the subjects in the placebo control group were not. Therefore, we can conclude that LLLT irradiation can reduce the level of muscle fatigue and increase the body's anti-fatigue ability, which can enable the body to achieve anti-fatigue. dos Santos Maciel et al. [25] investigated the effect of LLLT (light-emitting diode, 780 nm, 30 mW, 0.81 J/light source, spot area 0.2 cm^2, 27 s, 29 sources) pre-exposure of professional soccer players before resistance exercise on their exercise capacity, and found that LLLT significantly improved their exercise capacity and that LLLT was the most important factor in the improvement of their performance. The effects of irradiation on their exercise capacity revealed that LLLT significantly increased the mean power frequency (MPF) of the tibialis anterior muscle of the athletes. Meanwhile, the blood lactate level of the LLLT group remained relatively stable. Ribeiro et al. [26] found that the application of LLLT before muscle injury resulted in a decrease in muscle necrotic cells and inflammatory cells, an increase in blood vessels and immature muscle fibers, an increase in matrix metalloproteinase-2 activity, and a decrease in collagen deposition. In this study, there was a significant difference in thigh circumference between LLLT group and the Placebo group after exercise, suggesting that LLLT could reduce muscle fatigue, and the blood lactate level of the LLLT group was significantly lower than that of the Placebo group after exercise, suggesting that LLLT could improve the body's anti-fatigue ability.

Blood lactate a marker of fatigue, is widely used to monitor exercise intensity [27]. Lactate is largely generated and blood lactate concentrations increased significantly, especially during strenuous exercise. Elevated lactate levels can lead to a decrease in interstitial H^+ concentration and intracellular pH, causing acidification at the neuromuscular junction, which may subsequently impair neuromuscular transmission and muscle contraction [28]. LLLT can facilitate lactate transport, expedite lactate clearance, and mitigate the impact of hydrogen ions on muscle contraction and nerve cell excitability, thereby reducing the fatigue of skeletal muscles and the nervous system [29]. Research has shown that applying LLLT (655 nm, 500 J/cm^2, 100 s on each point) or (830 nm, 1785 J/cm^2, 50 s at each point) to volleyball players' biceps humeri muscles just before repeated contractions at 75% of maximum voluntary contraction (MVC) to exhaustion did not yield significant differences in blood lactate levels between the LLLT and placebo groups [30]. Consequently, they optimized the LLLT treatment process and discovered that pre-exercise irradiation of the biceps with LLLT (810 nm, 200 mW, 164.85 J/cm^2, 30 s on each point, 2 points, 30 J on each point, 60 J of total energy) increased the number of repetitions and the time until exhaustion, reduced blood lactate levels at 5 min post-exercise, but did not significantly affect blood lactate levels at 10, 15, or 20 min post-exercise. Our study's results suggest that LLLT (632.8 nm, 50 mW, 3 J/cm^2, 300 s on each point, 4 points, 15 J on each point, 60 J of total energy) administered prior to a 200-m breaststroke swim decreases blood lactate levels immediately following exercise. Compared with the above-mentioned studies, it is easy to find that, LLLT applied before exercise decreased lactate generation and may be mainly related to the total energy delivered. Leal Junior [31] carried out a randomized, double-blind study involving male professional volleyball players, requiring them to perform as many 75% MVC loaded contractions of the biceps brachii muscle as possible on days 1 and 8. Four points of LLLT (655 nm, 5 J, at an energy density of 500 J/cm^2) were given to the biceps muscle belly immediately before the start of the second part of the experiment on day 8, and the number of contractions of 75% MVC of the muscle and blood lactate concentration was determined. The findings indicated a notably higher count of contractions and a markedly lower level of blood lactate in the biceps muscle undergoing 75% MVC loading within the LLLT group. Leal Junior et al. [32] irradiated the quadriceps muscle of 9 volleyball players and 11 soccer players with LLLT (830 nm, 100 mW, 0.0028 cm^2, 3–4 J/loci, 5 loci of the quadriceps) before performing the Wingate test (loading of 7.5% of body weight, 30 s of exercise) on the subjects. The results showed a significant increase in anaerobic power and a significant decrease in both serum CK and lactate levels in the LLLT group compared with the placebo group ($p < 0.01$). During this 200-m breaststroke trial, it was observed that the pre-exercise lactate levels averaged at 2.21 mmol/L for the control group, while the group receiving LLLT had an average lactate level of 2.16 mmol/L. Following the exercise, the control group's lactate levels rose to an average of 13.4 mmol/L, whereas the irradiated group's levels were 11.6 mmol/L on average. The group that received post-exercise LLLT demonstrated a significant reduction in lactate concentration by 13.43% ($p < 0.05$) when compared to the placebo-controlled group. Therefore, we conclude that LLLT can allow the body to accelerate the rate of lactate clearance, thus promoting the recovery of body fatigue.

CK and LDH are intracellular enzymes, and serum CK and LDH are major markers of skeletal muscle injury. Leal et al. [33] indicated that the application of pre-exercise LLLT on individual muscle groups led to a reduction in post-exercise blood lactate levels, CK, and C-reactive protein, pointing towards a protective role against exercise-induced muscle damage. Dos Reis et al. [34] discovered that administering low-level laser (830 nm) therapy either prior to or following exercise led to a decrease in serum lactate and CK levels, thereby facilitating recovery from fatigue, with particularly notable effects in the group that received post-exercise treatment. Additionally, Lopes-Martins et al. [35] demonstrated that the application of low-level laser light (655 nm) to the tibialis anterior muscle prior to the induction of fatigue could prevent the development of fatigue caused by electrical stimulation at energy densities of 0.5 and 1 J/cm^2. Furthermore, energy densities of 1.0 and 2.5 J/cm^2 were found to markedly reduce muscle damage, as indicated by a significant decrease in serum CK levels. Leal Junior et al. [32] studied the occurrence and recovery of exercise fatigue in male professional volleyball players by low light exposure. Research indicates that low-intensity light therapy administered prior to biceps muscle fatigue postpones the onset of skeletal muscle fatigue, lowers post-exercise blood lactate levels [20], and curbs the release of CK and C-reactive protein. Similarly, a rat model study demonstrated that low-level laser treatment (1.0 J, 904 nm) immediately before exercise could delay muscle fatigue and reduce blood lactate and CK levels after exercise [33]. In the current study, pre-exercise LLLT also led to a decrease in CK and LDH levels, suggesting that LLLT can protect against muscle damage caused by intense 200-m breaststroke swimming. This protective effect may contribute to the improved performance observed. Our results are in line with previous research that utilized pre-exercise LLLT to mitigate muscle damage [32,33]. De Marchi et al. [11] noted that LLLT (810 nm, 200 mW, 30 J per site, 30 s of irradiation per site, 12 sites per lower limb) before progressive-intensity running exercise can enhance performance and reduce post-exercise damage to lipids and proteins, as well as the activity of CK and LDH enzymes, indicating that pre-exercise LLLT could be beneficial in lowering muscle damage induced by exercise.

5. Conclusion

Pre-exercise photobiomodulation by LLLT improved the exercise performance, reduced muscle fatigue and damage.

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