Effect of different time intervals of light emitting diode therapy application on muscle fatigue

Efecto de diferentes intervalos de tiempo de la aplicación de la terapia con diodos emisores de luz sobre la fatiga muscular

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ABSTRACT

This study aimed to identify the near-infrared (NIR) light emitting diode (LED) time response on isokinetic peak torque of elbow flexors. This was a prospective, randomized, single blind, pre–test post-test, and placebo-controlled crossover study. Forty-five healthy non-athletes were assigned at random to 3 groups equally: 5 minutes, 1-hour and 5-hours groups. Each group (n=15) had at the first session 890 nm wavelength NIR-LED, either in active or placebo conditions, prior to exercise that provokes fatigue (5 minutes, 1 hour, or 5 hours). After an interval of 7 days, the second NIR-LED condition was applied at the second session. The assessment of isokinetic peak torque was done at baseline and post exercise in each session using isokinetic dynamometer. There was no significant difference between groups and between conditions in isokinetic peak torque (p > 0.05). Isokinetic peak torque decreased significantly post treatment compared with pretreatment (p < 0.01) in all groups, but
the 5 minutes group showed the least percent of decrease. In conclusion, NIR-LED can most probably resist muscle fatigue with the parameters used in this study if it is employed 5 minutes before exercise.

KEYWORDS
Light emitting diode; infra-red wavelength; peak torque.

RESUMEN
Este estudio tuvo como objetivo identificar el momento idóneo de aplicación del diodo emisor de luz (LED) del infrarrojo cercano (NIR) en la potencia máxima isocinética de los flexores del codo. Este fue un estudio cruzado prospectivo, aleatorizado, simple-ciego, con pre-test y post-test, y controlado con placebo. Se asignaron al azar cuarenta y cinco no deportistas sanos a 3 grupos por igual: grupos de 5 minutos, 1 hora y 5 horas. Cada grupo (n = 15) tuvo en la primera sesión NIR-LED de 890 nm de longitud de onda, en condiciones activas o placebo, antes del ejercicio que provoca fatiga (5 minutos, 1 hora o 5 horas). Después de un intervalo de 7 días, se aplicó la segunda condición NIR-LED en la segunda sesión. La evaluación de la potencia máxima isocinética se realizó antes y después del ejercicio en cada sesión utilizando un dinamómetro isocinético. No hubo diferencias significativas entre los grupos y entre las condiciones en la potencia máxima isocinética (p > 0.05). La potencia máxima isocinética disminuyó significativamente después del tratamiento en comparación con el pretratamiento (p < 0.01) en todos los grupos, pero el grupo de 5 minutos mostró el menor porcentaje de disminución. En conclusión, lo más probable es que NIR-LED pueda resistir la fatiga muscular con los parámetros utilizados en este estudio si se emplea 5 minutos antes del ejercicio.

PALABRAS CLAVE
Diodo emisor de luz; longitud de onda infrarroja; potencia máxima.

1. INTRODUCTION

Muscle fatigue is identified as the reduction in the muscles’ capability to develop force or power throughout physical activities (Enoka & Duchateau, 2008). Muscle fatigue can be frequently experienced, although the complexity of this process is not fully distinguished. The usually detected attributes of skeletal muscle fatigue are declined muscle strength, deficient motor skills and consequent muscular pain (Green, Langberg, Skovgaard, Bülow & Kjær, 2000).
Muscle fatigue exposes operators to more risks of overexertion, and cumulative muscle fatigue may lead to musculoskeletal disorders (Chaffin, Andersson & Martin, 2006; Armstrong, Buckle, Fine, Hagberg, Jonsson & Kilbom et al., 1993). The additional reinforcing joint structures are subjected to extraneous loading because the fatigued muscles cannot produce the needed force. This may lead to failure of the tissues that support the joints and in consequence cause injuries. Altered neuromuscular control emerges with fatigue, which influences the joint sense of position and kinesthesia (Ballantyne & Shields, 2010).

Many interventions have been led to promote muscle functioning or impede muscle fatigue signals, such as massage, warm-up, use of compression garments and cryotherapy (Calleja-González, Terrados, Mielgo-Ayuso, Delextrat, Jukic & Vaquera, et al., 2016; LaBella, Huxford, Grissom, Kim, Peng, Christoffel, 2011; Weerapong, Hume, Kolt, 2005). However, the benefits of such methods are still uncertain and rather theoretical (LaBella, Huxford, Grissom, Kim, Peng, Christoffel, 2011; Weerapong, Hume, Kolt, 2005).

Photobiomodulation therapy (PBMT) comprises low-level laser therapy (LLLT), as well as light-emitting diode therapy (LEDT), which is a method extensively used by physiotherapists in the process of rehabilitation, and there are numerous indications of its positive influence on tissue repair (Pinheiro, 2009), pain (Fulop, Dhimmer, Deluca, Johanson, Lenz & Patel et al., 2010) and inflammation (Bjordal, Lopes-Martins, Joensen & Iversen, 2010).

Most of near infra-red (NIR) light therapy researches were focused on the ability of PBMT to support soft tissue healing (Larkin-Kaiser, Christou, Tillman, George & Borsa, 2015). There were several studies on the ability of NIR light therapy, during and after resistance exercise, in alleviating skeletal muscle fatigue and resisting strength loss (Baroni, Junior, De Marchi, Lopes, Salvador & Vaz, 2010; Leal Junior, Lopes-Martins, Dalan, Ferrari, Shabo & Generosi et al., 2008; Junior, Lopes-Martins, Baroni, De Marchi, Tauber & Manfro et al., 2009; Leal, Lopes-Martins, Frigo, De Marchi, Rossi & De Godoi et al., 2010; Leal Junior, Lopes-Martins, Vanin, Baroni, Grosselli & De Marchi et al., 2009). Some authors have shown evidence of light therapy's capability in blood circulation enhancement, resulting in an improvement in tissue oxygenation (Samoilova, Zhevago, Menshutina & Grigorieva, 2008; Maegawa, Itoh, Hosokawa, Yaegashi & Nishi, 2000; Larkin, Martin, Zeanah, True, Braith & Borsa, 2012).
2. LITERATURE REVIEW

It was noted that healthy untrained participants’ irradiation by PBMT prior to exercise significantly improved VO$_{2 \text{max}}$ both in absolute and in relative measures, in addition to the time to reach fatigue (De Marchi, Junior, Bortoli, Tomazoni, Lopes-Martins & Salvador, 2012). On the other hand, while its results are well established, there is a multifaceted variety of parameters as wavelengths, application patterns, dosages, and treatment time windows (Lanferdini, Bini, Baroni, Klein, Carpes & Vaz, 2018).

It was reported in literature that the main guideline for applying PBMT for muscle preconditioning was the muscle’s irradiation of 5 minutes before exercise, in order to resist fatigue and increase functioning (Baroni, Junior, De Marchi, Lopes, Salvador & Vaz, 2010; Baroni, Leal Junior, Geremia, Diefenthaler & Vaz, 2010; Ferraresi, de Brito Oliveira, de Oliveira Zafalon, de Menezes Reiff, Baldissera & de Andrade Perez, et al., 2011; Higashi, Toma, Tucci, Pedroni, Ferreira & Baldini, et al., 2013; Rossato, Dellagraana, Lanferdini, Sakugawa, Lazzari & Baroni, et al., 2016; Miranda, Vanin, Tomazoni, Grandinetti, de Paiva & Machado, et al., 2016). While positive gains have been identified, such as improved time to fatigue and maintenance of strength competence, the best implementation period for the procedure may not be immediately prior to the fatiguing exercise (Leal Junior, Lopes-Martins, Dalan, Ferrari, Sbabo & Generosi et al., 2008).

This perspective was reinforced when a muscular pre-conditioning protocol, delivered to qualified volleyball competitors 40 to 60 minutes before the formal match, was able to significantly reduce creatine kinase concentrations in the bloodstream, which reflects reduction of muscle damage. This study indicated that muscle pre-conditioning applied 3 to 5 minutes prior to the exercise might not necessarily be the best time interval (Ferraresi, Dos Santos, Marques, Zangrande, Leonaldo & Hamblin et al., 2015).

Furthermore, it was observed that PBMT applied 3 or 6 hours before evaluation improved the potential of the mitochondrial membrane and Adenosine Triphosphate (ATP) manufacture of myotubes of muscle cells of mouse in comparison to 5 minutes or 24 hours before assessment (Ferraresi, Kaippert, Avci, Huang, de Sousa & Bagnato et al, 2015). Additionally, it was recorded that PBMT applied 6 hours prior to exercise improved the findings during fatigue test and increased the content of ATP in an animal model experiment compared to PBMT applied 5 minutes, 3 hours, or 24 hours before evaluation (Ferraresi, de Sousa, Huang, Bagnato, Parizotto & Hamblin, 2015).
Governed by the results of these two previous experimental investigations, a clinical trial on healthy and physically active participants pointed out that PBMT applied immediately before (i.e. 5 minutes), and combined with 6 hours before a high-level intensity exercise protocol, was able to reduce the strength fall than when applied immediately or 6 hours before separately (Rossato, Dellagrana, Sakugawa, Lazzari, Baroni & Diefenthaeler, 2018). On the contrary, the time response of PBMT by LED was examined at 5 minutes, 1 hour, 3 hours, and 6 hours before exercise, showing that the PBMT using LEDs was not efficient in fatigue resistance of elbow flexor muscles at any of these time intervals, in healthy and substantially active males (Abreu, Dos Santos, Fonsati, Marques & Ferraresi, 2020).

These contradictory findings bring up some remarkable question that needs to be resolved by further research: What is the best time interval separating LED application and exercise? This query creates a knowledge gap in this area that prompts researchers’ and clinicians’ incapability to precisely detect the best time to apply PBMT, especially NIR LED, before exercise to improve muscle’s resistance capability. Therefore, the purpose of this study was to identify the effects of different time intervals of pre LEDT application (5 minutes, 1 hour and 5 hours) on isokinetic peak torque (IKPT) of elbow flexor muscles as an indicator of muscle’s fatigue resistance potential.

3. METHODS

3.1. Study design

A prospective, randomized, single blind, pre-test, post-test, placebo-controlled crossover study was carried out. Prior to commencing the study, ethical approval was fulfilled from the institutional review board at the Physical Therapy Faculty of Cairo University (NO: P.T.REC/012/002480). It was implemented between October 2020 and December 2021. The study protocol was recorded online on ClinicalTrials.gov (NCT04367727).

3.2. Participants

A sample of convenience, non-probability type, of forty-five participants, was enlisted through online announcement at the Faculty of Physical Therapy of Cairo University. Informed consent was taken from each participant after interpreting the research aims and procedures, ensuring their privacy through coding of all their data.

Participants were incorporated if their age was from 20 to 30 years, having a body mass index of less than or equivalent to 25 kg/m² (Mehta & Cavuoto, 2017) and if they were healthy physically
active and non-athletic. They were ruled out if they had a former musculoskeletal type of injuries in the wrist, elbow, or shoulder vicinities, if they were accustomed to strengthening exercise several times weekly or had used any category of complementary dietary nutrients or agents of pharmacological nature through the preceding 2 months (de Almeida, Lopes-Martins, De Marchi, Tomazoni, Albertini & Corrêa et al., 2012).

To avoid type II error, sample size calculation was performed using G*POWER statistical software (version 3.1.9.2; Franz Faul, Universitat Kiel, Germany) [F tests- MANOVA- special effects and interaction, α=0.05, β=0.2, effect size = 0.27] and revealed that the appropriate sample size for this study was N=45.

3.3. Randomization and cross over design procedures

Randomization was carried out by a simple pulling of a card, which determined the duration of the sessions (5 minutes, 1 hour or 5 hours), then pulling one more card to determine if active or placebo LEDT conditions should be given at the first session. At the second session participants were crossed over to receive whatever condition was not given at the first session.

Participants were crossed over in a two-session procedure, performed 7 days apart, to compare findings after active and placebo LEDT implementation for every participant. In this way, all participants underwent the same procedures, but in a different sequence, consistent with randomization procedures.

The code that was pulled was delivered to a researcher who prepared the LEDT sessions consequently to either an active or placebo LEDT mode. The researcher was instructed not to reveal the type of treatment given to the participants.

3.4. Interventions

Following randomization, procedures were carried out completely in the laboratory of isokinetic at the Faculty of Physical Therapy of Cairo university. The participants received active (60 Joules) or placebo (0 Joule using a switched off device) NIR-LED randomly before exercise that provokes fatigue: 5-minute group (n=15; NIR-LED employed 5 minutes before exercise), 1-hour group (n=15; NIR-LED employed 1 hour before exercise) and 5-hour group (n=15; NIR-LED employed 5 hours before exercise).
Consistent careful procedures were done in 2 separate sessions, which were spaced out by an interval of 7 days, so that each participant received one of the 2 LED conditions (active or placebo) at each session and, to minimize the impact of diurnal variations, all procedures were conducted at similar time of day and identical day of week. All study procedures are shown in Figure 1:

**Figure 1.** CONSORT chart showing the procedures of the study

LEDT was applied using 120 commercial diodes (Anodyne Therapy System, LLC, model 120, MH043463) arranged in parallel on 2 flexible array pads of neoprene material (Figure 2A) that produce a wavelength of 890 nm NIR. NIR-LEDs are commonly used in clinical physiotherapy practices (Enwemeka, 2009; Enwemeka, 2000). The parameters of application were selected in line with the most recent proposed parameters in the literature (Leal-Junior, Lopes-Martins, Bjordal, 2019). Table 1 shows the applied parameters.
Firstly, the overlying skin was cleaned with alcohol, then with participants’ arm rested, unilateral application of active or placebo NIR-LED was done over elbow flexors of the dominant upper limb, by the therapist, in direct skin contact via 2 flexible pads supported by straps at the center of the elbow flexor muscles ventral surface (Leal, Lopes-Martins, Frigo, De Marchi, Rossi & De Godoi, et al., 2010) (Figure 2B). Blinding of participants was sustained by using opaque glasses during implementation procedures, which also helped in eyes securing from LEDT irradiation.

**Table 1.** Light-emitting device optical parameters

- LEDs’ number :120
- Pads’ number: 2
- Frequency: 292 Hz
- Device’s overall optical power: 1800 mW (15 mW per diode)
- Diode’s area: 0.2 cm²
- Device’s efficient irradiation area: 45 cm²
- Diode’s power density: 75 mW/cm²
- Average power of the 2 pads: 960 mW
- Time of irradiation in seconds: 57 seconds.
- Energy density through 57 seconds: 1.33 J/cm² (2.5 J/diode)
- Device’s overall energy at 57 seconds: 63.06 J

*Hz: Hertz, mW: milliwatt, J: joule.*

Through the two sessions there were no variations in any of the procedures except for placebo state, which was done in the same way as active one, except with the device shut down.
A dynamometer (Biodex system 3; Biodex Medical, Shirley, New York), which was calibrated on a daily basis, was used for isokinetic peak torque assessment and fatigue induction, that is used to objectively assess muscle functioning or to evaluate the efficacy of different treatments frequently (Amaral, Marinho, Ocarino, Silva, Souza & Fonseca, 2014). Participants sat on the chair following the instructions’ manual, with the trunk secured by belts to counteract any offsetting movements. The dynamometer rotational axis was aligned with the elbow joint axis.

**Isokinetic peak torque (IKPT):** It was measured at base line (pre-LED application) and immediately post fatiguing exercise. In the isokinetic mode, five contractions were carried out. Participants were requested to move the elbow in flexion and extension through the complete motion range (0°-120°), as much as possible, strongly and quickly for each repetition, and to implement the greatest possible strength during elbow flexion and opposing elbow extension introduced by the dynamometer from the beginning to the end. The most powerful contraction of the 5 estimates was reported as the IKPT in newton meters and was considered the participant’s highest power generation capability at base line (pre-value). Verbal and visual feedbacks were provided throughout torque assessment to encourage maximal effort from all participants.

**Fatigue provoking exercise protocol (FPEP):** After IKPT assessment, active or placebo LED was applied, followed by fatigue provoking exercise protocol (FPEP). Force reduction was provoked using continued maximal concentric and eccentric elbow flexors’ actions. Participants were guided to do 3 sets of 20 repetitions of maximal concentric and eccentric contractions. This exercise protocol has been previously employed and imitates the cellular activities observed with acute exercise sessions (Borsa
The angular velocity was adjusted at 45 degrees/sec for concentric actions and 60 degrees/sec for eccentric actions. Each participant was guided to do repetitions “as powerful as possible”. Oral and visual feedbacks were given throughout the exercise protocol.

**Post fatigue procedures:** Participants underwent a post evaluation of IKPT exactly 30 seconds after the FPEP, following the same procedures as before FPEP with the same feedback given by the therapist. The maximum power generating capacity was considered as the participant’s after exercise value (post value). The change in torque was measured (change between the baseline and post-fatigue values) and was compared within and among groups at the 2 sessions.

### 3.5. Data analysis

Descriptive statistics and MANOVA test were done for comparison of participants characteristics among groups. Chi- squared test was used for comparison of sex allocation among the three groups. Normal distribution of data was checked using the Shapiro-Wilk test for all variables. Levene’s test for variances’ homogeneity was performed to examine the homogeneity among groups. Investigation of the effect of time (pre versus post), the effect of condition (active versus placebo) and the effect of treatment (among groups), as well as the interaction effect of values of the mean of isokinetic peak torque, was performed by 3 x 2 x 2 MANOVA of mixed design. Consequent multiple comparisons were assessed by tests of post-hoc type using the Bonferroni type of correction. The level of significance for all statistical investigations was adjusted at p < 0.05. All statistical exploration was operated through the statistical package for social studies (SPSS) version 20 for Windows (IBM SPSS, Chicago, IL, USA).

### 4. RESULTS

#### Participants’ characteristics

Table 2 shows the participants’ characteristics of the 5 minutes, 1 hour and 5 hours sessions. There was no significant variation among groups in age, weight, height, and BMI (p > 0.05). Also, there was no significant variation in sex distribution among groups (p > 0.05).
Table 2. Basic characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>5 minutes group</th>
<th>1-hour group</th>
<th>5 hours group</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.66±2.79</td>
<td>25.86±2.89</td>
<td>25.06±2.96</td>
<td>0.733</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.80±5.87</td>
<td>66.53±6.18</td>
<td>64.93±6.35</td>
<td>0.447</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.5±5.59</td>
<td>165.13±8.13</td>
<td>164.6±8.34</td>
<td>0.787</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.28±0.94</td>
<td>24.36±1.08</td>
<td>23.97±1.75</td>
<td>0.691</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>10 (67%)</td>
<td>8 (53%)</td>
<td>10 (67%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Males</td>
<td>5 (33%)</td>
<td>7 (47%)</td>
<td>5 (33%)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant level is set at alpha level <0.05.

Effect of time of application, treatment application and conditions on isokinetic peak torque

MANOVA of mixed design type revealed that there was non-significant interaction of time of application, treatment application and conditions (F = 1.29, p = 0.26). There was no significant main effect of time of application (F = 0.59, p = 0.73). There was a significant main effect of treatment application (F = 96.2, p = 0.001). There was no significant main effect of condition (F = 1.37, p = 0.26). Table 3 presented descriptive statistics of IKPT.

Within groups comparison

It revealed a significant decrease in IKPT in the three groups post treatment compared with that pretreatment in placebo and active conditions (p < 0.001). While the percent of decrease of IKPT in 5 minutes, 1 hour and 5 hours groups in placebo condition was 27.88%, 31.14% and 33.13% respectively, in active condition was 26.89%, 28.63% and 31.93% respectively, which demonstrated that the 5 minutes group showed the lowest percent of decrease in IKPT compared to the other 2 groups.
Among groups comparison

It showed a non-significant difference in IKPT at pre and post treatment in placebo and active conditions ($p > 0.05$) (Table 3).

Table 3. Descriptive statistics for isokinetic peak torque, pre- and post-LED, in placebo and active condition of the three groups

<table>
<thead>
<tr>
<th>5 minutes</th>
<th>1 hour</th>
<th>5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>20.64 ± 12.56</td>
<td>20.12 ± 6.18</td>
</tr>
<tr>
<td>% of change</td>
<td>27.88 %</td>
<td>31.14 %</td>
</tr>
<tr>
<td>p</td>
<td>$p = 0.001$</td>
<td>$p = 0.001$</td>
</tr>
<tr>
<td><strong>Active</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>28.93 ± 16.09</td>
<td>28.53 ± 8.70</td>
</tr>
<tr>
<td>% of change</td>
<td>26.89 %</td>
<td>28.63 %</td>
</tr>
<tr>
<td>p</td>
<td>$p = 0.001$</td>
<td>$p = 0.001$</td>
</tr>
</tbody>
</table>

5. DISCUSSION

There are few studies concerning photobiomodulation therapy (PBMT) and muscular preconditioning that focused on the effect of different application timing intervals of PBMT in clinical trials, with contradicting results (Rossato, Dellagrana, Sakugawa, Lazzari, Baroni & Diefenthaler, 2018; Abreu, Dos Santos, Fonsati, Marques & Ferraresi, 2020). So, the purpose of this study was to determine the optimal time between light emitting diode (LED) application and elbow flexors exercise and to determine effect of LED on muscle’s strength represented by isokinetic peak torque.

The primary findings of this study were that neither of the LED groups could maintain isokinetic torque level in the post test compared to pre-test in the active and placebo conditions significantly, but LED applied 5 minutes before exercise showed the least percent of diminishing isokinetic peak torques in the active condition, compared to the placebo condition, within the group and between groups, irradiated 1 hour and 5 hours prior to the exercise.
To the author’s knowledge, the current study is the first randomized controlled trial in human that investigates the time response of LEDT, focusing on studying a specific wavelength within the infrared range, since it was indicated that the light energy can be transferred from 2 to 4 cm across the skin interface by using diodes with wavelengths extending from 820 to 904 nm (Enwemeka, 2009; Enwemeka, Parker, Dowdy, Harkness, Harkness & Woodruff, 2004; Reddy, 2004). Therefore, they are the most appropriate for the management of deep soft tissues.

Another strength of the current study was that, at the beginning of the two sessions, the participants showed similar maximal torque capabilities and underwent the assessment with homogenous performance. Consequently, any variances in the post fatigue values could not be caused by possible pre-existing alterations in the two experimental conditions and the undesirable learning effect from the first and second treatment sessions can be ruled out.

Additionally, multi diode LEDT may have a privilege over single-diode, as they have larger beam divergence, so they do not have to be repositioned as frequently during treatment as the single-diode, while still incorporating a large muscle surface area and application points (Borsa, Larkin & True, 2013). Therefore, in the current study the multi diode LEDT was used with 120 diodes to irradiate a large number of points of the elbow flexor muscles, in contrast to many studies that relied on irradiating only a few number of points (Junior, Lopes-Martins, Baroni, De Marchi, Taufer & Manfro, et al., 2009; Leal, Lopes-Martins, Frigo, De Marchi, Rossi & De Godoi, et al, 2010; Borsa, Larkin & True, 2013).

One of the unique points in the present study was the use of isokinetic fatigue instead of the isometric protocol most commonly used, as isometric contractions may not be demonstrative of incidence of muscle action and fatigue during human activity (Green, 1995). This exercise procedure has been previously used and replicates the cellular and efficient processes detected with acute sessions of exercise (Borsa & Sauers, 2000).

The current study focused on evaluating the isokinetic peak torque, as the peak torque variable is addressed as the ‘‘golden criterion’’ estimate in isokinetic testing (Kannus, 1994; Miller, Pierson, Nickols-Richardson, Wootten, Selmon & Ramp et al., 2006). Additionally, peak torque has been the variable most widely used in the interpretation of isokinetic assessments (Amaral, Marinho, Ocarino, Silva, Souza & Fonseca, 2014). The 5 minutes group showed a higher capacity to preserve isokinetic peak torque, although not significant, which may be attributed to using several series of fatiguing exercise that veiled comparatively insignificant but noticeable effects.
The extensive inconsistency of findings documented from earlier studies may be due to the wide variability in the application parameters, diverse machines enclosing combined Laser and LED groupings or each type separately, and using PBMT by probes, pads, or clusters of variable sizing. Also, the wide range of parameters used by investigators, such as wavelengths, power, number of treated points, and energy per point. Further probable explanations involve differences in the fatigue provocation protocol type, the nature of different participants, and the different methods of assessment that were applied (Rossato, Dellagranra, Sakugawa, Lazzari, Baroni, Diefenthaler, 2018; Abreu, Dos Santos, Fonsati, Marques & Ferraresi, 2020; Beltrame, Ferraresi, Parizotto, Bagnato & Hughson, 2018). The present study was performed using NIR-LED of 890 nm, 60 J, 15 mW per diode, for 57 seconds irradiating 120 points by technique of direct contact overlaying 45 cm² of the elbow flexor muscles. These parameters were consistent with the most recent recommendations for the enhancement of post-exercise recovery process (Leal-Junior, Lopes-Martins & Bjordal, 2019).

One of the triggering mechanisms of the fatigue of skeletal muscle is the decline in ATP production within the muscles (Grassi, Rossiter, Zoladz, 2015). It was shown that NIR radiation can promote cellular mitochondrial photochemical and photophysical modifications (Ferraresi, de Brito Oliveira, de Oliveira Zafalon, de Menezes Reiff, Baldissera, de Andrade Perez, et al., 2011; de Brito Vieira, Ferraresi, de Andrade Perez, Baldissera & Parizotto, 2012). These modifications may initiate amplified mitochondrial membrane potential (MMP) (Passarella, Ostuni, Atlante & Quagliariello, 1988) and the maturity of giant mitochondria (Manteifel, Bakeeva & Karu, 1997) that boosted cellular ATP levels. This justifies the fatigue resistance potential of NIR-LED. Although the improved ATP generation produced by PBMT has been extensively recognized in the literature (Ferraresi, de Brito Oliveira, de Oliveira Zafalon, de Menezes Reiff, Baldissera, de Andrade Perez, et al., 2011, de Brito Vieira, Ferraresi, de Andrade Perez, Baldissera & Parizotto, 2012), there is still a demand for additional explanation of the ideal time interval during which this improvement happens (Rossato, Dellagranra, Sakugawa, Lazzari, Baroni & Diefenthaler, 2018).

It has been recorded that there is an increase in all complexes of the electron transport chain (ETC) of the mitochondria, specifically cytochrome c oxidase (CCO) enzyme action or complex IV, following radiation by NIR (Silveira, da Silva, Fraga, Freitas, Streck, Pinho, 2009). PBMT was recorded to excite CCO, starting from 5 minutes to 24 hours following treatment, as per the applied dosage and wavelength (Albuquerque-Pontes, de Paula Vieira, Tomazoni, Caires, Nemeth & Vanin, et al., 2015). An earlier in-vitro study has revealed that PBMT altered production of ATP and potential
of the mitochondrial membrane in hepatocytes, applying a timing interval of 3 minutes span between irradiation and measurement (Passarella, Casamassima, Molinari, Pastore, Quagliariello & Catalano et al., 1984).

In this regard, findings of the present study are in harmony with an earlier study which described that loss of muscle strength was diminished by using NIR laser treatment applied 5 minutes prior to exercise (Larkin-Kaiser, Christou, Tillman, George & Borsa, 2015), and also is consistent with a study that reported no impact of LED employed 30 minutes or 6 hours prior to exercise, on metabolism of aerobic type (Beltrame, Ferraresi, Parizotto, Bagnato & Hughson, 2018).

On the other hand, there is a conflict between the present results and the results of a study that recommended that the grouping of PBMT was utilized at 2 time intervals; 5 minutes before and 6 hours before the exercise triggered a smaller drop in peak torque values contrasted to placebo, control, and 5 minutes prior to treatment groups (Rossato, Delagrana, Sakugawa, Lazzari, Baroni & Diefenthaler, 2018). This conflict can probably be accredited to the use of collective red and infrared wavelengths at the same time, which possibly gives a privilege to the absorbing light chromophores in the cells (Ferraresi, Parizotto, Pires de Sousa, Kaippert, Huang & Koiso et al., 2015), predominantly CCO, causing much more ATP generation than individual application of either NIR or red wavelengths (Karu, 1999). On the contrary, the current study aimed to study NIR time reaction, which has not been investigated so far.

From another perspective, it has been reported that LED applied at different time intervals from 5 minutes to 6 hours before exercise showed no effect on torque values (Abreu, Dos Santos, Fonsati, Marques & Ferraresi, 2020), in spite of using the dose of 60 Joules, which is a highly recommended dose, and LED flexible arrays treating a large extent of elbow flexors (Leal-Junior, Lopes-Martins & Bjordal, 2019). This could be interpreted by using lower density of energy (0.35 J/cm²) than the advocated density by Rossato et al. (Rossato, Delagrana, Lanferdini, Sakugawa, Lazzari & Baroni, et al., 2016), who claimed that the small-sized and large-sized PBMT clusters showed equally positive outcomes on elbow flexors fatigue, so that it can be deduced that the effectual density of energy varies from 0.99 J/cm² to 4 J/cm². Taking this factor into account, the present study accredited an energy density of 1.33 J/cm², which clarifies the difference in our results from those of Abreu, Dos Santos, Fonsati, Marques & Ferraresi (2020).
6. LIMITATIONS

There is a number of limitations to be considered in the interpretation of the current results. A particular class of muscles was involved in the fatiguing exercise, to precisely control the study, thus this can limit the findings’ generalizability to further multidimensional training activities. Moreover, muscle fatigue blood markers as lactate were not measured prior to and following exercise, which is proposed to be done in upcoming studies.

7. CONCLUSION

The current study proposes that the most advantageous point of time for NIR LED application is 5 minutes before exercise to augment muscle’s potential to resist fatigue. This might facilitate achieving a systematized protocol of PBMT to reduce the unfavorable muscle fatigue consequences that influences workers’ and patients’ populations production and functioning commonly. Also, this could be valuable for patients through rehabilitation settings, maximizing LED efficacy prior to exercises and enhancing the process of recovery and the rehabilitation effects.

8. REFERENCES


**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

**FUNDING**

This research received no external funding.

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