# PHOTOBIOMODULATION TIME RESPONSE OF HUMAN SKELETAL MUSCLE FATIGUE

by

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**ABSTRACT**

Photobiomodulation therapy (PBMT) is a modality that is gaining popularity in the sports medicine field. By using light, specifically red and infrared wavelengths, PBMT can reduce inflammation, promote faster healing and recovery, treat neurological disorders and pain, and also prevent muscle damage following intense exercise. More recently, it has been shown that PBMT can also increase resistance to skeletal muscle fatigue by stimulating mitochondrial function in muscle cells. Photobiomodulation therapy improves ATP production in the cell for up to 24 hours post-irradiation. The purpose of this study was to determine the optimal time between PBMT application and an isometric knee extensor exercise to resist fatigue. A total of 42 participants were randomized into 4 different treatment groups: (1) PBMT applied 0 h (<10 minutes) prior to exercise, (2) PBMT applied 5 h prior to exercise, (3) PBMT applied 24 h prior to exercise, and (4) sham PBMT at 0 h, 5 h, or 24 h prior to exercise. The sham treatment used the same device, without powering the light patch. This study aimed to demonstrate that 5 hours prior to an exercise rather than immediately before will give the muscle the greatest resistance to fatigue. Peak quadriceps torque pre-fatigue to post-fatigue task (MVC), muscle fatigue, and acute muscle soreness perception (mVAS) were measured. None of the PBMT groups reached significance in their ability to reduce fatigue in any outcome measure, however the PBMT 0 h and 5 h treatment groups showed smaller decreases in peak quad torque compared to the sham treatment ($P = 0.2488$). The 5 h PBMT group also had slightly better muscle endurance during the fatigue test, averaging

4% more repetitions than the 0 h treatment (P = 0.9169). The results suggest that PBMT application 5 hours before a fatiguing exercise may enhance muscular endurance and prevent a decrease in maximal strength compared to an immediate application, however further investigation is necessary with an increased number of subjects.

## I: Introduction

Photobiomodulation therapy (PBMT), also referred to as low-level light therapy, is a modality that is gaining interest and popularity in different areas of medical practice. It is defined as a form of nonthermal light therapy, using non-ionizing forms of light, to elicit photophysical and photochemical events at various biological scales.[1] After the invention of the laser in 1960, researchers have demonstrated that PBMT can produce positive therapeutic effects. PBMT works by exposing the cells or tissues to light with low power to avoid temperature change.[2] The lower power allows the laser to be used therapeutically compared to other laser forms that are used for cutting and thermally coagulating tissue. PBMT was once thought to require the use of coherent (does not spread or diffuse) lasers for optimal effect, although in recent years, non-coherent light emitting diodes (LEDs) have shown to be just as effective and is a most cost effective alternative.[2]

There are a variety of uses for PBMT in medicine. Therapists may use PBMT to reduce inflammation and edema from injury or chronic joint disorders.[3] Superficial wounds, deeper injured tissues, and damaged nerves irradiated by red and infrared light promote quicker healing and recovery of function.[4,5] Photobiomodulation therapy is also used to treat neurological disorders and pain.[5,6] It has been shown to work prophylactically as well, by reducing the magnitude of muscle damage following intense exercise.[7] Decreases in muscle damage markers (lactate, creatine kinase) occur following PBMT treatment applied before and after exercise, however pre-exercise treatment may provide better outcomes.[7]

More recently, there has been evidence of using PBMT to enhance muscular endurance.[8] Muscle fatigue can be delayed when a pre-exercise PBMT treatment is applied.[2,7-17] Fatigue occurs when a muscle cannot maintain the expected force, or is unable to maintain the intensity or power required for a specific exercise.[18] Fatigue is very complex with many theories but is often attributed to when muscle activity exceeds tissue substrate regeneration and oxygenation capacity. The depletion of energy sources such as phosphocreatine and glycogen; a lack of required oxygen in the muscle; and slowed production of adenosine triphosphate (ATP), the cell's energy unit, are some contributors to the muscle's inability to generate force.[19]

There are increased injury rates with the accumulation of fatigue in athletes.[20] More injuries tend to occur towards the end of a sporting event, suggesting that they are a result of muscular fatigue experienced by the athletes.[21] Fatigued athletes have reduced voluntary force production in concentric, eccentric, and isometric contractions.[13,22] Because the fatigued muscles cannot produce the expected force required, the other supporting structures of the joint are exposed to excessive loading.[22] This can lead to failure of the tissue to support the joint, resulting in injury. Altered neuromuscular control occurs with fatigue, affecting joint position sense and joint kinesthesia.[22] Fatigue will cause an athlete to make changes in voluntary activation patterns which will affect the dynamic stability during complex movements and increases the risk of injury.[23]

It has been found that light from PBMT can reduce fatigue when a pre-exercise treatment is applied.[7,16,17,24] There are some proposed mechanisms in the effect PBMT has on muscle fatigue. Under certain wavelengths of light, PBMT increases cytochrome c oxidase (Cox) activity in the electron transport chain, by generating larger mitochondria

to produce more ATP, providing more energy to the muscle.[14,25] During high intensity exercise, ATP consumption is faster than the rate of phosphocreatine (PCr) re-synthesis, leading to an excess of tissue substrates which contributes to fatigue. With improved ATP production in the mitochondria, PBMT also improves PCr re-synthesis at the muscle contraction site providing an energy source for maximal strength tests or short high intensity exercise.[14] Blood lactate is a biomarker often used to measure muscle fatigue and is associated with increased lactate dehydrogenase (LDH) activity. LDH is the enzyme that converts pyruvate into lactate during anaerobic glycolysis when oxygen supply is insufficient to synthesize ATP. PBMT has been shown to modulate LDH activity during exercise, even when the supply of oxygen is to slow or insufficient for mitochondrial ATP synthesis.[14]

Effectiveness of PBMT is dependent on parameter and dosage.[7,12,26,27] PBMT has a biphasic dose response, meaning that treatment delivered at smaller doses tend to work better than larger doses of the same parameters.[28] There are many parameters to consider including wavelength, energy, and timing. In PBMT research, wavelengths can include blue, green, red, and infrared light.[29] When treating skeletal muscle tissue, wavelengths of red (630 – 700 nm) and infrared light (700 – 1200 nm) are typically used because their longer waves can penetrate deeper through the skin and affect muscle.[29] In a recent systematic review indicating success of PBMT on muscular performance improvements, wavelengths ranged from 655 – 950 nm in significant findings.[30] Wavelengths of blue light (400 – 470 nm) have been shown to reduce acne and treat other dermatological conditions,[31] however its effects on skeletal muscle has not been thoroughly investigated. Energy is indicated by how much power is used to transfer the light and is measured in

Joules (J). PBMT has been shown to be effective to reduce fatigue with energy doses between 20 – 60 J for small muscles, and between 60 – 300 J for larger muscles.[30] Depending on desired treatment outcomes, timing is important as well.[32] PBMT has been shown to have positive results when used either before or after an exercise, however a pre-exercise treatment may be the most effective to reduce fatigue.[7,30]

In most PBMT clinical studies that aim to reduce fatigue, the researcher will irradiate the muscle immediately before the exercise.[7,16,17,24] Although, there are positive benefits recorded such as increased time to exhaustion and the maintenance of strength capacity, application immediately before a fatiguing exercise may not be the optimal treatment application time. Red and infrared light increases mitochondria activity from immediately following (3-5 minutes) to 24 hours following PBMT irradiation.[25] A recent cell culture study measured mitochondrial membrane potential and ATP content in mouse muscle at 5 minutes, 3 hours, 6 hours, and 24 hours after receiving light emitting diode therapy.[33] Both outcome measures were greatest at 6 hours post-irradiation, followed by 3 hours, 24 hours, and 5 min, respectively. These data suggests an optimum time window of 3-6 hours to stimulate skeletal muscle cells to have the most improvement in ATP production.[33,34] This is consistent with an in vivo mouse study.[34] The mice in the study performed a fatiguing ladder climbing exercise 5 minutes, 3 hours, 6 hours, and 24 hours following PBMT irradiation. The 6 hour group performed the most repetitions followed by the 3 hour group and 24 hour group. A high positive correlation has been reported between the number of fatigue task repetitions and the muscular ATP content.[34] Although these two studies were performed in animal models, the results suggest that applying

PBMT at least 3 hours before an exercise may provide greater resistance to skeletal muscle fatigue.

**Purpose:**

The purpose of this study was to determine the optimal time between PBMT application and a knee extensor exercise to resist fatigue.

**Hypothesis:**

Muscles irradiated 5 hours prior to exercise will have the most resistance to fatigue. Muscles irradiated 5 hours prior to exercise will also have greater muscular endurance during the fatiguing knee extensor exercise.

**Operational Definitions:**

1. Photobiomodulation Therapy – A nonthermal therapeutic application of laser or light to improve tissue healing, reduce inflammation, reduce pain, and improve resistance to fatigue during exercise.
2. Dose – PBMT dose parameters are recorded by time, energy, and energy density.
    a. Irradiation Time – How long each point will receive light during treatment.
    b. Irradiance (Power Density) – The optical power as measured in Watts (W) or milliWatts (mW) per unit of treatment area. Typically measured in $W/cm^2$ or $mW/cm^2$.
    c. Energy – A measure of Power x Time measured in Joules (J). One Joule = 1 Watt x 1 Second.

d. Fluence (Energy Density) – The amount of energy per unit of treatment area, measured in $J/cm^2$

3. Wavelength – Light is an electromagnetic form of energy with a wave-like behavior.[8] Wavelength equals the distance between two wave crests or the number of waves that passes per second. Measured in nanometers (nm).

    a. Blue = 400 – 470 nm

    b. Red = 630 – 700 nm

    c. Infrared = 700 – 1200 nm

4. Fatigue – A failure to maintain the expected force, or the inability to maintain a given exercise intensity or power output level.[13]

5. Torque – A measure of how much force to cause a rotation. For the present study, how much force the participant exerted to perform the knee extension effort.

**Delimitations**

1. This study is delimited by only including healthy strength-trained individuals defined under ACSM guidelines (strength training at least 2x week).[35]

2. This study is delimited by only applying photobiomodulation immediately, 5 hours, and 24 hours prior to exercise.

**Assumptions and Limitations:**

1. It is assumed that all participants will give full effort throughout the exercises and procedure.

2. It is assumed that evidence from this study can be used in rehabilitation of injury.

3. This study is limited to fatigue resulting from isometric exercise.

**Significance of the Study:**

Photobiomodulation therapy can be used to pre-condition muscles prior to exercise to increase resistance to fatigue. [2,7-17,27] For healthy athletes, PBMT may be used to increase athletic performance and reduce the chance of injury by reducing fatigue in joint stabilizing muscles. Additionally, the increased energy production in the mitochondria may help sustain the amount of force required during dynamic muscular contractions throughout an athletic competition. PBMT may be useful by reducing fatigue between bouts of high intensity performances, such as during half time in sports like soccer, basketball, and football.[36] When retraining neuromuscular control during rehabilitation, proper voluntary muscle activation patterns are necessary to reduce the chance of re-injury.[37] Photobiomodulation therapy reduces fatigue, which may limit alterations in muscular activity and make exercises more efficient during rehabilitation.

To our knowledge this is the first study to compare different times of PBMT application prior to a fatiguing exercise *in vivo* in human skeletal muscle. Many researchers choose to irradiate muscles immediately prior to exercise to reduce fatigue, however there is evidence of the prophylactic effect outside of the typical treatment window.[25,33,34,38,39] Ferraresi et al observed this by giving PBMT to male volleyball players before their matches.[38] The team received PBMT to the lower extremity 40-60 minutes prior to each of their 4 matches. Blood samples were collected 1 hour before and 24 hours after each match to measure creatine kinase activity, a marker of muscle damage

and a contributor to muscle fatigue. The treatment prevented increases in creatine kinase activity, suggesting PBMT at an earlier time prior to fatiguing exercise may allow more time for the cellular response to occur.

## II: Review of Literature

Photobiomodulation Therapy is More Effective than Cryotherapy for Skeletal Muscle Recovery:

A Critically Appraised Topic.

***Clinical Scenario:*** Cryotherapy is one of the most commonly used modalities for post-exercise muscle recovery despite inconsistencies in the literature validating its effectiveness. With the need to find a more effective modality, photobiomodulation therapy (PBMT) has gained popularity because of recent research demonstrating its ability to accelerate the muscle recovery process. ***Focused Clinical Question:*** Is PBMT more effective than cryotherapy at reducing recovery time and decreasing delayed onset muscle soreness (DOMS) after strenuous exercise? ***Summary of Key Findings:*** Three moderate- to high quality double-blinded, randomized placebo-controlled trials and two low- to moderate quality translational studies performed on rats were included in this CAT. All 5 studies supported the use of PBMT over cryotherapy as a treatment for post-exercise muscle recovery following exercise. PBMT was superior in reducing creatine kinase, inflammation markers, and blood lactate compared to cryotherapy following strenuous/high intensity aerobic or strength muscular exercise. PBMT was also shown to improve post-exercise muscle performance and function more than cryotherapy. ***Clinical Bottom Line:*** There is moderate evidence to suggest the use of PBMT over cryotherapy post-exercise to enhance muscle recovery in trained and untrained athletes. Shorter recovery times and increased muscle performance can be seen 24 to 96 hours following PBMT application. ***Strength of Recommendation:*** Based on consistent findings from all 5 studies, there is grade B evidence to support the use of PBMT over cryotherapy for more effective post-exercise recovery of skeletal muscle performance.

## Clinical Scenario

Photobiomodulation therapy (PBMT) is a promising modality that has gained popularity in different areas of medical practice. Previously referred to as low-level laser therapy (LLLT) or light emitting diode therapy (LEDT), PBMT has effectively improved muscle performance by increased exercise times, and reduced muscle fatigue limiting post-exercise strength losses.[14] After intense exercise, PBMT confines the degree of exercise induced muscle damage, limiting the need for a large inflammatory process.[26] It also reduces patient-reported muscle soreness, modulates growth factors and myogenic regulatory factors, and increases the formation of new red blood cells locally.[26] These effects make PBMT a valuable treatment option for muscle recovery; however, PBMT has not become a mainstream tool for muscle recovery in clinical practice. For decades, cryotherapy has been a popular modality for post-exercise muscle recovery utilized by many athletes, coaches, and sports medicine practitioners, despite recent challenges to its effectiveness.[40] For these reasons, PBMT should be explored as a substitute to cryotherapy for post-exercise muscle recovery.

## Focused Clinical Question

Is PBMT more effective than cryotherapy at reducing muscle recovery time and decreasing delayed onset muscle soreness (DOMS) after strenuous exercise?

**Summary of Search, 'Best Evidence' Appraised, and Key Findings**

- The literature was searched for studies of level 2 evidence or higher (based on Oxford Centre of Evidence-Based Medicine 2011, Levels of Evidence) that compared PBMT vs. cryotherapy as a treatment for muscle recovery.
- Three moderate- to high quality double-blinded, randomized placebo-controlled trial studies[41,42] and two low- to moderate quality translational rat studies[36,43,44] were included in the critical appraisal.
- All five studies[36,41-44] supported the use of PBMT rather than cryotherapy as treatment for muscle performance recovery following exercise.

**Clinical Bottom Line**

There is moderate evidence to support the use of PBMT over cryotherapy when using this modality post-exercise for muscle recovery in trained and untrained athletes. Shorter recovery times, identified by a fast return to baseline muscle torque and subjective muscle soreness values, can be seen 24 to 96 hours following PBMT application. Lower markers of muscle damage, creatine kinase (CK), which lead to less inflammation markers, were found 24 to 96 h after PBMT treatments; however, CK levels after cryotherapy treatments followed similar patterns to placebo treatments.

**Strength of Recommendation**

Based on the Oxford Centre for Evidence-Based Medicine strength of recommendation there is grade B evidence to support the use of PBMT over cryotherapy for post-exercise

muscle recovery. The results were consistent across all five studies included in this appraisal.

**Search Strategy**

Terms used to guide Search Strategy:

- **P**atient/Population/Problem
    - Muscle recovery following strenuous exercise
- **I**ntervention
    - Photobiomodulation
- **C**omparison
    - Cryotherapy
- **O**utcome
    - Improve recovery time, decrease muscle soreness

Search Terms Used:

Searches included the key terms “photobiomodulation,” “low-level laser therapy,” “light-emitting diode therapy,” “phototherapy,” “cryotherapy,” “cold-water immersion therapy,” “muscle recovery,” and “muscle damage.”

Sources of Evidence Searched:

- MEDLINE
- SPORTDiscus

- Additional articles obtained through hand search of reference lists

**Inclusion and Exclusion Criteria**

Inclusion:

- Articles that investigated a direct comparison between PBMT and cryotherapy for muscle recovery after strenuous exercise
- Articles with treatment post-exercise
- Limited to articles in English
- Level 2 or higher level of evidence

Exclusion:

- Articles published before 2007

**Results of Search**

Five relevant studies met the inclusion criteria and are categorized in Table 2.1.

**Best Evidence**

The studies listed in Table 1 represent the best available evidence and were included in this critically appraised topic (CAT). The selection of studies was based on the following criteria: included a level of evidence rating of 2 or better, investigated a direct comparison between cryotherapy and PBMT application in relation to muscle recovery following strenuous exercise, and compared the effectiveness of the treatments post-exercise in terms of muscle performance recovery.[36,41-44]

**Table 2.1 Summary of Study Designs of Articles Retrieved**

| Level of Evidence | Study Design | Number located | References |
|---|---|---|---|
| 1 | Double-blinded, Randomized, Placebo-Controlled Clinical Trial | 3 | de Pavia et al.[4]<br>Leal Junior et al.[5]<br>de Marchi et al.[8] |
| 2 | Translational Rat Studies | 2 | Camargo et al.[6]<br>de Costa Santos et al.[7] |

**Implications for Practice, Education, and Future Research**

All 5 studies reviewed in this CAT support the use of PBMT over cryotherapy when treating trained and non-trained individuals post aerobic and strength exercise for muscle recovery.[36,41-44] There were no studies found in the literature search that supported cryotherapy over PBMT. Photobiomodulation therapy was more effective in preventing increases in CK levels,[36,41-44] blood lactate,[42] C-reactive protein,[36,42] and inflammation[36,43] after an exercise bout. In addition, PBMT was able to increase time to exhaustion[36] and better maintain muscular strength following strenuous exercise[41,44] compared to cryotherapy.

Training and competition in athletics can be stressful on an athlete's muscles requiring appropriate treatment to accelerate post-exercise recovery. A quick recovery can maintain muscular function when repeated performance is necessary. After completing an intense exercise, especially one that is unfamiliar, an athlete experiences physiological stress within the affected muscles. Muscle stress causes energy substrate depletion, such as glycogen and ATP, mechanical muscle damage, oxidative stress, inflammation, and neuromuscular fatigue.[45-47] Symptoms such as soreness and decreased muscle function are reported by athletes following strenuous exercise and results in

muscle fatigue.[48] Fatigue alters muscle proprioception and activation, which can limit muscular performance in subsequent sport competition or practice.[22]

Many athletes, coaches, and sports medicine professionals utilize cryotherapy as the primary modality for muscle recovery, especially following an intense training session. There continues to be widespread use of cryotherapy techniques post-exercise despite inconsistencies in the literature validating its effectiveness. Cryotherapy decreases the tissue metabolic rate,[49] promotes superficial vasoconstriction,[50] decreases vascular permeability[51] and leads to less edema formation.[48,52] A form of cryotherapy, cold water immersion therapy has an additional effect, due to hydrostatic pressure, at encouraging reabsorption of interstitial fluids found in the muscle after exercise.[53] Cryotherapy is able to improve subjective measures of recovery after intense exercise bouts such as self-reported muscle soreness; however, objective measures of muscle force, lactate, CK, and inflammatory markers are hindered.[40,54,55]

The physiological response resulting from photobiomodulation therapy on muscle recovery is quite different than cryotherapy. Photobiomodulation therapy affects the tissue at the cellular level by inducing a photochemical reaction within the cell. Red and infrared light is absorbed by 1 of 4 membrane-bound complexes within the mitochondria known as cytochrome c oxidase (Cox).[25] Also known as complex IV, Cox is a key chromophore in the respiratory electron transport chain that leads to the production of ATP in the mitochondria. An improvement of mitochondrial function and increase in ATP synthesis within the mitochondria is seen following PBMT application.[56,57] Photobiomodulation therapy has also been shown to reduce circulating reactive oxygen species (ROS) by stimulating an increase in antioxidants and nitric oxide release.[58]

Muscle fibers are damaged as a result from exercise, especially from prolonged or strenuous exercise.[20,59] As a response to this exercised induced muscle damage of the muscle, an inflammatory process occurs to heal and regenerate damage fibers.[48,59] Muscle damage was noted in the included studies following the exercise protocols. This was demonstrated by an increase of CK, blood lactate, and frequency of necrosis, measured by histological analysis, in placebo treatments,[41,42,44] an ice bag application,[41,44] and cold water immersion therapy.[36,42] Compared to placebo treated groups, cryotherapy demonstrated no difference in CK[41,42,44] or blood lactate[42] levels at any time points. Photobiomodulation therapy protected the muscle against damage, in the included studies, with significantly lower levels of muscle damage markers;[36,41-44] thus, inflammation markers of c-reactive protein[36,42,43] and leukocyte analysis[36,43] were also lower in groups treated with PBMT.

The ability to maintain muscle strength and function performance between bouts of exercise should be a factor when choosing a modality to promote post-exercise muscle recovery. Oxidative stress increases after intense exercise, decreasing contractile function.[60] Photobiomodulation therapy during repeated high intensity muscular exercise bouts aided in preventing a decrease in maximum voluntary contraction (MVC); however, cryotherapy treatment resulted in significant decreases in MVC.[41,44]

Future research is necessary to optimize treatments that clinicians and athletes use for muscle recovery. Although two of the studies utilized in this CAT were translational rat studies, the results offer valuable information that provides a foundation for future clinical research in human muscle. Additional unbiased *in vivo* human studies are needed to address the physiology behind cryotherapy and photobiomodulation and their

respective effects on muscle recovery post-strenuous exercise. Also, continual investigation into the proper treatment parameters for PBMT and cryotherapy is needed, as the various parameters used between studies may impact the study outcomes.

Photobiomodulation therapy research has shown positive results regarding the ability to aid in the recovery and improvement of muscular strength and function. Future research should continue to address optimal parameters, timing and dosage for PBMT, especially comparing high and low powered devices and parameters. All studies we included used low powered PBMT devices. Future photobiomodulation therapy research should also be compared to cryotherapy and other treatment modalities for its effects immediately after musculoskeletal injury. This CAT should be reviewed in 2 years to determine whether additional best-research evidence has been published that could aid in answering the focused clinical question.

**Table 2.2 Characteristics of Included Studies**

| Article | de Costa Santos et al.[7] | Camargo et al.[6] | de Marchi et al.[44] | de Pavia et al.[4] | Leal Junior et al.[5] |
|---|---|---|---|---|---|
| **Study Design** | Translational Study | Translational Study | Randomized, Double-blinded, Placebo-controlled Trial | Randomized, Double-blinded, Placebo-controlled Trial | Cross-over, Randomized, Double-blinded, Placebo-Controlled Trial |
| **Participants** | 29 male Wistar rats randomized into 4 groups: control (Co, n=6), exercised + passive recovery (PR, n=6), exercised + cryotherapy (Cryo, n=8), and exercised + LED therapy (LED, n=9) | 32 male Wistar rats randomized into 4 groups (n=8): control (Co), exercised (E), exercised + CWI (CWI), and exercised + LED phototherapy (LED). | 40 male volunteers aged between 19 and 29 years old. Randomized to 5 groups: Placebo (PG), Photobiomodulation therapy (PBMT), Cryotherapy (CG), Cryotherapy-PBMT (CPG), PBMT-Cryotherapy (PCG) | 50 untrained male participants aged between 18 and 25, randomized to 5 groups (n=10): Placebo, PBMT, Cryotherapy, Cryotherapy+PBMT, PBMT+Cryotherapy | 6 male professional futsal players from Brazil randomized to receive either CWIT, active LEDT, or placebo LEDT in a random manner after 3 exercise tests. |

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| **Intervention Investigated** | PD 300 Standard Photodiode Sensor (Ophir Optronics, Jerusalem, Israel). *Parameters for LEDT:* 940 nm wavelength and a spectral bandwidth of 45 nm in 4 min intervals, 4 J/cm$^2$ of energy intensity, 9.5 mW/cm$^2$ power density, 160 mW power output, 1 cm$^2$ irradiation area on each hind leg.<br><br>*Parameters for Cryotherapy:* hind legs immersed in 10°C for 10 min.<br><br>*Exercise Protocol:* Animals were submitted to 45 min of swimming exercise followed by 25 min of recovery and then a second | PD 300 Standard Photodiode Sensor (Ophir Optronics, Jerusalem, Israel). *Paramaters for LEDT:* 940-nm wavelength with a spectral bandwidth of 45 nm in intervals of 7 min and 15s to administer 4 J/cm$^2$ of energy intensity, 9.5 mW/cm$^2$ power density, 160 mW power output, 1 cm$^2$ irradiation area on each hind leg.<br><br>*Parameters for Cryotherapy:* hind legs were immersed in 10°C for 10 min.<br><br>*Exercise Protocol:* The exercise groups (E, CWI, LED) swam for 100 min in a plastic container | PBMT: 69 LED (34 red 660nm, 35 infrared 850nm) cluster probe (THOR® Photomedicine London, UK), continuous frequency, output power=10mW red, 30mW infrared, LED spot size=0.2 cm$^2$, total spot size=13.8 cm$^2$, power density=0.05 W cm$^{-2}$ (red), 0.15 W cm$^{-2}$ (infrared), energy=41.7J, 30s treatment time, 1 irradiation point per muscle.<br><br>Cryotherapy: muscle belly of biceps. Ice bag applicaton of 20 min.<br><br>*Muscle fatigue protocol*: On Biodex Systems 4 Pro isokinetic dynamometer, five sets of 10 eccentric/concentric contractions of the elbow flexors separated by 30s. Performed with an | PBMT: Cordless, portable GameDay™ device (Multi Radiance Medical, Solon, OH, USA). One super pulsed infrared 905nm laser, dose=0.375J; 4 red LEDS, dose=4.5J; 4 infrared LEDs, dose=5.25J; total dose per site=39.37J; irradiation time per site=300s; applied to 6 sites of quadriceps femoris.<br><br>*Eccentric exercise protocol*: On Biodex Systems 4 Pro isokinetic dynamometer, 75 eccentric isokinetic contractions in non-dominant leg (5 sets of 15, 30s rest between sets) at a velocity of 60° .seg$^{-1}$ in both flexion and extension of knee with a 60° range of motion | LEDT: Cluster probe with 34 LED diodes of 660 nm (red) and 35 LED diodes of 850 nm (infrared) (THOR® Photomedicine, London, United Kingdom), continuous frequency, optical output=10mW (red) and 30mW (infrared), spot size=0.2 cm$^2$, power density=0.05 W/cm$^2$ (red) and 0.15 W/cm$^2$ (infrared), energy=41.7 J each point, 10 irradiation points, 30s each point, 5 min total.<br><br>CWIT: Standing position with lower limbs immersed to the gonadal region 5 °C for 5 min.<br><br>*Fatigue test protocol*: at the 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ session of study, |

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| | bout of either 45 min or time to exhaustion. | | amplitude of 90° and speed of 90° .seg$^{-1}$ for eccentric and 180° .seg$^{-1}$ for concentric. | | subjects performed a Wingate test on a cycle ergometer. It consisted of cycling at maximum speed for 30s against a load of 7.5% of their respective body weight |

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| **Outcome Measures** | CK and CRP levels from blood samples, histology analysis (necrosis %, edema %, inflammation % and cell count), swimming performance (min) | Blood samples collected immediately after exercise for blood lactate measurement. Blood samples collected at 24 h for creatine kinase and hematological analysis. Histological analysis of soleus muscles to determine damaged muscle fibers, inflammatory cell infiltrate, and edema. | Maximal voluntary contractions were measured using the isokinetic dynamometer (Biodex System 4 Pro, Biodex Medical Systems, USA), DOMS soreness measured through the 100-mm visual analog scale (VAS), Blood samples were collected at 5min, 60min, 24h, 48h, and 72h to measure oxidative damage to protiens (carbonylated proteins nanomole of DNPH/gram/deciliter of proteins), CK levels, and oxidative damage to lipids (TBARS nmol/ml) | Blood samples were taken at 1min, 1h, 24h, 48h, 72h, and 96h after eccentric protocol to evaluate CK activity. A visual analog scale (VAS) of 100-mm was used to assess DOMS intensity. Maximal voluntary contractions were assessed utilizing the isokinetic dynamometer (System 4, Biodex®, USA). | Blood samples were collected 3min and 20min after exercise for blood lactate, CK, and CRP analysis. Peak Power and Mean Power were assessed with the Wingate Cycle Test. |
| **Main Findings** | 24 hours after exercise there was an increase of total leukocytes in PR and Cryo groups.<br>CK levels were only increased | LED group showed fewer areas of muscle damage and inflammatory cell infiltration than E and CWI groups. LED group also presented with lower levels of CK | Significant increases in MVC capacity and decrease in DOMS in PBMT, CPG, and PCG groups compared to PG and CG groups ($p<0.05$), | PBMT significantly increased MVC compared to placebo from 24h to 96h ($p<0.05$). PBMT+cryotherapy had similar outcomes to PBMT alone. However, | No significant differences in peak power or mean power among groups in the Wingate cycle test.<br>CK activity increased after each test but |

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| | significantly in the Cryo group.<br><br>CRP was more pronounced in the PR group.<br><br>PR group had increased areas with cell necrosis compared to control, the LED group had significantly less than the PR group.<br><br>PR and Cryo groups presented more areas of edema than control, the LED group did not show any signs of edema.<br><br>The Control group had the lowest frequency of fields of inflammatory cells followed by LED, PR, and Cryo groups, respectfully, with significant | activity than the E group. CWI and LED did not reduce edema areas. No significant effect on leukocyte counts in either treatment group. | no significant differences between CG and PG.<br><br>Significant decrease in TBARS concentration in PBMT, CPG, PCG groups compared to PG ($p<0.01$), CG had significant decreases at 1h ($p<0.01$), 48h ($p<0.05$), and 72h ($p<0.01$).<br><br>Significant decrease in PC concentrations in PBMT, CG, and PCG compared to PG ($p<0.01$); CPG had significant decreases in 24-72h ($p<0.01$).<br><br>Significant decrease in CK levels in PBMT compared to PG ($p<0.01$); the PCG and CPG presented significant decreases in 48h($p<0.05$) and 72h ($p<0.01$). | cryotherapy+PBMT and cryotherapy alone were not different from placebo.<br><br>Significant differences occurred between PBMT and placebo for DOMS at 1h to 96h after exercise; PBMT+cryotherapy was only significant between 1h to 48h compared to placebo ($p<0.05$).<br><br>The PBMT group did not have significant increases in CK levels compared to placebo from 24h to 96h. PBMT+cryotherapy was not as effective but still significantly better than placebo. Cryotherapy as a single treatment and crotherapy+PBMT were no different from placebo. | there were no differences between test sessions. Active LEDT decreased CK levels significantly compared to post-exercise values ($p=0.0065$). Placebo and CWIT did not significantly decrease CK levels.<br><br>Active LEDT significantly decreased blood lactate levels from post-exercise ($p=0.0044$), placebo and CWIT were not significant.<br><br>CRP levels did not significantly decrease after any treatment however, a tendency to decrease from baseline values was found for active LEDT. |

| | | | | | |
|---|---|---|---|---|---|
| | differences between each group.<br><br>The Cryo group showed the highest density of inflammatory cells per field.<br><br>There were no significant differences in CK levels between groups after 24 hours.<br><br>Performance was significantly better in LED and Cryo groups than PR. The LED group had the best performance. | | | | |
| **Level of Evidence** | 2b | 2b | 1b | 1b | 1b |
| **Validity Score (PEDRO)** | N/A (Animal Study) | N/A (Animal Study) | 7 | 9 | 8 |

| | | | | | |
|---|---|---|---|---|---|
| **Conclusion** | LED PBMT is more efficient at preventing muscle damage and inflammatory reactions than passive recovery or cryotherapy. LED and cryotherapy also improved exercise performance. | LED PBMT is more efficient than CWI in preventing muscle damage and local inflammatory reactions after exercise. This may be due to its anti-inflammatory effects and preservation of muscle fiber cell membrane integrity. | Isolated PBMT treatment is the best option to improve muscle recovery in both short term and long term. Isolated cryotherapy was unable to provide muscle recovery. Combined PBMT and cryotherapy treatments do not improve recovery effects. | PBMT as a single treatment was the best for post-exercise recovery and provided the greatest reduction in DOMS. | 5 min of LEDT was more effective than placebo to reduce levels of biochemical markers related to muscle recovery. CWIT was not significantly different from the placebo. |

**Table 2.3 Photobiomodulation Therapy Parameters**

| Article | da Costa Santos et al[7] | Camargo et al.[6] | de Marchi et al.[44] | de Pavia et al.[4] | Leal Junior et al.[42] |
|---|---|---|---|---|---|
| **Wavelength** | 940 nm (infrared) | 940 nm (infrared) | 660 nm (red), 850 nm (infrared) | 905 nm (super-pulsed infrared laser), 640 nm (red diodes), 875 nm (infrared diodes) | 660 nm (red), 850 nm (infrared) |
| **Irradiance Power Output** | 160 mW | 160 mW | 10 mW (red), 30 mW (infrared) | 1.25 mW (super-pulsed infrared laser), 15 mW (red diodes), 17.5 mW (infrared diodes) | 10 mW (red), 30 mW (infrared) |
| **Power Density** | 9.5 $mW/cm^2$ | 9.5 $W/cm^2$ | 0.05 $W/cm^2$, 0.15 $W/cm^2$ | 2.84 $mW/cm^2$ (super-pulsed infrared laser), 16.67 $mW/cm^2$ (red diodes), 19.44 $mW/cm^2$ (infrared diodes) | 0.05 $W/cm^2$ (red), 0.15 $W/cm^2$ (infrared) |
| **Treatment Time** | 240s | 435s | 30s | 300s | 300s |
| **Irradiation Area** | 1 $cm^2$ | 1 $cm^2$ | 13.8 $cm^2$ (red and infrared) | 7.64 $cm^2$ (super-pulsed infrared laser, red and infrared diodes) | 138 $cm^2$ (red and infrared) |

| | | | | | |
|---|---|---|---|---|---|
| **Number of Diodes** | Not specified | Not specified | 69 (34 red LEDs and 35 infrared LEDs) | 1 super-pulsed infrared laser, 4 red LEDs, 4 infrared LEDs | 69 (34 red diodes and 35 infrared diodes) |
| **Energy** | 4 J | 4 J | 41.7 J | 39.37 J | 417 J total (208.50 J each lower limb) |
| **Fluence Energy Density** | 4 J/cm$^2$ | 4 J/cm$^2$ | 1.5 J/cm$^2$ (red), 4.5 J/cm$^2$ (infrared) | 0.85 J/cm$^2$ (super-pulsed infrared laser), 5 J/cm$^2$ (red diodes), 5.83 J/cm$^2$ (infrared diodes) | 1.5 J/cm$^2$ (red), 4.5 J/cm$^2$ (infrared) |

# III: Methods

## Design

This study was completed as a randomized placebo-controlled clinical trial. The purpose of this study was to determine the optimal time between PBMT application and a knee extensor exercise to resist fatigue. Measured outcomes included maximum voluntary contractions (MVC), perceived muscle soreness, and repetitions to muscular fatigue during an isometric fatiguing exercise. Participants were randomized into 4 different treatment groups: (1) PBMT applied 0 h (<10 min) prior to exercise, (2) PBMT applied 5 h prior to exercise, (3) PBMT applied 24 h prior to exercise, and (4) sham PBMT at either 0 h, 5 h, or 24 h prior to exercise. Participants had 2 or 3 visits depending on group randomization. The PBMT 24 h and sham 24 h groups visited the lab 3 times. The PBMT 0 h and 5 h groups along with the sham 0 h and 5 h groups visited the lab 2 times. Visits were at least 72 hours but no more than 10 days apart. The 1$^{st}$ visit consisted of signing the consent form and recording baseline measurements. The 2$^{nd}$ visit included the PBMT or sham treatment followed by the post-treatment fatigue task depending on treatment group. Participants with 3 visits received PBMT treatment on the 2$^{nd}$ visit and returned to the lab 24 hours later for the post-treatment fatigue task on the 3$^{rd}$ visit.

## Participants

A total of 42 participants were included in this study: 0 h group (n=12), 5 h group (n=14), 24 h group (n=9), and sham group (n=7). Individuals consisted of healthy strength-trained men and women, aged 18-35 years. Strength trained was defined under ACSM guidelines[61] as individuals who resistance train at least 2 times per week for at least 2 consecutive months. We included strength trained participants to make the results

clinically relevant for athletes. Participants were recruited from Texas State University in San Marcos, Texas. Exclusion criteria included current or previous lower extremity injury that occurred within the last 3 months, previous lower extremity surgery, any tattoos in the areas of irradiation, and the use of nutritional supplements or pharmacological agents that are related to pain or muscle development (i.e. creatine, ibuprofen) on test and treatment days during the study. Participants were also not permitted to consume caffeine on test days during the study. Each participant was also screened for contraindications for photobiomodulation which include: cancer at the treatment site, immune-suppressed participants, photosensitive participants, and participants who recently underwent steroid or Botox treatments.[62] Each participant that met the inclusion/exclusion criteria was required to provide informed consent prior to participation in the study. The study procedures were approved by the Institutional Review Board (IRB) at Texas State University before enrollment of participants.

Participants were randomly assigned into 1 of the 4 treatment groups. Randomization was done with a simple drawing of numbers labeled 1-4 that corresponded to a specific treatment group.

**Treatment Intervention**

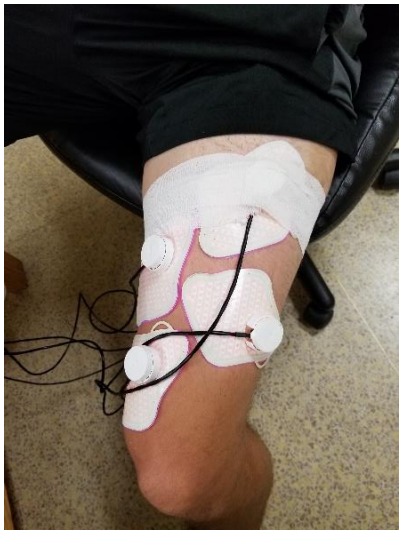
Photobiomodulation therapy was applied using LED light patches. The light patches provide pulsed light in the blue (450 nm) and red (630 nm) spectrum. Participants received a PBMT or sham treatment either 0 h, 5 h, or 24 h before the isometric muscle fatigue protocol, depending on randomization. Parameters of the light device are outlined in Table 3.1. Four light patches were placed over the quadriceps muscle group, as shown in Figures 3.1 and 3.2, for pre-exercise PBMT treatment. The sham treatment consisted

of the light patches being placed over the quadriceps, but the power was not turned on. It was explained to the participant that the treatment uses an infrared wavelength, thus they were unable to see the visible light. The participants were not told if they were in the sham treatment group.

**Table 3.1 PBMT Parameters for Study**

| | |
|---|---|
| Wavelength | Blue = 450 nm<br>Red = 630 nm |
| Dosage | 5.4 J/cm$^2$ |
| Average Power | 3 mW/cm$^2$ |
| Peak Power | 9 mW/cm$^2$ |
| Treatment Time | 1800s (30 min) |
| Application Mode | Pulsed - Duty cycle 33% |

**Figure 3.1 and 3.2.** Positioning of the 4 light patches placed on the quadriceps muscles



## Examination Procedures

Isometric Strength and Fatigue Task

The isometric strength test and fatigue task was performed using the Biodex Systems 4 Pro™ isokinetic dynamometer (Biodex System 4 Pro™, Biodex Medical Systems, Shirley, NY). Maximal voluntary contractions (MVC) were measured to determine muscular strength. The participant performed three 5 s MVCs with a rest of 5 s between each contraction. The participant was positioned at 60° of knee flexion of their dominant leg and at 100° of flexion at the hip to obtain maximal torque value. The Biodex positioning was consistent with previous studies.[10,11,13,24,32] Isometric strength measurements took place before and after the fatigue task on both the baseline and post-treatment visits. The change in MVC torque was measured (change between the pre-fatigue and post-fatigue task) and were compared between groups at baseline and post-treatment visits.

For the fatigue task, participants performed intermittent sub-maximal isometric contractions at 50% MVC of the knee extensor group to induce fatigue. The isometric contractions were performed for 20 s followed by a rest of 2 s and were repeated until their muscular force dropped below 45% of their MVC for ≥ 2 s. A monitor displaying the participant's torque data was placed in front of them with a target line at 50% MVC. This helped the participant visualize how much force was required during the exercise. The mean of the 3 pre-fatigue task MVC measurements from the baseline visit was used for the fatigue task target line during both visits. The percent change of the number of repetitions and the change of the time to exhaustion between pre-fatigue to post-fatigue

task were compared between groups. Verbal encouragement was given during MVC tests and the fatigue task to encourage full effort at all times.

Acute Muscle Soreness

To evaluate acute muscle soreness at both pre-fatigue and post-fatigue task, we used a modified visual analog scale (mVAS). The mVAS consisted of a 100 mm empty line with the words “no soreness” on the far left side and “extreme soreness” on the far right side. The line was presented horizontally to the participant and they were asked to mark their soreness intensity on the line. The researcher then measured the distance between the beginning of the line to the participant’s score, to quantify the soreness intensity. The change in score from pre-fatigue to post-fatigue task were compared between groups

## Procedures for Collecting Data

*Visit 1: Baseline Visit*

The first session was the consent and baseline measurement visit. Each participant was given time to read the informed consent form and ask questions about the study. After providing consent, the participant’s demographic data, such as sex, height, weight, and BMI, were recorded. The participant then randomly drew a number from 1-4, indicating their treatment group allocation. Following treatment assignment, the participant was given the opportunity to become familiarized with the Biodex. After being properly positioned on the Biodex, participants performed 3 short contractions of 5 s as they would during the MVC measurement. Once demonstrated correctly, the participant had a rest period of 15 min before starting the first MVC measurement.

The MVC test consisted of three 5 s isometric contractions at 60° of knee flexion to obtain maximum torque value. A 5 s rest period was provided between each contraction. Following the MVC measurement there was another 15-minute rest period prior to starting the fatigue test. The fatigue test involved repeated isometric contractions of 20s with a rest period of 2 s in between repetitions. This sequence was repeated until the participant's muscular force dropped below 45% of MVC for ≥ 2 consecutive seconds. The mean of the 3 pre-fatigue task MVC measurements from the baseline visit was used for the fatigue task target line during both visits. Immediately (< 2 min) after the fatigue test, a MVC was performed to measure the reduction in maximal torque and this served as a measure of muscle fatigue. Verbal encouragement was given the entire time during MVC and fatigue tests to encourage 100% effort throughout the protocol.

Following the fatigue protocol, the participant was scheduled for the next visit based on group placement. The next session was no sooner than 72 hours and no longer than 10 days from the first session. During this period, participants were told not to change in normal resistance training routine, though were asked to rest 24 hours prior to the PBMT treatment visit.

*Visit 2 – 3: PBMT and Post-Treatment Visit*

Participants had 2 or 3 visits depending on group randomization. The PBMT 24 h and sham 24 h groups visited the lab 3 times. The PBMT 0 h and 5 h groups along with the sham 0 h and 5 h groups visited the lab 2 times.

On the second session, all participants received the PBMT or sham treatment on the quadriceps muscle group in their dominant leg. Health history was revisited prior to

the treatment to ensure the participant was not disqualified from PBMT. For treatment, the participant sat in the treatment chair and had the light patches placed on the skin over the muscle belly as shown in Figure 3.1 and 3.2. The skin was cleaned with an alcohol wipe prior to light patch placement. The PBMT treatment was 30 min in duration and set with the parameters outlined in Table 3.1.

Participants in the 0 h group had the light patches removed following PBMT or sham treatment and were then correctly positioned on the Biodex as described above to begin pre-fatigue task MVCs for the post-treatment visit. Following the last MVC contraction there was a rest period of 15 min prior to starting the post-treatment visit fatigue task. The fatigue task was the same as it was during the baseline visit. The mVAS scale was given to the participant following the last MVC measurement to indicate the level of acute muscle soreness the participant felt.

The participants in the 5 h group were instructed to return back to the lab 4.5 h following the PBMT treatment. The fatigue task began exactly 5 h following the PBMT or sham treatment. Pre-fatigue task MVCs were measured as soon as the participant arrived at the lab to ensure a proper fatigue task start time. The fatigue task was administered the same as it was during the baseline visit. The 24 group was instructed to return back to the lab 23.5 h following the PBMT or sham treatment. The fatigue test began exactly 24 h following PBMT or sham treatment.

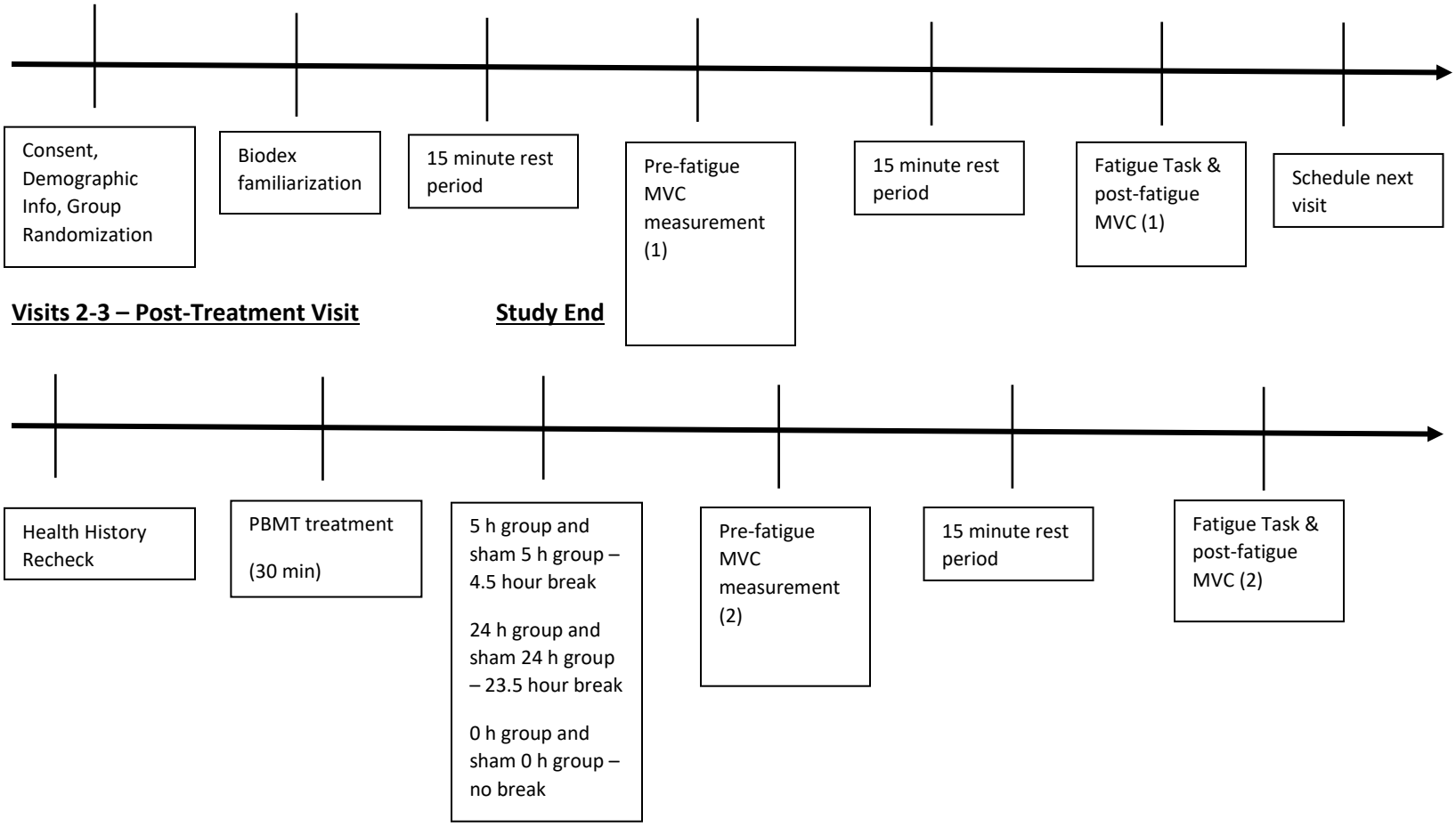
The participants in the 5 h, 24 h, and sham groups were given guidelines for the time between PBMT treatment and the fatigue task. Participants were asked to refrain from alcohol or recreational drug use, caffeine, exercising, walking long distances, and nutritional supplement or NSAIDs use.

## Data Analysis

Results for the fatigue task was calculated by the percent change in repetitions and the change in duration to muscle exhaustion. The percent change in isometric MVC from pre-fatigue to post-fatigue task was calculated for each participant for both the baseline and post-treatment visits. The MVC with highest torque from pre-fatigue and post-fatigue task were used. Acute muscle soreness was reported as the change in mVAS score from pre-fatigue to post-fatigue task. Data was reported for all measures as the Mean ± SD.

An analysis of variance (ANOVA) was used in all outcome measures to determine significance between groups. An *a priori* alpha level of 0.05 was utilized in the chosen statistical tests. If it was determined that there were statistical differences with $p \leq 0.05$, then a Tukey's post hoc test was utilized to determine statistical significance of the interaction effects between treatment groups. Statistical analysis was done using the JMP PRO 13 (SAS Inc., Cary, NC) software.

**Figure 3.3.** Timeline of Study Methods



## IV: Results

Volunteers in this study included both males (n=24) and females (n=18). The average age for males was 21.4 ± 2.38 y and average age for females was 20.7 ± 2 y. The average height for males was 177.57 ± 5.31 cm and for females was 164.18 ± 8.32 cm. The average weight for males was 85.11 ± 14.31 kg and for females was 62.21 ± 11.51 kg. The average BMI for males was 27.0 ± 4.72 and for females was 23.1 ± 4.08. The participants were randomized into four groups: 0 h (n=12), 5 h (n=14), 24 h (n=9), and sham (n=7).

### Fatigue Task

For the fatigue task, data representing the percent change of the number of repetitions completed between the baseline visit and treatment visit is organized in Table 4.1. Data representing the percent change of the duration of the fatigue exercise between baseline and post-treatment visits is organized in Table 4.2.

There were no differences between treatments in the percent change of repetitions (($F_{3,39}$ = 0.1687), P = 0.9169) or the change in duration (($F_{3,39}$ = 0.2171), P=0.8840) of the fatigue task between the baseline and treatment visits. Visually however, there was a small 3 – 5% increase in reps and a 3 – 12 second increase in duration in the 5 h group compared to the others, which shows a small but potential clinical benefit.

**Table 4.1 Percent Change of Fatigue Task Repetitions (Baseline Visit to Post-Treatment Visit)**

| Treatment Group | N | Mean %Δ | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|
| 0 h Before | 12 | 6.04 | 17.36 | 5.01 | -4.99 | 17.07 |
| 5 h Before | 14 | 11.80 | 24.19 | 6.25 | -1.60 | 25.20 |
| 24 h Before | 9 | 8.58 | 20.33 | 6.78 | -7.04 | 24.20 |
| Sham | 7 | 9.52 | 20.72 | 7.83 | -9.63 | 28.68 |

**Table 4.2 Change of the Duration (s) of the Fatigue Task (Baseline Visit to Post-Treatment Visit)**

| Treatment Group | N | Mean (s) | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|
| 0 h Before | 12 | 9.08 | 32.52 | 9.39 | -15.62 | 33.778 |
| 5 h Before | 14 | 21.27 | 46.87 | 12.11 | -0.82 | 43.363 |
| 24 h Before | 9 | 11.74 | 47.51 | 15.84 | -16.78 | 40.255 |
| Sham | 7 | 18.01 | 39.37 | 14.88 | -14.32 | 50.350 |

**Maximal Voluntary Contraction**

The results describing the percent change of peak quadriceps torque from pre-fatigue to post-fatigue task during the baseline visit are presented in Table 4.3, Figure 4.1. The results from the treatment visit are presented in Table 4.4 and Figure 4.2. The exercise was fatiguing enough to cause an average 18.56 ± 15.92% (Range: 14 – 29%) decline in MVC from pre-fatigue to post-fatigue task during the baseline visit without any treatment. On the treatment visit, an average 11.91 ± 13.79% (Range: 8 – 15%) decline in MVC was observed from pre-fatigue to post-fatigue task.

During the treatment visit, there were no significant differences between treatment groups ($F_{3,39}$ = 1.4295, P = 0.2488). However, there was a small trend that the 0 and 5 h treatments have better benefits for maintaining strength capacity. Both groups had similar percent decrease in peak quadriceps torque, roughly 6% less decline than the sham group and 10% less than the 24 h group. The 24 h group had the highest percent decline in torque after the treatment from pre-fatigue to post-fatigue task MVC torque values.

**Table 4.3 Percent Change in MVC After Fatigue Task (Baseline Visit)**

| Treatment Group | N | Mean % Δ | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|
| 0 h Before | 12 | -19.52 | 21.27 | 6.14 | -33.03 | -6.01 |
| 5 h Before | 14 | -14.21 | 10.89 | 2.91 | -20.50 | -7.92 |
| 24 h Before | 10 | -16.26 | 14.27 | 4.51 | -26.47 | -6.06 |
| Sham | 7 | -28.89 | 14.29 | 5.40 | -42.11 | -15.68 |

**Figure 4.1 Percent Change in MVC After Fatigue Task (Baseline Visit)**

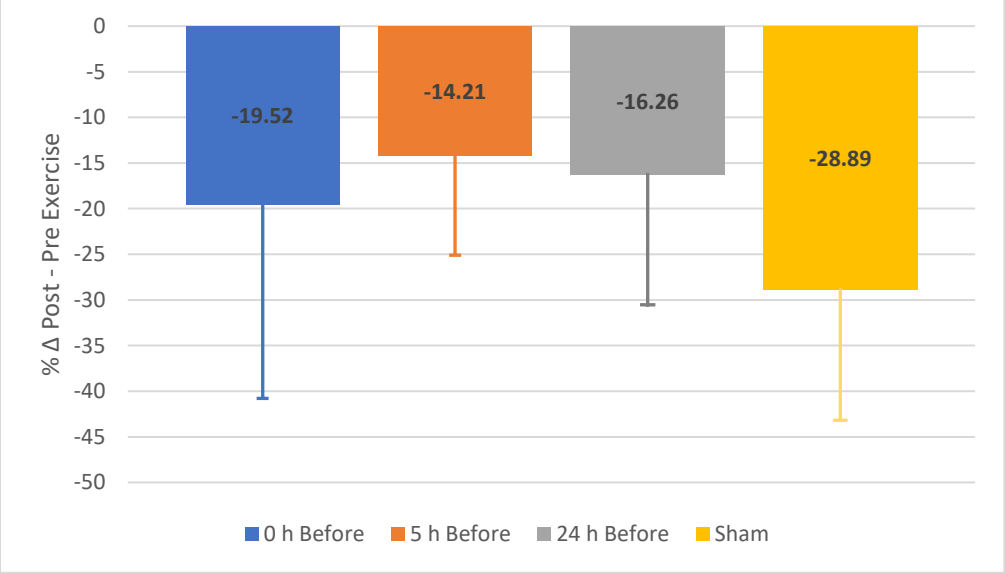



*Figure 4.1. The graph and error bars are presented as the means ± SD of percent change from pre to post fatigue exercise of isometric peak torque*

**Table 4.4 Percent Change in MVC After Fatigue Task (Post-Treatment Visit)**

| Treatment Group | N | Mean % Δ | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|
| 0 h Before | 12 | -8.21 | 15.46 | 4.46 | -18.03 | 1.61 |
| 5 h Before | 14 | -8.94 | 11.75 | 3.14 | -15.72 | -2.151 |
| 24 h Before | 10 | -18.64 | 9.81 | 3.104 | -25.66 | -11.621 |
| Sham | 7 | -14.57 | 17.82 | 6.74 | -31.05 | 1.911 |

**Figure 4.2 Percent Change in MVC After Fatigue Task (Post-Treatment Visit)**

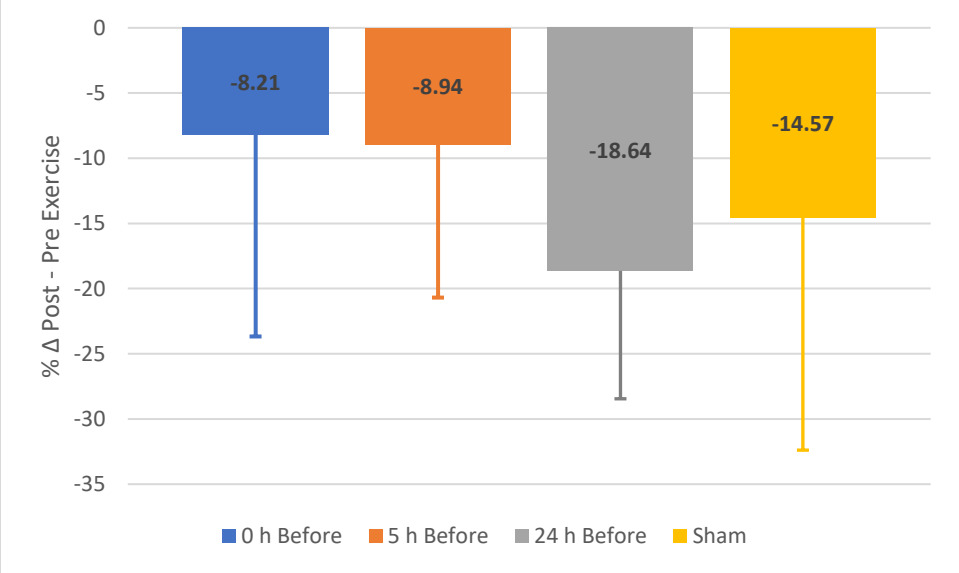



*Figure 4.2 The graph and error bars are presented as the mean ± SD of percent change from pre to post fatigue exercise of isometric peak torque*

## Acute Muscle Soreness

Subjective perceptions of acute muscle soreness were measured using a mVAS (100 mm line). Data representing the difference between pre-fatigue and post-fatigue task acute muscle soreness on the treatment visit is organized in Table 4.5 and Figure 4.3. Prior to the exercise, the average reported acute muscle soreness mVAS score was 9.35 mm ± 12.35 mm (Range: 4.30 –12.0 mm). The average increase in mVAS score from pre-fatigue to post-fatigue task was 56.58 mm ± 24.81 mm (Range: 46.30 – 61.86 mm).

This confirms that we created acute muscle soreness in the participants' quadriceps with the fatiguing exercise. With this subjective measurement we can assume that acute soreness is attributed to the muscle fatigue.

There were no differences between treatment groups in the amount of muscle soreness reported by the participants after the exercise on the post-treatment visit ($F_{3,39}$ = 1.4307), P=0.2485).

**Table 4.5 Change in mVAS Score on Post-Treatment Visit (pre-fatigue to post-fatigue task)**

| Treatment Group | N | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|---|---|---|---|---|---|---|
| 0 h Before | 12 | 60.58 | 28.83 | 8.32 | 42.27 | 78.90 |
| 5 h Before | 14 | 54.47 | 18.14 | 4.68 | 44.42 | 64.51 |
| 24 h Before | 10 | 46.30 | 28.10 | 8.89 | 26.20 | 66.40 |
| Sham | 7 | 61.86 | 31.49 | 11.90 | 32.73 | 90.98 |

**Figure 4.3 Change in mVAS Score on Post-Treatment Visit (pre-fatigue to post-fatigue task)**

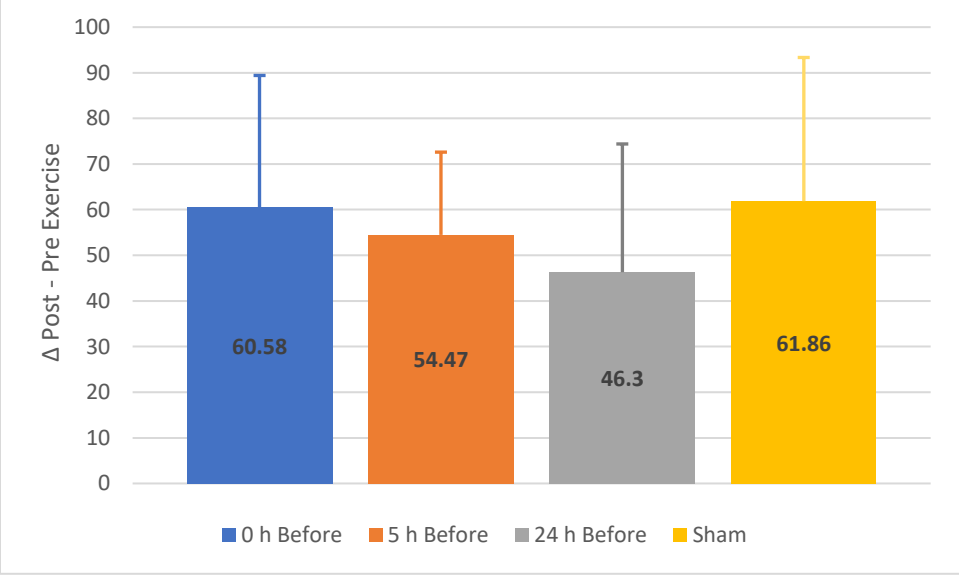



*Figure 4.3 The graph and error bars are presented as the means ± SD for change pre to post in mVAS score*

## V: Discussion

The purpose of this study was to determine the optimal time between PBMT application and a knee extensor exercise to resist fatigue. None of the PBMT groups were able to reduce fatigue in any outcome measure. Although the results were not significant, the PBMT 0 h and 5 h treatment groups showed roughly a 5% less decrease in peak quad torque compared to the sham treatment. The 5 h PBMT group also had slightly better muscle endurance during the fatigue test, averaging 4% more repetitions and lasting 12 s longer than the 0 h treatment.

Photobiomodulation therapy has been shown to delay the onset of skeletal muscle fatigue in humans using a pre-exercise treatment.[7,16,17,24] However, in many of these clinical studies, the researcher will irradiate the muscle immediately before the exercise. While producing positive results, it may not be the best time for treatment. During a previous animal cell culture study, Albuquerque-Pontes et al.[25] found that PBMT increases Cox activity up to 24 h post irradiation. The increase in Cox activity is thought to be one of the benefits PBMT has to prevent fatigue, because when stimulated, higher levels of cell respiration and ATP synthesis occurs along the electron transport chain.[14] Following the previous animal cell culture study, two additional animal studies were performed to measure the time response PBMT may have on skeletal muscle. In an *in vitro* animal study, Ferraresi et al.[33] found that mitochondrial membrane potential was greatest at 6 hours post-irradiation, followed by 3 hours, then 24 hours. All of which were significantly better than the immediate group. Additionally, the increase in mitochondrial membrane potential correlated with an increase of ATP content found in the skeletal muscle cells. Upon their discovery, the authors then performed an *in vivo* animal study with mice and found similar results. Mice that received PBMT treatment 3-6 hours prior

to a ladder climbing endurance exercise were able to perform the most repetitions, with the 6 h group performing the best.[34] As with the previous study, there was a high correlation between performance during the exercise and the amount of ATP found in the muscles irradiated.

Using evidence from the cell culture and animal studies,[25,33,34] we hypothesized that receiving PBMT treatment 5 h before an exercise would be the optimal time in the present study. We chose to include a 5 h group based on the 3 – 6 hour best treatment window discussed in the literature.[33,34] The 24 h group was included because the mitochondria has been shown to have increased ATP production up to 24 hours post-irradiation.[25] Although not significant, our results are consistent with previous research in that PBMT immediately before and 5 hours before a fatiguing exercise was able to promote an enhancement of muscular endurance.

Following PBMT treatment, all groups showed an improvement in the fatigue task compared to the baseline visit, yet the data was not statistically significant. A learning effect may be an explanation for the participants' improvement in the exercise, though we tried to eliminate such an effect through a familiarization period. The PBMT treatment had no significant effects to improve fatigue task performance between groups. There are plenty of studies that show that PBMT has an ergogenic and protective effect on skeletal muscle to prevent fatigue.[7,9,11-14,16,17,24,32,63-66] This is due to boosted intramuscular microcirculation,[24,67] the lower accumulation of blood lactate and creatine kinase,[16,17,42,68] increases in ventilation and $VO_2$ kinetics,[63,64,69] and the improvement in mitochondrial function.[2,8,14,70] However, the present study is also the first to incorporate an isometric knee extensor exercise. In previous PBMT studies, the fatiguing exercises

have been isokinetic or isotonic, using eccentric or concentric movements. During our study, the 5 h group showed slightly more improvement in the fatigue task compared to the other treatment groups, which could be indicative of a PBMT benefit consistent with our hypothesis. Previous animal studies have shown an advantage to using PBMT 3-6 hours prior to a fatiguing exercise.[33,34] Using such information we expected to see greater improvements with the 5 h group compared to the other treatment times. In the present study we involved human participants rather, so it is expected to have variations in results compared to previous PBMT time response papers. Nonetheless, this must still be further explored due to our unequal groups, small sample size, and exercise type.

In all treatment groups, the participants' peak quad torque declined from pre-fatigue to post-fatigue task on both the baseline and treatment visits. The 0 h before and 5 h before treatment groups were roughly 5% better at preventing a decrease in quadriceps peak torque compared to the sham group. Consistent with our trending results, PBMT has been shown to promote a smaller reduction in strength capacity following strenuous exercise.[11,13,24] In a study comparing 3 different PBMT devices, De Marchi et al.[13] found that both low powered pulsed and continuous cycle PBMT devices were able to maintain MVC compared to placebo treatments after a fatiguing eccentric knee extensor exercise. Baroni et al.[11] found in their 8-week knee extensor training program that PBMT treated groups had smaller decreases in strength following each resistance training session, which overall led to faster gains in muscular strength and hypertrophy compared to non-treated groups. It is hypothesized that the smaller decrease in strength capacity after each session allows for greater muscle work for each subsequent session which helps improve performance over time.

In the present study it was surprising that the 24 h group had the largest torque decrease, even compared to the sham group. The 24 h group declined roughly 10% more than the 0 h and 5 h groups, and about 4% more than the sham group. Knowing that MMP and ATP content are still increased up to 24 hours post-irradiation, we expected the 24 h group to have a smaller decline than the sham group. However, that was not the case. Albuquerque-Pontes et al.[25] noted that the MMP and ATP increases at 24 hours seem to be dose and wavelength dependent, which could be the cause of our inconsistent results. In the present study we used a combination of blue and red light for PBMT treatment which might not have been able to penetrate deep enough to the muscle or may be providing its photobiomodulation effects through different mechanisms with the addition of pulsed blue light irradiation. We used a novel light patch device with an ability to only change the power and duty cycle settings. In most PBMT studies, only red and infrared wavelengths are typically used.[7,30,65] Future studies may find significant results utilizing different parameters and incorporating infrared light for its ability to penetrate deeper to muscle tissue. Additionally, researchers should continue to investigate the potential effect of combining blue light with red and infrared light when treating skeletal muscle to reduce fatigue. To our knowledge, the present study is the first to measure peak torque decline after an exercise, 24 h post-treatment. Additional studies must continue to investigate the effects of PBMT 24 hours post-irradiation with different parameters and device types.

The participants' perception of fatigue, indicated by rating the level of soreness and exhaustion of the knee extensor group on the mVAS, were not affected by PBMT treatment. Though not significant, the 5 h and 24 h groups tended to perceive less

soreness than the 0 h and sham groups. The VAS is one of the most widely used scales to determine soreness perception in clinical studies. However, in various PBMT studies there have been conflicting results in the treatments' ability to reduce the perception of muscle soreness on the VAS. In some cases, the effect PBMT has on VAS appears to be dose dependent.[9,13,24] While not universally affecting subjective measures, PBMT has been shown to have a prophylactic effect on skeletal muscle damage that leads to muscle soreness. Muscle damage biomarkers such as blood lactate, CK, and inflammatory cytokines can be decreased when a pre-exercise PBMT treatment is applied.[9,13,15,17,24] In these studies however, the researchers irradiated the muscles immediately before the exercise. One recent study investigated the effect PBMT may have on CK activity on professional volleyball players before their matches.[38] The authors found that PBMT treatment 40-60 minutes before a volleyball match significantly decreased CK levels. This study serves as a precursor to the time response PBMT may have to prevent muscle damage and fatigue outside of a laboratory setting. Although we did not measure muscle damage biomarkers, the 5 h and 24 h PBMT groups were trending positive effects against acute muscle soreness. More investigation is necessary to show a potential effect of the time-response PBMT may have on the perception of muscle soreness and damage resulting from strenuous exercise.

The present study aimed to find a time response benefit of using blue and red PBMT with wearable light patches at time periods other than immediately before an exercise. While not statistically significant, our results may still prove clinically beneficial for practicing sports medicine professionals. Improvements were found in the 5 h treatment group, giving an early indication that PBMT can be beneficial when there is

more time between treatment and exercise. Recently, Rossato et al.[39] found that applying PBMT treatment at time points of both 6 hours and immediately before an exercise may also benefit athletes. While adding evidence that pre-exercise PBMT treatment may be effective beyond just immediately before an exercise, the authors note that it may be more beneficial to have both an earlier and immediate pre-conditioning session. More *in vivo* PBMT time response studies with human participants must be investigated.

There are limitations in the present study that should be considered when interpreting the results. First, it should be noted that we had limited participants resulting in a small sample size. We still expect our hypothesis to be true when the sample is increased, based on data showing the 5 h group with small improvements in muscle endurance and strength capacity maintenance. The exercise protocol successfully induced fatigue in the knee extensor muscle group, however the treatment was not able to improve exercise performance. This may be due to the exercise being more anaerobic than aerobic and difficulty of the exercise. Future studies should investigate PBMT time response on both aerobic and anaerobic type exercises. In the present study, the treatment had no effect on soreness perception scores. However, we only measured immediately before and after the exercise, only indicating acute soreness. It would be interesting to see how delayed onset muscle soreness developed between groups at different time points through to 72 hours post-exercise.

This study provides a basis for new research to determine best use parameters for PBMT. There are many questions to be answered regarding dosage, wavelengths, and time. With continual investigation into these, PBMT can become a valuable treatment option for all athletes.

## Literature Cited


1. Anders JJ, Lanzafame RJ, Arany PR. Low-level light/laser therapy versus photobiomodulation therapy. *Photomed Laser Surg.* 2015; 33(4): 183-184.
2. Chung H, Dai T, Sharma SK, Huang Y-Y, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. *Ann Biomed Eng.* 2012; 40(2): 516-533.
3. Tomazoni S, Leal-Junior E, Pallotta R, et al. Effects of photobiomodulation therapy, pharmacological therapy, and physical exercise as single and/or combined treatment on the inflammatory response induced by experimental osteoarthritis. *Lasers Med Sci.* 2017; 32(1): 101-108.
4. Yadav A, Gupta A. Noninvasive red and near-infrared wavelength-induced photobiomodulation: promoting impaired cutaneous wound healing. *Photodermatol Photoimmunol Photomed.* 2017; 33(1): 4-13.
5. Hashmi JT, Huang Y-Y, Osmani BZ, Sharma SK, Naeser MA, Hamblin MR. Role of low-level laser therapy in neurorehabilitation. *PM&R.* 2010; 2(12): S292-S305.
6. Chow RT, Armati PJ. Photobiomodulation: Implications for anesthesia and pain relief. *Photomed Laser Surg.* 2016; 34(12): 599-609.
7. Leal-Junior ECP, Vanin AA, Miranda EF, de Carvalho PdTC, Dal Corso S, Bjordal JM. Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. *Lasers Med Sci.* 2015; 30(2): 925-939.
8. de Freitas LF, Hamblin MR. Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE J Sel Top Quantum Electron.* 2016; 22(3): 348-364.
9. Aver Vanin A, De Marchi T, Silva Tomazoni S, et al. Pre-exercise infrared low-level laser therapy (810 nm) in skeletal muscle performance and postexercise recovery in humans, what is the optimal dose? A randomized, double-blind, placebo-controlled clinical trial. *Photomed Laser Surg.* 2016; 34(10): 473-482.
10. Baroni BM, Leal Junior ECP, Geremia JM, Diefenthaeler F, Vaz MA. Effect of light-emitting diodes therapy (LEDT) on knee extensor muscle fatigue. *Photomed Laser Surg.* 2010; 28(5): 653-658.
11. Baroni BM, Rodrigues R, Freire BB, Franke RDA, Geremia JM, Vaz MA. Effect of low-level laser therapy on muscle adaptation to knee extensor eccentric training. *Eur J Appl Physiol.* 2015; 115(3): 639-647.
12. Borsa PA, Larkin KA, True JM. Does phototherapy enhance skeletal muscle contractile function and postexercise recovery? a systematic review. *J Athl Train.* 2013; 48(1): 57-67.
13. De Marchi T, Schmitt VM, Danubia da Silva Fabro C, et al. Phototherapy for improvement of performance and exercise recovery: comparison of 3 commercially available devices. *J Athl Train.* 2017; 52(5): 429-438.
14. Ferraresi C, Hamblin MR, Parizotto NA. Low-level laser (light) therapy (LLLT) on muscle tissue: performance, fatigue and repair benefited by the power of light. *Photonics Lasers Med.* 2012; 1(4): 267-286.
15. Leal Junior EC, Lopes-Martins RA, Frigo L, et al. Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue

and changes in biochemical markers related to postexercise recovery. *J Orthop Sports Phys Ther.* 2010; 40(8): 524-532.
16. Leal Junior ECP, Brandão Lopes-Martins RÁ, Vanin AA, et al. Effect of 830 nm low-level laser therapy in exercise-induced skeletal muscle fatigue in humans. *Lasers Med Sci.* 2009; 24(3): 425-431.
17. Leal Junior ECP, Lopes-Martins RAB, Dalan F, et al. Effect of 655-nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans. *Photomed Laser Surg.* 2008; 26(5): 419-424.
18. Edwards RH. Human muscle function and fatigue. *Ciba Found Symp.* 1981; 82:1-18.
19. Grassi B, Rossiter HB, Zoladz JA. Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? *Exerc Sport Sci Rev.* 2015; 43(2): 75-83.
20. Appell H-J, Soares JMC, Duarte JAR. Exercise, muscle damage and fatigue. *Sports Med.* 1992; 13(2): 108-115.
21. Hawkins RD, Fuller CW. A prospective epidemiological study of injuries in four English professional football clubs. *Br J Sports Med.* 1999; 33(3): 196-203.
22. Ballantyne BT, Shields RK. Quadriceps fatigue alters human muscle performance during a novel weight bearing task. *Med Sci Sports Exerc.* 2010; 42(9): 1712-1722.
23. Nyland JA, Shapiro R, Stine RL, Horn TS, Ireland ML. Relationship of fatigued run and rapid stop to ground reaction forces, lower extremity kinematics, and muscle activation. *J Orthop Sports Phys Ther.* 1994;20(3):132-137.
24. Baroni BM, Leal Junior ECP, De Marchi T, Lopes AL, Salvador M, Vaz MA. Low level laser therapy before eccentric exercise reduces muscle damage markers in humans. *Eur J Appl Physiol.* 2010; 110(4): 789-796.
25. Albuquerque-Pontes GM, Vieira RdP, Tomazoni SS, et al. Effect of pre-irradiation with different doses, wavelengths, and application intervals of low-level laser therapy on cytochrome c oxidase activity in intact skeletal muscle of rats. *Lasers Med Sci.* 2015; 30(1): 59-66.
26. Alves AN, Fernandes KPS, Deana AM, Bussadori SK, Mesquita-Ferrari RA. Effects of low-level laser therapy on skeletal muscle repair: a systematic review. *Am J Phys Med Rehabil.* 2014; 93(12): 1073-1085.
27. Ferraresi C, Huang YY, Hamblin MR. Photobiomodulation in human muscle tissue: an advantage in sports performance? *J Biophotonics.* 2016; 9(11-12): 1273-1299.
28. Huang Y-Y, Chen ACH, Carroll JD, Hamblin MR. Biphasic dose response in low level light therapy. *Dose-Response.* 2009;7(4):358-383.
29. Barolet D. Light-emitting diodes (LEDs) in dermatology. *Semin Cutan Med Surg.* 2008; 27(4): 227-238.
30. Vanin AA, Verhagen E, Barboza SD, Costa LOP, Leal-Junior ECP. Photobiomodulation therapy for the improvement of muscular performance and reduction of muscular fatigue associated with exercise in healthy people: a systematic review and meta-analysis. *Lasers Med Sci.* 2018; 33(1): 181-214.
31. Alam MF, ul Hassan SK, Ashraf S. Efficacy of blue light in mild and moderate facial acne vulgaris. *JPAD.* 2017;27(4):320-323.

32. Vanin AA, Miranda EF, Machado CSM, et al. What is the best moment to apply phototherapy when associated to a strength training program? A randomized, double-blinded, placebo-controlled trial. *Lasers in Medical Science.* 2016;31(8):1555-1564.
33. Ferraresi C, Parizotto NA, Bagnato VS, et al. Low-level laser (light) therapy increases mitochondrial membrane potential and ATP synthesis in C2C12 myotubes with a peak response at 3-6 h. *Photochem Photobiol.* 2015; 91(2): 411-416.
34. Ferraresi C, de Sousa MVP, Huang Y-Y, Bagnato VS, Parizotto NA, Hamblin MR. Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice. *Lasers Med Sci.* 2015; 30(4): 1259-1267.
35. Pescatello LS, American College of Sports M. *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014.
36. da Costa Santos VB, de Paula Ramos S, Milanez VF, et al. LED therapy or cryotherapy between exercise intervals in wistar rats: anti-inflammatory and ergogenic effects. *Lasers Med Sci.* 2014; 29(2): 599-605.
37. Swanik CB, Lephart SM, Giannantonio FP, Fu FH. Reestablishing proprioception and neuromuscular control in the ACL-injured athlete. *J Sport Rehab.* 1997; 6(2): 182-206.
38. Ferraresi C, Parizotto NA, Bagnato VS, et al. Light-emitting diode therapy (LEDT) before matches prevents increase in creatine kinase with a light dose response in volleyball players. *Lasers Med Sci.* 2015; 30(4): 1281-1287.
39. Rossato M, Dellagrana RA, Sakugawa RL, Lazzari CD, Baroni BM, Diefenthaeler F. Time response of photobiomodulation therapy on muscular fatigue in humans. *J Strength Cond Res.* 2018.
40. Hohenauer E, Taeymans J, Baeyens J-P, Clarys P, Clijsen R. The effect of post-exercise cryotherapy on recovery characteristics: a systematic review and meta-analysis. *Plos One.* 2015; 10(9): e0139028.
41. de Paiva PR, Tomazoni SS, Johnson DS, et al. Photobiomodulation therapy (PBMT) and/or cryotherapy in skeletal muscle restitution, what is better? A randomized, double-blinded, placebo-controlled clinical trial. *Lasers Med Sci.* 2016; 31(9): 1925-1933.
42. Leal Junior EC, de Godoi V, Mancalossi JL, et al. Comparison between cold water immersion therapy (CWIT) and light emitting diode therapy (LEDT) in short-term skeletal muscle recovery after high-intensity exercise in athletes--preliminary results. *Lasers Med Sci.* 2011; 26(4): 493-501.
43. Camargo MZ, Siqueira CPCM, Preti MCP, et al. Effects of light emitting diode (LED) therapy and cold water immersion therapy on exercise-induced muscle damage in rats. *Lasers Med Sci.* 2012; 27(5): 1051-1058.
44. De Marchi T, Schmitt VM, Machado GP, et al. Does photobiomodulation therapy is better than cryotherapy in muscle recovery after a high-intensity exercise? A randomized, double-blind, placebo-controlled clinical trial. *Lasers Med Sci.* 2017; 32(2): 429-437.

45. Leeder J, Gissane C, van Someren K, Gregson W, Howatson G. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med.* 2012; 46(4): 233-240.
46. Barnett A. Using Recovery Modalities between training sessions in elite athletes: does it help? *Sports Med.* 2006; 36(9): 781-796.
47. Finsterer J. Biomarkers of peripheral muscle fatigue during exercise. *BMC Musculoskelet Disord.* 2012; 13: 218.
48. Cheung K, Hume PA, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med.* 2003; 33(2): 145-164.
49. Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train.* 2002; 37(2): 209-217.
50. Selkow N, Herman D, Liu Z, Hertel J, Hart J, Saliba S. Blood flow after exercise-induced muscle damage. *J Athl Train.* 2015; 50(4): 400-406.
51. Meeusen R, Lievens P. The use of cryotherapy in sports injuries. *Sports Med.* 1986; 3(6): 398-414.
52. Bleakley CM, Davison GW. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med.* 2010; 44(3): 179-187.
53. Wilcock I, Cronin J, Hing W. Physiological response to water immersion: a method for sport recovery? *Sports Med.* 2006; 36(9): 747-765.
54. Crystal NJ, Townson DH, Cook SB, Laroche DP. Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *Eur J Appl Physiol.* 2013; 113(10): 2577-2586.
55. Pointon M, Duffield R, Cannon J, Marino FE. Cold application for neuromuscular recovery following intense lower-body exercise. *Eur J Appl Physiol.* 2011; 111(12): 2977-2986.
56. Silveira PCL, Silva LAd, Fraga DB, Freitas TP, Streck EL, Pinho R. Evaluation of mitochondrial respiratory chain activity in muscle healing by low-level laser therapy. *Photochem Photobiol.* 2009; 95(2): 89-92.
57. Xu X, Zhao X, Liu TC-Y, Pan H. Low-intensity laser irradiation improves the mitochondrial dysfunction of C2C12 induced by electrical stimulation. *Photomed Laser Surg.* 2008; 26(3): 197-202.
58. Avni D, Levkovitz S, Maltz L, Oron U. Protection of skeletal muscles from ischemic injury: low-level laser therapy increases antioxidant activity. *Photomed Laser Surg.* 2005; 23(3): 273-277.
59. Armstrong RB, Warren GL, Warren JA. Mechanisms of exercise-induced muscle fibre injury. *Sports Med.* 1991; 12(3): 184-207.
60. Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L, West MS. Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J Appl Physiol.* 1992; 73(5): 1797-1804.
61. Pescatello LS, American College of Sports M. *ACSM's Guidelines for Exercise Testing and Prescription.* Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014.
62. Kneebone WJ. Practical applications of low level laser therapy. 2006.
63. Ferraresi C, Parizotto NA, Bagnato VS, et al. Muscular pre-conditioning using light-emitting diode therapy (LEDT) for high-intensity exercise: a randomized

double-blind placebo-controlled trial with a single elite runner. *Physiother Theory Pract.* 2015; 31(5): 354-361.
64. Miranda EF, Vanin AA, Tomazoni SS, et al. Using pre-exercise photobiomodulation therapy combining super-pulsed lasers and light-emitting diodes to improve performance in progressive cardiopulmonary exercise tests. *J Athl Train.* 2016; 51(2): 129-135.
65. Nampo F, Cavalheri V, Santos Soares F, et al. Low-level phototherapy to improve exercise capacity and muscle performance: a systematic review and meta-analysis. *Lasers Med Sci.* 2016; 31(9): 1957-1970.
66. Vaz M, Diefenthaeler F, Leal-Junior EC, et al. Effect of light-emitting diodes therapy (LEDT) on knee extensor muscle fatigue. *Photomed Laser Surg.* 2010; 28(5): 653-658.
67. Mak MC, Cheing GL. Immediate effects of monochromatic infrared energy on microcirculation in healthy subjects. *Photomed Laser Surg.* 2012; 30(4): 193-199.
68. Dos Reis FA, da Silva BA, Laraia EM, et al. Effects of pre- or post-exercise low-level laser therapy (830 nm) on skeletal muscle fatigue and biochemical markers of recovery in humans: double-blind placebo-controlled trial. *Photomed Laser Surg.* 2014; 32(2): 106-112.
69. De Marchi T, Leal Junior EC, Bortoli C, Tomazoni SS, Lopes-Martins RA, Salvador M. Low-level laser therapy (LLLT) in human progressive-intensity running: effects on exercise performance, skeletal muscle status, and oxidative stress. *Lasers Med Sci.* 2012; 27(1): 231-236.
70. Hayworth CR, Rojas JC, Padilla E, Holmes GM, Sheridan EC, Gonzalez-Lima F. In vivo low-level light therapy increases cytochrome oxidase in skeletal muscle. *Photochem And Photobiol.* 2010; 86(3): 673-680.