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The Effects of Acute Photobiomodulation on Anaerobic Exercise Performance

Jillian Danielle Forsey

West Virginia University, jdforsey@mix.wvu.edu

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The Effects of Acute Photobiomodulation on Anaerobic Exercise Performance

Jillian Danielle Forsey

Thesis to be submitted to the School of Medicine at West Virginia University in partial fulfillment of the requirements for the degree of:

Masters of Science in Exercise Physiology

Randall W Bryner, Ed.D, Committee Chair

Joshua Hagen, Ph.D

Paul D. Chantler, Ph.D

Department of Exercise Physiology

Morgantown, West Virginia

2020

Key words: Photobiomodulation, LLLT, red and near infrared light therapy, anaerobic exercise, performance

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ABSTRACT

The Effects of Acute Photobiomodulation on Anaerobic Exercise Performance

Jillian Forsey

Optimal performance in sport requires a balance between training stress and recovery. Therapeutic modalities that allow athletes to maintain this balance while training hard are in high demand, but many lack research. Photobiomodulation is a red and near-infrared light therapy that is proposed to enhance exercise performance and hasten recovery. The purpose of this study was to examine the effects of acute whole body photobiomodulation, applied pre-exercise, on anaerobic exercise performance.

Forty-eight healthy, active subjects participated in this single-blind, crossover study. Subjects visited the lab three times to complete a repeat Wingate test, with one week between each visit. All subjects completed baseline testing during their first visit and randomly received either the photobiomodulation or placebo condition before testing on the second visit, followed by the opposite condition on the third visit.

There was a significantly higher peak power on the first Wingate test following photobiomodulation ($p=0.046$) but no differences in peak power on the second, third, and fourth Wingate tests. There was also a significantly higher power drop on the first Wingate test ($p=0.045$) but no differences on the second, third, and fourth Wingate tests. There were no differences in average power on any of the Wingate tests. Blood lactate was significantly lower following photobiomodulation, before exercise ($p=0.0001$), trending higher after the first Wingate test ($p=0.077$), and significantly higher after the second Wingate test ($p=0.048$). Peak heart rate was significantly higher following photobiomodulation on the first ($p=0.009$), second ($p=0.022$), and fourth ($p=0.015$) Wingate tests. On the day following photobiomodulation, heart rate variability was higher ($p=0.0043$) but there were no differences in subjective recovery and stress scores.

Based on these findings, we concluded that acute photobiomodulation may enhance maximal anaerobic performance but does not attenuate fatigue. We also concluded that acute photobiomodulation may enhance recovery from maximal, anaerobic exercise.

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CHAPTER 1: INTRODUCTION

1.1 Background and Significance

Sport is an integral part of culture worldwide. While moderate to vigorous physical activity is necessary for health and longevity, sport serves a wider purpose (1,2,98). Sport connects communities and countries through shared interest and common goals. Sport has evolved significantly over the past 40 years with improving media coverage that provides access to a global audience (98). During this time period, federal governments put more money into development of sport in order to compete on the international stage, contributing to sportive nationalism (98). This is evident through the development of training and recovery centers such as the US Olympic Training Centre and the Australian Institute of Sport, that aim to produce some of the top athletes in the world (51,101,102).

Optimal performance in sport requires a balance between training stress and adequate recovery (95). Thus; in order to be their best, athletes must train and recover as efficiently as possible. To enhance their training and performance, athletes often use strategies such as hydration, nutrition, supplements, and therapeutic modalities to help delay the onset of fatigue (17,18,19,36).

Recovery is just as important as training, particularly because athletes are training at a higher volume than ever (97). In order to balance this high training stress, recovery strategies must also evolve. Elite training centers value recovery just as much as training, and often provide mobile recovery modalities that travel with their athletes to the Olympics and other major competitions (51). While adoption of modern recovery modalities by athletes is high, the research is still limited (3). Although elite coaches have

stated that they want more research to back up the recovery modalities that they are using, they continue to use unproven methods at a high rate (52). Popular recovery modalities among athletes include cold water immersion or cryotherapy, compression garments, massage therapy, electrostimulation, and photobiomodulation (3,33).

Photobiomodulation is a red and near-infrared light therapy that uses light-emitting diodes, lasers, or a combination of both, to apply light at wavelengths ranging between 600 and 1100 nm (4,5). Light in this wavelength range penetrates the skin and is absorbed by the mitochondria, where it speeds up cellular processes (specific mechanisms discussed in section 2.8 below) (6). This promising modality has been used for many years in medical settings to decrease inflammation, increase tissue repair, and apply analgesia (5,41). In the last two decades, there has been an increased use of photobiomodulation in sport settings (4). Pre-exercise, photobiomodulation may be used to enhance performance in aerobic events by improving time to exhaustion and VO_2 kinetics (59,60,61,75,78). In anaerobic events, photobiomodulation can improve force produced and fatigue index (69,73,74,81,82). Photobiomodulation can also be used as a recovery modality when applied pre- or post-exercise by attenuating increases in muscle damage and oxidative stress markers such as creatine kinase (CK), lactate dehydrogenase (LDH), protein carbonyls, and thiobarbituric acid-reactive substances (TBARS) (60,69,73,74,75,82,83,84,96). However, the vast majority of the research on photobiomodulation in sport settings has been done with custom made LED arrays generally targeting a single muscle group. For this research, this application is termed “targeted photobiomodulation”. Thus, there is very limited research on the use of whole body photobiomodulation. Due to the promising findings of targeted photobiomodulation

in previous literature, and the adoption of whole body devices by professional and collegiate sport teams, there is a significant need for research on the efficacy of whole body photobiomodulation as a performance enhancing and recovery modality.

1.2 Purpose and Specific Aims

The purpose of this study was to examine the effects of acute whole body photobiomodulation, applied pre-exercise, on anaerobic exercise performance. We hypothesized that acute photobiomodulation would enhance performance in an anaerobic exercise test by increasing peak power and average power and decreasing power decrement. We also hypothesized that acute photobiomodulation would attenuate the increase in blood lactate and increase work output, as defined through an increase in heart rate throughout the exercise test. Finally, we hypothesized that acute photobiomodulation would hasten the recovery process and result in lower blood lactate and resting heart rate and higher heart rate variability at 10 minutes post-exercise test, as well as decreased recovery and stress scores and increased heart rate recovery in the days following.

Specific Aim 1: To determine the effect of acute photobiomodulation on performance during an acute, maximal, anaerobic exercise test.

Hypothesis 1: Acute photobiomodulation will improve power dynamics achieved in a repeat Wingate test.

Hypothesis 2: Acute photobiomodulation will decrease blood lactate before, during, and following a repeat Wingate test.

Hypothesis 3: Acute photobiomodulation will increase work output, as defined by a higher heart rate during a repeat Wingate test.

Specific Aim 2: To determine the effect of photobiomodulation on recovery from an acute, maximal, anaerobic exercise test.

Hypothesis 1: Acute photobiomodulation will increase heart rate variability before and after a repeat Wingate test.

Hypothesis 2: Acute photobiomodulation will decrease stress and recovery scores on the day following treatment.

Hypothesis 3: Acute photobiomodulation will increase heart rate variability on the day following treatment.

CHAPTER 2: LITERATURE REVIEW

2.1 Fatigue and performance

Muscular fatigue is defined as the inability to maintain muscular contractile force for a period of time. It is developed through maximal exercise or sub-maximal exercise performed for a prolonged time period (8,10,14,15,21,34,37). Muscular fatigue typically affects muscular strength and motor control, and often causes some level of pain (10). The time of onset of fatigue is affected by fitness level, muscle fiber type, and intensity, type, and duration of exercise (34,37). However, if the individual is exercising at a high enough intensity, or for a long enough time, they will experience muscular fatigue at some point (10,34,37). Muscular fatigue is different from muscle damage because it is more easily reversible and may be considered a protective mechanism to prevent structural muscle damage from occurring (21). Management of fatigue is particularly relevant for athletes because an increase in muscular fatigue will increase risk of injury and decrease performance (16). Because of this, a significant amount of research has been dedicated to determining how to delay this fatigue response.

Fatigue can be quantified using a fatigue index, which measures resistance to fatigue. The fatigue index may measure the drop in peak force, torque, or power of muscle contraction (37). There are a number of tests that can measure fatigue resistance. An isokinetic dynamometer may be used to test the number of maximal effort repetitions before either exhaustion or a 50% reduction in torque output is reached (37). Another common test is the Wingate test, which assesses the difference between the peak and lowest power outputs during a 30-second maximal cycling bout (37, 38). It is common among both elite and recreational athletes to employ methods to improve their fatigue

index, or delay the onset of fatigue. These methods include hydration strategies, nutrition, supplements such as creatine and arginine, and therapeutic modalities such as photobiomodulation (10,17,18,19,36).

2.2 Mechanisms of muscular fatigue

Muscle fatigue can stem from a number of points in the motor pathway. Peripheral fatigue occurs due to changes in processes at or distal to the neuromuscular junction while central fatigue occurs at the central nervous system and decreases signaling to the muscle (10,21,100). Metabolic factors have a significant effect on peripheral fatigue. When muscle contracts, ATPases and glycolysis are activated, leading to increased levels of H^+ , lactate, inorganic phosphate (Pi), and reactive oxygen species (ROS). Accumulation of these metabolites interferes with cross-bridge cycling, thus affecting the muscle's ability to produce force (100). Pyruvate is a byproduct of glycolysis that feeds into the citric acid cycle for oxidation. When pyruvate cannot be oxidized as quickly as it is being produced, it gets converted into lactic acid, which is further broken down into lactate and H^+ . As H^+ accumulates, pH decreases and ATP is depleted (21,100). This process affects the release of Ca^{2+} from the sarcoplasmic reticulum, sensitivity of troponin C to Ca^{2+} , and cross-bridge cycling (100). Furthermore, creatine phosphate is hydrolyzed to creatine and Pi. Pi accumulation can decrease release of Ca^{2+} from the sarcoplasmic reticulum, leading to a deleterious effect on activation (21,100). Increasing exercise intensity also leads to increased production of reactive oxygen species (ROS) (100). This increase in ROS leads to an increase in oxidation of proteins, lipids, and nucleic acids as well as a decrease in antioxidant capacity (21). This

oxidation of cell proteins then inhibits Ca^{2+} release from the sarcoplasmic reticulum and decreases myofibrillar Ca^{2+} sensitivity (100).

2.3 Biomarkers related to fatigue

2.3.1 Lactate

One of the most commonly measured biomarkers to assess fatigue is blood lactate (35,100). Blood lactate is often used because it correlates well with increasing exercise intensity and is unrelated to age, sex, and physical fitness (100). Normal resting blood lactate levels generally fall in the 0.5-1.5 mM range, with 4 mM considered the onset of blood lactate accumulation (35). At exercise intensities above 50-60% of maximal aerobic capacity, there is an increase in blood lactate, with levels typically ranging from 10-20 mM (34). However, maximal intermittent exercise can produce higher blood lactate levels than continuous exercise, with values ranging up to 25 mM (34,35). This increase in blood lactate during high intensity exercise is because ATP production, through oxidative phosphorylation of ADP, cannot meet the energy requirement of the myocyte, so there is a shift in ATP production from aerobic processes to anaerobic processes (21). The anaerobic breakdown of carbohydrates creates lactic acid, which is broken down into lactate and hydrogen ions, causing acidosis. This debilitates muscle function by impairing calcium handling, decreasing metabolic enzyme activity, and inhibiting myosin ATPase (37,66,67). The lactic acid hypothesis states that, as lactate is accumulated at the working muscle, contractile processes are inhibited, directly or indirectly through metabolism, and there is a decline in exercise performance (37,39). This hypothesis is supported by Messonnier et al. (40), who found a strong correlation between pH levels and work capacity during supramaximal exercise in humans. Because

of the relation to fatigue, blood lactate monitoring is a common tool used in planning training programs in high-level sports (36).

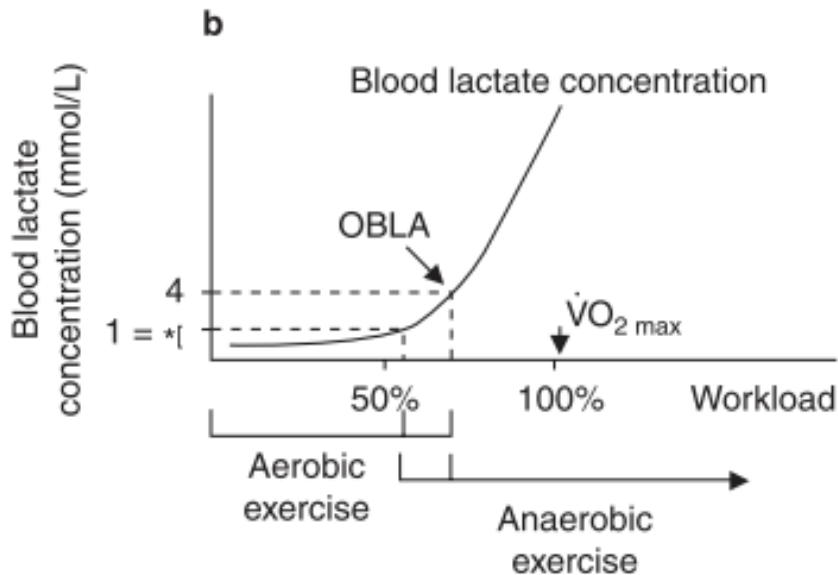


Figure 1. Blood lactate kinetics during exercise (20).

2.3.2 Reactive Oxygen Species (ROS)

When reactive oxygen species (ROS) are not reduced by antioxidants at the same rate at which they are produced by oxidation, oxidative stress occurs (13,24). Research has shown a correlation between ROS and decreased muscle function and the development of muscle fatigue (13,25,60). This correlation may be due to the excess electron held by ROS, which causes fatigue and damage to mitochondria (21). Effects of ROS on muscle function include a decrease in the sarcolemma's ability to depolarize, impaired calcium release and sensitivity, decreased actin-myosin interactions, inhibition of enzymes, and restriction of blood flow (21). Two enzymes that defend against oxidative stress are superoxide dismutase (SOD) and catalase (CAT) (62). SOD catalyzes a redox reaction to convert superoxide (O_2^-) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (62). Catalase (CAT) catalyzes the decomposition of H_2O_2 to water and

oxygen (21). Common biomarkers to examine oxidative stress include damage markers thiobarbituric acid-reactive substances (TBARS) and protein carbonyls, and antioxidant capacity markers SOD, CAT, glutathione (GSH), and total antioxidant capacity (TAC) (21). Following prolonged or maximal exercise, there is typically an increase in TBARS, protein carbonyls, SOD, CAT, GSH, and TAC but even short-duration, submaximal exercise can induce increases in SOD and TAC (64,65).

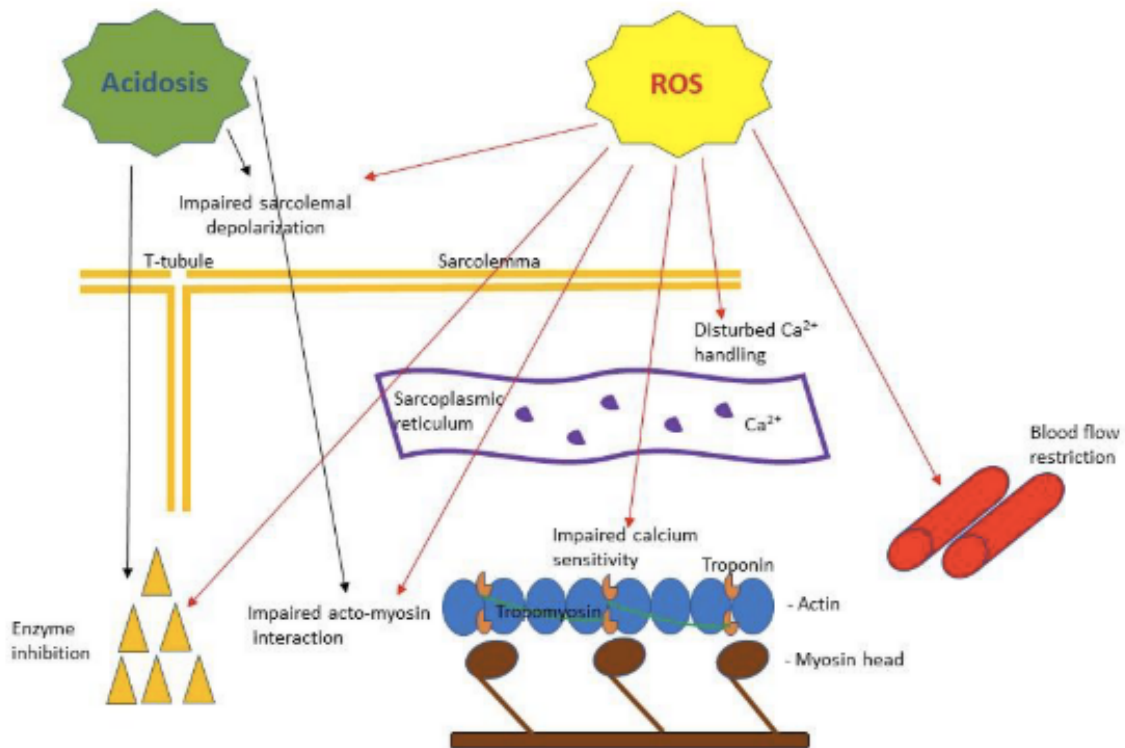


Figure 2. Demonstrates the effects of acidosis and ROS as they relate to muscle fatigue (37).

2.3.3 Inflammatory Biomarkers

Muscle contraction during exercise causes a protective inflammatory reaction, inducing a release of pro- and anti-inflammatory cytokines (21,63). This response is typically related to muscle damage that is fatiguing and contributes to delayed onset muscle soreness in the days following damaging exercise (69). This muscle damage

promotes a marked increase in pro-inflammatory cytokines Tumor Necrosis Factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β); interleukin-6 (IL-6), which acts as both a pro- and anti-inflammatory cytokine; cytokine inhibitors IL-1 receptor antagonist (IL-1ra) and TNF soluble receptors (sTNF-r1 and sTNF-r2); and anti-inflammatory cytokine interleukin-10 (IL-10) (63).

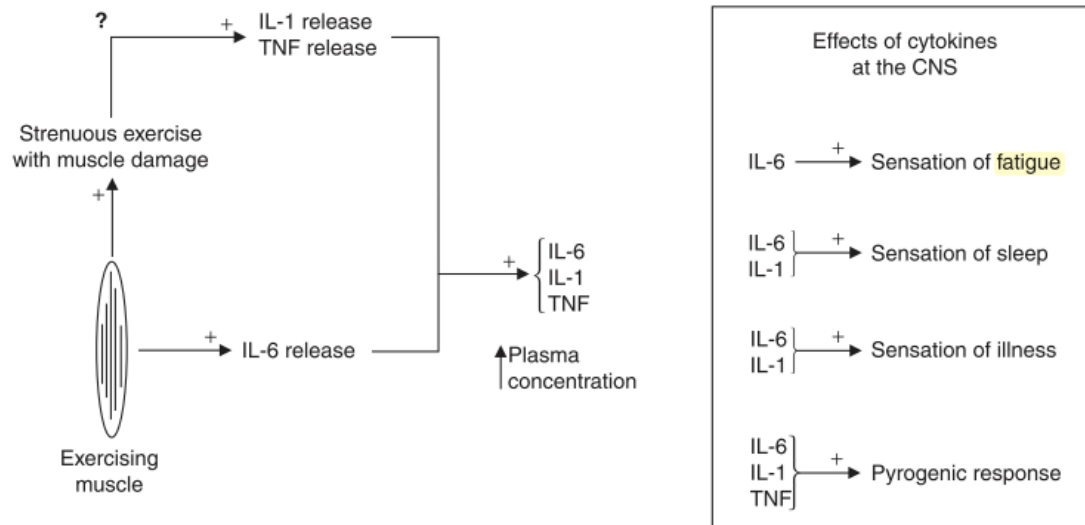


Figure 3. The interaction between exercise and cytokines (20).

2.4 Recovery and Performance

Optimal recovery following an intense training session or competition is essential to an athlete's performance (95). This is because rate of recovery from one session affects the effort an athlete is able to give in subsequent training sessions and competitions (51,52). If training stress is not balanced with adequate recovery, an athlete will experience under-recovery. As fatigue accumulates, this athlete becomes at risk for developing overreaching and overtraining, the latter of which can take months to years to recover from (95). There are different subjective and objective measures that can be taken into account when quantifying recovery status, which are detailed below.

2.5 Markers of recovery

2.5.1 Delayed Onset Muscle Soreness (DOMS)

A common measure of recovery status is delayed onset muscle soreness (DOMS). DOMS refers to exercise-induced muscle injury that is characterized by muscle pain, decreased maximal muscle strength, and increased serum creatine kinase (CK) (28,30,31,32). DOMS typically peaks 24-48 hours after exercise and subsides within 5-7 days (30,31). DOMS most frequently occurs following unfamiliar exercise, most often at the beginning of a training season, or exercise that involves a significant amount of eccentric contractions (28,30,31).

2.5.2 Creatine Kinase (CK)

Creatine Kinase (CK) is an enzyme present most abundantly in skeletal muscle, myocardium, and the brain. Hypoxia or injury causes release of CK into the circulation, thus CK has long been used as a marker of myocardial infarction; but can also be used as a marker of muscle damage (68). During exercise, the sarcolemma is disrupted, allowing CK to travel in the extracellular fluid, through the lymphatic system, and to the circulation (70). CK is an ideal muscle damage marker to measure because it has a substantial increase from baseline in response to exercise, is fairly inexpensive to measure, and can be measured effectively through capillary or venous samples (71,72). McLellan et al. (72) found that CK was elevated within 30 minutes of a competitive Rugby League match and still hadn't returned to baseline five days following the match, concluding that CK is a valuable monitoring tool for determining recovery status in athletes.

2.5.3 Subjective Wellness

Another common index of recovery is subjective wellness. Subjective wellness refers to perceived feelings of fatigue by an athlete and may be a better indicator of readiness and recovery than objective measures (46). There are a number of validated questionnaires that are used to measure subjective wellness. One of the most commonly used questionnaires is the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport), designed to measure how physically and/or mentally stressed an individual is (51). In a study examining the use of the RESTQ-Sport with professional football players, Laux et al. (47) found a significant relationship between the Fatigue, Sleep Quality, Disturbed Breaks, and Injury scales on the RESTQ-Sport and subsequent athlete injuries. They concluded that the RESTQ-Sport could effectively be used throughout the season to predict and prevent future injuries. The downside of the RESTQ-Sport is that it is a lengthy questionnaire, comprised of 77 items, which may be difficult to implement on a regular basis (51). Similarly, the Profile of Mood States (POMS) assesses the effect of training on mood and affective states (51). Kentta et al. (53) found that using the POMS during intense training periods in elite kayakers may allow them to avoid overreached and stale states. Like the RESTQ-Sport, the POMS is a longer questionnaire, with 65 items, and may be difficult to use consistently (51).

Fortunately, there are shorter subjective wellness questionnaires that have been validated and may be easier to use on a frequent basis. The Acute Recovery and Stress Scale (ARSS) and the Short Recovery and Stress Scale (SRSS) were developed by Hitzschke and colleagues (48,54,55) to efficiently measure recovery and stress status in athletes. The ARSS consists of 32 items whereas the SRSS consists of just 8 items to allow for frequent monitoring (56). These questionnaires were originally developed in

German but have since been translated and validated for English populations, with recovery and stress scores that correlate with those of the RESTQ-Sport (48,56). Flynn and colleagues (57) validated the SRSS during in-season volleyball training and found that the SRSS was an effective tool for monitoring recovery and stress states.

To measure an athlete's perceived effort during a single training session, Borg's Rating of Perceived Exertion (RPE) scale is a one-dimensional scale that asks individuals to rate their exertion on a scale of 6-20 (52). Borg's RPE scale has been validated and RPE strongly correlates to blood lactate and heart rate values, making this a credible measure of perceived effort during a single session (58).

2.5.4 Heart Rate Variability (HRV)

Recovery can also be monitored through resting heart rate (HR) and heart rate variability (HRV). Traditionally, resting HR has been used as a marker of recovery, with increased resting HR suggesting central nervous system fatigue and potential overreaching and low resting HR considered a sign of recovery (42,43). This relationship is dependent on the inotropic and chronotropic effects of the autonomic nervous system (ANS) (42,44). However, the problem with resting HR as a measure of recovery is that the changes are fairly minimal, usually only changing a few beats per minute, and can be influenced by other environmental factors (42,45). Heart rate variability is another marker of autonomic tone that may provide a better indication of recovery status than resting HR (42,43,44). HRV measures the time variation between two heart beats, referred to as the R-R interval. The most commonly used index of HRV is RMSSD, which is calculated as the root mean square successive difference between all consecutive R-R intervals and the number of contiguous R-R intervals with a difference greater than

50 ms, within the collection period (45). RMSSD is a time-domain measurement and is more influenced by the parasympathetic system than the sympathetic system (110). HRV can also be observed through frequency-domain measurements that estimate power distribution in four frequency bands (110). These bands include high frequency (HF), low frequency (LF), very low frequency (VLF), and ultra low frequency (ULF) bands (110). The HF band is influenced by respiration from 9-24 bpm, LF band by respiration of 3-9 bpm and rhythm periods from 7-25 seconds, and VLF band by rhythm periods from 25-300 seconds (110). The LF/HF ratio is often used to estimate the ratio of sympathetic to parasympathetic activity (110). Total power may be used as an HRV index during short-term recordings by summing the VLF, LF, and HF bands (110). Although HRV can also be affected by environmental factors, it is considered a better marker than HR because even when heart rate is consistent, HRV may show significant changes in beat to beat variation (45). Plews et al. (43) found that when monitoring elite triathletes for non-functional overreaching, HRV was a more sensitive marker than resting HR. From a broad health and wellness perspective, high HRV (generally RMSSD) is associated with high fitness levels (i.e. VO₂ max values) whereas low HRV is associated with increased mortality (45). HRV is individual so examining a person's deviation from their own baseline is the most effective way to evaluate HRV as an indicator of ANS status (43). HRV measurements taken upon awakening have shown to have a stronger correlation to nonfunctional overreaching than HRV measurements taken overnight, so when monitoring for chronic fatigue, morning HRV measurements are most beneficial (49). Due to individual differences and environmental factors, to adequately use HRV as a monitoring tool for fatigue and nonfunctional overreaching, HRV should be measured on

a consistent basis, at the same time of day, and under the same conditions (43). HRV may also be lowered following intense exercise due to activation of the sympathetic nervous system, and remain lowered for up to 24 hours (42,50).

2.6 Recovery Modalities

Recovery modalities can be used by everyone from recreational to elite athletes between training sessions to hasten their recovery from one training session or competitive event, thus preparing them for the next. The goal of recovery modalities is typically to decrease delayed onset muscle soreness, attenuate fatigue, and increase rate of lactate removal from the blood (3,33). Traditional recovery modalities include stretching, massage therapy, active recovery, and cold-water immersion (3). Currently, there is a wave of modern recovery modalities entering the market, such as cryotherapy, float therapy, compression garments, electrostimulation, and photobiomodulation (3,33). Athletes are quick to embrace these modalities, with the idea that quicker recovery allows them to train more and see greater improvements (51,52). However, in a survey of elite level coaches of Summer Olympic sports, a significant number of coaches stated that they would like to see more research on the effectiveness of these recovery modalities (52). This is because although interest in and adoption of these modalities is high, there is still limited or conflicting research proving the efficacy of many of these modalities (3,33). Massage therapy has shown to provide significant improvements to muscle soreness and perceived fatigue, compression and cold-water immersion have shown smaller but still significant positive effects, but there is limited research showing the other modalities to be effective (3,33). There is not yet any research on the efficacy of whole body photobiomodulation as a recovery modality.

2.7 Photobiomodulation Therapy

Photobiomodulation is also known as low level laser therapy (LLLT) or light emitting diode therapy (LEDT) (4). Photobiomodulation is administered through lasers, light-emitting diodes, or some combination of the two (4,5,22,41,93).

Photobiomodulation consists of red and near-infrared light therapy that applies light at wavelengths ranging from 600-1100 nm, most typically 660 and 850 nm, with a power density ranging from 1 mw-5 W/cm² (4,5,41). The output power is low enough that it does not cause any heating of tissue, thus there is no structural change (5,41). The ideal dose is 20-60 J for small muscle groups and 120-300 J for large muscle groups (93).

Although lasers have greater coherency and smaller spectral bandwidths with less divergence of light beams than LEDs, both methods provide the same effects to tissue as long as the dose is delivered according to the biphasic dose-response (41).

There are a variety of reported benefits of photobiomodulation, many of which that can be applied to both sports performance and medical use. Some of these uses include decreasing inflammation, increasing tissue repair, and applying analgesia (5,41).

When administered pre-exercise, photobiomodulation is proposed to act as a performance enhancer through increased force produced and delayed fatigue response (10,12,13). When administered pre-exercise, it may also act as a preventative measure against muscle damage during exercise (10,12,13). Acutely, photobiomodulation can be administered five minutes to six hours prior to exercise, but studies defining this window more precisely are lacking. For chronic effects in conjunction with strength or aerobic training, photobiomodulation is more effective when administered immediately before exercise (93). Photobiomodulation has also been studied as a recovery modality

employed post-exercise to decrease muscle damage and enhance recovery processes (26,27,28,29). These effects have been examined in both animal and human models.

2.8 Proposed Mechanism of Photobiomodulation

The red and near-infrared light administered during photobiomodulation therapy lies within the wavelength range that can transmit through the skin and tissues with minimal photon absorption and reach the mitochondria, where it is absorbed, all without the carcinogens and mutagens associated with ultraviolet light (6). Within the mitochondria, photobiomodulation increases activity of complexes I, II, III, IV, and succinate dehydrogenase. However, complex IV, also known as Cytochrome C Oxidase, is believed to act as the primary photoacceptor (5,6,7,22,23). Cytochrome C Oxidase is the terminal enzyme in the electron transport chain. Here, photobiomodulation increases electron availability for reduction of molecular Oxygen to water. This action increases the mitochondrial membrane potential, adenosine triphosphate (ATP) production, cyclic adenosine monophosphate (cAMP), and protection against reactive oxygen species (ROS) (5,41). Ferraresi et al. (9) tested this theory in C2C12 myotubes, a mouse myoblast cell line in vitro, and found that photobiomodulation applied to these cell cultures increased mitochondrial membrane potential and synthesis of ATP. Based on this data, it was concluded that photobiomodulation applied to muscle before exercise could result in improved exercise performance.

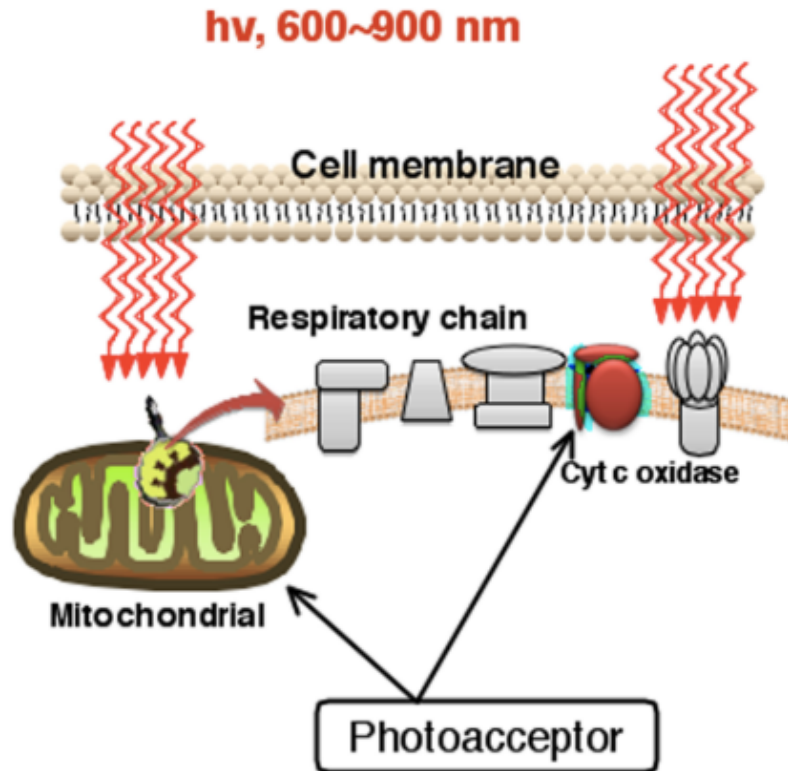


Figure 4. Mechanistic visual of the absorption of red and near-infrared light by cellular chromophores in the mitochondrial respiratory chain (41).

2.9 Photobiomodulation Therapy in Animal Models

2.9.1 Pre-exercise

In animal models, photobiomodulation has effectively reduced fatigue and decreased muscle damage when applied pre-exercise (10,12,13). Lopes-Martins et al. (10) and Leal Junior et al. (12) followed similar protocols by developing muscle fatigue in rats with induced tetanic contractions by electrical stimulation. These protocols administered photobiomodulation immediately before the first contraction and measured subsequent performance. Both groups found that when the rats received photobiomodulation treatment immediately before, peak force produced on the final contraction and mean performed work on the final contraction were significantly higher. Silva et al. (13) used a different protocol by inducing muscle fatigue through forced high-

intensity treadmill running and delivering photobiomodulation for three consecutive days prior to the treadmill task. These data suggested that in the photobiomodulation group, there was a significant improvement in time to exhaustion.

When photobiomodulation was administered pre-exercise, as a performance enhancer, it was also assessed as a recovery tool (10,12,13). Lopes-Martins et al. (10) examined plasma creatine kinase (CK) whereas Leal Junior et al. (12) looked at both plasma CK and lactate. Both teams found significantly lower CK levels following photobiomodulation as compared to the control and Leal Junior et al. (12) found significantly smaller changes in lactate as well (10). Silva et al. (13) looked at a broader scope of biomarkers of oxidative stress and muscle damage by measuring serum CK, protein carbonyls, and superoxide dismutase (SOD). Twenty-four hours following the protocol, the photobiomodulation group had significantly lower CK, protein carbonyls, and SOD as compared to the control group. The photobiomodulation group presented values that were comparable to baseline for all three biomarkers, showing that photobiomodulation may be effective at reducing oxidative stress in response to exercise.

2.9.2 Post-exercise

In animal studies where photobiomodulation was employed as a recovery modality post-exercise or following muscle injury, the focus was muscle damage markers and/or inflammatory markers (26,28,29,32). Although the protocols for these studies varied greatly, there were similar findings that could be of interest to athletic populations. Multiple studies found that photobiomodulation attenuated increases in serum CK as compared to a control (26,28,32). Both Liu et al. (28) and Sussai et al. (32) induced muscle damage in rats, with forced downhill running and swimming, respectively, and

found that at 24 and 48 hours post exercise, the group that received high dose photobiomodulation immediately after exercise had significantly lower serum CK levels than an exercise control. Furthermore, Liu et al. (28) found that the serum CK levels of the exercise group that received high dose photobiomodulation were not significantly different from those of the sedentary control. Liu et al. (28) also found that the high dose photobiomodulation group had significantly higher muscle SOD activity and significantly lower muscle MDA than the exercise control. Several studies also found that photobiomodulation attenuated the inflammatory response as compared to a control (26,28,29). Lui et al. (28) found an attenuated degree of inflammatory cell infiltrate in the gastrocnemius muscle in the photobiomodulation groups as compared to the control. Almeida et al. (29) compared the effects of photobiomodulation (to cryotherapy, diclofenac, and a control) on inflammatory cytokine levels following acute skeletal muscle injury. This group found that the group that received photobiomodulation had significantly lower levels of interleukin-1 β , interleukin-6, and tumor necrosis factor-alpha than all three other groups.

2.10 Photobiomodulation Therapy in Human Models

2.10.1 Pre- aerobic exercise

In human models, promising findings for the use of targeted photobiomodulation as a performance enhancer in aerobic events have been noted (59,60,61,75,78). Several studies have used similar progressive-intensity running protocols and found a beneficial effect of photobiomodulation on time to exhaustion and VO₂max (59,60,61). Miranda et al. (59) also found increased distance covered and decreased dyspnea following photobiomodulation. Mezzaroba et al. found higher peak velocity, lower maximum heart

rate and lower blood lactate at 13-15 minutes following photobiomodulation, as well (61). There have been similar findings in studies involving elite or competitive athletes (75,78). In a case study on an elite, female runner performing constant-load high-intensity running, Ferraresi et al. (75) found improvements in VO₂max kinetics, time to exhaustion, and O₂ deficit following photobiomodulation as compared to the placebo. Meanwhile, Lanferdini et al. (78) found that photobiomodulation effectively increased time to exhaustion in competitive cyclists. However, not all studies were this promising. Junior et al. (77) found a tendency towards increased time to exhaustion in an incremental treadmill test following photobiomodulation, but their findings were not significant. Beltrame et al. (76) found no statistical differences between photobiomodulation and placebo groups on VO₂ kinetics or blood lactate during maximal incremental cycle ergometer exercise. Machado et al. (79) employed photobiomodulation between two 40-minute running time trials that were separated by six hours and found there were no significant differences on performance in the second time trial or blood lactate. These discrepancies in findings may have to do with methodology or differences in photobiomodulation dose, and thus prompt the need for more performance-based research.

When photobiomodulation is administered pre-exercise, it has an effect on the recovery period, as well. Many studies that looked at the aerobic performance effects of pre-exercise photobiomodulation also looked at changes in muscle damage, oxidative stress, and recovery markers (60,75,79). De Marchi et al. (60) found attenuated increases in TBARS, protein carbonyls, CK, and LDH and increases in SOD activity in the photobiomodulation group as compared to the control. Ferraresi et al. (75) found lower

lactate, CK, and alanine levels in the elite runner following the photobiomodulation trial. Similar to their findings on performance, Machado et al. (79) found no differences in CK or HRV in the photobiomodulation trial, although there was a moderate effect size for attenuated DOMS.

2.10.2 Pre- anaerobic exercise

There has also been substantial research on the effects of photobiomodulation on anaerobic performance and, much like the literature on aerobic performance, there are promising but mixed findings (36,69,73,74,80,81,83,93). A common protocol that is used is an eccentric contraction fatiguing protocol followed by a maximal voluntary contraction (MVC) test to determine fatigue index (69,73,74). Three separate studies have found an improvement in performance following photobiomodulation, with subjects reaching a higher peak torque on the MVC than when they received the placebo (69,73,74). Similar studies have examined pre-match or pre-training photobiomodulation in team sports that can be highly anaerobic (81,82,93). Dornelles et al. (81) found that when photobiomodulation was given to soccer players before a simulated soccer match, there was an attenuation in loss of hamstring peak torque, hamstring to quadriceps torque ratio, and countermovement jump height. This finding was important, as hamstring injuries are common in soccer players, and often caused by fatigue. Thus, the attenuation in hamstring fatigue could indicate photobiomodulation as a promising preventative measure against injury. Pinto et al. (82) examined the use of photobiomodulation with high-level rugby players, utilizing a common field test, the Bangsbo Sprint Test. These researchers found a significant improvement in sprint time and fatigue index and attenuation in blood lactate changes and perceived fatigue, as compared to the placebo

condition. De Marchi et al. (93) looked at photobiomodulation applied 40 minutes before a professional futsal match and found that players stayed on the pitch for a significantly longer period of time and had significantly lower blood lactate pre, immediately post, and 48 hours post in the photobiomodulation trial as compared to the placebo trial. There were also studies that found no significant effects of photobiomodulation on anaerobic performance (36,80,96). Dos Reis et al. (36) administered photobiomodulation before a maximal knee extension exercise and found no difference in number of reps or duration of fatigue between the photobiomodulation and placebo groups. However, this study differed from other studies that used the eccentric contraction fatiguing protocol. Dos Reis et al. (36) used their fatiguing protocol as the performance assessment rather than having subjects complete an MVC test post-fatigue, as we've seen in previous literature (69,73,74). This discrepancy may explain the differing findings. Orsatto et al. (80) developed a protocol that induced fatigue with repeated bilateral countermovement jumps (CMJ) and assessed performance and fatigue with a CMJ test, echo intensity, and muscle soreness. These researchers found no significant differences between pre-exercise photobiomodulation and placebo trials for any of their measures. However, these researchers randomly selected one leg to receive photobiomodulation and one leg to receive the placebo for each participant, rather than having separate photobiomodulation and placebo trials, which may have affected their results. Leal Junior et al. (96) applied photobiomodulation or a placebo condition to male soccer and volleyball athletes before they performed a Wingate test and found no differences between groups for work performed or blood lactate at 3 and 10 minutes post exercise. However, at 15 minutes post exercise, blood lactate was significantly lower in the photobiomodulation group.

Photobiomodulation before anaerobic exercise also influences muscle damage, oxidative stress, and recovery markers. Following anaerobic exercise, pre-exercise photobiomodulation caused a decrease or attenuated increase in CK, LDH, and IL-6, in comparison to a placebo (69,73,74,82,94,96). Oleivera et al. (73) and De Marchi et al. (94) also found decreases in TBARS and carbonylated proteins following photobiomodulation as compared to the placebo. Furthermore, Oleivera et al. (73) found decreases in TNF- α and no differences in IL-1 β , CAT, or SOD, following photobiomodulation. The effect on muscle soreness is inconclusive as Oleivera et al. found a decrease in DOMS following photobiomodulation whereas Baroni et al. and Vanin et al. found no difference between groups (69,73,74).

2.10.3 Post-exercise

Photobiomodulation can also be used post-exercise solely as a recovery modality to attenuate muscle damage and loss of performance on proceeding days. Several researchers have compared the effects of photobiomodulation and cryotherapy following damaging exercise (83,84). De Marchi et al. (83) and de Paiva et al. (84) followed similar protocols in which a damaging eccentric exercise was performed before receiving treatment of either photobiomodulation (PBM), photobiomodulation followed by cryotherapy (PBM+CRY), cryotherapy alone (CRY), cryotherapy followed by photobiomodulation (CRY+PBM), or a placebo. In both studies, PBM significantly attenuated loss of MVC peak torque, increases in DOMS, and increases in CK at 24, 48, 72, and 96 hours post-exercise. PBM+CRY had lower but still significant, positive effects on all three measures while CRY by itself had similar effects to the placebo. The results of these studies suggest that photobiomodulation is an effective recovery modality but its

efficacy may be decreased by the addition of cryotherapy. Similarly, Borges et al. (85) found that photobiomodulation administered following an eccentric contraction protocol attenuated loss of muscle strength, loss of range of motion, and increased muscle soreness at 24, 48, 72, and 96 hours post-exercise. Although dos Reis et al. (36) found no significant effects for pre-exercise photobiomodulation, in the same study, they found decreases in lactate and CK following damaging exercise when photobiomodulation was administered post-exercise. However, not all findings were this positive. Malta et al. (86) conducted research comparing photobiomodulation (PBM), cold-water immersion (CWI), active recovery (AR), and placebo following a repeat Wingate test. These researchers found no significant effects on blood markers, DOMS, or CMJ performance for any of the recovery modalities at any time points.

2.10.4 Chronic Photobiomodulation therapy associated with training

Photobiomodulation can also affect performance when used in conjunction with training over a period of time (87,88,90,91). Viera et al. (88) found that in a 9-week cycle ergometer training program, the training group that received photobiomodulation following each training session had an improved fatigue index at the end of the study, as compared to the sedentary control and just training groups. Paolillo et al. (87) conducted a unique study in which subjects received photobiomodulation for 45 minutes twice a week for six months while they performed running training on a treadmill. This group was compared to a group performing the same training without photobiomodulation and a sedentary group. These researchers found improved time to exhaustion, METs, Bruce stage, heart rate, and RPE in both the photobiomodulation group and the exercise group but the differences in time to exhaustion, METs, and heart rate were significantly greater

in the photobiomodulation group. The photobiomodulation group also experienced a decrease in time to recovery. One issue with this study is the lack of placebo condition. The light was very visible when administered during training so subjects were keenly aware of which group they were in. In another aerobic training study, Peserico et al. (92) found that when subjects performed a 5-km training program for 8 weeks and received either photobiomodulation or a placebo before each training session, there were no significant differences between groups for peak velocity or 5-km time at the end of the training period. There was a possibly positive effect of photobiomodulation on peak velocity and 5-km time when using a magnitude-based inference analysis, so further research in this area should be conducted.

Several studies also examined photobiomodulation in conjunction with a strength-training program, but the results were mixed (89,90,91). Vanin et al. (90) separated subjects into four groups- photobiomodulation before training and placebo after, placebo before training and photobiomodulation after, photobiomodulation before and after training, and placebo before and after training. The group that received photobiomodulation before training and placebo after, had significantly greater improvements in MVC, leg press 1RM, and leg extension 1 RM, following 12 weeks of strength training, as compared to the other three groups. This finding suggests that the best moment to apply photobiomodulation to a strength-training program is pre-exercise. However, Almeida et al. (89) conducted a study in which participants received photobiomodulation or a placebo condition prior to strength training twice a week for eight weeks, and there were no differences between groups for muscle strength, functional performance, or quality of life. Cunha et al. (91) compared

photobiomodulation and neuromuscular electrical stimulation (NMES) applied during an eight-week training program for muscle strength and jumping skills. Although there was an increase in non-dominant lower limb strength in the photobiomodulation group compared to the control, NMES was more effective, with significant increases in both dominant and non-dominant lower limb strength, as well as global perceived effort, as compared to the control.

CHAPTER 3: METHODS

Experimental Approach: A randomized, single-blind, placebo-controlled, crossover design was used to examine the effect of photobiomodulation therapy on exercise performance in a repeat Wingate test. Order of photobiomodulation condition and placebo condition was randomized using a random number generator in Microsoft Excel software.

Subjects: Sample size determination was conducted with respect to only the primary aim. Variables of interest included differences between photobiomodulation and placebo with regards to: peak power, average power, and power decrement (fatigue index); blood lactate; and peak heart rate. Due to four comparisons being made, 80% power is achieved with 48 volunteers when significance level is $0.05/4 = 0.0125$ and Cohen's d is set to a medium effect of 0.5. The individuals were recruited from local running, triathlon, and crossfit training groups in Morgantown, WV and surrounding area through Facebook groups, email lists, and word of mouth. Subjects were selected to participate in the study if they were considered "low risk" by the ACSM Risk Stratification and met ACSM's minimum physical activity guidelines of at least 75 minutes of vigorous or 150 minutes of moderate aerobic activity per week.

Table 1. Participant Characteristics. Data shown are mean \pm standard deviation.

	N=48; F=30, M=18
Age (years)	34.65 (\pm 12.04)
Weight (kg)	70.04 (\pm 12.58)
Height (cm)	170.22 (\pm 9.89)

Body Fat Percentage (%)	19.76 (± 7.37)
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Procedures: Each volunteer had three visits to the laboratory, with each visit separated by a seven-day washout period. Each visit occurred on the same day of the week at the same time of day. The temperature inside the photobiomodulation room stayed within a range of $\sim 80^{\circ}\text{F}$ to $\sim 85^{\circ}\text{F}$ and the average temperature inside the testing room was $\sim 70^{\circ}\text{F}$ to $\sim 74^{\circ}\text{F}$. The first visit consisted of familiarization of procedures, completion of questionnaires, anthropometric measurements, and baseline testing. During familiarization of procedures, participants received a verbal description of the expectations of them for the duration of the study and the events that would take place during each visit. Participants were instructed to keep their diet, activity level, and sleep habits consistent throughout the duration of the study.

Photobiomodulation: Photobiomodulation was administered using the Novathor Whole Body Light Pod. This device delivers light at wavelengths of 660 and 850 nm with a power density of 25 mW/cm^2 . For the photobiomodulation condition, photobiomodulation was administered for 20 minutes immediately before the repeat Wingate test was performed. While in the bed, participants wore a blindfold and noise-cancelling headphones.

Placebo Condition: For the placebo condition, participants laid in the Novathor Whole Body Light Pod for 20 minutes with the device switched off, immediately before the repeat Wingate test was performed. Space heaters and a fan were turned on to maintain

the temperature in the same range of ~80°F to ~85°F that was reached during the photobiomodulation condition. Participants wore a blindfold and noise-cancelling headphones during both conditions to block out as much light and noise generated by the device as possible.

Questionnaires: During the initial visit, participants were instructed to complete three questionnaires at their own pace. In order to complete the questionnaires, subjects were asked to download the Smartabase application on their smartphone. These questionnaires included the Pittsburgh Sleep Quality Index (PSQI), GRIT scale, and Competitive State Anxiety Inventory- 2 Revised (CSAI-2R).^{103,104,105} These questionnaires were selected to determine sleep quality of participants before beginning the study, individual differences in levels of grit, and levels of competitive state anxiety that may pertain to performance in the exercise test administered throughout this study, respectively. Participants were also asked to complete two questionnaires each day for the duration of the study. The daily questionnaires consisted of the Short Recovery and Stress Scale (SRSS) questionnaire and the End of Day Questionnaire.⁵⁵ The SRSS questionnaire was completed each morning upon waking and consisted of eight scales pertaining to the participants' current states of recovery and stress. The purpose of this questionnaire was to determine whether the individual's stress level was consistent throughout the study and whether there was a difference in stress level following the first and second testing sessions. The End of Day questionnaire was completed each night before bed and was used to record the participants' daily training and nutrition rating. The purpose of this questionnaire was to determine whether participants maintained fairly consistent levels of

activity and similar dietary habits throughout the duration of the study. The purpose of examining homogeneity of stress, sleep, exercise, and diet habits throughout the study was to look for any outside factors that may affect a participant's performance in the exercise tests. Upon completion of the study, participants completed an exit questionnaire asking them to describe any differences they felt between conditions (photobiomodulation and placebo) to determine whether they could tell when they were receiving the photobiomodulation condition. This questionnaire also asked whether participants maintained consistent diet, sleep, and training habits throughout the study so that we could look for potential explanations for any inconsistencies in results.

Daily Heart Rate Variability (HRV): Participant HRV was tracked throughout the duration of the study using the Oura ring.¹⁰⁶ The Oura is a commercially available sleep device with the form factor of a ring, and gives information on sleep quantity and quality, as well as heart rate, heart rate variability (RMSSD), and body temperature. This technology was selected due to high data quality as assessed in an internal validation study (publication in preparation) as well as ease of use to the participant. During the initial visit, participants downloaded the Oura ring application on their smartphone and were sized for a ring using the Oura ring sizing kit. Participants were given a short tutorial on how to navigate the application, how to wear the ring, and when to wear the ring. Participants were instructed to wear the ring on the same finger each night with the sensor on the palmer side of the hand. Participants were instructed to put the ring on before going to bed and to wear it overnight. Upon awakening, participants were instructed to complete a 5-minute "Check Body Status" Moment in the application to

measure heart rate variability (RMSSD). During the Moment, participants were told to lie as still as possible. Following the Moment, participants were instructed to remove the ring and leave it on the charging base during the day.

Anthropometric measurements: Height, weight, and body fat percentage was determined for each participant during the initial visit. Height was measured using a Healthometer professional scale and weight was measured using a Welch Allen Scaletonix scale. Body fat percentage was measured with Lange skinfold calipers using the 7-site skinfold method. Each site was measured two times and an average of the two was recorded. The same study personnel measured each participant to remove any chance of poor inter-rater reliability.

Testing Day Heart Rate Variability: During the initial visit, HRV was measured immediately before the exercise test and ten minutes following completion of the last Wingate test. During the second and third visits, HRV was measured immediately before the exercise test, which fell immediately following reception of either the placebo or photobiomodulation condition, and ten minutes following completion of the last Wingate test. Heart rate variability was measured using a Polar heart rate strap and the Firstbeat Sports application on a smartphone. The sensors on the heart rate strap were moistened with OneStep Clear gel and the strap was placed just below the sternum. The participant was instructed to lie in a prone position on the turf track for 3 minutes while heart rate variability was measured with the Quick Recovery Test in the Firstbeat Sports application.

Repeat Wingate Test: The exercise test used to assess performance was a repeat Wingate test. This test was performed on the Monark 894E Wingate Testing Bike Ergometer. Seat height was determined based on hip height and affirmed by visual inspection of leg in a fully extended position. Participants were instructed to warm up for five minutes on the cycle ergometer at a self-selected pace, against no resistance. After cycling for 5 minutes, participants were instructed to accelerate to their maximum speed and then hit a button on the right handlebar, causing the weight to drop. The weight was set at 7.5% of the participant's body weight.¹⁰⁹ Once the weight dropped, participants cycled at maximal effort for 30 seconds. During the 30-second bout, participants were verbally encouraged at a consistent rate by the study personnel and were told when they had 15 and 5 seconds remaining. Following the 30-second bout, the weight was lifted and the participants cycled at an easy, self-selected pace against no resistance for two minutes. Immediately after the two-minute recovery period, participants sped up and started the next Wingate test. The participants did this a total of four times. Following the fourth and final 30-second bout, participants were instructed to stay on the bike and cycle at an easy, self-selected pace for five minutes followed by five minutes of easy walking on the turf track.

Heart Rate: To measure heart rate (HR), participants wore a Polar heart rate strap that was connected to the Firstbeat Sports application and collected heart rate data from the start of the warm-up until ten minutes following the final Wingate. The sensors on the heart rate strap were moistened with OneStep Clear gel and the strap was placed just below the sternum. HR was recorded manually by study personnel immediately before

starting the warm-up, within five seconds of completion of the first Wingate test, within five seconds of completion of the second Wingate test, within five seconds of completion of the third Wingate test, within five seconds of completion of the fourth Wingate test, and at ten minutes following completion of the fourth Wingate test.

Rating of Perceived Exertion: The Borg Rating of Perceived Exertion Scale (RPE) was used to measure participants' subjective perceived exertion. It was explained to participants that the RPE scale was a 6-20 scale, with 6 correlating to no exertion at all and 20 correlating to their maximum exertion. To help the participants gain a better understanding of these numbers, it was explained that 6-7 was similar to laying on the couch, 11-12 was similar to going for a light walk, 15-16 was similar to going for a run, and 18-19 was similar to the end of a race when running as hard as possible. Subjects were asked to provide their RPE immediately before starting the test, within five seconds of completion of the first Wingate test, within five seconds of completion of the second Wingate test, within five seconds of completion of the third Wingate test, within five seconds of completion of the fourth Wingate test, and at ten minutes following completion of the fourth Wingate test. These values were recorded by study personnel.

Blood Lactate: Lactate was measured using a Nova Biomedical Lactate Plus meter, Lactate Plus meter test strips, and Unistik 3 Comfort single-use 28-gauge 1.8 mm lancets. To test lactate, a test strip was inserted into the Lactate Plus meter, with the logo side up and gold side down. The index, middle, or ring finger was prepared for puncture by cleaning the tip with an alcohol swab and drying with gauze. To perform the finger prick,

the cap was twisted off the lancet, the lancet was placed against the pad of the finger, the release button was pressed, and the lancet was removed from the finger. The end of the test strip was placed to the blood until the test strip well was full and the test meter beeped. The result was provided in thirteen seconds and recorded manually by study personnel. Blood lactate was measured immediately before starting the warm-up, within thirty seconds of completion of the first Wingate test, within thirty seconds of completion of the second Wingate test, within thirty seconds of completion of the third Wingate test, within thirty seconds of completion of the fourth Wingate test, and at ten minutes following completion of the fourth Wingate test. The Nova Biomedical Lactate Plus meter was tested for quality control once a week using the Nova Lactate Plus level 1 and level 2 control solutions.

Power Dynamics: Power dynamics were assessed during each individual Wingate test. Power dynamics were calculated by the Monark Anaerobic Test Software. During the repeat Wingate test, peak power, average power, and power decrement were measured for each of four individual Wingate tests, allowing for observation of power dynamics for an individual Wingate test as well as changes in power dynamics across the four Wingate tests. Power dynamics were calculated unscaled as well as scaled for body weight. No power data was collected during warm-up, recovery periods, or cool-down.

Data analysis: All analyses were conducted using R software version 3.6.2. To investigate the effect of photobiomodulation with respect to the two aims listed in the introduction, two-way repeated measures ANOVAs were constructed using Condition

(PBM/Placebo), Time, and the Condition:Time interaction effect. Thus, the repeated measures model is of the form:

$$Y_{ijk} = \mu + \alpha_{i(j)} + \alpha_j + \alpha_k + (\alpha\alpha)_{jk} + \epsilon_{ijk}$$

In the above equation, Y_{ijk} represents the observation at the k^{th} time point from the i^{th} subject in group j (PBM or Placebo group), α_j represents the main effect of Condition, α_k represents the main effect of time, $(\alpha\alpha)_{jk}$ represents the interaction effect of Condition and Time, $\alpha_{i(j)}$ represents the nested effect of subject within Condition (since subjects will have different baselines for Lactate, HR, Peak Power, etc.), and ϵ_{ijk} represents the error term. This allowed for the examination of both the main effect of PBM, as well as the effect of PBM across each individual time point. For Condition, the null and alternative hypotheses can be arranged as follows:

H₀: $\mu_{\text{PBM}} = \mu_{\text{Placebo}}$, or equivalently, $\alpha_{\text{PBM}} = \alpha_{\text{Placebo}}$

H_A: $\mu_{\text{PBM}} \neq \mu_{\text{Placebo}}$, or equivalently, $\alpha_{\text{PBM}} \neq \alpha_{\text{Placebo}}$

For the interaction the hypotheses would be:

H₀: $(\alpha\alpha)_{jk}$ are equal for all levels of j (PBM/Placebo) and k (Time points)

H_A: some inequality in $(\alpha\alpha)_{jk}$

Following the Repeated Measures ANOVA, post-hoc tests were conducted by first calculating the matched pair differences between PBM and Placebo at each time point, followed by a paired t -test with the following hypotheses:

H₀: $\mu_{dk} = 0$

H_a: $\mu_{dk} \neq 0$, where μ_{dk} represents the average paired difference between PBM and Placebo at time point k .

The power analysis for each aim was conducted with a medium Cohen d effect size of 0.5 (medium effect), and a significance level of 0.05. Thus, any p-value less than 0.05 was considered statistically significant.

CHAPTER 4: RESULTS

The effects of acute photobiomodulation on power dynamics

A primary goal of this study was to determine the effect of acute, pre-exercise photobiomodulation on performance in an anaerobic exercise test. One question of interest within this study was to examine the differences in power dynamics during a repeat Wingate test. In the photobiomodulation trial, there was a significantly higher peak power achieved on the first Wingate test ($p=0.046$), but no differences in peak power on the second ($p=0.887$), third ($p=0.544$), or fourth ($p=0.207$) Wingate tests. There was also a significantly higher drop in power during the first Wingate test ($p=0.045$) following the photobiomodulation condition, with no differences for drop in power on the second ($p=0.187$), third ($p=0.858$), or fourth ($p=0.118$) Wingate tests (Figure 5, Tables 2,3,4). There was no difference in average power between conditions. There was a strong, positive correlation between the peak power achieved on the first Wingate test and the decrement in power during that test (Figure 6).

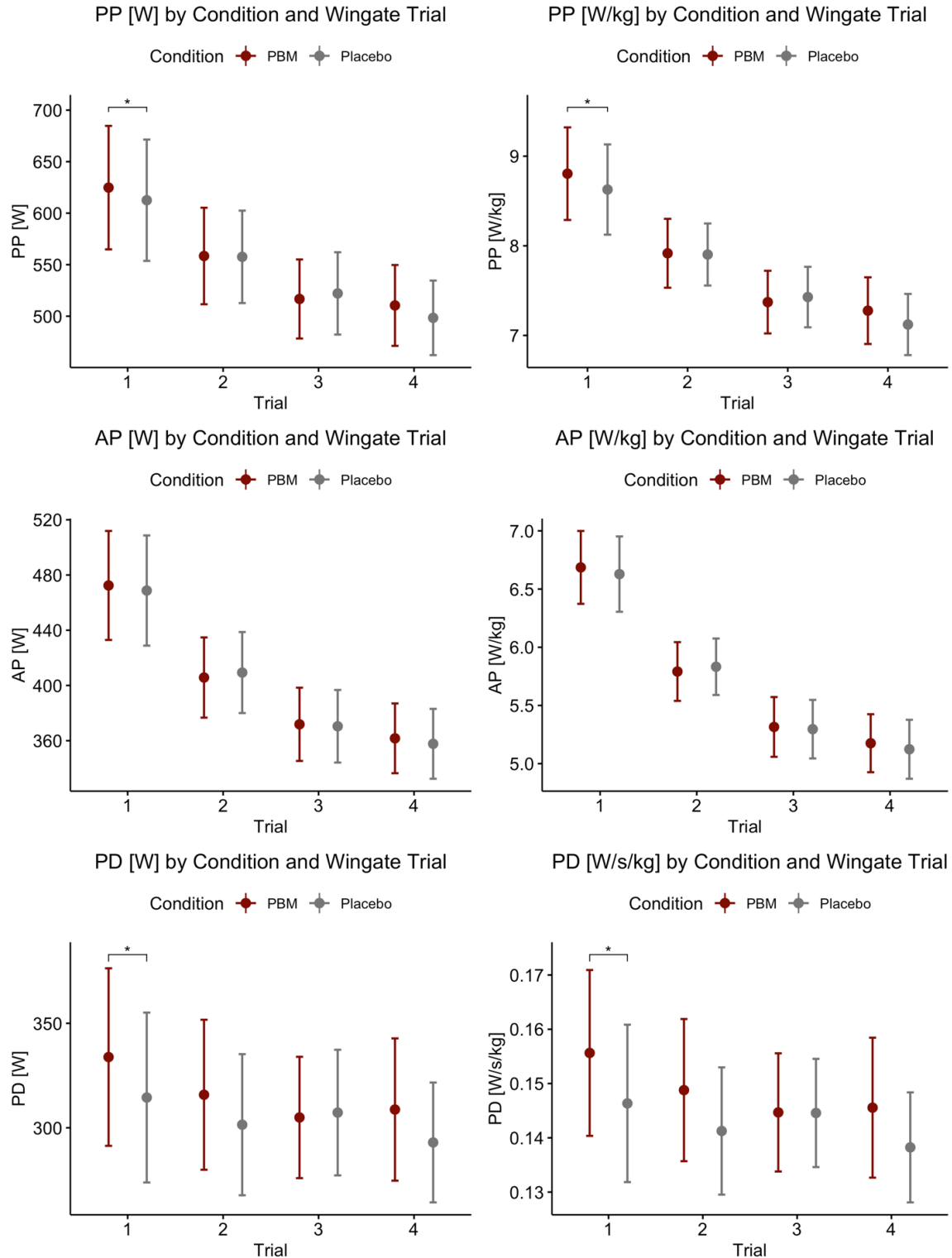


Figure 5. * $p < 0.05$. PP= peak power, AP= average power, PD= power decrement, PBM=photobiomodulation. Comparison of unscaled and scaled power dynamics across all four Wingate tests between photobiomodulation and placebo conditions. Y-axes represent unscaled [W] and scaled for bodyweight [W/s/kg] power dynamics. X-axes represent Wingate 1, 2, 3, and 4 in the repeat Wingate test.

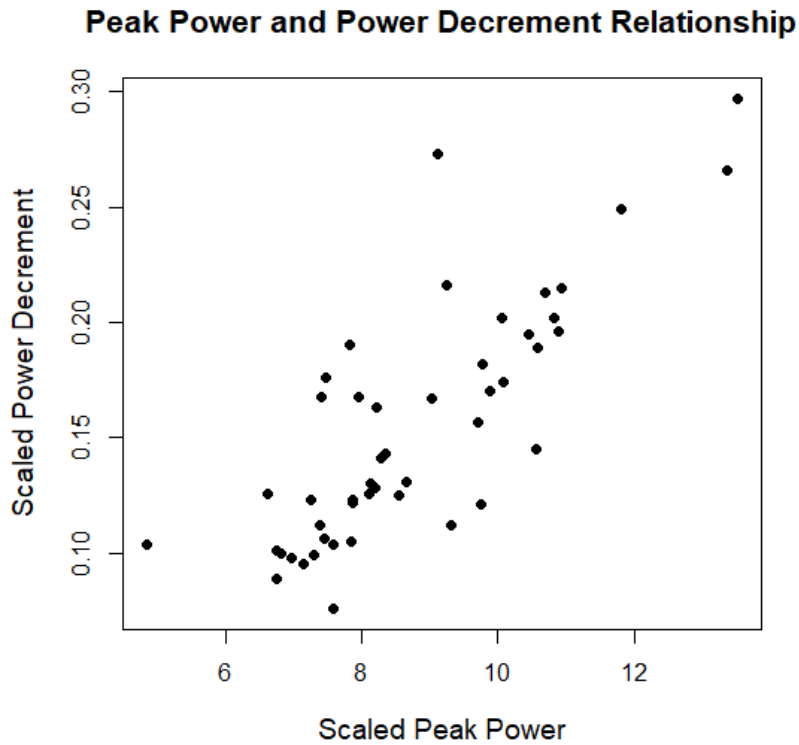


Figure 6. Comparison of scaled peak power and scaled power decrement on the first Wingate test.

Table 2. Minimum (Min.), Median, Mean, Maximum (Max.), and Standard Deviation (SD) for scaled [W/kg] peak power (PP) for photobiomodulation (PBM) and placebo trials in a repeat Wingate test (W1, W2, W3, W4).

Variable	Min.	Median	Mean	Max.	SD
W1 PBM Scaled PP (W/kg)	4.85	8.25	8.766	13.52	1.761
W2 PBM Scaled PP (W/kg)	4.32	7.805	7.886	12.12	1.312
W3 PBM Scaled PP (W/kg)	5.24	7.425	7.352	10.72	1.186
W4 PBM Scaled PP (W/kg)	4.91	7.235	7.258	10.99	1.259
W1 Placebo Scaled PP (W/kg)	4.75	8.215	8.599	13.21	1.709
W2 Placebo	5.17	7.78	7.903	10.83	1.179

Scaled PP (W/kg)					
W3 Placebo Scaled PP (W/kg)	4.84	7.165	7.419	9.96	1.139
W4 Placebo Scaled PP (W/kg)	4.08	6.955	7.131	10.02	1.152

Table 3. Minimum (Min.), Median, Mean, Maximum (Max.), and Standard Deviation (SD) for scaled [W/kg] average power (AP) for photobiomodulation (PBM) and placebo trials in a repeat Wingate test (W1, W2, W3, W4).

Variable	Min.	Median	Mean	Max.	SD
W1 PBM Scaled AP (W/kg)	4.16	6.53	6.659	8.76	1.072
W2 PBM Scaled AP (W/kg)	3.26	5.735	5.78	8.32	0.854
W3 PBM Scaled AP (W/kg)	3.46	5.235	5.303	8.21	0.869
W4 PBM Scaled AP (W/kg)	3.59	5.04	5.169	8.2	0.841
W1 Placebo Scaled AP (W/kg)	3.78	6.525	6.614	8.79	1.095
W2 Placebo Scaled AP (W/kg)	3.79	5.75	5.833	8.27	0.826
W3 Placebo Scaled AP (W/kg)	3.34	5.12	5.303	8.19	0.848
W4 Placebo Scaled AP (W/kg)	3.03	5.025	5.136	8.09	0.857

Table 4. Minimum (Min.), Median, Mean, Maximum (Max.), and Standard Deviation (SD) for scaled [W/kg] power drop (PD) for photobiomodulation (PBM) and placebo trials in a repeat Wingate test (W1, W2, W3, W4).

Variable	Min.	Median	Mean	Max.	SD
W1 PBM Scaled PD (W/kg)	0.076	0.142	0.154	0.297	0.052

W2 PBM Scaled PD (W/kg)	0.074	0.14	0.148	0.275	0.045
W3 PBM Scaled PD (W/kg)	0.082	0.141	0.144	0.26	0.037
W4 PBM Scaled PD (W/kg)	0.081	0.133	0.145	0.283	0.044
W1 Placebo Scaled PD (W/kg)	0.068	0.138	0.145	0.263	0.049
W2 Placebo Scaled PD (W/kg)	0.057	0.133	0.141	0.247	0.04
W3 Placebo Scaled PD (W/kg)	0.077	0.14	0.144	0.23	0.034
W4 Placebo Scaled PD (W/kg)	0.073	0.134	0.138	0.218	0.034

The effect of acute photobiomodulation on blood lactate

Another question of interest was the effect of photobiomodulation on blood lactate levels before, during, and following a repeat Wingate test. There was a significant decrease in blood lactate ($p=0.0001$) following photobiomodulation at rest, prior to beginning exercise (Figure 7, Table 5). There was a trend towards higher blood lactate level following the first ($p=0.077$) Wingate test and a significantly higher blood lactate level following the second ($p=0.048$) Wingate test. There were no differences in blood lactate between groups following the third and fourth Wingate tests and ten minutes following completion of the test.

Lactate AUC Mean and 95% CI across Time

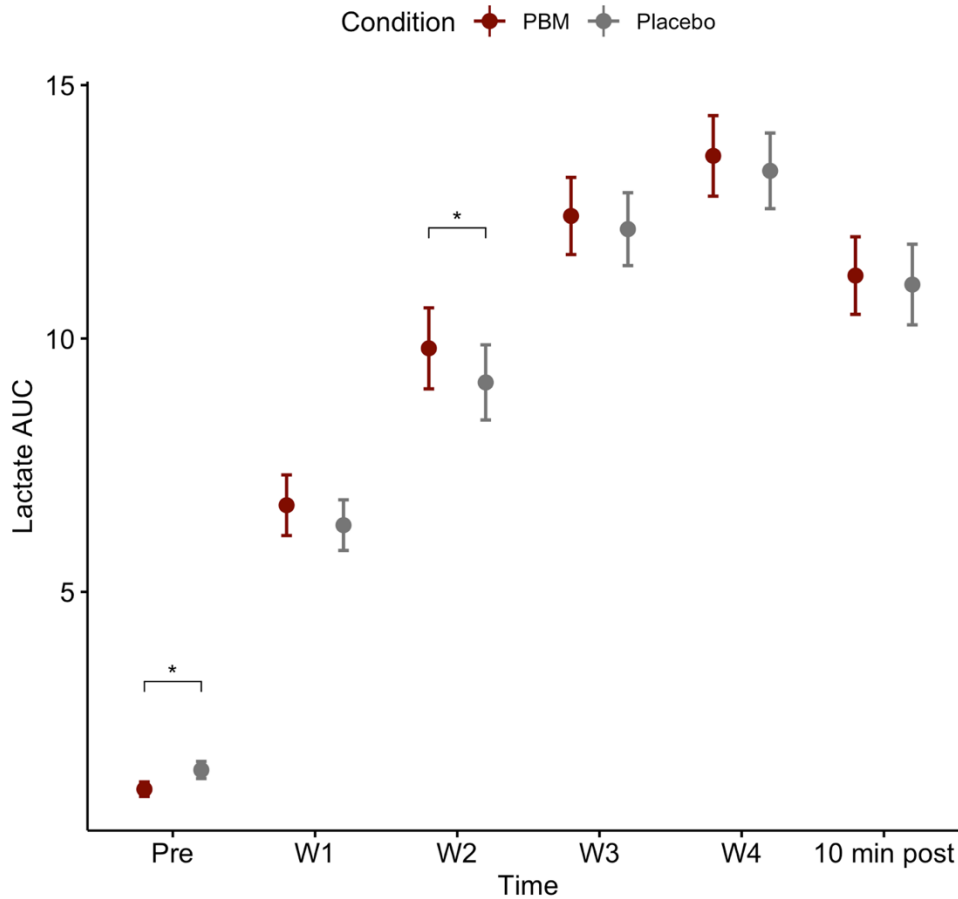


Figure 7. * $p < 0.05$. Lactate AUC= lactate area under the curve, PBM=photobiomodulation. Comparison of blood lactate levels between photobiomodulation and placebo trials. Y-axis represents blood lactate level. X-axis represents time points at which blood lactate was tested. Pre= pre-exercise, W1= immediately after first Wingate test, W2= immediately after second Wingate test, W3= immediately after third Wingate test, W4= immediately after fourth Wingate test, 10 min post= 10 minutes following completion of fourth Wingate test.

Table 5. Minimum (Min), median, mean, maximum (Max), and standard deviation (SD) for blood lactate values for photobiomodulation (PBM) and placebo trials at pre-exercise (Pre), Wingate 1 (W1), Wingate 2 (W2), Wingate 3 (W3), Wingate 4 (W4), and 10 minutes following completion of Wingate 4 (10 min post).

Time	Condition	Min	Median	Mean	Max	SD
Pre	PBM	0.3	1	1.106	2.9	0.495
Pre	Placebo	0.4	1.3	1.485	3.4	0.581
W1	PBM	2.8	6.2	6.71	11.1	2.059
W1	Placebo	3.2	6.15	6.317	11.2	1.721
W2	PBM	5.3	9.6	9.806	15.4	2.753

W2	Placebo	2.8	9.05	9.133	15.1	2.555
W3	PBM	7.9	12.45	12.419	18.5	2.622
W3	Placebo	7	12.5	12.158	16.5	2.477
W4	PBM	9.3	13.45	13.604	19.8	2.737
W4	Placebo	7.4	13.5	13.308	17.5	2.567
10 min post	PBM	5.8	11.45	11.242	17.1	2.631
10 min post	Placebo	5.6	11.05	11.065	16.6	2.742

The effect of photobiomodulation on peak heart rate

Another question of interest was the effect of photobiomodulation on peak heart rate during a maximal, anaerobic exercise test. Peak heart rate was significantly higher on the first ($p=0.009$), second ($p=0.022$), and fourth ($p=0.015$) Wingate tests following photobiomodulation (Figure 8, Table 6). There were no significant differences in peak heart rate pre-exercise ($p=0.077$), on the third Wingate test ($p=0.054$), or ten minutes following completion of the fourth Wingate test ($p=0.997$).

Heart Rate Mean and 95% CI across Time

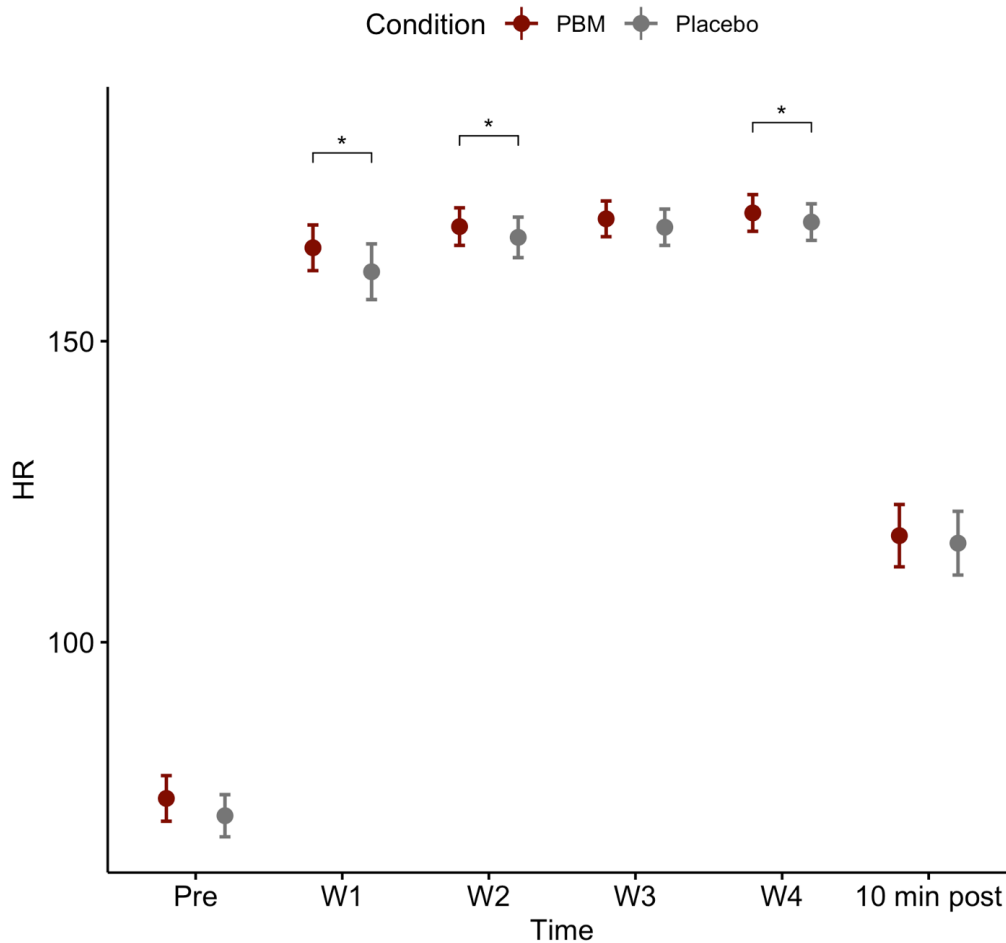


Figure 8. * $p < 0.05$. HR= heart rate, PBM= photobiomodulation. Plot shows the comparison of peak heart rate before, during, and after the repeat Wingate test between photobiomodulation and placebo trials. Y-axis represents heart rate. X-axis represents time points at which heart rate was recorded. Pre= pre-exercise, W1= immediately after first Wingate test, W2= immediately after second Wingate test, W3= immediately after third Wingate test, W4= immediately after fourth Wingate test, 10 min post= 10 minutes following completion of fourth Wingate test.

Table 6. Minimum (Min), median, mean, maximum (Max), and standard deviation (SD) for peak heart rate values for photobiomodulation (PBM) and placebo trials at pre-exercise (Pre), Wingate 1 (W1), Wingate 2 (W2), Wingate 3 (W3), Wingate 4 (W4), and 10 minutes following completion of Wingate 4 (10 min post).

<i>Time</i>	<i>Condition</i>	<i>Min</i>	<i>Median</i>	<i>Mean</i>	<i>Max</i>	<i>SD</i>
Pre	PBM	49	74.5	74.062	112	13.062
W1	PBM	143	165.5	165.521	204	13.066
W2	PBM	149	168.5	169.042	194	10.735
W3	PBM	152	170	170.333	192	10.217

W4	PBM	152	170	171.312	192	10.492
10 min post	PBM	85	118	117.708	156	17.84
Pre	Placebo	52	68	71.188	98	12.079
W1	Placebo	107	160.5	161.542	200	15.936
W2	Placebo	147	166	167.25	195	11.597
W3	Placebo	150	169	168.938	190	10.381
W4	Placebo	152	170	169.792	189	10.455
10 min post	Placebo	68	118	116.458	155	18.227

The effect of acute photobiomodulation on rating of perceived exertion

Another question of interest was the effect of acute photobiomodulation on rating of perceived exertion (RPE). There were no differences in RPE between photobiomodulation and placebo conditions at any time point (Figure 9).

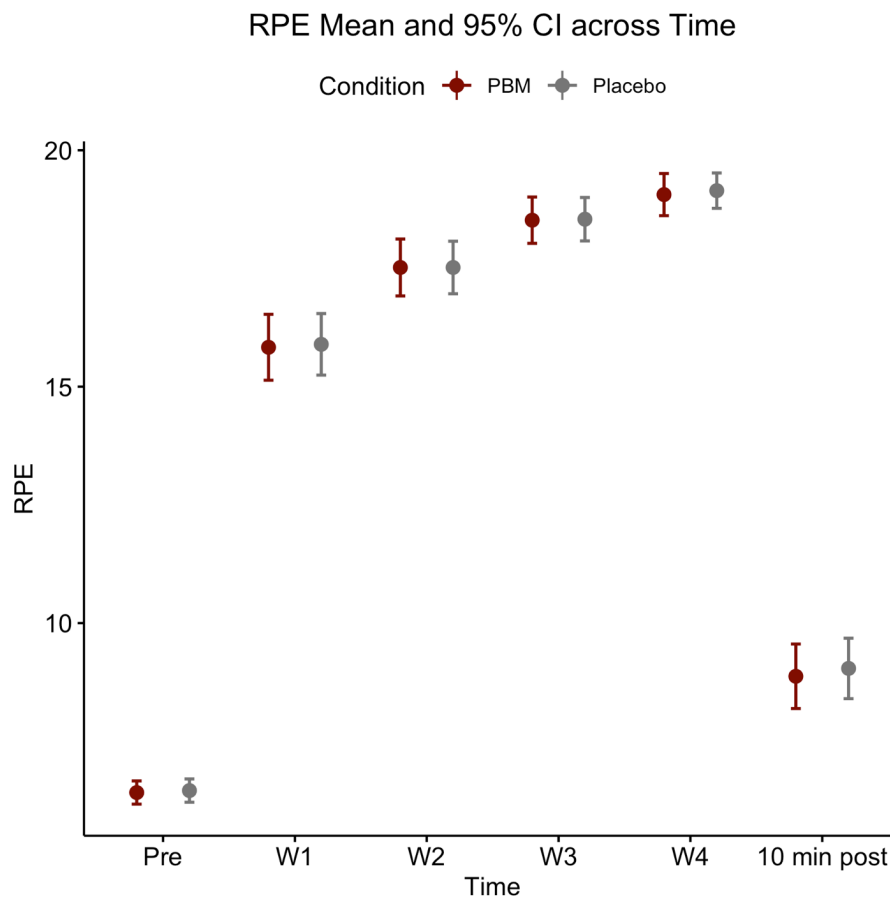


Figure 9. * $p < 0.05$. RPE= rating of perceived exertion, PBM= photobiomodulation. Plot shows the comparison between RPE during photobiomodulation and placebo trials during a repeat Wingate test. Y-axis represents RPE. X-axis represents time points at which RPE

was recorded. Pre= pre-exercise, W1= immediately after first Wingate test, W2= immediately after second Wingate test, W3= immediately after third Wingate test, W4= immediately after fourth Wingate test, 10 min post= 10 minutes following completion of fourth Wingate test.

The effects of acute photobiomodulation on recovery

A secondary goal of this study was to determine the effects of acute photobiomodulation on recovery from a repeat Wingate test. One index of recovery that was measured was heart rate variability. Heart rate variability was measured following the photobiomodulation or placebo condition at rest, before exercise and ten minutes following completion of the fourth Wingate test. Following photobiomodulation, prior to exercise, RMSSD ($p=0.0076$), HF average ($p=0.013$), LF average ($p=0.003$), and total power ($p=0.003$) were significantly lower. There were no differences in LF/HF ($p=0.675$) or VLF average ($p=0.072$). Ten minutes following the fourth Wingate test, there were no significant differences in any heart rate variability measurements (Figure 10).

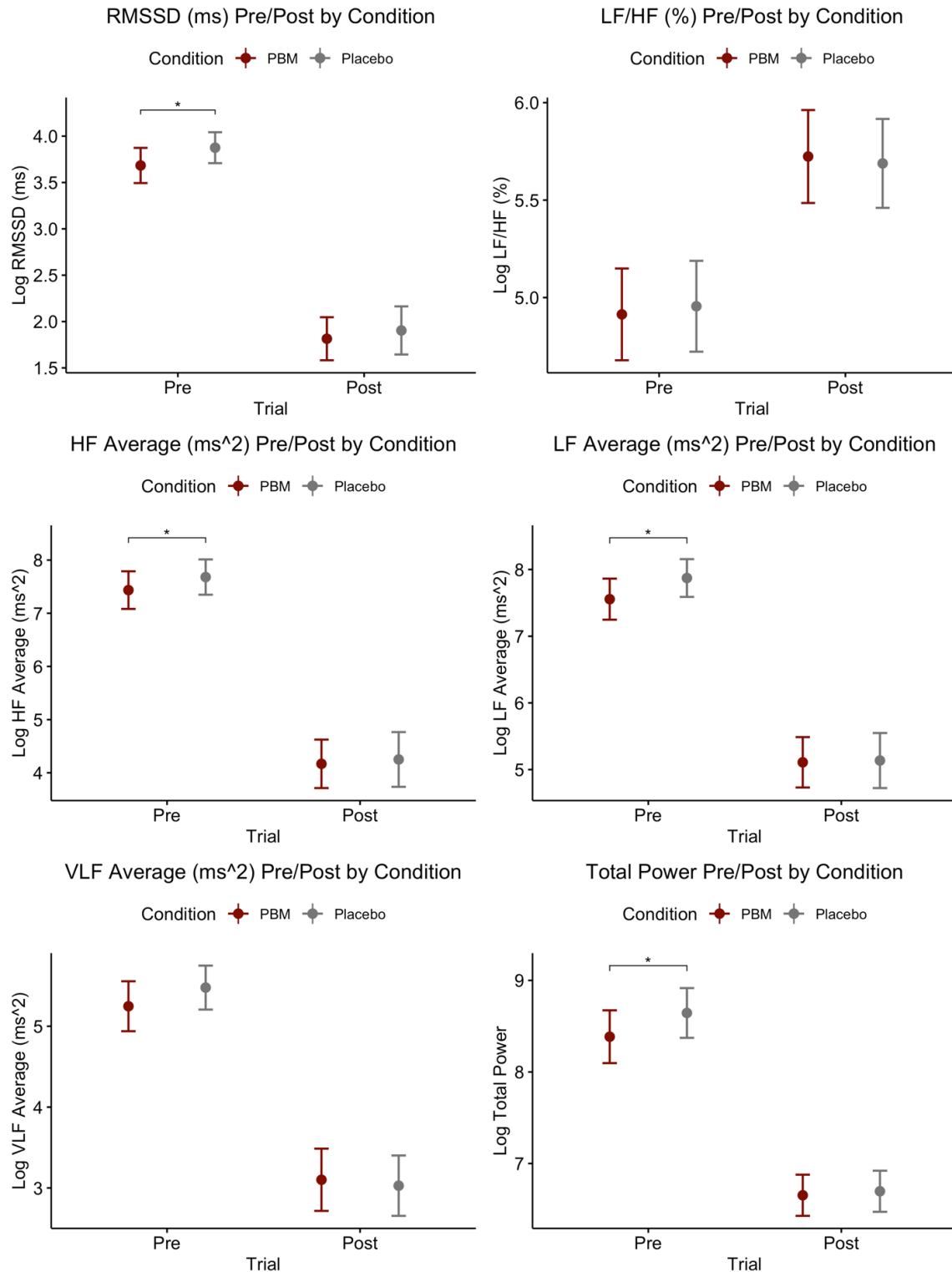


Figure 10. * $p < 0.05$. PBM=photobiomodulation, HF=high frequency band, LF=low frequency band, VLF= very low frequency band, LF/HF=the ratio of low frequency to high frequency power. Plots show comparison of heart rate variability measures following photobiomodulation or placebo conditions, before exercise. Y-axes represent log of each heart rate variability measurement. X-axes represent time point at which heart

rate variability was measured. Pre= immediately pre-exercise, Post= 10 minutes following completion of fourth Wingate test.

Recovery was also assessed on the day following the repeat Wingate test. For this assessment, we looked at participants who received photobiomodulation or placebo condition on the second week as separate groups. We compared changes in heart rate variability and Stress and Recovery scores on the day following the repeat Wingate test from baseline testing in the first week to either photobiomodulation or placebo condition testing in the second week. According to the data, subjects who received photobiomodulation in the second week had a significantly higher heart rate variability than those who received the placebo ($p=0.043$) (Figure 11). There were no differences between Recovery ($p=0.713$) or Stress ($p=0.978$) scores between the two groups (Figure 12).

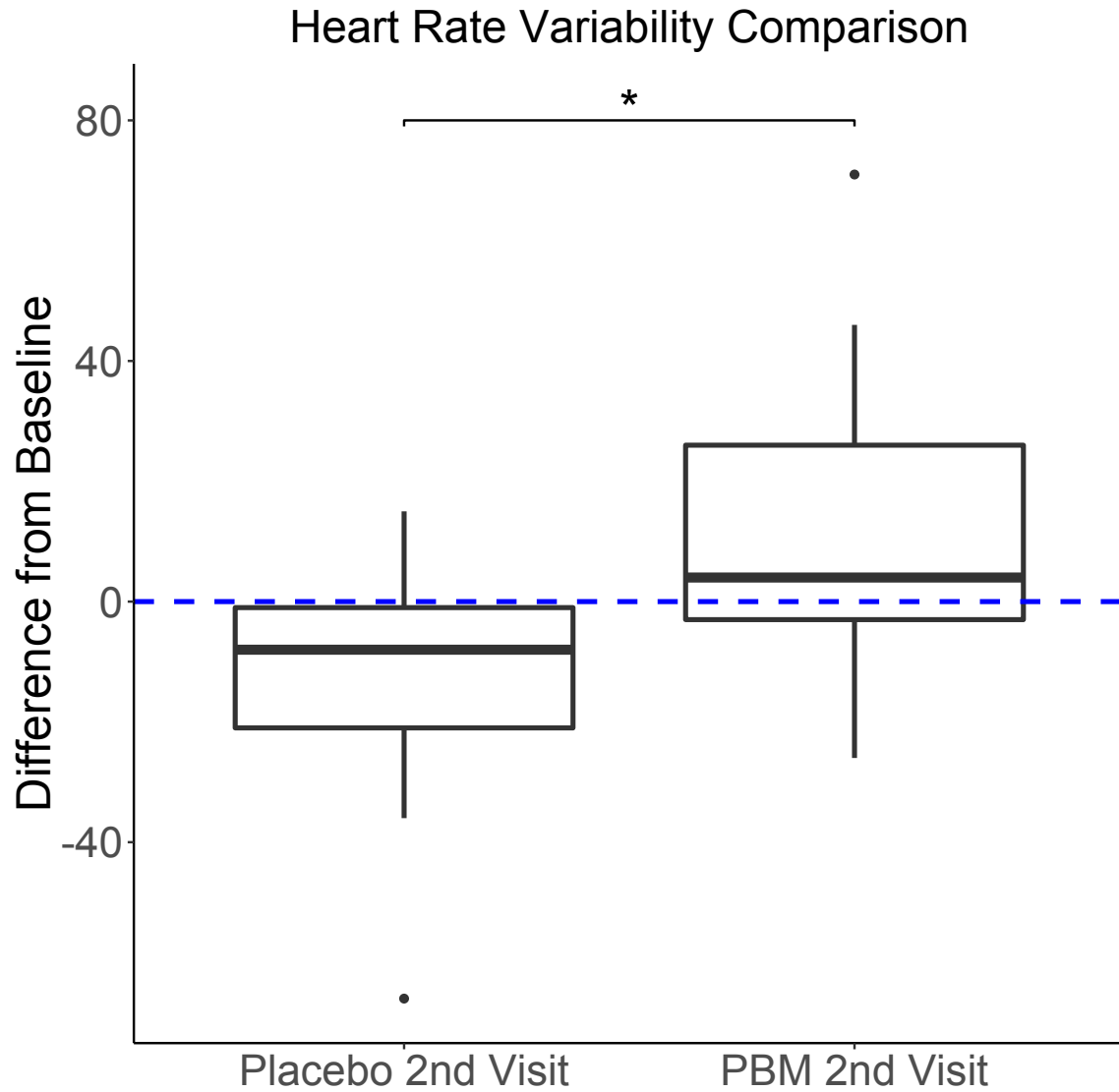


Figure 11. * $p < 0.05$. PBM= photobiomodulation. Plot shows the comparison between heart rate variability from baseline on the morning following the repeat Wingate test to the morning following either placebo or photobiomodulation condition and repeat Wingate test. Y-axis represents the difference between week two and week one. X-axis represents placebo and PBM groups.



Figure 12. * $p < 0.05$. Plot shows the comparison between Recovery and Stress scores from baseline to placebo or photobiomodulation condition. Y-axis represents the difference between recovery and stress scores in week two and week one. X-axis represents placebo and PBM groups.

The effectiveness of the placebo condition

To determine the effectiveness of the placebo condition, we looked at answers to a questionnaire completed following the end of study participation. 58.3% of participants stated that they could detect a difference between the conditions (Figure 13). When asked to describe the difference, 29% of subjects correctly identified which week they received the photobiomodulation condition.

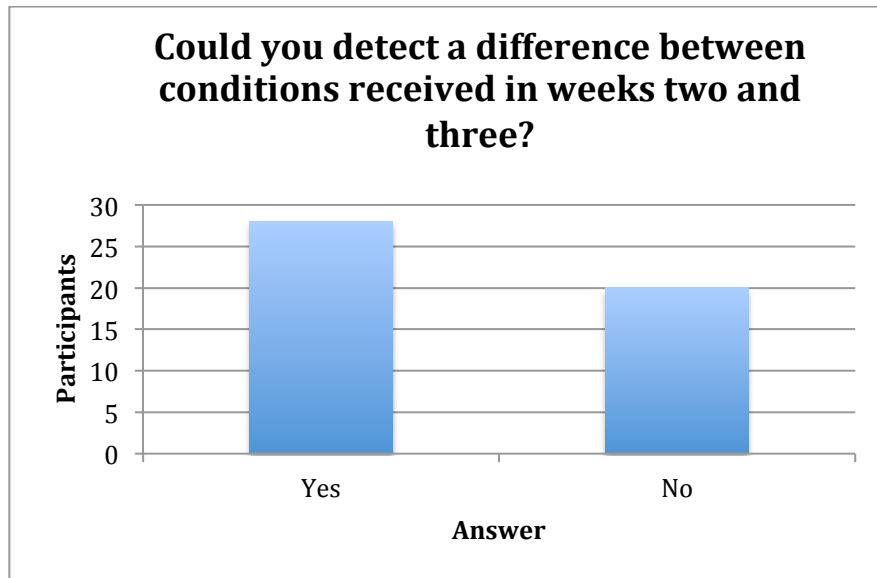


Figure 13. Answers to the question of whether participants could detect a difference between conditions. Y-axis represents the number of participants who answered. X-axis represents the answers, yes or no.

The familiarization effect of the repeat Wingate test on performance and stress and recovery scores

We also looked at whether there was a familiarization effect of the test that would result in changes in performance and stress and recovery scores from the first to second week, when photobiomodulation was not a factor. To examine this, we looked at only the participants that received the placebo condition on the second week of testing. There was a significantly higher peak power on the fourth Wingate test in the second week of testing (Figure 14). There was a great amount of variability in stress and recovery scores but no significant differences between the two weeks (Figure 15). Heart rate variability was significantly lower in week two than week one. Anecdotally, subjects were asked in informal interviews whether the test felt harder or easier on the second week and the majority of subjects responded that the test felt easier on the second week.

Second Visit Placebo Comparison to Baseline

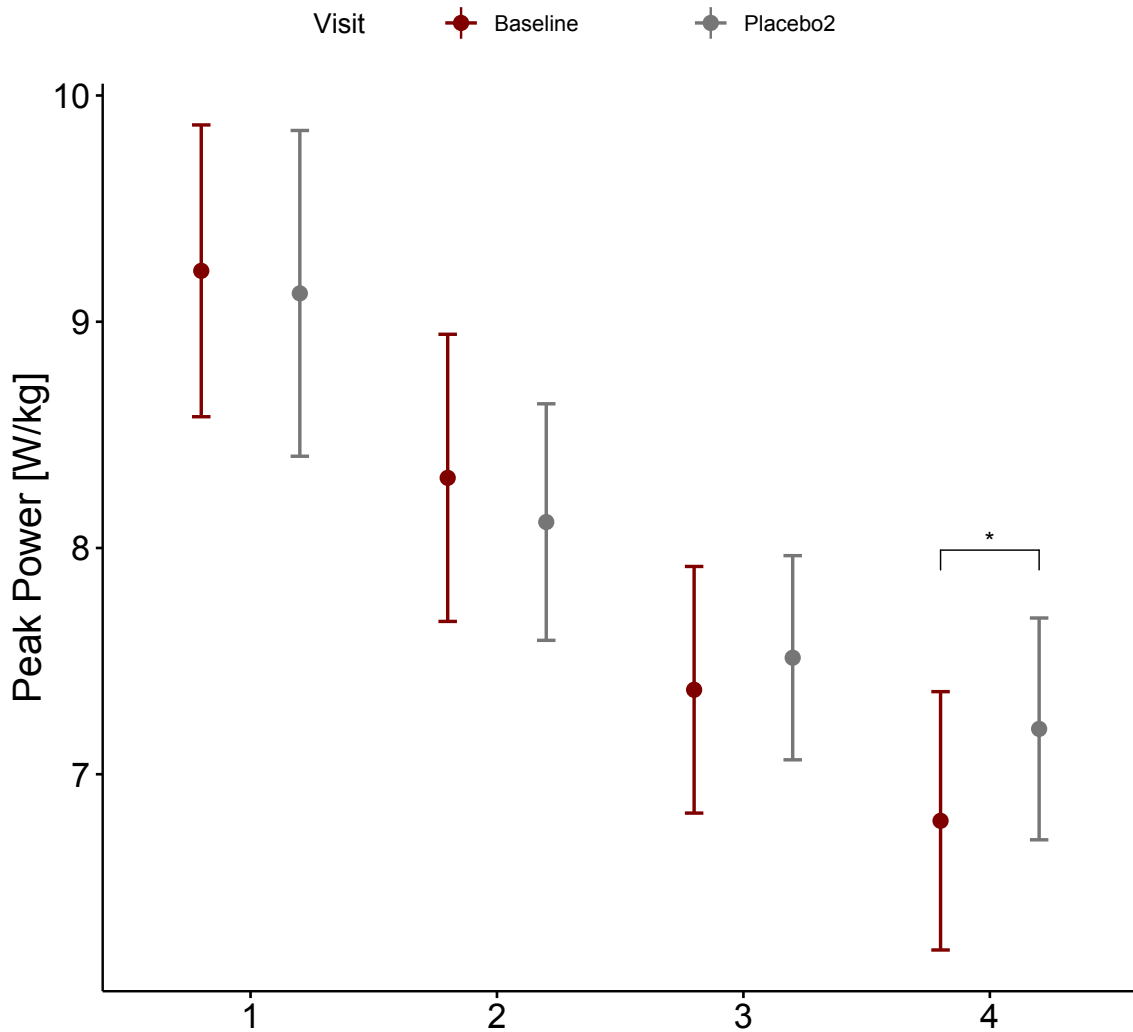


Figure 14. * $p < 0.05$. Plot shows comparison of scaled [W/kg] peak power across the sequence of four Wingate tests from baseline testing during week one and placebo group testing during week two. Y-axis represents scaled peak power [W/kg]. X-axis represents Wingate test number.

Table 7. Comparison of peak power across the sequence of four Wingate tests from baseline testing during week one and placebo group testing during week two.

<i>Variable</i>	<i>Min.</i>	<i>Median</i>	<i>Mean</i>	<i>Max.</i>	<i>t</i>	<i>p-value</i>
Wingate 1	-0.98	-0.23	-0.100	2.18	-0.724	0.476
Wingate 2	-4.92	-0.2	-0.195	2.04	-0.714	0.482
Wingate 3	-1.53	0.055	0.142	3.84	0.683	0.501
Wingate 4	-1.08	0.345	0.406	1.98	2.61	0.016

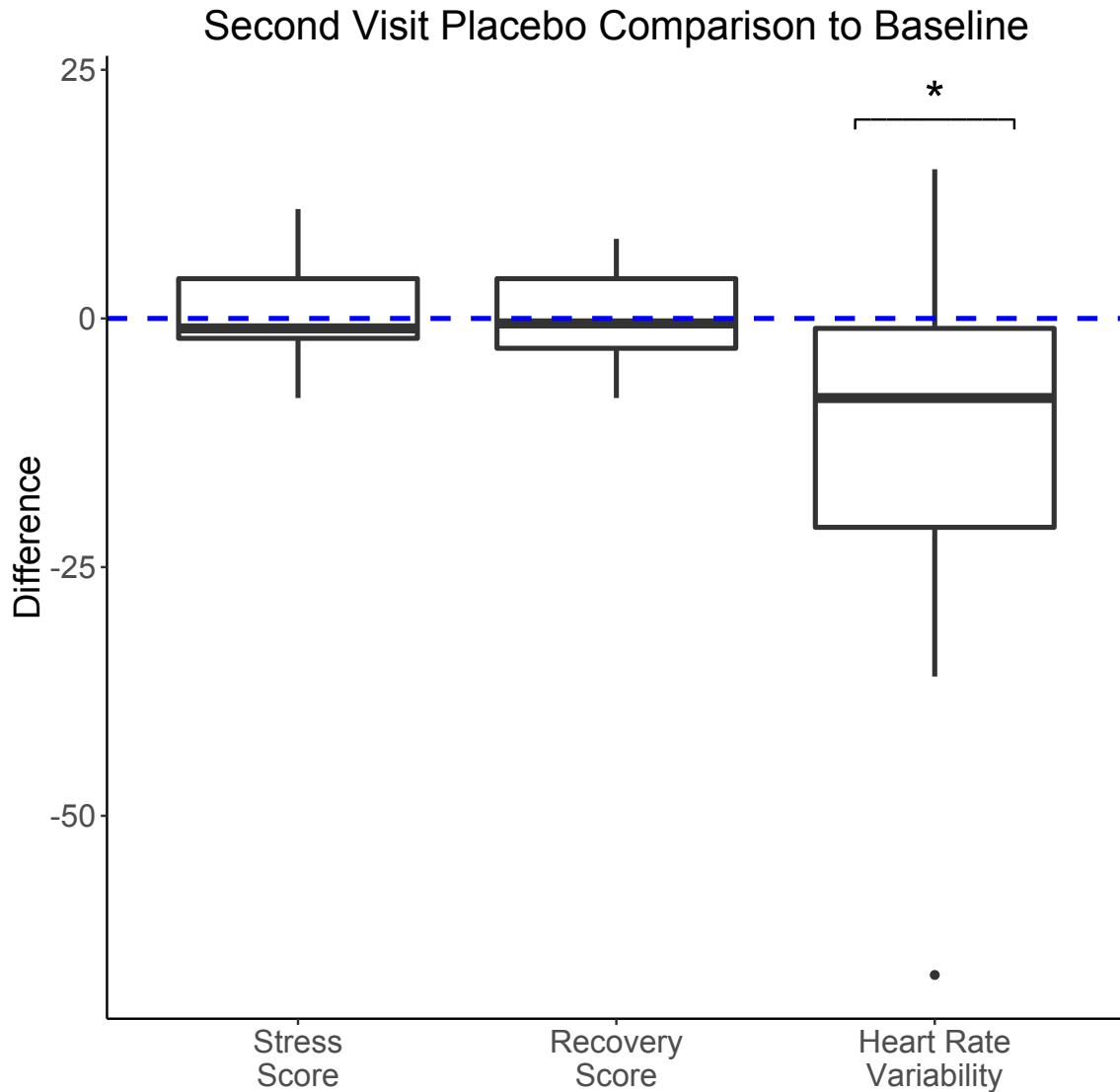


Figure 15. * $p < 0.05$. Comparison of stress, recovery, and heart rate variability scores following baseline testing in week one and placebo group testing in week two. Y-axis represents the difference between week two and week one. X-axis represents scores for stress, recovery, and heart rate variability.

Table 8. Comparison of stress, recovery, and heart rate variability Scores following baseline testing in week one and placebo group testing in week two.

<i>variable</i>	<i>Min.</i>	<i>Median</i>	<i>Mean</i>	<i>Max.</i>	<i>t</i>	<i>p-value</i>
Stress Score	-8	-1	1	11	0.927	0.365
Recovery Score	-8	-0.5	0.35	8	0.356	0.726
Heart Rate Variability	-66	-8	-13.18	15	-2.83	0.014

Variability in Activity and Stress Levels

Study participants were instructed to maintain consistent activity, sleep, and dietary habits throughout the study so we looked at the variability in training load and recovery and stress scores from questionnaires that the subjects filled out daily. There was a large amount of variation in both training load and stress and recovery scores within and between subjects (Figures 16-18).

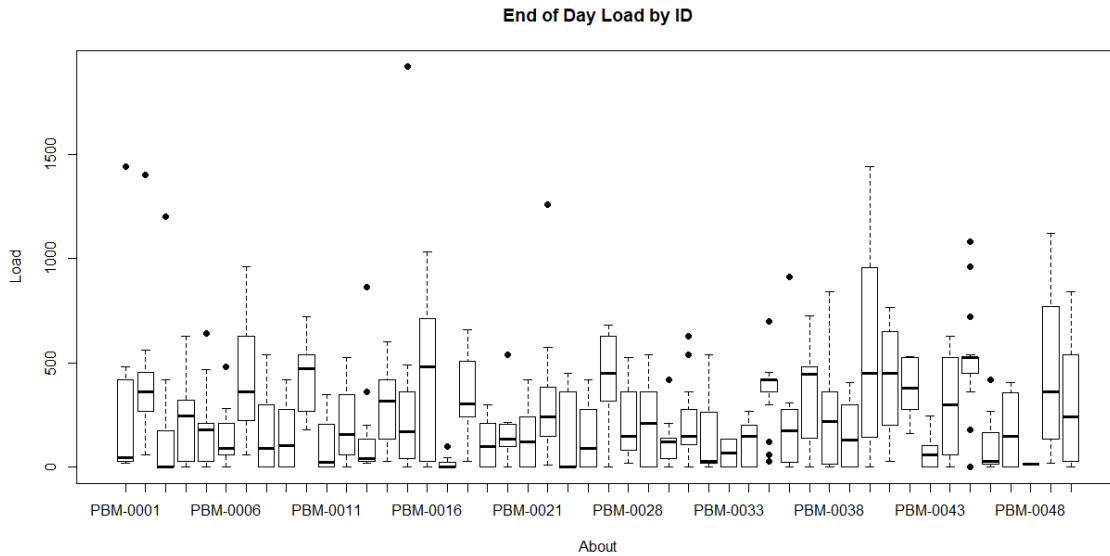


Figure 16. Variation in daily training load for each subject during the two-week duration of the study

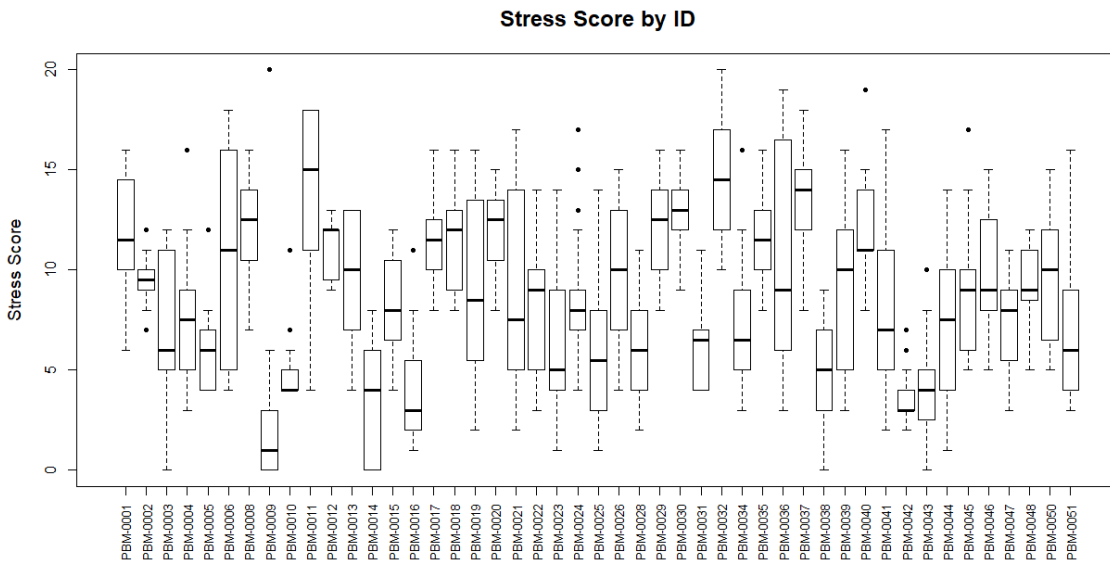


Figure 17. Variation in daily stress scores for each subject during the two-week duration of the study

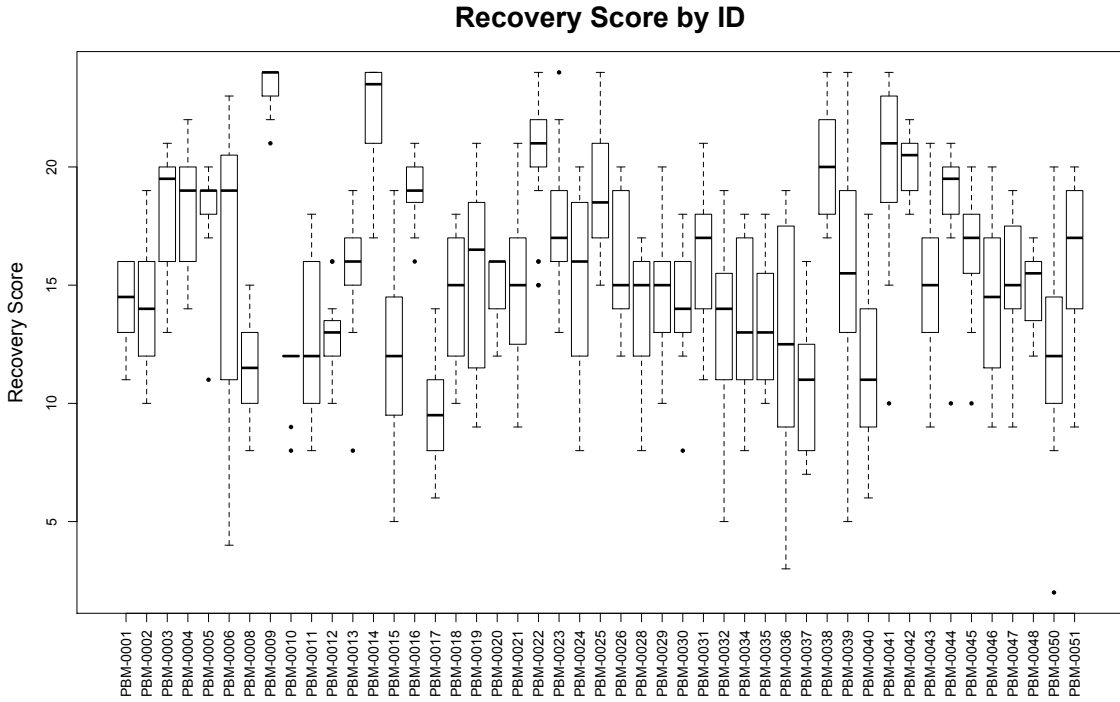


Figure 18. Variation in daily recovery scores for each student during the two-week duration of the study

CHAPTER 5: DISCUSSION

The purpose of this study was to determine the effects of acute photobiomodulation, pre-exercise, on maximal, anaerobic exercise performance. It was hypothesized that pre-exercise photobiomodulation would improve power dynamics in a repeat Wingate test. We found that on the first Wingate test in a series of four repeat Wingate tests, there was an increase in peak power achieved. There was also an increase in power decrement on the first Wingate test. The correlation between increased peak power and increased power decrement can account for the lack of change in average power on the first Wingate test. These results are consistent with the literature on targeted photobiomodulation that showed improvements in anaerobic power following acute photobiomodulation (69,73,74,81,82). However, the majority of these studies included a fatiguing protocol that took place after photobiomodulation and before the anaerobic performance assessment (69,73,74,81). Only Pinto et al. (82) examined the effect of acute, pre-exercise photobiomodulation on anaerobic performance without any sort of fatiguing protocol beforehand, much like the first Wingate test in our series of four Wingate tests. Based on the previous literature that showed better anaerobic performance following a fatiguing protocol when targeted photobiomodulation was employed pre-exercise, we expected that we would see better performance on all four Wingate tests in the series. We had this expectation because the repeat Wingate test serves as both a performance assessment and a fatiguing protocol. Thus, there is a higher degree of fatigue for each successive Wingate test. Based on the previous literature, it was expected that this fatigue would be attenuated following the photobiomodulation condition. Instead, we observed no differences in peak power, average power, or power decrement

between conditions on Wingate tests two, three, and four. There are a couple of potential explanations. The Wingate test is a 30-second, intense bout of cycling that relies primarily on immediate energy from ATP and phosphocreatine from the ATP-PC system (111). These energy compounds become depleted in about 20-30 seconds of maximal exercise (111). Based on this knowledge, it is possible that the increased ATP produced by the photobiomodulation therapy is depleted during the first Wingate test. Therefore, we don't see continued higher power output on the remaining Wingate tests because that ATP has already been depleted. Another potential factor could be due to differences in homogeneity among subjects. In the literature, subjects were fairly homogenous. In the studies that found improvements in anaerobic performance following a fatiguing protocol, subjects were all male and in the 18-36 age range (69,73,74,81). In this study, there was an uneven distribution of sex, with 30 females and 18 males; subjects ranged in age from 18 to 56; and subjects ranged in body fat percentage from 5.5% to 35.2%. Furthermore, despite the instruction to maintain consistent sleep, exercise, and dietary habits throughout the duration of the study, there was a large amount of variation in training load and stress scores between and within subjects. This subject group allows us to examine the effects of photobiomodulation in a broader population than those examined in the literature. This is important because although professional or elite athletes tend to be fairly homogenous, there are other population groups that are less homogenous and still have a need or desire for these types of devices. Therefore, these findings may be of interest to populations such as military high performers or recreational athletes. Furthermore, because the literature is dominated by male subject groups, our

subject group consisting of both sexes allows us to better apply our findings to the population at large.

It was also hypothesized that pre-exercise photobiomodulation would decrease blood lactate levels before, during, and after a repeat Wingate test. We found that following pre-exercise photobiomodulation, lactate was significantly lower before exercise, as expected. There were limited protocols in the literature that tested lactate following administration of photobiomodulation and before exercise but our findings were consistent with De Marchi et al. (94) who also found a significantly lower blood lactate level at this time point as compared to the placebo condition. Following the first Wingate test, lactate was trending higher, and following the second Wingate test, lactate was significantly higher. There were no examples in the literature that tested lactate during exercise. However, lactate increases with increasing workload (20,100). There was a higher peak power and peak heart rate achieved on the first Wingate test in the photobiomodulation condition, which could explain the higher blood lactate levels following the first and second Wingate tests. There were no differences between conditions following the third and fourth Wingate tests or ten minutes following the final Wingate test. Because there were no differences in power dynamics on the second, third and, fourth Wingate tests; it could be expected that lactate levels would even out between groups following the third and fourth Wingate tests. It was expected that there would be lower blood lactate levels in the photobiomodulation trial at ten minutes following the test because the literature consistently showed lower blood lactate levels post-exercise when photobiomodulation was administered pre-exercise (36,61,82,93,96). However, the higher blood lactate following the first and second Wingate tests may partially explain

this finding because lactate was already higher for the photobiomodulation condition and had further to drop. Another factor could be the time point at which we tested. Several studies found significantly or trending lower lactate levels immediately and ten minutes post-exercise, so we expected to see lower lactate at 10 minutes post (36, 82, 93). However, there were also several studies that did not find a significant difference until later (61,96). Leal Junior et al. (96) found blood lactate to be significantly lower at 15 minutes post exercise with no difference between photobiomodulation and placebo groups at 3 and 10 minutes post-exercise. Similarly, Mezzaroba et al. (61) found blood lactate to be significantly lower at 13 and 15 minutes post-exercise, but no difference between photobiomodulation and placebo groups at 0, 3, 5, 7, 9, or 11 minutes post-exercise. If we took a blood lactate measurement at 15 minutes post or another later time point, we may have seen more significant results.

We also hypothesized that peak heart rate would be higher throughout the repeat Wingate test for the photobiomodulation condition. Peak heart rate was significantly higher on the first, second, and fourth Wingate tests. This was expected because, much like blood lactate, heart rate increases with increasing exercise intensity (107). Therefore, as peak power trended higher on the first Wingate, we observed an increase in peak heart rate to correlate with the higher workload. There were no significant differences in RPE, which is likely due to the nature of the exercise test. Participants were consistently reporting very low RPE before the test and near maximal RPE by the end of the test. These results are consistent with the findings of Mezzaroba et al. (61).

A secondary purpose of this study was to examine the recovery effects of photobiomodulation. On testing days, we assessed heart rate variability immediately

before exercise at rest and ten minutes following completion of the last Wingate test. Following photobiomodulation, before exercise, RMSSD, HF average, LF average, and total power were significantly lower. At ten minutes following completion of the last Wingate test, there were no differences in any measures of heart rate variability. There were no protocols in the literature that measured heart rate variability at these time points. We know that heart rate variability is a measure that functions best as an index of recovery when tracked consistently over time and is most accurate when assessed in the morning (43,45,49). Therefore, it is likely that heart rate variability measurements taken immediately pre and post exercise are not good indicators of recovery status.

We also assessed recovery on the day following testing by separating the subjects into two groups: the group that received photobiomodulation in the second week of the study and the group that received the placebo in the second week of the study. The group that received photobiomodulation in the second week had a significantly higher heart rate variability than the group that received the placebo, when compared to baseline. This indicates that photobiomodulation may be an effective recovery tool when used pre-exercise because higher heart rate variability is indicative of better recovery (42,43,44). This is also consistent with the findings of Machado et al. (79), who found an increase in heart rate variability 48 hours following photobiomodulation administered between two 40-minute running time trials. There were no differences in recovery and stress scores from baseline. This is likely because these scores can be affected by other factors, such as general life stress, and were highly variable throughout the study. There was also a significant amount of variability in training load throughout the study. Depending on the timing of higher and lower training load days in respect to testing days, this could have

been a significant factor when looking at the effect of photobiomodulation as a recovery modality.

Following completion of the study, participants completed a questionnaire to help us understand our findings. One question asked was whether subjects maintained consistent activity, sleep, and dietary habits throughout the study. Only 17% of subjects replied that these habits were inconsistent. This conflicted with the variability we saw in daily training loads and recovery scores. Based on this inconsistency, it is possible that participants did not understand the importance of day-to-day consistency. Another question we asked was whether participants could detect a difference between the conditions they received before testing in weeks two and three. About 58% of subjects stated that they could detect a difference between the conditions they received on weeks two and three. However, when asked to describe the difference between the conditions received, only 29% of subjects correctly identified which week they received photobiomodulation. Based on these findings, we determined that our placebo condition was effective.

We also looked at the familiarization effect of the test by looking at changes in power dynamics of those who completed baseline testing the first week and received the placebo condition before testing the second week. Within this cohort, there was a significantly higher peak power on the fourth Wingate test in the second week. Based on these findings, we determined that the subjects were more likely to “save” more energy in the beginning of the test in the second week because they had a better understanding of how hard the test would feel. By starting more conservatively, they fatigued less and were able to reach a higher peak power on the fourth test. In an informal survey

following completion of the test on week two, subjects were asked whether it had felt harder, easier, or the same as the previous week. Most subjects replied that it felt easier in the second week, with many providing reasoning that they felt more mentally prepared. The feeling that it was easier in the second week was likely related to the trend towards lower peak power on the first and second Wingate tests, even if they were not aware that their peak power was lower. This finding is significant because the Wingate test is intended to be a true maximal test. Therefore, if subjects are starting more conservatively on the first Wingate test and have less fatigue accumulated by the fourth Wingate test, we may not be seeing a true picture of how photobiomodulation affects power dynamics in a highly fatigued state. There were no significant differences in stress or recovery scores on the day following testing between week one and two but heart rate variability was trending lower in week two. This lower heart rate variability is not expected based on differences in performance on the repeat Wingate test in weeks one and two. It is possible that these participants had higher activity levels in the days or week prior to the second testing session than the first that may have skewed the results to seem like they were not recovering as well from the test on the second week.

CONCLUSION

In conclusion, in a group of healthy, active adults, acute photobiomodulation may enhance maximal, anaerobic performance, as observed through increased peak power on a Wingate test. Because improvements in peak power were only seen on the first Wingate test in a cumulative series of four, we could not conclude that photobiomodulation attenuates fatigue, but this area deserves further research. Based on differences in heart

rate variability on the day following testing, pre-exercise photobiomodulation may enhance recovery.

LIMITATIONS AND FUTURE DIRECTIONS

The main limitation within this study was our inability to test a wide range of biomarkers. There were several promising biomarkers of interest tested in the literature; including muscle damage markers, reactive oxygen species, and inflammatory markers. These biomarkers are most accurate when tested through venipuncture, which is not a capability our lab had at the beginning of this study. Furthermore, in the literature, these biomarkers were typically tested at several time points up to 72 hours after administration of photobiomodulation. Given the high volume of study participants, short time frame for this study, and limited study personnel; testing this extensive would not have been possible. With increased resources, future research should include testing for muscle damage markers, such as CK and LDH; reactive oxygen species, such as CAT, SOD, TBARS, and carbonylated proteins; and inflammatory markers, such as TNF- α , IL-6, and IL-1 β . In the literature, targeted photobiomodulation had an effect on all of these biomarkers. We are interested in seeing whether whole body photobiomodulation has similar effects on these biomarkers as this will tell us more about the mechanistic effects of photobiomodulation.

Another limitation of this study was that we only tracked heart rate variability, training, and recovery and stress scores for two weeks rather than three. Participants returned the Oura rings and stopped completing the questionnaires on the last testing day, so we did not have recovery data for the day and week following the final testing session. Because of this limitation, when analyzing the recovery effects of photobiomodulation,

we had to split the participants into separate groups based on whether they received photobiomodulation or placebo in the second week, and compare them to baseline. The reasoning behind this decision was concerning resource management and participant compliance. However, in reflection, our analysis would have been stronger if we had maintained the crossover design for this aspect of the study. While the primary goal of this study was to examine the performance effects of acute photobiomodulation, future research should focus on the recovery effects by continuing to monitor recovery indices for a period following the final testing session. Future research focused on recovery should also compare pre and post exercise photobiomodulation to determine which time point has the greatest effect on recovery. Because we saw a lower resting blood lactate after photobiomodulation, pre-exercise, we are now interested in determining if we see a similar effect when photobiomodulation is administered immediately after exercise when blood lactate levels are high.

Another limitation of this study was the lack of consistency in the training and recovery habits of the participants. This lack of consistency likely affected the results both in the performance tests and recovery indices. Future research should provide stricter guidelines for participants to follow in terms of training and recovery to reduce the effect of these factors on study findings. Another limitation in relation to the participants was differing experience riding a bike. Some participants were not comfortable on a bike and thus had trouble giving a maximal effort, which likely affected our results. Future research should use more universal testing methods, such as a treadmill test; include more familiarization trials; or use stricter inclusion and exclusion criteria to insure that participants have experience on a bike.

We are also interested in future research looking at the effects of chronic photobiomodulation. We were interested in the effects of acute photobiomodulation because these effects can be related to the use of photobiomodulation pre-competition. However, the literature on chronic use of photobiomodulation is not as extensive as the literature on acute photobiomodulation. This is an area of interest because it would provide valuable information to athletes and sport teams interested in incorporating regular photobiomodulation into their training programs.

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