

Effects of photobiomodulation therapy (pulsed LASER 904 nm) on muscle oxygenation and performance in exercise-induced skeletal muscle fatigue in young women: a pilot study

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ABSTRACT

Photobiomodulation therapy (PBMt) has been used to increase muscle performance and improve recovery when applied before exercise. We aimed to evaluate the effects of PBMt using LASER on muscle oxygenation and performance. The study was a randomized, participant and assessor-blinded, within-subject crossover trial with placebo control to test the viability of the methods. Five physically active young women were randomly assigned to either placebo, or active PBMt (12 diode cluster probe; 904 nm; 60 mW; 250 Hz; 43.2 J per site, 129.6 J total) in contact over rectus femoris (RF) muscle of the dominant limb immediately before an isokinetic fatigue protocol. A one-week wash-out period preceded cross-over. Electromyography and isokinetic performance measures were evaluated. Absolute concentrations of deoxygenated haemoglobin and myoglobin (deoxy[Hb + Mb]) of the RF, an index of local microvascular fractional O₂ extraction, was monitored continuously by near-infrared spectroscopy (NIRS). Total haemoglobin concentration as an indicator of microvascular haematocrit was calculated as the sum of the deoxy[Hb + Mb] and oxy[Hb + Mb] signals. PBMt pre-conditioning reduced time to peak torque when compared to placebo (P<0.05). PBMt resulted in a noticeably reduced trend in deoxy[Hb + Mb] during exercise compared to placebo (P>0.05). PBMt before exercise improves indicators of muscle performance, potentially by increasing local matching of bulk and microvascular O₂ delivery relative to skeletal muscle O₂ utilisation. Further work is required to understand the effect of PBMt on haemodynamic and metabolic characteristics of muscle.

Keywords: photobiomodulation therapy, LASER therapy, muscle fatigue, deoxygenated haemoglobin, muscle performance, muscle oxygenation

1. INTRODUCTION

Muscle fatigue is defined as a decline in maximal force or power capacity of a muscle¹. The cause of muscle fatigue is multifaceted but the general consensus is that muscle fatigue occurs due to limitations in energy supply leading to the accumulation of metabolites that inhibit the ability of muscle fibres to generate force²⁻⁵. The amount of fatigue is task dependent and can relate to the intensity or duration of exercise, and whether the individual is trained or untrained in the task¹. A person's age or gender may also affect the measured experience of fatigue¹.

Many interventions have been tested to attenuate muscle fatigue. Among these interventions is photobiomodulation (PBM) using low-level LASER, which has emerged as a viable treatment to reduce muscle fatigue and improve muscle performance⁶⁻⁹. PBM therapy (PBMt) with LASER has been shown to reduce muscle fatigue and increase muscle performance in young males, young females and elderly females^{6,8,9}. Leal-Junior et al⁶ found that PBMt before a maximum voluntary contraction (MVC) fatigue protocol of the biceps brachii, enabled young male athletes to achieve more contractions compared to a placebo. Toma et al⁸ found that irradiation with PBM prior to an exercise protocol decreased the fatigue response in elderly women, allowing them to achieve a higher number of isotonic knee flexion-extension repetitions.

Although previous findings are highly promising, the precise mechanism by which PBM decreases muscle fatigue and increases muscle performance is not entirely understood. One theory of how PBM might reduce muscle fatigue is that the treatment increases adenosine triphosphate (ATP) synthesis through upregulation of the electron transport chain (ETC)¹⁰. A second theory for how PBM with LASER might reduce muscle fatigue is that the treatment protects muscle from exercise induced damage¹¹. By understanding the mechanisms, and the clinical outcomes of PBMt pre-conditioning of muscles prior to exercise in normal individuals, it may be possible to translate the findings to people with clinical conditions characterised by muscle fatigue. In particular, it would be useful to understand the effects of PBMt in real time in order to understand treatment latency, efficacy and time-dependent effects.

Previous studies using PBMt for mitigating muscle fatigue after exercise have used either infrared (e.g., 655, 808, 810, 830nm) or red (660nm) wavelengths of light¹¹. Although a range of PBMt wavelengths has been shown to be effective in reducing muscle fatigue, the authors saw the need to study 904nm PBM with LASER therapy due to the possibility of penetrating to deeper tissues as proposed by Joensen et al¹² and its effect on ATP synthesis and consumption of oxygen¹³ and to explore the utility of this wavelength not previously utilised in isolation in earlier studies. In order to better evaluate the effect of the 904nm wavelength in a standardised model as suggested by Leal-Junior et al¹¹, the authors aimed to replicate features from previous published research including the muscle fatigue protocol¹⁴ and associated muscle performance indicators.

In addition to determining the effect of 904nm PBMt on muscle performance characteristics and blood lactate, we aimed to assess the offloading of O₂ from haemoglobin using near-infrared spectroscopy (NIRS) as a means of understanding the possible mechanism of effect. We believe that this is the first time that NIRS has been used in this way to measure the real-time effects of PBMt in muscle tissue.

2. METHODS

The study was a randomized, participant-blinded, within-subject crossover trial with a placebo control. All participants were informed about the purpose and procedures of the study and signed an informed consent declaration before their participation. All procedures were conducted in the Biomechanics Laboratory (School of Allied Health Sciences) at Griffith University (Gold Coast, QLD, Australia) and approved by the Griffith University Human Research Ethics committee (Approval No: 2016/026).

2.1 Participants

Five healthy, female physiotherapy student volunteers (between the ages of 20 and 26) from Griffith University (Gold Coast campus, QLD, Australia) were recruited to participate in the study. Recruitment of volunteers was done via Facebook messenger. Volunteers with a current medical illness, previous musculoskeletal injury of the quadriceps femoris or the hamstring muscles, or pain preventing the conduct of the physical exercise protocol were excluded from the study. Performance athletes and individuals outside of the healthy BMI range (18.5-24.9) were also excluded. Prior to the experiment all volunteers undertook a physical activity readiness questionnaire (PAR-Q), screening questionnaire, and provided written consent.

2.2 Randomisation

Each participant was randomly allocated to begin with either a placebo or PBM treatment at the first session. Randomisation was decided by prior allocation, i.e., the first three participants to arrive at the first testing session, received the PBM treatment, and the placebo in the second testing session. The next participants received the placebo in the first session, and the PBM treatment in the second testing session.

2.3 Order of procedures

Prior to each testing session, the participant's height and weight was determined in order to calculate BMI. Before the protocol began, each participant underwent a test to determine leg dominance¹⁵. Each participant's baseline rating of perceived exertion (RPE), was recorded before and immediately after the fatigue protocol on a 6-20 BORG scale. A 5 minute warm-up period on a bicycle ergometer at 60-70RPM was then undertaken. Following familiarisation with the isokinetic dynamometer, a baseline blood lactate reading was obtained. Surface EMG electrodes were placed on the participant's dominant leg above and below the site of PBMt. A near-infrared spectrophotometer (NIRS) was secured at the site of PBMt. Immediately after the PBM or placebo intervention was applied, each participant commenced a fatigue protocol using an isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY, USA).

Immediately after the fatigue protocol, an RPE measurement was recorded and lactate readings were re-measured (with repeat measures at 3 and 6 minutes after the cessation of the exercise protocol). A one week wash-out period separated the PBMt and placebo arms of the study.

2.4 PBM protocol

A 12 diode ‘super-pulsed’ LASER (Mid-Laser, Irradia AB, Sweden) was used in order to expose a large area of RF muscle tissue during the active intervention. The LASER device parameters and mode of application are detailed in Table 1.

Table 1 PBM with low level laser characteristics and application mode

| Device variable | |
|---|--|
| Number of laser diodes | 12 |
| Wavelength | GaAs, $\lambda = 904 \pm 10$ nm |
| Frequency | Pulsed output |
| Optical Output | 60 mW each diode (total of 720 mW) |
| Spot Size | 0.5 cm ² each spot |
| Energy | 43.2 J on each point (3.6 J for each spot) |
| Treatment time | 60s at each point (180s of total treatment time) |
| Number of irradiation sites per lower limb | 3 |
| Number of irradiation points per lower limb | 36 |
| Total energy delivered per lower limb | 129.6 J |
| Application mode | Probe stationary in skin contact with minimal pressure and perpendicular to skin surface |

The midpoint of the RF muscle was taken as a measure between the anterior superior iliac spine and the centre of the superior border of the patella. Temporary ink markings were made to divide the area into three zones. The intervention was applied at the midpoint of the three zones, in the order of mid-muscle belly, distal third and proximal third of the RF muscle (Figure 1). The order of application enabled efficient application of EMG electrodes and NIRS optodes; and the application of EMG and NIRS was performed by the same researchers (MT & TC) at each session.

Researchers wore safety glasses for each trial for protection from laser irradiation. To ensure subject blinding, each participant wore a blindfold and was asked to close their eyes. To mimic all sounds and movements for each arm of the study, the same pulsed laser device was used for both the placebo and PBM treatment except that the manufacturer-supplied safety cap was used to cover the diodes for the placebo intervention.

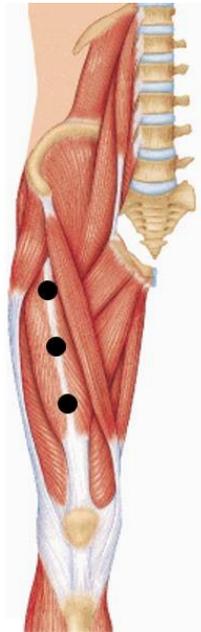


Figure. 1 Treatment points (black circles) over Rectus Femoris muscle

2.5 Fatigue protocol

The protocol for the isokinetic dynamometry was replicated based on previous studies¹⁴. Immediately following a warm-up period, the participants were positioned on the isokinetic dynamometer (System 4 Pro, Biodex Medical Systems, Inc., Shirley, NY, USA) and the final seating position was recorded (chair height, chair distance, back rest distance, and dynamometer base position) to ensure consistent seating positions of participants between sessions.

Once the participant's position had been recorded and confirmed, the dominant leg and torso were stabilised using Velcro straps. For familiarisation, each participant completed 5 trial submaximal voluntary muscle contractions on the isokinetic dynamometer. The muscle contractions were of full range, standardized and pre-programmed motion (90–20°) with a constant angular velocity of 180°/s. The PBMt was applied immediately prior to the fatigue protocol. The fatigue protocol consisted of a set of 60 concentric contractions of the quadriceps muscle in the full range of standardized range of motion (70°) at 180°/s. The participants were visually and verbally encouraged to maintain the exercise effort during the test. The muscle performance was assessed by the fatigue index, total work, average torque, peak torque, the time to peak torque, and average power and lactate production. Concentrations of oxy/deoxy-haemoglobin levels were determined throughout the duration of the exercise protocol using the NIRS.

2.6 Perceived muscular exertion

Participants rated perceived muscular exertion using the BORG rating of perceived exertion scale (range 6–20; 6 = no exertion at all, 9 = very light, 11 = light, 13 = somewhat hard, 20 = maximal exertion)¹⁶⁻¹⁸.

2.7 Blood lactate concentration

Samples of blood for lactate concentration were taken prior to, immediately after, and at 3 and 6 minutes after the fatigue protocol. The same researcher (TC) took lactate samples from the participants' right earlobe using a disposable lancet. The first drop of blood was discarded to avoid sample contamination and then 0.2 µl of capillary blood was analysed using a previously calibrated Lactate Scout+ Analyzer (SensLab GmbH, German). The samples were collected according to the manufacturer's instructions.

2.8 Locomotor skeletal muscle electromyography

Neuromuscular activity of the RF muscle was measured with surface EMG. A pair of silver-silver chloride surface electrodes (2 cm inter-electrode distance) was positioned longitudinally on the participant's dominant RF muscle. The

electrodes were positioned according to European Recommendations for Surface Electromyography of SENIAN project¹⁹ at the mid-point of the RF muscle on the line between the anterior superior iliac spine to the centre of the superior border of the patella. A reference electrode was positioned on the fibula head. Prior to electrode application, the skin was carefully shaved and cleaned with diluted ethanol to minimise skin impedance. The EMG signal was acquired with a bio-amplifier (Octal Bioamp, ML138, ADInstruments) with a bandwidth frequency ranging from 5 to 500 Hz (input impedance = 200 MV, common mode rejection ratio = 85 dB, gain = 1000), transmitted to a computer and analyzed with LabChart 8 software (ADInstruments). The SEMG visualization and digital signal processing were performed by single observer (BJ). The raw SEMG data was digitally filtered at frequency bandwidth of 10–500 Hz and the median frequency (MDF) of the signal used for analysis. The Fast Fourier Transform (FFT) was used to obtain the power spectral density of the EMG signal obtained during 60 reciprocal concentric contractions of the RF muscle. Thus, the values used for the analysis refer to the median frequency of the electromyographic signal (MDF).

The signal obtained during 60 contractions was analyzed in two parts. The values were used to calculate the index of electromyographic fatigue, using the following formula: $EFI = (\text{sum MDF of last contractions} / \text{sum MDF of prior contractions}) * 100$. Higher EFI scores indicate a larger attenuation of muscle fatigue.

2.9 Skeletal muscle deoxygenation and oxygenation

A continuous, near-infrared spectrophotometer (NIRS) (Oxymon Mk III, Artinis Medical Systems BV, Netherlands) was used to measure the local oxygenation and deoxygenation of the RF muscle. NIRS is a relatively inexpensive, non-invasive optical technique for measuring relative local changes in muscle oxygen saturation and blood flow including oxy-haemoglobin and deoxy-haemoglobin in exercising muscle. NIRS is based on the principle that O₂-dependent chromophores (i.e., haemoglobin, Hb; and, myoglobin, Mb) have relatively high light absorption potential in the near-infrared region. The NIRS system consists of two light sources (i.e., transmitters) and two receivers operating at discrete wavelengths (764 nm and 858 nm). Laser diodes (attached to optical fibres) continuously pulse near-infrared light through the tissue of interest (e.g., skeletal muscle), and the receivers transmit the near-infrared light from the tissue to a photon detector (avalanche photodiode) in the spectrophotometer. The discrete wavelengths of near-infrared light permit the differentiation between the relative forms of oxygenated and deoxygenated Hb and Mb (oxy[Hb + Mb] and deoxy[Hb + Mb], respectively). In the present investigation, the deoxy[Hb + Mb] NIRS signal was used to quantify the relative changes in local skeletal muscle deoxygenation.

The optical fibres of the NIRS were fixed in a plastic optode holder, and secured to the skin by adhesive tape. The NIRS optodes were covered by an optically-dense black vinyl sheet to minimise the intrusion of ambient light. The NIRS optodes were positioned longitudinally on the dominant RF muscle just above the EMG electrodes on the ipsilateral limb. The source-detector separation was fixed at 3.5 cm, which corresponded to a measurement depth of ~1.75 cm.

The modified Beer-Lambert law was used to determine the absolute changes in absorption of near-infrared light at each wavelength. The modified Beer-Lambert law incorporates a Differential Pathlength Factor (DPF) to account for the increase in optical path length due to scattering in biological tissue. A fixed DPF value of 4.0 was selected for the present study. This was done to resolve the concentration changes in oxy[Hb + Mb] and deoxy[Hb + Mb], and to accurately calculate the changes in total haemoglobin (tHb). Total haemoglobin is the summation of Δ oxy[Hb + Mb] and Δ deoxy[Hb + Mb]. NIRS data was sampled at 100Hz.

2.10 Statistical analysis

Participants were crossed over during the experiment to ensure bias was reduced between the placebo and PBM treatments. To evaluate if the order of allocation (placebo or PBM) had effects on the variables, EFI and isokinetic outcomes, data was checked for normality (Shapiro-Wilk test) and between-conditions and analysed using a Paired-Samples t-test. Two-way repeated measures ANOVA with Bonferroni adjustments were used to compare changes before and after treatment on the lactate concentration levels and on the ratings of perceived exertion.

The deoxy[Hb] signal was down-sampled to 10Hz in LabChart 8 and converted from mV to micromoles using a conversion factor of 1 volt = 40 micromoles. Resulting data was normalised against time (i.e., 0-100% on the x-axis) into 10 equal bins at 10% intervals. Relative change in absolute deoxy[Hb + Mb] from rest/baseline is reported. Results for PBM and control conditions were compared based on each participant's relative

change in the deoxy[Hb + Mb] signal averaged for each data bin. The significance level used for all comparisons was set at 5 % ($P < 0.05$).

3. RESULTS

PBMt pre-conditioning of the RF prior to a fatigue exercise protocol reduced time to peak torque muscle performance when compared to placebo ($P < 0.05$).

Analysis of lactate concentration levels and ratings of perceived exertion showed no statistically significant differences.

PBMt resulted in a reduced trend in deoxy[Hb + Mb] during exercise compared to placebo ($P > 0.05$) (Figure 2). Similar trends not shown herein were evident for reductions in absolute deoxy[Hb + Mb] and Total[Hb + Mb].

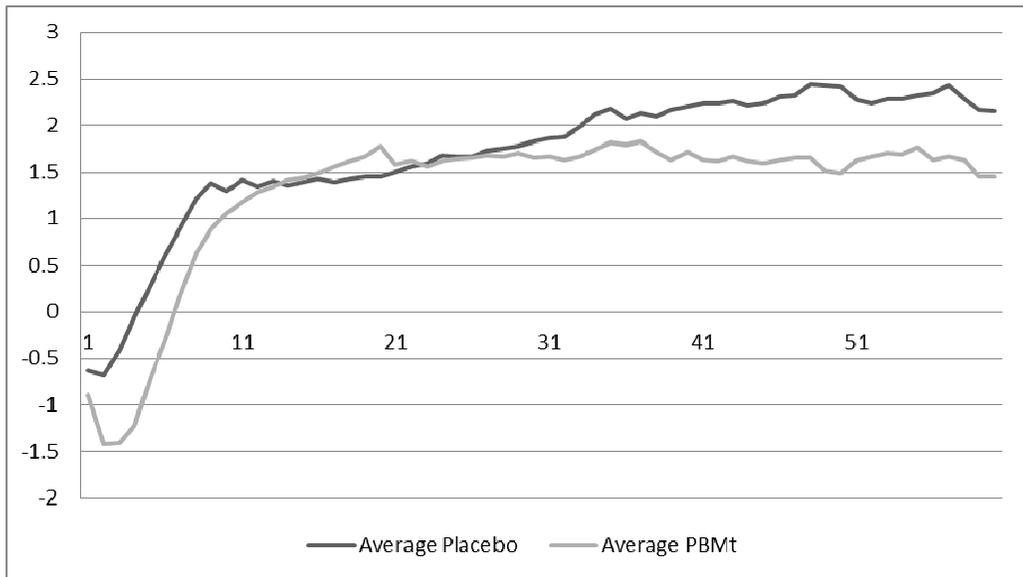


Figure. 2 Relative change (vertical axis) over time (horizontal axis) in deoxy-haemoglobin[Hb+Mb] measures from rest during 60 seconds of isokinetic muscle fatigue protocol.

4. DISCUSSION

This pilot study was designed to replicate previous exercise protocols and to specifically investigate the effects of 904 nm PBM with low level LASER therapy applied using a multiple-diode probe on RF muscle performance in young women. The results were suggestive of beneficial changes in muscle performance as well as providing information about the potential utility of NIRS as a method to investigate real-time physiological effects of PBMt.

The 904nm wavelength was chosen for investigation because it had not previously been used alone in studies analysing the efficacy of PBMt for pre-conditioning of muscles prior to a fatigue protocol. 904nm ‘super-pulsed’ lasers have reportedly demonstrated deeper penetration in animal tissues than some other wavelengths¹² although this has been disputed²¹. Given the diversity of effects seen with different wavelengths (and indeed other light treatment parameters), and the variety of PBM devices available in the marketplace, it is important to understand the capabilities of different parameters of PBM in order to assist in translating findings in to clinical populations.

In this pilot study, 'time to peak torque' performance was the sole indicator (amongst a range of measures that can be used to assess muscle performance²²) that demonstrated a statistically significant difference between the PBMt and placebo control arms. Although other muscle performance measures did not exhibit the definitive effects demonstrated in studies of other wavelengths of light^{e.g.6,8,9}, the difference in this measure between PBMt and placebo interventions, indicates that a physiological response to 904nm PBMt occurs when used to pre-condition muscles that are exercised to fatigue. The result suggests that further research using 904nm PBMt is warranted.

Albuquerque-Pontes et al²³ confirmed that the primary mechanism of increased cytochrome c oxidase expression occurs with the application of LASER PBMt to rat tibialis anterior muscle at a range of energies (1, 3 and 10J) and wavelengths (660, 830 and 905nm). Others have investigated the potential protective effects of PBMt thought to be due to reductions in lactate dehydrogenase (LDH) and creatine kinase (CK). For example, De Marchi et al⁷ found that participants receiving PBMt prior to a progressive exercise protocol had lower LDH levels post-exercise compared to a placebo intervention. LDH catalyses the reduction of pyruvate to lactate, so it would be reasonable to expect that lower LDH may be indicative of lower blood lactate in participants who received PBM with LASER prior to fatiguing exercise⁹. Reduced levels of lactate in response to pre-conditioning of muscle with PBMt have been verified in Leal-Junior's lab on several occasions¹¹. Antonialli et al²⁴ demonstrated that CK levels (as a marker of muscle damage) decreased compared to placebo in healthy untrained males exposed to a combination of PBMt wavelengths and at different energies prior to an isokinetic eccentric exercise protocol.

Our pilot study did not demonstrate any effect on lactate concentration measured from earlobe blood samples nor was there any statistically significant change in participant ratings of perceived exertion. Given that muscle time to peak torque performance was statistically different in the placebo and active PBMt arms of our study, the most logical explanation for the demonstrated lack of statistical difference for other measures of muscle performance is that the study was not powered sufficiently to demonstrate a difference. The authors acknowledge that greater participant numbers are required in order to improve the power of the analyses, and we have already moved in this direction.

Another explanation may be that 904nm PBMt does not have a protective effect which raises the possibility that another physiological mechanism may be contributing to the change in time to peak torque, that of increased ATP synthesis through upregulation of the ETC. Silveira et al¹⁰ found that the application of PBM to rat muscle increased the activity of mitochondrial complexes I, II, III and IV and the enzyme succinate dehydrogenase. The authors posited that if these components of the ETC are upregulated by PBM, then more ATP synthesis would occur and an increase in oxygen delivery to the affected muscle could be observed. Silveira's results added weight to Karu's¹³ findings where, in a study of rat liver, she found that irradiation with light increased ATP synthesis and the consumption of oxygen.

In a clinical setting, the ability to assess and treat effectively and efficiently is a goal for cost-restrained health services and a challenge for translational researchers. Despite changes noted in biochemical markers in previous studies, the ability to demonstrate more immediately and in real time how PBMt might upregulate the ETC in human mitochondria in a clinical setting has not been feasible until recently. With the advent of NIRS, this may now be possible.

In order to investigate real-time oxygen consumption factors as a potential mechanism of the PBM effect of low-level LASER, in our study absolute concentrations of deoxygenated haemoglobin and myoglobin (deoxy[Hb + Mb]) of the RF muscle, were used as an index of local microvascular fractional O₂ extraction, and were monitored continuously during exercise by near-infrared spectroscopy (NIRS). We found that 904nm PBMt resulted in a reduced trend in relative change in absolute deoxy[Hb + Mb] during exercise compared to placebo suggesting that muscle oxygenation changes after the PBM pre-conditioning protocol used in this study. Unexpectedly, the effect was in a negative direction and this outcome requires further investigation including NIRS evaluation of other wavelengths of PBMt.

It is not possible to be more definitive about our results due to the low participant numbers. If however, future research were to find that 904nm PBMt has a selective effect in mitigating certain characteristics of muscle fatigue, it raises the possibility that it (or other specific combinations of PBMt) could be used selectively for personalised medicine in clinical settings. This remains only a hypothetical yet tantalising possibility at this time.

Some methodological matters will require attention prior to further research using NIRS. These factors include knowledge of how the light wavelengths used in the NIRS device (764 nm and 858 nm) may affect muscle oxygenation.

All participants were exposed to the NIRS in small amounts suggesting that any effect from the NIRS device should have been similar across each arm of the study. Also, in attempting to replicate the exercise protocol used in earlier studies, we found that the inclusion of EMG electrodes in close proximity to the NIRS optodes, and the temporal spacing of PBMt with application of the NIRS optodes were difficult to standardise. The placement of the NIRS over the RF muscle also requires further consideration given the anatomical features of that muscle, specifically its tissue make-up and blood supply and distribution.

There are a number of other limitations to the use of NIRS for measuring oxygen delivery and skeletal muscle capacity to utilise oxygen²⁰ which require consideration in determining its further utility for evaluating the outcomes of PBMt. In the meantime however, NIRS is considered to have good reproducibility, sensitivity and agreement with gold standard clinical techniques against which it has been compared²⁰ and so it represents a cost-effective method to evaluate skeletal muscle oxygenation characteristics in dynamic settings.

Lastly, due to the size of NIRS optode field and to better understand the potential utility of NIRS, the authors chose to focus PBMt on the rectus femoris (RF) muscle. Despite using a 12 diode PBM device to focus on the superficial rectus femoris muscle of the quadriceps muscle complex, it is possible that we did not irradiate a sufficiently large amount of muscle tissue to generate a measurable PBM effect. Each of these factors needs to be addressed in future work, and the authors have already made changes in study design and procedures in readiness for further research.

5. CONCLUSION

This pilot study confirms previous work with other wavelengths of light demonstrating that 904nm PBMt preconditioning has an effect on muscle performance, in particular reduced time to peak torque when compared to placebo. Of note, 904nm PBMt resulted in a noticeably reduced trend in deoxy[Hb + Mb] during exercise compared to placebo. PBMt before exercise may affect local matching of bulk and microvascular O₂ delivery relative to skeletal muscle O₂ utilisation. Further work is required to understand the effect of PBMt on haemodynamic and metabolic characteristics of muscle, and NIRS may be an efficient and non-invasive technique for measuring such factors in real time.

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