Photobiomodulation Attenuates Skeletal Muscle Injury and Preserves Contractile Function Following Eccentric Exercise in Mice

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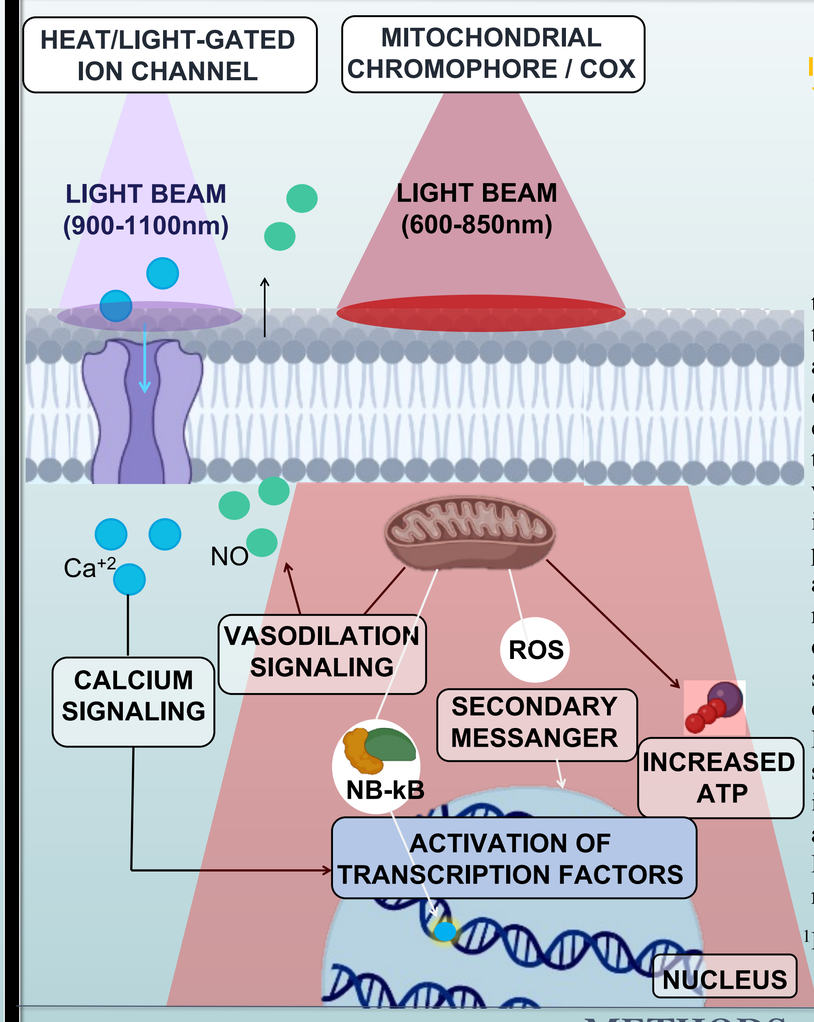
ABSTRACT

Eccentric muscle contractions induce structural damage and functional impairment, with severity influenced by age and exercise modality. Photobiomodulation therapy (PBMT), the application of near infra-red (NIR) light to induce therapeutic effects, has emerged as a potential therapeutic strategy to mitigate muscle; however, its efficacy across injury paradigms remains incompletely defined. The objective of this study is to evaluate PBMT effects on muscle damage following downhill exercise- and contraction-induced injury to the gastrocnemius muscle in mice. 8-week-old mice underwent 4 days of downhill running to induce mild muscle damage, while controlled eccentric contraction (ECC) injury was performed using an *in vivo* set-up to model moderate injury. Injury severity was assessed by plasma creatine kinase (CK), lactate dehydrogenase (LDH), histological analysis, and contractile force measurements. PBMT was applied post-injury. Age-dependent susceptibility was further evaluated in 13-week-old mice subjected to ECC injury. Downhill running in 8-week-old mice produced mild injury, characterized by increased CK without changes in LDH and minimal histological disruption with PBMT reducing CK levels. ECC injury demonstrated age-dependent susceptibility where 13-week-old mice had significant force reduction post injury compared to 8-week-old mice. In 13-week-old mice, PBMT prevented loss of muscle force, reduced CK/LDH to control levels, improved histological outcomes, with reduced myofiber damage and increased immune cell infiltration. In conclusion, PBMT effectively attenuates muscle injury, preserves contractile function, and enhances tissue recovery following eccentric contractions. These findings support PBMT as a therapeutic strategy for mitigating skeletal muscle damage, with potential implications for exercise physiology, aging, and injury rehabilitation.

PHOTOBIOMODULATION



P light **B** living cells **M** exert influence on

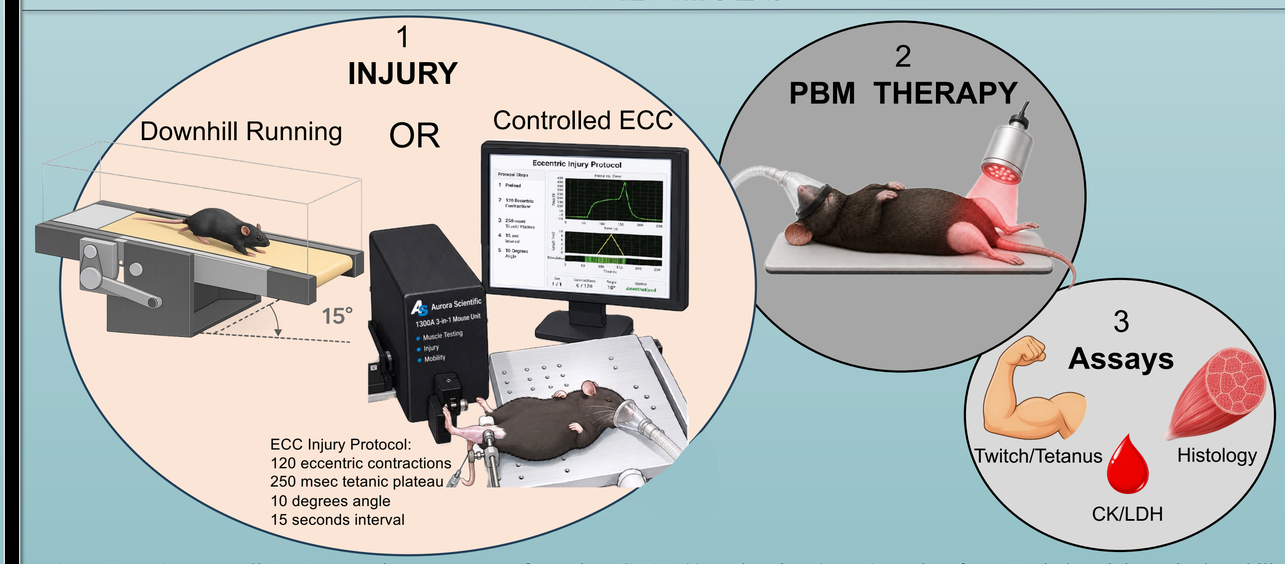
photobiomodulation

describes biochemical reactions that occur in living cells in response to light

The mechanism of PBM is mediated through chromophores present in the cells that absorb energy from light and become activated. The primary mitochondrial chromophore believed to mediate PBM effects, is cytochrome c oxidase (COX), in the electron transport chain, that absorbs wavelengths from 600-900 nm and increases ATP production. Another putative mechanism is energy being absorbed, from longer wavelengths (> 900 nm), by water and light-sensitive ion channels which open upon activation and stimulate cellular responses affecting energy and oxidative stress homeostasis[1]. PBM has become a common practice in sports medicine and is used to reduce inflammation, muscular injuries, fatigue and prevent loss in performance. However, the biological mechanisms are not clearly understood.

[1]Hamblin, M.R. (2018). *Photochem Photobiol*, 94 (2): 199-212. PMID: 29164625

METHODS



Animal Experiments: All *in vivo* experiments were performed on C57BL/6J male mice, 8 or 13 weeks of age. To induce injury via downhill running, mice ran **downhill at a 15 degree decline**. In acute sessions, time to exhaustion was noted. The point of exhaustion is defined when the mouse has been shocked 10 consecutive times on the shock grid and is unable to run further ahead or stays within the fatigue zone, the bottom area of the treadmill lane, for longer than 10 seconds. In training experiments, mice ran for 40 mins bouts for 4 days. **Eccentric contraction injury (ECC)** was induced via 120 ECC's stimulated by 3-1 mouse setup by Aurora Scientific Inc. For irradiation treatment we used OmniLuxPlus LED panels with **830nm LEDs**. Mice were anesthetized prior to treatment for irradiation and hair removal from the lower limbs to allow penetration of light into the muscles. Body temperature was monitored to keep it constant between control and treated mice. 24 hours post ECC injury, *in vivo* **twitch and tetanic force production** was measured for gastrocnemius muscle. Submandiubular **blood** draws were performed prior to euthanasia. Plasma was isolated and Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) activity was determined via Element DCFX Chemistry Analyzer. Soelus and gastrocnemius muscle was isolated for **histology**. For all experiments, N=4-6 mice and student t-tests and one way ANOVA when multiple groups present was performed. Statistical significance is indicated as $p < 0.05$, *p < 0.05, **p < 0.001, ***p < 0.0001; ns, not significant. All data is reported as mean ± SD.

RESULTS

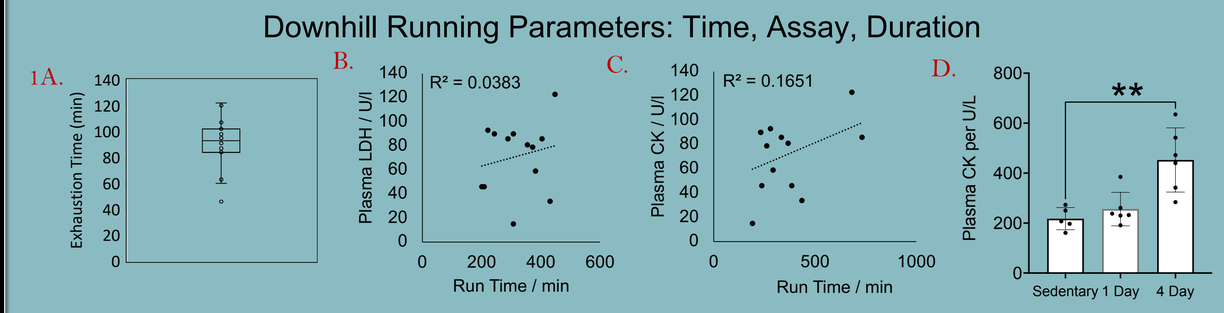


Figure 1. Downhill running for 40 mins sessions in 4 days increases plasma CK levels (A) 8-week-old mice were ran till exhaustion with a 1 bout downhill running session. The minimum run time was at 40 mins and maximum run time at 120 mins **(B-C)** LDH and CK plasma levels do not show any change with run time however, with CK, the lowest and highest CK levels correlated with downhill run times **(D)** Running time kept constant at 40 mins per bout in mice that did training for 4 days. Plasma CK levels increase with 4 consecutive days of running with no difference with 1 bout of running (i.e 1 Day).

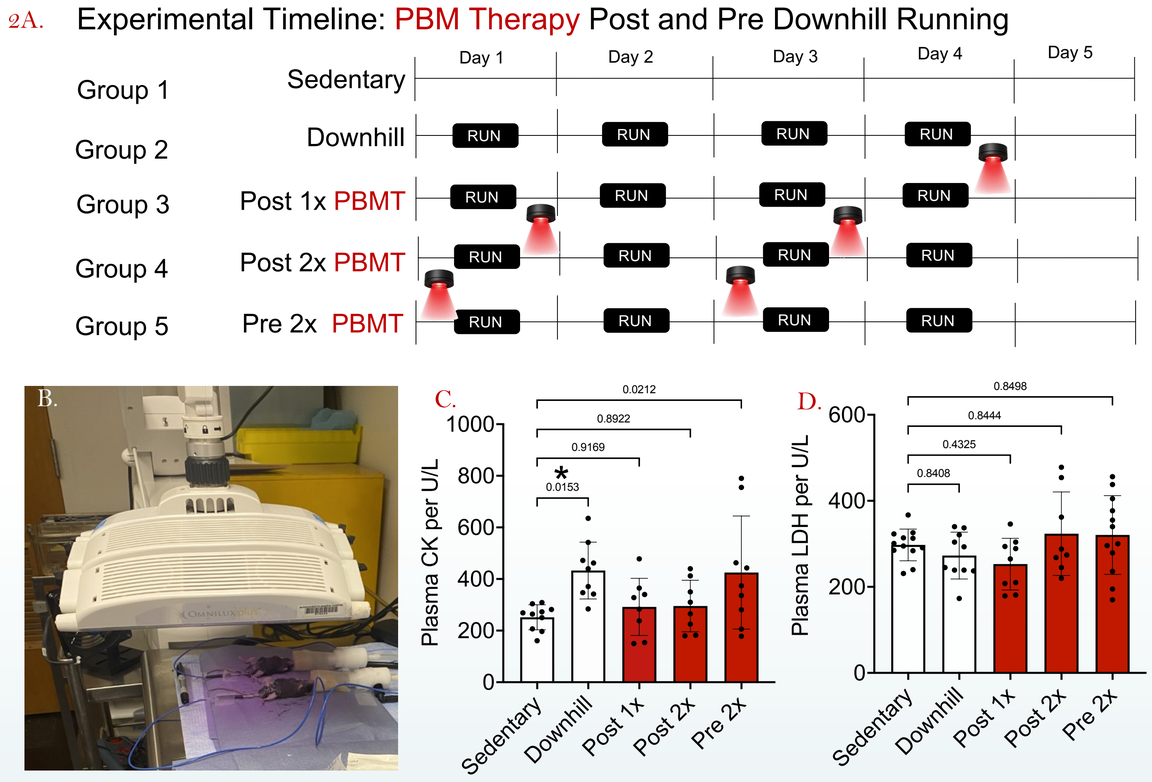


Figure 2. PBMT post run lowers plasma CK to basal levels with no changes to LDH in 8-week-old mice. (A) Schematic of experimental timeline and group allotment. Group 1 and 2 (non PBMT) were sedentary and downhill running. Groups 3-5 had PBMT pre or post running. Each run session per day was 40 mins. **(B)** The LED setup used for treatment. Mice were anesthetized and the hair from their lower limbs are removed by shaving/ hair removal cream. Mice were treated with the OmniLux panels at 25mW/cm2 for 20mins (30J/cm2). **(C-D)** Plasma CK and LDH levels in all groups **(E)** Mild histological damage in soleus of downhill running groups with no difference between downhill and PBMT.

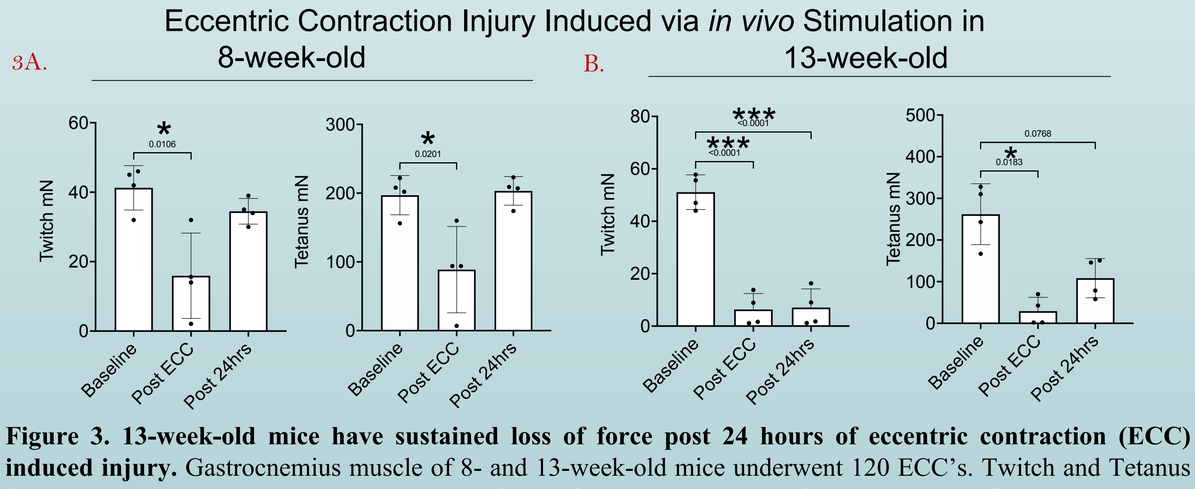


Figure 3. 13-week-old mice have sustained loss of force post 24 hours of eccentric contraction (ECC) induced injury. Gastrocnemius muscle of 8- and 13-week-old mice underwent 120 ECC's. Twitch and Tetanus force of gastrocnemius muscle was measured fir **(A)** 8-week-old and **(B)** 13-week-old mice. In both ages, force was measured for baseline pre-injury, immediately post ECC injury and 24 hours post ECC injury.

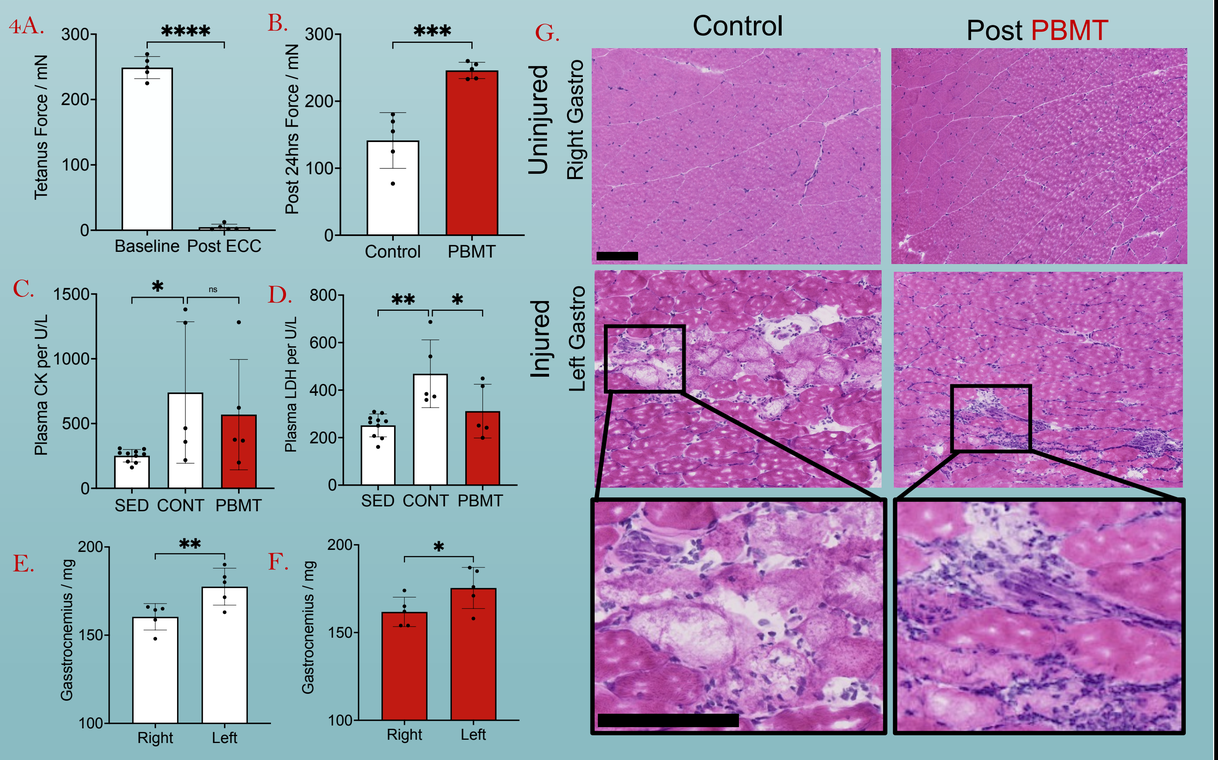


Figure 4. PBMT preserves muscle function, reduces LDH and myofiber disruption in ECC injury in 13-week-old mice. (A) ECC injury reduced tetanic force at 24 h post-ECC. **(B)** PBMT preserved tetanic force at 24 h compared with injured controls. **(C–D)** Plasma CK and LDH were elevated with injury when compared to sedentary (SED); PBMT lowered LDH and had no difference in plasma CK levels. **(E–F)** Injured left gastrocnemius was larger than uninjured right gastrocnemius, in control **(E)** and PBMT **(F). (G)** Representative H&E-stained show that injured left gastrocnemius from untreated controls have disrupted myofibers, inflammatory cell infiltration, and areas of degeneration, whereas PBMT has improved tissue architecture with reduced myofiber disruption. Scale bar is presented as 100um.

CONCLUSIONS

- Chronic downhill running in 8-week-old mice caused mild damage with increase in plasma CK and histological pathology. Post treatment of PBMT resulted in reduced plasma CK levels
- Controlled ECC injury in 13-week-old mice resulted in sustained loss of gastrocnemius force post 24hrs injury whereas 8-week-old mice recovered force generation
- PBMT in 13-week-old mice that underwent injury resulted in maintained force, reduced CK and prevented histological muscle fiber damage.

ACKNOWLEDGMENTS

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