# **SUPPLEMENTARY FILE 2**

**Photobiomodulation Therapy (PBMT)**

Photobiomodulation Therapy (PBMT), also known as photobiostimulation and phototherapy (1), is a nonthermal process in which light interacts with photo-signal transducers (i.e., chromophores), leading to photophysical and photochemical reactions within tissues (2, 3). PBMT uses non-ionizing light sources, including lasers (low-level laser therapy - LLLT), light emitting diodes (light emitting-diode therapy - LEDT), and broadband light from the visible to the infrared spectrum (4), to therapeutically assist in skin wound healing (5) and tissue regeneration in muscle (6), tendon (7), nerve (8), and cartilage (9). It may also positively influence both pain (10) and inflammatory responses (11), as well as reduce the central sensitization associated with chronic osteoarthritis (9). The magnitude of effect of PBMT is influenced by several factors, including the light wavelength, energy density (or fluence) and power density as well as the injury type and the photoreceptor absorption spectrum (12-14).

In addition to the healing process, studies in animal and human models have examined the effects of LLLT and LEDT applied before exercise on muscle performance and fatigue. Lopes-Martins et al. (15) observed reduced post-NMES muscle fatigue in isolated rat muscles exposed to phototherapy. Subsequent studies support these findings in frog (16) and mouse (17) muscles as well as in cell cultures (18). In humans, Leal-Junior et al. (19) found that individuals performed more elbow flexion-extension repetitions before task failure immediately after LLLT than placebo. Several studies subsequently assessed the effects of imposing LLLT and/or LEDT before performing isoinertial (20-23), isometric (24-26) and isokinetic exercise (27-29) as well as cycling exercise (30-33), treadmill running (34-36) and futsal (i.e. indoor soccer)(37). Positive effects of LLLT and/or LEDT before exercise on performance and/or recovery were consistently observed.

Muscle fatigue, an important consequence of NMES, is a complex process involving physiological, biomechanical, and psychological elements (38-43). The loss of muscle function results from changes at several sites within the muscle that contribute to impairment as well as to several intervening factors that may alter muscle fatigue. These include metabolic alterations such as substrate depletion (lack of ATP and glycogen), inorganic phosphate accumulation, increased oxidative stress and reactive oxygen metabolite-derived compounds, tissue hypoxia, blood acidification and reduced biological antioxidant potential (38, 44). Exercise intensity and duration as well as age, gender, motivation, and previous task knowledge/experience are important factors increasing the complexity of the fatigue process. Despite this, strategies that enhance the response to, or recovery from, NMES-evoked fatigue will be well important in the clinical context, as they should subsequently increase the chance of evoking positive outcomes from NMES use.

The effect of PBMT on muscle fatigue likely results from direct light-tissue interactions and the phenomena generated by this interaction on blood circulation and mitochondrial function. BPMT has been shown to improve peripheral microcirculation and promote arteriolar vasodilation, for example (45, 46). Consequently, increased muscle blood and oxygen supply may allow for improved performance in aerobic activities and reduce blood lactate accumulation (15, 21, 27, 28, 47), although a lack of effect of laser therapy on blood lactate accumulation after exercise has also been observed (19, 48). PBMT has been shown to improve mitochondrial function in several cell types and organelles. The interaction between LLLT and mitochondria has been a particular research focus, with a stimulatory effect on the mitochondrial capacity to generate ATP being observed (49-51).

According to Borsa et al. (52), chromophores absorb light particles (i.e., photons) at the plasma membrane or at cytosolic organelles (e.g., mitochondria). At the plasma membrane, chromophores act as photosensitizers that change membrane permeability and cellular transport, leading to intracellular changes in pH, ion concentrations, and membrane excitability (53). Photons that are able to penetrate the cell membrane enter the mitochondria where cytochrome enzymes (e.g., cytochrome c oxidase) absorb them. These enzymes, in turn, lead to the production of reactive oxygen species (ROS), and increase adenosine triphosphate (ATP) and protein synthesis rates (3, 51, 54). Evidence in support of the above mechanism has been provided by cell culture studies, which have shown that LLLT increases ATP synthesis, mitochondrial membrane potential and intracellular calcium levels, and stimulates ROS production and nitric oxide release with a biphasic pattern in normal murine cortical neurons (55-57). Animal models have also shown laser interventions to decrease oxidative stress (58, 59). Xu et al. (18), for example, verified that laser treatment significantly decreased ROS production and restored mitochondrial function. In addition to these functional changes at the cell membrane and cytosolic organelles, Manteifel et al. (60) observed giant mitochondria in human lymphocytes after laser irradiation that could produce greater ATP levels. Such results suggest that PBMT alters mitochondrial structure and function, allowing for an increase in cellular energy production that may consequently reduce fatigue or aid recovery.

Ferraresi et al. (61) raised several questions regarding the use of PBMT (e.g., wavelengths, best time to apply on muscles, best parameters, how many points to irradiate) for improving sports performance. Leal-Junior et al (4) proposed clinical and scientific recommendations for what they believe is the correct use of PBMT in exercise performance enhancement and post-exercise recovery. Their recommendations for healthy subjects bare repeating in the present Supplementary file for consideration in future research: (1) dose of 20-60 J for small muscle groups (i.e. biceps brachii or triceps surae), and 60-300 J for large muscle groups (i.e. quadriceps and hamstrings); (2) power of 50-200 mW per diode for single probes and 10-35 mW per diode for cluster probes; (3) wavelengths of 640 nm (red) - 950 nm (infrared), although most studies combine red and infrared wavelengths; (4) and either pulsed or continuous mode. The authors also suggest that therapy is used 5 min to 6 h before activity for acute effects (a single event), but 5 to 10 min before each exercise session for chronic effects associated with strength training. For chronic effects associated with endurance training (e.g. treadmill running), irradiation should be performed 5-10 min before and immediately after each exercise session; this may also be suitable for use in programs using low-force, prolonged functional electrical stimulation (FES)-based exercise, although this has yet to be tested scientifically. The minimum irradiation duration should be 30 s per site/point, with irradiation covering as much of the area as possible in most (if not all) involved muscle groups. Finally, when single probes are used, the distance between irradiation sites/points must be less than 2 cm.

# **REFERENCES**

1. Anders JJ, Lanzafame RJ, Arany PR. Low-level light/laser therapy versus photobiomodulation therapy. Photomed Laser Surg. 2015;33(4):183-4.

2. Mester E, Szende B, Gärtner P. [The effect of laser beams on the growth of hair in mice]. Radiobiologia, radiotherapia. 1968;9(5):621-6. 3. Chung H, Dai T, Sharma SK, Huang YY, Carroll JD, Hamblin MR. The nuts and bolts of low-level laser (light) therapy. Ann Biomed Eng. 2012;40(2):516-33.

4. Leal-Junior ECP, Lopes-Martins RAB, Bjordal JM. Clinical and scientific recommendations for the use of photobiomodulation therapy in exercise performance enhancement and post-exercise recovery: current evidence and future directions. Braz J Phys Ther.

5. Minatel DG, Frade MA, Franca SC, Enwemeka CS. Phototherapy promotes healing of chronic diabetic leg ulcers that failed to respond to other therapies. Lasers Surg Med. 2009;41(6):433-41.

6. Cressoni MD, Dib Giusti HH, Casarotto RA, Anaruma CA. The effects of a 785-nm AlGaInP laser on the regeneration of rat anterior tibialis muscle after surgically-induced injury. Photomed Laser Surg. 2008;26(5):461-6.

7. Oliveira FS, Pinfildi CE, Parizoto NA, Liebano RE, Bossini PS, Garcia EB, et al. Effect of low level laser therapy (830 nm) with different therapy regimes on the process of tissue repair in partial lesion calcaneous tendon. Lasers Surg Med. 2009;41(4):271-6.

8. Rochkind S, Geuna S, Shainberg A. Chapter 25 Phototherapy in Peripheral Nerve Injury: Effects on Muscle Preservation and Nerve Regeneration. Int Rev Neurobiol. 87: Academic Press; 2009. p. 445-64.

9. Balbinot G, Schuch CP, Nascimento PSD, Lanferdini FJ, Casanova M, Baroni BM, et al. Photobiomodulation Therapy Partially Restores Cartilage Integrity and Reduces Chronic Pain Behavior in a Rat Model of Osteoarthritis: Involvement of Spinal Glial Modulation. Cartilage. 2019:1947603519876338.

10. Chow RT, Johnson MI, Lopes-Martins RAB, Bjordal JM. Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials. The Lancet. 2009;374(9705):1897-908.

11. Lopes-Martins RA, Albertini R, Martins PS, Bjordal JM, Faria Neto HC. Spontaneous effects of low-level laser therapy (650 nm) in acute inflammatory mouse pleurisy induced by carrageenan. Photomed Laser Surg. 2005;23(4):377-81.

12. Huang YY, Sharma SK, Carroll J, Hamblin MR. Biphasic dose response in low level light therapy - an update. Dose Response. 2011;9(4):602-18.

13. Karu T. Photobiological fundamentals of low-power laser therapy. IEEE J Quantum Electr. 1987;23(10):1703-17.

14. Leal-Junior EC, Vanin AA, Miranda EF, de Carvalho Pde T, Dal Corso S, Bjordal JM. Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. Lasers Med Sci. 2015;30(2):925-39.

15. Lopes-Martins RA, Marcos RL, Leonardo PS, Prianti AC, Jr., Muscara MN, Aimbire F, et al. Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. J Appl Physiol. 2006;101(1):283-8.

16. Komatsu M, Kubo T, Kogure S, Matsuda Y, Watanabe K. Effects of 808 nm low-power laser irradiation on the muscle contraction of frog gastrocnemius. Lasers Surg Med. 2008;40(8):576-83.

17. Ferraresi C, de Sousa MV, Huang YY, Bagnato VS, Parizotto NA, Hamblin MR. Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice. Lasers Med Sci. 2015;30(4):1259-67.

18. Xu X, Zhao X, Liu TC, Pan H. Low-intensity laser irradiation improves the mitochondrial dysfunction of C2C12 induced by electrical stimulation. Photomed Laser Surg. 2008;26(3):197-202.

19. Leal Junior EC, Lopes-Martins RA, Dalan F, Ferrari M, Sbabo FM, Generosi RA, et al. Effect of 655-nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans. Photomed Laser Surg. 2008;26(5):419-24.

20. Leal Junior EC, Lopes-Martins RA, Baroni BM, De Marchi T, Taufer D, Manfro DS, et al. Effect of 830 nm low-level laser therapy applied before high-intensity exercises on skeletal muscle recovery in athletes. Lasers Med Sci. 2009;24(6):857-63.

21. Leal Junior EC, Lopes-Martins RA, Rossi RP, De Marchi T, Baroni BM, de Godoi V, et al. Effect of cluster multi-diode light emitting diode therapy (LEDT) on exercise-induced skeletal muscle fatigue and skeletal muscle recovery in humans. Lasers Surg Med. 2009;41(8):572-7.

22. Leal Junior EC, Lopes-Martins RA, Frigo L, De Marchi T, Rossi RP, de Godoi V, et al. Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. J Orthop Sports Phys Ther. 2010;40(8):524-32.

23. Vanin AA, Miranda EF, Machado CS, de Paiva PR, Albuquerque-Pontes GM, Casalechi HL, et al. What is the best moment to apply phototherapy when associated to a strength training program? A randomized, double-blinded, placebo-controlled trial : Phototherapy in association to strength training. Lasers Med Sci. 2016;31(8):1555-64.

24. de Almeida P, Lopes-Martins RA, De Marchi T, Tomazoni SS, Albertini R, Correa JC, et al. Red (660 nm) and infrared (830 nm) low-level laser therapy in skeletal muscle fatigue in humans: what is better? Lasers Med Sci. 2012;27(2):453-8.

25. Kelencz CA, Munoz IS, Amorim CF, Nicolau RA. Effect of low-power gallium-aluminum-arsenium noncoherent light (640 nm) on muscle activity: a clinical study. Photomed Laser Surg. 2010;28(5):647-52.

26. Miranda EF, Leal-Junior EC, Marchetti PH, Dal Corso S. Acute effects of light emitting diodes therapy (LEDT) in muscle function during isometric exercise in patients with chronic obstructive pulmonary disease: preliminary results of a randomized controlled trial. Lasers Med Sci. 2014;29(1):359-65.

27. Baroni BM, Leal Junior EC, De Marchi T, Lopes AL, Salvador M, Vaz MA. Low level laser therapy before eccentric exercise reduces muscle damage markers in humans. Eur J Appl Physiol. 2010;110(4):789-96.

28. Baroni BM, Leal Junior EC, Geremia JM, Diefenthaeler F, Vaz MA. Effect of light-emitting diodes therapy (LEDT) on knee extensor muscle fatigue. Photomed Laser Surg. 2010;28(5):653-8.

29. Baroni BM, Rodrigues R, Freire BB, Franke Rde A, Geremia JM, Vaz MA. Effect of low-level laser therapy on muscle adaptation to knee extensor eccentric training. Eur J Appl Physiol. 2015;115(3):639-47.

30. da Silva Alves MA, Pinfildi CE, Neto LN, Lourenco RP, de Azevedo PH, Dourado VZ. Acute effects of low-level laser therapy on physiologic and electromyographic responses to the cardiopulmonary exercise testing in healthy untrained adults. Lasers Med Sci. 2014;29(6):1945-51.

31. Leal Junior EC, de Godoi V, Mancalossi JL, Rossi RP, De Marchi T, Parente M, et al. Comparison between cold water immersion therapy (CWIT) and light emitting diode therapy (LEDT) in short-term skeletal muscle recovery after high-intensity exercise in athletes--preliminary results. Lasers Med Sci. 2011;26(4):493-501. Epub 11/19.

32. Lanferdini FJ, Bini RR, Baroni BM, Klein KD, Carpes FP, Vaz MA. Improvement of Performance and Reduction of Fatigue With Low-Level Laser Therapy in Competitive Cyclists. Int J Sports Physiol Perform. 2018;13(1):14-22.

33. Lanferdini FJ, Kruger RL, Baroni BM, Lazzari C, Figueiredo P, Reischak-Oliveira A, et al. Low-level laser therapy improves the VO2 kinetics in competitive cyclists. Lasers Med Sci. 2018;33(3):453-60.

34. De Marchi T, Leal Junior EC, Bortoli C, Tomazoni SS, Lopes-Martins RA, Salvador M. Low-level laser therapy (LLLT) in human progressive-intensity running: effects on exercise performance, skeletal muscle status, and oxidative stress. Lasers Med Sci. 2012;27(1):231-6.

35. Malta Ede S, De Poli RA, Brisola GM, Milioni F, Miyagi WE, Machado FA, et al. Acute LED irradiation does not change the anaerobic capacity and time to exhaustion during a high-intensity running effort: a double-blind, crossover, and placebo-controlled study : Effects of LED irradiation on anaerobic capacity and performance in running. Lasers Med Sci. 2016;31(7):1473-80.

36. Miranda EF, Vanin AA, Tomazoni SS, Grandinetti Vdos S, de Paiva PR, Machado Cdos S, et al. Using Pre-Exercise Photobiomodulation Therapy Combining Super-Pulsed Lasers and Light-Emitting Diodes to Improve Performance in Progressive Cardiopulmonary Exercise Tests. J Athl Train. 2016;51(2):129-35.

37. De Marchi T, Leal-Junior ECP, Lando KC, Cimadon F, Vanin AA, da Rosa DP, et al. Photobiomodulation therapy before futsal matches improves the staying time of athletes in the court and accelerates post-exercise recovery. Lasers Med Sci. 2019;34(1):139-48.

38. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008;88(1):287-332.

39. Cairns SP, Knicker AJ, Thompson MW, Sjøgaard G. Evaluation of Models Used to Study Neuromuscular Fatigue. Exerc Sport Sci Rev. 2005;33(1).

40. Enoka RM, Duchateau J. Muscle fatigue: what, why and how it influences muscle function. J Physiol. 2008;586(1):11-23.

41. Fitts RH. The cross-bridge cycle and skeletal muscle fatigue. J Appl Physiol (1985). 2008;104(2):551-8.

42. Szubski C, Burtscher M, Loscher WN. Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. Med Sci Sports Exerc. 2007;39(6):948-54.

43. Weir JP, Beck TW, Cramer JT, Housh TJ. Is fatigue all in your head? A critical review of the central governor model. Br J Sports Med. 2006;40(7):573-86; discussion 86.

44. Fukuda S, Nojima J, Motoki Y, Yamaguti K, Nakatomi Y, Okawa N, et al. A potential biomarker for fatigue: Oxidative stress and anti-oxidative activity. Biol Psychol. 2016;118:88-93.

45. Maegawa Y, Itoh T, Hosokawa T, Yaegashi K, Nishi M. Effects of near-infrared low-level laser irradiation on microcirculation. Lasers Surg Med. 2000;27(5):427-37.

46. Ihsan FR. Low-level laser therapy accelerates collateral circulation and enhances microcirculation. Photomed Laser Surg. 2005;23(3):289-94.

47. Leal Junior EC, Lopes-Martins RA, de Almeida P, Ramos L, Iversen VV, Bjordal JM. Effect of low-level laser therapy (GaAs 904 nm) in skeletal muscle fatigue and biochemical markers of muscle damage in rats. Eur J Appl Physiol. 2010;108(6):1083-8.

48. Leal Junior EC, Lopes-Martins RA, Vanin AA, Baroni BM, Grosselli D, De Marchi T, et al. Effect of 830 nm low-level laser therapy in exercise-induced skeletal muscle fatigue in humans. Lasers Med Sci. 2009;24(3):425-31.

49. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. J Photochem Photobiol B. 1995;27(3):219-23.

50. Oron U, Ilic S, De Taboada L, Streeter J. Ga-As (808 nm) laser irradiation enhances ATP production in human neuronal cells in culture. Photomed Laser Surg. 2007;25(3):180-2.

51. Silveira PC, Silva LA, Fraga DB, Freitas TP, Streck EL, Pinho R. Evaluation of mitochondrial respiratory chain activity in muscle healing by low-level laser therapy. J Photochem Photobiol B. 2009;95(2):89-92.

52. Borsa PA, Larkin KA, True JM. Does phototherapy enhance skeletal muscle contractile function and postexercise recovery? A systematic review. J Athl Train. 2013;48(1):57-67.

53. Klebanov GI, Kreinina MV, Poltanov EA, Khristoforova TV, Vladimirov YA. Mechanism of Therapeutic Effect of Low-Intensity Infrared Laser Radiation. Bull Exp Biol Med. 2001;131(3):239-41.

54. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol B. 1999;49(1):1-17.

55. Sharma SK, Kharkwal GB, Sajo M, Huang YY, De Taboada L, McCarthy T, et al. Dose response effects of 810 nm laser light on mouse primary cortical neurons. Lasers Surg Med. 2011;43(8):851-9.

56. Huang YY, Nagata K, Tedford CE, McCarthy T, Hamblin MR. Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. J Biophotonics. 2013;6(10):829-38.

57. Hamblin MR. Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. Photochem Photobiol. 2018;94(2):199-212.

58. Fillipin LI, Mauriz JL, Vedovelli K, Moreira AJ, Zettler CG, Lech O, et al. Low-level laser therapy (LLLT) prevents oxidative stress and reduces fibrosis in rat traumatized Achilles tendon. Lasers Surg Med. 2005;37(4):293-300.

59. Rizzi CF, Mauriz JL, Freitas Correa DS, Moreira AJ, Zettler CG, Filippin LI, et al. Effects of low-level laser therapy (LLLT) on the nuclear factor (NF)-kappaB signaling pathway in traumatized muscle. Lasers Surg Med. 2006;38(7):704-13.

60. Manteifel V, Bakeeva L, Karu T. Ultrastructural changes in chondriome of human lymphocytes after irradiation with He-Ne laser: Appearance of giant mitochondria. J Photochem Photobiol B. 1997;38(1):25-30.

61. Ferraresi C, Huang YY, Hamblin MR. Photobiomodulation in human muscle tissue: an advantage in sports performance? J Biophotonics. 2016;9(11-12):1273-99.