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PRE-CONDITIONING WITH LOW-LEVEL LASER (LIGHT) THERAPY: LIGHT BEFORE THE STORM

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□ Pre-conditioning by ischemia, hyperthermia, hypothermia, hyperbaric oxygen (and numerous other modalities) is a rapidly growing area of investigation that is used in pathological conditions where tissue damage may be expected. The damage caused by surgery, heart attack, or stroke can be mitigated by pre-treating the local or distant tissue with low levels of a stress-inducing stimulus, that can induce a protective response against subsequent major damage. Low-level laser (light) therapy (LLLT) has been used for nearly 50 years to enhance tissue healing and to relieve pain, inflammation and swelling. The photons are absorbed in cytochrome(c) oxidase (unit four in the mitochondrial respiratory chain), and this enzyme activation increases electron transport, respiration, oxygen consumption and ATP production. A complex signaling cascade is initiated leading to activation of transcription factors and up- and down-regulation of numerous genes. Recently it has become apparent that LLLT can also be effective if delivered to normal cells or tissue before the actual insult or trauma, in a pre-conditioning mode. Muscles are protected, nerves feel less pain, and LLLT can protect against a subsequent heart attack. These examples point the way to wider use of LLLT as a pre-conditioning modality to prevent pain and increase healing after surgical/medical procedures and possibly to increase athletic performance.

Key words: Pre-conditioning, Remote Ischemic Pre-conditioning, LLLT, Photobiomodulation, Mitochondria, Reactive Oxygen Species

INTRODUCTION

Many diseases and traumatic events involving tissue damage are injurious because of ischemia, a sudden or more gradual process characterized by deprivation of the tissues of life-sustaining oxygen. Ischemic heart disease and ischemic stroke account for the largest burden of mortality and morbidity in modern life. The term pre-conditioning (PC) was first applied to a regimen in which repetitive short episodes of ischemia and

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reperfusion could lead to the development of resistance of the myocardium to a subsequent ischemic insult (heart attack) (Das and Das, 2008). A novel treatment strategy to counter ischemic cardiac disease and stroke developed which was termed ischemic pre-conditioning IPC) (Koch *et al*, 2014). IPC rests on the basic premise that brief durations of ischemia induce intrinsic cellular defense systems, and lead to the tolerance of vital organs to subsequent more severe ischemia. In direct pre-conditioning, for example, brief occlusions of a coronary or carotid artery protects the heart or the brain from longer durations of ischemia and reduces the resulting infarct volumes after heart attack or stroke. In remote IPC, brief ischemia is deliberately induced in one organ, typically an easily accessible tissue such as a limb, and subsequently protects a distant organ (typically the brain or the heart) from more severe ischemia. This concept was originally discovered using a rabbit heart attack model, when an initial episode of mild ischemia followed by reperfusion made the heart more resistant to a subsequent lethal ischemic insult (Murry *et al*, 1986). Since then IPC has become a powerful experimental technique to combat ischemic insults in most organs, and has been extensively studied in many laboratories around the world (Narayanan *et al*, 2013).

In the brain, IPC responses are found to occur in specific time windows consisting of an early and a late phase. The early phase, if the duration between the initial pre-conditioning and the final insult is around one hour, has the maximum protective effect (Narayanan *et al*, 2013). In the second (late) phase the combination of released factors and activated pathways results in delayed pre-conditioning. If this late phase is extended to several days after the preconditioning insult, it may provide more robust and longer lasting neuroprotection than the early phase.

MECHANISMS OF IPC

Previously it was believed that the mechanism of IPC was mediated by increased flow through the coronary collateral circulation. However, this theory was abandoned after a study by Murray et al (1986) in which they measured tissue radioactivity after injecting radiolabeled microspheres into the left atrial chamber of the dog heart and found no difference in collateral blood flow between preconditioned dogs and controls. IPC represents a complex molecular process that is rapidly generated and multifactorial in nature, that is then transduced into an intracellular message and amplified to produce the final effector mechanism (Hawaleshka and Jacobsohn, 1998). In the response to a brief duration of sublethal ischemic insults, the receptors of the triggering factors may activate signaling pathways which consist of a well-organized series of events. These delayed pre-conditioning-activated signaling pathways govern gene expression resulting in cells expressing a phenotype that is highly resistant to ischemic insults. Locally released agonists such as adenosine, bradykinin,

catecholamines and opioids activate the protective response through various G- protein coupled receptors which, when stimulated, increase activity of phospholipases C and D (Yang *et al*, 2010b). Kinases such as protein kinase C tyrosine kinase p38MAPKinase contribute in the signaling pathway. Ytrehus *et al* (1994) demonstrated inhibition of the protection obtained after IPC by administration of PKC inhibitors in the rabbit heart model . It has also been shown that among the PKC isozymes, the PKC-ε isoenzyme specifically provides cardioprotection against ischemia/reperfusion damage (Dorn *et al*, 1999; Ping *et al*, 2002). Consistently, in all the animal models tested including mice, rats, rabbits and pigs the PKC-ε-selective activating peptide protected hearts, and inhibitors of PKC-ε inhibited ischemic/hypoxic or pharmacologic preconditioning (Yang *et al*, 2010b). On the other hand, deletion of cardiac PKC-ε resulted in failure to decrease infarct size in mice, suggesting a major role of PKC-ε activation in IPC (Inagaki *et al*, 2005). Another hypothesis for the underlying mechanism of IPC is by production of an inducible 70 kD stress or heat shock protein (HSP70i) (Currie *et al*, 1987). A variety of stimuli (including haemodynamic overload, myocyte stretch, hypertension, ischaemia, exercise, and oxidative stress) lead to an increase in the synthesis of HSP70i. After 24 hours of IPC, it has been shown that the levels of HSP70i were increased, and notably over-expression of the HSP70i gene in transgenic mice decreased the infarct size and improved myocardial recovery (Marber *et al*, 1995; Yellon *et al*, 1992). The mechanism of cardioprotection in IPC by HSP70i could be due to identification of damaged proteins and synthesis of new proteins under myocardial stress (Richard *et al*, 1996).

Another primary mediator in IPC could be activation of hypoxia inducible factor 1α (HIF1α) which is the means by which tissue and cells can sense hypoxia. An autonomous adaptive response by the cell to chronic hypoxia regulated by HIF-1 is decreased mitochondrial mass and/or metabolism. Fibroblasts deficient in HIF-1 when exposed to chronic hypoxia generate high levels of reactive oxygen species (ROS), leading to cell death (Semenza *et al*, 2011). The HIF-1α gene has been shown to produce cardioprotective effects during preconditioning by stimulating the secretion of the hormone erythropoietin (EPO) in the kidney (Wang and Semenza, 1995). HIF-1α knock-out (KO) mice exposed to alternating cycles of ambient hypoxia and re-oxygenation failed to develop the protective cardiac preconditioning as compared to the wild type mice (Cai *et al*, 2003). Direct administration of EPO to HIF-1α KO mice into perfused hearts resulted in acute protection against ischemia-reperfusion injury (Cai and Semenza, 2004). The levels of adenosine increased in the hearts subjected to ischemic pre-conditioning, and blocking the adenosine receptor inhibited the protective effects of ischemic preconditioning, whereas adenosine receptor agonists protected the heart (Marber *et al*,

1993; Thornton *et al*, 1992). The HIF-1 gene induced CD73, the enzyme that produces adenosine thus indirectly helping in ischemic preconditioning. On the other hand, HIF-1-regulates mitochondrial metabolism thus contributing to the protective effects of ischemic preconditioning (Thornton *et al*, 1992). Increased plasma IL-10 levels and decreased myocardial infarct size are observed in remote ischemic preconditioned wild type mice, but could not be demonstrated in HIF-1α KO mice. Injecting the active form of HIF-1α into the mouse hind limb muscle significantly increased plasma IL-10 levels and decreasedd myocardial infarct size. In conclusion, HIF-1 plays a major role in IPC thus protecting the myocardium (Cai *et al*, 2013).

OTHER MODALITIES FOR PRE-CONDITIONING

In addition to ischemic preconditioning, other effective preconditioning stimuli and regimens have been reported. These are both numerous and diverse (Stetler *et al*, 2014), including hypothermia, hyperthermia, physical exercise, hyperbaric oxygen, exposure to neurotoxins and pharmacological agents such as resveratrol, volatile anesthetics and xenon, and even the mild inflammation produced by endotoxin.

In aged animals, there is decrease in ischemic tolerance leading to the impairment of the cardioprotective effect of IPC. Heat preconditioning (HP) protects myocardial injury induced by 8 weeks of overload training. HP enables adjustment of the activities of creatine kinase (CK), catalase (CAT) and superoxide dismutase (SOD), regulates the expressions of apoptosis-related genes (Bcl-2, Bax, caspase-3, caspase-9),and adhesion molecules (ICAM-1, VCAM-1, and PECAM-1) resulting in enhanced capacity against overload training induced injury and reduced damage to the myocardium (Yang *et al*, 2013). Hypothermic preconditioning is induced by rapid cycles of cooling and warming which produces sub-lethal stress to the brain's microenvironment leading to the protection of the CNS from successive insults such as cerebral ischemia (Katz *et al*, 2009). Exercise preconditioning lessens the harmful effect of ischemia/reperfusion injury by increasing the expression of neurotrophic factors, the extracellular matrix (ECM) proteins, integrins, angiogenic factors, heat shock proteins (Hsp-70) and decreasing expression of matrix metalloproteinase (MMP-9) and Toll-like receptor-4. Chronic exercise pre-conditioning increases cerebral metabolism following periods of hypoxia by increasing the neuronal response by increasing ATP production (Ding *et al*, 2005; Dornbos and Ding, 2012).

Pre-conditioning with hyperbaric oxygen reduces the number of apoptotic neural cells and promotes the nerve functional recovery in rats after spinal cord injury (Lu *et al*, 2013). Additionally, pre-conditioning coronary artery disease patients with hyperbaric oxygen prior to on-pump cardiopulmonary bypass or coronary artery bypass graft (CABG) sur-

gery was capable of improving left ventricular function, and resulted in reduced myocardial injury, less intraoperative blood loss, shorter length of stay in ICU, fewer postoperative complications, and saved on patient costs, post-CABG (Yogaratnam *et al*, 2010). Lifelong caloric restriction has been found to modify the physiological and pathophysiological changes induced by aging and also increases life expectancy. In aged rats short term caloric restriction was found to provide cardioprotection by improving ischemic tolerance (Shinmura *et al*, 2005). Pre-treatment with perivascular or intramuscular neurotoxins such as botulinum toxin A (Btx-A) has a potential role in ischemic preconditioning of muscle flaps which is believed to be achieved through the release of substance P, calcitonin gene-related peptide (CGRP), and vascular endothelial growth factor (VEGF) (Akcal *et al*, 2013). Preconditioning with resveratrol has been found to induce brain tolerance against ischemia by suppression of the inflammatory response via regulation of NF-κB, COX-2 and iNOS (Simao *et al*, 2012). The administration of volatile anesthetics before a prolonged ischemic episode is known as anesthetic preconditioning, which has been developed as a cardioprotective modality in both animal and humans coronary disease models (Muntean *et al*, 2013). Moreover, pre-conditioning with anesthetics such as xenon gas can also exert neuroprotective and cardioprotective effects in different models suggesting the use of xenon preconditioning as an alternative strategy for the prevention of various neurological and cardiovascular diseases (Liu *et al*, 2013). Studies have shown that lipopolysaccharide (LPS) from Gram-negative bacteria used in pre-conditioning provides neuroprotection against successive cerebral ischemic injury. LPS activates Toll-like receptors and causes systemic inflammation. After stroke, mice pre-conditioned with LPS had a significant decrease in the levels of TNF-alpha, neuronal TNF-receptor 1 (TNFR1), and TNFR-associated death domain (TRADD). Additionally, to establish LPS-induced tolerance before ischemia, it was necessary to show upregulation of TNF-alpha, whereas if LPS was given during ischemia then suppression of TNF-alpha signaling was associated with neuroprotection after LPS preconditioning (Rosenzweig *et al*, 2007).

LOW-LEVEL LASER (LIGHT) THERAPY (LLLT)

Low-level laser (light) therapy or photobiomodulation has been known for almost 50 years since shortly after the discovery of lasers in 1960. For many years it was thought that there was something special about laser light, but it is now known that many wavelengths of visible light (especially in the red or near-infrared regions) produced from LEDs or other light sources are equally effective. Longer red and near-infrared wavelengths are much better at penetrating tissue than shorter blue/green wavelengths and are therefore preferred clinically. Although the mechanism of action of LLLT is still not fully understood, much infor-

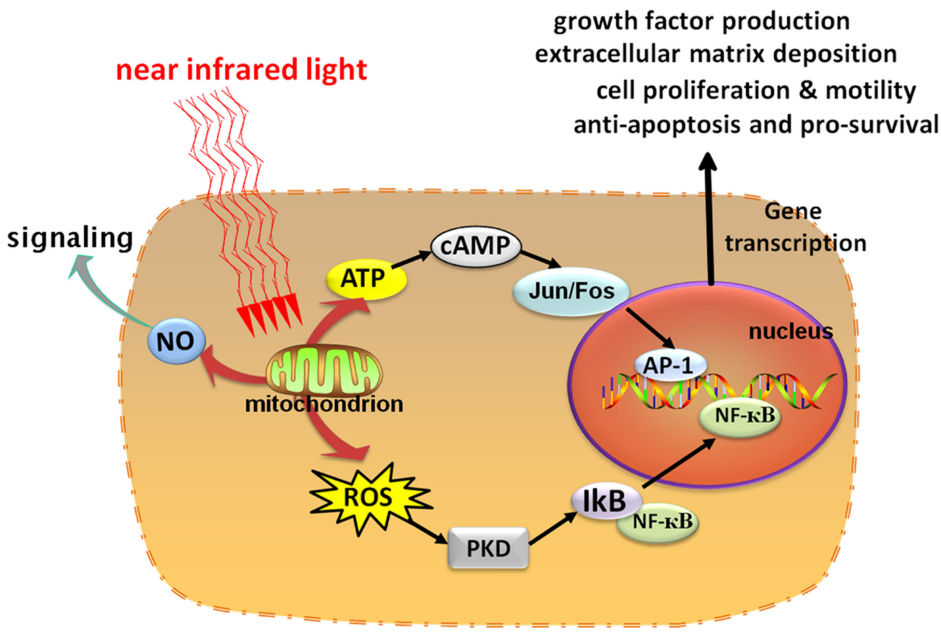

FIG. 1. Schematic depiction of the cellular signaling pathways triggered by LLLT. After photons are absorbed by chromophores in the mitochondria, respiration and ATP is increased but in addition signaling molecules such as reactive oxygen species (ROS) and nitric oxide (NO) are also produced (with permission from Huang *et al*, 2011).

mation is now known concerning the primary photoacceptor molecules, the immediate effectors, the signaling pathways, transcription factor activation and short and long-term effects of LLLT on cells and tissues. The most important photoacceptor is accepted to be cytochrome c oxidase (unit IV in the mitochondrial respiratory chain). Increase in mitochondrial membrane potential, oxygen consumption and ATP production are rapidly observed. Nitric oxide and ROS are transiently produced. Cyclic AMP is involved in signaling pathways. Transcription factors such as NF-kB are activated. Anti-apoptotic proteins, heat shock proteins, anti-oxidant defense pathways and anti-inflammatory cytokines are increased. In the long-term parameters involved in healing such as cell migration, cell adhesion, protein synthesis, and DNA synthesis are stimulated (Fig. 1) (Huang *et al*, 2011).

SIMILARITIES BETWEEN IPC AND LLLT

Part of the aim of this review is to draw comparisons between the hypothesized mechanisms of IPC and those of LLLT when used in a pre-conditioning or post-injury mode. It is in fact quite remarkable how many similarities that can be found between the two modalities, although of course, there are also some differences (Table 1). One of the unifying

TABLE 1. Comparison of cellular and molecular mechanisms that have been proposed for ischemic preconditioning and for LLLT.

Pathway or mediator	IPC	LLLT
Inflammation	Reduction in vascular permeability and neutrophil migration in rat paw (Souza Filho *et al*, 2009)	Reduction of COX-2 mRNA in rat paw and brain (Prianti *et al*, 2014)
Cytokines (IL10)	Upregulation of IL10 in plasma and heart (Cai *et al*, 2012)	Reduced IL6 and TNFa and increased IL10 in muscle of rats with heart failure (Hentschke *et al*, 2013)
Nitric oxide	Increases circulating nitrites in mice (Rassaf *et al*, 2014)	Increases venous nitric oxide levels (Mitchell and Mack, 2013)
Oxidative stress	Reduction of oxidative stress and downregulation of COX-2 expression in rat kidney (Sedaghat *et al*, 2013)	Reduction of oxidative and nitrative stress in injured tibialis anterior muscle in rat (Assis *et al*, 2012)
Heat shock proteins	Over-expression of the HSP70i gene in transgenic mice limits infarct size and improves post-ischaemic contractile recovery (Marber *et al*, 1995)	Decrease in the HSP27 phosphorylation thereby decreasing NF-κB p65 translocation human gingival fibroblast cells. (Lim *et al*, 2013)
MAP tyrosine kinase	Phosphorylation of p38 MAPK via mitochondria translocation of Bcl-xL in mice brain (Zhao *et al*, 2013)	Increase in MAP kinase activity leading to increase in VEGF levels in human granulosa cells. (Kawano *et al*, 2012)
Kinin receptors	Down-regulates kinin receptor expression in neutrophils of patients undergoing heart surgery. (Saxena *et al*, 2013)	Downregulates kinin receptors mRNA expression in the subplantar muscle of rat paw (Bortone *et al*, 2008)
Ion channels	K(+) inward rectifier subunits of the 6.2 isotype (Kir6.2) required for cardioprotection (Wojtovich *et al*, 2013)	Increased Ca2+ in oral tissue cells via TRPC1 ion channels (Chellini *et al*, 2010)
Cyclic AMP/CREB	CREB phosphorylation contributes to triggering preconditioning in rat heart (Marais *et al*, 2008)	Promotes the proliferation and osteogenic differentiation of human periodontal ligament cells via cyclic AMP (Wu *et al*, 2013)
VEGF	Overexpression of VEGF protects intrahepatic cholangiocytes after liver transplantation in rats (Zhou *et al*, 2010)	Increases angiogenesis in a model of ischemic skin flap in rats mediated by VEGF, HIF-1α and MMP-2 (Cury *et al*, 2013)
Hypoxia-inducible factor 1	HIF-1 activates IL10 gene transcription and is required for remote ischemic preconditioning in mice heart (Cai *et al*, 2013; Kalakech *et al*, 2013)	Reduces the overexpressions of HIF-1α, TNF-α, and IL-1β, and increases the amounts of VEGF, NGF, and S100 proteins in rats with chronic constriction injury (Hsieh *et al*, 2012)
TFG-beta	Downregulated TGFβ1 but upregulated smad7 in cardiac infarcts (Wang *et al*, 2013)	Increase in proliferation of vascular endothelial cells and decrease in VEGF and TGF-beta secretion. (Szymanska *et al*, 2013)

…continued

TABLE 1. Continued

Pathway or mediator	IPC	LLLT
Vasodilation and endothelial function	Prevents reduction in brachial artery flow-mediated dilation after strenuous exercise in humans. (Bailey *et al*, 2012)	Rapid increase of human skin microcirculation at the local and systemic levels(Samoilova *et al*, 2008)
Hemostasis	Prevents systemic platelet activation in humans (Pedersen et al, 2011)	Speeds wound healing in hemophilia by enhancing platelet procoagulant activity in humans (Hoffman and Monroe, 2012)
Stem cell activation	Increases the intestinal stem cell activities in the intestinal crypts in mice (Chen *et al*, 2014)	Induces autologous bone-marrow-derived mesenchymal stem cells in infarcted heart of rats (Tuby *et al*, 2011)

mechanisms is hypothesized to be at the level of mitochondria (Dirnagl and Meisel, 2008).

SKELETAL MUSCLE PRE-CONDITIONING THROUGH LIGHT

In acute conditions, skeletal muscle fatigue impairs the strength of muscle, its motor coordination and decreases the capacity of the muscle to perform work (Allen *et al*, 2008). Overall fatigue decreases the muscle function, which is believed to be a result of metabolic changes such as depletion of ATP and glycogen, oxidative stress, tissue hypoxia and blood acidification. Phototherapy has been shown to improve muscle fatigue and exhaustion (Leal Junior *et al*, 2010a). Studies have shown that pre-treatment with specific doses of phototherapy decreases inflammatory biomarkers and lactate levels in blood after strenuous upper and lower extremity exercise (Lopes-Martins *et al*, 2006b). On the basis of these studies we can infer that pre-conditioning the muscle with phototherapy increases the contractile function, decreases exercise induced muscle fatigue and improvement in post exercise recovery. We have tabulated the experimental studies on animals in Table 2, and the clinical studies in humans in Table 3.

There are two different phases of exercise-induced damage; one is primary and the other is secondary. The primary phase of muscle damage results from direct exercise-induced mechanical stress, and secondary muscle damage is due to the inflammatory response that follows after mechanical stress (Merrick *et al*, 2002). Pre-conditioning with phototherapy protects the muscle both from primary as well as secondary damage, while phototherapy when administered after injury protects cells from secondary damage only. In vivo studies have shown that pre-conditioning phototherapy to injured muscles produces anti-inflammatory and antioxidant effects, thus protecting the muscle from secondary damage (Avni *et al*, 2005).

TABLE 2. LLLT preconditioning and exercise: experimental animal models (adapted from Ferraresi *et al* 2012).

Ref	Light source / Wavelength	Spot area (cm2)	Performance characteristics	Muscle(s)	Exercise
Lopes-Martins *et al*, 2006a	Laser 655 nm	0.08	- 2.5 mW; 31.25 mW/cm^2 - 1 application point - Groups: 0.5 J/cm^2 (32 s) / 1 J/cm^2 (80 s) / 2.5 J/cm^2 (160 s)	Tibialis anterior	Neuromuscular electric stimulation
Leal Junior *et al*, 2009c	Laser 904 nm	0.2	- 15 mW, 75 mW/cm^2 - 1 application point - Groups: 0.1 J (7 s) / 0.3 J (20 s) / 1 J (67 s) / 3 J (200 s)	Tibialis anterior	Neuromuscular electric stimulation
de Almeida *et al*, 2011	Laser 904 nm	0.2	- 15 mW; 75mW/cm^2 - 1 application point - Groups: 0.1 J (7 s) / 0.3 J (20 s) / 1 J (67 s) / 3 J (200 s)	Tibialis anterior	Neuromuscular electric stimulation

An important parameter for the muscle to receive the optimum irradiation is that the irradiation points should be designed to cover the largest area and to better distribute the energy applied over the muscles (Ferraresi *et al*, 2012). Figures 2 and 3 demonstrate some examples of the number and distribution of the irradiation points on femoral quadriceps using LLLT or LEDT (Ferraresi *et al*, 2012).

Studies support that hypothesis that the ergogenic effects of phototherapy on muscles are due to increases in intramuscular microcirculation (Baroni *et al*, 2010b), decreases in the lactic acid production (Leal Junior *et al*, 2009a; Leal Junior *et al*, 2009b; Leal Junior *et al*, 2009d), improved mitochondrial function, improved antioxidant ability of the exercising muscles, enhanced contractile function, prevention of exercise-induced cell damage, and improved post exercise recovery of strength and function (Borsa *et al*, 2013; Leal Junior *et al*, 2011; Leal Junior *et al*, 2010b). Borsa *et al* (2013) critically evaluated these studies that address the ability of phototherapeutic devices, such as lasers and light-emitting diodes (LEDs), to improve skeletal muscle contractile

TABLE 3. LLLT preconditioning and exercise: clinical studies in humans (adapted from Ferraresi *et al* 2012).

Ref	Light source/ Wavelength	Spot area (cm2)	Performance characteristics	Muscle(s)	Exercise
de Almeida *et al*, 2012	Laser 660nm *versus* 830nm	0.0028	- 50 mW; 17.85 W/cm^2 - 5 J (100 s); 1.785 J/cm^2 - 4 application points: total energy 20 J	Biceps brachii	MVC per 60 s in Scott bench
Gorgey *et al*, 2008	Cluster with 4 diodes laser 808 nm		- 500 mW; 8.3mW/cm^2 - 3 J (5 min); 7J (10 min)	Femoral quadriceps	Isokinetic dynamometer
Leal Junior *et al*, 2008	Laser 655 nm	0.01	- 50 mW; 5 W/cm^2 - 5 J per diode (100 s); 500 J/cm^2 - 4 application points: total energy 20 J	Biceps brachii	Scott bench
Leal Junior *et al*, 2009e	Laser 830 nm	0.0028	- 100 mW; 35.7 W/cm^2 - 5 J (50 s); 1.785 J/cm^2 - 4 application points: total energy 20 J	Biceps brachii	Scott bench
Leal Junior *et al*, 2010b	Cluster with 5 diodes laser 810 nm	0.0364	- 200 mW; 5.495 W/cm^2 - 6 J per diode (30 s); 164.85 J/cm^2 - 30 J per application point (5 × 6 J) - 2 application points: total energy 60 J	Biceps brachii	Scott bench
Baroni *et al*, 2010a	Cluster with 5 diodes laser 810 nm	0.029	- 200 mW; 6.89 W/cm2 - 6 J per diode (30 s); 206.89 J/cm2 - 30 J per application point (5 × 6 J) - 6 application points: total energy 180 J (2 points on vastus medialis, 2 points on vastus lateralis, 2 points on rectus femoris)	Femoral quadriceps	Isokinetic dynamometer

continured…

TABLE 3. Continued

Ref	Light source/ Wavelength	Spot area (cm2)	Performance characteristics	Muscle(s)	Exercise
De Marchi *et al*, 2012	Cluster with 5 laser diodes 810 nm	0.0364	- 200 mW; 5.495 W/cm^2 - 6 J per diode (30 s); 164.85 J/cm^2 - 30 J per application point (5 × 6 J) - 12 application points: total energy 360 J (2 points on rectus femoris, 2 points on vastus medialis, 2 points on vastus lateralis, 4 points on hamstrings, 2 points on gastrocnemius)	Femoral quadriceps, hamstrings, gastrocnemius	Running on treadmill until exhaustion
Leal Junior *et al*, 2009a	Laser 810 nm *versus* Cluster with 69 LEDs 660/850 nm	0.036 *versus* 0.2	- 200 mW; 5.50 W/cm^2 - 6 J per diode (30 s); 164.84 J/cm^2 - 2 application points: total energy 12 J *versus* - 34 LEDs 660nm; 10 mW; 1.5 J/cm^2 ; 0.05 W/cm^2 - 35 LEDs 850 nm; 30 mW; 0.015 W/cm^2; 4.5 J/cm^2 - 0.3 J LED 660nm (30 s) - 0.9 J LED 850nm (30 s) - 41.7 J per application point (30 s) - 2 application points: total energy 83.4 J	Rectus femoris	Wingate test
Leal Junior *et al*, 2009d	Cluster with 69 LEDs 660/850 nm	0.2	- 34 LEDs 660nm; 10 mW; 1.5 J/cm^2 ; 0.05 W/cm^2 - 35 LEDs 850 nm; 30 mW; 0.015 W/cm^2; 4.5 J/cm^2 - 0.3 J LED 660nm (30 s) - 0.9 J LED 850nm (30 s) - 41.7 J per application point (30 s) - 1 application point	Biceps brachii	Scott bench

continured…

TABLE 3. Continued

Ref	Light source/ Wavelength	Spot area (cm2)	Performance characteristics	Muscle(s)	Exercise
Baroni *et al*, 2010b	Cluster with 69 LEDs 660/850 nm	0.2 cm^2	- 34 LEDs 660nm; 10 mW; 1.5 J/cm^2; 0.05 W/cm^2 - 35 LEDs 850 nm; 30 mW; 0.015 W/cm^2; 4.5 J/cm^2 - 0.3 J LED 660nm (30 s) - 0.9 J LED 850nm (30 s) - 41.7 J per application point (30 s) - 3 application points: total energy 125.1 J (2 points on rectus femoris, 2 points on vastus medialis, 2 points on vastus lateralis)	Femoral quadriceps	Isokinetic dynamometer

function, decrease exercise-induced muscle fatigue, and enable post exercise recovery.The main outcome measures included total lapsed time to fatigue, number of repetitions to fatigue, overall work performed, maximal voluntary isometric contraction (strength), electromyographic activity, and post exercise biomarker levels. Ten of the randomized controlled trials met the inclusion criteria including 32 data sets. In all the studies Borsa et. al(2013) consistently found that phototherapy administered before resistance exercise provided ergogenic and prophylactic benefits to skeletal muscle.A randomized double-blind study using 22 untrained male volunteers was performed by De Marchi et al (2011) . The subjects were pre-conditioned with LLLT for 30 seconds on a lower-limb before a standardized exercise protocol on a treadmill until they were exhausted. Figures 4 and 5 show pre- and post-exercise activity of markers of muscle damage-lactate dehydrogenase (LDH) and creatine kinase (CK). De Marchi et al (2011) observed that preconditioning with LLLT before exercise also decreased the post-exercise improvement in the activities of CK and LDH compared to the placebo group, demonstrating that pre-treatment with LLLT can protect skeletal muscle against exercise-induced damage in long-duration exercises (De Marchi *et al*, 2012). In a similar study, male volleyball players were preconditioned with: 1) an active LEDT cluster-probe (660nm=10mW, 850nm=30mW); (2) a placebo cluster-probe with no output; and (3) a single-diode 810-nm 200-mW laser on the rectus femo-

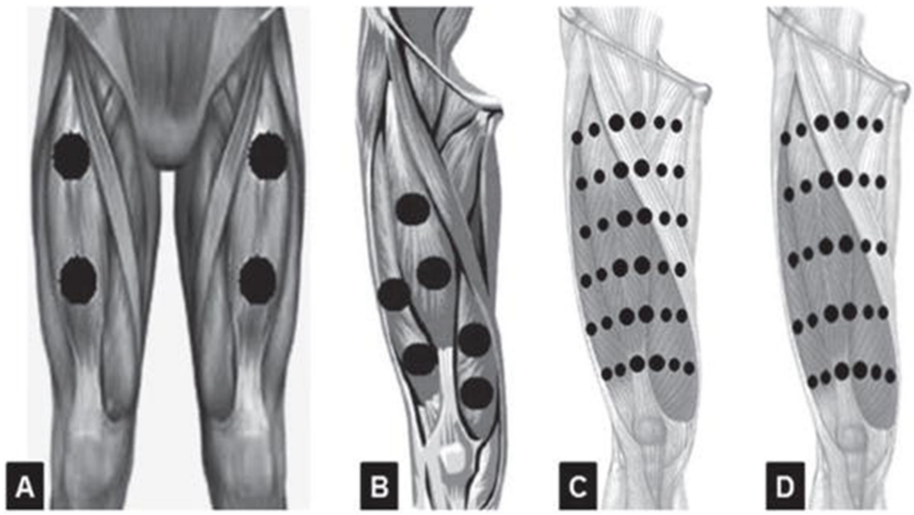

FIG. 2. Number of LLLT or LEDT radiation points applied on femoral quadriceps muscle (with permission from Ferraresi *et al*, 2012).

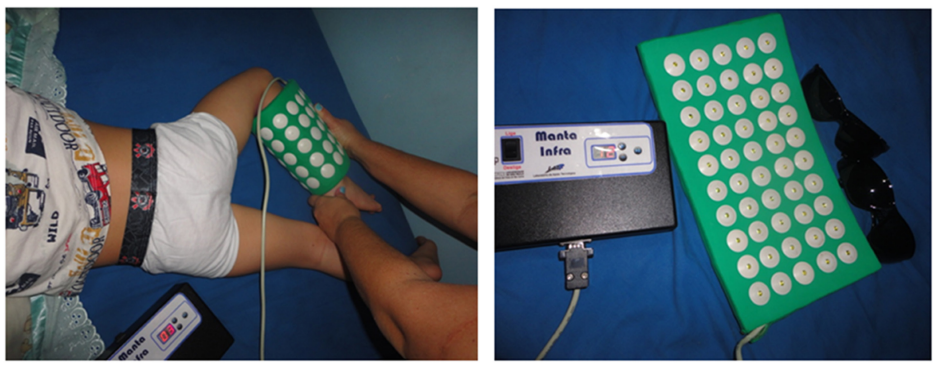

FIG. 3. LEDT array on a patient to improve muscle function (with permission from Ferraresi and Parizotto, 2014).

ris muscle and then asked to perform three Wingate cycle tests. It was observed that post exercise CK levels were significantly lower in active LEDT cluster group compared to the placebo cluster or single diode group (Leal Junior *et al*, 2009a). (Fig. 6).

Conversely, preconditioning with LEDT in healthy male volunteers did not improve maximal knee extensor muscle isokinetic voluntary contractions (preMVC) performed before the isokinetic fatigue test as compared to the placebo group. However, higher torque values were observed in post-MVC subjects pre-conditioned with LEDT compared to the placebo group (Fig. 7). Pretreatment with LEDT increased the exercise performance by producing higher isometric torque after high-intensity concentric isokinetic exercise (Baroni *et al*, 2010b).

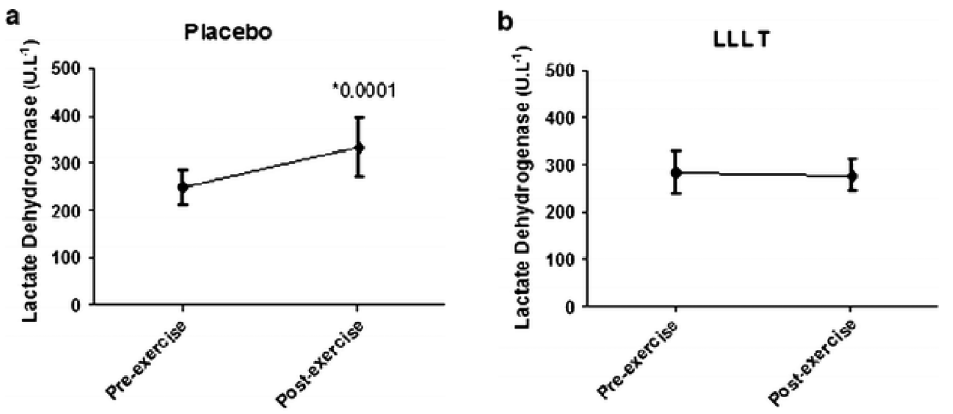

FIG. 4. Pre and post-exercise activity of lactate dehydrogenase (a) in placebo (b) and in active LLLT group. * Statistical significance from pre to post-exercise levels using the software SPSS 18.0 for Windows (with permission from De Marchi *et al*, 2012).

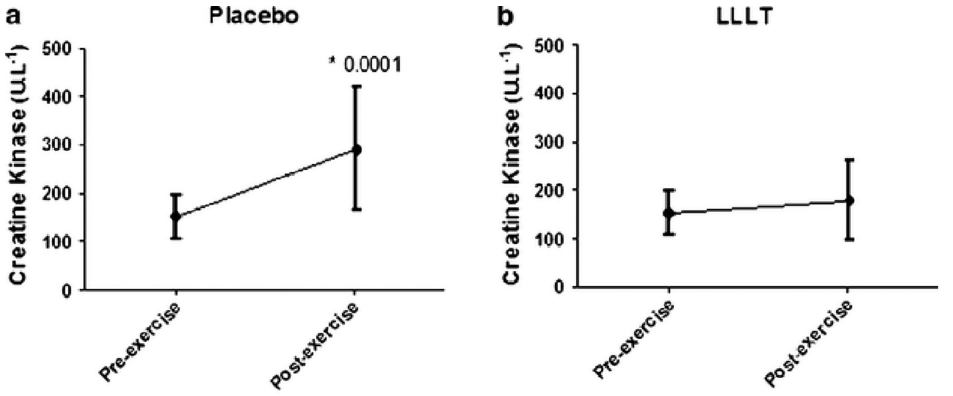

FIG. 5. Pre and post-exercise activity of creatine kinase (a) in placebo (b) and in active LLLT group. * Statistical significance from pre to post-exercise levels using the software SPSS 18.0 for Windows (with permission from De Marchi *et al*, 2012).

Leal-Junior *et al.* (2013) performed a meta-analysis to explore the effects of phototherapy applied before, during and after exercises. Data from thirteen randomized control trial was assessed based on number of repetitions and time until exhaustion for muscle performance, and creatinine kinase (CK) activity to assess risk for exercise-induced muscle damage. The meta-analysis concluded that preconditioning skeletal muscles with phototherapy (lasers and LEDs) improves muscular performance and accelerates recovery.

PRECONDITIONING WITH LIGHT IMPROVES INFLAMMATION AND ANALGESIC EFFECT:

In temporomandibular joint disorders pain is usually a very common finding. Barretto *et al* (2013) studied the effect of LLLT of pain and

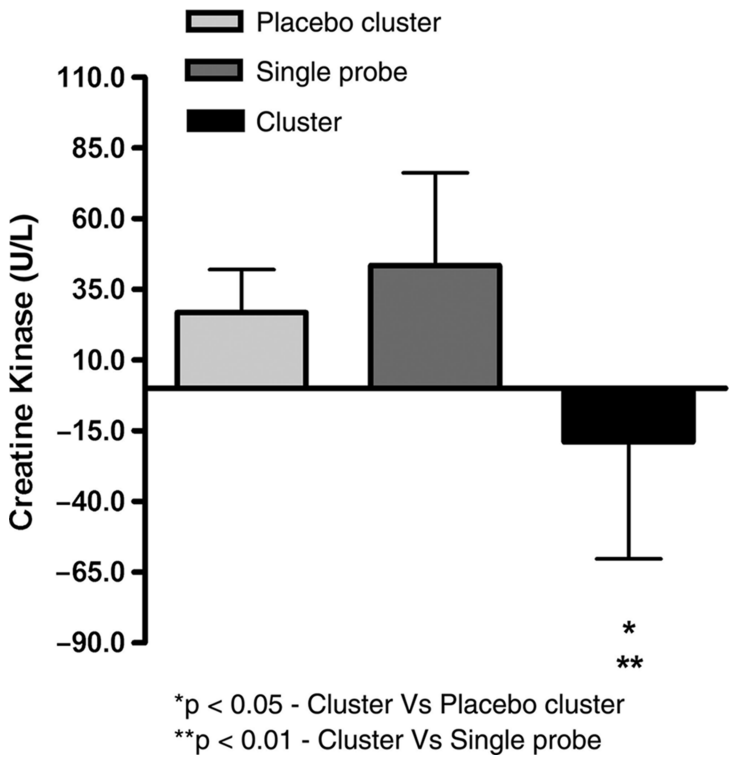

FIG. 6. Changes in creatine kinase levels post-exercise. (with permission from Leal Junior *et al*, 2009a).

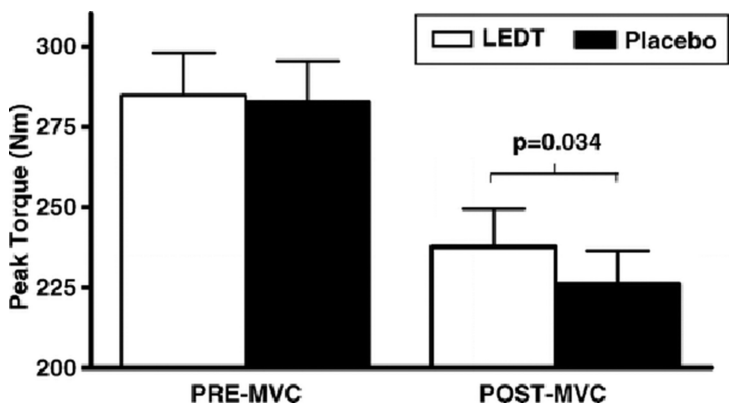

FIG. 7. PRE-MVC and POST-MVC knee-extensor maximal isometric torques for LEDT and placebo treatments (mean SEM). The bracket indicates differences in POST-MVC between LEDT and placebo (p value above the bracket) (with permission from Baroni *et al*, 2010b).

inflammation on temporomandibular joint (TMJ) of rats. For the assessment of nociceptive response, formalin and carrageenan was injected to induce inflammation into the rat TMJ. Nociceptive responses were quantified by assessing behavioral responses categorized by rubbing the

orofacial region and flinching the head quickly. Barretto et al observed that rats pretreated with diclofenac sodium- (10mg/kg i.p.) and LLLT infrared (LST group, 780nm, 70mW, 30s, 2.1J, 52.5J/cm^2 GaAlAs), significantly reduced the formalin nociceptive response. The inflammatory response was assessed by the presence of infiltrate rich in neutrophils, presence of liquefactive necrosis and interstitial edema, hemorrhagic areas, or enlargement of the joint space on the region on histomorphological analysis. The rats pretreated with LLLT showed significantly less inflammatory response and exuberant granulation tissue with higher vascularization, and formation of new collagen fibers. The study established that the pre-conditioning LLLT had an anti-nociceptive and anti-inflammatory effect on the inflammation induced in the TMJ of rodents (Barretto *et al*, 2013).

A similar study, revealing the effect of infrared LLLT in modulating the inflammatory processes and immunological responses was done on the mouse hind paw. Carrageenan inoculation (CGN) was used to induce edema into the plantar surface of left hind paw in male mice. Animals were distributed into five groups: CGN (control), no treatment; Diclo: sodium diclofenac; Paw: LLLT on the paw; Ly: LLLT on the inguinal lymph nodes; and Paw+Ly: LLLT on both paw and lymph nodes. They were also subdivided by time of irradiation: A: 1 h and 2 h before CGN, B: 1 h and immediately before CGN, C: 1 and 2 h after CGN, and D: 3.5 and 4.5 h after CGN. Figure 8 shows the average of the volume of edema at time A. The mice were preconditioned with irradiation 2 and 1 h before edema induction. The study showed that on the second hour there was a steady decrease in the edema of Paw+Ly group and was lower than all irradiated groups on the fourth hour, suggesting that Paw+Ly group was the best irradiation option when preconditioned 2 and 1 h before edema induction. Figure 9 shows the average of the volume of edema at time B where preconditioning was performed 1 h and immediately before the edema induction. The Ly group was the best group to prevent edema 1 h and immediately before the induction and the edema reduction was 44.9 % compared with CGN group. These results suggest that LLLT preconditioning produced both anti-inflammatory and pro-inflammatory effects depending on to the site and timing of irradiation (Meneguzzo *et al*, 2013).

LLLT has been implicated in reducing neuropathic pain by releasing local neurotransmitters such as serotonin (Walker, 1983), stimulating release of endorphins and reduce inflammatory cells (Albertini *et al*, 2007; Hagiwara *et al*, 2008; Lopes-Martins *et al*, 2005). As a mode of peripheral endogenous opioid analgesia the opioid-containing immune cells migrate to inflamed sites and release endorphins to inhibit pain. Pre-conditioning of blood by LLLT on peripheral endogenous opioid analgesia in a rat model of inflamed paw tissue induces analgesia that was transiently antag-

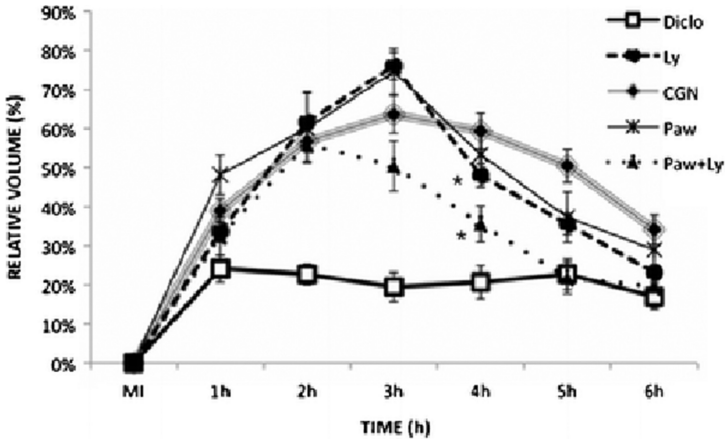

FIG. 8. Average of the volume of edema (%) ± SEM of moment A. . The irradiation was carried out 2 and 1 h before the injection of carrageenan (MI); N=27 for CGN group, N=13 for Diclo group, and N=5 for the other groups. Statistically significant differences compared to CGN are indicated by an asterisk ($p<0.05$) (with permission from Meneguzzo *et al*, 2013).

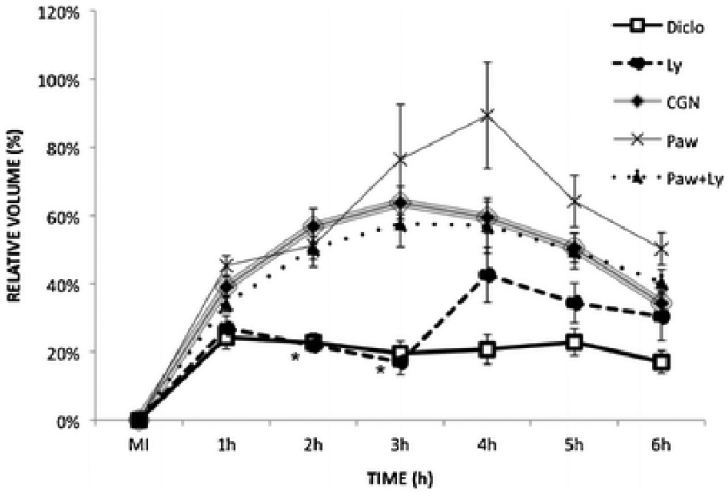

FIG. 9. Average of the volume of edema (%) ± SEM of moment B. The irradiation was carried 1 h and immediately before the injection of carrageenan at the initial moment (MI); N=27 for CGN group, N=13 for Diclo group, and N=5 for the other groups. Statistically significant differences compared to CGN are indicated by an asterisk ($p<0.05$) (with permission from Meneguzzo *et al*, 2013).

onized by naloxone. An upregulation in the expression of peripheral opioid precursor including endorphin precursors, pro-opiomelanocortin and corticotrophin releasing factor in blood cells, suggesting a direct induction by LLLT to mediate analgesia (Hagiwara *et al*, 2008).

Another study, exploring the effect of pre-conditioning with light to reduce pain in rats was done by Yang *et al* (2010) They pre-treated with 650 nm laser and moxibustion in a visceral traction pain model of rats. The differences in the pain score, systolic pressure, activity of acetylcholin-

esterase (AChE), leu-enkephaline (LEK) and the positive index of c-Fos protein and glial fibrillary acidic protein (GFAP) were assessed. The study determined that the pre-conditioned group that received both laser at 650nm and moxibustion could inhibit visceral traction pain by a mechanism that might be due to decreased activity of AChE and increasing the activity of LEK thereby decreasing the expression of c-Fos protein and GFAP.

The above studies suggest that preconditioning with phototherapy might reduce pain and inflammation. Although the mechanism needs to be further explored, this gives us a hope in clinical practice for various pain syndromes.

PRE-CONDITIONING WITH LLLT REDUCES DAMAGE AFTER HEART ATTACK

The beneficial effect of LLLT has been revealed in the infarcted heart model of rat and dogs, which showed reduction of 50–70% of infarct size in 4–6 weeks post-infarction (Oron *et al*, 2001a; Oron *et al*, 2001b; Yaakobi *et al*, 2001). A study by Tuby *et al.* (2006) irradiated the heart with LLLT in both intact and post infarction rats. Myocardial infarction (MI) was induced by occlusion of left anterior descending artery. The myocardium was irradiated for 2 minutes at an energy density of 5 and 12 mW/cm^2. The myocardium was preconditioned with LLLT and after 7 days myocardial infarction was induced and rats were euthanized 21 days post- infarction. Figure 10 shows the effect of preconditioning with LLLT before MI, on the development of infarct post-MI. Tuby et al. (2006) observed that laser

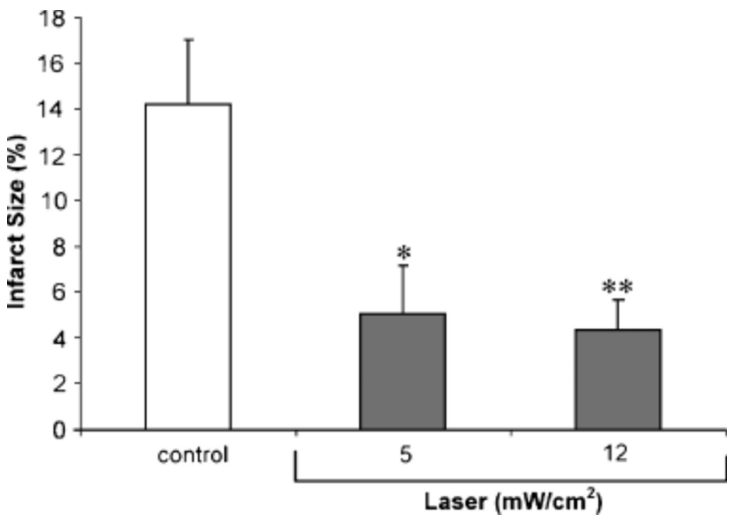

FIG. 10. Effect of LLLT, 7 days before myocardial infarction, on the reduction of infarct size. Infarct size was measured as the relation between the infarct size area to the left ventricle area. Each column represents meanSEM of 7–9 rats; *P<0.05 or **P<0.01. (with permission from Tuby *et al*, 2006).

irradiated pre-conditioned hearts with a power density of 5 mW/cm^2 had 64% less infarct size, and with a power density of 12 mW/cm^2 had 69% less infarct size compared to those that were not pre-conditioned with laser irradiation prior to MI-induction. The expression of genes (iNOS and vascular endothelial growth factor (VEGF)) responsible for angiogenesis and cardioprotection was analyzed. Pre-conditioning the heart with laser treatment before infarction induction resulted in increased levels of VEGF and iNOS in the rats heart and a significant reduction in the infarct size Another study by Zhang *et al.*(2010) studied the effect of pre-conditioning the infarcted myocardium with LLLT prior to cell transplantation. Cell transplantation has emerged as a promising method to regenerate the injured myocardium. Myocardium infarction was induced by ligating the left anterior descending artery of rats. Pre-conditioning to the myocardium was performed by irradiating with a 635 nm, 5mW diode laser and energy density of 0.96 J/cm^2 for 150 seconds. After three weeks bone marrow mesenchymal stem cells (BMSCs) were injected to rats with or without LLLT pretreatment. After LLLT pre-conditioning, there was an increased protein and mRNA expression of VEGF, glucose-regulated protein 78 (GRP78) as well as increased activity of superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) in the infarcted myocardium. LLLT pre-conditioning increased cell survival rate, decreased apoptotic percentage of implanted BMSCs and increased angiogenesis. The study established that LLLT pre-conditioning is a unique non-invasive approach for cell transplantation given intraoperatively to augment cell survival and therapeutic prospective (Zhang *et al*, 2010).

PRECONDITIONING WITH LLLT PROTECTS CELLS FROM TOXINS.

In mammalian tissues, the major photoacceptor molecules that absorb light in NIR range are hemoglobin, myoglobin, and cytochrome c oxidase (Liu *et al*, 2001). Among these, only cytochrome c oxidase (EC 1.9.3.1) has been implicated in the production of energy. Cytochrome c oxidase, a photoacceptor in the NIR range plays an important role in therapeutic photobiomodulation (Liu *et al*, 2001). In primary neuronal cultures, light-emitting diode (LED) 670 nm in NIR range was able to reverse the effect of tetrodotoxin (TTX) (voltage-dependent sodium channel blocker), that impedes neuronal impulse activity, decreases ATP demand, and down-regulates cytochrome c oxidase activity. The treatment with LED 670 nm not only brought the cytochrome c oxidase back to control levels but it also up-regulated enzyme activity of normal neurons above control levels (Liu *et al*, 2001). LED treatments improved the retinal function in rats intoxicated with methanol, by removing the inhibition by formic acid on cytochrome c oxidase (Eells *et al*, 2003) and thus plays a significant role in the therapeutic process of photobiomodulation. Based on these findings, Wong-Riley *et al.* (2005) studied the effect of the cytochrome

c oxidase inhibitor, potassium cyanide (KCN) on primary cultured neurons, and whether KCN could reduce the beneficial effect of LED treatment. They observed that LED treatment partially restored cytochrome c oxidase activity which had been blocked by KCN (10-100 microM) and significantly reduced neuronal cell death (induced by KCN at 300 microM) from 83.6 to 43.5%. Pre-conditioning of the primary neuronal cells enhanced the protective action of LED during KCN exposure (10-100 microM). LED pre-treatment for 10 min using a total energy density of 30 J/cm^2, effectively reduced the number of neurons exhibiting cell death after 300 μM KCN by 48%. Thus, pre-conditioning of neuronal cells by photobiomodulation upregulatd cytochrome c oxidase that led to increased energy metabolism in neurons and promoted wound healing and reversed intoxication by various toxins that inactivate cytochrome c oxidase in vitro (Wong-Riley *et al*, 2005).

Liang *et al* (2006) investigated the effect of NIR-LED preconditioning on primary neuronal cultures to test if it could inhibit apoptotic cell death induced by KCN. The primary neuronal cells were cultured from postnatal rat visual cortex and were pre-treated with LED for 10 min at a total energy density of 30 J/cm2 and were then exposed to potassium cyanide (100–300 μM) for 28 h. The neural cell death via apoptosis was confirmed by electron microscopy Hoechst 33258, single-stranded DNA, Bax, and active caspase-3. LED pre-conditioning applied to the primary neuronal cells significantly reduced apoptosis representing a 50.3% (100μM KCN) and 32.8% (300μM KCN) reduction. LED pretreatment significantly decreased the expression of KCN-induced caspase-3, reversed the increased expression of Bax and decreased expression of Bcl-2 to control levels. The study concluded that preconditioning with light partly protected the neuronal cells from cyanide by a mechanism most likely involving decrease of ROS production, down-regulation of pro-apoptotic proteins, activation of anti-apoptotic proteins and by increasing energy metabolism in the neurons (Liang *et al*, 2006).

PRECONDITIONING WITH LLLT IN WOUND HEALING:

The penetration of NIR is deeper than UV or visible light and is harmless to living tissue. Therapeutic devices using NIR have been successfully used for the treatment of various injuries chiefly infected, ischemic, and hypoxic wounds (Conlan *et al*, 1996; Sommer *et al*, 2001; Whelan *et al*, 2001). Studies have shown that NIR promotes wound healing but the mechanisms are poorly understood.

Wound healing is a complex process consisting of multiple physiologic events which are controlled by an infinite array of signaling mechanisms (Barolet and Boucher, 2010). Any dysregulation in the process of wound healing leads to abnormal scarring and might lead to the development of keloids and hypertrophic cells. Treatment of these abnormal scars is

really challenging to the clinicians and is associated with low self-esteem and impaired quality of life in affected individuals. Recent studies have implicated a major role of transforming growth factor beta-I (TGF-β1) in the formation of hypertrophic scars and keloids. A higher expression or dysregulation of TGF-β1 is associated with fibroblastic proliferation and excess collagen deposition (Bouzari *et al*, 2007; Wolfram *et al*, 2009). LLLT with red light and with NIR has been shown to promote wound healing including modulation of TGF-β1 making it useful in scar treatment. A study by Barolet and Boucher (2010) used LED pre-conditioning with NIR irradiation (805nm at 30mW/cm^2) for 30 days in three patients prone to development of hypertrophic scars or keloids after surgery. They found a significant improvement on the NIR-treated areas compared to the control scars. The enhancement of wound healing process might be due to modulation of proteins involved in regulation of wound repair such as TGF-β1 platelet- derived growth factor (PDGF), interleukins (IL-6, 13, 15) and matrix metalloproteinases (MMPs) by LLLT. However large patient samples are needed for evaluation of this promising technique.

PRECONDITIONING WITH LLLT IN THE CENTRAL NERVOUS SYSTEM

The function of brain is highly dependent on cerebral bloodflow (CBF) (Balestreri *et al*, 2006; Ling and Neal, 2005). In diseases with reduced CBF such as traumatic brain injury, degenerative diseases such as Parkinson's disease and Alzheimer's disease, improving the CBF has shown to not only prevent these diseases but also increases the response to treatment of these diseases (Uozumi *et al*, 2010; Wolfson *et al*, 1985). Photobiostimulation effects of near-infrared (NIR) laser irradiation is known for a long time. It has also been shown that NIR laser irradiation is very efficient for reducing cerebral ischemia both in vivo and clinically. The NIR laser irradiation produces vasodilatory effects which might be mediated by nitric oxide (NO) (Hamblin, 2008). Uozumi *et al.* (2010) studied the effect of 808 nm laser irradiation at three different power densities (0.8, 1.6 and 3.2 W/cm^2) on CBF in mice and measured directly NO in brain tissue during NIR laser irradiation using an amperometric NO-selective electrode. They also observed the influence of NO and a neurotransmitter, glutamate, to the regulation of CBF by using a nitric oxide synthase (NOS) inhibitor, nitro-L-arginine methyl ester hydrochloride (L-NAME), and an N-methyl-D-aspartate (NMDA) receptor blocker, MK-801, respectively. The protective effect of NIR laser irradiation on transient cerebral ischemia using a model of transient bilateral common carotid artery occlusion (BCCAO) in mice was also investigated. As compared to the control mice, the NIR laser irradiation (1.6 W/cm^2 for 15-45 minutes) significantly increased local CBF and cerebral NO concentration. Pre-conditioning the brain with NIR laser irradiation increased the residual CBF by 30% and significantly decreased the numbers of apoptot-

ic cells in the hippocampus in mice subjected to BCCAO and neuronal damage. These results can be partly explained by the fact that pre-conditioning with NIR laser irradiation led to the alleviation of NO surge after the reperfusion.

Low-energy laser irradiation in the far red to NIR range not only has vasodilatory effects but can also modulate many biological processes (Karu, 1999) by increasing mitochondrial respiration, or ATP synthesis (Karu *et al*, 1995; Passarella *et al*, 1984) and promoting cell survival (Shefer *et al*, 2002). NIR is believed to accelerate the electron transfer in the respiratory chain and activation of photoacceptors, including cytochrome c oxidase in the mitochondria (Beauvoit *et al*, 1994). Cyanide toxicity has been implicated in the cell death by inhibition of cytochrome oxidase (Bhattacharya and Lakshmana Rao, 2001) and compromising energy status (Wong-Riley *et al*, 2005). Moreover, depending on the concentration (Prabhakaran *et al*, 2004) or the susceptibility of brain (Mills *et al*, 1999) cyanide produces neuronal cell deathy either by apoptosis or necrosis. Exposure to cyanide produces apoptosis in cortical neurons but necrosis in mesencephalic cells (Prabhakaran *et al*, 2002).

PRECONDITIONING WITH LLLT PROTECTS SKIN FROM ULTRAVIOLET DAMAGE.

Ultraviolet radiation (UVA, 290-320nm; UVB 320-400 nm) exposure, damages skin cells which leads to photoaging and cancer of the skin (Calles *et al*, 2010; Sinha and Hader, 2002). The mechanisms responsible for the UV damage to the skin could be due to the breakdown of collagen fibers, free radical formation, DNA damage and inhibition of the immune system (Schroeder *et al*, 2010). The effects of UV radiation can be minimized by avoiding sun exposure or the use of sunscreens. Conversely, the exposure to sun cannot be avoided in certain occupations and the use of sunscreen has many limitations such as compliance, allergy and possible harmful effects of nanoparticles that are contained in most sunscreens (Kimura *et al*, 2012). Recent studies have shown protective effects of infrared (IR) exposure against harmful effects of UV-irradiation to the skin (Avci *et al*, 2013).

The light in the early morning is predominantly composed of visible and NIR solar wavelengths and at noon contains substantial amounts of UVB and UVA radiation, suggesting a natural defense mechanism for mammals that involves pre-conditioning the skin with red/NIR light in the morning to protect against impending damaging UV radiation later in the day (Barolet and Boucher, 2008). Patients with polymorphous light eruption (PLE) and healthy controls were exposed to LED treatments (660 nm) on experimental anterior thigh region. Baseline minimal erythema doses (MED) were then determined. UV radiation was thereafter performed on both experimental and control areas and digital pictures of

the MED TEST areas were taken prior to UV exposure and immediately, 24 hours, 7 and 14 days after. Figures 11 and 12 show Erythema Index at 24 hours as a function of MEDs for the LED pre-treated and control sides. There was a significant reduction in UV-B induced erythema reaction in both control and patients suffering from PLE preconditioned with LED. Overall, less redness was observed for each tested MED on the

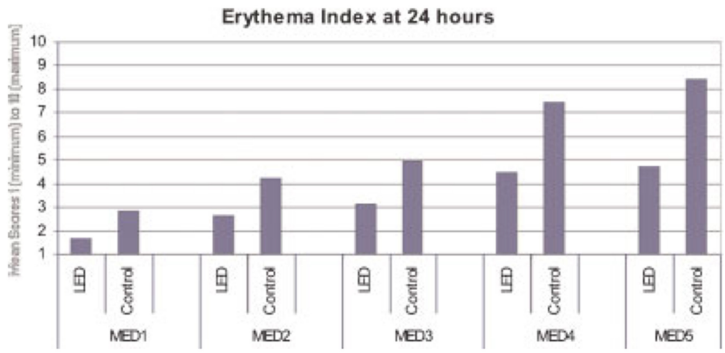

FIG. 11. Mean scores for the Erythema Index at 24 hours as a function of MEDs for the LED pre-treated and CONTROL sides. (with permission from Barolet and Boucher, 2008).

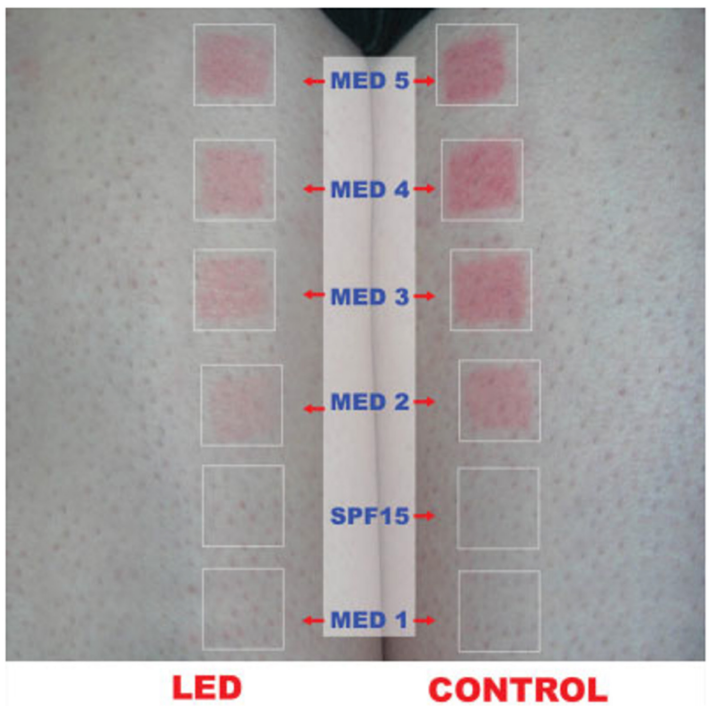

FIG. 12. MED responses for the LED and CONTROL side at 24 hours for MED 1–MED 5 for a subject from Group II. (with permission from Barolet and Boucher, 2008).

preconditioned experimental areas compared to control areas. These results suggest that pre-conditioning with LED prior to UV exposure provided significant protection against UV-B induced erythema (Barolet and Boucher, 2008).

Illumination of the skin with non-coherent NIR (700- 2000 nm) generated a strong cellular defense mechanism against solar UV cytotoxicity and induced cellular mitosis without increasing skin temperature. This protection against UV light was long lasting (at least 24 hours) and cumulative effect (Menezes *et al*, 1998). Furthermore, another study proposed that pre-conditioning with IR radiation protects human fibroblasts cells by inducing resistance to the harmful effects of UV light by affecting the genes of mitochondrial apoptotic pathway. IR preconditioning of human fibroblasts inhibited UVB activation of caspase-9 and -3 and partial release of cytochrome c and Smac/Diablo, decrease proapoptotic proteins (ie, Bax), and increase antiapoptotic proteins (ie, Bcl-2 or Bcl-xL), contributing the cell to resist UVB-triggered apoptosis. This effect could be mediated most likely by the induction of heat shock protein Hsp27 by IR, which prevents apoptosome assembly (Frank *et al*, 2004).

CONCLUSION

Pre-conditioning regimens are increasingly receiving more attention in the biomedical arena. The concept that low levels of stress can induce a protective response against all manner of subsequent insults is inherently attractive. One of the problems preventing wider acceptance is that of striking a balance between the degree of preconditioning stress needed, the severity of the possible adverse consequence and its likelihood of happening. For instance being subjected to hypoxia, caloric restriction, hypothermia or hyperthermia might be considered too much of a price to pay, to protect oneself against a slim chance of relatively mild extra damage in a heart bypass operation. The same cannot be said about light therapy. LLLT has many advantages in that it is painless, inexpensive if LEDs are employed, and free of potential side effects, making it a “no-brainer” before surgery or potentially hazardous adventures. Moreover the multiple demonstrations of LLLT effectiveness for preconditioning of muscles before exercise, suggests that it will soon be taken up by the general public, and may in fact have to be regulated by the athletic authorities as a concern that could be called “light-doping”.

One topic that is of great interest to all investigators working in the pre-conditioning field, is the similarities and differences between the molecular and cellular mechanisms that have been proposed to explain the effects of each different modality. It is quite clear that there is not going to be a single “unified field theory” that will explain every pre-conditioning modality that has been reported. However, it is equally clear that there an intriguing number of overlaps between the various modal-

ities. Mitochondria, reactive oxygen species, HIF1α, HSF have all been implicated in more than one modality of preconditioning and there may yet be more common pathways left to discover. As the progression of preconditioning therapy into the mainstream of clinical medicine continues we expect that much interesting science will be reported.

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