

## Low-level laser therapy modulates musculoskeletal loss in a skin burn model in rats<sup>1</sup>

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DOI: <http://dx.doi.org/10.1590/S0102-86502015002000002>

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### ABSTRACT

**PURPOSE:** To investigate the effectiveness of low-level laser therapy (LLLT) on *gastrocnemius* muscle morphology and MyoD immunoexpression in a model of dorsal burn in rats.

**METHODS:** Sixteen male Wistar rats were distributed into two groups: control group (CG): rats submitted to scald burn injury without treatment and laser treated group (LG): rats submitted to scald burn injury and treated with laser therapy. Fourteen days post-surgery, *gastrocnemius* muscle was evaluated being the specimens stained with HE and morphometric data was evaluated. MyoD expression was assessed by immunohistochemistry.

**RESULTS:** The results showed that laser treated animals presented more organized tissue morphology compared to the non-treated animals, with a higher number of nucleus in the fibers. Also, the cross sectional area of the fibers and the MyoD immunoexpression in the laser treated groups was higher.

**CONCLUSION:** Low-level laser therapy had positive effects on *gastrocnemius* muscle, improving tissue muscle morphology, increasing cross sectional area and MyoD immunoexpression.

**Key words:** Burns. Laser Therapy, Low-Level, MyoD Protein. Rats.

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## Introduction

Burns are severe injuries associated with tissue destruction, infection, pain or even death<sup>1</sup>. It is estimated that more than 1 million burn injuries occur every year in the United States, resulting in more than 500,000 emergency department visits<sup>2</sup>.

Depending on the extension of the injury, perturbations in metabolism affecting different physiological system occur and result in inflammatory and hypermetabolic stress response<sup>3</sup>. Also, severe burn leads to a significant increase in the levels of peripheral catecholamine and cortisol, and severe skeletal muscle wasting<sup>3</sup>.

Although the muscle skeletal tissue is one of the most affected, little attention has been given to the physiological modifications occurred on this system after severe burns<sup>4</sup>. Patients with burns greater than 40% are always catabolic<sup>3</sup>, which will affect their metabolic derangements and persist at least one year after injury in most body tissues<sup>3</sup>. These thermal injuries result in both local and systemic responses in skeletal muscle distant from the site of thermal injury<sup>5,6</sup>.

Muscle catabolism, marked loss of protein, redistribution of proteins from muscle to burned tissue and modification in body composition are common consequences of thermal injury<sup>3,4,7</sup>. Moreover, muscle mitochondria activity is also affected by burn, with a reduction in mitochondrial cytochrome C levels, increase in the concentration of cytochrome C in the cytosol and altered membrane potential, contribution with a severe alteration of skeletal muscle bioenergetic<sup>3,8</sup>.

In this context, it is extremely important to develop new clinical innovative approaches aiming to reduce and preventing damages related to skin burn, restoring normal physiological function and improving quality of life in patients<sup>3</sup>. Low-level laser therapy (LLLT) is a technology that has been demonstrating positive effects on the stimulation of cell activities involved in the wound healing process<sup>9-11</sup>. The action of LLLT is based on the absorption of the light by tissues, which will generate modifications in cell metabolism<sup>12,13</sup>. Studies have shown that laser irradiation increased mitochondrial respiration and adenosine triphosphate (ATP) synthesis in isolated cells in culture<sup>14</sup>. Other studies have shown that laser light affects calcium exchange through the cell membrane, causing transient changes in the cytoplasmic calcium level<sup>15,16</sup>. These modifications can increase DNA, RNA and cell-cycle regulatory proteins synthesis, stimulating cell proliferation which could therefore be beneficial for the reestablishment of connective tissue during tissue repair and wound healing<sup>17-19</sup>.

Bayat *et al.*<sup>19</sup> evaluated the effects of helium–neon laser on second-degree burns in rats and found a significant

increase in the number of macrophages and an amount of new formed skin tissue (new epidermis) in the laser treated group in comparison with control at the site of the lesion. Other authors found similar results such as reduction of inflammatory process, increase in granulation tissue and fibroblast proliferation, resulting in the acceleration of third-degree burn healing after laser photoestimulation<sup>2,13</sup>.

Although the positive effects of this therapeutic intervention on the acceleration of burned skin healing, there is a lack of information about the interaction of LLLT on skeletal muscle modifications in this clinical condition. In this context, the hypothesis that LLLT would affect mitochondria activity in skeletal muscles in the presence of severe burn, increasing tissue bioenergetic and metabolism and preventing muscle loss and atrophy was raised. Thus, the aim of this study was to determine the effectiveness of LLLT on *gastrocnemius* muscle morphology and MyoD immunoeexpression in a model of dorsal burn in rats.

## Methods

Research approved by the Experimental Animal Use Committee of Federal University of Sao Paulo (0344/12). All animal handling and surgical procedures were strictly conducted according the Guiding Principles for the Use of Laboratory Animals.

Sixteen Wistar male rats (weighing  $300 \pm 20$  g) were used. They were maintained under controlled temperature ( $22 \pm 2^\circ\text{C}$ ), light-dark periods of 12 hours, and with free access to water and commercial diet.

The animals were randomly distributed into two groups of eight animals each: control group (CG): rats submitted to scald burn injury without treatment and laser treated group (LG): rats submitted to scald burn injury and treated with laser therapy. Animals were sacrificed 14 days post-surgery.

### *Scald burn injury*

Animals were anesthetized (Ketamine 50 mg/Kg and Xylazin 10 mg/Kg) and had their dorsum and ventral regions shaved and cleaned. A nonlethal scald burn injury was administered by immersing 45% of each rat's body –in  $87^\circ\text{C}$  water as firstly described by Walker and Mason<sup>20</sup>. As a result, each animal had 30% of its dorsal and 15% of its ventral area exposed to scald burn for 10 and 3 seconds, respectively. Every rats were injected subcutaneously with the analgesic buprenorphine (0.2 mg/kg) immediately following scald injury and again 24h later. Animals were returned to their cages and the status of each animal was checked by recording the body weight and food intake of each daily.

### Laser therapy

Laser irradiation initiated immediately after the surgery and it was performed daily. A low-energy GaAlAs laser (Photolase DMC Ltda<sup>®</sup>), 808 nm, continuous wavelength, 30 mW, 3.57 W/cm<sup>2</sup>, beam diameter 0,028 mm, 1.5°, fluency of 50 J/cm<sup>2</sup>, irradiation time of 48 seconds (energy per point 1.44J). The irradiation was performed through the punctual contact technique, at one point, on the middle of the muscle belly of the *gastrocnemius* muscle.

At the end of the experimental period, rats were sacrificed individually by carbon dioxide asphyxia and muscles were removed for analysis.

### Histological procedures

After harvesting the specimens of all experimental group, the middle part of the *gastrocnemius* muscles was washed immediately with saline and fixed in 10% buffered formalin (Merck, Darmstadt, Germany) for 24 hours, followed by dehydration in a graded series of ethanol and embedding in paraffin. In the transversal axis of the muscle, thin sections (4 µm) were prepared using a microtome (Leica Microsystems SP 1600, Nussloch, Germany). Six sections of each specimen were stained with hematoxylin and eosin (HE) (Merck) and examined using light microscopy (Axio Observer.D1 Zeiss<sup>®</sup>, Germany).

### Immunohistochemistry

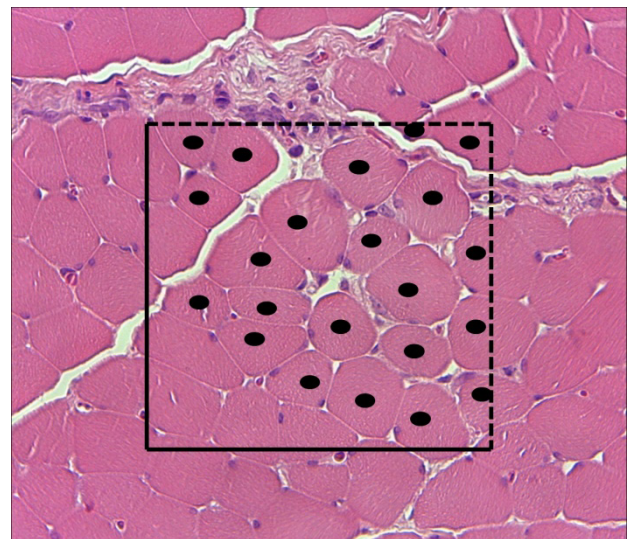
Paraffin was removed with xylene from serial sections of 4 µm and the sections were rehydrated in graded ethanol, then pretreated in a microwave with 0.01 M citric acid buffer (pH 6) for three cycles of 5 min each at 850 W for antigen retrieval. The material was pre-incubated with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) solution for 5 min for inactivation of endogenous peroxidase and then blocked with 5% normal goat serum in PBS solution for 10 min. The specimens were then incubated with anti-MyoD polyclonal primary antibody (MyoD-5.8A, SC-32758; Santa Cruz Biotechnology, USA) at a concentration of 1:100. Incubation was carried out overnight at 4°C within the refrigerator. This was followed by two washes in PBS for 10 min. The sections were then incubated with biotin conjugated secondary antibody anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) at a concentration of 1:200 in PBS for 1 h. The sections were washed twice with PBS followed by the application of preformed avidin biotin complex conjugated to peroxidase

(Vector Laboratories) for 45 min. The bound complexes were visualized by the application of a 0.05% solution of 3-3'-diaminobenzidine solution and counterstained with Harris hematoxylin. For control studies of the antibodies, the serial sections were treated with rabbit IgG (Vector Laboratories) at a concentration of 1:200 in place of the primary antibody. Additionally, internal positive controls were performed with each staining bath.

### Morphometric evaluation

Six photomicrographs, stained with HE, were obtained at 40x magnification and were evaluated by light microscopy with an attached camera (AxioCam HRc, Zeiss<sup>®</sup>, Germany). Using an image software (Axiovision 4.8, Zeiss<sup>®</sup>, Germany) two parameters were investigated: the cross sectional area of muscle fibers and cell density (cells/mm<sup>2</sup>) of transverse section of muscle.

The cross sectional area of muscle fibers was measured (µm<sup>2</sup>) in thirty fibers randomly selected of the same photomicrographs used for cell density. A blind procedure was used for measurements. Cell density was analyzed following the guidelines described by Mandarin-de-Lacerda<sup>21</sup> in six random fields obtained in each animal. Counting was carried out using a test frame (100µm x 100µm at 40x magnification) and the muscle fibers were counted provided entirely within an counting frame of the inclusion lines (dashed lines, Figure 1) of a counting frame but not any of the exclusion lines (Figure 1).



**FIGURE 1** - Test frame utilized to determine cell density. Inclusion lines (dashed lines) and exclusion lines (solid lines). Black points represent the muscle fibers counted.



Statistical analysis

Student t test were performed to compare the morphometric assessment (cell density and cross sectional area of muscle fibers) between groups. Statistica version 7.0 (data analysis software system - StatSoft Inc.) was used to carry out the statistics analysis. Values of  $p < 0.05$  were considered statistically significant.

RESULTS

Histopathological analysis

The analysis with HE revealed in CG histopathological changes such as muscular fascicles with heterogeneous distribution and increased interfibrillar space with cells exhibiting some irregular contours different from characteristic polygonal contour of skeletal muscle fibers (Figure 2a). In addition, CG showed increased amount of connective tissue (Figure 2c).

In Figure 2b, the treated animals showed muscular fascicles and fibers equidistantly distributed. The quantity of nuclei was increased in LG (Figure 2b, d) when compared with CG (Figure 2a, c). There were great variation in size of muscle fibers and fiber splitting (Figure 2d).

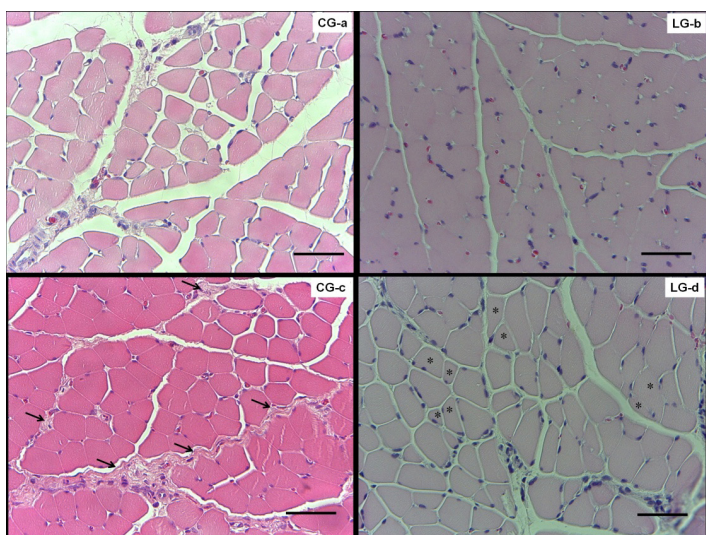


FIGURE 2 - Photomicrographs of *gastrocnemius* muscle cells of CG (a, c) and LG (b, d). In a, muscular fascicles with heterogeneous distribution and increased interfibrillar space with cells with irregular contours, different from treated animals (b) with muscular fascicles and fibers equidistantly distributed. CG (c) shows increased amount of connective tissue (arrows) and LG (d) fiber splitting (\*). HE stain, barr=50µm.

Immunohistochemical data

MyoD expression has been detected in the nuclei of cells (Figure 3). LG showed increased quantity of immunopositive nuclei when compared with C.

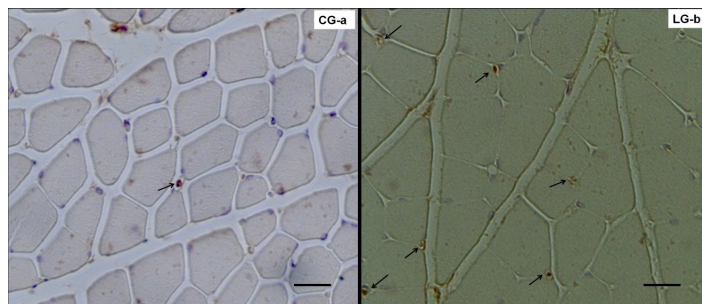


FIGURE 3 - Immunoeexpression of MyoD. (a), control group. (b), laser group. (HE stain).

Morphometry

Cross sectional area (CSA) evaluation of muscle cells revealed that the laser therapy was effective because treated group had increased cross sectional area when compared with control group ( $p < 0.05$ ) (Figure 4). Nevertheless, there were no significant differences in cell density across all experimental groups (Figure 5).

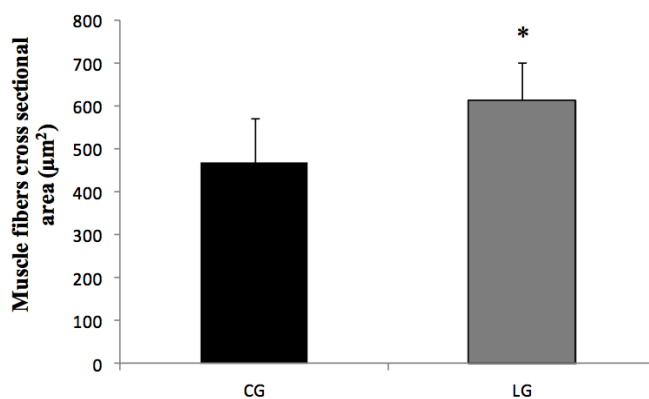


FIGURE 4 - Means and stand deviation of muscle fibers cross sectional area (µm²). \* $p < 0.05$ .

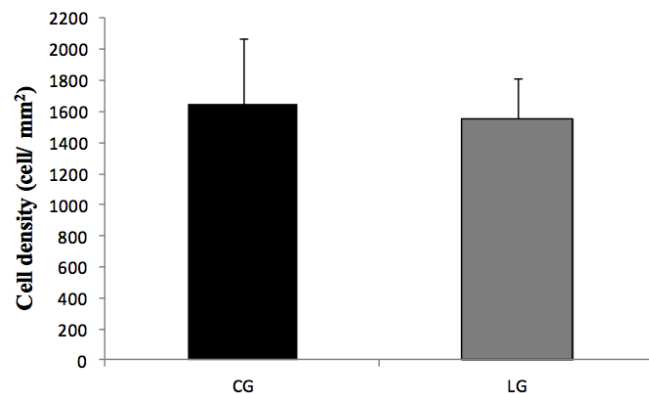


FIGURE 5 - Means and stand deviation of muscle fibers density (cells/mm²).

## Discussion

This study aimed to evaluate the effects of *in vivo* muscle tissue response of photolasertherapy on *gastrocnemius* muscle in burned rats. The main findings showed that laser treated animals presented more organized tissue morphology compared to the non-treated animals, with a higher number of nucleus in the fibers. Also, the CSA of the fibers and the MyoD immunoexpression in the laser treated groups were higher. Interestingly, similar findings in the cell density were observed for both groups.

The exact mechanisms underlying the therapeutic effects of LLLT are not yet well-established<sup>11,22</sup>. It seems that LLLT acts at the molecular, cellular and tissue levels, stimulating mitochondria and increasing ATP production<sup>22</sup>. As described above, laser energy is absorbed by chromophores in the cells, mainly cytochrome *c* oxidase, increasing the mitochondrial products such as ATP, NADH, protein, and RNA, as well as a reciprocal augmentation in oxygen consumption<sup>11,22</sup>. All these physiological modifications induced by LLLT may have stimulated the impaired function of the mitochondrias due to the skin burn, decreasing the levels of muscle catabolism and loss of protein, which may result in the more organized muscle tissue and increased number of fiber nucleus observed after laser irradiation.

In this study, MyoD immunoexpression was increased compared to the CG. Muscle regeneration is controlled by muscle regulatory factors (MRFs), including MyoD<sup>23</sup>. MyoD is related to satellite cell proliferation at the beginning of the muscle healing<sup>24</sup> and the arrival of satellite cells is crucial once they will turn into myoblasts to replace the impaired fibers muscle<sup>25</sup>. These statements corroborate the findings of Rodrigues *et al.*<sup>26</sup>, who found an upregulation in RNA of MyoD expression after laser irradiation in a model of cryolesion in tibial muscle of rats. The results of this study suggest that laser therapy may activate satellite cells which were responsible by the stimulation of new muscle fibers formation.

Also, the CSA of the fibers were significantly increased in the irradiated animals. Previous studies have already demonstrated that LLLT increased the number of cells per muscle fiber and enlarged muscle fiber diameter in different experimental models<sup>27-30</sup>. In a clinical study, Ferraresi *et al.*<sup>31</sup> demonstrated an increase of the muscle volume of the thigh of the trained irradiated limb compared to the trained limb only. It seems that laser therapy acts on the activation of muscle satellite cell (myogenic regulatory precursor cell) and modulates myogenic transcription factors expression, such as MyoD e Myogenin, markers of muscle growth and hypertrophy. This fact led us to infer that the regulation of these

events by LLLT may contribute to new muscle fiber formation and promote the increase in muscle hypertrophy in the burned rats.

Burn injury is associated with muscle wasting and represents a model of cachexia<sup>32</sup>. The cachectic state is observed in many pathological conditions such as cancer. According to Antoun *et al.*<sup>33</sup> skeletal muscle density predicts prognosis in patients with metastatic renal cell carcinoma treated with targeted therapies, i.e., high densities of muscle fiber are related with muscle wasting because when the profile of fibers decreases results in increased fiber density. In the present study, no difference was found in the measurement in fiber density. Considering that burn injury causes increase of fiber density because decreased muscle fiber cross sectional, the laser therapy interestingly do not induced decreased of fiber density despite induced higher CSA. New studies using different periods post-burn are needed to confirm the effects of LLLT in muscle fiber density.

## Conclusion

Low-level laser therapy had positive effects on *gastrocnemius* muscle, improving tissue muscle morphology, increasing cross sectional area and MyoD immunoexpression.

## References

- Garcia VG, de Lima MA, Okamoto T, Milanezi LA, Júnior EC, Fernandes LA, de Almeida JM, Theodoro LH. Effect of photodynamic therapy on the healing of cutaneous third-degree-burn: histological study in rats. *Lasers Med Sci.* 2010 Mar;25(2):221-8. doi: 10.1007/s10103-009-0694-z.
- Vasheghani MM, Bayat M, Rezaei F, Bayat A. Effect of low-level laser therapy on mast cells in deep second-degree burns of rats. *Photomed Laser Surg.* 2008 Feb;26(1):1-5. doi: 10.1089/pho.2007.2103.
- Porter C, Hurren NM, Herndon DN, Børsheim E. Whole body and skeletal muscle protein turnover in recovery from burns. *Int J Burns Trauma.* 2013;3(1):9-17. PMID: 23386981.
- Cree MG, Fram RY, Herndon DN, Qian T, Angel C, Green JM, Mlcak R, Aarsland A, Wolfe RR. Human mitochondrial oxidative capacity is acutely impaired after burn trauma. *Am J Surg.* 2007 Aug;196(2):234-9. doi: 10.1016/j.amjsurg.2007.09.048.
- De Oliveira F, Bevilacqua LR, Anaruma CA, Boldrini Sde C, Liberti EA. Morphological changes in distant muscle fibers following thermal injury in Wistar rats. *Acta Cir Bras.* 2010 Dec;25(6):525-8. doi:10.1590/S0102-86502010000600012.
- De Oliveira BC, De Oliveira F, Martini DT, Prisco CR, Da Silva Rigueti MM, Liberti EA, De Campos Boldrini S. The relative effects of severe burn injury and pre- and post-natal protein deprivation on mandibular condyle morphology. *Histol Histopathol.* 2010 Jan;25(1):45-54. PMID: 19924640.
- Gore DC, Chinkes DL, Wolf SE, Sanford AP, Herndon DN, Wolfe RR. Quantification of protein metabolism in vivo for skin, wound, and muscle in severe burn patients. *JPEN J Parenter Enteral Nutr.* 2006 Jul-Aug;30(4):331-8. doi: 10.1177/0148607106030004331.

8. Yasuhara S, Perez ME, Kanakubo E, Yasuhara Y, Shin YS, Kaneki M, Fujita T, Martyn JA. Skeletal muscle apoptosis after burns is associated with activation of proapoptotic signals. *Am J Physiol Endocrinol Metab.* 2000 Nov;279(5):E1114-21. PMID: 11052967.
9. Dortbudak, O, Hass R, Mailath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. *Clin Oral Implants Res.* 2000 Dec;16: 540-5. doi: 10.1034/j.1600-0501.2000.011006540.x.
10. Medrado AR, Pugliese LS, Reis SR, Andrade ZA. Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. *Lasers Surg Med.* 2003 Feb;32(3):239-44. doi: 10.1002/lsm.10126.
11. Karu T, Tiphlova O, Samokhina M, Diamantopoulos C, Sarantsev VP, Shveikin V. Effects of near-infrared laser and superluminous diode irradiation on *Escherichia coli* division rate. *IEEE J Quantum Electron.* 1990;26(12):2162-5. doi: 10.1109/3.64353.
12. Bossini PS, Fangel R, Habenschus RM, Renno AC, Benze B, Zuanon JA, Neto CB, Parizotto NA. Low-level laser therapy (670 nm) on viability of random skin flap in rats. *Lasers Med Sci.* 2009 Mar;24(2):209-13. doi: 10.1007/s10103-008-0551-5.
13. Meireles GC, Santos JN, Chagas PO, Moura AP, Pinheiro AL. Effectiveness of laser photobiomodulation at 660 or 780 nanometers on the repair of third-degree burns in diabetic rats. *Photomed Laser Surg.* 2008 Feb;26(1):47-54. doi: 10.1089/pho.2007.2051.
14. Coombe AR, Ho C, Philips JR, Chapple CC. The effects of low level laser irradiation on osteoblastic cells. *Clin Orthod Res.* 2006 Feb;4:3-14. doi: 10.1034/j.1600-0544.2001.040102.x.
15. Rezende SB, Ribeiro MS, Nunes SC, Garcia VG, Maldonado EP. Effects of a single near-infrared laser treatment on cutaneous wound healing: biometrical and histological study in rats. *J Photochem Photobiol B.* 2007 Jun;87(3):145-53. doi: 10.1016/j.jphotobiol.2007.02.005.
16. Shinzawa H, Takeda A, Sone Y, Murashita K, Uchinuma E. Wound healing process of a full-thickness skin wound model in rats. *Int Surg.* 2007 Mar-Apr; 92(2):63-72. PMID: 17518246.
17. Sasaki GH, Pang CY. Hemodynamics and viability of acute neurovascular island skin flaps in rats. *Plast Reconstr Surg.* 1980 Feb;65:152-8. PMID: 7352155.
18. Kaufman T, Angel MF, Eichenlaub EH, Levin M, Hurwitz DJ, Futrell JW. The salutary effects of the bed on the survival of experimental flaps. *Ann Plast Surg.* 1985 Jan;14:64-73. PMID: 3883875.
19. Bayat M, Vasheghani MM, Razavi N. Effect of low-level helium-neon laser therapy on the healing of third-degree burns in rats. *J Photochem Photobiol B.* 2006 May;83(2):87-93. doi: 10.1016/j.jphotobiol.2005.12.009.
20. Walker HL, Mason JAD. A Standard animal burn. *J Trauma.* 1968 Nov;8:1049-51. PMID: 5722120.
21. Mandarim-de-Lacerda, CA. Stereological tools in biomedical research. *An Acad Bras Cienc.* 2003 Dec;75:469-86. doi: 10.1590/S0001-37652003000400006.
22. 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 Feb;40(2):516-33. doi: 10.1007/s10439-011-0454-7.
23. Liu XG, Zhou YJ, Liu TC, Yuan JQ. Effects of low-level laser irradiation on rat skeletal muscle injury after eccentric exercise. *Photomed Laser Surg.* 2009 Dec;27(6):863-9. doi: 10.1089/pho.2008.2443.
24. Herman JH, Khosla RC. Nd:YAG laser modulation of synovial tissue metabolism. *Clin Exp Rheumatol.* 1989 Sep-Oct;7(5):505-12. PMID: 2591125.
25. Marsh DR, Criswell DS, Carson JA, Booth FW. Myogenic regulatory factors during regeneration of skeletal muscle in young, adult, and old rats. *J Appl Physiol.* 1997 Oct;83(4):1270-5. PMID: 9338436.
26. Rodrigues NC, Brunelli R, de Araújo HS, Parizotto NA, Renno AC. Low level laser therapy (LLLT) (660nm) alters gene expression during muscle healing in rats. *J Photochem Photobiol B.* 2013 Mar;120:29-35. doi: 10.1016/j.jphotobiol.2013.01.002.
27. Gigo-Benato D, Russo TL, Tanaka EH, Assis L, Salvini TF, Parizotto NA. Effects of 660 and 780 nm low-level laser therapy on neuromuscular recovery after crush injury in rat sciatic nerve. *Lasers Surg Med.* 2010 Nov;42(9):673-82. doi: 10.1002/lsm.20978.
28. Shefer G, Barash I, Oron U, Halevy O. Low-energy laser irradiation enhances de novo protein synthesis via its effects on translation-regulatory proteins in skeletal muscle myoblasts. *Bioch Biophys Acta.* 2003 Feb;1593(2-3):131-9. doi: 10.1016/S0167-4889(02)00350-6.
29. Nakano J, Kataoka H, Sakamoto J, Origuchi T, Okita M, Yoshimura T. Low-level laser irradiation promotes the recovery of atrophied gastrocnemius skeletal muscle in rats. *Exp Physiol.* 2009 Sep;94(9):1005-15. doi: 10.1113/expphysiol.2009.047738.
30. Assis L, Moretti AI, Abrahao TB, de Souza HP, Hamblin MR, Parizotto NA. Low-level laser therapy (808 nm) contributes to muscle regeneration and prevents fibrosis in rat tibialis anterior muscle after cryolesion. *Lasers Med Sci.* 2013 May;28(3):947-55. doi: 10.1007/s10103-012-1183-3.
31. Ferraresi C, de Brito Oliveira T, de Oliveira Zafalon L, de Menezes Reiff RB, Baldissera V, de Andrade Perez SE, Matheucci Júnior E, Parizotto NA. Effects of low level laser therapy (808 nm) on physical strength training in humans. *Lasers Med Sci.* 2011 May;26(3):349-58. doi: 10.1007/s10103-010-0855-0.
32. Pedroso FE, Spalding PB, Cheung MC, Yang R, Gutierrez JC, Bonetto A, Zhan R, Chan HL, Namias N, Koniaris LG, Zimmers TA. Inflammation, organomegaly, and muscle wasting despite hyperphagia in a mouse model of burn cachexia. *J Cachexia Sarcopenia Muscle.* 2012 Sep;3(3):199-211. doi: 10.1007/s13539-012-0062-x.
33. Antoun S, Lanoy E, Iacovelli R, Albiges-Sauvin L, Lorient Y, Merad-Taoufik M, Fizazi K, di Palma M, Baracos VE, Escudier B. Skeletal muscle density predicts prognosis in patients with metastatic renal cell carcinoma treated with targeted therapies. *Cancer.* 2013 Sep;119(18):3377-84. doi: 10.1002/cncr.28218.

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Received: Oct 15, 2014

Review: Dec 17, 2014

Accepted: Jan 12, 2015

Conflict of interest: none

Financial source: National Council for Scientific and Technological Development (CNPq) and Sao Paulo Research Foundation (FAPESP)

<sup>1</sup>Research performed at Laboratory of Biomaterials and Biocompatibility, Biosciences Department, Federal University of Sao Paulo (UNIFESP), Santos-SP, Brazil.