

Muscle Regeneration

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Low-level laser irradiation promotes the recovery of atrophied gastrocnemius skeletal muscle in rat.

[Nakano J](#), [Kataoka H](#), [Sakamoto J](#), [Origuchi T](#), [Okita M](#), [Yoshimura T](#).

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Low-level laser (LLL) irradiation promotes proliferation of muscle satellite cells, angiogenesis and expression of growth factors. Satellite cells, angiogenesis and growth factors play important roles in the regeneration of muscle. The objective of this study was to examine the effect of LLL irradiation on rat gastrocnemius muscle recovering from disuse muscle atrophy. Eight-week-old rats were subjected to hindlimb suspension for 2 weeks, after which they were released and recovered. During the recovery period, rats underwent daily LLL irradiation (Ga-Al-As; 830 nm; 60 mW; total, 180 sec) to the right gastrocnemius muscle through the skin. The untreated left gastrocnemius muscle served as the control. In conjunction with LLL irradiation, 5-bromo-2.deoxyuridine (BrdU) was injected subcutaneously for labeling of nuclei of proliferating cells. After 2 weeks, myofiber diameters of irradiated muscle increased in comparison with those of untreated muscle, but didn't recover back to normal levels. Additionally, in the superficial region of muscle, the number of capillaries and fibroblast growth factor levels in irradiated muscle exhibited meaningful elevation relative to those of untreated muscle. In the deep region of muscle, BrdU-positive nuclei of satellite cells and / or myofibers of irradiated muscle increased significantly relative to that of the untreated muscle. The results of this study suggested that LLL irradiation can promote recovery from disuse muscle atrophy in association with proliferation of satellite cells and angiogenesis.

[Lasers Med Sci.](#) 2009 Jun 25. [Epub ahead of print]

Low-level laser therapy attenuates creatine kinase levels and apoptosis during forced swimming in rats.

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Studies suggest that high-intensity physical exercise can cause damage to skeletal muscles, resulting in muscle soreness, fatigue, inflammatory processes and cell apoptosis. The aim of this study was to investigate the effects of low-level laser therapy (LLLT) on a decrease in

creatinase kinase (CK) levels and cell apoptosis. Twenty male Wistar rats were randomly divided into two equal groups: group 1 (control), resistance swimming; group 2 (LLLT), resistance swimming with LLLT. They were subjected to a single application of indium gallium aluminum phosphide (InGaAlP) laser immediately following the exercise for 40 s at an output power of 100 mW, wavelength 660 nm and 133.3 J/cm². The groups were subdivided according to sample collection time: 24 h and 48 h. CK was measured before and both 24 h and 48 h after the test. Samples of the gastrocnemius muscle were processed to determine the presence of apoptosis using terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick end labeling. (There was a significant difference in CK levels between groups ($P < 0.0001$) as well as between the 24 h and 48 h levels in the control group, whereas there was no significant intra-group difference in the LLLT group at the same evaluation times. In the LLLT group there were 66.3 +/- 13.2 apoptotic cells after 24 h and 39.0 +/- 6.8 apoptotic cells after 48 h. The results suggest that LLLT influences the metabolic profile of animals subjected to fatigue by lowering serum levels of CK. This demonstrates that LLLT can act as a preventive tool against cell apoptosis experienced during high-intensity physical exercise.

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Low-level laser (LLL) irradiation promotes proliferation of muscle satellite cells, angiogenesis and expression of growth factors. Satellite cells, angiogenesis and growth factors play important roles in the regeneration of muscle. The objective of this study was to examine the effect of LLL irradiation on rat gastrocnemius muscle recovering from disuse muscle atrophy. Eight-week-old rats were subjected to hindlimb suspension for 2 weeks, after which they were released and recovered. During the recovery period, rats underwent daily LLL irradiation (Ga-Al-As; 830 nm; 60 mW; total, 180 sec) to the right gastrocnemius muscle through the skin. The untreated left gastrocnemius muscle served as the control. In conjunction with LLL irradiation, 5-bromo-2-deoxyuridine (BrdU) was injected subcutaneously for labeling of nuclei of proliferating cells. After 2 weeks, myofiber diameters of irradiated muscle increased in comparison with those of untreated muscle, but didn't recover back to normal levels. Additionally, in the superficial region of muscle, the number of capillaries and fibroblast growth factor levels in irradiated muscle exhibited meaningful elevation relative to those of untreated muscle. In the deep region of muscle, BrdU-positive nuclei of satellite cells and / or myofibers of irradiated muscle increased significantly relative to that of the untreated muscle. The results of this study suggested that LLL irradiation can promote recovery from disuse muscle atrophy in association with proliferation of satellite cells and angiogenesis.

Effect of 655-nm low-level laser therapy on exercise-induced skeletal muscle fatigue in humans.

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OBJECTIVE: To investigate if development of skeletal muscle fatigue during repeated voluntary biceps contractions could be attenuated by low-level laser therapy (LLLT). **BACKGROUND DATA:** Previous animal studies have indicated that LLLT can reduce oxidative stress and delay the onset of skeletal muscle fatigue. **MATERIALS AND METHODS:** Twelve male professional volleyball players were entered into a randomized double-blind placebo-controlled trial, for two sessions (on day 1 and day 8) at a 1-wk interval, with both groups performing as many voluntary biceps contractions as possible, with a load of 75% of the maximal voluntary contraction force (MVC). At the second session on day 8, the groups were either given LLLT (655 nm) of 5 J at an energy density of 500 J/cm² administered at each of four points along the middle of the biceps muscle belly, or placebo LLLT in the same manner immediately before the exercise session. The number of muscle contractions with 75% of MVC was counted by a blinded observer and blood lactate concentration was measured. **RESULTS:** Compared to the first session (on day 1), the mean number of repetitions increased significantly by 8.5 repetitions (+/- 1.9) in the active LLLT group at the second session (on day 8), while in the placebo LLLT group the increase was only 2.7 repetitions (+/- 2.9) ($p = 0.0001$). At the second session, blood lactate levels increased from a pre-exercise mean of 2.4 mmol/L (+/- 0.5 mmol/L), to 3.6 mmol/L (+/- 0.5 mmol/L) in the placebo group, and to 3.8 mmol/L (+/- 0.4 mmol/L) in the active LLLT group after exercise, but this difference between groups was not statistically significant. **CONCLUSION:** We conclude that LLLT appears to delay the onset of muscle fatigue and exhaustion by a local mechanism in spite of increased blood lactate levels.

Effect of 830 nm low-level laser therapy applied before high-intensity exercises on skeletal muscle recovery in athletes.

[Leal Junior EC](#), [Lopes-Martins RA](#), [Baroni BM](#), [De Marchi T](#), [Taufer D](#), [Manfro DS](#), [Rech M](#), [Danna V](#), [Grosselli D](#), [Generosi RA](#), [Marcos RL](#), [Ramos L](#), [Bjordal JM](#).

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Our aim was to investigate the immediate effects of bilateral, 830 nm, low-level laser therapy (LLLT) on high-intensity exercise and biochemical markers of skeletal muscle recovery, in a

randomised, double-blind, placebo-controlled, crossover trial set in a sports physiotherapy clinic. Twenty male athletes (nine professional volleyball players and eleven adolescent soccer players) participated. Active LLLT (830 nm wavelength, 100 mW, spot size 0.0028 cm², 3-4 J per point) or an identical placebo LLLT was delivered to five points in the rectus femoris muscle (bilaterally). The main outcome measures were the work performed in the Wingate test: 30 s of maximum cycling with a load of 7.5% of body weight, and the measurement of blood lactate (BL) and creatine kinase (CK) levels before and after exercise. There was no significant difference in the work performed during the Wingate test ($P > 0.05$) between subjects given active LLLT and those given placebo LLLT. For volleyball athletes, the change in CK levels from before to after the exercise test was significantly lower ($P = 0.0133$) for those given active LLLT (2.52 U l⁻¹ +/- 7.04 U l⁻¹) than for those given placebo LLLT (28.49 U l⁻¹ +/- 22.62 U l⁻¹). For the soccer athletes, the change in blood lactate levels from before exercise to 15 min after exercise was significantly lower ($P < 0.01$) in the group subjected to active LLLT (8.55 mmol l⁻¹ +/- 2.14 mmol l⁻¹) than in the group subjected to placebo LLLT (10.52 mmol l⁻¹ +/- 1.82 mmol l⁻¹). LLLT irradiation before the Wingate test seemed to inhibit an expected post-exercise increase in CK level and to accelerate post-exercise lactate removal without affecting test performance. These findings suggest that LLLT may be of benefit in accelerating post-exercise recovery.

[Photomed Laser Surg.](#) 2006 Apr;24(2):111-20.

Photoengineering of tissue repair in skeletal and cardiac muscles.

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This review discusses the application of He-Ne laser irradiation to injured muscles at optimal power densities and optimal timing, which was found to significantly enhance (twofold) muscle regeneration in rats and, even more, in the cold-blooded toads. Multiple and frequent (daily) application of the laser in the toad model was found to be less effective than irradiation on alternate days. It was found that in the ischemia/reperfusion type of injury in the skeletal leg muscles (3 h of ischemia), infrared Ga-Al-As laser irradiation reduced muscle degeneration, increased the cytoprotective heat shock proteins (HSP-70i) content, and produced a twofold increase in total antioxidants. In vitro studies on myogenic satellite cells (SC) revealed that phototherapy restored their proliferation. Phototherapy induced mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) phosphorylation in these cells, probably by specific receptor phosphorylation. Cell cycle entry and the accumulation of satellite cells around isolated single myofibers cultured in vitro was also stimulated by phototherapy. Phototherapy also had beneficial effects on mouse, rat, dog and pig ischemic heart

models. In these models, it was found that phototherapy markedly and significantly reduced (50-70%) the scar tissue formed after induction of myocardial infarction (MI). The phototherapeutic effect was associated with reduction of ventricular dilatation, preservation of mitochondria and elevation of HSP- 70i and ATP in the infarcted zone. It is concluded that phototherapy using the correct parameters and timing has a markedly beneficial effect on repair processes after injury or ischemia in skeletal and heart muscles. This phenomenon may have clinical applications.

[J Appl Physiol.](#) 2006 Jul;101(1):283-8. Epub 2006 Apr 20.

Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats.

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We investigated whether low-level laser therapy (LLLT) can reduce muscular fatigue during tetanic contractions in rats. Thirty-two male Wistar rats were divided into four groups receiving either one of three different LLLT doses (0.5, 1.0, and 2.5 J/cm²) or a no-treatment control group. Electrical stimulation was used to induce six tetanic muscle contractions in the tibial anterior muscle. Contractions were stopped when the muscle force fell to 50% of the initial value for each contraction (T50%). There was no significant difference between the 2.5 J/cm² laser-irradiated group and the control group in mean T50% values. Laser-irradiated groups (0.5 and 1.0 J/cm²) had significantly longer T50% values than the control group. The relative peak force for the sixth contraction in the laser-irradiated groups were significantly higher at 92.2% (SD 12.6) for 0.5 J/cm², 83.2% (SD 20.5) for 1.0 J/cm², and 82.9% (SD 18.3) for 2.5 J/cm² than for the control group [50% (SD 15)]. Laser groups receiving 0.5 and 1.0 J/cm² showed significant increases in mean performed work compared with both the control group and their first contraction values. Muscle damage was indirectly measured by creatine kinase levels in plasma. A distinct dose-response pattern was found in which 1.0 and 2.5 J/cm² LLLT groups had significantly lower creatine kinase levels than the 0.5 J/cm² LLLT group and the control group. We conclude that LLLT doses of 0.5 and 1.0 J/cm² can prevent development of muscular fatigue in rats during repeated tetanic contractions.

[Photomed Laser Surg.](#) 2005 Jun;23(3):273-7

Protection of skeletal muscles from ischemic injury: low-level laser therapy increases antioxidant activity.

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OBJECTIVE: The aim of this study was to investigate the effect of low-level laser therapy (LLLT) on ischemic-reperfusion (I-R) injury in the gastrocnemius muscle of the rat. **BACKGROUND DATA:** Ischemic injury in skeletal muscle is initiated during hypoxia and is aggravated by reoxygenation during blood reperfusion and accumulation of cytotoxic reactive oxygen superoxides. LLLT has been found to biostimulate various biological processes, such as attenuation of ischemic injury in the heart. **MATERIALS AND METHODS:** The injury was induced in the gastrocnemius muscles of 106 rats by complete occlusion of the blood supply for 3 h, followed by reperfusion. Another group of intact rats served to investigate the effect of LLLT on intact nonischemic muscles. Creatine phosphokinase, acid phosphatase, and heat shock protein were determined 7 days after I-R injury and antioxidant levels 2 h after reperfusion. **RESULTS:** Laser irradiation (Ga-As, 810 nm) was applied to the muscles immediately and 1 h following blood supply occlusion. It was found that laser irradiation markedly protects skeletal muscles from degeneration following acute I-R injury. This was evident by significantly ($p < 0.05$) higher content of creatine phosphokinase activity and lower ($p < 0.05$) activity of acid phosphatase in the LLLT-treated muscles relative to the injured non-irradiated ones. The content of antioxidants and heat shock proteins was also higher ($p < 0.05$) in the LLLT-treated muscles relative to that of injured non-irradiated muscles. **CONCLUSION:** The present study describes for the first time the ability of LLLT to significantly prevent degeneration following ischemia/reperfusion injury in skeletal muscles, probably by induction of synthesis of antioxidants and other cytoprotective proteins, such as hsp-70i. The elevation of antioxidants was also evident in intact muscle following LLLT. The above phenomenon may also be of clinical relevance in scheduled surgery or microsurgery requiring extended tourniquet applications to skeletal muscle followed by reperfusion.

[Lasers Med Sci](#). 2006 Dec;21(4):238-44. Epub 2006 Oct 11

Low-level laser therapy can reduce lipopolysaccharide-induced contractile force dysfunction and TNF-alpha levels in rat diaphragm muscle.

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Our objective was to investigate if low-level laser therapy (LLLT) could improve respiratory function and inhibit tumor necrosis factor (TNF- α) release into the diaphragm muscle of rats after an intravenous injection of lipopolysaccharide (LPS) (5 mg/kg). We randomly divided Wistar rats in a control group without LPS injection, and LPS groups receiving either (a) no therapy, (b) four sessions in 24 h with diode Ga-AsI-Al laser of 650 nm and a total dose of 5.2 J/cm², or (c) an intravenous injection (1.25 mg/kg) of the TNF- α inhibitor chlorpromazine (CPZ). LPS injection reduced maximal force by electrical stimulation of diaphragm muscle from 24.15 \pm 0.87 N in controls, but the addition of LLLT partly inhibited this reduction (LPS only: 15.01 \pm 1.1 N vs LPS+LLLT: 18.84 \pm 0.73 N, P<0.05). In addition, this dose of LLLT and CPZ significantly (P<0.05 and P<0.01, respectively) reduced TNF- α concentrations in diaphragm muscle when compared to the untreated control group.

J Cell Sci. 2002 Apr 1;115(Pt 7):1461-9.

Low-energy laser irradiation promotes the survival and cell cycle entry of skeletal muscle satellite cells.

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Low energy laser irradiation (LELI) has been shown to promote skeletal muscle cell activation and proliferation in primary cultures of satellite cells as well as in myogenic cell lines. Here, we have extended these studies to isolated myofibers. These constitute the minimum viable functional unit of the skeletal muscle, thus providing a close model of in vivo regeneration of muscle tissue. We show that LELI stimulates cell cycle entry and the accumulation of satellite cells around isolated single fibers grown under serum-free conditions and that these effects act synergistically with the addition of serum. Moreover, for the first time we show that LELI promotes the survival of fibers and their adjacent cells, as well as cultured myogenic cells, under serum-free conditions that normally lead to apoptosis. In both systems, expression of the anti-apoptotic protein Bcl-2 was markedly increased, whereas expression of the pro-apoptotic protein BAX was reduced. In culture, these changes were accompanied by a reduction in the expression of p53 and the cyclin-dependent kinase inhibitor p21, reflecting the small decrease in viable cells 24 hours after irradiation. These findings implicate regulation of these factors as part of the protective role of LELI against apoptosis. Taken together, our findings are of critical importance in attempts to improve muscle regeneration following injury.

[Anat Rec.](#) 1995 Jan;241(1):123-8.

Regeneration in denervated toad (*Bufo viridis*) gastrocnemius muscle and the promotion of the process by low energy laser irradiation.

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BACKGROUND: It is known that while denervated skeletal muscles have the ability to regenerate, maturation of regenerated myofibres does not take place under these conditions. Denervation also causes elevation of "invasive" and satellite cells, but the role of these cells in the regeneration process after injury to the denervated muscle is still unknown. Low energy lasers have recently been found to modulate and accelerate physiological processes in cells. The aim of the present study was to compare regeneration in denervated and innervated amphibian muscles and to investigate whether this process in denervated muscles can be stimulated by low energy laser irradiation prior to injury in these muscles. **METHODS:** Denervated gastrocnemius muscles of toads were irradiated with He-Ne laser (6.0 mW, 31.2 J/cm²) 7 days postdenervation (control muscle received red light irradiation at the same wavelength). Nine days after denervation cold injury was performed on the site of irradiation of both groups of muscles. At 14 days postinjury all muscles were removed and processed for histology and histomorphometric analysis of mononucleated cells, myotubes, and young myofibres in the regenerated zone. **RESULTS:** The volume fraction (percent of total injured zone) of the various histological structures in the injured zones 14 days after cold injury in the denervated (9 days prior to injury) muscles did not differ from innervated injured muscles at the same time interval postinjury. The mononucleated cells and myotubes in the laser irradiated muscles comprised 49 +/- 4% and 6 +/- 1% of the injured area, respectively, which was significantly lower than their volume fraction (67 +/- 2% and 11 +/- 2%, respectively) in the control muscles. The young myofibres populated 34 +/- 4% of the total injured area in the denervated and laser irradiated muscles which was significantly higher than their volume fraction (12 +/- 2%) in control denervated muscles. **CONCLUSIONS:** It is concluded that initial stages of regeneration can also take place in skeletal denervated and injured muscles of amphibians. The kinetics of the regeneration process are identical in denervated and innervated muscles. The process of regeneration in denervated muscles can be markedly enhanced if the muscle is irradiated by low energy laser prior to injury, probably by activation (stimulation of proliferation and/or differentiation) cells in the muscles that are "recruited" and participate in the process of regeneration.

[Lasers Med Sci.](#) 2001;16(1):44-51

Dose-dependency of low-energy HeNe laser effect in regeneration of skeletal muscle in mice.

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We evaluated the effect on mice skeletal muscle regeneration of different doses (2.6, 8.4, and 25 J/cm²) of HeNe laser (lambda 632.8 nm; power, 2.6 mW; spot size, 0.007 cm²) applied directly to intact skin of injured muscle. Muscle injury was induced in both right and left Tibialis anterior (TA) muscles by ACL myotoxin (5 mg/kg). Right TA muscles were irradiated daily for 5 days while contralateral muscles received a sham treatment. Only the 2.6 J/cm² dose resulted in changes such as increased mitochondrial density and muscle fibre in the TA muscles as compared to sham groups (3280 +/- 704 microns² versus 2110 +/- 657 microns², p = 0.02). We concluded that the HeNe effect on mouse muscle regeneration is dose-specific: only 2.6 J/cm² increased muscle fibre area and mitochondrial density.

[Radiats Biol Radioecol](#). 2002 May-Jun;42(3):315-21.

[Histogenetic, metabolic, and immunologic aspects of the effect of infrared laser radiation on injured skeletal muscles from irradiated and nonirradiated rats]

[Article in Russian]

[Zubkova SM](#), [Buliakova NV](#), [Mikhailik LV](#), [Varakina NI](#), [Azarova VS](#).

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Using biochemical, histological, morphometric and cytogenetic methods, it was shown that low-intensive infrared laser radiation (total dose 3.6 J/cm²), applied to the injured rat skeletal muscles, stimulated metabolism and regeneration more efficiently in the muscles locally exposed to 20 Gy X-rays compared to the unexposed muscles. The laser irradiation promoted postradiative recovery in bone marrow cells, but did not provide normalization in thymus lymphocyte activity.

[Vopr Kurortol Fizioter Lech Fiz Kult](#). 2000 Nov-Dec;(6):8-11.

[Experimental study of the effect of pulsed infrared laser radiation on skeletal muscle regeneration after mechanical trauma]

[Article in Russian]

[Buliakova NV](#), [Zubkova SM](#), [Azarova VS](#), [Mikhaïlik LV](#), [Varakina NI](#).

Impulse infrared laser radiation (0.89 microm) was examined for effect on regeneration of cross-cut musculus gastrocnemius in adult rats (ten 3-min exposures, 2-3 procedures weekly for one month after the operation, modulating frequency 10 Hz, total course dose 3.6 J/cm²). Histological, morphometric and biochemical parameters of muscular regenerates as well as biochemical serum indices show enhanced regeneration of the skeletal muscle. Formation of the muscular organ ends earlier vs untreated muscles. This was accompanied with more active sclerotization of the muscular regenerate.

[Vopr Kurortol Fizioter Lech Fiz Kult](#). 1998 Nov-Dec;(6):11-6.

[A comparative analysis of the action of red and infrared laser radiation and ultrasound on the regeneration of locally irradiated skeletal muscle and on immune system function in the rat]

[Article in Russian]

[Subkova SM](#), [Buliakova NV](#), [Mikhaïlik LV](#), [Varakina NI](#), [Azarova VS](#).

The study was made of a regeneration ability of the rat skeletal muscle which had been mechanically injured and exposed to a 20 Gy irradiation, of the thymus and bone marrow condition after local exposure of the muscle to red and infrared laser radiation and ultrasound. These factors were found to stimulate regeneration of the injured soft tissues and to reduce cytogenetic damage to bone marrow cells in the irradiated zone under growing immunosuppression. The stimulation was most potent in prolonged exposure to the ultrasound.

[Biull Eksp Biol Med](#). 1989 Mar;107(3):345-7.

[Effect of helium-neon laser rays on the processes of postradiation recovery in the skeletal muscles of old rats]

[Article in Russian]

[Buliakova NV](#).

The present experiments were conducted to determine the stimulant effect of helium-neon laser on the postradiation recovery in irradiated uninjured skeletal muscle of rats

aged 2-2.5. This was indicated by a restored ability of the muscle for posttraumatic regeneration. The both hind rat legs were exposed to local irradiation of 20 Gy and following laser therapy (8-9 procedures at 3 min each, impulsive or continuous one). Then both musculus gastrocnemius were cut across 30 days after irradiation. It was shown that laser therapy employed before injury of the irradiated muscle accelerated fibrin resorption and improved connective tissue, but slightly stimulated muscular tissue. Impulse laser therapy was more favourable for state of skin and healing of the skin wound after irradiation.