



Excerpts from Literature on Mechanism of Action for Infrared Therapy for Soft Tissue Injury Healing

Infrared light therapy has been shown to trigger release of Nitric Oxide, a small endogenous molecule with multiple effects on body systems. Nitric oxide is involved in a broad array of biological functions including vasodilatation, immune response, and neurotransmission.¹ Among the key effects in sports medicine are recovery from soft tissue injury (with outcomes in the form of reduced swelling and inflammation, and accelerated repair of tissues such as muscle, tendons and ligaments), and recovery from fractures. The excerpts from published literature below highlight just some of the scientific and clinical investigations into the effects of infrared light, suggesting mechanisms of action and pointing to potential clinical outcomes.

1) Application of infrared light to body tissues causes release of nitric oxide.

- a. Studies have demonstrated infrared photochemical generation of nitric oxide by two-photon excitation of precursor molecules such as porphyrin complexes.²

2) Nitric Oxide is known to trigger vasodilatation.

- a. Studies of aortic tissues demonstrated the presence of a chemical substance termed "endothelium-derived relaxing factor" (EDRF). This substance was subsequently revealed to be nitric oxide.³

3) Nitric oxide causes increases in lymphatic drainage.

- a. There is also evidence that the presence of infrared light, by increasing lymphatic circulation, does so by virtue of an increase in the diameter of the lymphatic vessels, not just by increased flow rates within the vessel at an unchanged diameter. This diameter increase, if definitively present, would also explain the presence of large diameter protein cells within the normal bone circulation that cannot be attributed to the vascular circulation and would additionally explain a facilitated process for removal of debris and larger protein cells passing out of traumatized areas that is additionally stimulated by the use of infrared light therapy.⁴
- b. Infrared light, with its known general effects and specific direct effects on the lymphatic system, would act to stimulate mitochondria ATP that increases cellular and circulatory motility as well as directly influencing lymphatic flow. It also promotes increased permeability in interstitial tissue and facial layers (Gabel 1995) reducing stagnation and blockage.⁵

4) Nitric Oxide and Infrared-induced Nitric Oxide affects tendon repair.

a. *Achilles Tendon Repair*

The authors investigated the role of nitric Oxide (NO) in tendon healing. NO synthase activity and immunoreactivity was absent in un-injured rat Achilles tendon. After surgical division there was a five-fold increase in NO synthase activity and immunoreactivity within the healing tendon at day 7, with a return to near baseline levels at day 14. Inhibition of NO synthase activity with oral administration of Nw-nitro-L-arginine methyl ester (L-NAME) resulted in a significant reduction in cross-sectional area (30% at day 7, $p < 0.01$, 50% at day 15, $p < 0.001$) and failure load (24% at day 7 $p < 0.01$) of the healing Achilles tendon constructs. These results indicate that nitric oxide synthase is induced during tendon healing and inhibition of nitric oxide synthase inhibits this tendon healing.⁶



b. *NO and Rotator Cuff Tendon Disorders*

Tendon disorders with a chronic nature, including the rotator cuff, are extremely common, and represent a major clinical problem. Mechanical overload has been proposed as an important etiologic factor in tendinopathies. Nitric oxide (NO), a free radical produced by nitric oxide synthases (NOSs), is a potent regulator and stimulator of biological processes including tendon degeneration and healing. It is also involved in response to mechanical stimuli in different tissues. In an animal model of acutely injured tendon healing temporal and differential expression of NOS isoforms has been demonstrated, suggesting that different patterns of NOSs expression may have different biological functions. Therefore, we hypothesized that tendon overuse may result in a differential upregulation of NOSs, particularly iNOS. An animal model of supraspinatus tendon overuse was utilized, which consisted of treadmill running. A group of animals of the same strain and age subjected to normal cage activity were used as controls. Following a 4-week exercise protocol supraspinatus tendons were harvested, RNA was extracted, and subjected to competitive reverse transcription and polymerase chain reaction (RT-PCR) to determine the expression levels of inducible-, endothelial-, and neuronal-NOS isoforms (i-, e-, and nNOS). The mRNA expression of all three NOS isoforms increased in the supraspinatus tendons as a result of overuse exercise. iNOS and eNOS mRNA expression increased fourfold ($p < 0.01$), and there was an increase, but statistically not significant, in nNOS mRNA expression in the overused tendons when compared with the controls. This study is the first to show that NOS isoforms are upregulated in rotator cuff tendon as a result of chronic overuse, and suggests the involvement of nitric oxide in the response of tendon tissue to increased mechanical stress.⁷

5) Nitric Oxide modulates ligament repair via enhanced circulation

a. *Blood Supply and Ligament Healing*

Functional outcomes of anterior cruciate ligament (ACL) injury are generally poorer than those of medial collateral ligament (MCL) tears. Following ligament damage, all phases of ligament healing require an adequate blood supply. We hypothesized that the differences in healing properties of the ACL and MCL would reflect their vascular responses to joint injury. This study examined the long-term changes in blood flow and vascular volume of rabbit knee ligaments after direct injury, and under conditions of chronic joint instability induced by section of the posterior cruciate ligament (PCL). Standardized injuries were surgically induced in adult rabbit knee ligaments: partial MCL transection, partial ACL transection, or complete PCL transection (joint instability). Sixteen weeks later the blood flow and vascular volume of the ACL and MCL were measured and compared to control and sham-operated animals. Direct ligament injury induced significant increases in standardized blood flow and vascular volume of both ACL and MCL after 16 weeks; however, the vascular volume of the ACL was not higher than the control levels in the MCL. We conclude that direct injury to both the anterior cruciate and MCLs induces long-term physiological responses. Joint laxity is a common sequel to PCL injury. Chronic joint laxity failed to induce adaptive vascular responses in the ACL, while the MCL shows significant amplification of blood supply. Although both MCL and ACL showed increased weight after PCL transection, the lack of a long-term vascular response in the ACL may be a major factor in its the diminished healing potential.⁸

6) Nitric Oxide modulates muscle repair

a. *Role for Nitric Oxide in Muscle Repair via direct satellite cell signaling*



Muscle satellite cells are quiescent precursors interposed between myofibers and a sheath of external lamina. Although their activation and recruitment to cycle enable muscle repair and adaptation, the activation signal is not known. Evidence is presented that nitric oxide (NO) mediates satellite cell activation, including morphological hypertrophy and decreased adhesion in the fiber-lamina complex. Activation *in vivo* occurred within 1 min after injury. Cell isolation and histology showed that pharmacological inhibition of nitric oxide synthase (NOS) activity prevented the immediate injury-induced myogenic cell release and delayed the hypertrophy of satellite cells in that muscle. Transient activation of satellite cells in contralateral muscles 10 min later suggested that a circulating factor may interact with NO-mediated signaling. Interestingly, satellite cell activation in muscles of *mdx* dystrophic mice and NOS-I knockout mice quantitatively resembled NOS-inhibited release of normal cells, in agreement with reports of displaced and reduced NOS expression in dystrophin-deficient *mdx* muscle and the complete loss of NOS-I expression in knockout mice. Brief NOS inhibition in normal and *mdx* mice during injury produced subtle alterations in subsequent repair, including apoptosis in myotube nuclei and myotube formation inside laminar sheaths. Longer NOS inhibition delayed and restricted the extent of repair and resulted in fiber branching. A model proposes the hypothesis that NO release mediates satellite cell activation, possibly via shear-induced rapid increases in NOS activity that produce "NO transients."⁹

- b. Modified muscle use or injury can produce a stereotypic inflammatory response in which neutrophils rapidly invade, followed by macrophages. This inflammatory response coincides with muscle repair, regeneration, and growth, which involve activation and proliferation of satellite cells, followed by their terminal differentiation. Recent investigations have begun to explore the relationship between inflammatory cell functions and skeletal muscle injury and repair by using genetically modified animal models, antibody depletions of specific inflammatory cell populations, or expression profiling of inflamed muscle after injury. These studies have contributed to a complex picture in which inflammatory cells promote both injury and repair, through the combined actions of free radicals, growth factors, and chemokines. Recent discoveries concerning the interactions between skeletal muscle and inflammatory cells clearly show a role for neutrophils in promoting muscle damage soon after muscle injury or modified use. New evidence also shows that muscle cells can release positive and negative regulators of inflammatory cell invasion, and thereby play an active role in modulating the inflammatory process. In particular, muscle-derived nitric oxide can inhibit inflammatory cell invasion of healthy muscle and protect muscle from lysis by inflammatory cells *in vivo* and *in vitro*. On the other hand, muscle-derived cytokines can signal for inflammatory cell invasion, at least *in vitro*. The immediate challenge for advancing our current understanding of the relationships between muscle and inflammatory cells during muscle injury and repair is to place what has been learned *in vitro* into the complex and dynamic *in vivo* environment.¹⁰
- c. *NO can prevent neutrophil-mediated damage of muscle cell membranes*
Nitric oxide (NO) can function as either a pro-inflammatory or anti-inflammatory molecule, depending upon its concentration and the microenvironment in which it is produced. We tested whether muscle-derived NO affects muscle inflammation and membrane lysis that occur in modified muscle use. Transgenic mice with muscle-specific over-expression of neuronal NO synthase (nNOS) were generated in which transgene expression was driven by the human skeletal muscle actin promoter. Transgenic mice and non-transgenic littermates were



subjected to hindlimb muscle unloading followed by reloading, which causes muscle inflammation and membrane lysis. NOS expression decreased in transgenic and non-transgenic mice during muscle unloading. Muscle inflammation was assessed by immunohistochemistry after 24 h of muscle reloading following 10 days of unloading. Soleus muscles of non-transgenic mice showed significant increases in the concentrations of neutrophils (4.8-fold) and macrophages (11.3-fold) during reloading, compared to mice that experienced unloading only. Muscles of transgenic mice showed 51 % fewer neutrophils in reloaded muscles than those of non-transgenic mice, but macrophage concentrations did not differ from non-transgenic mice. Muscle membrane damage was determined by measuring influx of an extracellular marker dye. Significantly more membrane damage occurred in muscles of non-transgenic mice experiencing reloading than in ambulatory controls. However, membrane damage in the reloaded muscles of transgenic mice did not differ from that in ambulatory mice. In vitro cytotoxicity assays confirmed that mouse neutrophils lyse muscle cell membranes, and showed that inhibition of NOS in muscle and neutrophil co-cultures significantly increased neutrophil-mediated lysis of muscle cells. Together, these data show that muscle-derived NO can function as an anti-inflammatory molecule in muscle that experiences modified loading, and that NO can prevent neutrophil-mediated damage of muscle cell membranes in vivo and in vitro.¹¹

- d. *The role of nitric oxide in muscle inflammation during modified muscle use*
The objective of this study was to determine the role of nitric oxide in muscle inflammation, fiber necrosis, and apoptosis of inflammatory cells in vivo. The effects of nitric oxide synthase (NOS) inhibition on the concentrations of neutrophils, ED1+ and ED2+ macrophages, apoptotic inflammatory cells, and necrotic muscle fibers in rats subjected to 10 days of hindlimb unloading and 2 days of reloading were determined. Administration of NOS inhibitor N(omega)-nitro-L-arginine methyl ester (L-NAME) significantly reduced the concentrations of neutrophils, ED1+ and ED2+ macrophages, and necrotic fibers in soleus muscle relative to water-treated controls. The concentration of apoptotic inflammatory cells was also significantly lower for L-NAME-treated animals compared with water-treated controls. However, the proportion of the inflammatory cell population that was apoptotic did not differ between L-NAME-treated and control animals, suggesting that L-NAME treatment did not decrease inflammatory cell populations by increasing the frequency of apoptosis. Thus, nitric oxide or one of its intermediates promotes muscle inflammation and fiber necrosis during modified muscle use and plays no more than a minor role in the resolution of muscle inflammation by inducing apoptosis of inflammatory cells.¹²
- e. *Nitric Oxide Synthase plays a role in muscle repair*
Following muscle injury, satellite cells are recruited and activated to enable muscle repair. Previous studies using nitric oxide synthase (NOS) inhibition, mdx dystrophy mice and NOS-I knock-out mice have suggested that nitric oxide release may mediate satellite cell activation. PURPOSE: To assess the presence of NOS in strain-induced muscle injury in conjunction with two muscle regulatory factors: MyoD and myogenin. Using female Sprague Dawley rats, muscle strains were produced by manually stretching (50 repetitions) an activated plantar flexor group through its normal range of motion. Muscle biopsy samples were evaluated 48- hours after strain injury for the presence of nitric oxide synthase, MyoD, and myogenin using Western immunoblot analysis. Transcript levels for neuronal (n) NOS, endothelial (e) NOS, inducible (i) NOS, MyoD and myogenin



were assessed using reverse transcriptase polymerase chain reaction (RT-PCR) and kinetic PCR. RESULTS: An increase in myogenin transcripts and a decrease in nNOS transcript expression was observed 48- hours post-strain injury by RT-PCR. Western immunoblot analysis revealed increased eNOS, MyoD, and myogenin protein levels 48- hours post-strain injury, but a decrease in nNOS protein levels. CONCLUSION: NOS has been reported to play a role in muscle repair. Although nNOS expression was decreased, eNOS expression increased providing support for other NOS isoforms in muscle repair.¹³

- f. *NO contributes to the regulation of vascular conductance within muscles*
One study sought to determine the functional role of nitric oxide (NO) in regulating vascular conductance during high intensity dynamic exercise in skeletal muscles composed of all major fibre types. In a rat study the administration of nitric oxide synthase inhibitor, L-NAME, reduced vascular conductance in 20 of the 28 individual hindlimb muscles or muscle parts examined during high speed treadmill exercise. These reductions in vascular conductance correlated linearly with the estimated sum of the percentage of slow twitch oxidative (SO) and fast twitch oxidative glycolytic (FOG) types of fibres in each muscle ($\Delta\text{conductance} = -0.0082(\%SO + \%FOG) - 0.0105$; $r = 0.66$; $P < 0.001$). However, if the reduction in vascular conductance found in the individual hindquarter muscles or muscle parts was expressed as a percentage decrease from the pre-L-NAME value ($\% \Delta = (\text{pre-L-NAME conductance} - \text{post-L-NAME conductance}) / \text{pre-L-NAME conductance} \times 100$), then the reduction in vascular conductance was similar in all muscles examined (average $\% \Delta = -23 \pm 2\%$). These results suggest that NO contributes substantially to the regulation of vascular conductance within and among muscles of the rat hindquarter during high intensity exercise. When expressed in absolute terms, the results suggest that the contribution of NO to the regulation of vascular conductance during high intensity exercise is greater in muscles that possess a high oxidative capacity. In contrast, if results are expressed in relative terms, then the contribution of NO to the regulation of vascular conductance during high intensity exercise is similar across the different locomotor muscles located in the rat hindlimb and independent of the fibre type composition.

Human Trials of Infrared Light Treatment of Soft Tissue Injury

1) Infrared light treatment can reduce edema in sprains.

- g. Combining the NO-induced enhancement of arterial/venous circulation with lymphatic drainage, a possible application is suggested for soft tissue injury. Enhanced arterial circulation delivers more nutrients to the site of injury while increase venous circulation and lymphatic drainage reduce swelling and increase debridement of damaged tissue. The following prospective clinical study is illustrative.
- h. Forty-seven soccer players with second degree ankle sprains, selected at random, were divided into the following groups: The first group (n = 16) was treated with the conventional initial treatment (RICE, rest, ice, compression, elevation), the second group (n = 16) was treated with the RICE method plus placebo laser, and the third group (n = 15) was treated with the RICE method plus an 820-nm GaAlAs diode laser with a radiant power output of 40 mW at 16 Hz. Before the treatment, and 24, 48, and 72 h later, the volume of the edema was measured. RESULTS: A three by three repeated measures ANOVA with a follow up post hoc test revealed that the group treated with the RICE and an 820-nm GaAlAs diode laser presented a statistically significant reduction in the



volume of the edema after 24 h (40.3 +/- 2.4 mL, $p < 0.01$), 48 h (56.4 +/- 3.1 mL, $p < 0.002$), and 72 h (65.1 +/- 4.4 mL, $p < 0.001$).¹⁴

2) Nitric Oxide modulates ligament repair via enhanced circulation

a. Tendonitis

One hundred two patients were treated for trapezius tendonitis/tendonosis (TS), splenius capitus tendonitis/tendonosis (SCS), representing 200 total cases treated. Of the 102 patients, 94 (92.0%) had some type of previous treatment for these conditions. Superluminous infrared diodes emitting pulsed near-infrared irradiation were employed. Treatment times were prescribed at 30 min daily for one week, corresponding to a radiant exposure of 36.0 J per treatment. Patient responses to treatment with skin-contact monochromatic infrared irradiation were as follows: 176 (88.0%) of the cases treated had total to excellent relief from the treatment in 1-12 applications. Twenty-two total cases (11.0%) had fair to poor relief, and 2 cases (1.0%) had no relief.¹⁵

Human Case Studies / Testimonials of LumiWave Treatment of Soft Tissue Injuries

1) Treatment of Anterior Cruciate Ligament Deficient Knee

"I am currently participating in a product study to determine the effectiveness of the LumiWave device for pain relief. My expectations when I agreed to participate in this study were at best, limited. I say this not because I questioned the effectiveness of the product (in fact I have used it successfully for temporary pain relief on various body parts when participating in senior softball tournaments) but because I did not feel that the LumiWave (or anything else short of surgery) would help the condition for which I have been using it in the study. I have been ACL deficient in my right knee for forty-seven years. While this condition has never prevented me from participating in vigorous activities it has made it more painful to do so as the years go by. In addition, I have had constant tightness and a lack of stability in the knee that periodically causes pain when climbing stairs, or other activities that result in abnormal stress on my knee.

I am both surprised and pleased to report that during the five plus weeks that I have been participating in this study I have experienced noticeable improvement in flexibility, and stability of my knee as well as decreased tightness. It feels as if there has been a reduction in what may have been a permanent swelling in the knee. While I have no idea how the LumiWave device has effected this improvement, the results are definite. Although I have not put my knee through a rigorous test such as snow skiing or softball, I am very much looking forward to doing that as I have noticed much improvement when jogging."

Jack Davis
Chief Executive Officer
Advanced Surface Technologies
January 24, 2006

2) Treatment of Frozen Shoulder (Adhesive Capsuliti)

"I was diagnosed with frozen shoulder, or adhesive capsuliti, a condition that causes loss of motion in the shoulder joint. The diagnosis of frozen shoulder is often used for any painful shoulder condition associated with a loss of motion, but it is important to understand the cause of the symptoms in order for treatment to proceed effectively.

The orthopedics doctors prescribed a cortisone shot directly to the shoulder. They then told me that I needed to have frequent physical therapy to get the



shoulder moving again. They also told me that if I didn't do the therapy they would recommend that I be anesthetized and my shoulder forcibly moved to "break" the crustiness that was continuing (hence the stiff shoulder).

I started the physical therapy. It was painful! Towards the end of my therapy, I started using LumiWave, a photo-therapy treatment. I'm using it on a daily basis AND I'm back swimming 3000 meters every day!! (And does it ever feel good!)"

Erika Hanson Brown
Founder
Stellar Connections, LLC
January, 2006

3) Treatment of 3rd Degree Ankle Sprain

"I was diagnosed with a 3rd degree ankle sprain with an expectation of 6-8 weeks recovery time. After 2 weeks of LumiWave treatments I made my first visit to the physical therapist. The ankle was nearly healed already. The Physical Therapist said she had never seen a 3rd degree sprain heal so quickly."

Deanne Lee, Highlands Ranch resident
August, 2006

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