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BRIEF REPORT

# Low-Level Laser Treatment with Near-Infrared Light Increases Venous Nitric Oxide Levels Acutely

## A Single-Blind, Randomized Clinical Trial of Efficacy

## ABSTRACT

Mitchell UH, Mack GL: Low-level laser treatment with near-infrared light increases venous nitric oxide levels acutely: a single-blind, randomized clinical trial of efficacy. Am J Phys Med Rehabil 2013;92:151–156.

**Objective:** The use of near-infrared light in the form of low-level laser therapy (LLLT) has become more popular in the treatment of a variety of conditions where increased peripheral blood flow is desired. The hypothesis behind its working mechanism is its purported ability to generate nitric oxide (NO) in the treated area. We tested the hypothesis that the efficacy of near-infrared light lies in its ability to generate NO at the treatment site.

**Design:** We conducted a single-blind, placebo-controlled, randomized clinical trial to measure NO, by its metabolites nitrite and nitrate, in venous blood draining from tissue receiving LLLT. Fifteen healthy subjects received LLLT to the forearm, and blood samples were taken immediately before treatment; at 1, 5, 15, and 30 mins; as well as 15 mins after the treatment to check for NO content.

**Results:** We found a significant treatment effect ($F = 15.75$, $P = 0.003$). A post hoc test showed that minutes 1, 5, and 15 were different compared with the baseline measures ($P$'s $< 0.05$). The area under the treatment curve was significantly larger than the area under the sham treatment curve ($t = 2.26$, $P = 0.037$). A limitation of this study was that the data were collected from healthy subjects.

**Conclusions:** LLLT increased NO levels in venous blood draining from the treatment site in healthy subjects. The peak increase in NO occurred 5 mins into the treatment, after which it slowly waned. Further research is necessary to assess NO increases with LLLT in patients with pathologies.

**Key Words:** NIR, Phototherapy, Nitric Oxide, LLLT

Monochromatic near-infrared (NIR) light therapy in the form of low-level laser therapy (LLLT) is used to treat a variety of conditions where increased peripheral blood flow has some therapeutic benefit.[1–11] LLLT involves a noninvasive placement of a device that delivers NIR light at an 880–890 nm wavelength. The ability of NIR light therapy to improve peripheral circulation has been attributed

to the deposition of energy (heat) to the treatment area and/or to an increase in the bioavailability of nitric oxide (NO).[12] NO is endogenously synthesized by various isoforms of the enzyme nitric oxide synthase[13] and is known to dilate blood vessels.[14] In addition, NO can be scavenged by red blood cells where the molecule is bound to the heme moiety of the hemoglobin molecule.[15,16] As such, it can then be transported to distant tissue, where it can again dissociate from the heme moiety and alter local vascular function.[15] It is known that NO can be dissociated from hemoglobin in response to absorption of photon energy.[17–21] As such, LLLT may improve the bioavailability of NO and thereby improve peripheral blood flow. NO has been known to reversibly bind to cytochrome oxidase, hindering cell respiration.[22] Cytochrome oxidase catalyzes the last step in the electron transfer chain, where chemical energy is generated in the form of adenosine triphosphate. Bound NO slows down this process and thereby hampers tissue oxygenation. It is theorized that phototherapy is able to not only dissociate NO from the enzyme and thus facilitate respiration but also affect the enzyme without NO presence.[23] Other biologic effects of LLLT include increased adenosine triphosphate and mitochondrial function, prevention of cell death, induction of cell migration, and tissue repair (see Hamblin and Deidova[20] for review).

Although the proposed working mechanism of LLLT involves, in part, an increase in NO bioavailability in the blood, evidence supporting this hypothesis is limited. Arnall et al.[24] were unable to demonstrate an increase in NO bioavailability in patients with diabetes after 12 wks of NIR light therapy. However, it is possible that the endothelial dysfunction seen in patients with diabetes[25] will limit the impact of phototherapy on NO bioavailability. Arnall et al.[24] measured the blood levels of NO metabolites 30 mins after LLLT. Considering the short half-life of NO[26] and the rapid scavenging ability of hemoglobin,[15,16] it is likely that they missed the transient rise in NO bioavailability during phototherapy. The purpose of this study was to demonstrate the effectiveness of LLLT in augmenting the bioavailability of NO. The bioavailability of NO was assessed by monitoring the time course of changes in the metabolic by-products of NO in venous blood draining from the NIR light–treated tissue.

## METHODS

This measurement-focused study used a single-blind, placebo-controlled, randomized design. Measuring NO availability in tissue or blood is difficult because NO has a very short half-life of only a few seconds or less.[26] It is quickly oxidized to nitrite ($NO^{2-}$), which, in turn, is further oxidized to nitrate ($NO^{3-}$).[26] Therefore, detection of the blood levels of NO was performed indirectly by measuring its metabolites (i.e., nitrite and nitrate).[27]

### Participants

The participants were healthy adults (21–27 yrs old) recruited from a local college campus. Volunteers were excluded if they reported any history of lower limb peripheral vascular disorders, surgery, or injury to a lower limb within the preceding 3 mos; loss of lower limb sensation; or taking any prescription medication or if they smoked. In addition, the subjects were requested to abstain from caffeinated drinks within the preceding 8 hrs. To detect a statistical increase in venous NO, or its metabolites, at a two-sided 5% significance level and a power of 90%, a total sample size of 15 was required.

Fifteen subjects volunteered for this study: five women (mean [SD] height, 166.4 [7.6] cm; mean [SD] body mass, 62.1 [6.8] kg) and ten men (mean [SD] height, 183.7 [5.3] cm; mean [SD] body mass, 87.3 [15.6] kg).

Each participant provided a written informed consent before participating in this study, which was approved by the institutional review committee.

### Study Setting

This study took place at the modalities laboratory of the Exercise Sciences Department from April to May 2010.

### Intervention

All 15 subjects participated in the experimental trials involving LLLT. In addition, 5 of the 15 participants were randomly selected to participate in an additional sham-treatment trial. These subjects were blinded to the order of treatment (sham or NIR light), and the treatment order was randomized.

Each participant lay comfortably on a plinth in a quiet room at a room temperature of approximately 22°C. A venous catheter was placed in a large antecubital vein. Four flexible therapy pads, each containing 60 gallium aluminum arsenide diodes (Anodyne Therapy, Tampa, FL), were placed on the right forearm. The area of the light-emitting diodes per therapy pad was $3 \times 7.5$ cm, yielding a treatment area of 90 $cm^2$. The Anodyne Therapy system delivers pulsed light at 292 Hz with a wavelength of 890 nm and uses a 50% duty cycle. Using an

oscilloscope (Model TDS 220; Tektronix, Beaverton, OR), we confirmed the duty cycle and the frequency. Using a spectrometer (USB4000 Miniature Fiber Optic Spectrometer; Ocean Optics, Dunedin, FL), we measured the power delivered by six randomly chosen diodes selected from four pads and found an average of 2.65 mW per light-emitting diode, yielding 159 mW per pad, therefore 636 mW per four pads. The intensity used for this study was set at maximum.

After a 5-min resting period, a baseline blood sample was drawn, and then, the NIR light system was turned on. Sham NIR light trials (controls, $n = 5$) consisted of the same setup, but no light energy was emitted. Venous blood samples were drawn at 1, 5, 15, and 30 mins of the treatment, as well as 5 mins after the treatment.

## Blood Samples

The blood samples were transferred to nonheparinized vacutainers and allowed to clot at room temperature for 15 mins. The samples were centrifuged at 4°C in a clinical centrifuge at 1500 × g for 15 mins, after which the serum was separated into sample collection vials and stored frozen at −80°C until analysis.[28,29] After thawing, the serum was ultrafiltered through a 10-kDa molecular weight cutoff filter that had been prerinsed with UltraPure water. The nitrate and nitrite levels in the filtered serum were determined by combining a nitrate reductase reaction with a Griess reagent system[28,29] (Nitrate/nitrite colorimetric assay kit; Cayman Chemicals). Total serum (nitrate/nitrite) levels were calculated from the calibration curve performed with each assay kit. The sensitivity of this assay is approximately 2 μM nitrate, with an interassay coefficient of variability of 2.7%. The total nitrate/nitrite concentration in the serum samples is an index of the nitric oxide synthesis.[29]

## Data Analysis

We used repeated measures analysis of variance for data analysis. The NO time-response curve was plotted, and a general linear modeling two-way (treatment and time) repeated measures analysis of variance was used to compare the NO time course of response. A Tukey HSD [honestly significant difference] post hoc test was used to determine where the significant differences were found and to establish the minimal significant difference for NO increase at a significance level of $P < 0.05$. In addition, the area under the response curve was calculated for each subject, providing an estimate of the net gain or loss of NO during a treatment. The mean areas under the response curves were compared using a Student's unpaired $t$-test. The significance level was set at $P < 0.05$.

# RESULTS

The total serum NO before the NIR light or the sham treatment had a mean (SD) of 3.73 (0.52) μM (mean of all subjects; range, 0.64–7.60 μM). In response to the LLLT total serum, the NO was higher during the 30-min treatment period compared with

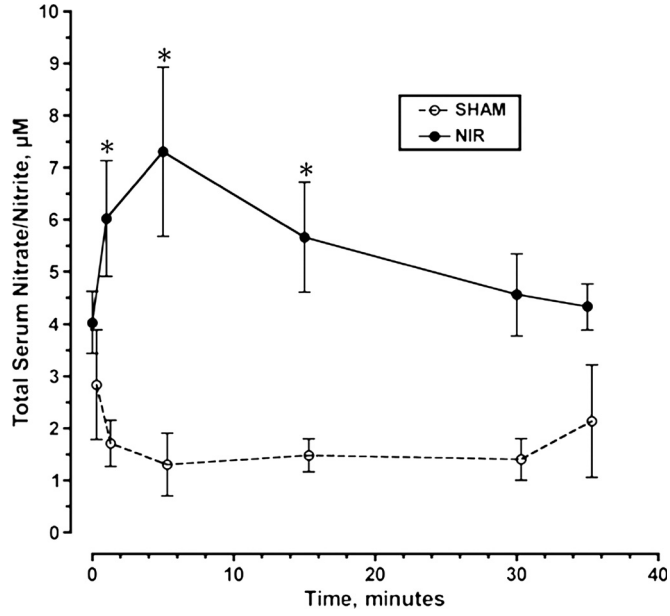


**FIGURE 1** *Time course of change in plasma NO levels as monitored by NO metabolite (nitrate and nitrite) levels during 30 mins of LLLT. NO indicates nitric oxide; LLLT, low-level laser therapy.*

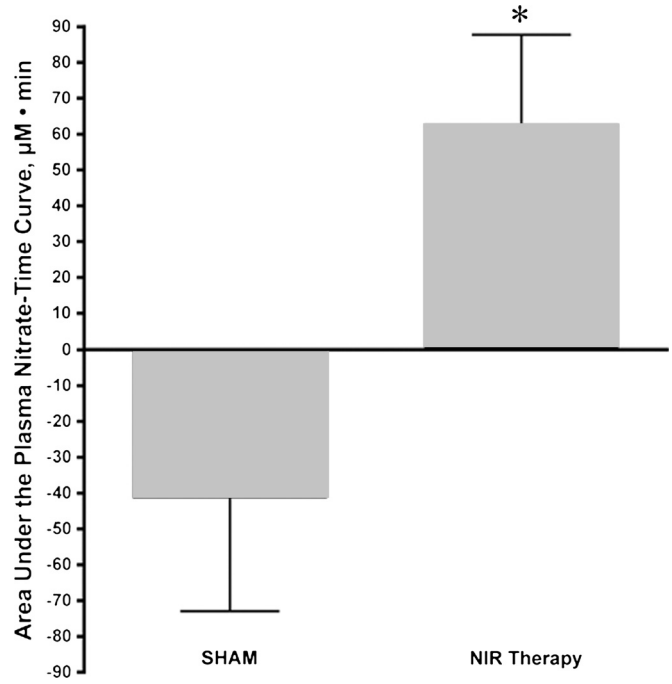


**FIGURE 2** *Area under the plasma NO time curve during 30 mins of LLLT. LLLT indicates low-level laser therapy; NIR, near-infrared light; NO, nitric oxide.*

the sham NIR light trial ($F$ =15.75, $P$ = 0.003; Fig. 1). The post hoc analysis (adjusted for unequal sample sizes) showed that the differences were at minutes 1 ($P < 0.005$), 5 ($P < 0.005$), and 15 ($P < 0.005$) of the treatment. The area under the serum NO time-response curve was significantly greater for the LLLT than for the sham trial ($t$ = 2.26, $P$ = 0.037; Fig. 2).

## DISCUSSION

The significant novel finding of this study was that monochromatic NIR light therapy increased the total concentration of nitrate and nitrite in venous blood draining from the treated tissue and the total amount of nitrate/nitrite metabolites (area under the concentration-time curve) released during the treatment. We interpreted the increase in NO metabolites as a by-product of increased NO bioavailability and eventual metabolism during phototherapy. In contrast, the sham-treated tissue showed no change in venous blood NO metabolites.

The success of LLLT in improving circulation, decreasing pain[1] and symptoms associated with Willis-Ekbom disease,[11] accelerating wound healing,[10] and increasing cutaneous pressure sensation in patients with peripheral neuropathy[2–9] has been largely attributed to the generation of NO. NO is an important omnipresent biologic-signaling molecule that has been implicated in a variety of physiologic control systems, such as in the vasoregulation, neurotransmission, signal transduction, and host defense.[30–32] It is an endogenously synthesized free radical gas, a by-product from the conversion of arginine to citrulline by various nitric oxide synthase isoforms.[30] Our study demonstrated for the first time that NIR light at 880 and 890 nm can significantly increase NO amounts in venous blood in healthy subjects.

Our findings are in contrast with recently published work[24] where patients with type 1 and type 2 diabetes received NIR light treatments (three times a week for 8 wks). The subjects (20 patients with type 2 diabetes and 2 patients with type 1 diabetes) underwent an 8-wk treatment protocol receiving NIR light at 880 nm and 584 Hz three times a week for 30 mins each to the dorsal and plantar aspect of the foot. Blood was drawn from the foot veins while the foot was still exposed to the NIR light after 4 wks and at the end of the treatment protocol. No increase in venous NO was observed. There are several possible reasons why increases in venous NO could not be shown. First, there may have been no increase in NO. This could be because of the population (diabetic patients with neuropathy) used in that study. Diabetic patients with significant peripheral neuropathy have demonstrated a reduction in endothelial function and, particularly, impaired flow-mediated dilation associated with the endothelial nitric oxide synthase production of NO.[25,33] Second, it could also be a function of the time of blood collection. In our

study, the peak of NO availability was not at the end of the 30-min treatment, but rather at 5 mins into the treatment. Because we collected the blood at the end of the treatment time (30 mins), the transient peak in blood NO may have been missed. Third, the area treated was different in the studies. We used the forearm, which offers a smooth surface for the pads, whereas Arnall et al.[24] applied the pads to the dorsal and plantar side of the foot. The tissue characteristics are different and might have affected photon penetration.

### Limitations

The limit of detection for the Griess reagent assay is around 2 μM nitrite. The values for serum nitrite after the nitrate reductase reaction were in the range of 3–5 μM. Thus, our determination of the total nitrate/nitrite in the serum was close to the limit of detection of the Griess reagent assay. The data were collected from healthy college-aged subjects.

### Implications

This study corroborated the hypothesis on NIR light's working mechanism. The goal of this research was to show the efficacy of NIR light treatment and, consequently, to allow physical therapists to expand the use of NIR light to areas other than the ones named above. Potential future diagnoses indicated for NIR light treatment could include postsurgical wound healing, tendonitis, and edema reduction. It is even conceivable that NIR light could be used for the treatment of lesions of peripheral nerves, such as neuroapraxia or axonotmesis. This supposition is based on the assertion that increased amounts of NO could potentially improve neurotransmission and signal transduction because it is a neurotransmitter itself.

## CONCLUSIONS

LLLT produced a transient increase in NO levels in venous blood draining from the treatment site in healthy subjects. The data from this study indicate that LLLT can impact the underlying tissue and promote a transient increase in NO levels. The mechanism by which LLLT might increase blood NO is unknown. In addition, whether this response is similar in individuals with pathologies remains to be determined.

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