Limb Blood Flow After Class 4 Laser Therapy

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Context: Laser therapy is purported to improve blood flow in soft tissues. Modulating circulation would promote healing by controlling postinjury ischemia, hypoxia, edema, and secondary tissue damage. However, no studies have quantified these responses to laser therapy.

Objective: To determine a therapeutic dose range for laser therapy for increasing blood flow to the forearm.

Design: Crossover study.

Setting: Controlled laboratory setting.

Patients or Other Participants: Ten healthy, college-aged men (age = 20.80 ± 2.16 years, height = 177.93 ± 3.38 cm, weight = 73.64 ± 9.10 kg) with no current history of injury to the upper extremity or cardiovascular conditions.

Intervention(s): A class 4 laser device was used to treat the biceps brachii muscle. Each grid point was treated for 3 to 4 seconds, for a total of 4 minutes. Each participant received 4 doses of laser therapy: sham, 1 W, 3 W, and 6 W.

Main Outcome Measure(s): The dependent variables were changes in blood flow, measured using venous occlusion plethysmography. We used a repeated-measures analysis of variance to analyze changes in blood flow for each dose at 2, 3, and 4 minutes and at 1, 2, 3, 4, and 5 minutes after treatment. The Huynh-Feldt test was conducted to examine differences over time.

Results: Compared with baseline, blood flow increased over time with the 3-W treatment ($F_{3,9} = 3.468$, $P < .011$) at minute 4 of treatment (2.417 ± 0.342 versus 2.794 ± 0.351 mL/min per 100 mL tissue, $P = .032$), and at 1 minute (2.767 ± 0.358 mL/min per 100 mL tissue, $P < .01$) and 2 minutes (2.657 ± 0.369 mL/min per 100 mL tissue, $P = .022$) after treatment. The sham, 1-W, and 6-W treatment doses did not change blood flow from baseline at any time point.

Conclusions: Laser therapy at the 3-W (360-J) dose level was an effective treatment modality to increase blood flow in the soft tissues.

Key Words: therapeutic modalities, circulation, musculoskeletal injuries

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Key Points

- Using a class 4 laser in a human clinical model, we found a protocol-response effect: a 3-W protocol at a 50% duty cycle applied to the biceps brachii muscle was the most effective for increasing blood flow to the distal forearm.
- Laser therapy is an effective, noninvasive treatment modality to improve blood flow and perhaps tissue healing in the clinical setting.

The use of laser as a clinical modality has increased greatly over the past decade. Positive effects of laser therapy for the treatment of acute and chronic musculoskeletal disorders include pain control[1,2] and improved tissue repair.[3,4] However, the underlying mechanisms and clinical effectiveness of laser therapy remain poorly understood.

Lasers are classified by power level and their ability to produce eye injury. These power and beam characteristic ratings are established by the American National Standards Institute and the International Electrotechnical Commission. Most therapeutic lasers available for use in clinical practice are classified as 3B or 4. Class 3B lasers emit power of 5 to 500 mW, whereas class 4 lasers emit power of more than 500 mW. A few therapeutic laser manufacturers offer divergent-beam power outputs greater than 10000 mW. Class 3B level emitting lasers are known as low-level, low-intensity, and cold lasers because they generate no significant thermal effect in the superficial tissue during irradiation. Class 4 lasers are known as high-power and hot lasers because they can produce rapid increases in superficial tissue temperatures when maximum permissible exposure limits are exceeded. Recent trends in laser therapy show a preference for class 4 lasers in patient care settings.[5] Class 4 lasers can emit greater photonic energy in a shorter period of time than class 3B lasers without producing an appreciable rise in tissue temperature under normal treatment protocols.[5] This higher power becomes important when treating injuries to deeper tissues such as ligaments, muscles, tendons, and cartilage.

Authors of most published clinical studies on laser therapy to treat musculoskeletal injuries have used class 3B low-power lasers. Several published reports[6,7] have questioned the ability of low-power lasers to effectively transmit energy beyond the skin into deep musculoskeletal tissues. Excessive beam scattering and attenuation within the skin limit the potential biostimulative effects of laser in the deeper target tissues because of several factors related to dosimetry, such as subthreshold optical power, insufficient treatment durations, and varied treatment frequencies.[8,9] Therefore, it is relevant and timely to study the dosimetric responses of specific infrared wavelengths of high-power class 4 lasers and their ability to modulate the physiologic effects that are conducive to healing.

Positive therapeutic effects of laser have been attributed to increased blood flow in soft tissues and, coincidentally, the

limb.[10–12] Maegawa et al[10] showed that laser therapy induced arteriolar dilation and vastly improved blood flow in the microvascular bed of the rat mesentery. Moreover, Ihsan[11] observed accelerated collateral circulation and increased microcirculation in rabbits exposed to laser therapy after femoral artery occlusion. Laser therapy–induced changes in blood flow and microcirculation promote healing by controlling ischemia, hypoxia, and edema after injury, thereby limiting the zone of secondary tissue damage.[10,13] In addition, increased circulation in and around the injury zone creates a favorable environment for biological repair after musculoskeletal injury.[11,13]

An important factor associated with the therapeutic effectiveness of laser is dose.[3,14,15] Achieving a therapeutic dose without understimulating or overstimulating the target tissues is often the most difficult component of clinical laser therapy practice.[15] The Arndt-Schultz principle has been adopted from early toxicology studies of yeast culture to explain the optimal therapeutic dosage level of low-level laser. Optimal doses have been experimentally established in cell and tissue cultures. This therapeutic laser dosage or level of photostimulation must be attained; otherwise "no reactions or changes can occur in body tissues if the amount of energy absorbed is insufficient to stimulate the absorbing tissues."[16(379)] This positive stimulatory effect of specific wavelengths of light on tissues has been called *photobiomodulation*. The threshold level of laser biostimulation for many human tissues is not known; however, results from cell and animal studies suggest that the therapeutic effects of laser are dose dependent and are most pronounced during the acute phase of injury. This initial therapeutic window may be within the first hours to days after soft tissue injury.[14,17,18] These studies suggest that dose dependency also exists when treating humans, but except for superficial tissues and some dental applications, standard treatment doses for deep musculoskeletal tissues have yet to be firmly established in human clinical populations. Calculating an appropriate dose range for humans from studies using cell and animal models is challenging. No known transformation function can be used to calculate a comparable dose range in humans from those used in cell and animal models. Most authors have based dosage calculations on a 2-dimensional measurement (joules per square centimeter) of surface area exposure for laser. However, the actual dosage for deep tissues must take into consideration the 3-dimensional irregular Gaussian distribution of the scattered and absorbed laser beam. Without an adequate understanding of laser wavelength interactions with tissue chromophores, pigments, and tissue turbidity, this complex calculation can only be estimated. These factors dynamically influence the 3-dimensional pattern of absorption, scatter, reflectance, or transmittance of the laser beam in deep tissues. Therefore, our primary objective was to determine a therapeutic dose range using a class 4 laser to increase vasodilation and limb blood flow to the soft tissues of the forearm for treatment of the biceps brachii in humans.

METHODS

Participant Characteristics and Inclusion Criteria

Ten volunteers were recruited for participation in the study: healthy men of any racial or ethnic background (white = 8, Hispanic = 2), aged 18–35 years (age = 20.80 ± 2.16 years, height = 177.93 ± 3.38 cm, weight = 73.64 ± 9.10 kg). This age range represents a young yet mature study population readily available at our institution. Only men were recruited for this study

to eliminate any influence certain phases of the menstrual cycle may have on vasoactivity.[19] If a study candidate met the eligibility requirements and volunteered to participate, then he was randomly assigned to a treatment order group. Each group was given an identification code that linked the participant to the treatment order. Institutional review board approval was obtained before candidate recruitment. Written informed consent was obtained from all participants.

Exclusion Criteria

Candidates were excluded if they (1) had a recent injury (within 6 months) to the left upper extremity; (2) regularly participated in upper extremity weight training, defined as any weight (resistance) training more than once per week in the past 8 weeks; (3) took pain or prescription medication or nutritional supplements on a daily basis; (4) had any tattoos on or around the area of treatment; (5) had high blood pressure (greater than 140/90 mm Hg) or any known cardiovascular conditions (eg, coronary artery disease or peripheral arterial disease); or (6) had a history of any of the following conditions: malignant tumor in the upper extremity area, kidney or liver dysfunction, lupus or any other autoimmune disease, blood clots or phlebitis, anemia, skin allergies, severe muscle weakness, serious neurologic injury, or any disorder that might affect blood flow.
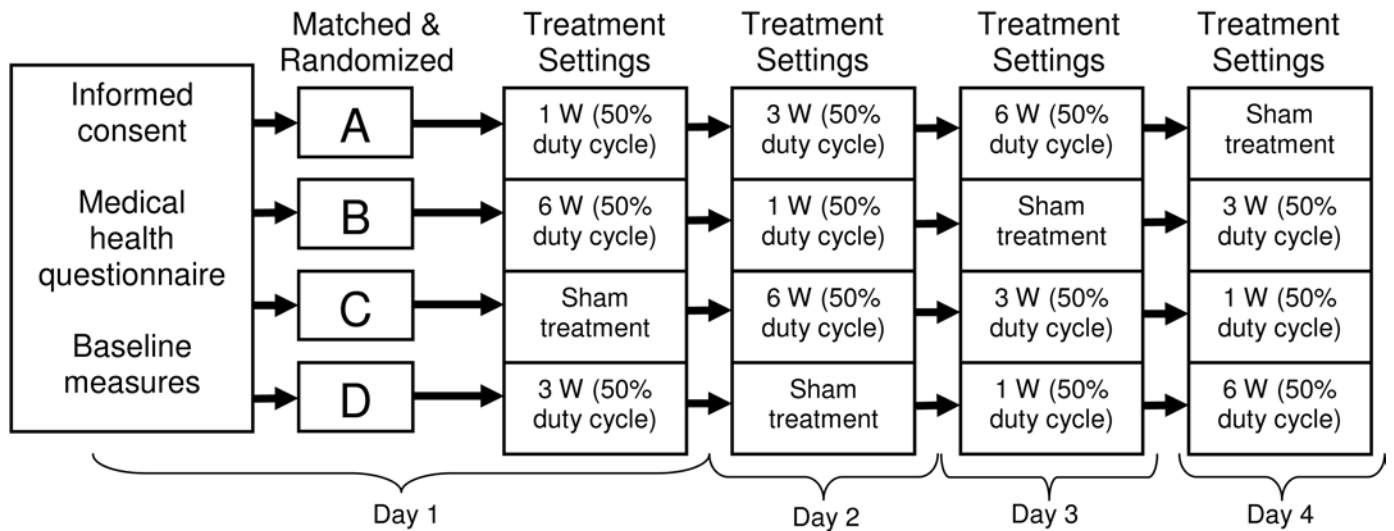
Experimental Design

A crossover, repeated-measures design was used to measure blood flow changes in the forearm before (baseline), during, and after a 4-minute laser treatment session. Participants were randomly assigned to 1 of 4 treatment order groups, and each group received the same 4 treatment doses in randomized order (Table). Each participant served as his own control (Figure 1). Laser treatments were administered during all 4 sessions, accompanied by venous occlusion plethysmography (VOP) measurements. The primary outcome measure for the experiment was percentage change in the circulation of the forearm (mL/min per 100 mL tissue). Data masking was performed to eliminate investigator, technician, and participant bias. The participant and VOP technician were blinded to the treatment order; the laser technician was blinded to the VOP measures obtained during and after treatments.

Experimental Procedures

Participants were instructed not to consume any caffeine, alcohol, theophylline (found in tea leaves), or other substances that might affect pain perception or hydration status for 24 hours before each testing session.

All participants underwent 4 testing sessions on separate days, with 24 hours between sessions. Each participant completed all testing sessions at the same time of day to control for circadian rhythm. During the first session (day 1), participants read and signed the informed consent document; completed a brief demographic (eg, age, weight), medical health, and medical history questionnaire; and underwent random assignment to a treatment order group (Figure 1).

At each treatment session, the examiner placed an occlusion cuff on the participant's upper left arm and performed VOP (model EC-6; D.E. Hokanson, Inc, Bellevue, WA) using calibrated mercury strain gauges. A strain gauge was applied to the left forearm 5 cm below the antecubital space. The participant

Figure 1. Experimental protocol.

was instructed to lie supine for 20 minutes with the left arm passively elevated above the right atrium of the heart. This position was used to obtain stable baseline measurements of forearm blood flow (FBF). The wrist cuff was inflated to 200 mm Hg to occlude hand circulation during FBF measurements for the remainder of the study after the 20-minute baseline stabilization period. To measure FBF, the upper arm cuff was inflated to 40 mm Hg for 7 seconds every 15 seconds using a rapid cuff inflator to prevent venous outflow.[20,21] Total occlusion time for the wrist cuff was 14 minutes. The FBF was measured continuously from baseline until 5 minutes after treatment.

All participants, along with the laser technician and VOP technician, wore safety eyewear (K-LaserUSA, Franklin, TN) to prevent eye damage from the laser light. The safety eyewear filtered laser light up to 1070 nm. All laser treatments were performed in a confined room; only those involved in the study were allowed in the room. In addition, a warning sign was hung on the door outside the room in which the laser treatments were performed to prevent unauthorized people from entering the room during laser applications.

Laser treatments were initiated once baseline measurements were taken and determined to be stable. A commercially available, US Food and Drug Administration–approved phototherapeutic device (K-LaserUSA) was used for all laser treatments. Laser treatments were performed using a light-touch grid pattern covering the muscle belly of the biceps brachii in order to achieve total energy absorption for the assigned treatment dose. Each grid point was treated for 3 to 4 seconds. The FBF was recorded by the plethysmograph during the laser treatment at 0 seconds, 2 minutes, and 4 minutes. Follow-up measure-

ments were taken at 1, 2, 3, 4, and 5 minutes after treatment (Figure 2). All laser treatments were administered by the lead author (K.A.L.), who is a certified therapist for K-LaserUSA and completed a 4-hour course on laser safety and operational techniques.

The FBF output signal was transmitted to the NIVP3 software program (D.E. Hokanson, Inc) loaded on a desktop computer. The FBF for each time point was calculated as the average of the first 3 plethysmographic measurements recorded during that minute.[20,21] Forearm vascular resistance was calculated as the ratio of mean arterial pressure divided by FBF and expressed as mm Hg·mL/min per 100 mL tissue.

Statistical Analysis

All data analyses were performed using SPSS for Windows (version 16.0; SPSS Inc, Chicago, IL). A repeated-measures analysis of variance was used to assess changes in blood flow during laser treatments at 2, 3, and 4 minutes and 1, 2, 3, 4, and 5 minutes after treatment. We used the Huynh-Feldt test to adjust the degrees of freedom for potential violations of the sphericity assumption in the analysis of variance to produce more accurate significance (P) values. An α level of ≤.05 was the criterion for statistical significance.

RESULTS

The 3-W condition was the only treatment dose to show an increase in FBF from baseline over time ($F_{3,9} = 3.468$, $P < .011$). Increases in FBF occurred at minute 4 of treatment

Table. Dosing Protocol by Treatment[a]

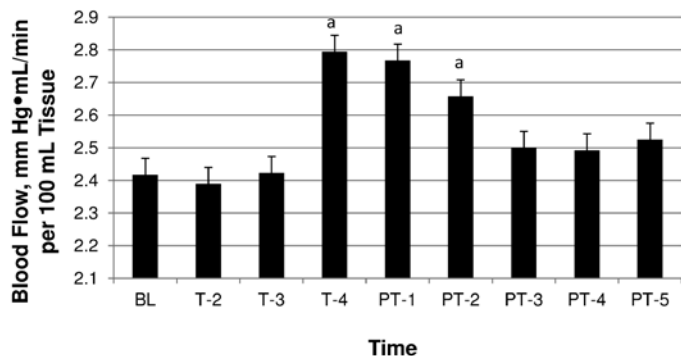| Output Power, W	Duty Cycle, %	Average Power/Treatment, W	Dose/Treatment, J
Sham	None	None	None
1	50	0.5	180
3	50	1.5	360
6	50	3.0	720

[a] Each treatment lasted 4 minutes.

Figure 2. 3-W dose response over time. Abbreviations: BL, baseline; T-2, mean during minute 2 of treatment; T-3, mean during minute 3 of treatment; T-4, mean during minute 4 of treatment; PT-1, mean at posttreatment minute 1; PT-2, mean at posttreatment minute 2; PT-3, mean at posttreatment minute 3; PT-4, mean at posttreatment minute 4; PT-5, mean at posttreatment minute 5. [a] Significant increase in blood flow from baseline.

Figure 4. 6-W dose response over time. Abbreviations: BL, baseline; T-2, mean during minute 2 of treatment; T-3, mean during minute 3 of treatment; T-4, mean during minute 4 of treatment; PT-1, mean at posttreatment minute 1; PT-2, mean at posttreatment minute 2; PT-3, mean at posttreatment minute 3; PT-4, mean at posttreatment minute 4; PT-5, mean at posttreatment minute 5.

$(2.417 \pm 0.342$ versus 2.794 ± 0.351 mL/min per 100 mL tissue, $P = .032$) and at 1 minute $(2.417 \pm 0.342$ versus 2.767 ± 0.358 mL/min per 100 mL tissue, $P < .01$) and 2 minutes $(2.417 \pm 0.342$ versus 2.657 ± 0.369 mL/min per 100 mL tissue, $P = .022$) posttreatment. At 3 minutes after treatment, FBF had returned to normal (Figure 2).

The sham, 1-W, and 6-W groups did not display any increases in FBF at any time point. However, the sham group demonstrated changes in FBF that mirrored those of the 1-W group (Figure 3), which may indicate an expectant placebo response during exposure to the sham laser treatment. Yet the 6-W group did not exhibit these changes, possibly because of overstimulation and suppression of physiologic responses (Figure 4).
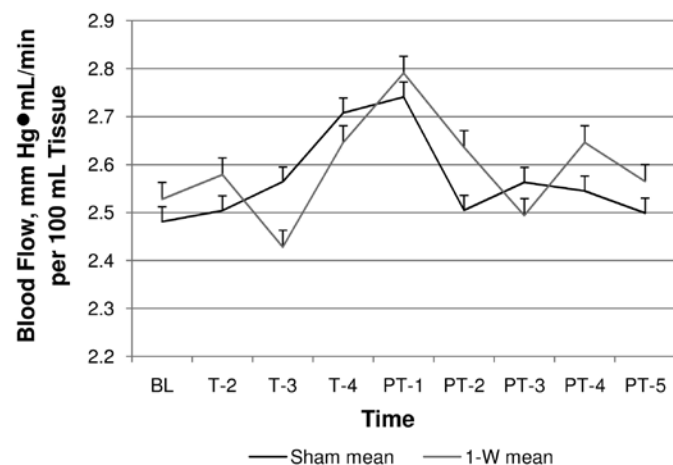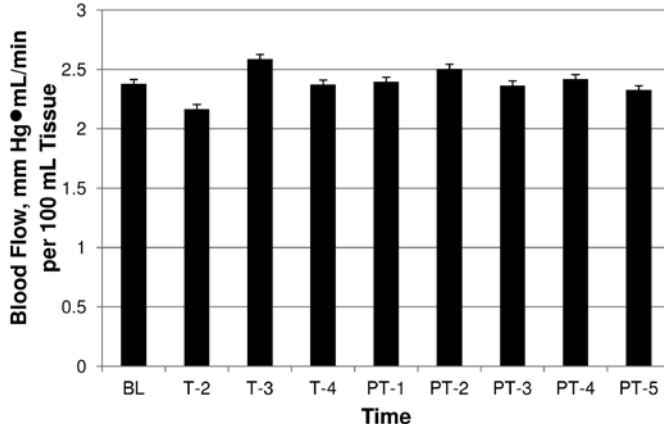

Figure 3. 1-W versus sham dose response over time. Abbreviations: BL, baseline; T-2, mean during minute 2 of treatment; T-3, mean during minute 3 of treatment; T-4, mean during minute 4 of treatment; PT-1, mean at posttreatment minute 1; PT-2, mean at posttreatment minute 2; PT-3, mean at posttreatment minute 3; PT-4, mean at posttreatment minute 4; PT-5, mean at posttreatment minute 5.

DISCUSSION

Improved circulation is considered one of laser therapy's greatest contributions to soft tissue healing after injury.[10–12] To the best of our knowledge, no authors have directly examined the effect of class 4 lasers in vivo on blood flow in the limb distal to the treatment area. Our study objective was to determine a therapeutic dose range using a class 4 laser to increase vasodilation and blood flow to the soft tissues in the forearm after treating the biceps brachii. We found a biphasic dose-response effect of laser therapy on FBF, with the 3-W protocol showing the greatest increase in FBF. The 3-W dosing protocol delivered 360 J of light energy over the 4-minute treatment period, whereas the 1-W dose delivered 180 J, and the 6-W dose delivered 720 J. Given this biphasic dose-response pattern, the 1-W dose was probably too weak to stimulate an increase in FBF; the 6-W dose was probably too strong and therefore suppressed any vasodilatory response.[22]

For the 3-W dose, increases in FBF from baseline levels were observed at minute 4 of treatment and persisted for up to 2 minutes after treatment. We did not observe any changes in blood flow for the 6-W, 1-W, or sham doses during or after laser treatment. Two factors that establish an effective treatment dose are optical output power (in watts) and energy delivery (in joules or watts over time). Optical power, or beam intensity, has a direct effect on light transmission through tissue and the depth of penetration.[23] Beam intensities greater than 1 W greatly improve light transmission through soft tissues when compared with lower beam intensities (0.5 W).[23] Therefore, class 4 lasers, using beam intensities greater than 1 W, may have an advantage over class 3 lasers in transmitting light to deeper tissues. In addition, using a laser with greater output power allows more light energy to be delivered to soft tissues in a shorter amount of time. Our results show that the combination of an output power of 3 W and an energy delivery of 360 J was adequate to induce a vascular response that improved FBF. The increased blow flow was transient, beginning at minute 4 of exposure and lasting 2 minutes after treatment. Short-term improvements in blood flow are helpful in therapeutic situations when cellular repair and edema control can facilitate heal-

ing as an adjunct with rehabilitative therapy. Improvements in blood flow may also aid in therapeutic exercise by delaying the onset of fatigue and improving muscle function, thereby allowing more work to be performed.[24]

Evidence from basic science research supports our findings. In studies in vivo, vascularized tissues exposed to laser displayed increased arteriolar dilation and increased microcirculation.[11] Nitric oxide (NO) signaling has been implicated as playing an important role in the process of light-induced changes to vascular structures. Maegawa et al[10] and Chertok et al[25] investigated the effect of laser therapy on rat mesenteric microcirculation in vivo. Laser therapy vastly improved vasomotor relaxation and arterial blood flow; however, these effects were completely abolished with prior inhibition of NO synthase activity.[10,25] Similarly, Samoilova et al[12] reported that human skin exposed to visible light showed increased microcirculation in an NO synthase–dependent mechanism. Sources of NO in soft tissues include activated macrophages,[26] vascular endothelial cells,[11] and protein complexes such as hemoglobin, myoglobin, and cytochrome c oxidase.[27,28] Activated macrophages and endothelial cells upregulate the synthesis of NO,[11,26] whereas hemoglobin, myoglobin, and cytochrome c oxidase, once photonically stimulated, release NO via photolysis.[27,29]

Interestingly, the blow flow response for the sham and 1-W groups followed similar patterns of microcirculatory change during and after laser treatment. This finding may suggest a placebo response resulting from the participants' beliefs and expectation that the laser would modulate blood flow. Previous authors[30] have shown that participants who believe they are receiving an active treatment display greater physiologic responses than those who believe they are not receiving an active treatment. In our case, the strength of the participants' beliefs that the laser modality would improve FBF may have caused a similar vascular response as the subthreshold 1-W dose. In addition, we did not observe a placebo response with the 6-W dose and believe this is due to overstimulation and subsequent suppression of the physiologic processes that would lead to an increase in FBF. These findings indicate the importance of blinding a patient's previous treatment outcomes as a way of controlling the placebo response.

We used VOP to analyze the responses of 4 treatment doses and the subsequent changes to FBF in real time. This technique is a sensitive and noninvasive method of measuring limb blood flow that has been used in humans for decades.[20] For our study, the benefit of VOP was that it allowed us to measure FBF change in vivo noninvasively in combination with class 4 laser therapy. Although the biceps brachii muscle was our target organ for laser irradiation, FBF changes were measured as an outcome of downstream vascular responses.

Our results provide a scientific basis for establishing dosing guidelines for future studies. The ability to provide a biostimulative dose will be beneficial to future researchers studying the mechanistic effects of laser to better understand its benefits in the healing of musculoskeletal injuries. Ultimately, these new data will offer clinicians better dosing and treatment guidelines to use in health care practice settings.

Limitations and Future Studies

We did not directly monitor local tissue temperature changes during laser treatment; however, we suggest that the vascular responses were caused by photochemical changes in the tissues and were not due to thermal effects. Heating of the superficial tissue layers can be easily avoided by selecting and using a proper duty cycle and application technique. We used a 50% duty cycle and followed a grid pattern, so that each point on the skin was irradiated for a brief period of time.

Furthermore, we tested only healthy participants, as opposed to patients. Future authors should investigate the long-term effects and benefits of laser therapy in clinical populations with musculoskeletal conditions. In addition, we measured blood flow for a short time after laser treatment. It would be beneficial to measure blood flow changes for prolonged periods of time after treatment. Outcomes from such studies would provide great insight for developing appropriate clinical dosing protocols.

Information on tissue oxygenation and perfusion would also offer beneficial information. The use of a near-infrared spectrometer on the irradiated muscle tissue would allow us to measure tissue oxygenation and saturation levels of both hemoglobin and myoglobin. This information would supply additional mechanistic justification for appropriate dosing in both healthy and injured populations.

CONCLUSIONS

Our results show a dose-response effect of class 4 laser therapy in a human clinical model, similar to what has been observed in the animal model. Specifically, a 3-W dose, along with a 50% duty cycle, applied to the biceps brachii proved to be the most beneficial protocol for increasing FBF. This suggests that a properly designed laser treatment protocol with appropriate dosing guidelines is a viable therapy to increase limb blood flow. Laser therapy's ability to increase blood flow in a dose-response manner is therefore an effective, noninvasive treatment modality to improve blood flow and may promote tissue healing in the clinical setting.

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