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Low-Level Laser Therapy as a Non-Invasive Approach for Body Contouring: A Randomized, Controlled Study

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Background and Objective: Transmission electron microscopic images have demonstrated the formation of transitory pores in adipocyte cell membranes followed by the collapse of adipose cells subsequent to laser irradiation of 635 nm. The objective is to evaluate the application of a 635 nm and 17.5 mW exit power per multiple diode laser for the application of non-invasive body contouring of the waist, hips, and thighs.

Study Design/Patients and Methods: Double-blind, randomized, placebo-controlled trial of a 2-week non-invasive laser treatment conducted from May 2007 to June 2008 across multiple-private practice sites in the United States of America. Sixty-seven volunteers between the ages of 18–65 with a body mass index (BMI) between 25 and 30 kg/m^2 and who satisfied the set inclusion criteria participated. Eight of the 67 subjects did not have circumference measurements recorded at the 2-week post-procedure measurement point. Participants were randomly assigned to receive low-level laser treatments or a matching sham treatment three times per week for 2 weeks. Reduction in the total combined inches of circumference measurements of the waist, hip and bilateral thighs from baseline to the completion of the 2-week procedure administration phase was assessed.

Results: Participants in the treatment group demonstrated an overall reduction in total circumference across all three sites of −3.51 in. ($P < 0.001$) compared with control subjects who revealed a −0.684 reduction ($P < 0.071745$). Test group participants demonstrated a reduction of −0.98 in. ($P < 0.0001$) across the waist, −1.05 in. ($P < 0.01$) across the hip, and −0.85 in. ($P < 0.01$) and −0.65 in. ($P < 0.01$) across the right and left thighs from baseline to 2 weeks (end of treatment). At 2 weeks post-procedure, test group subjects demonstrated a gain of 0.31 total inches collectively across all three sites.

Conclusion: These data suggest that low-level laser therapy can reduce overall circumference measurements of specifically treated regions. Lasers Surg. Med. 41:799–809, 2009. © 2009 Wiley-Liss, Inc.

Key words: adipocyte; adipose panicle; emulsification; photobiomodulation; transitory pore

INTRODUCTION

The emergence of non-invasive modalities targeting subcutaneous fat to achieve a slimming effect continues to gain interest amongst physicians and patients. Numerous delivery mechanisms have been developed to achieve adipocyte destruction including, ultrasound, infrared, and radio frequency [1–5]. The external application of photonic energy at high intensities can generate significant adverse events if not properly utilized; therefore, all parameters must be properly explored in order to identity which delivery mechanism yields the most desirable results while minimizing adverse events.

In recent years, there has been an upsurge in the application of low-level laser therapy (LLLT) across myriad neurologic, dental, ophthalmic, dermatologic disorders, and injuries [6–10]. LLLT has been proven to be a safe and effective therapeutic option in clinical and histological trials; yet, a great deal of skepticism still remains regarding the efficacy of this modality at the clinical level.

Numerous studies have exhibited laser therapy's ability to induce an assortment of cellular reactions in non-photosynthetic cells. Laser therapy has been shown to preserve the membrane and genetic material of cells that are nutritionally starved [11]; regenerate erythrocytes enhancing their oxyphoric function [12]; enhance fertilization potential of spermatozoa [13]; stimulate the differentiation of satellite stem cells [14–16]; reduce the extent of myocardial infarctions and ischemic strokes [17]; and improve wound healing and modulate chronic inflammation [18]. A continually growing body of evidence suggests that laser therapy can alter cell bioenergetics, consequentially influencing the functional biochemical properties intracellularly, culminating in an observable diverse clinical effect.

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Understanding the photobiomodulatory principles of laser therapy, Neira et al. [19] applied laser therapy at 635 nm to cultured adipocytes and revealed an ability to emulsify the targeted tissue. In a separate study, Neira et al. [20] examined the effect of LLLT at 635 nm with 10 mW intensity on human adipose tissue taken from lipectomy samples. Tissue samples were irradiated for 6 minutes and compared with non-irradiated samples. Utilizing scanning and transmission electron microscopy (SEM and TEM), more than 180 images were collected, and revealed that 99% of the cellular content including fat was released from the adipocyte, a phenomenon not observed within the control samples [20]. Further, TEM images of the adipose tissue were taken at 60,000× and revealed the formation of a transitory pore and complete deflation of adipocytes [20]. The cells within the interstitial space and capillaries remained intact demonstrating that the photochemical event was unique to the adipocytes [20]. It was concluded that the disruption of the adipocyte membrane is what enabled the liberation of the stored fatty material; thus, prompting the collapse of the adipocyte [19–21].

To confirm the histological findings and assess the depth of penetration of LLLT within the subcutaneous layer, Neira et al. [22] assessed T1 and T2 MRI sequences to evaluate any radiological changes subsequent to laser irradiation. The T2 sequence following 6 minutes of laser irradiation exhibited a less defined superficial adipose layer, less defined septae, and a much more coalescent adipose tissue. The study confirmed a change in fatty density and organization of both superficial and deep fat while supporting Neira's histological work. The morphologic changes of deep subcutaneous fat cannot be attributed to direct photostimulation; however, studies have revealed a systemic effect associated with LLLT in which non-irradiated adjacent cells become stimulated via intercellular communicators [23–25]. Therefore, the observable changes within the deep subcutaneous layers may be based upon the system effect found subsequent to LLLT.

Based upon the histological evidence, Jackson et al. [25] applied LLLT externally several minutes prior to the aspiration phase of lipoplasty in order to evaluate the impact adipocyte disruption could have on the procedure and for patient recovery. Jackson et al. [25] noted that for those patients receiving LLLT a greater volume of fat was able to be extracted and reduction in post-operative edema and pain was observed. Blinded physicians were asked to rate on a visual analog scale (VAS) from 1 to 100 their assessment of ease of extraction with 100 being the hardest to extract. Dr. Jackson noted that non-irradiated patients averaged an ease of extraction score of 73.84 compared with laser-treated patients averaging an extraction score of 12.88 [25]. Jackson concluded that laser-induced emulsification was observable at the clinical level based upon the ease of extraction scores for laser-treated subjects.

Although multiple studies have been published highlighting adipocyte modifications subsequent to laser therapy and its ability to serve as an adjunctive tool for liposuction, the purpose of this institutional review board study was to evaluate the clinical use of LLLT as an independent modality in reducing total combined circumference measurements of waist, hip, and thighs. This investigation attempts to position LLLT as a safe and effective modality for non-invasive body contouring building upon numerous histological studies. The device utilized in this investigation possesses the same wavelength and a similar intensity to the instruments analyzed by Neira and Jackson.

METHODS

Participants

Seventy-seven individuals were evaluated for study suitability; all 77 qualified and were enrolled. Sixty-seven of the qualified and enrolled subjects attended the initial pre-treatment phase and completed study participation through the study endpoint.

All subjects deemed eligible for participation in this clinical study satisfied each of the following inclusion criteria: subject is candidate for liposuction of the waist, hips and bilateral thighs; willing and able to abstain from partaking in any treatment other than the study procedure to promote body contouring and/or weight loss throughout the course of study; willing and able to maintain regular diet and exercise regimen without effecting significant change in either direction during study participation; and were between the ages of 18–65 years.

Subjects had none of the following exclusionary conditions: body mass index (BMI) of 30 kg/m^2 or greater; diabetes mellitus dependent on insulin or oral hypoglycemic medication; known cardiovascular disease such as cardiac arrhythmias and congestive heart failure; history of cardiac surgery such as coronary artery bypass, heart transplant surgery, and pacemakers; excessive alcohol consumption (more than 21 alcoholic drinks per week); prior surgical intervention for body sculpting/weight loss, such as liposuction, abdominoplasty, gastroplasty, lap band surgery, etc.; medical, physical, or other contraindications for body sculpting/weight loss; current use of medications known to affect weight levels and/or to cause bloating or swelling and for which abstinence during the course of study participation is not safe or medically prudent; medical condition known to affect weight levels and/or to cause bloating or swelling; diagnosis of, and/or taking medication for, irritable bowel syndrome; active infection, wound or other external trauma to the areas to be treated with the laser; pregnant, breast feeding, or planning pregnancy prior to the end of study participation; serious mental health illness such as dementia or schizophrenia; psychiatric hospitalization in past 2 years; developmental disability or cognitive impairment that would preclude adequate comprehension of the informed consent form and/or ability to record the necessary study measurements; involvement in litigation and/or a worker's compensation claim and/or receiving disability benefits related to weight-related and/or body shape issues; and participation in a clinical study or other type of research in the past 90 days.

All subjects were recruited from the assessment investigators' patient base: who presented for liposuction

consultation, signed the informed consent form, and satisfied all of the study eligibility criteria. Subjects were not offered any form of compensation to participate in the clinical trial, nor were they charged for the cost of the laser procedure or related evaluations.

Randomization and Blinding

The clinical study was a prospective, controlled double-blind parallel group three-center design. Sixty-seven participating subjects, 35 were randomized to the active treatment group and 32 were randomized to the sham-treatment group. Subject randomization was performed by a third party and was computer generated.

Intervention

Subjects assigned to the test group were treated with a multiple head low-level diode laser consisting of five independent diode laser heads each with a scanner, each emitting 635 nm (red) laser light with each diode generating 17 mW output (The Erchonia® LipoLaser, manufactured by Erchonia Medical, Inc.). Sham-treatment group participants were treated with a multiple head non-laser red light-emitting diode (LED) consisting of five independent red diode light heads each with a scanner, each emitting 635 nm (red) light with each diode generating 2.5 mW power. Both the sham treatment light and real laser devices were designed to have the same physical appearances, including the appearance of any visible light output.

Study Design

The circumference in inches (in.) of the subject's waist, hip, and each of the left and right thighs were measured and recorded across all time points. The hip circumference measurement was made such that both hip bones were encircled. The waist circumference measurement was the distance in inches from the top of the hip bone to the point at which the circumference of the waist was measured (the subject's natural waist formation). Finally, bilateral thigh circumference was the distance in inches from the hip bone down the point at which the circumference of the thighs were measured. Furthermore, the same individual at each test site was responsible for all circumference measurement recordings for all subjects at that test site to preserve study consistency removing the potential of inter-investigatory variability.

The circumference in inches for participant's waist, hips, and each of the left and right thighs along with their BMI's were measured at four different times: pre-procedure; end of first procedure week; end of second procedure week; and 2 weeks post-procedure.

The treatment phase of the study commenced immediately following the pre-procedure circumference measurements. The treatment phase extended over two consecutive weeks, with each subject receiving six total treatments with the laser or sham light scanning device across the consecutive 2 weeks; three procedures per week, each treatment two days apart. Each procedure took place at the investigators' test sites.

The procedure protocol required that subjects entered the procedure room and were placed in a comfortable supine position upon the treatment table. Subjects were fitted with blindfolds. The center diode of the laser or sham light scanning device was positioned at a distance of 6.00 in. above the participant's abdomen, centered along the body's midline and focused on the navel. The four remaining diodes were positioned 120° apart and tilted 30° off the center light source of the center diode. The scanner device was activated for 20 minutes.

Following anterior stimulation, the participant was then placed in a prone position upon the treatment table. The center diode of the laser scanner was positioned at a distance of 6.00 in. above the subject's back, centered along the body's midline and focused on the equivalent spot to the navel's location on the stomach. The four remaining diodes were positioned 120° apart and tilted 30° off the center light source of the center diode. The scanner device was activated for 20 minutes. The total laser energy that the subjects randomized to actual laser treatment received, front and back treatments combined, was approximately 6.60 J/cm^2.

Data Analysis

The primary efficacy outcome measure was defined as the change in total combined inches in circumference measurements (waist, hips, and bilateral thighs) from baseline (pre-procedure) to following completion of the 2-week procedure administration phase (end of week 2).

Individual subject success criteria was defined as at least 3.0 in. reduction in combined circumference measurements for the waist, hip, and bilateral thighs from baseline to after completion of the 2-week study procedure administration protocol phase. The overall study success criterion, established by Food and Drug Administration (FDA), was defined as at least a 35% difference between treatment groups, comparing the proportion of individual successes in each group. It was determined by the FDA that a reduction of at least 3.0 in. was clinically meaningful. In addition to the analysis of circumferential reduction as a means to determine a clinically meaningful outcome, participants were asked to assess their level of satisfaction pertaining to their overall change in body shape at the completion of the treatment administration phase. Patients were asked to record a rating on a 5-point scale of very satisfied, somewhat satisfied, neither satisfied nor dissatisfied, not very satisfied, not at all satisfied.

Data were analyzed according to the intention-to-treat principle, including all subjects who had been randomized to treatment groups were included provided they had circumference measurements recorded at baseline. Drop-outs, terminated subjects, and so forth were included by carrying forward the last observation for all time points following Last Observation Carried Forward (LOCF) method. Eight of the 67 subjects did not have circumference measurements recorded at the 2 week post-procedure measurement point: 4 of these subjects who had been randomized to the test group and 4 of these subjects who had been randomized to the sham-treatment group. For these eight subjects, the (LOCF) method was employed,

TABLE 1. Pre-Procedure Body Mass Index Measurements for Treatment Groups ($n = 67$)

BMI (kg/m^2)	Test group ($n = 35$)	Control group ($n = 32$)	Difference
Mean	25.74	26.05	0.31

BMI, body mass index.

such that the subject's week 2 circumference measurement was carried forward as the 2 weeks post-procedure measurement.

RESULTS

At baseline, the differences in subject pre-procedure BMI recordings between experimental groups were not found to be statistically significant ($t = -0.48$; df = 64; $P = 0.647$ [$P > 0.05$]) (Table 1). Moreover, the differences in subject pre-procedure body circumference measurements between treatment groups were not found to be statistically significant for any body area or for the total number of inches of all body areas combined ($t = -1.18$; df = 65; $P = 0.240$ [$P > 0.05$]) (Table 2).

Of the 32 sham light treated group participants, 6.38% (2 subjects), demonstrated a total decrease in combined circumference measurements from pre-procedure to study endpoint of −3.0 in. or greater, while 22 (62.9%) of the 35 enrolled test group participants demonstrated a reduction of −3.0 in. or greater, a significant difference between both groups ($P < 0.0001$).

Fifty-seven percent more test group participants than sham light treated group participants showed a total decrease in combined circumference measurements from pre-procedure to study endpoint of 3.0 in. or greater (Table 3). This outcome exceeded the pre-established target of 35% difference between treatment groups by 22%.

Comparison of the two independent group means for the continuous variable of mean change in total combined circumference (total number of inches) from study baseline to endpoint demonstrated a mean difference of −2.837 (Table 4). The difference was found to be statistically significant ($t = -7.30$; df = 65; $P < 0.0001$).

Compared with baseline, the total combined circumference measurements for test subjects were significantly lower at all three subsequent evaluation points: −2.06 in. at week 1 ($P < 0.01$), −3.52 in. at week 2 ($P < 0.01$), and −3.21 in. at 2 weeks post-procedure ($P < 0.01$). Sham light treated group subjects from baseline to 2 weeks post-procedure produced an overall reduction in total combined circumference measurements of −0.62 in. ($P > 0.05$). Moreover, sham light treated group participants compared with baseline demonstrated statistically insignificant changes in total combined circumference measurements across all three subsequent evaluation points ($P > 0.05$) (Fig. 1).

Test group participants from week 2 to 2 weeks post-procedure revealed an overall gain in total circumference measurements of +0.30 in., which was not statistically significant ($P > 0.05$).

Compared with baseline, the changes in total circumference measurements between groups were statistically significant at all three subsequent evaluation points: −1.794 in. at week 1 ($t = -3.83$; df = 65; $P = 0.00029$ [$P < 0.0005$]), −2.838 in. at week 2 ($t = -7.30$; df = 65; $P < 0.0001$), and −2.593 in. at 2 weeks post-procedure ($t = -6.66$; df = 65; $P < 0.0001$) (Table 5).

Participants in the test group demonstrated an overall reduction in circumference of −0.98 in. across the waist from baseline to week 2 ($P < 0.0001$). Compared with baseline, circumference measurements of the waist were significantly lower at all three subsequent evaluation points: −0.56 in. at week 1 ($P < 0.01$), −0.98 in. at week 2 ($P < 0.0001$), and −1.08 in. at 2 weeks post-procedure ($P < 0.001$). Subjects assigned to the sham light treated group revealed insignificant changes in waist circumference measurements across all evaluation points ($P > 0.05$) (Fig. 2).

For test group participants, compared with baseline, circumference measurements for the hip were significantly lower at all three subsequent evaluation points: −0.73 in.

TABLE 2. Pre-Procedure Circumference Measurements Between Treatment Groups ($n = 67$)

Mean circumference (in.)	Test group ($n = 35$)	Control group ($n = 32$)	P-value
Waist	33.94	34.95	> 0.05
Hip	38.99	39.88	> 0.05
Right thigh	23.80	24.12	> 0.05
Left thigh	23.59	24.14	> 0.05
Total	120.31	122.99	> 0.05

In., inches.

TABLE 3. The Total Number and Percentage of Treatment Group Participants Meeting the Individual Success Criteria ($n = 67$)

	Test subjects ($n = 35$)	Control subjects ($n = 32$)
Number of participants meeting success criteria	22	2
% Meeting success criteria	62.86%	6.25%

TABLE 4. Mean Change in Total Combined Circumference Measurements From Baseline to Endpoint for Treatment Groups ($n = 67$)

	Test group ($n = 35$)	Control group ($n = 32$)
Mean reduction in total circumference (in.)	−3.521	−0.684

In., inches.

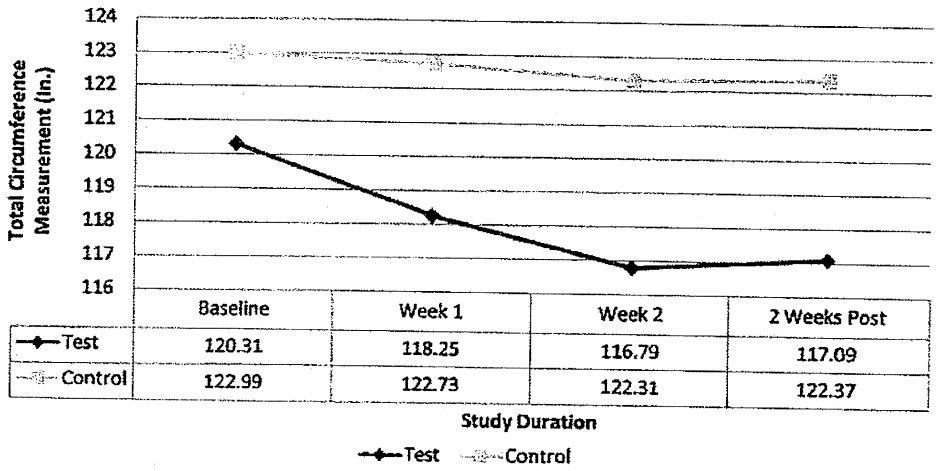


Fig. 1. Total circumference measurements across study duration for all participants ($n = 67$).

at week 1 ($P < 0.01$), −1.05 in. at week 2 ($P < 0.01$), and −0.70 in. at 2 weeks post-procedure ($P < 0.01$). Sham light treated group participants demonstrated insignificant changes in hip circumference measurement across all evaluation points ($P > 0.05$) (Fig. 3).

Compared with baseline, circumference measurements of the right thigh for test subjects were significantly lower at all three subsequent evaluation points: −0.49 in. at week 1 ($P < 0.01$), −0.85 in. at week 2 ($P < 0.01$), and −0.78 in. at 2 weeks post-procedure ($P < 0.01$). Participants of the sham light treated group revealed insignificant changes in right thigh circumference measurements across all measurement points ($P > 0.05$) (Fig. 4).

Compared with baseline, circumference measurements of the left thigh for test subjects were significantly lower at all three subsequent evaluation points: −0.29 in. at week 1 ($P < 0.05$), −0.65 in. at week 2 ($P < 0.01$), and −0.67 in. at 2 weeks post-procedure ($P < 0.01$). For subjects assigned to the sham light treated group, the changes in left thigh circumference measurement across all measurement points were not statistically significant for any interval ($P > 0.05$) (Fig. 5).

Of the total 67 study participants, 61 responded to the satisfaction survey. Thirty of the 35 test subjects and 31 of the 32 sham light treated subjects assessed their satisfaction level subsequent to the treatment administration phase. Twenty-one test group participants (70%) and eight sham light group participants (26%) recorded a "satisfied" rating. (Fig. 6) Moreover, 1 test group participant and 11 control group participants recorded a "dissatisfied" rating (Fig. 6). The difference of the rating score between the two treatment groups was found to be statistically significant ($P < 0.0005$).

DISCUSSION

In this double-blind, controlled, randomized trial, we observed that low-level laser of the appropriate wavelength applied three times per week for 2 weeks can significantly reduce the circumference at specifically targeted tissue sites due to reduction in the adipose layer. To fully appreciate these results, further scientific exploration is required to gain a better understanding of the role the lymphatic and circulatory systems may play in the absorption of the released triglycerides, fatty acids, and other adipocyte stored material evacuated following the laser induced formation of the transitory pore. Further, a study must be conducted to assess the long-term maintenance of the circumferential loss. A non-randomized, non-controlled study assessing the alteration of serum triglyceride and cholesterol levels using the same

TABLE 5. The Difference in Change in Total Circumference Measurements Between Evaluation Time Points Between Treatment Groups ($n = 67$)

Mean reduction (in.)	Test group ($n = 35$)	Control group ($n = 32$)	Difference between groups
Baseline—week 1	−2.06	−0.27	−1.794
Baseline—week 2	−3.52	−0.68	−2.838
Baseline—2 weeks post	−3.21	−0.62	−2.953
Week 1—week 2	−1.46	−0.42	−1.044
Week 1—2 weeks post	−1.15	−0.36	−0.799
Week 2—week 4	+0.31	+0.06	+0.245

In., inches.

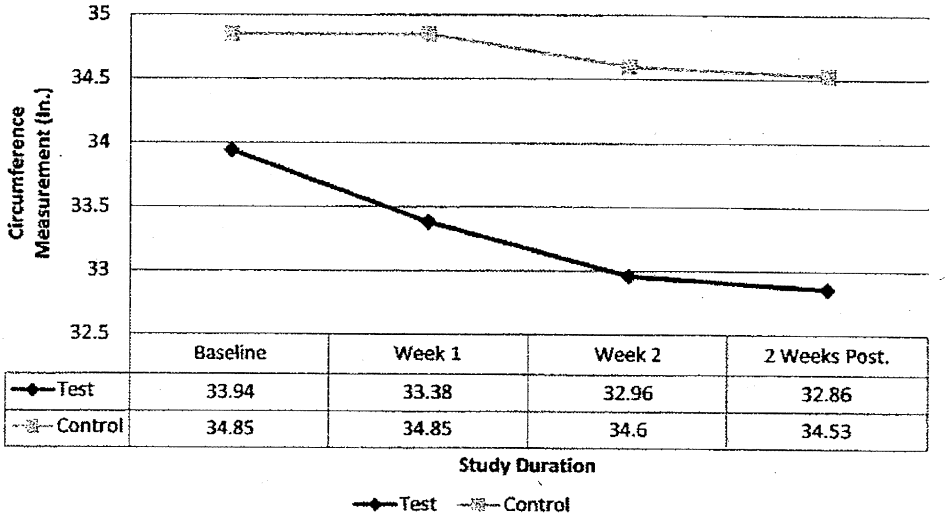


Fig. 2. Circumference measurements of the waist at each evaluation point for all participants ($n = 67$).

treatment parameters as used in this study was recently completed, the study will be published with preliminary results revealing an overall reduction in both triglyceride and total cholesterol levels following 2 weeks of laser therapy [26]. Further, no adverse events were reported in this clinical investigation. Punch biopsies were not performed during this clinical investigation as the mechanism of adipose tissue reduction has been previously demonstrated in the literature by Neira's work which provided compelling evidence that the application of laser therapy at 635 nm with output intensity between 7 and 20 mW consistently induces the formation of a transitory pore within the membrane of adipocytes provoking their collapse.

This ability to modulate cellular metabolism and provoke diverse biologic responses is strongly dependent on the intensity, wavelength, and frequency of light being emitted. Moreover, the very same biological response induced by a specific wavelength can be further optimized or inhibited depending whether the radiation characteristic is pulsatile or constant wave (CW) [27]. The parameters of laser light are important in the emulsification of adipocytes. Dr. Neira [20] noted that greater intensities of laser light did not achieve the same biological response that lower energy output devices did. Across multiple laser applications, studies indicate a greater induction of cellular modulation is readily attained utilizing low-energy laser devices [18,28].

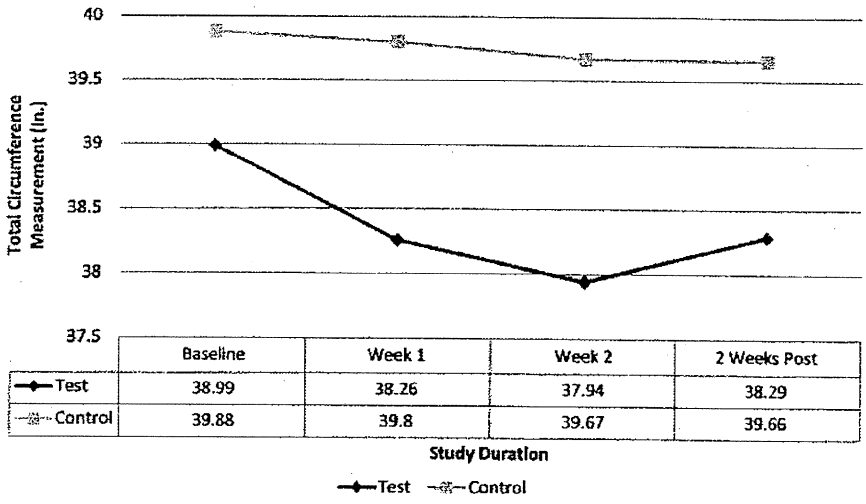


Fig. 3. Circumference measurements of the hip at each evaluation point for all participants ($n = 67$).

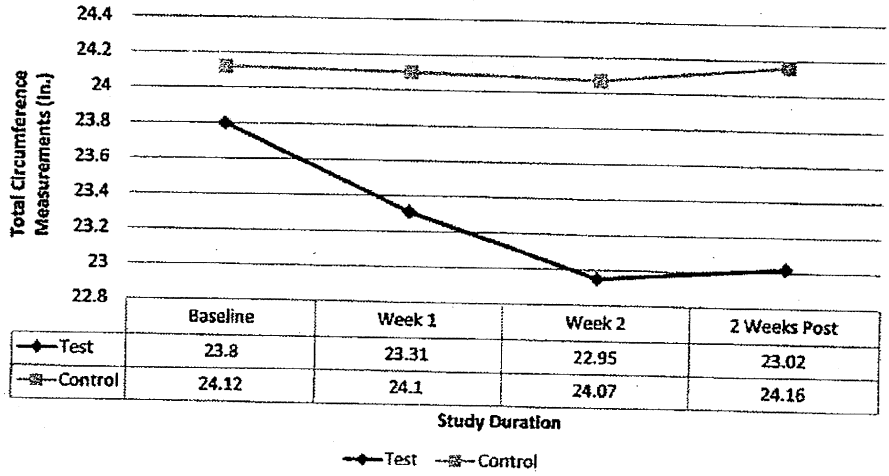


Fig. 4. Circumference measurements of the right thigh at each evaluation point for all participants ($n = 67$).

In accordance to the first law of photochemistry, the observable biological effect following LLLT can only transpire in the presence of a photoacceptor molecule, a molecule capable of absorbing the photonic energy emitted [27]. No photothermal or photoacoustic mechanisms are associated with this device; therefore no macroscopic heating or sensation is observed. An identified target of laser therapy is a highly specialized enzyme, cytochrome *c* oxidase, which plays a crucial role in the bioenergetics of the cell. Cytochrome *c* oxidase is a multicomponent membrane protein that contains a binuclear copper center (Cu_A) along with a heme binuclear center (a_3–Cu_B) both which facilitate the transfer of electrons from water soluble cytochrome *c* to oxygen [29–31]. This respiratory chain enzyme, due to the presence of transition metals, has been shown to absorb photonic energy-identifying cytochrome *c* oxidase as a photoacceptor molecule [32]. Studies indicate that following laser irradiation at 633 nm, the mitochondrial membrane potential and proton gradient increases, causing changes in mitochondria optical properties increasing the rate of adenosine diphosphate/adenosine triphosphate (ADP/ATP) exchange [33]. It is suggested that laser irradiation increases the rate at which cytochrome *c* oxidase transfers electrons from cytochrome *c* to dioxygen [34,35]. Moreover, it has been proposed that laser irradiation reduces the catalytic center of cytochrome *c* oxidase, making more electrons available for the reduction of dioxygen [36,37].

The upregulation of ATP following LLLT is also coupled with transient increases in reactive oxygen species (ROS) which then participates in intracellular signal transduction [18,27]. The modulation of cellular metabolism and

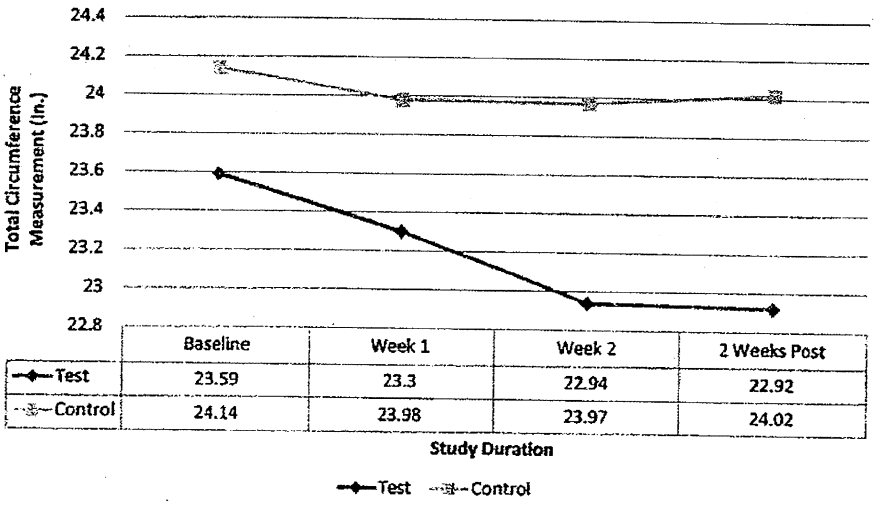
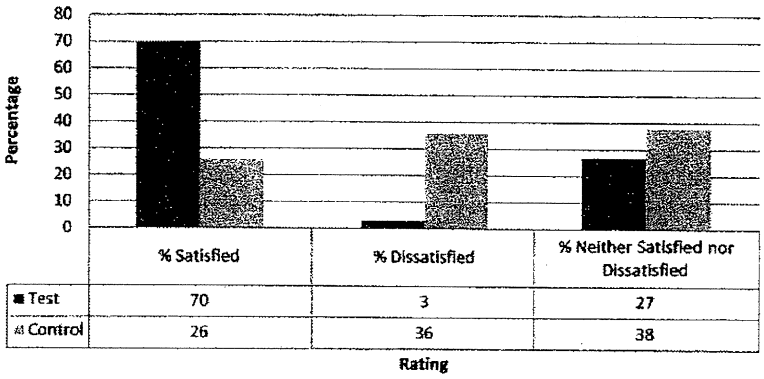


Fig. 5. Circumference measurements of the left thigh at each evaluation point for all participants ($n = 67$).



"Satisfied" included (very + somewhat satisfied) and "Dissatisfied" included (not very + not at all satisfied) for each treatment group.

Fig. 6. Percentage of test and placebo participants who were satisfied and dissatisfied ($n = 61$).

signal transduction has been found to alter gene expression [38], cellular proliferation [39–43], intra-cellular pH balance [44], mitochondrial membrane potential [45], generation of transient reactive oxygen species [46–49] and calcium ion level [46,50,51], proton gradient [52] and consumption of oxygen. Modulation of cell metabolism has also been associated with an increase in lipid peroxidation. Lipid peroxidation is the oxidative degradation of membrane bound cholesterol resulting in a deleterious effect on membrane structure and function [53]. Studies have exhibited that cells subsequent to low-energy laser irradiation can induce the upregulation of secondary free radical reactions resulting in lipid peroxidation [54,55]. The photo-induced excitation of lipid peroxidation has been demonstrated within blood leukocytes following low-level laser irradiation and perhaps is occurring within adipocytes. Selected hydroperoxides of cholesterol could be used an indicator to confirm lipid peroxidation within adipocytes following LLLT [56].

Further, the upregulation of ROS can directly impact the cellular redox state which can affect the expression of genes via the activation of specific cellular signaling pathways including redox-sensitive transcription factors and phospholipase A_2 [57]. There are two well-defined transcription factors, nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1), which become activated following an intracellular redox shift to an oxidized state [58,59]. Calkhoven and Ab [59] demonstrated that reductants generally suppressed transcription factors, preventing the expression of genes. LLLT provokes a shift of the intracellular redox state towards an oxidative state, activating redox-sensitive transcription factors such as NF-κB and AP-1, upregulating the expression of genes [61]. Perhaps the activation or suppression of specific transcription factors can influence membrane-related proteins altering the permeability of adipocytes. It is unclear at this time what cellular components of the adipocyte allow for this unique laser induced endpoint, which appears to be distinctive to adipocytes and a small group of other non-photosynthetic cells.

Controversy exists regarding the photostimulatory similarities between LED's and laser diodes. Although the biological effects when stimulating superficial surfaces with an LED or laser diode are the same under similar parameters; deep tissue photobiomodulation however, such as emulsifying subcutaneous adipose panicles, requires a coherent laser diode device [62–64]. The placebo device used in this controlled study delivered non-coherent LED light, and based upon the results obtained from the placebo group participants LED did not generate a statistically significant reduction in the circumference measurement in inches across all treatment sites at each subsequent evaluation point.

It is unclear at this time whether the transitory pore induced by laser therapy is the direct result of upregulated gene expression via transcription factor activation, lipid peroxidation by increased superoxide production, or an exocytosis-like event. Discussing the basic principles of laser therapy as described above could help guide further investigations towards uncovering the exact mechanism employed by laser therapy that ultimately results in the formation of the adipocyte membrane aperture. The findings in this study demonstrated laser light's efficacy of reducing the circumference measurements at each treated region across all evaluation points. The statistically significant difference between test and sham treatment groups identified the potential for laser therapy to serve as an adjunctive or independent treatment for subcutaneous fat reduction. Moreover, the higher study outcome satisfaction ratings reported by subjects in the test group than by subjects in the sham treatment group is statistically significant indicating that the efficacy of the application of LLLT for body contouring is clinically meaningful. Further studies analyzing the long-term effects should be conducted. Moreover, an investigation of the long-term benefits of improved nutrition and exercise stimulated by

the positive motivation of rapid circumferential reduction should be performed. Although a concerted effort must exist amongst multiple medical practitioners to properly educate the patient on the importance of healthy choices, non-invasive body-contouring tools like LLLT, may play a vital role in encouraging patients to adhere to new lifestyle changes.

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Mr. Maloney had full access to the clinical data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; however, Mr. Maloney was not at any time involved in the collection and analysis of data. Mr. Maloney was provided the full clinical data from an independent regulatory firm. Mr. Maloney takes responsibility that the data provided to him from the independent regulatory firm was not altered, manipulated, or changed in any way. Ryan Maloney is the Medical Director for Erchonia Medical, Inc., the sponsor and provider of the low-level laser device utilized in the clinical study. Mr. Maloney had no contact with the study participants, clinical investigators, or the independent regulatory firm responsible for processing and analyzing the clinical data. Mr. Maloney is a paid consultant for Erchonia Medical, Inc., and shares the patent with Erchonia for the test device utilized in the study. Statistical analysis of all collected clinical data was performed by an independent regulatory consulting firm, Regulatory Insight, Inc. Elvira Walls, MS, was the independent statistician for this clinical investigation. Elvira Walls was compensated for her time. At no time did she communicate with any of the participants of the clinical trial or with Ryan Maloney. Elvira Walls had full access to the clinical data and takes responsibility for the integrity of the data and the accuracy of the data analysis. The sponsor of the clinical trial, Erchonia Medical, Inc., was the manufacture of the device used in the clinical trial. The role of the sponsor was simply to provide the real treatment device and the placebo device. No patients or investigators were provided funding for their participation in this clinical investigation. No employed member of Erchonia Medical, Inc., was involved in the recruitment or the clinical investigator process. Dr. Robert Jackson, MD, was the primary clinical investigator. Dr. Robert Jackson was involved in the assessment phase of the clinical trial. Dr. Jackson was not aware of patient group assignments nor during any part of the clinical trial was he made aware of a patient's group assignment. Dr. Jackson was not provided any compensation for his participation in the clinical trial. Dr. Jackson was not involved in the writing of this manuscript; he however did read and edit this piece. Dr. Greg Roche served as an investigator for this study and was directly involved in the assessment phase of the clinical trial. Dr. Roche was not aware of patient group assignments nor during any part of the clinical trial was he made aware of a patient's group assignment. Dr. Roche was not provided any compensation for his participation in the clinical trial. Dr. Roche was not involved in the writing of this manuscript; he however did read and edit this piece. The same applies for Dr. Dedo, he served as an investigator and was involved in the assessment phase of the clinical trial.

REFERENCES

1. Wanner M, Avram M, Gagnon D, Mihm MC, Zurakowski D, Watanabe K, Tannous Z, Anderson RR, Manstein D. Effects of non-invasive, 1,210 nm laser exposure on adipose tissue: Results of a human pilot study. Lasers Surg Med 2009; 41(6):401–407.
2. Kim KH, Geronemus RG. Laser lipolysis using a novel 1,064 nm Nd:YAG laser. Dermatol Surg 2006;32:241–248.
3. O'Dey DM, Prescher A, Poprawe R, Gaus S, Stanzel S, Pallua N. Ablative targeting of fatty-tissue using a high powered diode laser. Lasers Surg Med 2008;40:100–105.
4. Moreno-Moraga J, Valero-Altes T, Martinez-Riquelme A, Isarria-Marcosy MI, De La Torre JR. Body contouring by noninvasive transdermal focused ultrasound. Lasers Surg Med 2007;39:315–323.
5. Narins RS, Tope WD, Pope K, Ross EV. Overtreatment effects associated with radiofrequency tissue tightening device: Rare, preventable, and correctable with subcision and autologous fat transfer. Dermatol Surg 2006;32:115–124.
6. Stonecipher KG, Kezirian GM. Wavefront-optimized versus wavefront-guided LASIK for myopic astigmatism with the ALLEGRETTO WAVE: Three-month results of a prospective FDA trial. J Refract Surg 2008;24(4):S424–S430.
7. Zins JE, Alghoul M, Gonzalez AM, Strumble P. Self-reported outcome after diode laser hair removal. Ann Plast Surg 2008;60(3):233–238.
8. Katz B, McBean. J. The new laser liposuction for men. Dermatol Ther 2007;20(6):448–451.
9. Zouari L, Bousson V, Hamze B, Roulot E, Roqueplan F, Laredo JD. CT-guided percutaneous laser photocoagulation of osteoid osteomas of the hands and feet. Eur Radiol 2008; May 24.
10. Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: Mechanism and efficacy. Dermatol Surg 2005;31(3):334–340.
11. Carnevalli CM, Soares CP, Zangaro RA, Pinheiro ALB, Silva NS. Laser light prevents apoptosis on Cho K-1 cells line. J Clin Laser Med Surg 2003;21:193–196.
12. Siposan DG, Lukacs A. Relative variation of the received dose of some erythrocyte and leukocyte indices of human blood as a result of low-level laser irradiation: An in vitro study. J Clin Laser Med Surg 2001;19:89–103.
13. Cohen N, Lubart R, Rubinstein S, Breitbart H. Light irradiation of mouse spermatozoa stimulation of in vitro fertilization and calcium signals. Photochem Photobiol 1998; 68:407–413.
14. Ben-Dov N, Schefer G, Irintchev A, Wernig A, Oron U, Halevy O. Low-energy laser irradiation affects satellite cell proliferation and differentiation in vitro. Biochem Biophys 1999;1448:372–380.
15. Shefer G, Oron U, Irintchev A, Wernig A, Halevy O. Skeletal muscle cell activation by low-energy laser irradiation: A role for the MAPK/ERK pathway. J Cell Physiol 2001;187:73–80.
16. 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. Biochem Biophys 2003;1593:131–139.
17. Streeter JD, Taboada L, Oron U. Mechanisms of action of light therapy for stroke and acute myocardial infarction. Mitochondrion 2004;4:569–576.
18. Tafur J, Mills PJ. Low-intensity light therapy: Exploring the role of redox mechanisms. Photomed Laser Surg 2008; 26(4):323–328.
19. Neira R, Solarte E, Isaza C, et al. Effects of the electric laser diode beam on in vitro human adipose tissue culture. Congreso Bolivariano de Cirugia Plastica Reconstructiva 2001.

20. Neira R, Arroyave, Ramirez H, et al. Fat liquefication: Effect of low-level laser energy on adipose tissue. Plast Reconstr Surg 2002;110:912–922.
21. Neira R, Arroyave J, Solarte E, et al. In vitro culture of adipose cells after irradiating them with a low-level laser device. Congreso Bolivariano de Cirugia Plastica Reconstructiva 2001.
22. Neira R, Jackson R, Dedo D, Ortiz CL, Arroyave A. Low-level-laser assisted lipoplasty: Appearance of fat demonstrated by MRI on abdominal tissue. Am J Cosmet Surg 2001;18(3): 133–140.
23. Rochkind S, Rousso M, Nissan M, Villarreal M, Barr-Nea L, Rees DG. System effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds, and burns. Lasers Surg Med 1989;9(2):174–182.
24. Schindl A, Heinze G, Schindl M, Pernerstorfer-Schon H, Schindl L. Systemic effects of low-intensity laser irradiation on skin microcirculation in patients with diabetic microangiopathy. Microvasc Res 2002;64:240–246.
25. Jackson R, Roche G, Butterwick KJ, Dedo DD, Slattery K. Low-level laser-assisted liposuction: A 2004 clinical trial of its effectiveness for enhancing ease of liposuction procedures and facilitating the recovery process for patients undergoing thigh, hip, and stomach contouring. Am J Cosmet Surg 2004; 21(4):191–198.
26. Maloney R, Shanks S, Jenney E. The reduction in cholesterol and triglyceride serum levels following low-level laser irradiation: A non-controlled, non-randomized pilot study. Laser Surg Med 2009;21S:66.
27. Karu T. *Ten* lectures on basic science of laser phototherapy. Grangesberg, Sweden: Prima Books AB; 2007.
28. Lubart R, Eichler M, Lavi R, Friedman H, Shainberg A. Low-energy laser irradiation promotes cellular redox activity. Photomed Laser Surg 2005;23(1):3–9.
29. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S. Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8Å. Science 1995;269:1069–1074.
30. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa. The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8Å. Science 1996;272:1136–1144.
31. Iwata S, Ostermeirer C, Ludwig B, Michel H. Structure of 2.8A resolution of cytochrome c oxidase from *Paracoccus denitrificants*. Nature 1995;376:660–669.
32. Karu TI, Afanasyeva NI. Cytochrome oxidase as primary photoacceptor for cultured cells in visible and near IR regions. Doklady Akad. Nauk (Moscow) 1995;342:693–695.
33. Alexandratou E, Yova D, Handris P, Kletsas D, Loukas S. Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. Photochem Photobiol Sci 2002;1:547–552.
34. Terenin AN. Photochemistry of dyes and other organic compounds. Moscow, Leningrad. Acad Sci Publ 1947.
35. Marcus RA, Sutin N. Electron transfer in chemistry and biology. Biochem Biophys 1985;811:265–322.
36. Konev SV, Belijanovich LM, Rudenok AN. Photoreactivations of the cytochrome oxidase complex with cyanide: The reaction of heme a3 photoreduction. Membr Cell Biol (Moscow) 1998;12:743–754.
37. Byrnes KR, Wu X, Waynant RW, Ilev IK, Anders JJ. Low power laser irradiation alters gene expression of olfactory ensheathing cells in vitro. Lasers Surg Med 2005;37:161–171.
38. Snyder SK, Byrnes KR, Borke RC, Sanchez A, Anders JJ. Quantification of calcitonin gene-related peptide mRNA and neuronal cell death in facial motor nuclei following axotomy and 633 nm low power laser treatment. Lasers Surg Med 2002;31:216–222.
39. Broadley C, Broadley KN, Disimone G, Reinisch L, Davidson JM. Low energy helium–neon laser irradiation and the tensile strength of incisional wounds in the rat. Wound Rep Reg 1995;3:512–517.
40. Allendrof JDF, Bessler M, Huang J, Kayton ML, Laird D, Nowygrod R, Treat MR. Helium–neon laser irradiation at fluences of 1, 2 and 4 J/cm2 failed to accelerate wound healing as assessed by both wound contracture rate and tensile strength. Lasers Surg Med 1997;20:340–345.
41. Lowe AS, Walker MD, O'Byrne M, Baxter GD, Hirst DG. Effect of low intensity monochromatic light therapy (890 nm) on a radiation impaired, wound-healing model in murine skin. Lasers Surg Med 1998;23:291–298.
42. Walker MD, Rumpf S, Baxter GD, Hirst DG, Lowe AS. Effect of low-intensity laser irradiation (660 nm) on a radiation-impaired wound-healing model in murine skin. Lasers Surg Med 2000;26:41–47.
43. Lubart R, Wollman Y, Friedman H, Rochkind S, Laulicht I. Effects of visible and near-infrared lasers on cell culture. J Photochem Photobiol 1992;12:305–310.
44. Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD. Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. Lasers Surg Med 2005;36:8–12.
45. Alexandratou E, Yova D, Handris P, Kletsas D, Loukas S. Human fibroblasts altercations induced by low power laser irradiation at the single cell level using confocal microscopy. Photochem Photobiol Sci 2002;1:547–552.
46. Grossman N, Schneid N, Reuveni H, Halevy S, Lubart R. 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: Involvement of reactive oxygen species. Lasers Surg Med 1998;22:212–218.
47. Lubart R, Eichler M, Lavi R, Friedman H, Shainberg A. Low-energy laser irradiation promotes cellular redox activity. Photomed Laser Surg 2005;1:3–9.
48. Lin Y, Berg AH, Iyengar P, Lam TKT, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilai N, Rhodes CJ, Fantus IG, Brownlee M, Scherer PE. The hyperglycemia-induced inflammatory response in adipocytes: The role of reactive oxygen species. J Biol Chem 2005;280: 4617–4626.
49. Lubart R, Friedman H, Levinshal T, Lavie R, Breitbart H. Effect of light on calcium transport in bull sperm cells. J. Photochem Photobiol 1992;15:337–341.
50. Tong M, Liu YF, Zhao XN, Yan CZ, Hu ZR, Zhang ZH. Effects of different wavelengths of low level laser irradiation on murine immunological activity and intracellular Ca2+ in human lymphocytes and cultured cortical neurogliocytes. Lasers Med Sci 2000;15:201–206.
51. Gordon SA, Surrey K. Red and far-red action on oxidative phosphorylation. Radiat Res 1960;12:325–339.
52. Passarella S, Casamassima E, Molinari S, Pastore D, Quagliariello E, Catalano IM, Cingolani A. Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium–neon laser. FEBS Lett 1984;175:95–99.
53. Geiger PG, Korytowski W, Lin F, Girotti AW. Lipid peroxidation in photodynamically stressed mammalian cells: Use of cholesterol hydroperoxides as mechanistic reporter. Free Radic Biol Med 1997;23(1):57–68.
54. Klebanov GI, Chichuk TV, Osipov AN, Vladimirov YA. The role of lipid peroxidation products in the effect of He-Ne laser on human blood leukocytes. Biofizika 2005;50:862–866.
55. Vladimirov IuA, Klebanov GI, Borisenko GG, Osipov AN. Molecular and cellular mechanisms of the low intensity laser radiation effect. Biofizika 2004;49(2):339–350.
56. Geiger PG, Korytowski W, Girotti AW. Photodynamically generated 3-beta-hydroxy-5 alpha-cholest-6-ene-5-hydroperoxide: Toxic reactivity in membranes and susceptibility to enzymatic detoxification. Photochem Photobiol 1995;62:580–587.
57. Gius D, Botero A, Shah A, Curry HA. Intracellular oxidation/reduction status in the regulation of transcription factors NF-κB and AP-1. Toxicol Lett 1999;106:93–106.
58. Sun Y, Oberley LW. Redox regulation of transcriptional activators. Free Radic Biol Med 1996;21:335–348.
59. Calkhoven CF, Ab G. Multiple steps in the regulation of transcription factor level and activity. Biochem J 1996;317: 329–342.
60. Haddad JJ. Oxygen-sensing mechanisms and the regulation of redox-responsive transcription factors in development and pathophysiology. Respir Res 2002;3:26–53.

61. Zhang Q, Piston DW, Goodman RH. Regulation of corepressor function by nuclear NADH. Science 2002;295:1895–1897.
62. Karu TI, Kalendo GS, Letokohov VS, Lobko VV. Dependence of biological action of low-intensity visible light upon HeLa cells on irradiation parameters: Coherence, dose, wavelength, and irradiation mode. Sov J Quantum Electron 1982;12:1134–1138.
63. Sazonov AM, Romanov GA, Portnoi LM, Odinokova VA, Karu TI, Lobko VV, Letokhov VS. Low intensity noncoherent red light in the complex treatment of peptic ulcers. Sov Med 1985;12:42–45.
64. Karu TI, Kalendo GS, Letokhov VS, Lobko VV. Biostimulation of HeLa cells by low intensity visible light. Stimulation of DNA and RNA synthesis in a wide spectral range. Nuovo Cimento 1984;3:309–318.