

ORIGINAL SCIENTIFIC PRESENTATION

Reduction in Cholesterol and Triglyceride Serum Levels Following Low-Level Laser Irradiation: A Noncontrolled, Nonrandomized Pilot Study

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Introduction: In the United States, millions of people older than 20 years have cholesterol serum levels greater than 200 mg/dL. To reduce the risk of coronary heart disease that is associated with high cholesterol serum levels, statins have been prescribed to inhibit the enzyme responsible for cholesterol synthesis. It has been proposed that Low-level laser therapy (LLLT) may suppress cholesterogenesis and thereby reduce cholesterol and triglyceride serum levels by altering gene expression and inducing cellular modifications. In this article we present a nonrandomized, noncontrolled pilot study to assess the efficacy of laser therapy in the noninvasive reduction of cholesterol and triglyceride serum levels.

Materials and Methods: Nineteen individuals were enrolled in the study. Participants were treated with a 5 independent diode laser scanner device emitting 635 nm (red) laser light. Standard blood draw was performed prior to the laser administration. A standard lipid panel was studied before the procedure to establish a baseline and at the end of the second procedure week. The cholesterol and triglyceride levels before and after the LLLT were compared.

Results: Eighty-four percent of 16 study participants demonstrated an overall reduction in total cholesterol serum levels when comparing baseline and study endpoint levels. Fifteen percent of study participants revealed an increase in overall cholesterol levels after 2 weeks.

Discussion: Although a significant majority of participants revealed a reduction in total cholesterol levels, several participants did reveal an increase that could be the result of several factors. The biosynthesis of cholesterol is strongly controlled by transcription factors, and a laser-induced

alteration of these regulatory transcription factors may play a vital role in the suppression of cholesterogenesis. The potential application of LLLT for cholesterol reduction merits a proper study to accurately analyze the therapeutic utility.

Nonfamilial hypercholesterolemia is defined by the American Heart Association as a serum cholesterol concentration exceeding 200 mg/dL.¹ Nonfamilial hypercholesterolemia is the most common form of elevated serum cholesterol concentrations. The primary etiology for an overwhelming majority of patients is caused by an unknown genotype and further provoked by the excessive intake of saturated fat, trans-fatty acids, and cholesterol. Lipoprotein subpopulations including low-density lipoproteins (LDL), very low-density lipoproteins, triglycerides, and high-density lipoproteins (HDL) are evaluated to assess patient risk.

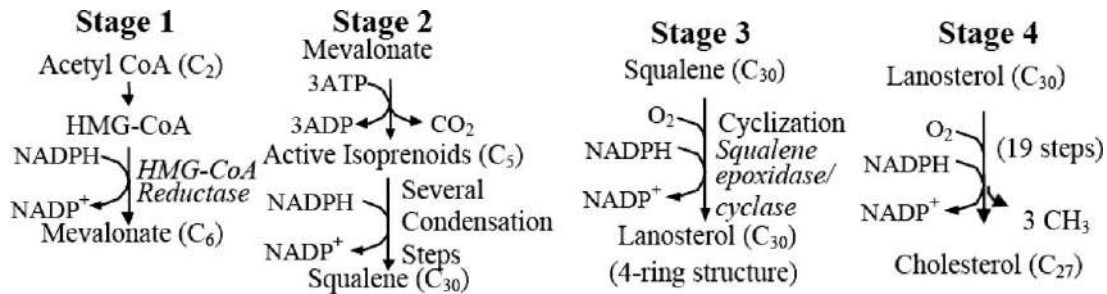
In the United States, 106.7 million people older than 20 years have cholesterol serum levels greater than 200 mg/dL, with 37.2 million people having levels exceeding 240 mg/dL.¹ Several epidemiological studies have demonstrated a relationship between increased serum cholesterol concentrations and coronary heart disease events and coronary heart disease mortality rates.¹ Moreover, elevated cholesterol levels have been linked to comorbidities such as atherosclerosis, stroke, and myocardial infarction.¹

The treatment regimen for patients with hypercholesterolemia is based on LDL, HDL, and triglyceride levels as modified by risk factors and history of previous coronary heart disease or risk equivalents including cigarette smoking, hypertension, age, and previous myocardial infarction or stroke. Current medical treatment involves pharmacologic therapy in addition to lifestyle modifications. With most

Received for publication April 3, 2009.

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Courtesy of University of Arizona, School of Medicine (2008).

Figure 1. The 4 rate-limiting steps of cholesterol synthesis.

cholesterol within the body arising via biosynthesis, clinicians aim to reduce cholesterol levels by prescribing hydroxyl methylglutaryl-CoA (HMG-CoA) reductase inhibitors, also referred to as statins, to inhibit HMG-CoA reductase, the enzyme responsible for cholesterol synthesis regulation. The formation of mevalonate from HMG-CoA is a rate-limiting and regulated step in the biosynthesis of cholesterol (Figure 1).

All statins are competitive inhibitors of HMG-CoA reductase, therefore effectively inhibiting the enzyme by blocking the activation site. Statins decrease the synthesis of cholesterol by the liver and other tissues. This action in the liver decreases the availability of cholesterol for the synthesis of very low-density lipoprotein particles that eventually become LDL particles. A multitude of studies have demonstrated the overall effectiveness of statins to lower cholesterol levels and reduce the risk of coronary heart disease and other comorbidities associated with hypercholesterolemia.²⁻¹⁰ Statins have become one of the most widely used classes of medications in the United States. Although serious side effects are rare, it is common to experience a less serious event.¹¹⁻¹³ Approximately 5% of patients will report experiencing some muscle pain.¹⁴ The American Heart Association defines 3 types of muscle disorders associated with statins: myalgia-muscle pain or weakness, myositis-pain or weakness, and rhabdomyolysis with creatine kinase elevation.¹⁴ In clinical trials, transaminase levels were elevated in patients taking statins; however, serious liver toxicity is rare and remains a controversial topic among medical professionals.^{15,16} Although the more serious side effects are rare, the risk of mild and even severe events can occur when taking statins.

In recent years, an innovative technology using low-level laser light has garnered an exceptional level of interest across myriad medical disciplines because

of its unique ability to modulate cellular metabolism, therefore inducing beneficial clinical effects. Low-level laser therapy (LLLT) has been found to alter gene expression,¹⁷ cellular proliferation,¹⁸⁻²² intracellular pH balance,²³ mitochondrial membrane potential,²⁴ generation of transient reactive oxygen species²⁵⁻²⁸ and calcium ion level,^{25,29,30} proton gradient,³¹ and cellular oxygen consumption.³² With laser light able to induce cellular modulation, it has been proposed that LLLT may be able to serve as a subtle, noninvasive instrument in the reduction of serum cholesterol levels. It is proposed that laser therapy may suppress cholesterologenesis by altering the transcription factors responsible for the expression of essential genes involved in the biosynthetic process. A nonrandomized, noncontrolled pilot study was performed to assess the efficacy of laser therapy in the noninvasive reduction of cholesterol and triglyceride serum levels.

Materials

Nineteen individuals were enrolled in this non-blinded, nonrandomized study; all 19 participants qualified and were enrolled. All participants deemed eligible for participation in this clinical study satisfied each of the following inclusion criteria: participant indicated for liposuction or use of liposuction techniques specifically for the indication of body contouring in the areas 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 the study; willing and able to maintain a regular diet and exercise regimen without effecting significant change in either direction during study participation; and between the ages of 18 and 65 years.

Participants had none of the following exclusive conditions: body mass index of 30 kg/m² or greater;

diabetes dependent on insulin or oral hypoglycemic medication; known cardiovascular disease such as cardiac arrhythmias and congestive heart failure; cardiac surgeries such as cardiac bypass, heart transplant surgery, or pacemakers; excessive alcohol consumption (more than 21 alcoholic drinks per week); prior surgical intervention for body sculpting/weight loss, such as liposuction, abdominoplasty, stomach stapling, lap band surgery, and so forth; 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 was 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 the 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 participants were recruited from the assessment investigator's normal pool of patients who came to their clinics for evaluation for liposuction, signed the informed consent form, and satisfied all of the study eligibility criteria. Participants 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.

Intervention

Participants assigned to the test group were treated with a 5 independent diode laser scanner device, emitting 635 nm (red) laser light, with each diode generating 17 mW output (Erchonia Zeron, Erchonia Medical Inc, McKinney, Tex).

Study Design

Standard blood draw was performed prior to the laser administrative phase. A standard lipid panel was studied to establish a baseline. Lipid panels were

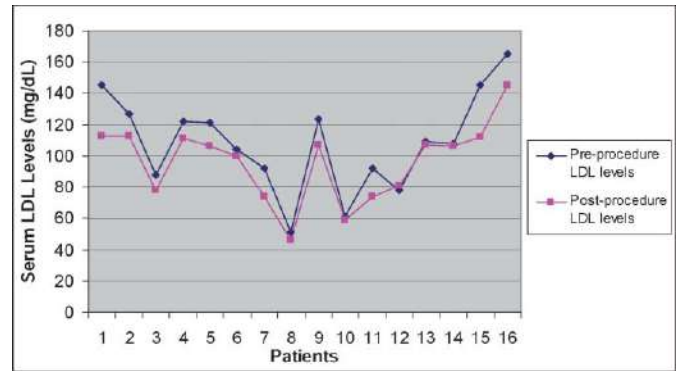


Figure 2. Low-density lipoprotein levels for participants demonstrating a reduction in overall cholesterol levels (n = 16).

Table 1. Preprocedure and Postprocedure Cholesterol Serum Levels for All Participants (n = 19)

| Participant (n = 19) | Preprocedure Cholesterol Level (mg/dL) | Postprocedure Cholesterol Level (mg/dL) | Change in Cholesterol Levels (mg/dL) |
|----------------------|--|---|--------------------------------------|
| 1 | 214 | 187 | -27 |
| 2 | 229 | 198 | -31 |
| 3 | 191 | 169 | -22 |
| 4 | 235 | 212 | -23 |
| 5 | 190 | 165 | -25 |
| 6 | 165 | 164 | -1 |
| 7 | 188 | 164 | -24 |
| 8 | 117 | 112 | -5 |
| 9 | 209 | 179 | -30 |
| 10 | 173 | 179 | +6 |
| 11 | 144 | 154 | +10 |
| 12 | 165 | 196 | +31 |
| 13 | 137 | 125 | -12 |
| 14 | 188 | 164 | -24 |
| 15 | 284 | 269 | -15 |
| 16 | 201 | 200 | -1 |
| 17 | 177 | 175 | -2 |
| 18 | 214 | 188 | -26 |
| 19 | 261 | 229 | -32 |

performed at 2 different time points: preprocedure and at the end of the second procedure week.

The procedure administration phase of the study commenced immediately following the preprocedure blood draw. The procedure administration phase extended over 2 consecutive weeks, with each participant receiving 6 total procedure administrations with the laser scanner across the consecutive 2 weeks (3 procedures per week, each one 2 days apart). Each procedure took place at the investigators' test sites.

The procedure administration protocol required that participants enter the procedure room and lie

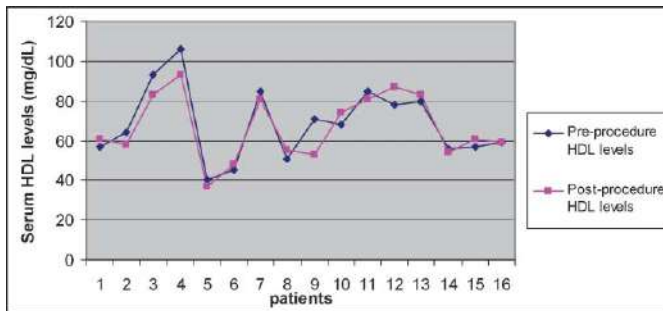


Figure 3. High-density lipoprotein levels for participants demonstrating a reduction in cholesterol serum levels ($n = 15$).

Table 2. Mean and Standard Deviation of Total Cholesterol for Participants at Baseline and Study End

| Total Cholesterol ($n = 19$) | Baseline | Study End |
|--------------------------------|----------|-----------|
| Average | 191.11 | 178.79 |
| SD | 43.34 | 36.46 |

Table 3. Mean and Standard Deviation of Low-Density Lipoprotein (LDL) for Participants at Baseline and Study End

| LDL ($n = 19$) | Baseline | Study End |
|------------------|----------|-----------|
| Average | 103.68 | 96.53 |
| SD | 31.53 | 25.69 |

Table 4. Mean and Standard Deviation of High-Density Lipoprotein (HDL) for Participants at Baseline and Study End

| HDL ($n = 19$) | Baseline | Study End |
|------------------|----------|-----------|
| Average | 65.53 | 64.63 |
| SD | 18.07 | 16.52 |

comfortably flat on their back. Participants were fitted with blindfolds. The center diode of the laser scanner device was positioned at a distance of 6.00 inches above the participant's abdomen, centered along the body's midline, and focused on the navel. The 4 remaining diodes were positioned 120° apart and tilted 30° off the centerline of the center diode. The scanner device was activated for 20 minutes.

Following anterior stimulation, the participant was advised to then lie flat on his or her stomach. The center diode of the laser scanner was positioned at a distance of 6.00 inches above the participant's back, centered along the body's midline, and focused on the equivalent spot to the navel's location on the stomach.

The 4 remaining diodes were positioned 120° apart and tilted 30° off the centerline of the center diode. The scanner device was activated for 20 minutes. The total laser energy that the test group participants received, front and back treatments combined, was approximately 6.60 J/cm².

The primary efficacy outcome measure was defined as demonstrated reduction in cholesterol serum levels. The purpose of this nonrandomized, noncontrolled study was to demonstrate a change in cholesterol serum levels from baseline to after completion of the 2-week procedure administration phase (end of week 2).

Results

Eighty-four percent or 16 study participants demonstrated an overall reduction in total cholesterol serum levels when comparing baseline and study endpoint levels (Figure 2) (Table 1).

For participants demonstrating a reduction in total cholesterol levels, an average reduction of -18.8 points with a range of -1.0 to -32.0 mg/dL was recorded. Moreover, 7 participants demonstrated a preprocedure cholesterol level greater than 200 mg/dL, and of those 7 participants, 57.1% demonstrated a reduction significant enough to lower the cholesterol level to an acceptable range below 200 mg/dL.

Fifteen percent of study participants revealed an increase in overall cholesterol levels after 2 weeks. The average increase of those participants was 15.6 points.

For all participants, the average baseline cholesterol measurements when compared with the study endpoint draw revealed a significant decrease of -12.32 points, represented by $P < .01$ (Table 2).

Evaluation of LDL measurements when comparing the mean change from baseline to study endpoint for all participants produced a reduction of -7.15 points, which was detected as a significant change ($P < 0.05$; Table 3). Seventy-three percent of participants demonstrated an LDL point reduction, with a range of -2.0 to -33.0 points.

The HDL levels for all participants revealed a statistically insignificant mean change of -0.895 between study baseline and endpoint ($P > 0.05$; Table 4). Of the total participants, 42.1% demonstrated an overall reduction in HDL levels, with a range of -2.0 to -18.0 points (Figure 3).

When comparing the baseline ratio of LDL to HDL concentrations to the study endpoint ratio, a favorable improvement was revealed, with LDL levels decreasing

Table 5. Preprocedure and Postprocedure Triglyceride Serum Levels for All Participants ($n = 19$)

| Patient | Preprocedure Triglyceride Level (mg/dL) | Postprocedure Triglyceride Level (mg/dL) | Change in Levels (mg/dL) |
|---------|---|--|--------------------------|
| 1 | 62 | 68 | +6 |
| 2 | 191 | 133 | -58 |
| 3 | 51 | 44 | -7 |
| 4 | 33 | 44 | +11 |
| 5 | 146 | 116 | -30 |
| 6 | 81 | 80 | -1 |
| 7 | 57 | 45 | -12 |
| 8 | 61 | 57 | -4 |
| 9 | 77 | 93 | +16 |
| 10 | 106 | 49 | -57 |
| 11 | 54 | 74 | +20 |
| 12 | 67 | 50 | -17 |
| 13 | 39 | 40 | +1 |
| 14 | 57 | 45 | -12 |
| 15 | 106 | 69 | -37 |
| 16 | 48 | 52 | +4 |
| 17 | 67 | 82 | +15 |
| 18 | 62 | 58 | -4 |
| 19 | 180 | 105 | -75 |

by -7.15 points and HDL numbers reducing by just -0.895 points; however, the improvement in the LDL to HDL ratio was not significant ($P > 0.05$).

Of the 19 participants, 63.1% or 12 subjects revealed a reduction in serum triglyceride levels from baseline to study endpoint (Table 5).

Of all participants, none demonstrated an increase in triglyceride levels that positioned the subject into an unsafe range exceeding 150 mg/dL. The mean change of triglyceride levels comparing baseline with the study endpoint produced a significant reduction of -11.68 points ($P < 0.05$; Table 6).

Three patients were identified at preprocedure lipid assays as having high triglyceride levels (146.0 mg/dL, 180.0 mg/dL, and 191.0 mg/dL). Following laser irradiation, the levels were decreased by more than -30.0 points, reducing the triglyceride levels to acceptable parameters.

Table 6. Mean and Standard Deviation of Triglycerides for Participants at Baseline and Study End

| Triglycerides ($n = 19$) | Baseline | Study End |
|----------------------------|----------|-----------|
| Average | 80.37 | 68.68 |
| SD | 45.96 | 26.88 |

Discussion

These data reveal a significant reduction in cholesterol and triglyceride levels following the administration of laser therapy with well-defined parameters regarding wavelength, intensity, and frequency. Although a statistically significant majority revealed a reduction in total cholesterol and triglyceride levels, several participants did reveal an increase that could perhaps be attributed to the procedure itself, reveal a variability in cholesterol levels, or perhaps indicate a drastic modification in an individual's dietary or lifestyle habits. In any case, this early work provides an interesting perspective regarding the potential application for this modality and merits the completion of a proper study to accurately analyze the therapeutic utility.

Although no histological work outlines the specific mechanism of action that contributes to the reduction of cholesterol levels or inhibition of cholesterol synthesis, studies have revealed the modulatory capacity of laser therapy on transcription factors and gene expression.³³ Jackson and coworkers³⁴ have identified more than 20 transcription factors that are regulated by the intracellular redox state. It is proposed that laser therapy, by means of altering the intracellular redox state, could affect the function of transcription factors tightly associated with cholesterol synthesis. In this nonrandomized, noncontrolled study, it was observed that LLLT 3 times per week for 2 weeks can reduce cholesterol levels—more importantly, reduce LDL levels while preserving HDL levels.

The first law of photochemistry states that the observable biological effects following LLLT can transpire only in the presence of a photoacceptor molecule, a molecule capable of absorbing the photonic energy being emitted.³⁵ Studies have revealed that cytochrome C oxidase serves as a photoacceptor molecule. Cytochrome C oxidase is a multicomponent membrane protein that contains a binuclear copper center (Cu_A) along with a heme binuclear center ($a_3\text{-Cu}_B$), both of which facilitate the transfer of electrons from water-soluble cytochrome c oxidase to oxygen.³⁶⁻³⁹ Cytochrome C oxidase is a terminal enzyme of the electron transport chain and plays a vital role in the bioenergetics of a cell. Studies indicate that following laser irradiation at 633 nm, the mitochondrial membrane potential and proton gradient increases, causing changes in mitochondria optical properties and increasing the rate of ADP-ATP exchange.⁴⁰ It is suggested that laser irradiation increases the rate at which cytochrome C oxidase transfers electrons from cytochrome

C to dioxygen.^{41,42} 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.^{43,44} The photoactivation of terminal enzymes may be the primary response that induces the suppression of cholesterologenesis.

The initial physical and/or chemical changes of cytochrome C oxidase have been shown to alter the intracellular redox state.⁴⁵ It has been proposed that the redox state of a cell regulates cellular signaling pathways that control gene expression.⁴⁶⁻⁴⁸ Modulation of the cellular redox state can activate or inhibit signaling pathways such as redox-sensitive transcription factors and/or phospholipase A₂.⁴⁹⁻⁵² Two well-defined transcription factors, nuclear factor Kappa B and activator protein-1, are regulated by the intracellular redox state; moreover, nuclear factor Kappa B and activator protein-1 become activated following an intracellular redox shift to a more alkalized state.^{51,52} Subsequent to laser irradiation, a gradual shift toward a more oxidized (alkalized) state has been observed; more importantly, the activation of redox-sensitive transcription factors and subsequent gene expression has been demonstrated.^{46,53,54}

The induction of cholesterologenesis is a complex and tightly regulated process that is strongly dependent on the activities of multiple transcription factors.⁵⁵⁻⁵⁷ The HMG-CoA reductase gene contains a regulator DNA sequence in its promoter region that requires the binding of the sterol regulator element binding protein (SREBP) in order to be actively transcribed. SREBPs are transcription factors of the helix-loop-helix family that undergo a maturation process regulated by cholesterol present in cell membranes.⁵⁸ Maturation of these proteins requires the activation of a chaperone protein, SREBP cleavage-activating protein.⁵⁹ Under sterol-depleted conditions, SREBP cleavage-activating protein escorts SREBPs from endoplasmic reticulum to Golgi for proteolytic processing, enabling SREBPs to stimulate cholesterol synthesis.⁵⁹ The mature forms of SREBPs bind to the sterol response element of targeted genes, enabling the expression and transcription of those genes. It has been proposed that different cofactors may assist SREBPs in identifying the preferential target genes. The SREBP maturation process serves an important role regarding cholesterol homeostasis.

The biosynthetic process of cholesterol synthesis is strongly controlled by transcription factors, and a laser-induced alteration of these regulatory transcription factors via modulation of the intracellular redox

state may play a vital role in the suppression of cholesterologenesis.

Acknowledgments

Dr Robert Jackson, Dr Kevin Wisler, and Dr Gregory Roche do not possess any financial interest with the device manufacturer. All data were collected and assessed by a third-party agency, Regulatory Insight Inc, who had no contact with the participants or investigators during the treatment phase. The authors would like to thank Ryan Maloney, medical director of Erchonia Corporation, for elucidating the complexities of photochemistry and how it perhaps contributes to the outcome recorded.

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