Introduction:

The prevalence of obesity continues to increase in the United States, now affecting the lives of more than half of its citizens. [1] In adults, obesity is defined using the body mass index (BMI), which is the ratio of weight in kilograms divided by the height in meters squared. [2] Overweight is defined as a BMI between 25.0 and 29.9 kg/m^2, and obesity is defined as a BMI higher than 30.0 kg/m^2. [3] Sixty-three percent of men and 55% of women in the United States have a body mass index of 25 kg/m^2 or greater. Moreover, the Center for Disease Control (CDC) reports obese individuals are at increased risk for numerous diseases and medical conditions, including the following: hypertension, osteoarthritis, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, gall bladder disease, and even some cancers. [4] A recent study conducted by Adams and colleagues (2006) examined the relationship between mortality and weight and revealed within a cohort of more than 500,000 Americans that mortality in both men and women who were overweight increased by 20-40%. The occurrence of obesity related comorbidities has sparked a concerted effort to prevent and treat obesity rather than its associated comorbidities.

Recent studies have made tremendous progress understanding the physiologic regulation of body weight and the genes that are associated with weight gain. However, the highly complex nature of the multiple organ systems that conspire to regulate weight gain presents an overwhelming task to develop future pharmacotherapies. The wide assortment of treatment options for obese patients has always and continues to be a byzantine issue. The moderate weight loss associated with a range of dietary approaches and the rigors to sustain high levels of physical activity to simply maintain are often what compel patients to endure highly invasive surgical treatment. Although recent articles suggest weight loss surgery can provide significant reductions in mortality due to the decrease of comorbidities such as cardiovascular disease and some cancers, the risk of serious and life-threatening side-effects is a harsh and difficult reality.

In recent years, photobiomodulation has garnered an extraordinary level of interest across a myriad of medical disciplines. This innovative technology employs low-level laser light and serves as a subtle instrument that can modulate cellular metabolism inducing beneficial clinical effects. The applications for phototherapy across areas of medicine and biotechnology are immense and have been well published.

Studies conducted by Neira and coworkers (2000) demonstrated that cold laser at 635nm emulsified isolated adipose panicles. Employing scanning and transmission electron microscopy (SEM and TEM), Dr. Neira revealed a significant collapse of round-shaped fat-filled adipocytes in panicle arrangements following 6 minutes of laser irradiation. Dr. Neira discussed the adipocyte collapse as a consequence to the disruption of the phospholipid membrane induced by laser irradiation. The aperture or transitory pore formed within the bilipid membrane enabled the fatty contents housed within the adipocytes to seep across the membrane and into extracellular space, resulting in the deflation of the adipose panicles.

Later work published by Dr. Solarte (2002) studying the visible light transmission spectra for different dissolution concentrations of adipocytes, observed changes in the

optical transmittance of irradiated samples-denoting that these observations supported the idea that morphological changes of adipocytes are induced by irradiation.

Dr. Jackson and co-workers in (2002) applied low-level laser therapy as an adjuvant instrument for lipoplasty, externally applying laser irradiation several minutes prior to the aspiration phase. Jackson and colleagues discussed the greater volume of fat able to be extracted following the application LLL.

Although several studies have since been published highlighting laser therapy as an adjunctive tool in liposuction, the purpose of this institutional review board study was to evaluate the clinical effectiveness low-level laser therapy could provide as sole instrument in reducing the volume of subcutaneous adipose tissue; perhaps, demonstrating the potential for laser light to serve as a safe and non-invasive alternative to treating obese patients.

Methods:

Seventy-seven participants were randomly subdivided into two groups. Sixty-seven of the qualified and enrolled subjects participated in the initial pre-treatment phase and participated to at least the study endpoint. Of the 67 participating subjects, 35 patients were assigned to the active treatment group and 32 were randomized to the placebo group. The study was designed to have a minimum of 32 subjects completing participation to study endpoint in each group. All subjects who were qualified as eligible for participation in this clinical study satisfied the necessary inclusion and exclusion criteria.

All subjects who qualified as eligible for participation in this clinical study satisfied each of the following inclusion criteria: subject 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 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 to 65 years.

Subjects had none of the following exclusive conditions: BMI of 30kg/m^2 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, and 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, etc; medical, physical, or other contraindications for body sculpting/weight loss; current use of medications know 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 two 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 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. Subjects were not offered any form of compensation to participate in the clinical trial, nor were they charged for the cost the laser procedure or related evaluations.

The clinical study was a double-blind design, such that neither the subject nor the investigator were aware of to which group an individual subject was assigned to until after the clinical study was complete. Subjects assigned to the test group were treated with a 5 diode laser scanner device, emitting 635nm red laser light with each diode generating 17mW output (The Erchonia® LipoLaser, manufactured by Erchonia Medical Inc.). Placebo group participants were treated with a 5 light emitting diode (LED) scanner device, emitting 635nm red light with each diode generating 2.5mW power. Both the placebo and real laser devices were designed to have the same physical appearances, including the appearance of any visible light output.

The circumference in inches (ins.) for the subject's waist, hips, 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 circumference measurement was distance in inches from 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, it was imperative that 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 any 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 time points: pre-procedure; end of first procedure week; end of second procedure week; and two-weeks post-procedure.

The procedure administration phase of the study commenced the same day following immediately following the pre-procedure circumference measurements. The procedure administration phase extended over two consecutive weeks, with each subject receiving six total procedure administrations with the laser scanner across the consecutive two-procedure administration phase; three procedures per week, each one at least two days but no more than three days apart. Each procedure took place at the investigators test site.

The procedure administration protocol required that subjects enter the procedure room and lie comfortably flat on their back. Subjects were fitted with blindfolds. The center diode of the laser scanner device was positioned at a distance of 6 inches above the participant's abdomen, centered along the body's midline and focused on the navel. The four remaining diodes were positioned 120 degrees apart and tilted 30 degrees 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 their stomach. The center diode of the laser scanner was positioned at a distance of 6.00 inches 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 degrees apart and tilted 30 degrees 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/cm^2.

Eight of the 67 subjects did not have circumference measurements recorded at the two-weeks post-procedure measurement point: 4 subjects who had been randomized to the test group and 4 subjects who had been randomized to the placebo group. For these 8 subjects, the last observation carried forward (LOCF) procedure was employed, such that the subject's week 2 circumference measurement was carried forward as the week 2 post-procedure measurement.

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Results:

Table 1: Individual success criteria met by both the test and placebo groups.

	Test subjects	Placebo subjects
n	35	32
n meeting success criteria	22	2
% meeting success criteria	**62.86%**	**6.25%**

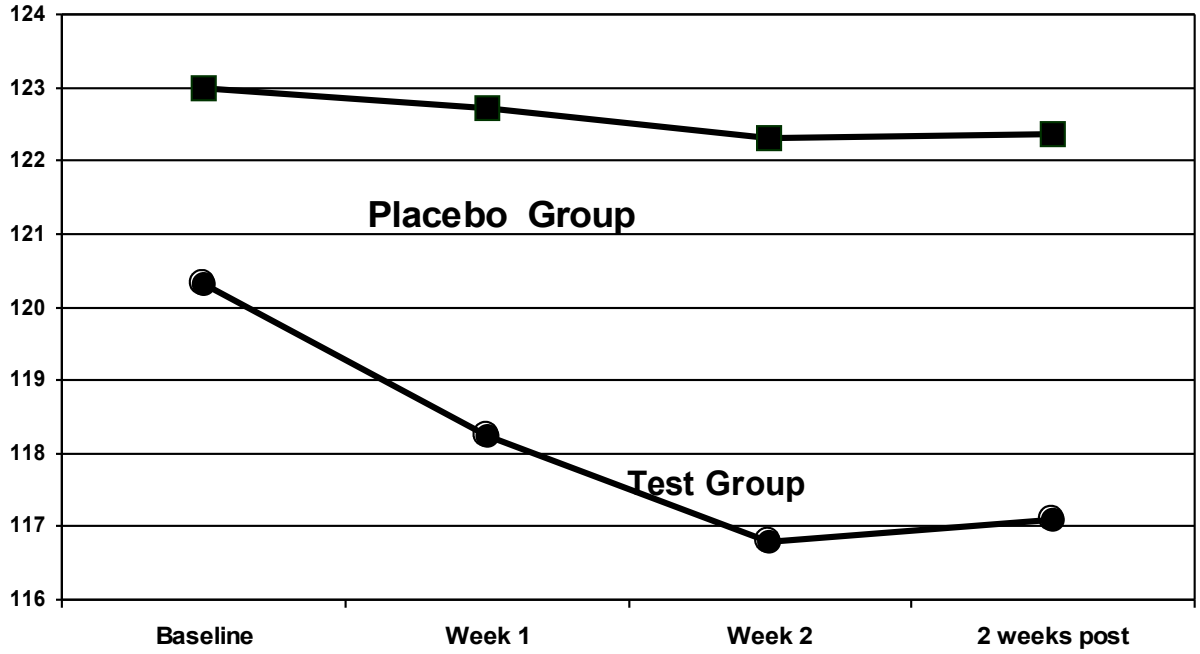
Fifty-seven percent more test group participants than placebo group participants showed a total decrease in combined circumference measurements from pre-procedure to study endpoint of 3 inches or greater, exceeding the pre-established target of 35% difference between treatment groups by more than 30%. The difference was found to be statistically significant at $p<0.00001$.

Table 2: Comparison between test and placebo subjects regarding overall inches lost.

	Test subjects (n=35)	Placebo subjects (n=32)
Mean	-3.521	-0.684
SD	1.854	1.233

Table 2 shows the mean and standard deviation of the magnitude of the change in combined circumference measurements from pre-procedure to study endpoint comparing both the test and placebo subjects. The test group participants lost on average -2.837 inches more than the placebo group participants. The difference was found to be statistically significant at $p<0.0001$.

Figure 1: Total circumference measurements across study duration between test and placebo groups for entire study population



Compared with the baseline, the total circumference measurements were statistically lower at all three subsequent evaluation points: week 1, week 2 and 2 weeks post procedure. The test group participants from baseline to 2 week post-procedure saw an overall decrease in total circumference measurements of -3.22 inches, statistically significant P<0.0001. Placebo group subjects from baseline to 2 week post-procedure produces an overall decrease in total circumference measurements of -0.62 inches (P<0.71745).

Table 3: Mean and standard deviation of the change in total circumference measurements between evaluation periods for test and placebo groups

	Test Group			Placebo Group		
	n	**Mean**	**St. Dev.**	**n**	**Mean**	**St. Dev.**
Baseline – week 1	35	-2.06	1.94	32	-0.27	1.89
Baseline -week 2	35	-3.52	1.85	32	-0.68	1.23
Baseline – 2 weeks post	35	-3.21	1.87	32	-0.62	1.21
Week 1 – week 2	35	-1.46	1.70	32	-0.42	2.20
Week 1 – 2 weeks post	35	-1.15	2.86	32	-0.36	1.95
Week 2 – week 4	35	0.31	2.10	32	0.06	1.05

Table 3 shows the change in total circumference measurements between evaluation points across test and placebo groups. The differences between placebo and test groups at each evaluation point regarding the change in inches demonstrated statically significant differences. Test group participants demonstrated a change of -3.52 inches from baseline to week 2, a difference of 2.84 inches when compared with the placebo group, a statistically significant difference of P<0.0001.

Table 4 below reveals the statistically significant difference between test and placebo groups between the following evaluation periods.

Table 4: Statistical significance between placebo and test groups between the evaluation points

Evaluation Points	**P value**
Baseline to week 1	P<0.0005
Baseline to week 2	P<0.0001
Baseline to 2 weeks post-procedure	P<0.0001

Table 5: Individual treatment area circumference measurements across study duration by test and placebo groups

	Test Group			**Placebo Group**		
Waist	**n**	**Mean**	**St. Dev.**	**n**	**Mean**	**St. Dev.**
Baseline	35	33.94	3.63	32	34.85	3.83
Week 1	35	33.38	3.39	32	34.85	3.76
Week 2	35	32.96	3.51	32	34.60	3.93
2 weeks post	35	32.86	3.65	32	34.53	3.81
Hip	**n**	**Mean**	**St. Dev.**	**n**	**Mean**	**St. Dev.**
Baseline	35	38.99	2.87	32	39.88	3.77
Week 1	35	38.26	3.71	32	39.80	3.57
Week 2	35	37.94	3.60	32	39.67	3.73
2 weeks post	35	38.29	2.83	32	39.66	3.66
Right thigh	**n**	**Mean**	**St. Dev.**	**n**	**Mean**	**St. Dev.**
Baseline	35	23.80	1.52	32	24.12	2.04
Week 1	35	23.31	1.41	32	24.10	2.09
Week 2	35	22.95	1.40	32	24.07	2.10
2 weeks post	35	23.02	1.49	32	24.16	2.05
Left thigh	**n**	**Mean**	**St. Dev.**	**n**	**Mean**	**St. Dev.**
Baseline	35	23.59	1.40	32	24.14	1.95
Week 1	35	23.30	1.34	32	23.98	2.02
Week 2	35	22.94	1.27	32	23.97	2.11
2 weeks post	35	22.92	1.28	32	24.02	1.96

Table 5 shows the mean and standard deviation circumference measurements for each of the following individual treatment areas, by group, at each of the four time points. Test group participants demonstrated a change (decrease) in circumference measurements for the waist ($p<0.0001$). Compared with baseline, circumference measurements for the waist were statistically significantly lower at all three subsequent evaluation points: week 1, week 2 and 2 weeks post-procedure.

A significant change (decrease) in circumference measurements for the hip was detected within the test group participants, $p<0.001$. Compared with baseline, circumference measurements for the hips were statistically significant lower at three subsequent evaluation points: week 1 ($p<0.01$), week 2 ($p<0.01$) and 2 weeks post-procedure ($p<0.01$).

Subjects assigned to the test group demonstrated a significant change (decrease) in circumference measurements for the right thigh, $p<0.0001$. Compared with baseline, circumference measurements for the right thigh were statistically significantly lower at all three subsequent evaluation points: week 1 ($p<0.01$), week 2 ($p<0.01$) and 2 weeks post procedure ($p<0.01$).

Participants assigned to the test revealed a significant change (decrease) in circumference measurements for the left thigh, $p<0.0001$. Compared with baseline, circumference measurements for the left thigh were statistically significantly lower at all three subsequent evaluation points: week 1 ($p<0.05$), week 2 ($p<0.01$) and 2 weeks post-procedure ($p<0.01$).

Discussion:

The photobiostimulatory effects of low-level laser therapy on adipocyte cells are well documented. However, it is not yet fully understood why laser therapy promotes the formation of the transitory pore within the bilipid membrane of adipocytes. Laser therapy has been shown to preserve membrane and genetic material of cells that are nutritionally starved [3]; revitalize erythrocytes enhancing their oxyphoric function [4]; enhance fertilization potential of spermatozoa [5]; stimulate the differentiation of satellite stem cells [6-8] and reduce the extent of myocardial infarctions and ischemic strokes. [9] However, the ability to modulate cellular metabolism and provoke a diverse biological response is highly dependent on the intensity, wavelength, and frequency being emitted by the particular device. Moreover, the very same biological response induced by a specific wavelength can be further optimized or inhibited depending whether the radiation is pulsed or constant wave (CW).

In accordance to the first law of photochemistry, the observable biological affect following LLLT can only transpire in the presence of a photoacceptor molecule, a molecule capable of absorbing the photonic energy emitted. [1] Cytochrome c oxidase is a terminal enzyme that plays a crucial role in the bioenergetics of a cell. Cytochrome c oxidase is 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 oxidase to oxygen. [11-13 The] 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. Studies indicate that following laser irradiation at 633nm, the mitochondrial membrane potential and

proton gradient increases, causing changes in mitochondria optical properties increasing the rate of ADP/ATP exchange. [17] It is suggested that laser irradiation increases the rate at which cytochrome c oxidase transfers electrons from cytochrome c to dioxygen. [19, 20] 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. [21, 22]

Controversy exists regarding the photobiological similarities between a light emitting diode (LED) and a laser diode. Although the biological effects at superficial surfaces are identical, when attempting to target deep tissue however, such as subcutaneous fat, it is absolutely essential that a coherent laser diode is applied. [25-28]

LLLT has been shown to promote a transient shift in the intracellular redox potential in the way of greater oxidation. [1] Redox potential of a cell refers to the balance of stable and unstable reducing and oxidizing equivalents for three coenzymes; nicotinamide adenine dinucleotide (NAD^+), glutathione (GSH), and flavin adenine dinucleotide (FAD^+). [1, 10] Cellular signaling pathways that control gene expression are regulated by the intracellular redox state. [31, 32, 33] Furthermore, a shift towards a more oxidative state stimulates various cellular signaling systems. Modulation of the cellular redox state affects gene expression via certain mechanisms of cellular signaling including; redox-sensitive transcription factors and phospholipase A_2. [34] There are two well-defined transcription factors, nuclear factor Kappa B (NF-κB) and activator protein-1 (AP-1) which becomes activated following an intracellular redox shift to an oxidized state. [35, 36] Calkhoven and Ab (1996) demonstrated that reductants generally suppressed transcriptions, preventing the expression of genes. [37] LLLT provokes a shift of the intracellular redox state towards an oxidative one, activating redox-sensitive transcription factors such as NF-κB and AP-1 upregulating expression of genes. [38, 39]

It is unclear at this time whether the transitory pore induced by laser therapy is the direct result of upregulated gene expression or formed by the production of reactive oxygen species (ROS) or nitric oxide (NO), both of which become elevated following laser irradiation. The concerted effort to reduce the number of overweight and obese individuals in the United States must provide a way to safely and non-invasively reduce adipose tissue volume. LLLT has been proven to safely treat a myriad of neurologic, dental, ophthalimic, sports injury, and dermatologic disorders. [16-20] Although further studies analyzing low-level lasers efficacy at emulsifying adipose tissue-and the role both the lymphatic and circulatory systems play in the absorption of the fatty cellular debris created following LLLT are warranted, it was overwhelmingly demonstrated that LLLT can safely and effectively reduce adipose tissue volume for overweight and obese patients.