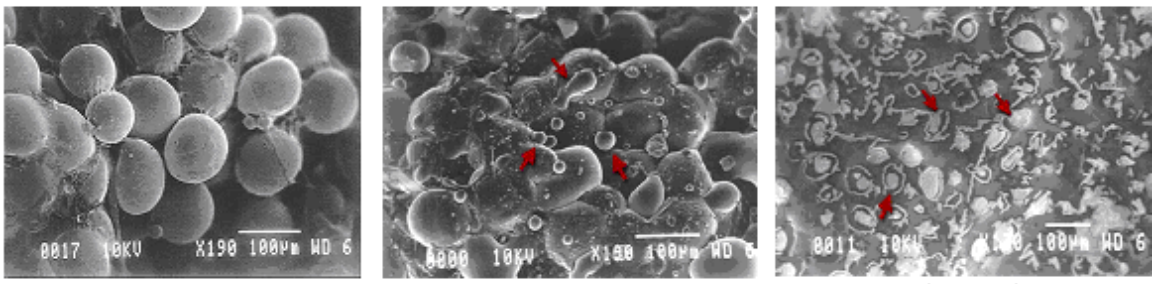
FDA STUDY FINDINGS

PRIMARY BIOCHEMICAL MECHANISM OF LOW-LEVEL-LASER THERAPY FOR THE NON-INVASIVE REDUCTION OF SUBCUTANEOUS ADIPOSE TISSUE.

Introduction:

Several published studies have revealed a unique biological effect in adipocytes following low-level laser stimulation. Dr. Rodrigo Neira and coworkers (2000) were able to demonstrate that low-level laser light at 635nm emulsified isolated adipose panicles.[1,2] Scanning and transmission electron microscopy (SEM and TEM) revealed the collapse of fat-filled adipocytes in panicle arrangements following 6 minutes of laser irradiation (Figure 1). [1, 2]

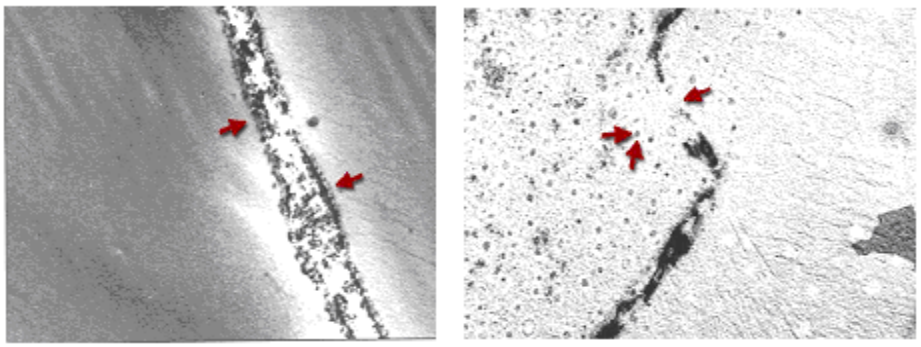
Figure 1: Emulsification of adipose panicles subsequent to laser irradiation



Untreated adipose panicle | Laser treated at 2 minutes | Laser treated at 6 minutes

Dr. Niera identified the formation of an aperture or transitory pore within the membrane of adipocytes following LLLT, and showed the movement of stored adipocyte contents across the membrane and into the extracellular space (figure 2). [3]

Figure 2: Formation of a transitory pore within the membrane of an adipocyte.



Untreated adipocyte | Laser treated adipocyte- aperature identified

Dr. Niera concluded that adipocyte collapse was the result of the disrupted adipocyte membrane induced by laser irradiation.[1-3] Work published by Dr. Solarte (2002) studied the visible light transmission spectra for different dissolution concentrations of adipocytes and observed changes in the optical transmittance of irradiated samples, and confirmed that morphological changes of adipocytes were the result of laser therapy. [4]

Dr. Jackson and co-workers (2004) applied low-level laser therapy as an adjuvant instrument for liposuction, externally administering laser irradiation several minutes prior to the aspiration phase.[5-7] Jackson and colleagues noted that for those patients receiving LLLT a greater volume of fat was able to be extracted and reduction in edema and pain was observed.[5-7]

Several studies have been published highlighting laser therapy as an adjunctive tool in liposuction; although, a placebo-controlled, randomized, double-blind clinical investigation revealed that low-level laser irradiation could serve as an independent, non-invasive instrument for the reduction of subcutaneous fat tissue.

The question that immediately arises is, "How can light, when externally applied, be capable of inducing such a phenomenal effect at the cellular level?" According to quantum theory, light radiation energy is absorbed as discrete units called photons, and at the molecular level, it is this photon-induced chemistry that ultimately gives rise to the observable effect at the biological level.[8] The first law of photochemistry states that the observable biological effects subsequent to LLLT can only transpire in the presence of a photoacceptor molecule, a molecule capable of absorbing the photonic energy being emitted.[8] A molecule capable of photonic absorption usually contains a light absorbing center referred to as a chromophore. Light absorbing centers often house transition metals, elements that are readily identified by their incomplete d subshell.[9] Based on physicist Niel-Bohrs model; subshells of an atom identify the possible quantum states in which an individual electron can reside depending on its energy level. [9] Electrons are capable of undergoing quantum leaps, where an electron transitions between quantum states, shifting from one energy level to another following the absorption or emission of a photon. [9] The shift from a lower energy state to a higher state is referred to as the excitation of an electron, the change from an occupied orbital to a given unoccupied orbital. Regarding transition elements,

such as copper (Cu) or iron (Fe), these elements are more susceptible to an electron shift because of their unique electron configuration. The photoacceptor molecules responsible for the photobiological effects subsequent to laser irradiation contain transition metals. The photon absorption is followed by a rapid vibrational relaxation which causes the molecule to reach an equilibrium geometric configuration corresponding to its electronic excited state.[8] This change may modulate the biological behavior of photo-absorbing molecules.

Studies have revealed that cytochrome c oxidase serves as a photoacceptor molecule. Cytochrome c oxidase is a multi-component 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. [10-13] 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 633nm, the mitochondrial membrane potential and proton gradient increases, causing changes in mitochondria optical properties increasing the rate of ADP/ATP exchange. [14] It is suggested that laser irradiation increases the rate at which cytochrome c oxidase transfers electrons from cytochrome c to dioxygen. [15,16] Moreover, it has been proposed that laser irradiation reduces (gain of electrons) the catalytic center of cytochrome c oxidase, making more electrons available for the reduction of dioxygen. [17, 18] The photo-activation of terminal enzymes, like cytochrome c oxidase, play a vital role in the activation of the diverse biological cascade observed subsequent to laser irradiation.

The peak absorption of cytochrome c oxidase is found in the red to near-infrared spectrum. [19-21] Therefore, optimal biological stimulation can be achieved utilizing a device that emits light within the red spectrum. Furthermore, to ensure proper depth penetration and deep tissue stimulation, the use of a coherent laser source is absolutely vital. [22-24] Biologically speaking, the difference between a light emitting diode (LED) and laser diode are negligible at extremely superficial surfaces; however, when attempting to target deep tissue such as subcutaneous adipocytes, it is essential that a coherent laser source is administered. [22-25]

The initial physical and/or chemical changes of cytochrome c oxidase have been shown to alter the intracellular redox state. [26] It has been proposed that the redox state of a cell regulates

cellular signaling pathways that control gene expression.[27-29] Modulation of the cellular redox state can activate or inhibit signaling pathways such as redox-sensitive transcription factors and/or phospholipase A_2.[30-33] Two well defined transcription factors, nuclear factor Kappa B (NF-κB) and activator protein-1 (AP-1), are regulated by the intracellular redox state; moreover, NF-κB and AP-1 become activated following an intracellular redox shift to a more alkalized state.[32,33] Subsequent to laser irradiation, a gradual shift towards a more oxidized (alkalized) state has been observed; more importantly, the activation of redox-sensitive transcription factors and subsequent gene expression has been demonstrated [27,34,35]

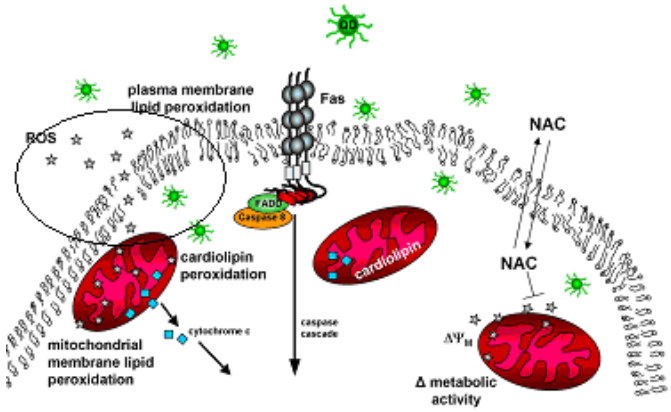
Based on its ability to modulate cellular metabolism and alter the transcription factors responsible for gene expression, low-level laser therapy (LLLT) has been found to alter gene expression, [36] cellular proliferation, [37-41] intra-cellular pH balance, [42] mitochondrial membrane potential, [43] generation of transient reactive oxygen species [44-47] and calcium ion level, [44, 48, 49] proton gradient [50] and consumption of oxygen [51]. Moreover, the proliferation of keratinocytes and fibroblasts has been reported in the literature for extremely low doses of laser irradiation. [52, 53]

The modulation of transcription factors has become a common therapeutic strategy to prevent or provoke the expression of specific genes, and the approach could potentially provide a means to treat a wide assortment of medical disorders. Jackson and coworkers (2002) identified more than twenty transcription factors are regulated by the intracellular redox state. [54] It is proposed that laser therapy, because it has been identified to alter the intracellular redox state, could affect the function of transcription factors associated with the formation and maintenance of adipocyte membranes. To support this claim, further studies are highly warranted. However, there is enough evidence to support that laser irradiation within the red spectrum does play a unique role in the expression of specific genes, and is plausible that the transitory pore observed following LLLT could result from the alteration in gene expression.

As discussed earlier, laser therapy activates the electron flow in the respiratory chain resulting in a greater production of ATP. In mitochondrial electron transport the superoxide radical is produced. Superoxides can stimulate or inhibit cell proliferation. [55] Dmitriev and coworkers

(1990) discussed that mitochondrial ATP synthesis can be inhibited and activated by the generation of superoxides subsequent to light radiation. [56] An increase in the production of superoxides results in an increase in calcium levels, release of arachidonic acid, activation of sodium and hydrogen antiport and calcium-ATPase, and alteration of sodium and calcium exchange. [57-59] A process known as lipid peroxidation is the degradation of lipids via superoxides. Lipids found in the membranes of cells are broken down due to the highly reactive nature of superoxides. Olban and coworkers (1998) demonstrated that laser irradiation between 1-5J stimulated lipid peroxidation and superoxide production (figure 1). [60]

Fig 1: Disruption of the bilipid membrane induced by superoxides



It is proposed that the up-regulation in superoxides may result in the degradation of lipids in the membranes of adipocytes; therefore resulting in the temporary formation of the transitory pore.

Laser therapy provides the medical community with an alternative therapeutic regime for the reduction of subcutaneous tissue volume. Although the biochemical mechanism is not yet fully understood, histological studies clearly and effectively identify the formation of the transitory pore and subsequent cell collapse immediately following laser irradiation. More importantly, the placebo-controlled, randomized, double-blind clinical investigation with 67 enrolled participants revealed a statistically significant reduction of overall circumference measurements of the waist, hip, and thighs in two weeks.

Laser therapy operates under the principle of photochemistry, activating and/or suppressing natural biochemical processes. Because LLLT does not induce cellular apoptosis, there is no up-

regulation of pro-inflammatory cytokines nor is there a large burden placed on the lymphatic system. The fatty material secreted from the adipocytes following laser irradiation are absorbed by the lymphatic system, broken down by the liver, and naturally secreted. Fatty acids released are bound to albumin, and are transported through the circulatory system to the liver to undergo fatty acid oxidation. The triglycerides released are bound as lipoproteins and transported to the liver to be processed. Moreover, a lipase known as lipoprotein lipase, has been demonstrated to breakdown emulsified triglycerides; therefore breaking the molecule down into three fatty acids and one glycerol molecule.

The most important aspect of laser therapy is that cellular apoptosis is not induced. The fatty material being evacuated from the cell must be absorbed by the lymphatic system, and because laser therapy does not destroy the cell, the complex organelles and the entire cell structure will not be absorbed by the lymphatic system. It is not well understood how much the lymphatic system can absorb; therefore, creating a mass amount of cellular debris by destroying adipose tissue may result in serious long term effects. Moreover, adipose tissue is not just composed of fat storing cells; there is also a collection of immune cells, fibroblasts, vessels, and stromal stem cells. By creating the transitory pore in the fat storing cell only, we are preserving the viability of the surrounding, non-fat storing cells.

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