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Original Article

Analysis of Laser Lipolysis Effects on Human Tissue Samples Obtained from Liposuction

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Abstract.
Background: The use of the neodymium: yttrium-aluminum-garnet (Nd:YAG) laser as an auxiliary tool for lipoplasty has refined the traditional technique. The primary limitation of the traditional technique—large volume blood loss—is addressed by the use of the Nd:YAG laser. This report describe's the technique of laser- assisted liposuction, then reviews and compares the histologic and morphometric effects with those of the traditional method.
Methods: The Nd:YAG laser was used to produce the desired injury to the adipose tissue after sufficient contact. Laser lipolysis was performed on the patient's right flank in two different areas, with the application of 1,000 and 3,000 J of accumulated energy (∈), respectively. Subsequently, another cannula was introduced to suction the liquid content obtained by laser lipolysis. This tissue product was sent to the pathology laboratory for morphologic and morphometric analysis, and for comparison with material obtained from traditional lipoplasty performed on the left flank of the same patient.
Results: The histopathologica examination of adipose tissue after laser lipolysis showed cell swelling and less bleeding, as compared with the traditional method. The morphometric analysis showed that the mean diameter of the major adipocye was 95.69 μm with laser lipolysis using 1,000 J (∈), 82.63 μm using 3,000 J (∈), and 84.54 μm with the traditional method
Conclusions: The traditional method produced less reversible cellular damage (swelling) than laser lipolysis using 1,000 J (∈). The area receiving 3,000 J (∈) showed major irreversible damage (cytoplasmatic retraction and disruption of membranes). For this reason, the mean diameter of the adipocyte was less.

Key words: Laser lipoplasty—Nd:YAG—Laser lipolysis—Laser-assisted liposuction

Liposuction is considered a safe and effective procedure for definition of the body's contours. Excessive blood loss constitutes the primary limiting factor in the volume of fatty tissue to be removed. For this reason, most surgeons limit traditional liposuction to the aspiration of 2,000 g of tissue and/or autologous blood replacement postopertively as a means for minimizing the reduction of total blood and fluid volume.

In an effort to reduce blood loss and improve the aesthetic results of liposuction, we introduced the use of the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser as an ancillary instrument in combination with the traditional technique.

This report aims to compare the morphometric and histologic changes between laser-assisted liposuction and conventional liposuction performed in the flank area.

Materials and Methods

Patient

Our patient was a 26-year-old, Moreno woman with a body mass index (BMI) of 24.3 kg/m^2, a fat body mass of 13%, and a lean body mass of 55.52%. Other specific measures included cutaneous subscapular

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fold (16. 4 mm), tricipital (26.5 mm), left suprailiac (27 mm), right suprailiac (23.5 mm), and abdominal wall (17.8 mm). In consultation with the department of plastic surgery, she presented with the main complaint of a poor aesthetic aspect to her flanks, caused by bilaterally adipose tissue deposits. The patient gave informed consent for the described procedure to be performed.

Laser

Lipolysis was performed with an Nd:YAG laser using a wavelength of 1,064 μm, energy of 150 millijoules mJ, a frequency of 20 Hz, and a potency of 4 W continuous mode. This laser also features a helium–neon (HeNe) red aiming beam. The HeNe beam can be seen through the skin, allowing the surgeon to identify the target.

Surgical Procedure and Processing of the Samples

The patient underwent conventional liposuction in the left flank area and laser-assisted liposuction in the right flank area. In the superior portion, 3,000 J of energy was applied, and the inferior portion received 1,000 J of energy. In the left flank, 300 g of material was obtained, and in the right flank, 200 g of material was obtained in the superior portion and 100 g in the inferior portion.

The technique of laser lipolysis was performed as follows. After local anesthesia of the right flank, tumescent saline solution (0.9%) was infiltrated with adrenaline at a concentration of 1:500.000. A cannula 1 mm in diameter was used, through which a fiber optic was inserted. The cannulas for the treatment beam and the aiming beam were inserted through microincisions of 2 to 3 mm. The rectilinear cannula was inserted to the depths necessary to reach all the layers of the fatty tissue (superficial, medium, and deep), and to reach the deep dermis of the patient's right flank.

With this techniques, laser–tissue interaction is produced, starting with a soft, continuous back-and-forth motion, the laser energy radiating from the distal tip of the cannula. The action of the laser is controlled through a panel that expresses the energy quantitatively, allowing for delivery of 1,000 J (inferior) and 3,000 J (superior), in the right flank. Other cannulas ranging from 2 to 3 mm were introduced for the aspiration of the fatty tissue liquefied by the laser lipolysis.

In left flank area, the liposuction procedure was performed using the traditional method. The area was infiltrated with the same tumescent solution as used in the right flank, with a 0.5 cm skin incision for the introduction of a metallic cannula. Through back-and-forth movements, the adipocytes were mechanically removed from the subdermal tissue, and later, the material was aspirated through the cannula with negative pressure.

For a control, adipose tissue untreated and undamaged by either laserlipolysis or liposuction was obtained. This tissue originated from a single specimen obtained during an abdominoplasty performed later. A flap of fatty tissue (1 × 1 cm) underwent the same treatments as the other tissue samples in this experiment.

The tissue samples from the conventional liposuction (left side), the laser-assisted liposuction (right side) with 1,000 J (inferior portion) and 3,000 J (superior portion) of energy, and the control area were fixed with 10% formalin solution, included in a paraffin block, split up in slices of 4 μm, and dyed with hematoxylin and eosin. Two sections were prepared at two different levels for each material in the study.

Method of Analysis

Histologic Analysis. The control and experimental tissue samples, were analyzed for main morphologic features such as hemorrhage, tumefaction (reversible cellular damage), and cellular lysis (irreversible cellular damage). The faulty sections were ignored, and the best levels were selected, so that the morphometric analysis was not skewed.

Morphometric Analysis. Morphometric analysis was used to evaluate the largest diameter of complete adipocytes. For each group of this study 240 measures were performed. Later, for the graphic and statistical analysis, about 40 measures were inconsistent with the experimental and control groups, and the measures with the larger standard deviation of the average were selectively removed from the study.

Results

Histologic Results

The control fatty tissue demonstrated no histopathologic change. In the histologic analysis of the fatty tissue treated with the laser, we observed areas of reversible cellular damage (tumefaction), irreversible cellular damage (lysis), and a reduced intensity of bleeding, as compared with the tissue products of conventional liposuction (Figs. 1 and 2). The degree of tumefaction and lysis varied proportionally with the intensity of energy accumulated to the target. This fact is better noted by the morphometric following results.

Morphometric Results

For the morphometric analysis, the criteria were the average of the largest diameters of the adipocytes in

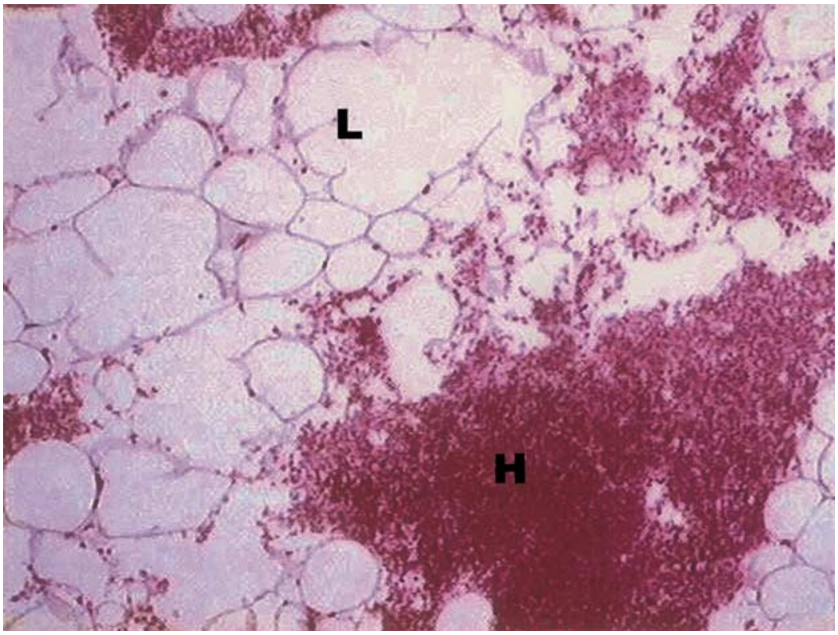

Fig. 1. Panoramic view showing damage of adipocytes (L) and extensive hemorrhage areas (H) resulting from conventional liposuction.

the control samples, the samples from conventional liposuction, and the samples from laser-assisted liposuction at the two levels of delivered energy (1,000 J and 3,000 J). The average of the 200 analyzed measures obtained in the control material was 73.48 ± 22.77 μm. With the conventional procedure, the average was 84.54 ± 18.17 μm. The average of the 200 measures obtained in the procedure using laser energy of 1,000 J was 95.69 ± 14.06 μm. The average of the 200 measures obtained in the procedure using laser energy of 3,000 J was 82. 63 ± 11.20 μm. The average of the largest adipocyte diameters for each technique used and their standard deviations are represented in Fig. 3.

Discussion

Beginning in the 1980s, an innovative surgical technique that sought to eliminate localized fat deposits was introduced to the plastic and reconstructive surgery. This technique was developed by the French surgeon Yves-Gérard Illouz, considered to be the father of liposuction [6, 8].

The conventional liposuction technique is performed beginning with the introduction of a metal cannula into the subcutaneous cellular tissue. Through microincisions 1 cm in length, the cannula is used in a back-and-forth motion, and the hypodermis is reached, separating the damaged adipocytes harmed by the mechanical motion and later aspirated.

The peristaltic system comprises the infusion of tumescent solution containing lukewarm physiologic serum (1,000 ml), lidocaine to 2% (30 ml), adrenaline 1/1,000 (1 ml), and bicarbonate of sodium 10% (15 ml), followed by aspiration of the adipose tissue through negative pressure. The use of this tumescent technique, developed by Klein, seeks to reduce blood and fluid loss and related postoperative morbidity [12, 14].

The success of liposuction is fundamentally contingent upon the proper selection of patients who fit the following criteria: good physical condition (within 30% of his or her ideal weight), localized areas of lipodistrophy resistant to lifestyle changes such as diet and exercise, capacity for retraction of the skin, and the patient's and doctor's clear understanding and acceptance of this procedure limits.

As with any surgical procedure, liposuction has its contraindications. Blood loss, skin flaccidity, and anatomic topography are limiting factors in the selection for the conventional technique and its success.

In 1990, Dressel [9], seeking to enhance the conventional liposuction procedure, conducted an experiment with 51 patients undergoing Nd:YAG lasers-assisted lipoplasty for approval by the Food and Drug Administration (FDA). This technique demonstrated disadvantages attributable to technical problems. However, the laser fiber was inside the cannula, and ended up doing mostly mechanical damage, quite similar to that of conventional liposuction, in which the tissue injury is largely mechanical. The action of the laser in this experiment was primarily delivered to the already-suctioned fat. Additionally, constant cooling of the cannula was required because of overheating and burning of target and collateral tissue by the laser fiber. Dressel was unable to demonstrate a clear and significant benefit of his procedure, as compared with conventional liposuction.

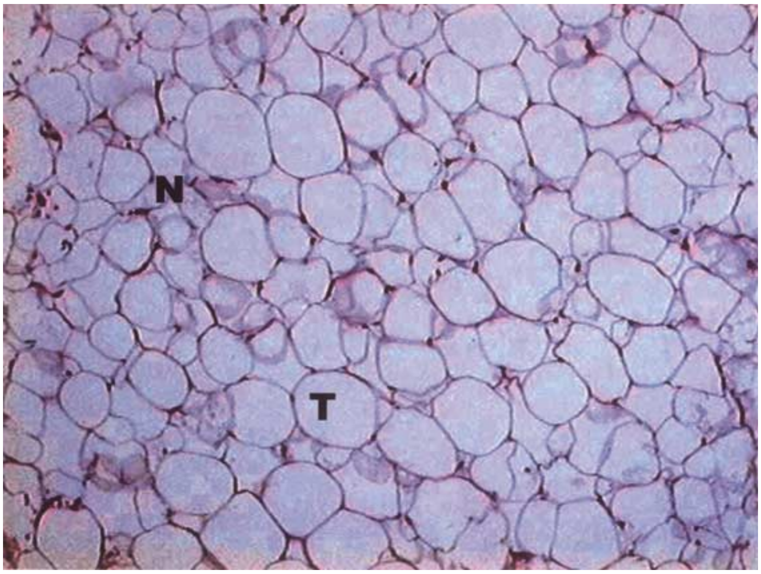

Fig. 2. Panoramic view showing swelled adipocytes (T) and areas of cellular lyis or necrosis (N) resulting from laserlipolysis.

The interaction of the laser with the tissue is achieved by the absorption of the laser energy by the receptive chromophores, thus producing sufficient heat to cause the desired thermal damage. The heat acts on the fatty cell, the extracellular matrix, and the microcirculation to produce both reversible and irreversible cellular damage, which facilitates the liposuction through less trauma and bleeding. On the basis of this theory, a new method for laser lipolysis has been proposed. Using this new method, the cannula of the laser is in contact with the subcutaneous tissue and positioned outside the aspiration cannula, rather than inside it, eliminating the need for cooling it. Laser lipolysis can cause several kinds of thermal damage, including coagulation of collagen fibers, microcirculation thrombosis, damage to nerve endings and tumefaction, and lysis of fatty cells.

First, the reversible damage comprises the tumefaction of the adipocytes. The heat generated by the laser alters the balance of sodium and potassium of the cellular membrane, allowing the free transport of extracellular liquid to the intracellular atmosphere. We see, initially, in optical microscopy, clear intracytoplasmic vacuoles that culminate with tumefaction of the cytoplasm. This phenomenon was observed in the samples of the fatty tissue irradiated with 1,000 J of energy that presented average adipocyts diameters of 95.69 μm, as compared with an average diameter of 73.48 μm for adipocytes in their normal state and a diameter of 84.54 μm for adipocytes after conventional liposuction. The luminous energy produced by the laser was absorbed and transformed to heat, which generated protein denaturation in he cellular membrane. This process culminated with cellular tumefaction and increased size of the cells. This also happened with conventional liposuction, however, to a smaller degree. The diameters of the adipocyte from conventional liposuction were larger than the diameters of the adipocytes from the control tissue.

Second, after the basic physiopathology of cellular damage, a lingering and intense time of the incentive (laser) culminates in irreversible damage with coagulative necrosis and cellular lysis [13]. In general, most of the infra- and extracellular proteins are denatured at temperatures between 40° and 100°C, Dependent upon time and temperature, the laser provokes cellular death through coagulative necrosis. The denaturization of the cellular structure and enzymatic proteins blocks proteolysis. In effect, there is immediate tissue contraction, although the basic cellular outline is maintained.

This process of thermal injury was evident in the tissues irradiated with 3,000 J of energy, which presented smaller average diameters of adipocytes (82.63 μm) than those from conventional liposuction (84. 54 μm) or from 1,000 J laser-assisted liposuction (95.69 μm). The localized delivery of energy was so intense that it provoked protein denaturization and cellular contration (i.e., irreversible damage). It also was observed that the average of the largest adipocyte diameters from the control (73.48 μm) was even smaller than that observed with the laser at 3,000 J (82.63 μm). It may be that at the 3,000 J energy level, although some adipocytes experienced cellular contraction indicative of irreversible damage, others experienced only reversible tumefaction damage, because the final average of their diameters was larger than that of the control.

In the sequence of the effects produced by the action of the laser, the liquefactive necrosis or cellular lysis is the last effect of the extreme thermal damage to the irradiated adipose tissue. This occurs when the membranes of the fatty cells break up. Lipases liberated by the adipocyte, are responsible for the liq-

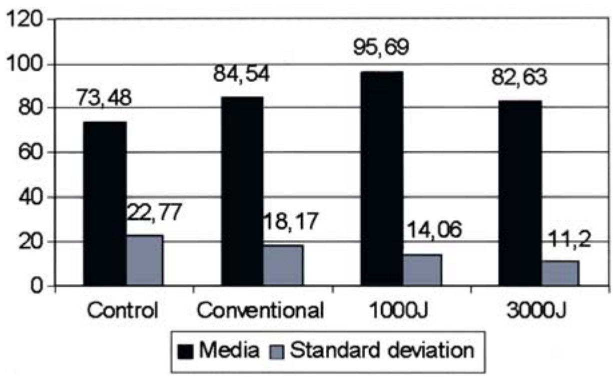

Fig. 3. Graphic representation of the averages (μm) and standard deviations of the diameters of the adipocytes from the groups in this study. control, adipocytes of the tissue control; conventional, adipocytes of the conventional liposuction; 1000J; adipocytes of the laserlipolysis with 1000 J of energy; 3000J, adipocytes of the laserlipolysis with 3,000 J of energy

uefaction of the tissue, which further facilitates the subsequent aspiration.

The use of the laser as an adjuvant in liposuction decreases trauma to the tissue targeted for aspiration. Through the liquefactive effect of the laser, the fat is loosened without the abrupt and repetitive back-and-forth motion of the cannula as performed in the conventional technique.

In 2000, Grippaudo et al. [11] performed a biochemical analysis of the material obtained from ultrasonically assisted liposuction. The results of this experiment can be compared with the results from the use of the laser, because in the same way that the ultrasound uses resonant energy to promote heating and subsequent cellular rupture, the laser uses luminous energy to achieve the same objective. However, larger values of triglycerides were observed in the samples undergoing ultrasound. That result denotes the irreversible cellular damage of liquefactive necrosis observed in samples submitted to laser application. We were unable to find any article that documented these high values of triglycerides in the aspirated material. However, because of the similarity in the final effect achieved by the laser, as compared with ultrasound, such a parallel might be drawn.

Liposuction removes significant amounts of fat, serum, and blood. In cases wherein large amounts of fatty tissue are to be removed, a physiologically significant loss of blood can provoke metabolic alterations. Laser lipolysis may be indicated in these situations, in that the decreased trauma and the microangiopathic coagulation reduces bleeding, often avoiding the need for transfusion, whether planned autologous transfusion or homologous transfusion, and the risk of blood-borne diseases. In this way, laser-assisted liposuction offers the advantage of removing larger volumes of fat without hemodynamic repercussions.

Commons et al. [7] studied patients between 17 and 24 years old, with American Society of Anesthesiology (ASA) classification 1 and 2. In this study, over the course of 1 year, they evaluated the safety of removing large fat volume (3–17). They concluded that large-volume liposuction can be a safe and effective procedure when strict principles, such as careful patient selection and appropriate surgical and anesthetic techniques, are adequately applied.

Goddio [10] studied the influence of the area undergoing conventional liposuction on the ability of the skin to retract during the first 3 days and 2 months of the postoperative period. They concluded that the treatment site is a significant influence on the potential for skin retraction. This conclusion was supported by the decreased pattern of positive results in the body, neck, and face. There were other contributing factors, including age and the amount of adipose tissue removed, but the suction site independently emerged as a determinant factor of the skin's ability to remodel. Given this finding, laser lipoliysis may be an alternative method for these anatomical area in which skin remodeling is deficient.

Apfelberg et al. [1,2] submitted 51 patients to laser lipolysis. Fifteen of these subjects underwent laser-assisted liposuction on one side and conventional liposuction on the opposite side. His results did not show a benefit of laser-assisted liposuction over conventional liposuction. This conclusion was based on the persistence of such clinical signs as edema and ecchymosis, which were evidenced equally with the traditional technique and the laser-assisted technique in the follow-up period at 8 weeks.

Badin et al. [3–5] performed laser lipolysis on 245 patients. The technique was different from that used by Dressel. The fiber was outside the cannula. As a result, the interaction between the laser and the fat produced direct cellular destruction before the suction, as well as remodeling of the collagenous tissue, with clinically evident skin retraction. The collagen denaturing performed in the deep reticular dermis and the conjunctive septum of the subcutaneous tissue constituted a proinflammatory stimulant followed by vascular proliferation and collagen neosynthesis.

Some weeks after laser treatment, we observed early skin retraction because of maturation and organization of the fibrous tissue [13], yielding an improvement in postoperative recovery. The regular and rapid adherence of the skin and underlying dermal tissue to the superficial fascial plane improved the degree of local flaccidity, and the patient was generally able to return to work in 6 days and to light exercise in 7 days. Our conclusions included improvement of postoperative morbidity through the reduction of swelling, yielding good contours. This method demonstrated benefits in areas of moderate or potential flaccidity, in which the ability to use traditional liposuction was restricted.

Conclusion

The study allowed us to conclude that the conventional method produces less reversible damage (tumefaction) than laser lipolysis with 1,000 J of energy. The area that received 3,000 J of energy presented significant irreversible damage (cytoplasmatic retraction and disruption of membranes), as indicated by a smaller average of the largest adipocyte diameters, compared with the area that received 1,000 J.

The thermal damage produced by the Nd:YAG laser (1064 nm) in the adipose tissue promoted better hemostasis, better wound healing, and less surgical trauma. Besides the anatomopathological evidence, the clinical evaluation showed improved postoperative recovery, resulting in a more rapid return to daily activities with an excellent aesthetic result.

The use of the laser in liposuction offers freedom from some of the current limitations faced by plastic surgeons using this procedure. Additional studies enrolling larger numbers of patients and evaluating all the variants are required to determine optimal energy parameters and the most suitable topography for this procedure. Such additional information would aid in the development of a more exact and safe method.

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