OPEN FORUM

3 Therapeutic

Body Contouring

Preliminary Report

Histopathological Analysis of Abdominal Skin and Fat After In Vivo Hyperthermia-Induced Lipolysis With a 1064 nm Diode Laser, Radiofrequency, and Electromagnetic Field: A Pilot Study

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Abstract

Background: Hyperthermic laser lipolysis safely reduces unwanted fat through controlled thermal injury of adipocytes.

Objectives: To assess the effects of 1064 nm laser, single vs multiple treatments, with and without radiofrequency (RF) and pulsed electromagnetic field (PEMF) energy on abdominal tissue.

Methods: Nine volunteers scheduled for elective abdominoplasty were divided into 1 of 3 arms: (1) laser alone, (2) laser with and without RF and PEMF, and (3) multiple vs single laser treatments. Untreated (control) and treated tissues were collected at various time points following treatment. Paraffin sections from surgically excised pannus were evaluated with Masson's trichrome, apoptosis (TUNEL), Collagen 1, Collagen 3, Elastin, and CD68 macrophage markers.

Results: All study arms showed subdermal adipose tissue lysis without compromising epidermal or dermal integrity. Apoptotic adipocytes and macrophage infiltration were present in areas of structural damage. Adjuvant RF and PEMF showed increased macrophage infiltration after 14 days. Multiple 1064 nm treatments induced apoptosis in subcutaneous adipocytes. There were no significant changes in dermal Collagen 1, Collagen 3, and elastin abundance.

Conclusions: The 1064 nm diode laser can destroy adipocytes without harming overlying epidermis. RF enhances lipolytic effects of the laser with increased inflammation and tissue remodeling, whereas PEMF therapy uses electromagnetic fields to stimulate cell function and tissue repair. These findings suggest the 1064 nm diode laser can achieve intended effects.

Level of Evidence: 3 (Therapeutic)

Noninvasive body contouring procedures continue to increase in popularity, surging by 77% from 2019 (386,557) to 2022 (682,932).[1-3] Although noninvasive devices cannot replace the transformative effects of surgery, they expand a plastic surgeon's toolbox, offering innovative avenues to optimize patient outcomes in appropriately selected patients.

Diode lasers, high-intensity focused ultrasound, pulsed electromagnetic field (PEMF) therapy, radiofrequency (RF), and cryolipolysis are notable energy-based technologies for noninvasive fat removal.[4-8] The 1064 nm diode lasers target subcutaneous adipose tissue

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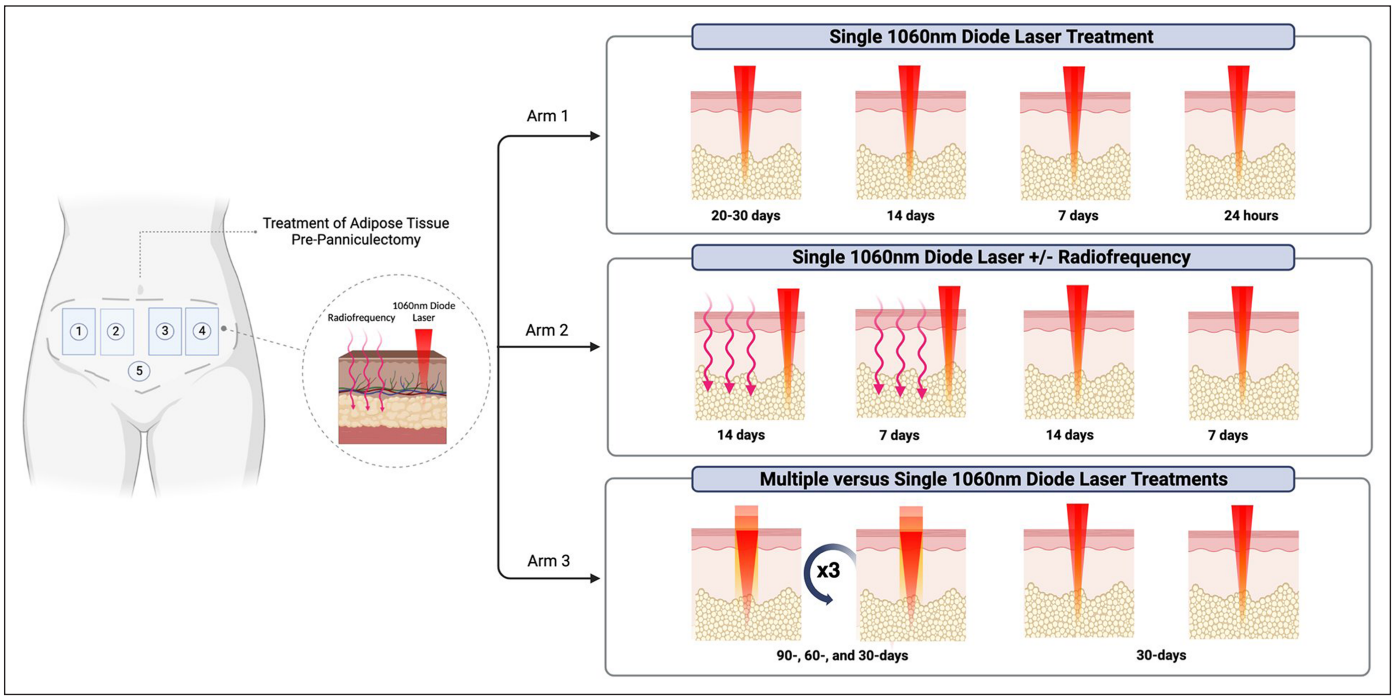

Figure 1. Treatment schematic.

and heat local adipocytes to the hyperthermic range of 42 to 47 °C. The induced hyperthermic injury and subsequent adipocyte injury trigger an immune response leading to the elimination of necrotic and apoptotic adipose cells.[9] Triglycerides released from fat breakdown are transported through lymphatics and blood to the liver for decomposition.[10] Previous clinical studies demonstrate the 1060/1064 nm diode laser as a safe and efficacious method for noninvasive fat reduction.[11-18]

Our study aims to contribute to the understanding of mechanisms of noninvasive fat reduction. Here, we evaluated histological structural changes in human abdominal subdermal fat and overlying skin following 1064 nm diode laser treatments with and without RF/PEMF in vivo.

METHODS

Study Design

This triple-arm study conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval (IRB:STU-2023-0661) from the Institutional Review Board at the University of Texas Southwestern Medical Center. Recruitment began in September 2019 and ended in November 2023. Patients over 18 years with scheduled abdominoplasty who were willing to receive treatment of their lower abdomen (pannus) were recruited. Exclusionary criteria included pregnancy in the prior 3 months, liposuction in the planned area of treatment, trauma or tattoos in the treatment area, and skin disorders. Informed consent was obtained from all patients.

The investigational device (Venus Bliss, Venus Concept, Toronto, Canada) consists of 4 diode laser applicators operating at a wavelength of 1064 nm and 1 applicator employing vacuum-assisted combination RF and PEMF energy. The diode lasers are cleared by the US FDA for noninvasive lipolysis of the abdomen and flanks in individuals with a BMI of ≤30, with the RF/PEMF component cleared for temporary reduction in cellulite appearance. The device utilizes a water-based cooling system to minimize collateral thermal damage.

Treatment Protocol

Nine patients were enrolled and assigned to 1 of 3 treatment arms and received 25 min sessions with the 1064 nm laser. Patients were positioned supine with their abdominal area exposed. A belt equipped with 4 frames to secure the 4 laser applicators was fastened to the abdomen, creating 4 distinct treatment areas (Sites 1-4) and control (Site 5) within the area to be excised and collected during standard-of-care abdominoplasty in all treatment arms (Figure 1). Each site measured 6 × 6 cm, the size of the emitting window of the applicator.

Arm 1: Single 1064 nm Diode Laser Treatment

Sites 1, 2, 3, and 4 each received a single treatment at 4 respective preoperative time points (20-30 days, 14 days, 7 days, and 24 h; Figure 1).

Arm 2: 1064 nm Diode Laser and RF

Sites 1 and 2 were treated 14 and 7 days preexcision, respectively, with the 1064 nm diode laser followed by RF/PEMF. Sites 3 and 4 were treated 14 and 7 days preexcision, respectively, with the 1064 nm diode laser alone (no RF/PEMF; Figure 1).

Arm 3: Multiple 1064 nm Diode Laser Treatments

Sites 1 and 2 underwent 3 treatments with the 1064 nm diode laser at 90, 60, and 30 days preoperatively, whereas Sites 3 and 4 received a single treatment at 30 days preoperatively. All arms had an untreated area (Figure 1, Site 5) serving as a control (Figure 1).

Device Settings

The active treatment duration was 25 min with a power of 1.4 W/cm^2. Settings were lowered to 1.2 W/cm^2 for patients receiving sequential RF/PEMF. Anesthesia was not indicated for this device and not used in this study.

Safety Assessment

Patients assessed procedural pain immediately following treatment using the Wong–Baker pain assessment scale ranging from 0 (no pain) to 10 (worst pain). Adverse events were recorded during follow-up visits.

Histology

Full-thickness skin with subdermal fat from treated and control areas was harvested from excised pannus. Samples were immediately fixed in 4% paraformaldehyde (Sigma-Aldrich, St Louis, MO) in phosphate-buffered saline for 48 h at room temperature with gentle shaking. Histological staining with hematoxylin–eosin and Masson's trichrome was performed.[16] Paraffin-embedded samples were then processed for immunohistochemistry and immunostaining, as described previously,[16-19] using primary antibodies specific for Collagen I (ab6308; Abcam, Cambridge, MA), Collagen III (ab7778; Abcam), elastin (ab2160; Abcam), and CD68 and Prolong Gold containing DAPI (Thermo Fisher, Waltham, MA) to stain cell nuclei. Collagen and elastin sections were subject to antigen retrieval with boiling in sodium citrate buffer (10 mM, pH 6) for 20 min prior to blocking. TUNEL staining for apoptotic cells was done according to the Promega DeadEnd Fluorometric TUNEL System protocol (Promega, Madison, WI). Apoptotic cells were labeled with fluorescein and counterstained with propidium iodide. Images were obtained using a Zeiss Axiscan7 microscope (Zeiss, White Plains, NY). Brightfield images were taken using NanoZoomer microscope (Hamamatsu Slide Scanner, Hamamatsu City, Japan), and NIH ImageJ software was used to generate individual images and quantification.

Data Analysis

The *t* test was used to test the null hypothesis using Microsoft Excel software (Redmond, WA). Significance was assessed at the $P = .05$ level.

RESULTS

Patient demographics are shown in Table 1. Most of the patients were Caucasian ($n = 6$) followed by individuals of Black/African ($n = 2$) and Asian descent ($n = 1$). All patients identified as non-Hispanic. Two patients were on weight loss medication (Ozempic, Wegovy).

Table 1. Patient Demographics

Characteristic	Value ($n = 9$)
Age	48.44 ± 6.56
Sex, *n* (%)	Female: 8 (89%); male: 1 (11%)
BMI	27.66 ± 4.45 kg/m^2
Power setting (W/cm^2)	1.2 ± 0.2 (range 1.0-1.4)
Mean pain score	2.11 ± 1.85

Descriptive statistics for subject demographics are shown as mean ± standard deviation.

Comorbidities included hypertension ($n = 5$), high cholesterol ($n = 3$), diabetes ($n = 1$), anxiety ($n = 3$), lupus ($n = 1$), hypothyroidism ($n = 1$), and chronic kidney disease ($n = 1$).

Single 1064 nm Diode Laser Treatment

Structural changes following a single laser treatment primarily involved subdermal fat, with little to no effect on the dermis or epidermis. As shown in Figure 2, extravasated red blood cells were scattered in subdermal fat at Day 1. At Day 7, there was partial breakdown of subdermal fat with increased collagen deposition. By Days 14 and 20, subdermal fat appeared disorganized and reduced, whereas subdermal collagen deposition increased, especially perivascularly (Figure 2). Collagen I, Collagen 3, and elastin expression were similar in the treated samples compared with the control based on specific immunofluorescence staining (data not shown). Apoptosis in subdermal fat started at Day 1 and gradually increased to the highest level at Day 20 post treatment (Figure 3).

1064 nm Diode Laser and RF/PEMF Treatment

The addition of RF/PEMF to the 1064 laser treatment resulted in ultrastructural changes in all layers of the skin and subdermal fat as shown in the trichrome images (Figure 4). At 7 days posttreatment, the laser–RF/PEMF combo treatment altered dermal collagen structure as collagen (blue) was denser in the papillary dermis compared with the control. By Day 14, the breakdown of subdermal fat resulted in increased collagen deposition and muscle cell formation. Hair follicles were also damaged in the skin treated with RF/PEMF and laser treatment (Figure 4).

The structural alteration was accompanied by increased macrophage infiltration (data not shown) and apoptosis (green staining, Figure 5) in the laser-treated sites.

In contrast to changes in collagen organization, Collagen 1, Collagen 3, and elastin abundance in the dermis and subdermal fat exhibited no statistically significant difference in the treated skin compared with the control (data not shown).

Multiple 1064 nm Diode Laser Treatments

Both single and multiple treatments caused varying degrees of structural damage to the hair follicles with reduction or complete

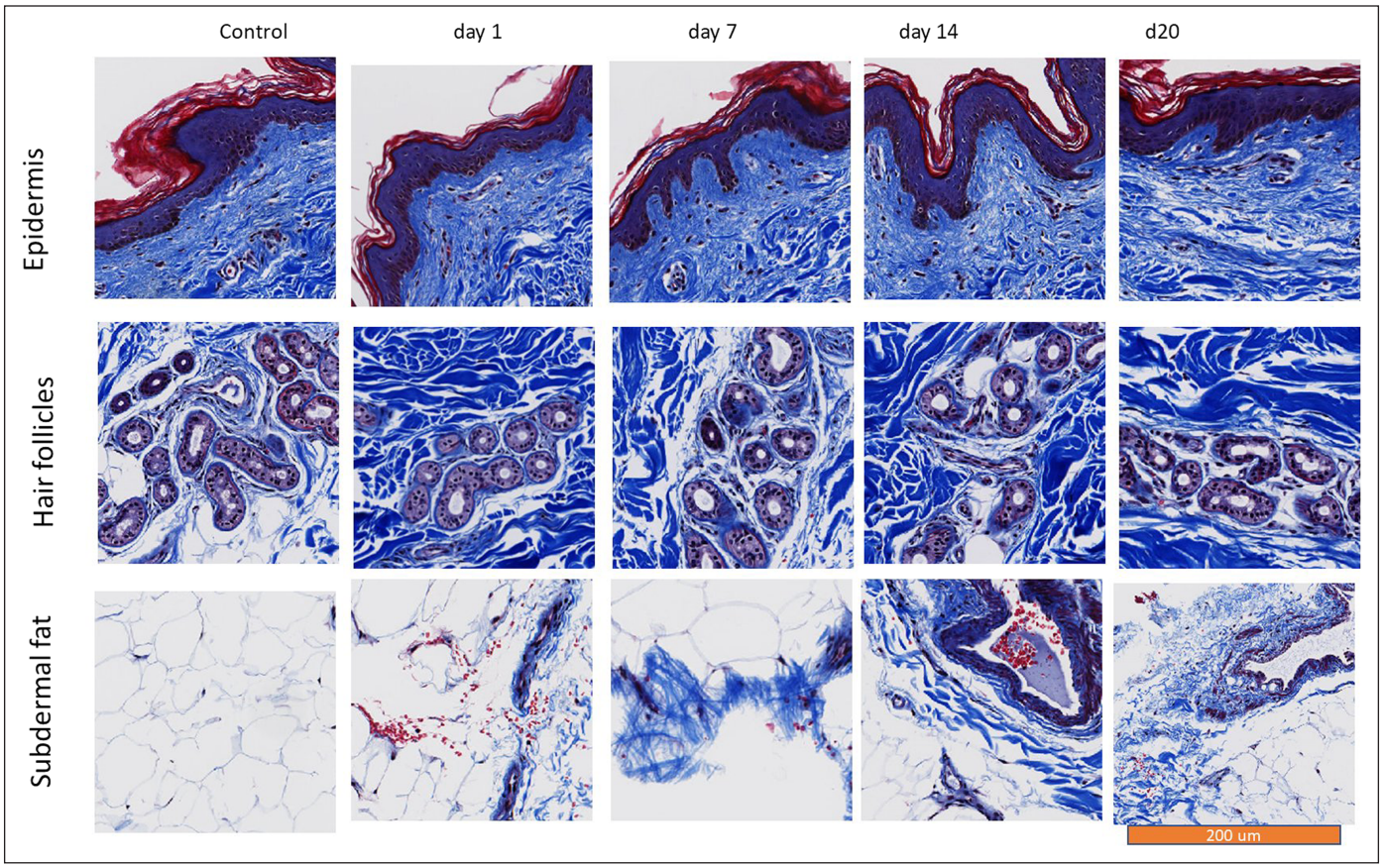

Figure 2. Masson's trichrome showing collagen (blue) and muscle cytoplasm, erythrocytes, keratin (red) at 1, 7, 14, and 20 days after single laser treatment (Arm 1).

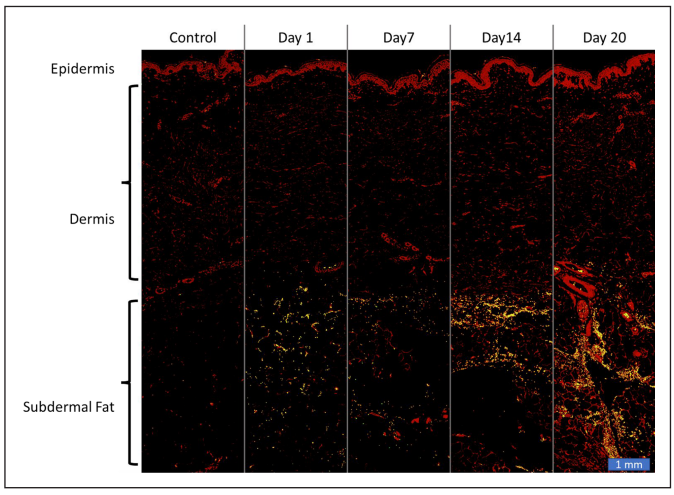

Figure 3. TUNEL immunofluorescence showing apoptosis (green/yellow) and nuclei (red) (Arm 1).

obliteration of subdermal fat in some areas (Figure 6). Collagen structure in the dermal hypodermal area appeared disorganized with scattered erythrocytes following complete subdermal fat loss in some of the laser-treated skin samples (Figure 6, last panel). The TUNEL staining showed apoptotic cells (green) in subdermal fat and bordering the dermis (Figure 7).

Adverse Events

Two subcutaneous fat nodules were reported during this study. One in Treatment Arm 2 resolved prior to surgery. The second nodule in Treatment Arm 1 (1064 nm Diode Laser only) was extracted with the abdominoplasty for histological evaluation. The nodule appeared in the lateral abdomen 1 week following treatment, described as non-painful, well-circumscribed, and palpable on clinical examination. Initial measurement was 7×8 cm at 1-week posttreatment, then 6×7 cm at 2 weeks and 5×4 cm prior to surgery. Trichrome staining showed extravasation of erythrocytes and an aberrant, dense collagen deposition (Figure 8). Fat necrosis was indicated by a high abundance of apoptotic cells based on the TUNEL staining (green staining, Figure 8, right panel).

Pain Assessment

Patients reported a pain level of 2.11 ± 1.85 (mean $\pm$ SD), considered "mild pain." The highest pain rating reported was a 5; however, this was reported in only 1 patient.

DISCUSSION

Clinically, 1064 nm diode laser has demonstrated safety and efficacy as a noninvasive method for reducing fat with significant reduction in

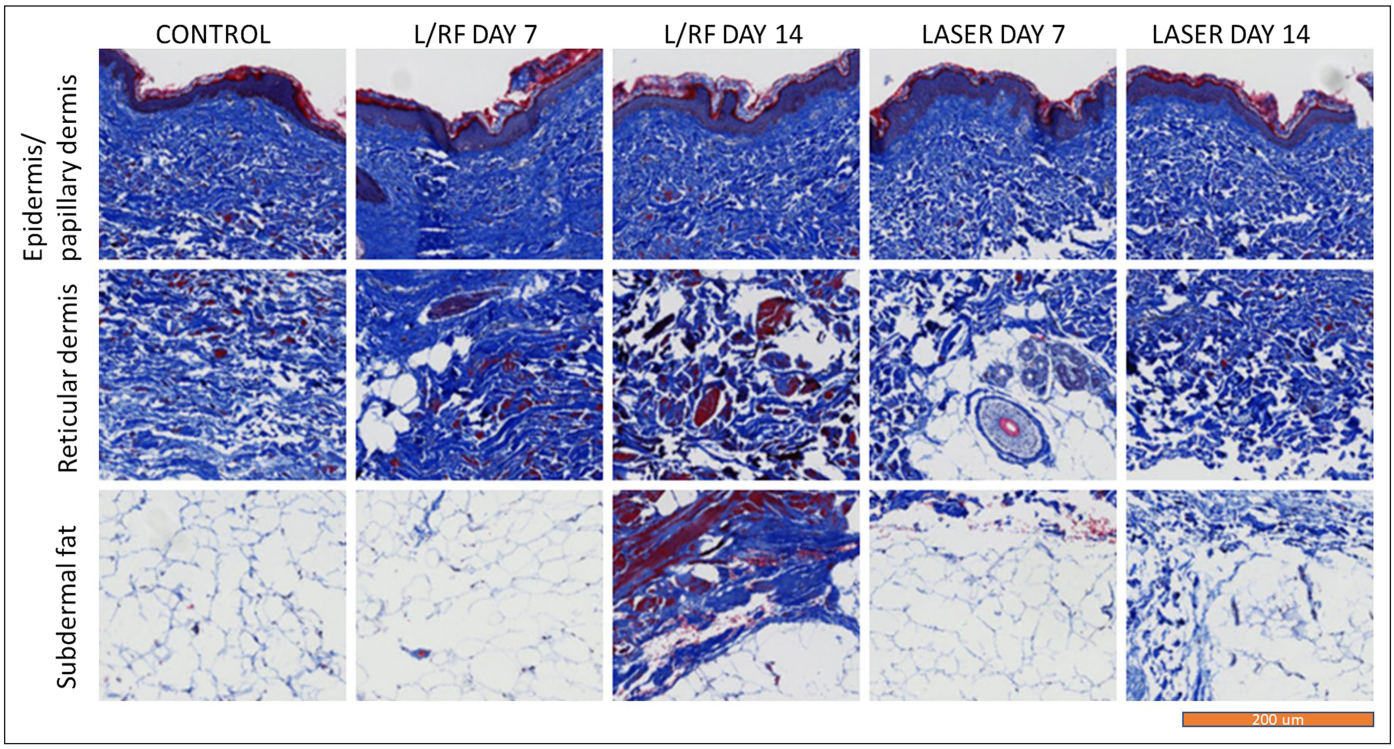

Figure 4. Masson's trichrome staining showing collagen (blue) and muscle cytoplasm, erythrocytes, keratin (red) at 7 and 14 days after laser and laser–radiofrequency (RF) and pulsed electromagnetic field treatments (Arm 2).

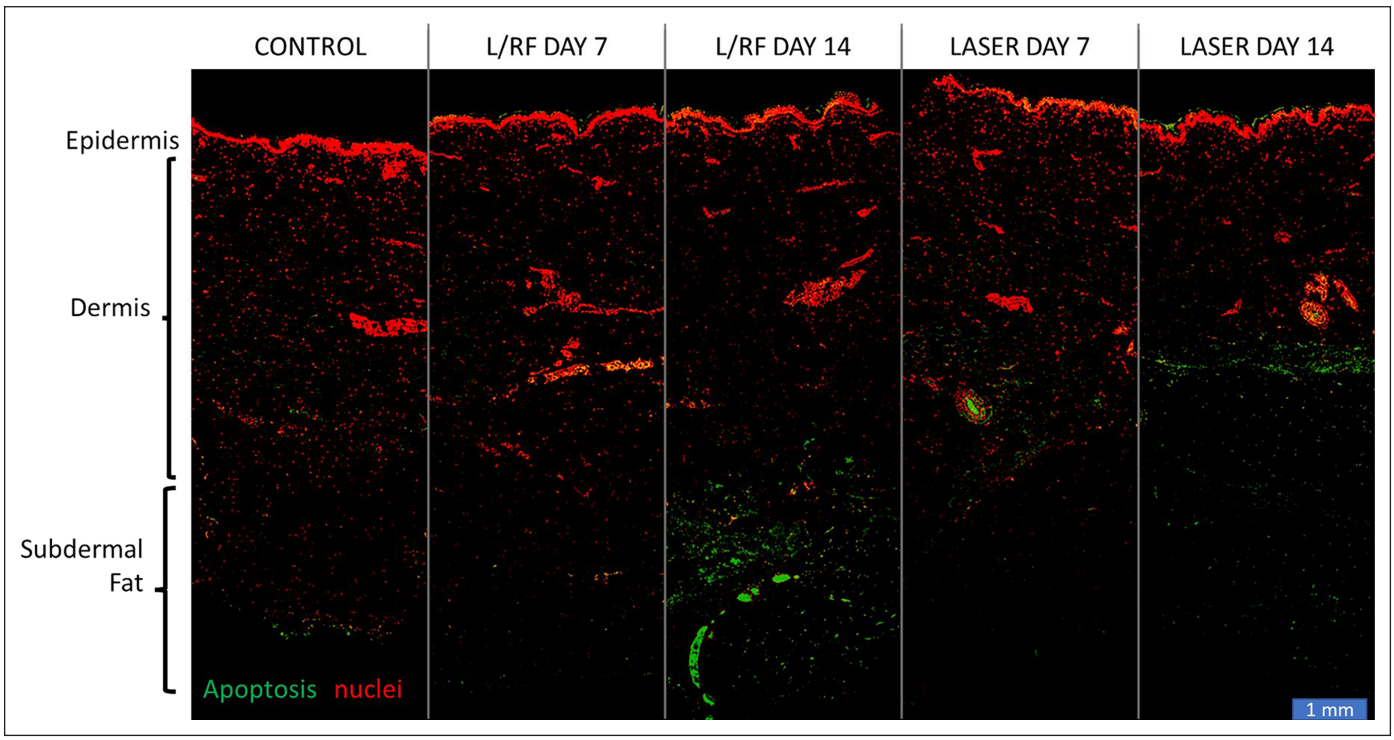

Figure 5. TUNEL immunofluorescence showing apoptosis (green/yellow) and nuclei (red) after laser or laser–radiofrequency (RF) and pulsed electromagnetic field treatment (Arm 2).

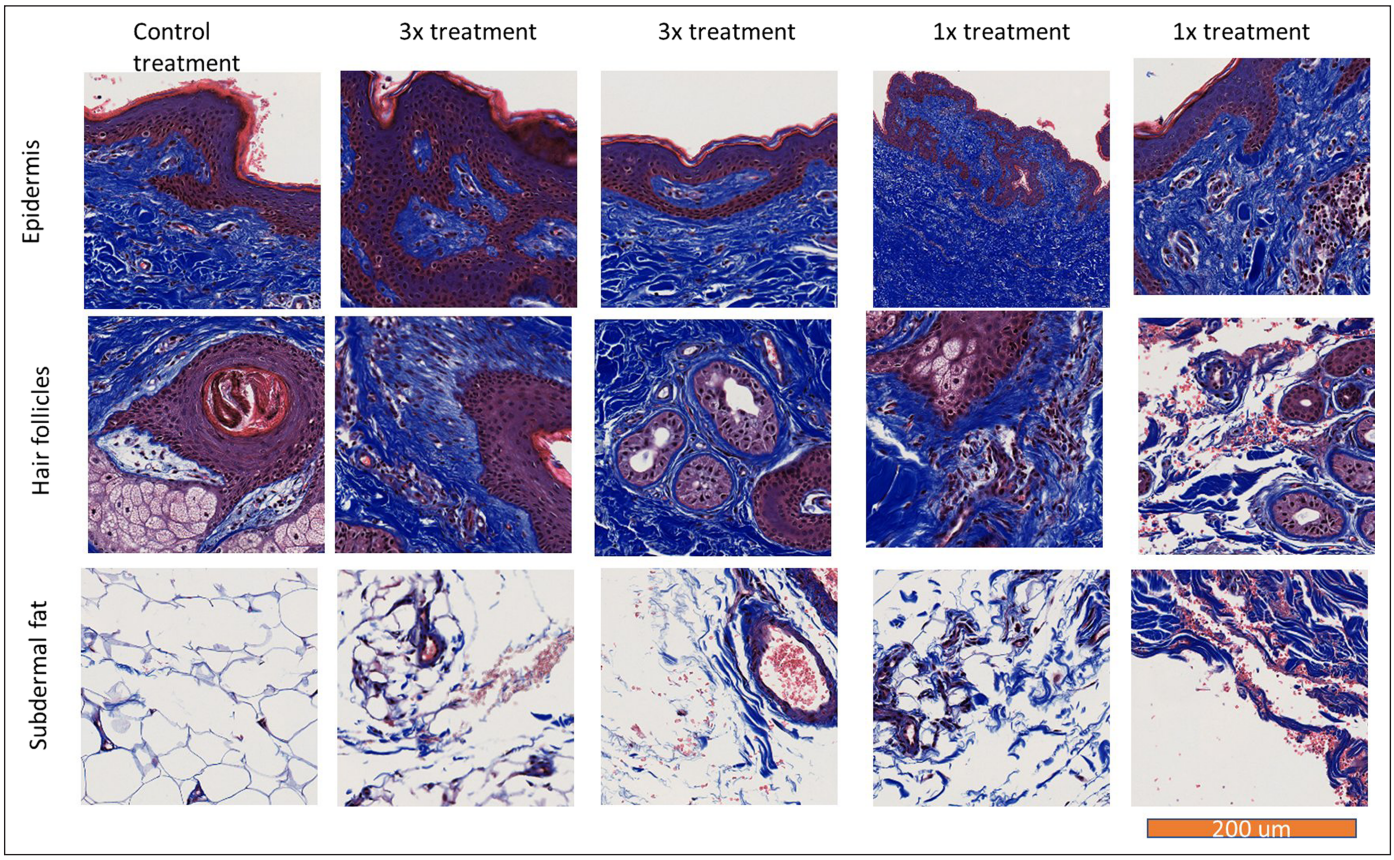

Figure 6. Masson's trichrome staining showing collagen (blue) and muscle cytoplasm, erythrocytes, keratin (red) after 1 and 3 laser treatments (Arm 3).

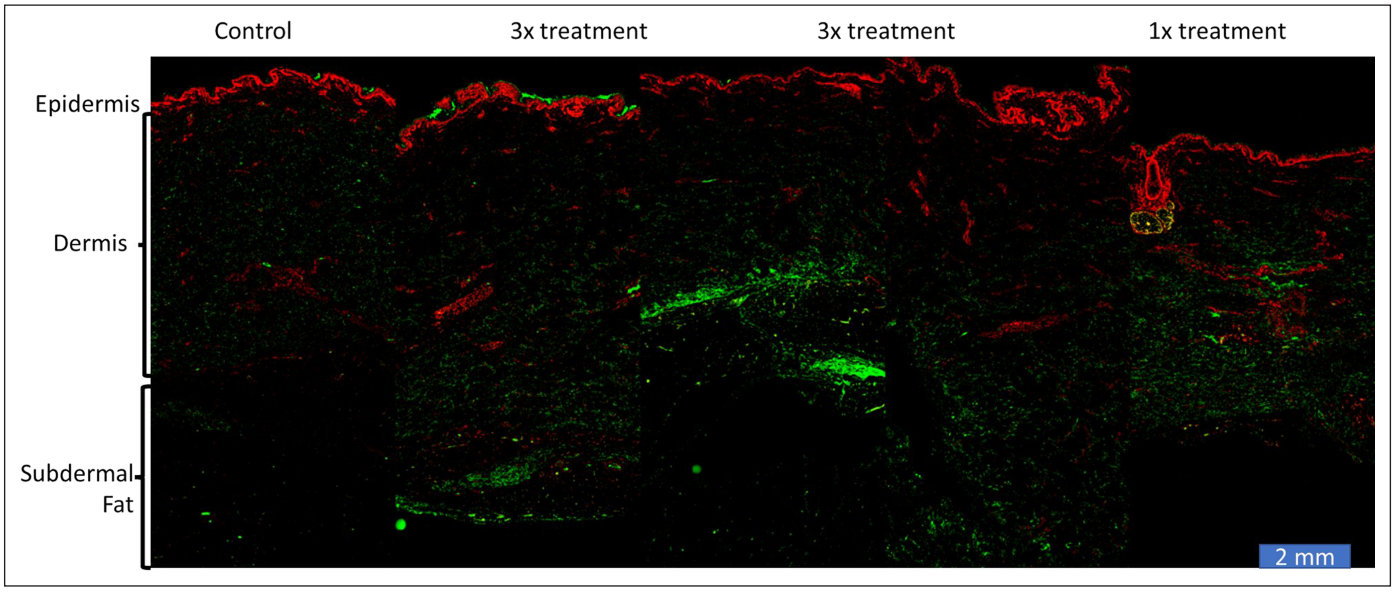

Figure 7. CD68 immunofluorescence showing macrophages (green) and nuclei (blue) after single laser or 3× laser treatment (Arm 3).

adipose thickness up to 12 weeks after a single session.[11-18] Here, we have evaluated the effect of the Venus Bliss laser with and without RF and PEMF energy on the skin to understand the effects of this treatment. In our study, all treatment arms of laser-alone and laser–RF/PEMF combo treatments resulted in apoptosis of adipocytes in the subdermis and tissue remodeling around the fat necrosis. The laser–RF/PEMF combo treatment led to the highest damage to the subdermal fat along with dermal collagen structure and hair

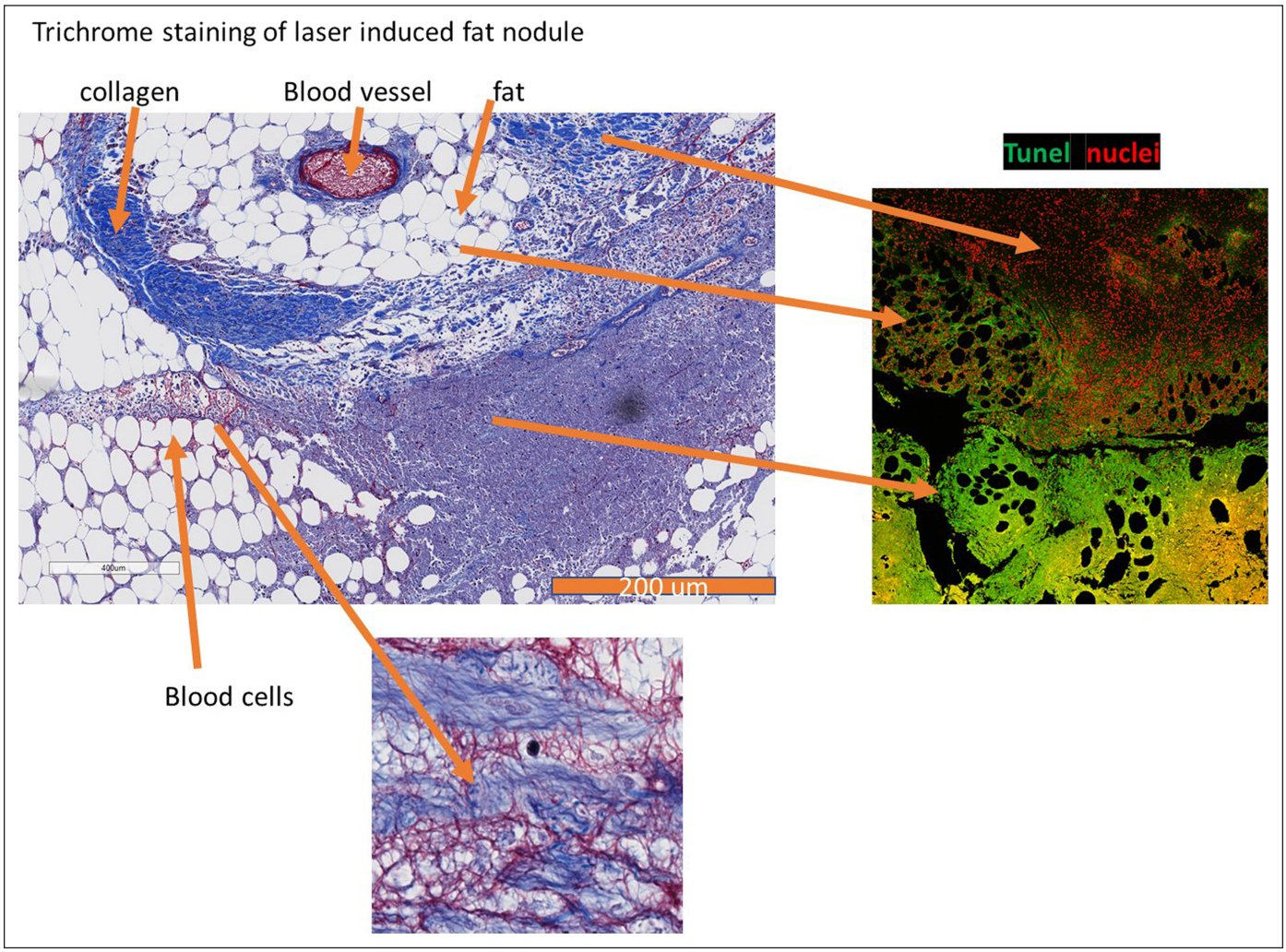

Figure 8. Masson's trichrome staining of a laser-induced fat nodule (left image with high magnification) and TUNEL staining (right image: apoptosis—green, nuclei—red).

follicles. In contrast, the laser-alone treatments achieved subdermal fat necrosis with little effect on dermal collagen. Apoptosis, along with macrophage populations, was highest in the areas of tissue damage based on TUNEL and CD68 macrophage immunofluorescence staining.

Here, we show that single-laser treatment caused erythrocyte extravasation, abundant macrophages, and apoptotic cells, suggesting damage to microvessels in subdermal fat and at the dermal–hypodermal junction. Localized hyperthermia causes fat cell destruction by heating tissue to 42 to 47 °C and subsequent macrophage recruitment (inflammatory response) as evidenced by our findings and previous histologic studies.[16,20,21] Mechanisms such as water-based cooling aim to minimize collateral damage while achieving targeted effects on subdermal fat.[10] In our study, all treatment groups had localized abdominal fat tissue lysis with minimal changes to epidermal and dermal integrity.

Multiple laser treatments resulted in fat tissue reformation with a denser dermal collagen appearance (Figure 6, 3× treatments). Although collagen appeared dense in localized areas, the overall collagen abundance in the dermis was not affected based on immunofluorescence staining of Collagens 1 and 3 (data not shown). Collagen structure may be altered due to loss of other large Extracellular matrix molecules such as hyaluronic acid without affecting the collagen abundance. Complete hyaluronic acid depletion in hyaluronan synthase triple knockout mice resulted in increased collagen density without affecting collagen abundance in the connective tissue, uterine cervix.[22] Despite some collagen accumulation in subdermal fat, dermal collagen and elastin levels remained unchanged. Therefore, these findings in dermal collagen and elastin may not imply clinical benefit.

The 1060/1064 nm diode laser safely induces localized subdermal fat reduction.[8-15,23,24] Enhancements to this mechanism include PEMF energy and RF. PEMF generates a magnetic field and promotes collagen synthesis by changing cyclic-AMP metabolism and affecting fibroblast surface receptors.[25] RF provides thermal stimulation that enhances the rate of fat breakdown (lipase degradation of triglycerides to glycerol and free fatty acid). As expected, the laser–RF/PEMF combination caused extensive damage to subdermal fat, affecting dermal collagen structure and hair follicles. In contrast, the laser-alone treatments achieved subdermal fat necrosis with little effect on dermal collagen.

Although multiple treatments with laser and RF/PEMF were not executed in this study, the treatment would likely amplify the observed

effects, leading to enhanced apoptosis of adipocytes and more extensive fat necrosis and tissue remodeling. This could result in greater fat loss and collagen restructuring, potentially improving skin firmness but also increasing the risk of collagen damage and hair follicle destruction over time, especially with the laser–RF/PEMF combo treatment. Repeated sessions would likely sustain or elevate macrophage activity, contributing to prolonged tissue repair and inflammation. The laser-alone treatments, while still causing fat necrosis, would have a more selective impact, preserving dermal collagen better than the combo treatment. Therefore, careful control of treatment parameters would be essential to maximize results while minimizing adverse effects.

The laser power settings in this study were at 1.4 or 1.2 W/cm^2, the latter for patients receiving RF and PEMF energy. Based on an initial numerical model, a porcine study, and clinical trials[26-31] the authors and device manufacturers determined these parameters optimal for inducing adipocyte damage while preserving skin cells, leading to programmed adipocyte death within the subcutaneous tissues.

Subcutaneous fat nodules are the known and expected side effects, occurring in up to 12% of laser treatments.[12,13] The 2 subcutaneous fat nodules reported in this study either resolved or reduced in size with expected resolution, which aligns with previous clinical studies investigating the 1060 nm laser. Katz and Doherty reported that such nodules typically resolve within 32 to 78 days,[14] and Decorato et al observed that similar nodules were transient and resolved within 2 weeks.[16] Kislevitz et al documented that nodules either resolved or significantly reduced in size by study completion,[12] with further resolution expected over subsequent months. Masson's trichrome showed extravasation of erythrocytes, indicating significant damage to small vessels in the fat tissue with aberrant, dense collagen and adipocyte apoptosis (Figure 8) further confirming the mechanism of action of the laser treatment on fat tissue.

Limitations

This pilot study consisted of a limited number of patients (3 patients per arm). Our findings are also constrained by the variability observed among individuals. These differences within individuals may be attributed to variations in muscle structure between the lateral and medial lower abdomen. Another limitation is that 2 of the patients in Arm 3 treatment group reported using semaglutide drugs ($n = 2$), which is linked to fat and muscle reduction.[32,33] Although these patients' semaglutide usage duration and their impact on subdermal fat are unknown, the patients' abdominal subdermal fat structure appears normal based on trichrome-stained control sections. Other factors such as water content (hydration), genetics, inherent dermal thickness, fat thickness, and immune response differences may contribute to the differences observed histologically among individuals. The next steps include exploring long-term effects and clinical implications in a larger cohort of patients and employing methods such as microbiopsies to minimize trauma to samples.

CONCLUSIONS

These results demonstrate that the 1064 nm diode laser device can be safe and effective for noninvasive fat reduction, as it destroys adipocytes without harming the overlaying skin tissue. The RF/PEMF treatment enhances the lipolytic effect of the laser, but also induces more inflammation and tissue remodeling. These findings have implications for the clinical application and optimization of these technologies for body contouring and aesthetic purposes.

Disclosures

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