OPEN FORUM

Body Contouring

The Impact of the Number and Duration of Treatments With a 1064 nm Diode Laser on Adipocyte Apoptosis: Implications for Noninvasive Fat Reduction Strategies

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Abstract

Background: Noninvasive treatment with a 1064 nm diode laser, generating hypodermal temperatures between 42 and 47 °C, induces adipocyte cell death, offering a solution to reduce fat in problematic areas.

Objectives: The objective of the authors of this study is to determine whether a 15 min treatment provides similar efficacy as the standard 25 min treatment.

Methods: Pigs underwent a 15 or 25 min 1064 nm laser treatment on 6 × 6 cm areas of abdominal skin. Single treatments were administered 14 or 28 days before biopsy, whereas dual treatments were performed at both 14 and 28 days prebiopsy. Hematoxylin and TUNEL staining were used to detect histological changes and apoptosis in harvested skin tissue.

Results: Pig skin subjected to a 25 min diode laser treatment exhibited nearly twice the apoptotic counts in the hypodermis compared with the 15 min treatment (10.5 vs 5.7) at 28 days following treatment. The degree of apoptosis increased with time following the treatment, with nearly twice the apoptosis counts at 28 days than at 14 days following a single 25 min treatment (10.5 vs 5.8). When testing 1 vs 2 treatments, 2 treatments (28 and 14 days before harvesting the tissue) did not result in significantly higher apoptosis than 1 treatment 28 days before harvesting. Apoptosis was primarily localized to adipocytes in the hypodermis and the dermis/hypodermis junction. There were no adverse side effects in the animals.

Conclusions: The degree of apoptosis following a 1064 nm laser treatment was proportional to the treatment duration. The 25 min treatment produced significantly more apoptosis than the 15 min treatment. The process of apoptosis continued over several weeks.

Level of Evidence: 4 (Therapeutic)

Recent advancements in noninvasive fat reduction methods offer patients alternatives to invasive lipolysis or surgical procedures like liposuction. Heat- or cold-based device treatments, typically lasting less than an hour, have emerged as effective solutions for nonobese individuals seeking to eliminate localized fat deposits. Using a wide range of technologies, such as laser, radiofrequency, cryolipolysis, and high-intensity focused electromagnetism, these devices enhance the patient's appearance by reducing localized fat deposits without the associated risks and downtime of surgery.[1-8]

The 1064 nm diode laser noninvasively induces adipocyte apoptosis by elevating temperatures (42-47 °C) within the adipocyte and periadipocyte environments. A period of elevated temperature leads to the breakdown of triglycerides into fatty acids and glycerol, facilitating their elimination from the cell through the fatty acid

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transporter.[9] Once bound to albumin, these triglycerides are transported throughout the body where they become available for energy metabolism by other cells. Additionally, temperatures exceeding 42 °C induce denaturation or hamper the function of proteins vital to the cell's survival, which initiates a cascade of factors, ultimately culminating in the cell's apoptosis or programmed cell death.[10,11]

Although authors of in vitro studies suggest that a 45 °C, 3 min exposure induces adipocyte death in more than half the cells within 72 h, in vivo, a 15 min exposure to 43 to 45 °C has been observed to result in delayed apoptosis and necrosis evident only at Day 9.[10]

The typical treatment duration for 1064 nm lasers is 25 min.[9,12-14] Longer treatments consume more staff time and clinic space, potentially limiting patient throughput. Furthermore, although safety concerns about treatment durations >25 min were raised by Decorato et al, who showed that a treatment duration of 30 min or longer resulted in instances of nodules in some patients,[12] little information is available about the efficacy of treatments shorter than 15 min. In light of this, the objective of the authors of this study is to evaluate the efficacy of a shortened 15 min noninvasive 1064 nm laser diode treatment on pig abdominal tissue through a histological analysis of fat cell apoptosis, comparing it with the impact of the conventional 25 min treatment duration.

METHODS

Device

The Venus Bliss (Venus Al, Toronto, ON, Canada) device is equipped with four 1064 nm diode laser applicators and an $(MP)^2$ applicator that combines radiofrequency (RF) and pulsed electromagnetic field (PEMF) technology. Utilizing a user-friendly graphical interface, operators can manage device settings. In clinical settings, a belt normally secures the laser applicators on the treatment area, ensuring continuous skin contact, although the belt was not used on pigs in this study. Each laser applicator includes a water-cooled window to prevent skin overheating, and 4 touch sensors guarantee proper placement and contact during laser emission. The treatment window covers a 60 × 60 mm area.

The $(MP)^2$ applicator boasts a dual multipolar electrode crown design, featuring a unique array of electrodes that enable swift and efficient treatment of large areas. Beyond skin tightening and circumference reduction, it addresses cellulite, resulting in smoother, firmer skin.[15] Leveraging PEMF and RF technology, this applicator achieves a substantial treatment depth, reaching up to 4.5 cm for enhanced efficacy. The device used in this study had a maximum RF output power of 125 W. The PEMF has a magnetic field of 15 G. The applicator is also able to provide pulsed suction at the surface of the skin. In this study, suction was set at Level 2. All Venus Bliss treatments are noninvasive.

Animal Model and Study Design

This study was approved by the National Council of Animal Experimentation (Number NPC-La-IL-2208-191-4). The animals were housed and the study was conducted at The Institute of Animal Research (Kibbutz Lahav, Israel) following Good Laboratory Practice guidelines.

Table 1. A Treatment Schematic Showing the Duration (25 or 15 min) and Schedule of Treatments for Each Study Arm

Treatment arm label	25 min 2×	15 min 2×	25 min 1×	15 min 1×	25 min 1×	15 min 1×
Treatment 1 (Day 28)	25 min	15 min	25 min	15 min	None	None
Treatment 2 (Day 14)	25 min	15 min	None	None	25 min	15 min

Tissue biopsies were collected after 14 or 28 days following the first treatment. The double treatment group (2×) received the second treatment 14 days after the first treatment.

Two female domestic large white pigs (*Sus scrofa domesticus*), aged ~4 months, were employed in the study. Areas on the abdomen were tattooed to demarcate areas for treatment. Anesthetization was done with 10 mg/kg ketamine and 2 mg/kg xylazine, followed by 1% to 3% in 100% oxygen isoflurane inhalation (3-4 L/min) administered by face mask. Following intravenously catheterization through the lateral ear vein, 5 to 10 mg/animal of diazepam was used. The animals were intubated with an endotracheal tube. Morphine (0.5 mg/kg) was injected intramuscularly (IM) and anesthesia was maintained throughout the procedure by isoflurane inhalation (1%-3% in 100% oxygen); supplemental ketamine and xylazine IM were provided according to the veterinarian's discretion. Each pig had all treatment arms and a negative control area. The treatment areas were treated with the 1064 nm laser applicator for the standard 25 min or for a shortened time of 15 min. Two treatment areas had single treatment sessions either 14 or 28 days before the scheduled tissue harvesting (Table 1). In other treatment areas, treatments occurred both 28 and 14 days before tissue harvesting. As a reference point, a site on each pig was intentionally left untreated, serving as a negative control for the study. At each treatment, the pigs also received treatment with the RF/PEMF applicator. This was not varied, and all treatment arms received RF/PEMF treatment. Commonly used presets were used for the treatment, with the laser set at 1.4 W/cm^2, whereas the $(MP)^2$ applicator power was set at 87.5 W to deliver energy to keep the skin temperature between 42 and 45 °C for 15 min. The vacuum level was set at 2. All areas received the same treatment with the $(MP)^2$ applicator.

Throughout the 28 day duration of the study, the animals were consistently monitored. They were provided with ~1.8 kg of commercial pig diet per day, with free access to water. The housing facilities were maintained at 16 to 27 °C with 30% to 70% humidity. Observations for morbidity, mortality, and adverse events were conducted at least 2 times daily. Additionally, detailed clinical examinations were carried out on the animals at least once a week to ensure a comprehensive assessment of their health and well-being.

On Day 0 (28 days after the first treatment), the pigs underwent anesthesia (as described above) for the collection of tissue samples. Full-thickness biopsies, obtained using a 1 cm diameter punch, were collected from each treatment area and the control site. Each punch biopsy was fixed using paraformaldehyde and then embedded in paraffin for further analysis. These biopsies were subsequently stained with TUNEL, a marker of apoptosis, and hematoxylin and eosin stain (H&E) for tissue structure and cell phenotype.

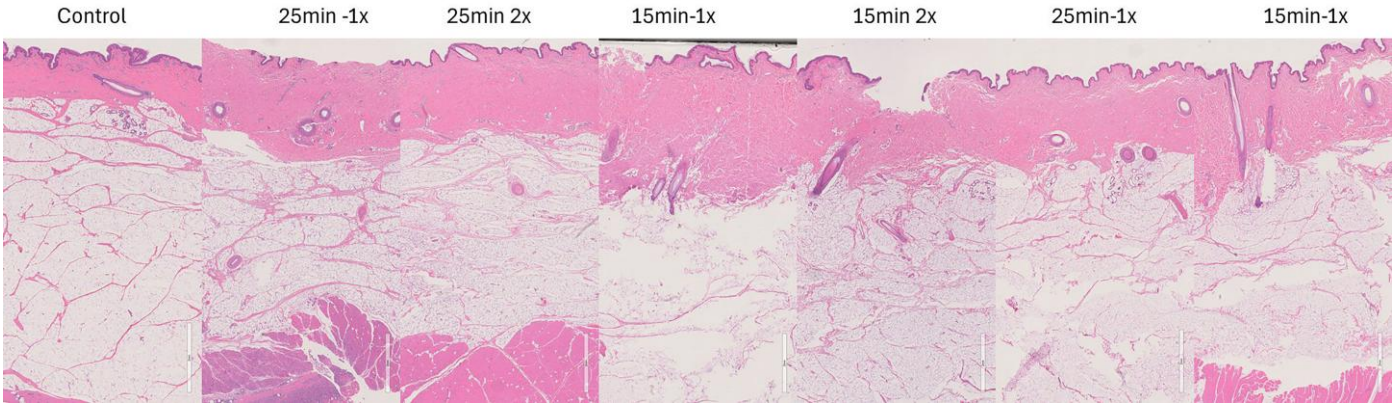

Figure 1. Hematoxylin and eosin staining in pig skin following Venus Bliss (Venus Al, Toronto, ON, Canada) treatment shows structural changes in subdermal fat or hypodermis. Tissues were collected 28 days after first treatment on both single (1×) and double (2×) treatments (second treatment was received 14 days after the first treatment) for 25 and 15 min compared with the untreated control area.

Histology

Slices ~5 µm thick were obtained from the paraffin-embedded blocks (University of Texas Southwestern Medical Center Histo Pathology Core). These slices underwent labeling for apoptosis, utilizing the TUNEL method as described previously.[16-18] Briefly, TUNEL staining for apoptotic cells was done using paraffin sections (5 µm thick), according to the protocol supplied with the Promega DeadEnd Fluorometric TUNEL System (Promega, Madison, WI). Apoptotic cells were labeled with fluorescein, and the sections were counterstained with propidium iodide. Images were obtained using a Zeiss Axioscan 7 microscope (Zeiss, White Plains, NY). Paraffin processing, embedding, sectioning, and histological staining with H&E were performed, as described previously.[19,20] Brightfield images were taken using a NanoZoomer microscope (Hamamatsu Slide Scanner, Hamamatsu City, Japan), and NIH ImageJ software (National Institutes of Health) was used to generate individual images and perform quantification. The images were obtained by the University of Texas Southwestern Medical Center Whole Brain Microscopy Core Facility (RRID:SCR_017949). Each pig provided 4 to 5 tissue samples for each treatment area/study arm.

RESULTS

Clinical Evaluation

Clinical evaluation revealed no observable abnormal signs in the animals throughout the entire study duration. Moreover, all animals demonstrated the anticipated weight gain throughout the study, indicating their overall well-being and normal physiological response to the experimental conditions. Importantly, no adverse side effects, including hyper- or hypopigmentation, burns, purpura, or scarring, were observed in the pigs, indicating the safety of the treatment.

Histological Evaluation of the Treated Sites

All of the treatments resulted in subdermal adipose tissue breakdown (Figure 1) and apoptosis in the dermal subdermal junction and subdermal fat (Figure 2, green/yellow staining). At 28 days following treatment, the 25 min treatment resulted in more apoptosis in the hypodermis compared with the 15 min treatment (Figure 2).

For the single treatment, areas treated for 25 min exhibited nearly 2-fold higher apoptosis compared with those treated for 15 min (10.48 ± 1.36 vs 5.70 ± 0.43) based on TUNEL staining of full-thickness skin sections containing subdermal fat (Figure 3). Additionally, a time-dependent trend was observed, with higher apoptosis 28 days post-treatment compared with 14 days after treatment. For the 25 min treatment, apoptosis at 28 days surpassed that at 14 days, reaching ~2-fold (10.48 ± 1.36 compared with 5.76 ± 0.72). Similarly, for the 15 min treatment, apoptosis at 28 days was ~1.7 times higher than at 14 days post-treatment. An additional treatment 14 days before tissue harvesting did not significantly elevate apoptosis compared with a single treatment 28 days before tissue analysis (10.48 ± 1.36 vs 9.75 ± 1.97 for the 25 min treatment and 5.70 ± 0.43 vs 5.14 ± 1.41 for the 15 min treatment).

DISCUSSION

In this study, the authors illuminate a key finding: a 15 min treatment duration with the noninvasive 1064 nm laser results in diminished adipocyte apoptosis compared with a conventional 25 min treatment. Also, apoptosis is an ongoing process and continues for weeks after treatment as shown by the doubling in the number of apoptotic cells between Day 14 and Day 28 after treatment.

The hyperthermia induced by the laser triggers protein denaturation and aggregation, halting critical cellular processes, such as the cell cycle, protein synthesis, DNA synthesis, DNA repair processes, and cell membrane integrity.[21] This disruption of normal cellular function ultimately initiates an irreversible, controlled process of cell death. A shorter hyperthermia duration (15 min) may inadequately arrest these processes, leading to fewer TUNEL-positive cells or a lower apoptosis rate.

Decorato et al previously looked at fat tissue responses to various laser dosages and times.[12] They reported no fat damage in patients who underwent 15 min treatment. Damage was seen only after at least 20 to 25 min of treatment. However, they did not use any markers of apoptosis, but rather observed cells with an H&E stain, which would not be as sensitive as the TUNEL method used in this study.

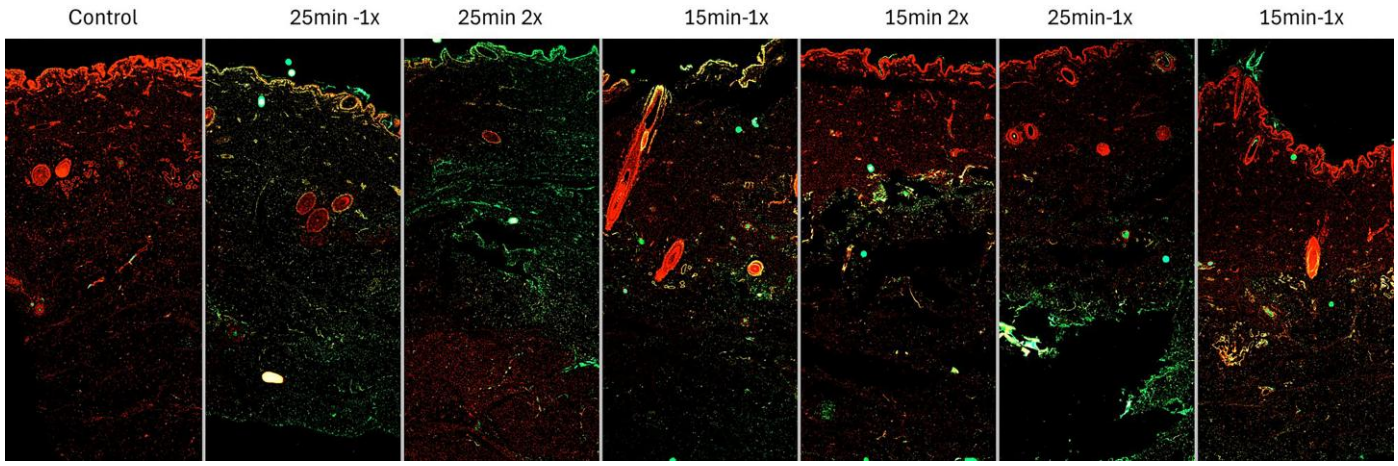

Figure 2. Apoptosis in pig skin following single (1×) or double (2×) Venus Bliss (Venus AI, Toronto, ON, Canada) treatment at 28 and 14 days after first treatment for 25 or 15 min. TUNEL (green) and nuclei (red) stains were used. The 25 min treatment leads to greater levels of apoptosis, especially in adipocytes, which are found in the hypodermis. The process of apoptosis continues over several weeks, with more apoptosis seen 28 days after treatment than at 14 days after treatment.

Here, TUNEL showed adipocyte cell death even with the shorter, 15 min treatment, although at a reduced rate compared with the 25 min treatment. Given that the apoptosis-inducing temperature of 42 to 45 °C is typically reached in the hypodermis within 3 to 5 min of the start of treatment (Venus AI's internal company data), it would suggest that around 20 min at these temperatures is necessary to produce enhanced levels of apoptosis in adipocytes seen in the 25 vs 15 min treatment.

Decorato et al also investigated the kinetics of changes following 1064 nm laser treatment.[12] By Day 14 following treatment, they had noticed changes in the adipocytes consistent with early damage. Their findings match the results of our study, in which using TUNEL, we had found a significant number of adipocytes undergoing DNA fragmentation by Day 14, indicating an ongoing apoptotic process. Given that the number of TUNEL-positive adipocytes was greater at 28 days posttreatment compared with 14 days posttreatment suggests that in some cells, the apoptotic process had started during the laser treatment but had not yet reached the DNA-fragmentation stage. Interestingly, following a treatment 28 days before tissue harvesting with one 14 days before did not result in a further increase in apoptotic cells beyond that shown with just a single treatment. This suggests that there was insufficient time for the second treatment effects to become apparent. Apoptosis from the first treatment may mask the effects and/or delay the time for the second treatment effects to be visible. A slightly longer follow-up period (4 weeks) may have been sufficient to show the effects of the second treatment and increased apoptosis counts. Typically, treatments with the 1064 nm laser are done approximately once every 4 weeks, which may produce better results given the longer intertreatment interval.

Although the authors of this study did not assess changes in fat layer thickness, the authors of previous studies in which the same 1064 nm laser device was used found an average 8% to 9% adipose reduction in ultrasound-measured fat layer thickness, with some patients seeing up to a 27% reduction following a single 25 min treatment.[9,14] Given the current findings, one might anticipate a lower percentage reduction with a shorter 15 min treatment. This prompts the consideration that multiple 15 min sessions may yield efficacy comparable to the standard three 25 min sessions, analogous to findings with other fat reduction devices.[22] The effects of RF and PEMF were not analyzed in this study. All treatment arms received this treatment. RF acts mainly in the dermis, leading to collagen denaturation, followed by new collagen synthesis.[23-25] PEMF further contributes to collagen synthesis by inducing fibroblast growth factor 2.[26,27] Together, RF and PEMF promote skin tightening, which is helpful in patients aiming to lose fat. Because the effects of PEMF are mostly restricted to the dermis, its effect on fat cells is limited and not expected to have much impact in increasing their apoptosis in the hypodermis.

Given its structural and functional similarities to human skin, porcine skin is considered an excellent model for (human) skin studies. The skin sublayers, composition, thickness, and pigmentation, as well as the paucity of hair, in a domesticated pig are similar to that of humans. The thickness of the epidermis of the pig (70-140 µm) favorably compares to the thickness of the human epidermis (50-120 µm).[28] Both pig and human skin have an epidermis and dermis thickness of a combined 1 to 3 mm.[29] Pig epidermis is composed of keratinocytes, and the underlying dermis has a well-defined capillary body, nicely distributed fibroblasts, and an extracellular matrix composed of 95% collagen and 2% elastic fibers that are also comparable to that seen in humans.[30-32] Although per subanatomies, the thickness of porcine skin and tissue laxity may be slightly different from that of humans,[30-33] the thin and loose skin on the abdomen and flanks of the pig is most similar to that of humans and is commonly used as a model in studies. Besides structural similarities, porcine adipocytes undergo apoptosis following exposure to high temperatures and follow the same sequence of physiological processes as human skin postinjury.

The limitations of this study include the examination of only 2 treatment durations. Intermediate durations (eg, 20 min) and durations longer than 25 min would provide further insights into how the heat produced by the 1064 nm diode laser affects adipocyte apoptosis or potentially leads to increased side effects with further extended treatments. Similarly, a more extended follow-up period, such as 2 or 3 months, could provide a more comprehensive understanding of the adipocyte cell death and clearance processes. The 4-week follow-up may not have been sufficient to gauge the full extent of apoptosis,

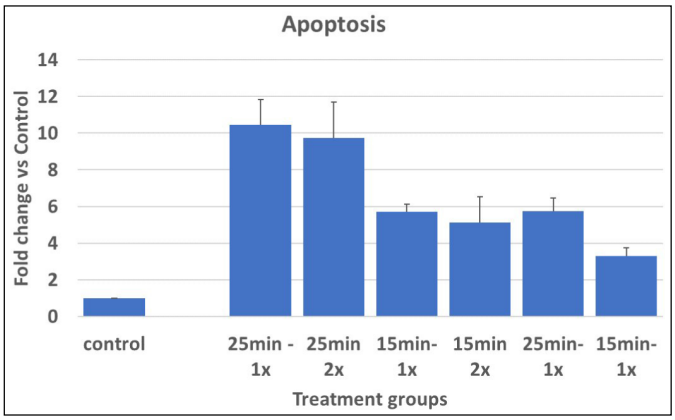

Figure 3. A quantification of TUNEL-stained biopsied skin from pigs treated with Venus Bliss (Venus AI, Toronto, ON, Canada), as shown in Figure 2. A biopsy from an untreated area was used as the control. Longer treatment times and a greater time after treatment show a greater apoptosis of adipocytes.

especially in the 2-treatment arm. Although only 2 pigs were used in this study, the results were consistent between pigs both in this and in previous studies. Thus, to minimize animal harm, we kept the number of pigs low. Furthermore, at least 3 biopsies were harvested from each treatment area, providing greater power to the results.

CONCLUSIONS

In summary, in this study, we establish a clear relationship between treatment duration with the 1064 nm laser and the induction of adipocyte apoptosis. Longer treatment durations, within the range tested, are associated with a greater extent of apoptosis induction, and this process continues in the weeks following treatment. The findings suggest a temporal relationship between the duration of hyperthermia, its related delivered energy, and the subsequent apoptotic response in adipocytes. More than 2 weeks to follow-up may be needed to observe the effects of a second treatment.

Looking forward, a promising avenue for future research involves exploring the effects of multiple treatments, both longer and shorter in duration, on the extent of adipocyte apoptosis. Investigating the cumulative impact of repeated sessions could provide valuable insights into the optimization of treatment protocols for enhanced fat reduction outcomes. Through this study, the authors could potentially further refine the readers' understanding of the dynamics of adipocyte response to laser therapy and guide the development of more effective and patient-tailored noninvasive fat reduction strategies.

Acknowledgments

The raw and processed data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to privacy concerns, some data may be restricted and require appropriate permissions for access.

Disclosures

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