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ORIGINAL RESEARCH

Efficacy of 1060 nm Diode Laser for Non-Invasive Subcutaneous Fat Reduction in Mini-Pigs

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Purpose: To evaluate the efficacy and safety of abdominal fat reduction in mini-pigs, utilizing at 1060 nm diode laser with a wavelength of 1060 nm.

Patients and Methods: The laser system non-invasively disrupts adipose tissue; its effectiveness and safety were evaluated by ultrasound imaging and histological analysis. Laser irradiation was performed with various powers, and the cooling function was activated to prevent skin surface damage.

Results: The dermal tissue temperature increased to at least 43°C during laser exposure, leading to a decrease in abdominal fat thickness after 30 days. Blood tests revealed no significant changes in kidney and liver function but showed increased blood levels of nonessential free acids (NEFAs), likely due to the release of fatty tissue-derived free fatty acids. Histological evaluation demonstrated rapid transformation of adipose tissue into collagen, muscle fibers, and intracellular fibrous tissue.

Conclusion: The 1060 nm laser showed promise as a non-invasive and safe tool for reducing abdominal fat.

Keywords: diode laser, fat, abdominal, non-invasive, preclinical

Introduction

Liposuction is one of the most frequently performed cosmetic procedures worldwide.[1] Through direct removal of fat from the body, liposuction reduces fat and corrects body shape. However, complications after surgery can include skin adhesion, skin necrosis, and pigmentation; cosmetic side effects such as unevenness and sagging skin can also occur.[2] Alternative methods involve localized fat destruction using low-power lasers, high-intensity focused ultrasound, and selective cryolysis.[3–10]

There are various fat reduction devices, including cryolipolysis, radiofrequency treatments, and ultrasonic fat reduction. Cryolipolysis uses extreme cold to freeze fat cells, whereas laser lipolysis breaks down fat cells using laser energy. Cryolipolysis fat reduction treatments generally take longer than laser lipolysis treatments. Both methods are effective for fat reduction, but laser liposuction, which includes a skin-tightening effect, is considered more suitable for areas where both fat reduction and skin improvement are needed.[11] Both radiofrequency and ultrasonic lifting deliver thermal energy to the deeper layers of the skin. The difference is that radiofrequency is effective for larger areas, while ultrasound is particularly effective for targeted areas such as wrinkles. Laser liposuction with skin-tightening effects may be more suitable for areas that require both fat reduction and skin improvement. On the other hand, the precision of ultrasonic liposuction makes it an excellent choice for individuals seeking minimal disruption to delicate areas or surrounding tissues.[12]

Laser lipolysis was first introduced in 1994 using a fiber laser inserted into a thin cannula. It is minimally invasive and causes minimal bleeding[13,14] In the 2000s, Blugerman, Schavelzon, and Goldman established the principles and techniques of fat disruption using a laser.[13] Since liposuction received marketing authorization from the United States

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Food and Drug Administration in October 2006, its effectiveness in the removal of fatty tissue has been continuously evaluated.[3,13] Compared to traditional liposuction, this method allows for selective heating of fat areas through minimal incisions, reducing thermal damage to surrounding tissues and leading to an increased frequency of use. In particular, with lasers, the energy is absorbed by the fat cells and aqueous chromophores, which is then converted into heat, causing damage to the fat area.[15,16] To determine more effective methods for laser-assisted lipolysis, comparative studies have been conducted to observe the effects based on characteristics such as the type and wavelength of the laser. Recently, efforts have been made to develop methods for fat reduction without the need for incisions.

Laser lipolysis is a minimally invasive procedure that uses a 1–2-mm-diameter fiber optic cannula to deliver laser energy to the treatment area, heating the tissue to break down fat.[17,18] Such heating damages the membranes of fat cells, causing their contents to be released into the extracellular space; this process induces triglyceride leakage from the fat cells, leading to decreased size, necrosis, and/or apoptosis. Next, fat is removed through a suction cannula inserted into the tissue. This approach has resulted in a > 10% reduction in fat volume.[5,18,19] Laser fat reduction prevents bleeding by coagulating blood vessels, reduces scar formation to shorten recovery time, and induces collagen production to strengthen the skin.[13] Lasers of various wavelengths have been evaluated in terms of the level of energy delivered to the target.[17] A wavelength of 924 nm showed the greatest difference in absorption by fat compared with skin, but it did not tighten the skin.[13] In contrast, a 1064 nm laser had a better rate of absorption by fat, penetrated > 900 nm of tissue, and could target subcutaneous fat while protecting dermal tissue.

The 1064 nm wavelength had a smaller absorption coefficient than 980 nm, it penetrated deeper into the fat tissues and could be more effectively used in laser lipolysis. The main mechanism of action of the 1060nm laser for lipolysis is heat, which increases the local rate of decomposition of fat cells. This increase in heat breaks down triglycerides into free fatty acids and glycerol, which are then transported out of the cell via fatty acid transporters. They then enter the blood and bind to albumin, allowing them to be transported throughout the body and metabolized by cells as needed.[15,20]

In this study, we non-invasively evaluated the effectiveness and safety of fat reduction in mini-pigs using a 1060 nm diode laser.

Materials and Methods

Animals

Three 12-month-old male mini-pigs (M-pig, *Sus scrofa domestica*; 70–80 kg body weight) were procured from Cronex (Cheongju, South Korea). The mini-pigs were raised in an AAAALAC-certified animal-breeding facility under controlled environmental conditions of 12-h cycles with a temperature of 20–24°C, relative humidity of 50 ± 20%, and illumination intensity of 150–300 lux. To anesthetize the mini-pigs, 10 mL of a mixture of Zoletil (Virbac; a general anesthetic) and Rompun (Bayer), a muscle relaxant, were injected into the muscle at a ratio of 6:4. Each mini-pig was anesthetized and positioned dorsally on the operating table for an abdominal irradiation laser. To maintain anesthesia, a mixture of isoflurane and oxygen gases was administered via inhalation at a ratio of 2:1. Laser irradiation was performed on four randomly selected abdominal areas on all three mini-pigs, each of which had been tattooed with a 6 × 4 cm rectangle. Ethical approval was obtained from the Ethics Committee of the Institutional Animal Care and Use Committee of CRONEX Inc. (Cheongju, Korea; CRJONEX-IACUC:201708002). All in vivo experiments using minipigs were performed in accordance with the Institutional Animal Care and Use Committee and the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Laser System and Irradiation Protocol

We compared the equipment specifications of laser devices already used in previous studies (Table 1). We used a Slimus device (Hironic, South Korea), which features an InGaAs diode laser with a central wavelength of 1060 nm The laser device was a Slimus instrument (Hironic, South Korea), which comprises an InGaAs diode laser with a central wavelength of 1060 nm. The laser device is cooled to prevent damage to the skin surface (Table 2). Laser irradiation was performed by marking four positions on the abdominal region of the mini-pig (Figure 1B). The three mini-pigs were assigned to Groups I, II, and III, and irradiated with 0, 1.0, 1.1, and 1.2 W/cm^2 (Table 3) with the cooling function activated.

Table 1 Summary of Laser Lipolysis Research

Study	Laser type	Wavelength	Power	Pulse duration	Total energy
Alberto Goldman. (2006)[21]	Nd:YAG (Deka, Florence)	1,064 nm	6 W (40-Hz frequency)	100 μs	-
William G. Stebbins et al (2011)[22]	Diode (Lipotherme^{TM}, Osyris Medical)	980 nm	25 W (50–60 Hz frequency)	Continuous	-
Kwan Chul Tark et al (2009)[23]	Nd:YAG (AccuSculpt^{TM}, Lutronic Corporation)	1,444 nm	8 W (40 Hz frequency)	200 μs	240 J
Mikaela Kislevitz et al (2021)[20]	Diode (Venus Bliss)	1,060 nm	1.1 W/cm^2, 1.4 W/cm^2	-	-

Table 2 Characteristics of 1060 nm Diode Laser Devices

Item		Laser
Wavelength		1060 nm ± 10% nm
Power density		Max. 1.4 W/cm^2
Power step		0.05 W/cm^2
Operating mode		Continuous with duty cycle
Laser spot size		4 cm × 6 cm
Electrical Requirement		AC 220 V, 50/60 Hz, under 4 kVA
Size (W × D × H)		560 × 1075 × 1545 mm
Weight		120 kg
Touch display		10.4”
Water Tank		1.0 L
Water Jacket		5 °C/W Max water temp=30 °C
Hand piece	Number of applicators	4EA@Multiple applicators
	Treatment	4 × 6 cm
	Weight	1.63 kg/EA
	Safety function	Four contrast sensors
		Contrast cooling

Evaluation of Abdominal Temperature and Fat Thickness

Thermal changes on the surface and within the skin were measured using an infrared thermometer (Fluke) and a thermocouple (Extech). The skin surface temperature was measured before and after laser irradiation for 25 min. The skin in the irradiated area was incised, and the temperature in the skin was measured. Next, the inner temperature was measured by placing a thermocouple 20 mm from the skin surface. As a result of examining subcutaneous fat thickness based on obesity level, the thickness was less than 20.4 mm in men and more than 20.4 mm in women, so the depth was set at 2 cm in this study.[24,25] Eight ultrasound images were acquired before and 1, 7, 14, 30, 60, and 90 days after laser irradiation. The thickness of the fatty part of the abdomen was measured using ultrasound images.

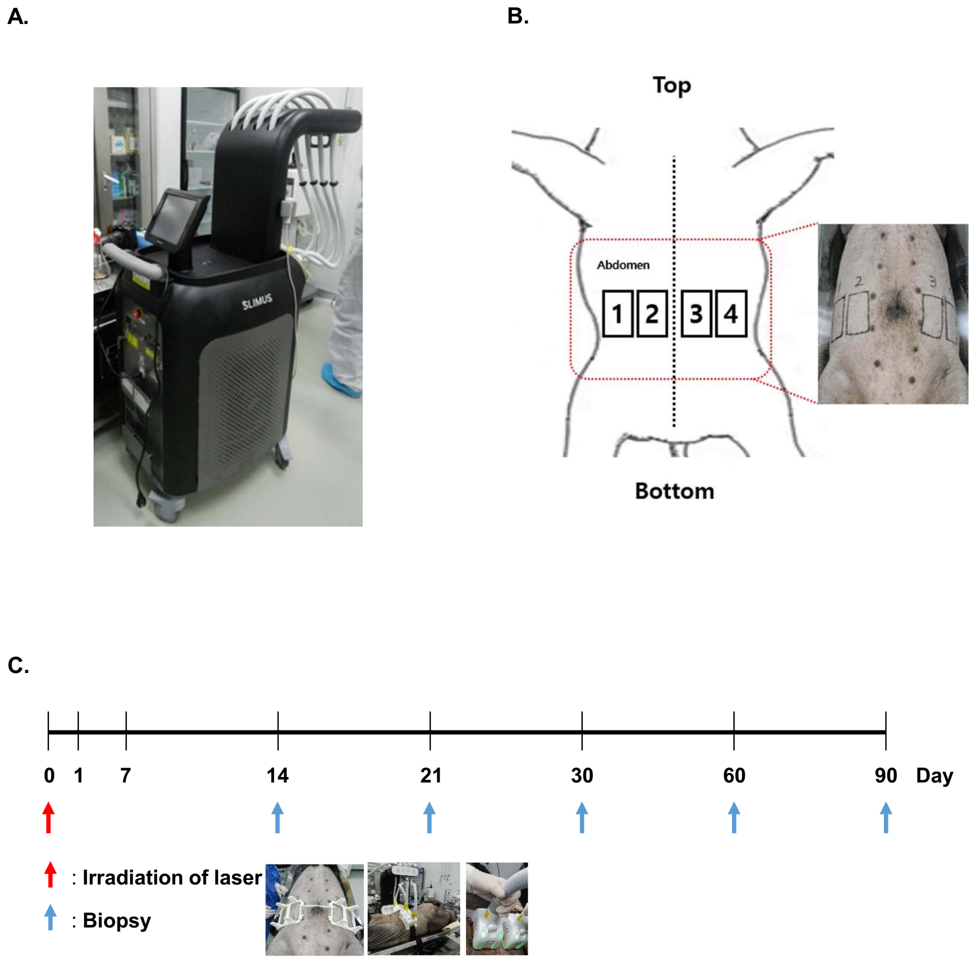

Figure 1 Laser device (1060 nm) and irradiation protocol. The 1060 nm wavelength InGaAs diode laser system (Slimus, Hironic) has four irradiation ports **(A)**. Laser irradiation was performed at four arbitrarily selected points on the abdomen of each mini-pig **(B)**. Visual observation and histological analysis via biopsy were performed at 14, 21, 30, 60, and 90 days after laser irradiation **(C)**.
Abbreviations: AUC, area under the curve; LS, least squares; NE, not estimable.

Observation of Abdominal Skin Surface

Skin surface erythema, swelling, inflammation, and pustules were visually evaluated before and after irradiation with the 1060 nm laser. Changes in the abdominal skin were classified based on the Banff criteria (Table 4).[26,27]

Table 3 Protocol for Laser Irradiation

Group	Position of laser irradiation	Protocol of laser irradiation
1	1	1.0 W/cm^2 (cooling x)
	2	1.1 W/cm^2 (cooling O)
	3	1.2 W/cm^2 (cooling O)
	4 (control)	0 W/cm^2

(*Continued*)

Table 3 (Continued).

Group	Position of laser irradiation	Protocol of laser irradiation
II	1	1.0 W/cm^2 (cooling O)
	2	1.1 W/cm^2 (cooling O)
	3	1.2 W/cm^2 (cooling O)
	4	0 W/cm^2
III	1	1.0 W/cm^2 (cooling O)
	2	1.1 W/cm^2 (cooling O)
	3	1.2 W/cm^2 (cooling O)
	4	0 W/cm^2

Table 4 Skin Rejection Classification (Modified Banff Criteria)

Grade	Findings
0	None
0.5	Rare (less swelling, inflammation, and pustules)
1	Mild (mild swelling, inflammation, and pustules)
2	Moderate (moderate swelling, inflammation, and pustules)
3	Severe (severe swelling, inflammation, and pustules)

Blood Tests

Blood was collected at 0, 2, 4, 8, 12, 24, 48, and 72 h after laser irradiation. To evaluate kidney and liver function and lipid levels, serum was separated by centrifugation at 12,000 rpm and 4°C. Subsequently, the serum values of total protein (TP), albumin (ALB), globulin (GLB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin/globulin (A/G), total bilirubin (T-BIL), cholesterol (CHO), blood urea nitrogen (BUN), calcium (CA), inorganic phosphate (IP), total triglyceride (TG), creatinine (CREA), HDL-cholesterol, LDL-cholesterol, and nonessential free fatty acids (NEFAs) were measured (Table 5).

Histological Evaluation

For histological evaluation, biopsies were performed at 14, 30, 60, and 90 days after laser irradiation; the tissues were stored in 10% (v/v) formaldehyde. Subsequently, they were embedded in paraffin and sectioned at 4 µm thickness using a microtome (RM2125, Leica). The sections were stained with hematoxylin and eosin (H&E) and Masson's Trichrome (MT) to visualize collagen deposition; they were stained with Oil Red O to observe changes in adipocytes caused by necrosis within fat tissue.

Statistical Analysis

Data are presented as means ± standard deviations. Data analysis was conducted using Prism ver. 9 software (GraphPad Software, Inc., La Jolla, CA, USA).

Table 5 Blood Parameters

Group		TP	ALB	GLB	ALP	AST	ALT	A/G	T-BIL	CHO	BUN	CA	IP	TG	CREA	HDL-C	LDL-C	NEFA
		g/dL			IU/L			RATIO	mg/dL									mEq/L
I	**0 h**	5.5	3.1	2.4	95	36	45	1.3	0.01	66	8.9	8.5	4.7	14	1.24	28	37	264
	2 h	6.6	3.6	3.0	97	43	54	1.2	0.00	**79**	9.8	9.5	5.2	15	1.30	32	43	97
	4 h	6.2	3.5	2.7	91	44	51	1.3	0.02	76	11.0	8.7	5.0	12	1.31	31	42	78
	8 h	6.8	3.8	3.0	102	74	57	1.3	0.00	77	15.4	9.3	5.9	17	1.58	31	46	61
	12 h	7.2	4.0	3.2	105	75	62	1.3	0.00	80	15.4	10.1	5.7	19	1.62	32	47	67
	24 h	7.1	3.9	3.2	84	77	66	1.2	0.03	87	10.1	8.9	4.6	11	1.48	32	56	222
	48 h	7.0	3.8	3.2	78	102	64	1.2	0.00	94	8.8	10.0	5.2	17	1.46	36	59	121
	72 h	6.9	3.8	3.1	98	62	61	1.2	0.00	98	9.2	9.9	5.7	16	1.44	36	65	31
II	**0 h**	5.8	3.3	2.5	35	63	44	1.3	0.10	57	5.0	7.8	5.2	12	1.09	20	36	836
	2 h	6.8	3.9	2.9	45	64	54	1.3	0.08	71	6.4	9.3	5.1	11	1.36	25	43	102
	4 h	7.5	4.2	3.3	48	88	61	1.3	0.03	73	7.2	9.7	5.0	12	1.38	27	48	116
	8 h	6.7	3.9	2.8	40	77	56	1.4	0.07	64	7.4	9.2	5.1	12	1.36	22	43	130
	12 h	7.0	4.0	3.0	42	49	56	1.3	0.12	67	7.1	8.9	5.0	18	1.40	23	43	280
	24 h	7.6	4.3	3.3	44	64	62	1.3	0.05	78	8.9	9.6	5.1	16	1.60	25	53	196
	48 h	7.3	4.2	3.1	47	36	57	1.4	0.03	72	7.8	10.2	5.7	34	1.40	28	47	63
	72 h	7.1	4.0	3.1	55	29	51	1.3	0.04	68	8.1	10.0	5.1	39	1.20	27	40	37
III	**0 h**	5.4	3.3	2.1	37	31	51	1.6	0.03	60	4.5	4.5	4.5	24	1.06	25	32	412
	2 h	6.9	4.2	2.7	50	68	68	1.6	0.05	78	5.6	5.6	5.4	11	1.25	32	43	137
	4 h	6.7	4.1	2.6	50	46	66	1.6	0.03	75	7.4	7.4	5.4	18	1.42	33	43	155
	8 h	6.9	4.2	2.7	48	37	65	1.6	0.03	79	7.8	7.8	6.1	13	1.36	31	50	222
	12 h	7.5	4.5	3.0	52	47	71	1.5	0.11	82	9.0	9.0	6.0	14	1.51	32	54	284
	24 h	7.3	4.5	2.8	56	47	71	1.6	0.04	75	12.0	12.0	6.0	14	1.54	28	53	139
	48 h	7.7	4.6	3.1	53	67	69	1.5	0.03	83	9.7	9.7	6.0	43	1.33	33	52	51
	72 h	7.4	4.6	2.8	43	28	62	1.6	0.13	86	6.8	6.8	6.0	22	1.25	35	53	141

Abbreviations: TP, total protein; ALB, albumin; GLB, globulin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; A/G, albumin/globulin; T-BIL, total bilirubin; CHO, cholesterol; BUN, blood urea nitrogen; CA, calcium; IP, inorganic phosphate; TG, total triglyceride; CREA, creatinine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, nonessential free fatty acid.

Results

Abdominal Temperature

The abdominal surface temperature before laser irradiation was 32°C; during irradiation by the 1060 nm laser, the temperatures were 25.0 ± 0.3°C (1.0 W/cm^2), 25.6 ± 0.7°C (1.1 W/cm^2), and 24.4 ± 0.7°C (1.2 W/cm^2) (Figure 2). After laser irradiation, the temperatures were 24.1 ± 0.3°C (1.0 W/cm^2), 23.1 ± 0.7°C (1.1 W/cm^2), and 23.7 ± 0.0°C (1.2 W/cm^2). When the temperature in adipose tissue exceeds 40°C, injury and inflammatory responses occur in the affected tissue area.[28,29]

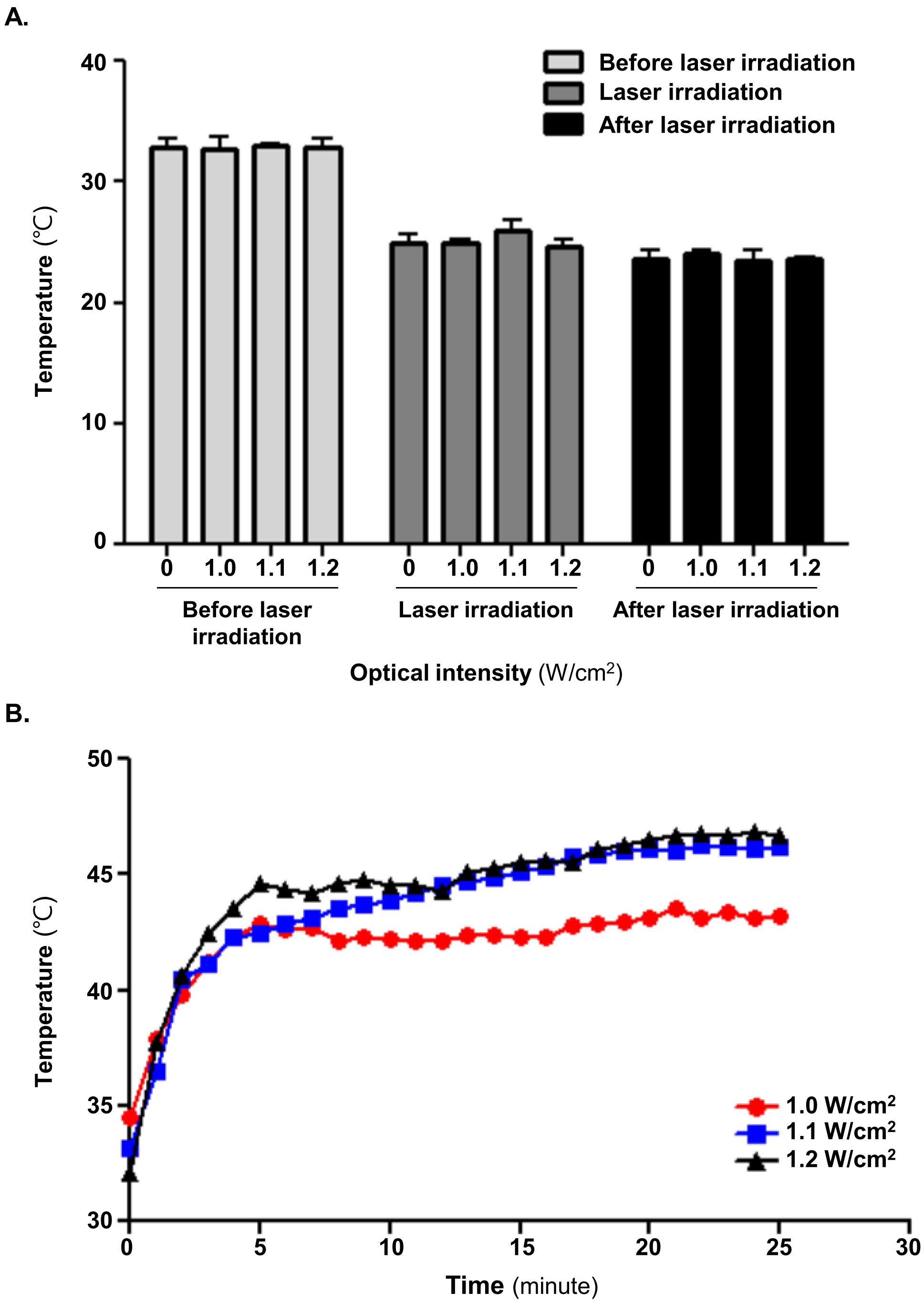

Figure 2 Temperature variation in the skin surface and subcutaneous tissue before and after 1060 nm laser irradiation (1.0, 1.1, and 1.2 W/cm^2) with cooling. The skin surface temperature was 32°C before laser irradiation, compared with 24–26°C during and after laser irradiation (**A**). The subcutaneous tissue temperature was > 43°C after irradiation with 1.0, 1.1, and 1.2 W/cm^2 (**B**).

The internal abdominal temperature was measured by making an incision and placing a thermocouple 20 mm deep from the surface. When irradiated with 1.0 W/cm^2, the temperature increased from 34.5°C to 42.6°C within 6 min and was 42.7 ± 0.5°C at 25 min. Similarly, when irradiated with 1.1 and 1.2 W/cm^2, the temperature increased from 33.2°C to 42.9°C (1.1 W/cm^2) and from 32.1°C to 44.4°C (1.2 W/cm^2) after 6 min. The temperatures were 45.1 ± 1.1°C and 45.6 ± 0.1°C at 25 min.

Fat Thickness

To assess the effect of laser irradiation on abdominal thickness, ultrasound examinations were performed before laser irradiation (day 0), and at 7, 14, 30, 60, and 90 days after irradiation. At 7 and 14 days after laser irradiation, ultrasound revealed increases in abdominal fat thickness (Figure 3). After irradiation with 1.1 W/cm^2, abdominal thickness decreased by mean proportions of 40% and 30% at 60 and 90 days, respectively, compared with before irradiation.

Skin Surface Abnormalities

In Group I, burns were observed on skin that had been irradiated with 1.0 W/cm^2 for 25 min without cooling. After 30 days, skin surface abnormalities ranged from rare (0.5) to severe (3). In contrast, in Groups II and III, no abnormalities were identified on skin that had been irradiated at the same intensity with cooling (Figure 4).

Histological Analysis

H&E staining showed destruction of fat tissue and formation of fibrous tissue 14 days after laser irradiation (Figure 5A). Fibrous tissue can form between fat tissues in a manner dependent on the energy intensity and surrounding vasculogenesis. At 60 and 90 days after irradiation, cytoplasmic and intracellular fibrous tissue had formed in adipose tissue.

The intensity of the irradiation increased in all three groups, leading to the formation of collagen (visualized by MT staining), myofibrils, and intracellular fibrous tissue (Figure 5B). We observed an increase in collagen after 30 days.

Oil red O staining suggested that fat cells were destroyed 14 days after laser irradiation (Figure 5C); the destroyed fat cells gradually recovered at 30, 60, and 90 days.

Blood Parameters

There were no changes in the values of TP, ALB, GLB, ALP, AST, ALT, A/G, T-BIL, CHO, BUN, CA, IP, TG, CREA, HDL-C, LDL-C, or NEFA before laser irradiation. However, in Groups I, II, and III, the NEFA level increased to 222 mEq/L at 24 h (3.6-fold increase), 280 mEq/L at 24 h (2.1-fold), and 222 mEq/L at 8 h (1.3-fold) after irradiation, respectively. Therefore, after irradiation, triglycerides are broken down into free fatty acids, increasing free fatty acid levels. However, this level decreases after a certain period. Consequently, it is estimated that the free fatty acids from the fat tissue broken down by laser irradiation were detected in the blood.

Discussion

We performed ultrasound imaging and histological analysis to evaluate the effectiveness and safety of lipolysis in minipigs using a 1060 nm laser.

During laser irradiation, thermal burns occurred on the skin surface in Group 1 when irradiated without cooling. However, there was no evidence of damage or thermal injury to the abdominal skin surface during laser irradiation with cooling enabled. These findings were attributed to the effect of the 1060 nm laser on water and fat in dermal tissue, which includes an impact on the epidermis.[30,31] To mitigate this effect, contact cooling is performed to dissipate heat.[32] In the present study, the use of cooling during laser irradiation maintained a skin surface temperature of 22–24°C, preventing damage to the skin surface.

The 1060 nm laser is absorbed by subcutaneous adipose tissue, which leads to the heating of fat cells at 42°C to 47°C, triggering apoptosis and subsequent fat reduction.[30–34] In the present study, although the temperature varied according to the laser energy applied, the dermal tissue temperature increased to 43°C and remained elevated during laser irradiation. Ultrasound showed that abdominal fat thickness increased at 14 to 30 days after laser irradiation, likely due to the

A.

B.

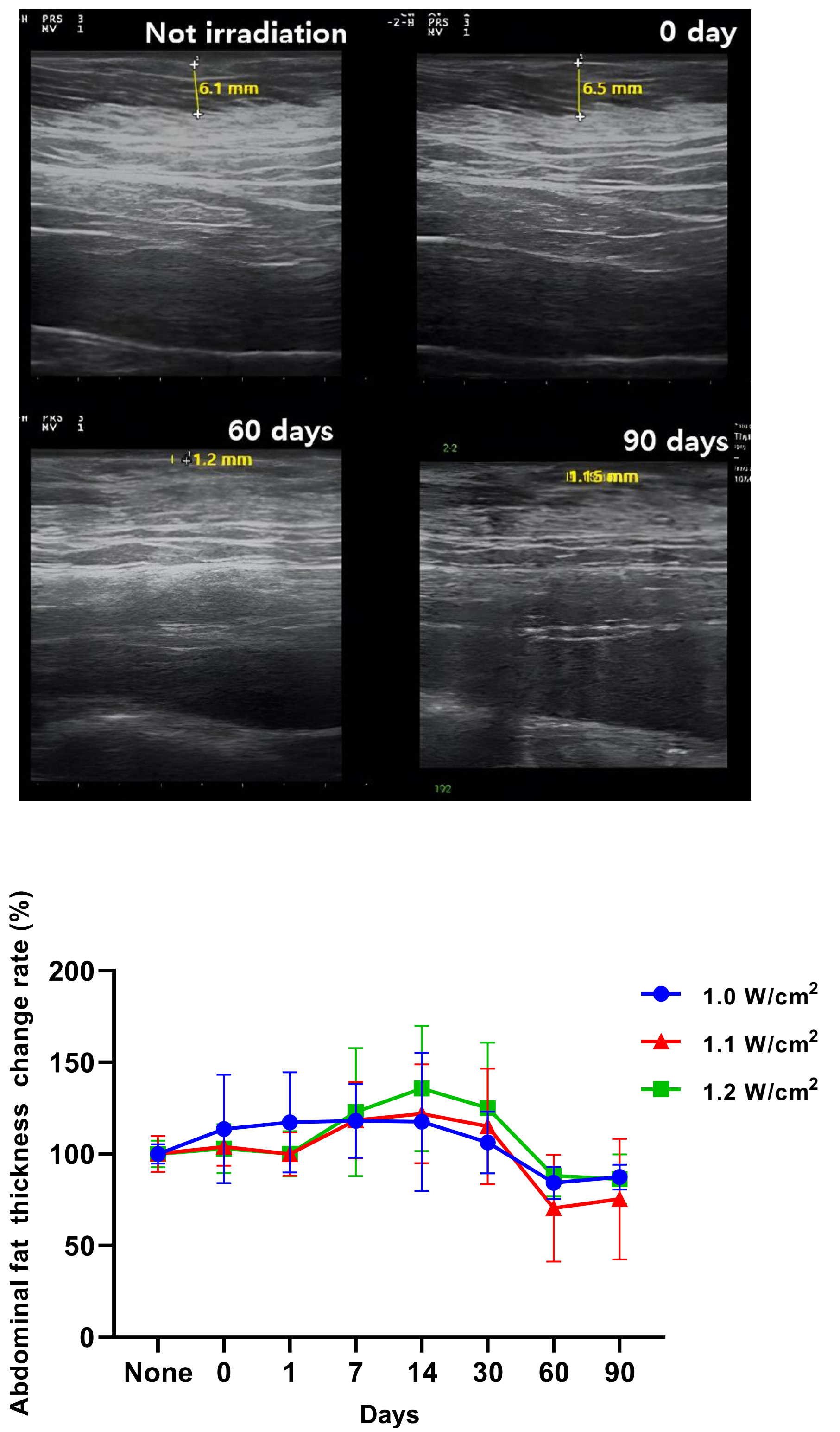

Figure 3 Ultrasound measurements before and 60 and 90 days after laser irradiation **(A)**. Change in fat layer thickness after laser irradiation **(B)**; error bars indicate standard deviations. Ordinary two-way analysis of variance; Tukey's test for multiple comparisons; n = 9.

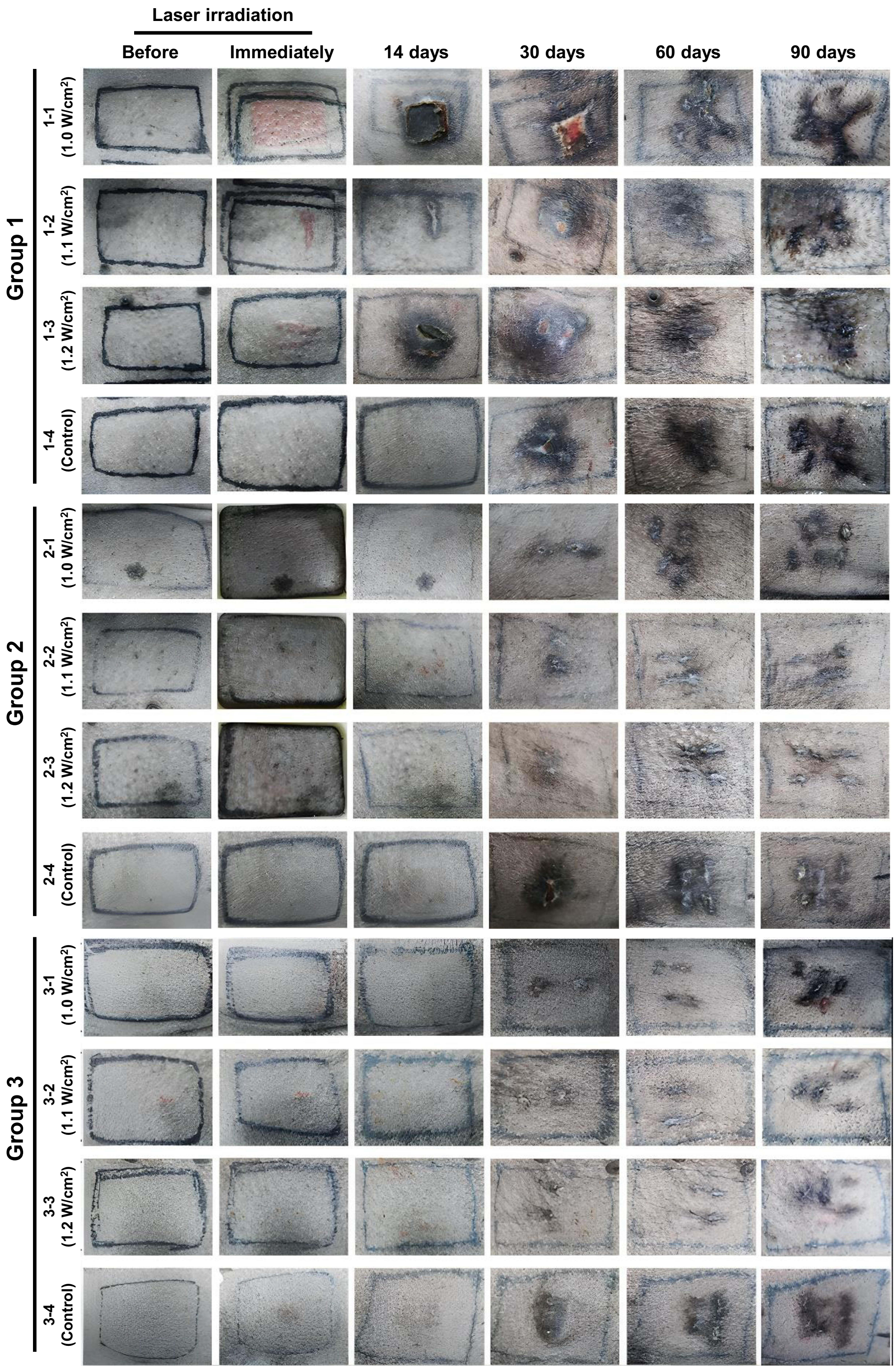

Figure 4 Visual observations of the abdominal skin surface after irradiation with 1.0, 1.1, and 1.2 W/cm^2, with or without cooling. In the absence of cooling (Group 1–1), severe (3) skin surface abnormalities were evident after 30 days. Conversely, no abnormalities were observed when cooling was used (Groups 2 and 3).

hardening of abdominal fat. Further research is required to confirm this finding. However, fat thickness decreased after 30 days; at 60 and 90 days, it remained significantly lower than in the control condition.

The absence of significant changes in blood parameters suggested that laser irradiation did not affect kidney and liver function. However, the NEFA levels increased after irradiation. It that an increase in heat breaks down triglycerides into free fatty acids and glycerol, which are then transported out of the cell via fatty acid transporters, enter the bloodstream, bind to albumin, and are carried throughout the body to be metabolized by cells as needed. Subsequently, they decreased over time, likely because of the detection of adipose tissue-derived free fatty acids in blood, which were released due to

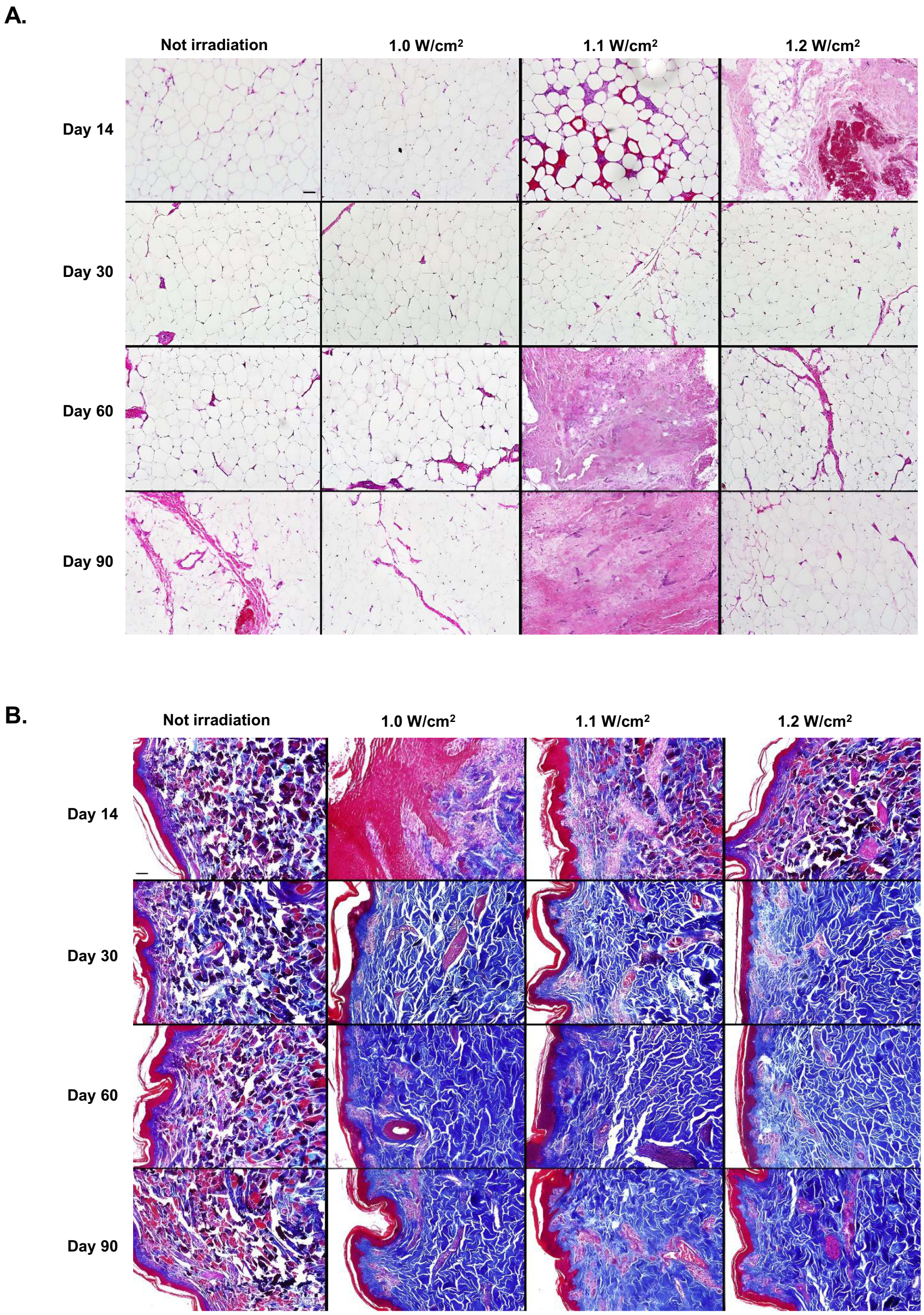

Figure 5 Continued.

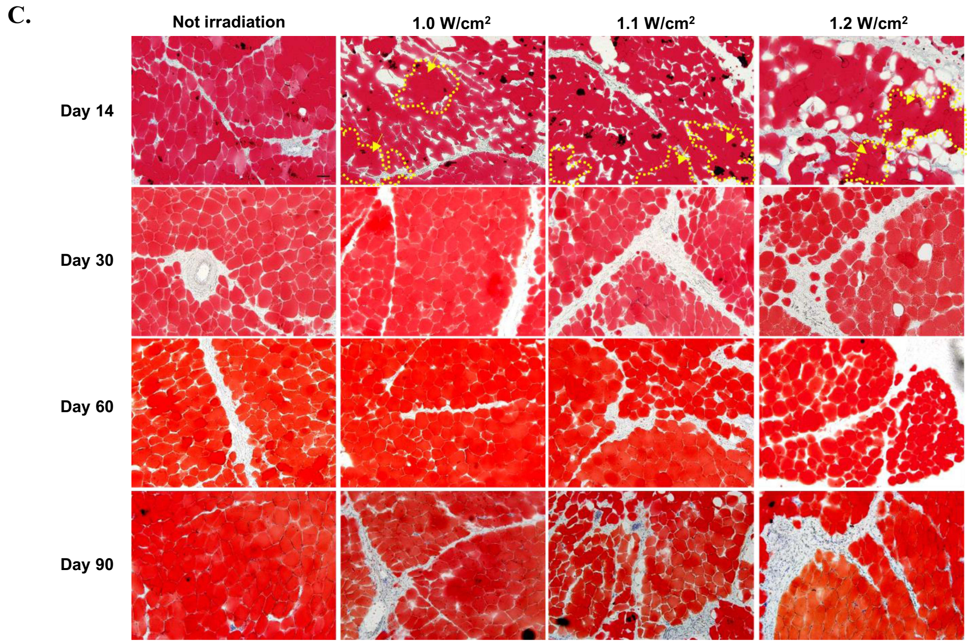

Figure 5 Hematoxylin and eosin (H&E), Masson's trichrome (MT), and Oil red O staining. H&E staining **(A)** indicated that adipose tissue was destroyed at 14 days; at 60 days, cytoplasmic and intracellular fibrocytes were observed. MT staining showed After 90 days, adipose tissue was absent **(B)**. Oil red O staining showed that at 14 days, adipocyte morphology was disrupted; at 90 days, adipocyte formation was evident **(C)**. Original magnification, ×100.

laser irradiation of fat tissue.[8,35] The laser-irradiated area exhibited an altered fat cell morphology; over time, cytoplasm, intracellular fibrous tissue, and muscle fibers were detected in areas treated with the laser.

The 1060 nm laser demonstrated optimal conditions for observing changes in collagen, muscle fibers, and intracellular fibrous tissue, along with changes in adipose tissue, at an irradiation energy of 1.1 W/cm^2.

Conclusion

We conducted a preclinical study in mini-pigs to evaluate the effectiveness and safety of abdominal fat dissolution using a 1060 nm laser. Ultrasound imaging and histological analysis results showed that 1.1 W/cm^2 laser irradiation reduced the volume of adipose tissue by 40% and 30% at 60 and 90 days, respectively. Histological evaluations and blood tests confirmed the safety of lipolysis using the 1060 nm laser. Therefore, non-invasive laser treatment can safely reduce abdominal fat.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflict of interest in this work.

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