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A Focused Monopolar Radiofrequency Causes Apoptosis: A Porcine Model

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ABSTRACT

Background: The purpose of this study was to demonstrate the effect of monopolar, focused radiofrequency (RF) with embedded cooling on subcutaneous skin structures. Specifically, the study was to prove that the monopolar RF with cooling can selectively heat fat, causing disintegration of adipocytes and programmed cell death (apoptosis) of the subcutaneous fat cells.

Methods: A non-invasive monopolar RF device with cooling (Exilis Elite, BTL Industries, Framingham, MA) was used to reduce abdominal fat in a porcine model. The study was done on 3 Vietnamese pigs in a certified veterinary facility. The treatment was delivered to an area the size of 20 x 10cm. The treatment duration was 11 minutes, 30 seconds. Biopsy samples were taken before the first treatment, 1 hour post each treatment, as well as 8 and 20 hours post each treatment. Programmed cell death (apoptosis) was monitored using the TUNEL method. The temperature was measured on the skin surface by an infrared thermal imager and built-in IR thermometer, and by an internal probe inserted into various depths of the subcutaneous layer. The internal probe placement was monitored by diagnostic ultrasound examination.

Results: The temperature in the treated adipose tissue was higher compared to the skin surface temperature. The average temperature gradient observed was 3.1°C. Due to the temperature gradient the skin surface remained intact, while subcutaneous layers showed significant changes. The TUNEL method proved large-scale apoptosis of fat cells after each treatment. The apoptotic index increased from 7% before the first treatment to an average of 53.4%, 39.6%, 40.2%, and 44.7% respectively for each treatment. In the three-month follow up the apoptotic index dropped back to 11.7%. Histology, blood biochemistry and hematology samples showed mild to no signs of inflammation in the treated area.

Conclusion: The study has shown that use of monopolar, focused radiofrequency can induce substantial apoptotic process in a porcine model. The data suggests that the monopolar, focused radiofrequency device can be used for reduction of fat and body shaping.

J Drugs Dermatol. 2014;13(11):1336-1340.

INTRODUCTION

The demand for safe and effective devices for non-invasive body shaping and reduction of fat has steadily risen over the last decade. Many modalities have been developed to target adipocytes, including ultrasound, radiofrequency, and various cooling and light based devices.[1,2,5]

In this study, we evaluated the ability of a monopolar focused radiofrequency device to induce apoptosis in the subcutaneous fat. The device delivers uniform heating at controlled depths to the subcutaneous tissue, due to its adjustable built-in cooling system. The clinical efficacy was intended to safely and efficiently deliver maximum power and speed of high frequency radio waves using an active cascade of hardware and software safety elements.

METHODS

This study was carried out in a veterinary and a laboratory certified to Good Laboratory Practices (GLP) standards. Animal care was in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and with the law on the Protection of Animals Against Cruelty. The protocol of the study was approved by the Institutional Animal Care and Use Committee (IACUC) and the Committee for Animal Protection of the Ministry of Agriculture of the Czech Republic. Procedures used conformed to accepted practices and to minimize or avoid causing pain, distress, or discomfort to the animals. In those circumstances in which study procedures were likely to cause more than momentary or slight pain or distress, the animals received appropriate analgesics or anesthetics. During anesthesia the life functions and pain perception of treated animals were monitored to assure full insensibility during painful treatment and correct recovery. The number of animals selected for use in this study was considered to be the minimum (OECD Principles) number necessary to meet scientific and regulatory guidelines for this type of study.

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Study Design Justification: Swine is a suitable animal model due to the similarity between human and swine dermal and subcutaneous structures. Additional anatomical similarities with humans include renal morphology, eye structure, skin, and tooth development. The pig is also one of few animals that will voluntarily eat to obesity.[7,8] The 3 study animals were housed individually and were continuously monitored by cameras. The room temperature was maintained at 20°C. Cleaning of the stall and surrounding area was performed on a daily basis. Food feeders were sanitized twice a week. During the acclimation and study period, animals were fed with complete cereal diet for swine (CDP), in the amount of 25g per kilogram of the body weight of the CDP provided per animal per day. The quality of the water was monitored during the whole study period. The acclimation period was 13 days. No prophylactic or therapeutic treatment was needed during the acclimation or study periods. Only animals in good health were used for the study.[6]

"Induction of the death of adipocytes through apoptosis is emerging as a promising strategy for the prevention and treatment of excess fat due to the destruction of adipocytes via this mechanism, resulting in reduced body fat."

Treatment Procedure: The area (20 x 10cm) of the skin was selected on the experimental animal in the abdominal region and labeled with a pen-marker (See Figure 1).

The treatment was administered 4 times with a week interval between treatments. The initial output setting was 95Watts with applicator tip cooling set at 10°C (See figure 10). The desired skin temperature was 42.5°C, minimum exposure temperature level was 39°C, and maximum exposure temperature level was 43°C. The surface temperature was measured continuously and output power was adjusted according to the temperature measurement. The internal tissue temperature was periodically measured by thermal probe during the exposure. Anesthesia was administered during each treatment, and during biopsy. Blood samples were taken before the first treatment, after each treatment, and after the three-month follow-up period. Tissue samples for the TUNEL method were taken from the treated area before the first treatment, after each treatment (3 samples after each treatment – 1 hour after therapy, 8 hours after therapy, and 20 hours after therapy), and after the three-month follow-up period. (For the location and summary of samples see Figure 2: Biopsy location in the treatment area). Autopsy and histological samples of skin, liver, kidney, and lungs were taken at the end of the recovery period.

FIGURE 1. Application area.

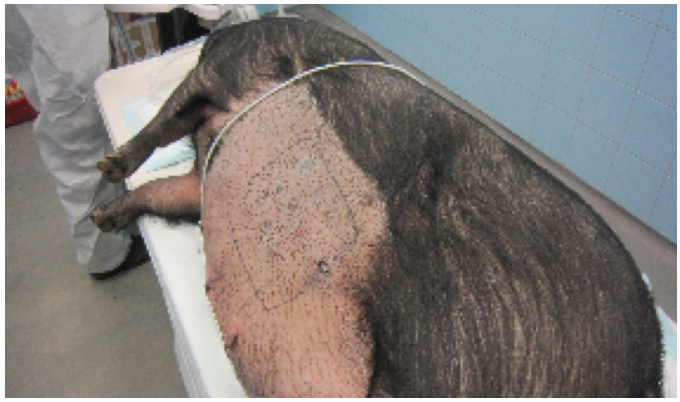
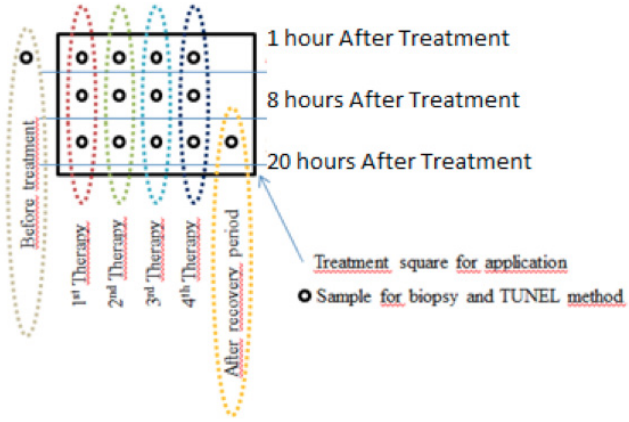


FIGURE 2. Biopsy location in the treatment area.



Clinical Observations

All swine were observed for clinical signs, morbidity, or mortality once a day during acclimation and during the treatment period. Onset, duration, and severity of any signs were recorded. The investigation included: changes of skin, eyes, and mucous membranes, respiratory, circulatory, and autonomic and central nervous system, somatomotor activity, and behavior pattern, changes in gait, posture, and response to handling, and the presence of clonic or tonic movements and stereotypes.

Clinical Procedures

The temperature of superficial structures was monitored by an infrared thermo imager during each treatment. The type of thermo imager used was the FLUKE Ti32. (FLUKE Corp., Everett, WA) The temperature of the cutaneous and subcutaneous tissue layers was measured by the TC-08 8-channel T-probe needle thermometer manufactured by Pico Technology Limited, UK, and placed under the control of USG Mindray M5Vet. Output power and other settings were recorded for each treatment.

Preservation of Samples

The full-blood samples were evaluated immediately, centrifuged sera was deep frozen, and punch biopsies and autopsy

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FIGURE 3. Thermal probe inserted within the subcutaneous tissue.

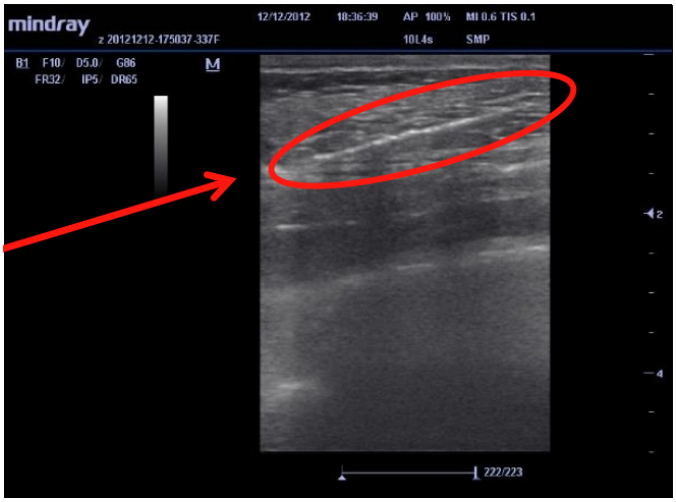
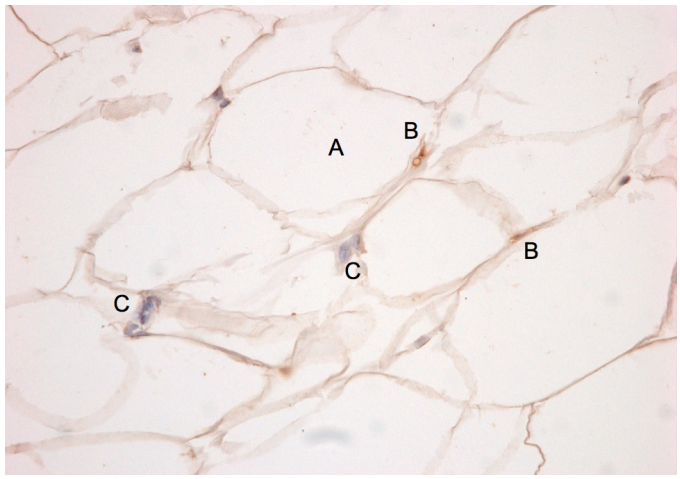


FIGURE 4. Adipocyte (A) apoptotic nucleus is stained brown (B), other nuclei are blue (C) (400x).



samples of skin, liver, kidney, and lung were preserved in formalin and prepared for paraffin-embedded tissue sections for further research. For stereological analysis the samples were fixed in 3% glutaraldehyd in 0.1M cacodylate buffer, pH 7.2, containing 7% sucrose.

Histological Examination

The tissue specimens were submitted to the fixation by 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline) for tissue preservation, processing (dehydration), clearing and infiltrating the tissue with paraffin wax, embedding the specimen in a cube and finally sectioning by a microtome to be placed on a microscope slide. The specimens were further stained by haematoxylin eosin.

Cell Death Monitoring

For apoptosis (programmed cell death), formaldehyde-fixed and paraffin-embedded tissue sections were analyzed by in situ TdT-mediated dUTPX nick-end labeling (TUNEL) staining, ie, visualizing the DNA fragmentation by TUNEL kit (Apoptosis Detection Kit), S7100, Scintilla. The results were evaluated and calculated in percentage of stained cells.

RESULTS

Apoptotic Index

Apoptotic index describes the percentage of the stained cells in the specimen, which were marked as apoptotic cells. The described TUNEL method indicates apoptotic cells through color change of nucleus in the histology sample. The cells with a brown nucleus indicate apoptosis while cells with a blue nucleus indicate viable cells (See Figure 4).

Before the treatment, the average apoptotic index average was 7.0%. The apoptotic index reached average levels of 53.4%, 39.6%, 40.2%, and 44.7%, respectively, in four consecutive treatments, from tissue samples taken at 1, 8, and 20 hours after the therapy. At the three-month follow-up the average dropped to 11.7%. The average, minimum and maximum apoptotic index two weeks before therapy, during each therapy and at the three month follow up is shown in Table 1 and Figure 5.

FIGURE 5. Graph of average apoptotic index.

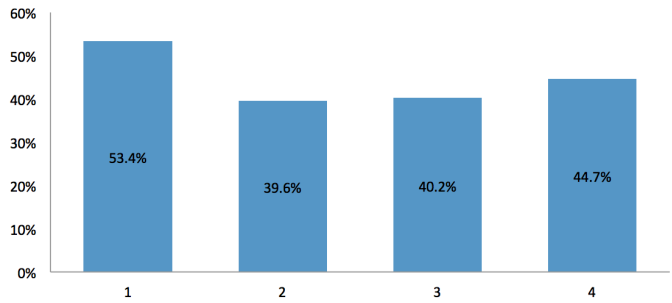


TABLE 1.

Apoptotic Index Results (%) Two Weeks Before Therapy, During Each Therapy, and at the Three-Month Follow-up

Treatment	Measurements (n)	Average Apoptosis (%)	Minimum	Maximum	St. Dev
2 weeks before	3	7.00	4.00	10.00	3.00
1	9	53.44	34.00	64.00	9.72
2	9	39.56	27.00	50.00	6.46
3	9	40.22	17.00	57.00	14.32
4	9	44.67	32.00	60.00	11.32
follow up	3	11.67	5.00	18.00	6.51

The apoptotic index values in percentages from individual animals are shown in Table 2 and Figure 6.

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FIGURE 6. Graph of apoptotic index values (%) in individual animals.

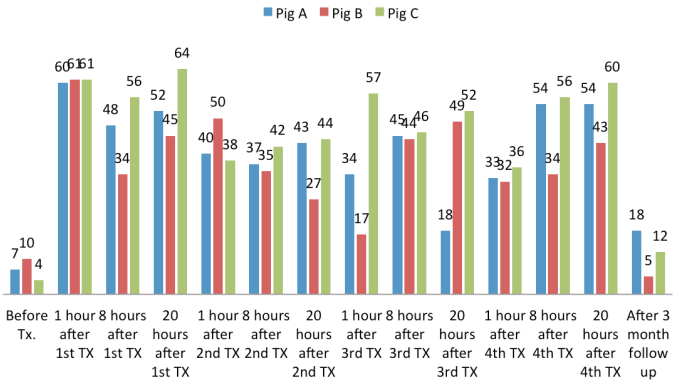

TABLE 2.

Apoptotic Index Values (%) in Individual Animals

TUNEL Test							
Time after Treatment (h)	Animal	2 Weeks Before	1	2	3	4	Follow up
1	A	7	60	40	34	33	18
1	B	10	61	50	17	32	5
1	C	4	61	38	57	36	12
8	A		48	37	45	54	
8	B		34	35	44	34	
8	C		56	42	46	56	
20	A		52	43	18	54	
20	B		45	27	49	43	
20	C		64	44	52	60	

The course of an apoptotic index of each animal after each treatment did not show significant differences, ie, there are not great rises and falls in average values of separate treatments. The comparison before (before any treatment) and after (just after the last treatment, up to 20 hours after) mean showed statistically significant results at nearly all treatments at $P \leq 0.05$ and very close to $P \leq 0.01$ in some cases.

Histology

The fragmentation of fibrous tissue within the cutaneous stroma and subcutaneous structures was visible in the histology specimens. The following images show intact adipocyte cell walls pre- treatment and decomposed or disrupted cell walls post-treatment four.

Thermal Profile Measurement

The temperature of the subcutaneous layers was measured using the 8-channel T-probe needle thermometer inserted to a maximum depth of 3cm within the tissue. Location of the probe was verified using diagnostic ultrasound. The surface temperature and deep tissue temperature measurement results are recorded in Figure 9: Graph of results of skin and fat temperature in time.

The measurement verified that the adipose tissue was heated more than skin during the therapy. The thermal gradient of adipose tissue and skin surface increased in time until saturation. The average thermal gradient (from 2:30 till 11:30 min) was 3.1°C. Maximum skin temperature was 43.0°C and maximum fat temperature was 45.6°C.

FIGURE 7. Histology samples.

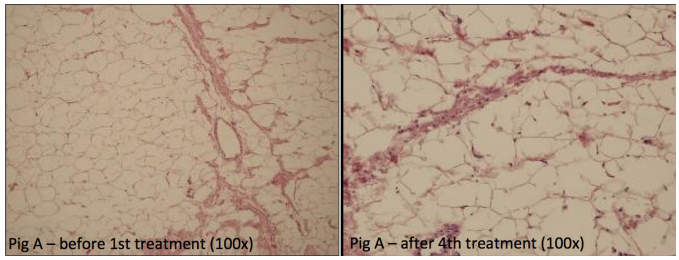

FIGURE 8. Thermal probe position verification using ultrasound measurement.

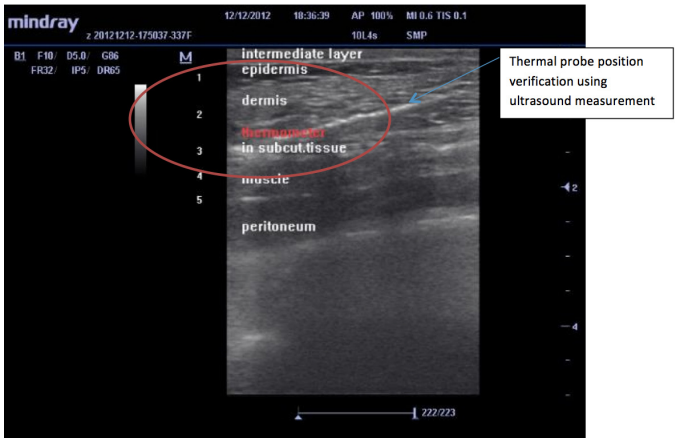

FIGURE 9. Graph of results of skin and fat temperature in time.

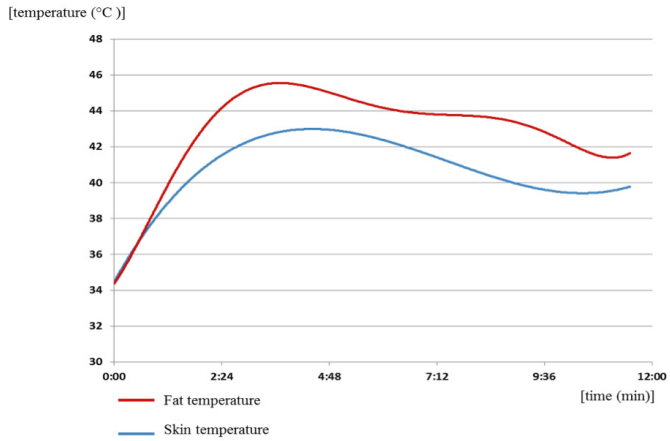

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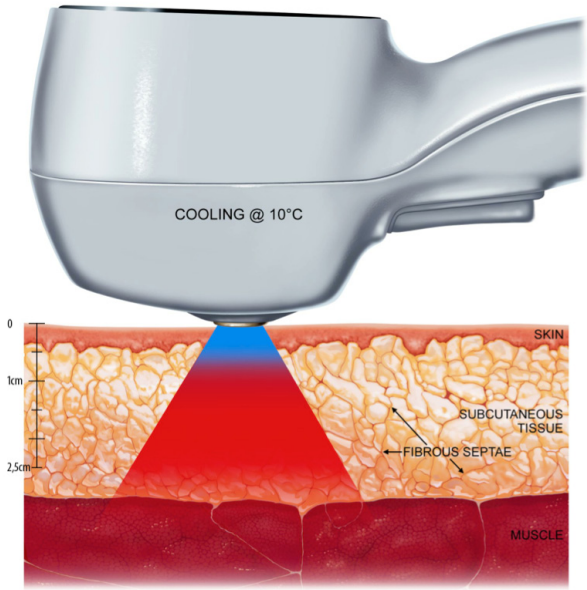
FIGURE 10. Cooling with corresponding depth of heat penetration.



Histology Results

Samples of skin from the treated area, liver, kidney and lung from all three swine for histopathological examinations were taken. No abnormalities were observed in the inner organs during pathology examination. Histology, blood biochemistry and hematology samples showed mild to no signs of inflammation in the treated area.

DISCUSSION

Apoptosis is the main mechanism for regulating cell death in many tissues. Induction of the death of adipocytes through apoptosis is emerging as a promising strategy for the prevention and treatment of excess fat due to the destruction of adipocytes via this mechanism, resulting in reduced body fat.[9]

The aim of this study was to demonstrate the effect of a monopolar radiofrequency device with embedded cooling on the cutaneous and subcutaneous structures in a porcine model. Multiple treatments of the skin and subcutaneous tissue demonstrated apoptosis in the adipose tissue. The apoptotic index increased from an average of 7% prior to start of the treatment to an average of 53.4%, 39.6%, 40.2%, and 44.7% after each of the four treatments. The thermal gradient (difference in temperature between the skin and the adipose tissue) was on average 3.1 degree Celsius and confirmed that the temperature was higher in subcutaneous tissue compared to the skin surface. The laboratory, histological or pathological analyses did not indicate any safety risks or side effects

In conclusion, the results of this study support the hypothesis that a focused monopolar radiofrequency treatment can induce apoptosis in adipose tissue via heat activation. Based on the findings of this preclinical animal study, human clinical studies looking at the improvement in body shaping as well as the potential for an effective approach for the prevention of excess body fat, is warranted.

DISCLOSURES

The authors have not disclosed any conflict of interest.

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