Impact of Contactless Apoptosis-Inducing RF on Temperature of Human Skin Surface and Subcutaneous Layer as well as Porcine Histology: A Pilot Study

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Background and Objectives

Radiofrequency (RF) technology has been developed as a noninvasive method to reduce subcutaneous abdominal fat. The aim of this study was to measure the changes of human skin surface temperature and human subcutaneous fat layer temperature, as well as to evaluate the histologic change in porcine adipocytes during and after treatment with contactless apoptosis-inducing RF device.

Materials and Methods

A single pig was treated with RF device for 30 minutes at maximum power of 200 Watts. The skin was biopsied and evaluated immediately after the therapy. A female human volunteer was also treated with RF device for 45 minutes. The temperatures of the skin surface and subcutaneous fat layer were measured during the therapy.

Results

Skin biopsy specimens from the pig revealed changes in the adipocyte shape and size. Many of the adipocytes had shrunken, with a few showing condensed chromatin and fragmented nuclei, reflecting signs of adipocyte apoptosis. In the human volunteer, subcutaneous fat layer maintained a temperature of 43-45°C, while the skin surface temperature did not reach 43°C during the treatment.

Conclusion

The contactless selective RF device achieved the ideal temperature for fat reduction in subcutaneous fat layer during the treatment, while maintaining skin surface temperature below the threshold of heat-induced pain for humans. Apoptosis of subcutaneous adipocytes was confirmed in porcine skin. Further clinical trials are necessary to evaluate the efficacy and safety.

Key words

Radiofrequency; Subcutaneous abdominal fat; Adipocyte; Apoptosis

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INTRODUCTION

Demands for an ideal body shape are rapidly increased. A fixed stereotype of beauty in mass media and health policies to fight against obesity have reinforced a slim figure as ideal. As a result, cosmetic procedures to remove subcutaneous adipose tissue have gained tremendous popularity in recent years. In all demographic groups, interventions to reduce subcutaneous abdominal fat are some of the most sought after aesthetic procedures.

A number of treatment options for subcutaneous abdominal fat removal are available. Although tumescent liposuction is still regarded as the gold standard,¹ many less and noninvasive methods for eliminating subcutaneous abdominal fat have merged as popular alternatives.² These include injection of phosphatidylcholine and deoxycholic acid, high-intensity focused ultrasound, low-frequency focused ultrasound, cryolipolysis and radiofrequency (RF) technologies.³ All of these methods seek to selectively destroy adipose tissue via a variety of different mechanisms.

Recently, contactless apoptosis-inducing RF technology was introduced as a safe and effective method to reduce subcutaneous abdominal fat.⁴ In this study, we aimed to investigate temperature changes of human skin surface during the treatment and the histologic changes in porcine subcutaneous adipocyte with newly developed contactless apoptosis-inducing RF device.

MATERIALS AND METHODS

For the animal study, a 61-month-old, female, solid white swine was used. The pig was housed individually under controlled environmental conditions (temperature, 18–22°C; relative air humidity, 30–70%; 15 air changes/h; and 12:12 hour light-dark cycle). General anesthesia was induced with an intramuscular bolus injection of

tiletamine/zolazepam (5 mg/kg) and xylazine (2 mg/kg). Then, endotracheal intubation was performed, and the pig was connected to a ventilator. The lungs were ventilated with oxygen, and anesthesia was maintained with 2% isoflurane. Intravenous hydration with normal saline was maintained through a superficial auricular vein (25 ml/hour). The back of the experimental pig was shaved with an electrical razor and the applicator of RF device (en-Curve™, Lutronic Corporation, Goyang, South Korea) was positioned as shown in Fig. 1A. A maximum power of 200 Watts (W) was applied for 30 minutes. An infrared thermal camera (Flir Systems, Wilsonville, USA) was utilized to measure skin surface temperatures before and during treatment (Fig. 1B, C). To examine changes in adipocytes of the subcutaneous fat layer, skin samples were biopsied immediately after the RF treatment, fixed in 10% buffered formalin, and embedded in paraffin. The tissue blocks were serially cut along the longitudinal plane at 4-μm thickness and stained with hematoxylin and eosin (H&E) stain.

A 42-year-old, female subject volunteered to receive RF treatment. The subject was positioned in the supine position, and the applicator for the RF device was positioned approximately 1 cm above the skin. A maximum power of 200 W was applied for 45 minutes. Skin surface temperatures were measured with an infrared thermal camera (Flir Systems) throughout the treatment (0, 10, 15, 20, 30, 45 minutes). Temperatures of the subcutaneous fat layer were measured each minute during treatment using a thermocouple (Omega Engineering, Stamford, USA).

RESULTS

At 30 minutes after RF treatment, skin surface temperatures of the pig were increased where the applicator was positioned (Fig. 1C). However, the surface temperature remained below 44°C, a temperature that would

Fig. 1. (A) Photographs of the porcine skin during RF treatment. Infrared thermal images (B) before and (C) 30 minutes after RF treatment.

not produce burn injury within 1 hour of exposure.⁵ The biopsy specimens, acquired immediately after RF treatment, revealed distortion of subcutaneous fat structures and changes in adipocytes (Fig. 2A). Shrinkage of many adipocytes was observed without any inflammatory cell infiltration (Fig. 2B). Under a high-power field, apoptotic nuclei, apparent as chromatin condensation and nuclear fragmentation, were observed (Fig. 2C). The histologic changes suggested the initiation of adipocytes apoptosis. As induction of adipocyte apoptosis is slow and full effect of apoptosis is seen weeks or months after the treatment, the histologic change is meaningful.

In the female volunteer, skin surface temperatures were measured with an infrared thermal camera (Fig. 3). Four fixed points around the umbilicus were selected,

and skin surface temperatures were precisely measured. The average temperatures were 33.2°C before treatment and 39.2°C, 40.3°C, 41.1°C, 41.3°C and 42.3°C after 10, 15, 20, 30 and 45 minutes of treatment, respectively (Fig. 4). Skin surface temperatures did not exceed 43°C throughout the RF treatment. Temperatures in subcutaneous fat increased with initiation of RF treatment, and reached 43°C after 10 minutes and 45°C after around 20 minutes of treatment (Fig. 4). Temperatures in subcutaneous fat layer remained steady at around 43–45°C for about 35 minutes until the end of treatment. Although the temperature spiked at 45.8°C at 23 minutes, the subject did not complain of an unpleasant heating sensation.

Fig. 2. (A) Histopathologic findings in the porcine subcutaneous fat layer after RF treatment (H&E staining, $\times 20$). (B) Many shrunken adipocytes are visible in fat layers with absent inflammatory cell infiltration ($\times 40$). (C) A few apoptotic nuclei show nuclear fragmentation between adipocytes ($\times 100$).

Fig. 3. Temperatures along the skin surface of a human volunteer were monitored during RF treatment for 45 minutes, and temperatures were measured at four selected points around the umbilicus.

Fig. 4. Temperatures of the subcutaneous abdominal fat (red line) and skin surface (blue line) during RF treatment are depicted.

DISCUSSION

Among various noninvasive fat reduction modalities, those utilizing RF technology are most commonly applied. RF technology is used in the medical field to generate heat in targeted tissue.⁶ Heat is generated when the RF energy emitted to tissue, and the impedance of the targeted tissue converts the energy to heat.⁷ Skin, subcutaneous fat, and muscles are dielectric substances. When placed in an electromagnetic field, unlike conductor materials, dielectric materials do not facilitate the flow of electric charges, thus an electric current is not generated. However, these materials do contain electric dipoles that rotate in electromagnetic fields and become inner polarized. If the electromagnetic field is alternated, electric dipoles oscillate to produce heat energy. Permittivity is the measure of resistance in a dielectric substance upon encountering an electromagnetic field. Greater electric flux is generated in a material with a low permittivity. The permittivity of fat is much lower than that of skin, thus fat generates greater electric flux than skin.⁷ Thereby, RF technology can be utilized to deliver energy selectively to subcutaneous fat tissue, avoiding unnecessary energy distribution to the epidermis, dermis and muscles.

Adipocytes are heat sensitive to temperatures of 50°C and 45°C for 1 and 3 minutes, respectively. Thermal exposures to 43–45°C for at least 15 minutes induces adipocyte cell death *in vivo*.⁸ According to the literature, the threshold temperature for skin damage is approximately 50°C.⁹ However, previous studies have shown that the heat-pain threshold for normal subjects is about 43°C.^{10,11} In addition, researchers have also reported that 43°C is a safe temperature for prolonged exposure to a hot object.⁵

Therefore, the ideal fat reducing RF device would selectively heat subcutaneous fat to 43–45°C, while limiting temperature of skin surface below 43°C.

The RF device used in this study emits 27.12 MHz radiofrequency energy with a maximum power of 200 W. It comprises multipolar applicator panels that can generate an electromagnetic field without contacting the subject. In our study, the RF device selectively generated heating of the subcutaneous fat layer at a constant temperature of around 43–45°C. Meanwhile, however, the temperature of skin surface did not reach 43°C during the study. Thus, we suggest that the contactless apoptosis-inducing RF device enCurve™ utilized in the present study could be of use as a safe and effective device with which to eliminate subcutaneous abdominal fat. Indeed, signs of adipocyte apoptosis were observed in porcine skin immediately after exposure to electromagnetic field over 30 minutes in this study. Induction of adipocyte apoptosis has proven to be an effective and irreversible method for removing subcutaneous fat, compared to weight loss, which results in a decreased volume of adipocytes without decreases in actual fat cells number.^{12,13}

The majority of RF devices on the market require the application of electrode panels to the skin. The RF device used in this study is contactless, helping to further meet demands for more noninvasive subcutaneous fat reduction therapies. The new RF device also comprises a unique Personalized Impedance Synchronization Application system, which provides real-time feedback on changes in impedance and automatically adjusting power level to maximize the amount of energy delivered. This would theoretically help overcome the critical drawbacks of using RF technology, such as variation in impedance between and within individuals, although further evaluation in a larger population is needed to definitely determine its efficacy and safety. Nevertheless, the selective RF device achieved ideal temperatures in human skin for inducing adipocyte apoptosis in subcutaneous fat layers during treatment, while maintaining skin surface temperatures below the threshold of heat-induced pain for humans, suggests its potential use in body contouring to reduce subcutaneous fat.

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