

Cryolipolysis for Noninvasive Fat Cell Destruction: Initial Results from a Pig Model

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BACKGROUND Liposuction is one of the most frequently performed cosmetic procedures in the United States, but its cost and downtime has led to the development of noninvasive approaches for adipose tissue reduction.

OBJECTIVE To determine whether noninvasive controlled and selective destruction of fat cells (Cryolipolysis) can selectively damage subcutaneous fat without causing damage to the overlying skin or rise in lipid levels.

METHODS Three Yucatan pigs underwent Cryolipolysis at 22 sites: 20 at cooling intensity factor (CIF) index 24.5 (−43.8 mW/cm²), one at CIF 24.9 (−44.7 mW/cm²), and one at CIF 25.4 (−45.6 mW/cm²). Treated areas were evaluated using photography, ultrasound, and gross and microscopic pathology. Lipids were at various times points. One additional pig underwent Cryolipolysis at various days before euthanasia.

RESULTS The treatments resulted in a significant reduction in the superficial fat layer without damage to the overlying skin. An inflammatory response triggered by cold-induced apoptosis of adipocytes preceded the reduction in the fat layer. Evaluation of lipids over a 3-month period following treatment demonstrated that cholesterol and triglyceride values remained normal.

CONCLUSIONS Cryolipolysis is worthy of further study because it has been shown to significantly decrease subcutaneous fat and change body contour without causing damage to the overlying skin and surrounding structures or deleterious changes in blood lipids.

The research was funded by Zeltiq Aesthetics. Drs. Brian Zelickson, Barbara Egbert, and Robert Rhoades are paid consultants to Zeltiq. Dr. Dieter Manstein receives royalty payments from Zeltiq related to licensing of the Cryolipolysis technology. Dr. Jessica Preciado, Dr. John Allison, and Kevin Springer are employees of Zeltiq Aesthetics.

Liposuction is a frequently performed aesthetic operation. It has been used extensively and is generally safe and well tolerated,^{1–4} but patient and physician interest in less invasive methods to reduce body fat has resulted in significant research effort directed toward the development of less invasive alternative and complementary procedures for fat removal.

High-intensity focused ultrasound (HIFU) has been evaluated as a noninvasive alternative for liposuction but is currently considered an adjunct to, rather than

a replacement for, this procedure.^{5,6} Radiofrequency methods may also be employed for removal of adipose tissue, but these techniques have been associated with complications that include appearance of second-degree burns, persistent erythema, scarring, edema, and fat atrophy.⁷ Infrared light, alone or in combination with other methods, has also been employed to remove fat, with limited success.^{8,9} Laser-assisted lipolysis with a medium-pulse 1,064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) system, a new method of removing

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localized areas of fat and tightening skin, is also being evaluated¹⁰ but still requires tumescent anesthesia and liposuction to remove the damaged fat. Additional laser systems (e.g., CoolTouch CoolLipo 1,320, CoolTouch Corporation, Roseville, CA and the Palomar 920, Palomar Medical Technologies, Inc., Burlington, MA) have also been developed.^{11,12} Subcutaneous injection of phosphatidylcholine and deoxycholate has also been tested for fat dissolution.¹³ Although these procedures may represent significant treatment advances, they are still invasive and may not provide a significant improvement in risk–benefit ratio over liposuction.

Results from several studies have suggested that fat cells may be more sensitive to cold than other tissues.^{14,15} Results from studies exploring the effect of cold on tissue destruction have suggested that temperatures as high as 1°C can decrease the viability of adipocytes.^{16–18} All of these findings suggest that application of cold to the skin may provide a non-invasive approach to fat removal.

The objective of the present study was to test noninvasive cold exposure regimens to determine whether selective damage to subcutaneous fat can be induced without damage to the overlying skin and without causing a harmful rise in lipid levels.

Methods

Experimental Animals

The study was conducted in three Yucatan pigs (A, B, and C; 24–30 months of age, body weight approximately 115 kg) and one Yorkshire pig (D; 96 kg).

Determination of Energy Withdrawal

The cooling intensity factor (CIF) setting on the proprietary research device is a numerical value that regulates a heat extraction (cooling) rate that translates to milliwatts per centimeter squared (mW/cm^2). The higher the CIF, the more rapid the rate of energy extraction. The other major treatment variable was time over which the research device, set to a chosen CIF value, was applied to the skin surface.

Treatment

All animals were treated using a three-section cooling applicator with individual cooling sections connected to a prototype Zeltiq cooling control device (Figure 1) (Zeltiq, Pleasanton, CA). To ensure consistent thermal conduction from skin to applicator, a single-use protective sleeve with nine embedded thermistors and thermal coupling gel (Figure 1) were used to couple the applicator to the skin surface in the treatment area. For each pig, a template was traced onto the area to be treated. The skin was scored with a marker along the top and right edges of the location where the template contacted the body surface so the exact locations treated could be relocated and assessed during follow-up. The placement of treatment areas (Figure 2) and the treatment regimens varied somewhat for each pig. Pigs A, B, and C received a single treatment 90 days before euthanasia, and pig D was treated at multiple sites 90, 60, 30, 14, 7, and 3 days and immediately (30 minutes) before euthanasia. Approximately 25% to 30% of the total body surface area was treated in each animal.

Pig A was treated at three sites with the rate of energy extraction set to a CIF value of 24.5 ($-43.8 \text{ mW}/\text{cm}^2$). For each of the treated areas, the cooling applicator extracted heat from the underlying tissue for 60 minutes. A mechanical vibration of the cooling applicator was used to massage the tissue



Figure 1. Photograph of two prototype Zeltiq cooling applicators being used to treat Pig A.

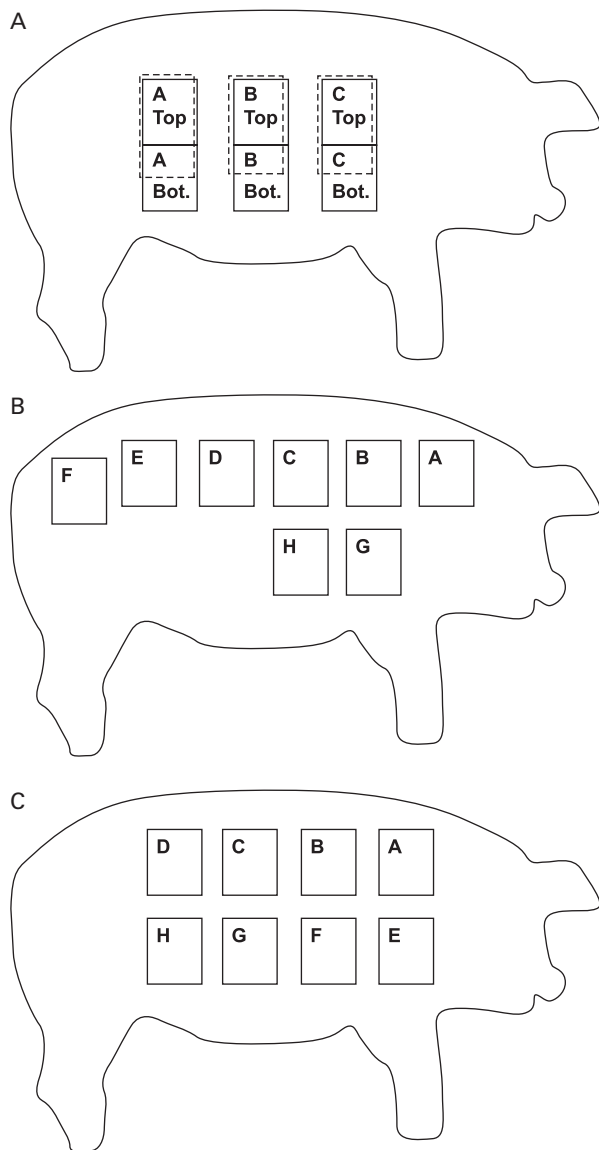


Figure 2. Treatment layouts for pigs (A–C). Each rectangle represents one treatment site. Each animal was treated on only one side of its body to produce a region of large surface area that extended over a uniform fat layer.

gently for 5 minutes during each cooling treatment. Pig B underwent cooling treatment at eight sites (Figure 2, Pig B, A–H) using a cooling applicator and a prototype cooling control device. The rate of energy extraction for this pig was set to a CIF value of 24.5. Sites A and B were treated for 60 minutes, including a 5-minute period of massage. Each of the other sites was treated for 45 minutes, including a 5-minute period of massage. Each site on pig C was treated with the rate of energy withdrawal set to a CIF value of

24.5. Each site was treated for 45 minutes, including a 5-minute period of tissue massage. Pig D was treated at 20 sites with the CIF set at 21.5 (-36.8 mW/cm^2). Each site was treated for 15 minutes.

Assessments

Treated and adjacent areas were evaluated using standardized flash photography and diagnostic ultrasound (SonoSite 180 [SonoSite Inc., Bothell, WA] with a 7.5-MHz linear transducer) assessments 3 months after treatment. At the time of necropsy, tissue was collected for gross pathologic and standard histologic evaluation. Histological sections were stained with hematoxylin and eosin and evaluated microscopically to assess the level of fat damage and any damage to the dermis or epidermis. Lipid panels were performed for each animal using blood samples collected after a 12-hour fast before treatment and 1 day, 1 week, and 1, 2, and 3 months after treatment.

Results

Evidence of Efficacy

Visual inspection of the Yucatan pigs 3 months after treatment revealed noticeable smooth inward contour changes on the surface of the treated areas (Figure 3). These contour changes correlated to decreased thickness of the fat layer as measured using ultrasound, with the greatest reductions measured in tissue areas that received the more intense and longer duration treatment. The changes in surface contour shown in Figure 3 reflect a substantial decrease in adipose tissue underlying the contoured area. The reduction in thickness of the upper layer of the subcutaneous fat is demonstrated in the gross pathology photographs shown in Figure 4.

Reduction in the fat layer thickness for each animal was assessed using ultrasound (Figure 5) and measurement of pathologic specimens. Results for Pig A indicated a reduction in the superficial fat layer of 33% (from 2.1 to 1.4 cm); those for Pig B indicated a reduction of 33% (from 1.8 to 1.2 cm). These data were not collected for pig C.

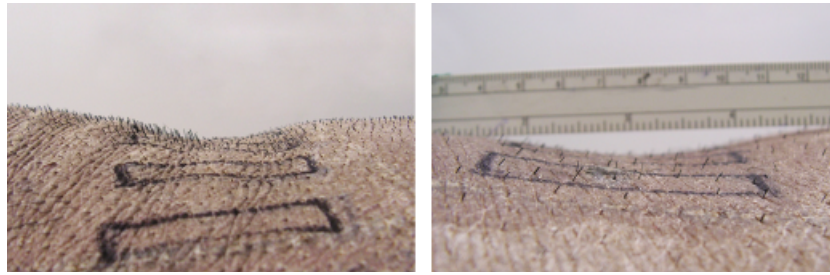


Figure 3. Photographs of treatment areas from two animals showing skin surface contour changes 3 months after treatment.

Examination of specimens collected for gross pathologic analysis (Figure 6) also demonstrated reductions in superficial fat in treated areas. Results from one pig indicated a decrease in the thickness of this layer of 53% (from 1.9 to 0.9 cm); those from a second pig indicated a decrease of 50% (from 2.0 to 1.0 cm). These data were not collected for the third pig.

Histologic analysis of tissues taken from Pig 4 demonstrated that Cryolipolysis resulted in the death of adipocytes that macrophages subsequently engulfed and digested (Figure 7). The progression of the inflammatory process that resulted in the phagocytosis of lipids is illustrated in Figure 8. Immediately after treatment, there are no changes in subcutaneous fat. By 3 days after treatment, there is evidence that an inflammatory process stimulated by adipocyte apoptosis has begun, as reflected by an influx of inflammatory cells. This inflammatory process became increasingly apparent at 7 and 14 days after treat-

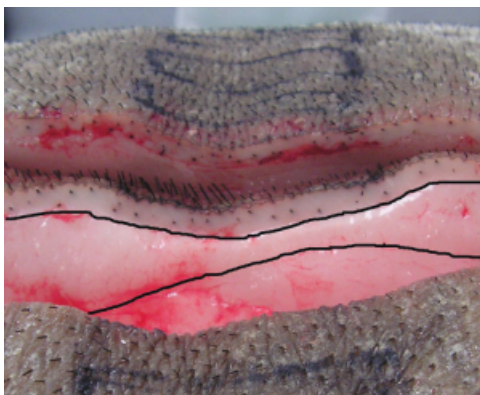


Figure 4. Gross pathology photograph showing an example of fat layer reduction responsible for the visible skin contour changes shown in Figure 3.

ment. Between 14 and 30 days after treatment, phagocytosis of lipids is apparent. By 30 days after treatment, the inflammatory process had begun to decline, and by 60 days, it appears that the thickness of interlobular septa has increased. The inflammatory process declined further by 90 days after treatment, and the increase in the thickness of septa was also pronounced at that time. This is believed to be the result of selective removal of adipocytes, reducing the thickness of the tissue and thereby increasing the proportion of collagen in the adipose tissue. Also apparent in the sequenced photomicrographs in Figure 8 is the substantial loss of fat cells in the treated area. Histologic analysis indicated no discernable damage to the dermis or epidermis in any of the areas treated. There was no ulceration or necrosis of the epidermis or dermis. In addition, no necrosis was observed in appendageal structures, such as hair follicles or sweat glands (Figure 9).

Erythema was observed immediately after treatment and resolved within 30 minutes. The skin was cold to the touch, although not hard or icy, after treatment. There was no evidence of edema, bruising, purpura, or scarring at the time of any follow-up examination or on the day of necropsy.

Changes in Lipids

Assessment of lipids over the 3-month follow-up period demonstrated some variation over time, but these small changes remained within the bounds of normal reported for these animals¹⁹ (Table 1).

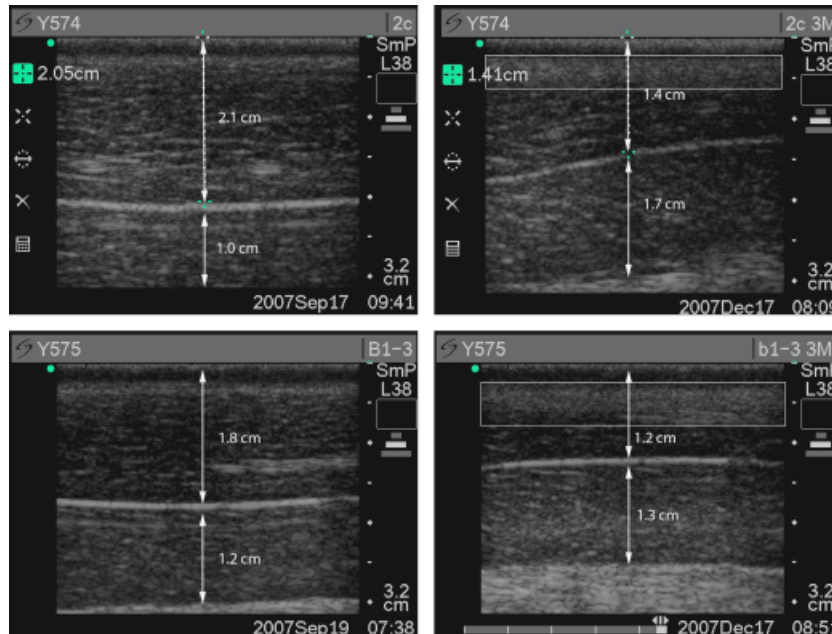


Figure 5. Ultrasound measurements of fat layer thickness demonstrating reductions in the superficial fat layer for two of the three Yucatan pigs included in this study. In each pair of images, that on the left was taken before treatment, and that on the right was taken 3 months after treatment.

Discussion

Results from this preliminary study indicate that Cryolipolysis induced using controlled exposure to cold can induce selective damage to the subcutaneous fatty tissue, resulting in subsequent changes in

body contour and reductions in the superficial fat layer of pigs. Although the cold was applied to the skin surface, the damage was confined to the fat, and no damage to the epidermis or dermis could be found. Evaluation of lipids at multiple time points after treatment indicated that lipids remained within the bounds of normal variation.

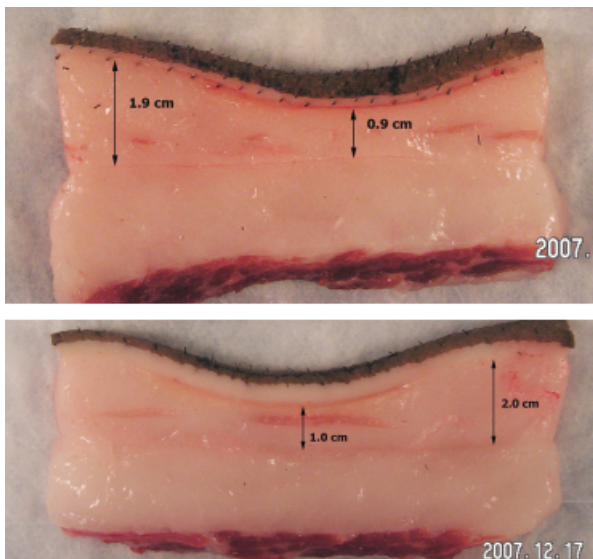


Figure 6. Gross pathology sections showing reductions in superficial fat layer 90 days after treatment.

Evaluation of histologic specimens collected after treatment indicated that the reduced thickness of the fat layer was associated with an inflammatory

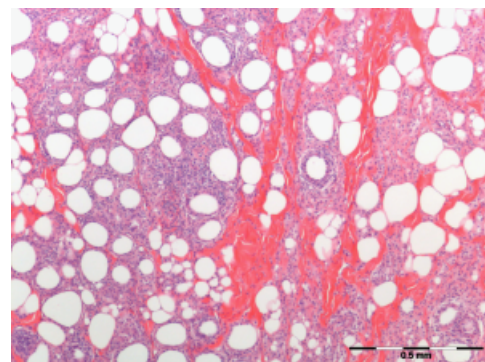


Figure 7. The natural inflammatory response to cold exposure in one area treated.

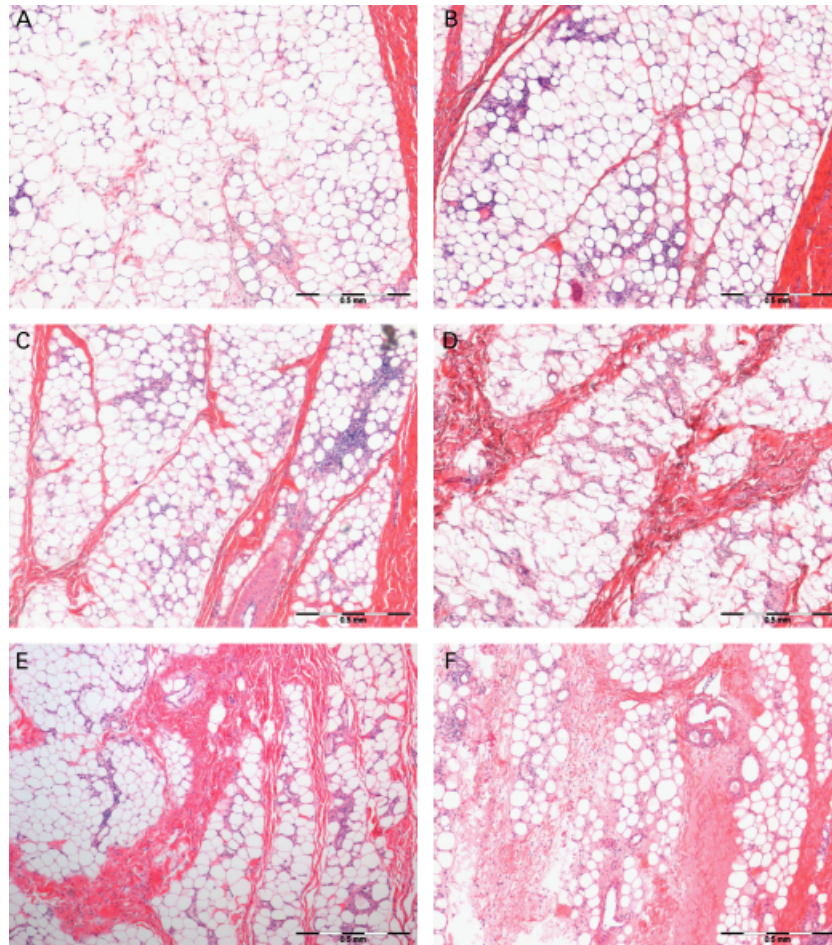


Figure 8. Progression of inflammatory response to cold exposure in tissue taken from pig D: (A) 3 days, (B) 7 days, (C) 14 days, (D) 30 days, (E) 60 days, (F) 90 days.

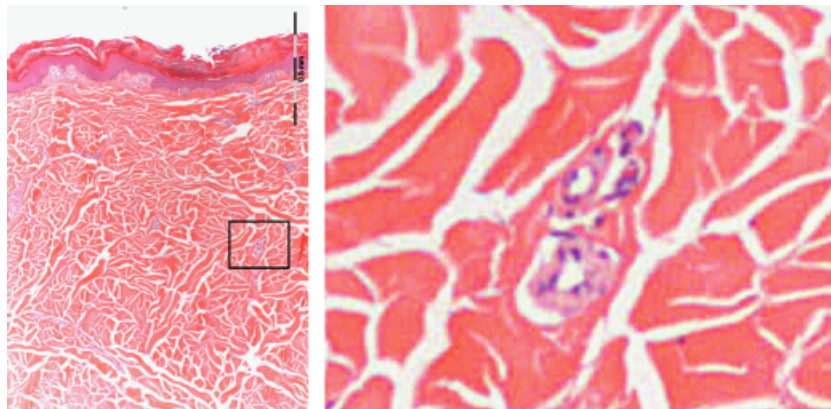


Figure 9. Tissue section from the epidermis and dermis of the same treatment site shown in Figure 7 (tissue taken 90 days after treatment).

TABLE 1. Posttreatment Changes in Lipids from Baseline in Pigs A to C

Lipid	Posttreatment Day			
	7	28	59	89
Cholesterol				
A	11.6	16.3	20.9	16.3
B	-7.7	-23.1	0	-9.9
C	5.6	5.6	0	7.4
Low-density lipoprotein cholesterol				
A	16.7	72.2	50.0	50.0
B	25.8	-51.6	0	16.1
C	41.2	17.6	0	11.8
High-density lipoprotein cholesterol				
A	40.0	-30.0	-5.0	-5.0
B	-2.9	-20.0	0	-2.9
C	7.7	-7.7	0	30.8
Triglycerides				
A	-22.2	-14.8	3.7	-33.3
B	-55.9	5.5	0	-52.8
C	-53.7	16.7	0	-51.8

Days for evaluation in Pig C were 8, 29, 59, and 90.

response. This response peaked approximately 1 month after treatment and then declined. Although the inflammatory response had clearly declined by 3 months after treatment, the modest residual inflammation at the time when the pigs were euthanized suggests that greater decreases in the fat layer may have been observed with longer survival times. Studies in which animals survive for longer periods after treatment are needed to determine the full time courses of the inflammatory processes and remodeling that follow Cryolipolysis. Two mechanisms of fat cell loss have been described in the literature (dedifferentiation and apoptosis),²⁰ and the results presented are consistent with the conclusion that exposure to cold induces apoptosis of fat cells. Review of the literature related to cryosurgery, a much more aggressive procedure than Cryolipolysis, indicates that cell death associated with cryosurgery represents apoptosis rather than necrosis,^{21,22} and it seems reasonable to suggest that apoptosis is also the mechanism underlying fat cell death in the present study. The extraction of heat from adipose tissue may also set the stage for ischemia-reperfusion injury that has been shown to result in apoptosis.^{23,24} The

selective loss of fat cells without damage to the overlying skin and associated structures may reflect the fact that adipocytes are more sensitive to cold than other cell types.^{14,15} Reperfusion of adipose tissue rendered ischemic by cold exposure may result in oxidative stress, including elevation of lipid peroxidation and reduced glutathione levels, ultimately resulting in cell death.^{25,26}

These results suggest that adipocyte apoptosis provided the stimulus for the observed inflammatory response, although it is likely that this response also contributes to the total number of adipocytes killed. The inflammatory response observed appeared similar to that reported previously in subcutaneous fat after exposure to intense cold.²⁷ As noted in the Introduction, exposure of fat cells to cold in situations in which an inflammatory response is not likely (e.g., storage of isolated cells) results in adipocyte death.^{16,17} These findings also support the view that the observed inflammatory response is a consequence rather than a cause of adipocyte death.

Study results indicated that application of cold did not result in any damage to the epidermis or dermis. The reason for the lack of effect of cold exposure on skin or associated structures is most probably the carefully controlled condition under which heat is being extracted from the adipose tissue—the prototype device used in this study regulates the rate of energy extraction from the skin (measured in mW/cm^2) through monitoring of several electronic thermistor sensors on the skin surface. In other situations, cold exposure can result in significant inflammation of the skin,²⁸⁻³⁰ but fat appears to be more susceptible to cold exposure and to ischemia and ischemia-reperfusion injury than other tissues, including the skin.^{25,31}

Cryolipolysis relies on natural thermal diffusion to realize a gradual and tapered effect within the fat layer. Controlling the rate of energy extraction and duration of treatment may be used to limit the amount of tissue subject to Cryolipolysis according to treatment goals.

The use of Cryolipolysis for reducing the superficial fat layer may have cosmetic applications in humans, although it is not known whether the levels of cold exposure necessary for Cryolipolysis pose a unique risk to patients with rare conditions such as cryoglobulinemia, paroxysmal cold hemoglobinuria, or cold urticaria. Future study of Cryolipolysis in humans should address these potential risks.

In conclusion, Cryolipolysis, a new method of controlled energy extraction (cooling) from adipose tissue, has been evaluated as a means of predictably destroying adipocytes while preserving the skin and surrounding structures. This noninvasive procedure achieves fat loss through heat extraction applied for an extended period of time. The results indicate that Cryolipolysis treatment merits further study because it leads to significant changes in body contour and can decrease subcutaneous fat without damaging the overlying skin or causing harmful changes in blood lipids.

References

- Heymans O, Castus P, Grandjean FX, Van Zele D. Liposuction: review of the techniques, innovations and applications. *Acta Chir Belg* 2006;106:647–53.
- Stewart KJ, Stewart DA, Coghlan B, et al. Complications of 278 consecutive abdominoplasties. *J Plast Reconstr Aesthet Surg* 2006;59:1152–5.
- Yoho RA, Romaine JJ, O'Neil D. Review of the liposuction, abdominoplasty, and face-lift mortality and morbidity risk literature. *Dermatol Surg* 2005;31:733–43.
- Coldiron BM, Healy C, Bene NI. Office surgery incidents: what seven years of Florida data show us. *Dermatol Surg* 2008;34:285–91; discussion 291–2.
- Rohrich RJ, Beran SJ, Kenkel JM, et al. Extending the role of liposuction in body contouring with ultrasound-assisted liposuction. *Plast Reconstr Surg* 1998;101:1090–102.
- Pine JL, Smith LJ, Haws MJ, Gingrass MK. Ultrasound-assisted lipoplasty. *Plast Surg Nurs* 2003;23:101–8.
- de Felipe I, Del Cueto SR, Pérez E, Redondo P. Adverse reactions after nonablative radiofrequency: follow-up of 290 patients. *J Cosmet Dermatol* 2007;6:163–6.
- Nootheti PK, Magpantay A, Yosowitz G, et al. A single center, randomized, comparative, prospective clinical study to determine the efficacy of the VelaSmooth system versus the Triactive system for the treatment of cellulite. *Lasers Surg Med* 2006;38:908–12.
- Sadick N, Magro C. A study evaluating the safety and efficacy of the VelaSmooth system in the treatment of cellulite. *J Cosmet Laser Ther* 2007;9:15–20.
- Katz B, McBean J. The new laser liposuction for men. *Dermatol Ther* 2007;20:448–51.
- Dierickx CC, Khatri KA, Tannous ZS, et al. Micro-fractional ablative skin resurfacing with two novel erbium laser systems. *Lasers Surg Med* 2008;40:113–23.
- Yang CH, Chou HS, Lo YF. Incompetent great saphenous veins treated with endovenous 1,320-nm laser: results for 71 legs and morphologic evolution study. *Dermatol Surg* 2006;32:1453–7.
- Schuller-Petrovic S, Wölkart G, Höfler G, et al. Tissue-toxic effects of phosphatidylcholine/deoxycholate after subcutaneous injection for fat dissolution in rats and a human volunteer. *Dermatol Surg* 2008;34:529–43.
- Wiandrowski TP, Marshman G. Subcutaneous fat necrosis of the newborn following hypothermia and complicated by pain and hypercalcaemia. *Australas J Dermatol* 2001;42:207–10.
- Diamantis S, Bastek T, Groben P, Morrell D. Subcutaneous fat necrosis in a newborn following icebag application for treatment of supraventricular tachycardia. *J Perinatol* 2006;26:518–20.
- Lidagoster MI, Cinelli PB, Leveé EM, Sian CS. Comparison of autologous fat transfer in fresh, refrigerated, and frozen specimens: an animal model. *Ann Plast Surg* 2000;44:512–5.
- Wolter TP, von Heimburg D, Stoffels I, et al. Cryopreservation of mature human adipocytes: in vitro measurement of viability. *Ann Plast Surg* 2005;55:408–13.
- Manstein D, Laubach H, Watanabe K, Farinelli W, Zurakowski D, Anderson RR. Selective cryolysis: a novel method of non-invasive fat removal. *Lasers Surg Med* 2008;40:595–604.
- Rispat G, Slaoui M, Weber D, et al. Haematological and plasma biochemical values for healthy Yucatan micropigs. *Lab Anim* 1993;27:368–73.
- Avram MM, Avram AS, James WD. Subcutaneous fat in normal and diseased states: 1. Introduction. *J Am Acad Dermatol* 2005;53:663–70.
- Baust JG, Gage AA. The molecular basis of cryosurgery. *BJU Int* 2005;95:1187–91.
- Yang WL, Addona T, Nair DG, et al. Apoptosis induced by cryoinjury in human colorectal cancer cells is associated with mitochondrial dysfunction. *Int J Cancer* 2003;103:360–9.
- Rentsch M, Beham A, Iesalnieks I, et al. Impact of prolonged cold ischemia and reperfusion on apoptosis, activation of caspase 3, and expression of bax after liver transplantation in the rat. *Transplant Proc* 2001;33:850–1.
- Tanaka M, Mokhtari GK, Terry RD, et al. Prolonged cold ischemia in rat cardiac allografts promotes ischemia-reperfusion injury and the development of graft coronary artery disease in a linear fashion. *J Heart Lung Transplant* 2005;24:1906–14.
- Coban YK, Kurutas EB, Ciralik H. Ischemia-reperfusion injury of adipofascial tissue: an experimental study evaluating early histologic and biochemical alterations in rats. *Mediators Inflamm* 2005;2005:304–8.

26. Werker PM, Kon M, Green CJ, et al. Oxidative processes and free radical scavengers in ischaemia-reperfusion injury in adipocutaneous flaps: in vitro lipid peroxidation assessment. *Br J Plast Surg* 1995;48:590-6.
27. Ter Poorten JC, Hebert AA, Ilkiw R. Cold panniculitis in a neonate. *J Am Acad Dermatol* 1995;33:383-5.
28. Page EH, Shear NH. Temperature-dependent skin disorders. *J Am Acad Dermatol* 1988;18:1003-19.
29. Ozyazgan I, Tercan M, Melli M, et al. Eicosanoids and inflammatory cells in frostbitten tissue: prostacyclin, thromboxane, polymorphonuclear leukocytes, and mast cells. *Plast Reconstr Surg* 1998;101:1881-6.
30. de Souza RC, Cunha JM, Ferreira SH, et al. Different inflammatory mediators induce inflammation and pain after application of liquid nitrogen to the skin. *Cryobiology* 2006;53:319-29.
31. Donski PK, Franklin JD, Hurley JV, O'Brien BM. The effects of cooling on experimental free flap survival. *Br J Plast Surg* 1980;33:353-60.

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