Clinical Efficacy of Noninvasive Cryolipolysis and Its Effects on Peripheral Nerves

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Received: 23 September 2008 / Accepted: 11 November 2008 © Springer Science+Business Media, LLC and International Society of Aesthetic Plastic Surgery 2009

Abstract

Background Cryolipolysis provides a method for noninvasive fat reduction that significantly reduces subcutaneous fat in a pig model without apparent damage to skin and surrounding structures. This study aimed to determine whether fat reduction in humans caused by cold exposure is associated with alteration in local sensory function or nerve fibers.

Methods In this study, 10 subjects were treated with a prototype cooling device. Fat reduction was assessed in 9 of the 10 subjects via ultrasound before treatment and at the follow-up visit. Sensory function was assessed by neurologic evaluation ($n = 9$), and biopsies ($n = 1$) were collected for nerve staining.

Results Treatment resulted in a normalized fat layer reduction of 20.4% at 2 months and 25.5% at 6 months after treatment. Transient reduction in sensation occurred in six of nine subjects assessed by neurologic evaluation. However, all sensation returned by a mean of 3.6 weeks after treatment. Biopsies showed no long-term change in nerve fiber structure. There were no lasting sensory alterations or observations of skin damage in any of the subjects evaluated.

Conclusion Noninvasive cryolipolysis results in substantial fat reduction within 2 months of treatment without damage to skin. The procedure is associated with modest reversible short-term changes in the function of peripheral sensory nerves.

Keywords Cryolipolysis · Efficacy · Fat reduction · Neurologic effects · Noninvasive

A new method of noninvasive fat layer reduction called cryolipolysis has been shown to reduce fat layer thickness significantly in a Yucatan pig model. A cold-induced inflammatory mechanism gradually reduces fat thickness in 90 days after a 30- to 60-min cold plate exposure to the skin surface (data on file) [1]. In vitro examination of the adipocyte response to cold showed that cooling of adipocytes to temperatures above freezing but below normal body temperature results in apoptosis-mediated cell death [2], which suggests that cryolipolysis produces an apoptotic injury in the adipose tissue. Furthermore, the subsequent inflammatory response may cause additional damage to those adipocytes not immediately affected by the cold exposure.

The results from the aforementioned animal study indicated that cryolipolysis caused a 30% to 50% reduction in fat layer thickness, with no damage to skin or associated structures and without causing changes in lipid profiles, including total cholesterol, low- and high-density lipoprotein cholesterol, and triglycerides (data on file) [1, 3]. There was no histologic evidence of necrotic or inflammatory damage to nerves.
Preliminary reports from early human studies of temporary altered sensation after the cryolipolysis procedure suggested potential transient effects on sensory nerve function that could not be evaluated in the animal model. To assess the effects of cryolipolysis on sensory function in humans, a study was conducted for a subset of subjects to confirm the efficacy of cryolipolysis for fat reduction and to document the occurrence and duration of altered sensory function after cryolipolysis.

Methods

Subjects

Individuals eligible for inclusion in this trial were men or women older than 18 years with visible fat on the flanks (love handles) and no weight changes exceeding 10 lb during the preceding month. All the subjects provided informed written consent under an institutional review board–approved protocol.

Potential subjects were excluded if they had recently undergone liposuction or another surgical procedure, had a history of subcutaneous injections into the area of intended treatment within the preceding 6 months, or had a known history of cryoglobulinemia, cold urticaria, or paroxysmal cold hemoglobinuria. Individuals unable or unwilling to comply with the study requirements, those with dermatologic conditions or scars within the location of the test sites that could interfere with the treatment or evaluation, those taking methylxanthines, and those currently enrolled in a clinical study of any other unapproved investigational drug or device were excluded as well. Exclusions from the study also involved women who were pregnant or intending to become pregnant in the following 9 months, women who were lactating or had been lactating in the prior 9 months, and individuals with any other condition or laboratory abnormality that could, in the opinion of the investigator, potentially affect response or participation in this clinical study or pose an unacceptable risk to the subject.

Device

The Zeltiq System (Zeltiq Aesthetics, Pleasanton, CA, USA) consists of a control console and an umbilical-style cable connecting the console to a cooling applicator cup applied to the area of desired treatment. The “love handle” tissue is drawn into the applicator head using a mild vacuum that positions the tissue between two cooling panels within the cup and holds it in place for the 30- to 60-min exposure of the procedure. The selected energy extraction rate (cooling) is modulated by thermoelectric cooling cells that directly contact the skin and monitor the heat flux out of the tissue according to predetermined values.

Treatment

Precisely controlled cooling was applied to the treatment area on one of two contralateral flanks or “love handles,” as illustrated in Figs. 1 and 2. One side was left untreated as a control condition. To ensure consistent thermal contact during the procedure, a proprietary coupling gel (Zeltiq Aesthetics) was applied to the skin surface before attachment of the cooling device to the treatment area. The cooling device was adhered to the treatment area with a moderate vacuum, regulated to ensure minimal discomfort during the procedure according to the subject’s feedback. Two to three application sites per love handle were required for each treatment to cover the area of desired reduction.

Two subject groups were treated as part of this sensory study. The first group was assessed for nerve sensation (discussed further in the Methods section) and treated with the Zeltiq clinical prototype device set to a cooling intensity factor of 33, which corresponds to an average energy extraction rate of 63.6 mW/cm² during treatment. The total treatment time per application site for this first group of subjects was 60 min.

The second group was assessed for histologic changes in the treated area at specific times after treatment (further discussed in the Methods section). The subject in the second group was treated with the Zeltiq clinical prototype device set to a cooling intensity factor of 37, which corresponds to an average energy extraction rate of 68.3 mW/cm² during treatment. The total treatment time per application site for this second group was 45 min.

Posttreatment Assessments

Efficacy Assessment

Several measures were used to assess the efficacy of the cryolipolysis procedure. These included visible change in the surface contour or fat volume based on clinical assessment of treated versus matched contralateral untreated areas, photographic assessment of baseline untreated area versus the same area after treatment, and reduction of the fat layer thickness in the treated area comparing baseline and posttreatment thickness as demonstrated by measurement with ultrasound. Similar ultrasound measurements of the contralateral untreated areas were used to normalize fat layer reduction measurements of the treated areas, accounting for possible variations in subject weight during the study.

Before treatment, baseline subject photographs were acquired according to a standardized photography setup.
and parameters (Nikon D200, Nikon 24- to 120-mm lens, DynaLite strobes). Both treated and untreated contralateral areas were photographed with the subject in a standing position at a series of viewing angles spaced one every 22.5°, starting at 0° and sequencing to 360°. This resulted in 16 images of each subject. At follow-up visits, photographs were repeated using the same setup and procedure.

All photographs were captured in Nikon raw format and processed in Adobe Photoshop so that all processing of images was recorded. Adjustments made during processing were limited to exposure and white balance only. Comparisons of pre- and posttreatment follow-up photos were accomplished by creating matched angle sets from which visual efficacy could be assessed.

Flank fat layer reduction, as demonstrated by fat layer thickness changes measured by ultrasound, was confirmed by comparing pretreatment and posttreatment images, in which each ultrasound image pair corresponded to the same anatomic area. A portable ultrasound system (SonoSite 180) was used with a 7.5-MHz high-resolution linear transducer to acquire images of the fat layer. A Small Part Imaging program was selected to optimize image quality for near-field imaging down to 3 to 4 cm.

A transparency of the treated and control sides was created for each subject to align ultrasound measurement sites to anatomic features (e.g., moles) during the course of the study. A series of up to 12 evenly spaced ultrasound pretreatment images were acquired through both the control and treated areas. A transparency was used to ensure that the pre- and posttreatment images were aligned in the same image plane including the same anatomic structures.

At the time of posttreatment follow-up evaluation, a template registered with the transparency was used for consistent placement of each ultrasound image location. In addition, posttreatment images were acquired using the corresponding pretreatment image from the same site as a

Fig. 1 Front and side views showing reduction in the love handle area (circled) of subject LH RIO 012. Pretreatment photographs are on the left, and 6-month posttreatment photographs are on the right. A ring over the treated area indicates the treated area reduced in volume over the follow-up period.

Fig. 2 Number of subjects reporting a sensory deficit to three different stimuli: pin prick (pain), light touch, and temperature (cold). Subjects also were assessed for two-point discrimination, but no subjects reported changes for this stimulus at any of the follow-up visits. As is evident from the graph, all reported sensory deficits returned within 7 weeks of cryolipolysis treatment.
reference to match the anatomic structures in both images. Common features found in both the pre- and posttreatment images were used to ensure consistent image alignment and measurement accuracy.

To avoid compression of the dermis and fat layer, a generous layer of ultrasound coupling gel was used to couple the ultrasound transducer acoustically to the subject. A series of up to 12 posttreatment images were acquired during each follow-up visit, which occurred at 2 and 6 months.

A percentage change in fat layer thickness was determined for the control side to account for subject weight variation during the study. A percentage change in fat layer thickness was determined for the treated side to account for fat layer reduction due to cryolipolysis and subject weight variation during the study. Overall fat layer thickness changes were normalized by subtracting the control side percentage change from the treated side percentage change to remove the influence of weight variations. For each subject, up to 12 fat layer reduction measurements were obtained and averaged to determine an overall average fat layer thickness change.

**Neurologic Assessment**

Baseline sensory evaluation by a board-certified neurologist, performed within 7 days of the procedure, consisted of a brief neurologic history and standard measures of sensory function including light touch evaluated with a soft tissue, two-point discrimination, temperature sensitivity (cold temperature sense), and pain sensitivity (assessed with a pinprick). Subjects also reported any subjective impressions of changes in sensory function.

Posttreatment sensory evaluations, performed at approximately weekly visits after treatment, included all the tests carried out at the baseline assessment. Subjects’ responses were not quantified other than to determine whether an aspect of normal sensory function was observed with each assessment or not.

**Nerve Biopsy Assessment**

Epidermal nerve fibers are responsible for heat, cold, and pain (pin prick) sensations associated with reported hypoesthesis symptoms [4]. Skin biopsies were collected from the love handle of one subject 3 and 6 weeks after treatment, in addition to a contralateral control at each time point. These 3-mm skin biopsies were processed by Neurology Ltd. (Minneapolis, MN, USA) using standardized methods [5] to assess epidermal nerve fiber density and sample morphology. The biopsies were sectioned at 60 μm and stained for double immunofluorescent localization of the basement membrane with type 4 collagen (Chemicon, Temecula, CA, USA) and of the nerves with anti-PGP 9.5 (Biogenesis, UK). Independent reviewers from Neurology Ltd. blinded to sample-identifying information evaluated the samples for several morphologic features that are indicators of sensory impairment.

**Results**

This report includes efficacy and sensory information collected for nine subjects over a period of 6 months after treatment (Table 1). All the subjects completed the weekly neurologic assessment until symptoms resolved. At 6 months, photographic assessments were completed for seven of nine subjects, and ultrasound assessments were completed for six of nine subjects.

**Clinical and Photographic Assessment Results**

Treatment sites were clinically evaluated immediately after treatment for any epidermal, dermal, or subcutaneous findings. Clinical observations documented immediately after treatment were consistent with those anticipated for local inflammation (e.g., edema, minor pain, erythema), the majority of which resolved within a few days after treatment.

Erythema was observed in all 25 treatment areas of the nine subjects who received cold exposure. Numbness was reported at 24 of the 25 treatment sites. At the phone follow-up assessment conducted 1 week after treatment, the observations of erythema and numbness were improved if not resolved. No clinical findings were reported for any of the treatment sites at the 2- or 6-month follow-up visits.

An adverse event was reported for one subject in the study. After the treatment on the medial portion of the love handle was completed, the cooling device was applied to

<table>
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<th>Table 1 Summary of study group clinical data</th>
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*Subjects 3, 4, and 9 did not complete the 6-month follow-up period; treatment was not completed for subject 3.
the left anterior portion of the love handle. After several minutes, the subject reported pain, and the treatment was aborted. At the phone follow-up assessment 5 days after treatment, the subject reported slight skin redness that resolved within 6 h of the treatment. Numbness was reported as absent.

For the 6 subjects with follow-up data available on the evaluation of efficacy, the cryolipolysis procedure visibly reduced the size of the love handle or the body surface contour treated, as demonstrated by ultrasound measurements and photography. Figure 1 shows photos of a subject from the sensory study taken at baseline and 6 months after treatment. Three independent blinded reviewers compared pretreatment and 4-month posttreatment images with the same viewing angle of the treated area of five subjects for whom 4-month data were available. Collectively, the three reviewers correctly identified the baseline image from a pair of images 93% of the time.

Quantitative ultrasound measurements of six subjects for whom 2- and 6-month follow-up data were available indicate that each subject demonstrated a reduction in fat layer after a single treatment. The normalized fat layer reduction at 2 months ranged from 11.5% to 26.3% in the study group, with an average reduction of 20.4% across the treated area, and normalized with the control condition to account for subject weight change. At 6 months, the normalized fat layer reduction ranged from 10.7% to 37.5% in the study group, with a larger average normalized reduction of 25.5% (Table 2). There is no apparent correlation between the measured fat reduction and the weight variations recorded during the study.

Sensory Results

Results from the sensory testing for nine subjects are summarized in Fig. 2. For three subjects, sensory testing indicated no changes from baseline in light touch, two-point discrimination, temperature sensitivity, or response to pain or pinprick at any time during the follow-up period. Four subjects had changes in response to light touch, which became apparent 1 to 2 weeks after treatment and typically lasted 1 to 2 weeks. All these changes resolved 2 months after treatment. There were inconsistent effects on two-point discrimination in four subjects that became apparent 1 to 3 weeks after treatment and typically lasted 1 to 2 weeks. Changes in temperature sensation were noted for one subject 1 and 2 weeks after treatment. The sensory changes noted most often were for pain or pinprick, which occurred for six subjects. Reductions in sensitivity to stimuli were noted 1 week after treatment in all these subjects and lasted 1 to 6 weeks. All reductions in pain sensitivity had resolved by 2 months after treatment.

Nerve Biopsies

Figure 3 is a photo of a subject from the nerve biopsy study taken before treatment and 3 months after treatment to demonstrate efficacy. The initial set of skin biopsy samples (treated and control subjects at the height of hypoesthesia symptoms) were evaluated. The number of epidermal nerve fibers could not be quantified in this sample due to a tissue fixation artifact. However, the subepidermal nerve plexi (containing the major nerve bundles of the dermis) for both samples were determined to be qualitatively equal. The morphologic results for the control and 6-week posttreatment sample (resolved hypoesthesia symptoms) seen in Fig. 4, show that both samples contain an equal and normal number of epidermal nerve fibers, indicating that the subject-reported resolution of hypoesthesia symptoms corresponds to a resolved tissue biopsy sample identical to the control sample. Finally, the subepidermal nerve plexi for both the control and resolved biopsies (approximately 6 weeks after treatment) also were deemed qualitatively equal. These results suggest that cryolipolysis does not

<table>
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<th>Subject</th>
<th>Weight change (kg) (2 months)</th>
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<th>Weight change (kg) (6 months)</th>
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Table 2 Fat layer reduction efficacy resulting from cryolipolysis as measured by ultrasound for 6 subjects with follow-up data at 2 and 6 months

* Of the 10 subjects enrolled in this arm of the study, 4 subjects did not complete the 6-month follow-up evaluation including ultrasound assessment

* Normalized reduction is an average of all measured sites within the treated area. Some measurement sites located outside the treatment area showed little to no change as expected and were excluded from the determination of the average fat layer reduction
cause any long-term change to the structure or functionality of either the epidermal nerve fibers or nerve plexi in the dermis [6].

Discussion

These study results are the first to demonstrate the efficacy of cryolipolysis for fat removal in human subjects and the neurologic response. Although cold exposure can cause a transient change in assessed sensory function, this change is not associated with long-term damage to epidermal nerve fiber structure or function. The 2- and 6-month follow-up data for six subjects treated with this cryolipolysis method confirmed measurable reductions in fat thickness of treated areas via ultrasound measurement. This measured fat reduction also results in a visible change in body contour, as judged by blinded independent reviewers of baseline and posttreatment photographs showing the treated area. Extraction of heat from tissue inducing cryolipolysis resulted in a 1-to 6-week reduction in sensation (e.g., light touch, temperature sensitivity, or pain sensitivity) in the treated areas for 6 of 9 treated subjects. The changes in sensation all were transient and resolved by 2 months. None of the subjects evaluated experienced any permanent deficits.

The fat reductions observed in this study are consistent with results from a study with pigs, which indicated that cryolipolysis reduces subcutaneous fat without damage to overlying skin or associated structures (data on file). They also are consistent with the results from studies suggesting that fat cells may be more sensitive to cold than other tissues [7, 8].

Results from pig studies exploring the effect of cold on tissue destruction have suggested that temperatures as high as 1°C can decrease the viability of adipocytes [1]. Evidence in the literature [9] further supports that the temperatures and times used by this device are not damaging to tissues other than fat.
Full recovery of the total number of nerve fibers after approximately 6 weeks, demonstrated by the immunofluorescent staining of nerve biopsies, suggests minimal damage to epidermal nerve fibers (which are responsible for pain, hot and cold sensation) after cryolipolysis. Studies comparing the epidermal nerve fiber count of biopsies after topical dermal treatment with capsaicin showed an 82% loss of fibers after 3 weeks. However, these capsaicin-affected nerve fibers regenerated within the epidermis over a 6-week period, with subjects showing normal levels of sensation except for cold [6]. A full recovery of epidermal nerve fiber counts after approximately 6 weeks, as seen after cryolipolysis, implies that the number of nerve fibers affected by cryolipolysis is small and that the loss of sensory function is transient in nature. Future biopsies, taken at earlier posttreatment times, should aid in clarifying whether epidermal nerve fiber loss and regrowth may be taking place during the follow-up period.

Exposures to cold temperatures above freezing for extended periods can result in degeneration of peripheral nerve fibers [10]. Very long (>10 h) cold exposure at higher temperatures also may cause long-lasting alterations in nerve function [11–13]. Cold damage insufficient to result in rapid anterograde degeneration of nerve fibers still may lead to ischemia-reperfusion injury that results in many of the changes detailed in the preceding statements [14, 15]. Ischemia times shorter than 1 h typically are associated with rapid recovery of function, whereas times 3 h or longer result in permanent damage [16]. Our results indicate that the intensity of heat extraction and the time used for the cryolipolysis effect fall below the threshold for significant nerve damage.

Conclusion

In conclusion, cryolipolysis treatment of humans causes substantial reductions in subcutaneous fat volume and changes in the contour of the treated love handle without damage to the skin. This noninvasive procedure is associated with modest short-term changes in the function of peripheral nerve fibers that resolve shortly after treatment. Biopsy results also confirmed the minimal effects of cryolipolysis on the density of epidermal nerve fibers in the skin. The current results support the conclusion that cold exposure sufficient to achieve significant cryolipolysis is not associated with discernable nerve injury. Additional studies may further characterize the transient neurologic response to this novel method for reducing fat noninvasively.

Acknowledgments The authors thank William Kennedy, MD, of the Kennedy Laboratory, Department of Neurology at the University of Minnesota for study guidance and histologic analysis, and Eric Okamoto, MD, of Fremont Plastic and Cosmetic Surgery, California, and Jeffrey Riopelle, MD, of Laser Advantage (Medi Spa) at San Ramon, California, for contributing subjects to the study. This research was funded by Zeltiq Aesthetics.

References

Cryolipolysis: A Historical Perspective and Current Clinical Practice

H. Ray Jalian, MD, and Mathew M. Avram, MD, JD

Dermatologists have long used cold-based therapeutic approaches for a variety of applications. Based on the differences in chemical composition, it is possible to selectively target certain tissues rich with lipid, while sparing the surrounding tissue predominantly containing water. With historical observations of cold-induced panniculitis suggesting the feasibility of this strategy, cryolipolysis has emerged as a new methodology using controlled cooling to selectively target fat. Both preclinical and clinical studies have established the safety and efficacy of cryolipolysis for noninvasive body contouring. This review will focus on the evolution of cryolipolysis from initial case reports of cold-induced panniculitis, to preclinical and clinical studies, and the current clinical practice.

Semin Cutan Med Surg 32:31-34 © 2013 Frontline Medical Communications

KEYWORDS cryolipolysis, cryotherapy, body contouring, fat, panniculitis

Cryotherapy has a storied history in dermatology. From the early use of carbon dioxide (“dry ice”) to the current benchmark, liquid nitrogen, dermatology as a specialty has long recognized the utility of cold-based therapies in the nonselective destruction of tissue. Indeed, cryotherapy is used for destruction of actinic keratoses, verrucae, and superficial skin tumors routinely in dermatology.1 While these approaches with liquid nitrogen at a temperature of −196°C have largely relied on nonspecific cryoinjury, technology tailored to selectively target fat at far warmer temperatures was recently introduced. In 2007, Manstein et al2 reported a novel noninvasive method for fat reduction, termed “cryolipolysis.” This concept was built on several cues from clinical observations.

The first report of adipose tissue and its sensitivity to cold injury dates back to 1902 by Hochsinger.3 He described firm nodules under the chin in young children, what he deemed an “acute freezing reaction.” It was not until 1941 that Haxthausen4 published a case series of 4 young children and a teenager who had developed what he termed “adiponecrosis e frigore.” He observed that these lesions occurred in the winter after exposure to extreme cold. Reports from 1940 to 1970 echoed these original findings, with red indurated nodules indicative of cold-induced panniculitis occurring in a variety of clinical situations, including in children and adults, after various cold insults.5-7 In 1970, Epstein and Oren8 coined the term “popsicle panniculitis” after reporting the presence of a red indurated nodule followed by transient fat necrosis in the cheek of an infant who had been sucking on a popsicle. Ice cube exposure on the buttocks of this child produced the same lesions. These observations led to the concept that lipid-rich tissues are more susceptible to cold injury than the surrounding water-rich tissue. The susceptibility is even more refined. In most of these cases, children and infants were more frequently affected than adults. These reports point to some clues that confirm the fundamental biochemistry that we observe on a daily basis in our kitchen. Saturated fats, such as butter, are solid at room temperature, whereas their less-saturated counterparts, such as olive oil, are in a liquid state at the same temperature. Indeed, babies’ and young children’s fat is a bit more like butter, with more saturated fats such as palmitic and stearic acid in their adipose tissue.9 This has been experimentally confirmed in animal models when young pigs fed saturated fats were more likely to have cold-induced lipoatrophy than those fed unsaturated fats.10

Preclinical Studies

With these historical observations in mind, a pilot clinical study sought to determine the feasibility of fat reduction using the external application of cold. A single Yucatan pig was exposed to a copper plate cooled to −7°C with circulating
antifreeze solution. Firm pressure was used to ensure contact as well as to decrease perfusion, facilitating a more rapid rate of cooling. Three months after exposure, all 10 sites demonstrated visible indentation with a measurable decrease in superficial fat layer thickness. A subsequent study confirmed this finding in 3 swine, with >30% reduction in the thickness of the superficial fat layer in the treatment area, as measured by ultrasonography. In both these studies, there was limited incidence of transient hyperpigmentation that resolved within 1 week. No ulceration or hypopigmentation was noted. Moreover, no significant change in serum lipids or liver function was noted.

In vitro studies on adipocyte cell cultures suggest that cold-induced adipocyte apoptosis is partially responsible for the clinical effect. An alternate mechanism is reperfusion injury of cryosensitized adipocytes, leading to inflammation, generation of reactive oxygen species, and cell death. Histology reveals a reproducible sequence of events after treatment. Immediately after treatment, there is no histologic evidence of adipocyte damage. This is in stark contrast with heat-based treatments that rely primarily on either selective or nonselective thermal damage and immediate thermal coagulation or necrosis. As early as 2 days after treatment, an inflammatory infiltrate is observed that quickly culminates into a predominantly lobular panniculitis 2-4 weeks after treatment (Fig. 1). This inflammation may last up to 3 months after treatment. Macrophages present within the infiltrate are thought to ingest and clear apoptotic adipocytes. During the 3 months after treatment, there is a gradual clearance of adipocytes and apparent widening of fibrous septae, which is concomitant with the clinical end point of reduction in the fat layer.

**Clinical Studies**

The current clinical device used for cryolipolysis is composed of a cup-shaped applicator that uses a vacuum to draw the target area into the applicator and position it between 2 cooling plates. The vacuum reduces blood flow to the area, facilitating cooling, while the cup-shaped applicator allows for more optimal contouring ability. A cooling intensity factor is then selected, a value that represents the rate of heat efflux out of the tissue. Treatment duration is 60 minutes. Toward the end of the treatment, a massage cycle engages to facilitate homogeneity of crystallization within the treatment site. Various hand pieces are available to tailor the treatment to a specific contour. After removal of the applicator, the immediate clinical end point is apparent as a solid block of tissue in the shape of the applicator (Fig. 2). This quickly resolves, and limited clinical studies support the use of postprocedure manual massage to increase treatment efficacy.

A series of clinical studies confirmed the efficacy of cryolipolysis for improvement in localized adiposity. Clinical trials first demonstrated improvement in adiposity of the flanks, so-called “love handles,” in 32 subjects. Subjective improvement, measured both by subject and investigator assessment, was evident. Ten subjects who underwent ultrasonography examination demonstrated a 22.4% average reduction of fat-layer thickness. A subsequent larger prospective study of 50 subjects confirmed this subjective improvement. Three blinded physician investigators were able to differentiate between pretreatment vs posttreatment sites in 82% of the cases. Based on these data, the device gained Food and Drug Administration clearance for the flanks in 2007. Since its initial clearance, multiple studies have confirmed safety and efficacy of the device, including in the setting of multiple repeat treatments and darker skin phototypes. Cryolipolysis recently gained Food and Drug Administration clearance for use on the abdomen in 2012.

Many practitioners have performed cryolipolysis treatment in patients with focal adiposity in other sites. Any provider using the device in “off-label” locations should pay close attention to ensure sufficient adiposity for efficacy. Care should also be taken to avoid areas with superficial nerve bundles, such as the upper arm, as this can result in temporary dysesthesia distal to the treatment area (see discussion of side effects later in text).
Patient Selection

As with any device-based treatment, patient selection is paramount. One should obtain a thorough medical history, including medications, rheumatologic history, and surgical history, particularly prior to abdominal surgery. Physical examination should aim at determining whether the patient is a good candidate. Areas with focal adiposity should be easily lifted from the underlying musculature. There should be a sufficient fat layer, otherwise the device may not attach correctly with the vacuum applicator. In those with previous abdominal surgeries, physical examination should focus on palpating for a hernia both in the recumbent position and also while the patient performs a Valsalva maneuver, as there is a potential for hernia incarceration with the vacuum suctioning.

Patient counseling is also an important predictor of satisfaction. Potential patients should be made aware of the moderate efficacy of the device. Patients should also be informed that the results are delayed and can take up to 3 months to notice a difference. There is usually a clear improvement, however this treatment does not approach the efficacy of liposuction. Cryolipolysis is not a substitute for diet and exercise, and treatment is largely cosmetic, offering minimal health benefits. This is not a weight-loss device, and it is not suitable for those who are looking to achieve global weight loss. Moreover, those with predominantly visceral fat are poor candidates and should not have this treatment. Figure 3 shows a representative outcome after a single application to the right flank before and 2 months after treatment.

There are some relative contraindications. The device manufacturer recommends caution when treating those with cold-sensitive disorders, including Raynaud’s phenomenon, cold urticaria, cryoglobulinemia, and paroxysmal nocturnal hemoglobinuria. Because of the temporary neurologic effects (discussed later in the text), it may be prudent to exercise caution in those with known neurologic disease (eg, multiple sclerosis).

Adverse Events

There have been roughly 450,000 cryolipolysis treatments since its introduction. In addition to efficacy, clinical experience points to the relative safety of this device. Immediately after treatment, there is expected edema and erythema that can last for up to 72 hours. Ecchymosis secondary to the vacuum applicator is not uncommon, especially in those on anticoagulation medications. In addition to these transient effects, decreased cutaneous sensation is common. Nearly all patients experience some sort of dysesthesia in the treatment.
site, which largely resolves within 1 week. However, there can be limited residual decrease in sensation that can last up to 2 months. No reports exist of cases of permanent sensory alteration after cryolipolysis treatment. Similar to the animal studies, no significant change in triglyceride levels or liver function tests was reported in the human studies.

Perhaps in connection with these transient neurologic connections, rare reports of severe pain emerged after cryolipolysis treatments. With an incidence of 1 in 1500 patients, the pain is described as severe shooting and jabbing in the treatment area 1 week after treatment. The incidence appears to be higher when using the larger treatment applicator. The mechanism remains unclear but may be related to hyperalgesia associated with transient nerve damage and subsequent regeneration or perhaps a more robust panniculitis. In the initial report of 23 patients, adequate pain control was achieved with topical or oral analgesics. All cases resolved spontaneously within 1–4 weeks.

This is an intriguing new technology, but both physicians and patients should be aware that there are important limitations. Because of the size of the applicator, currently only focal collections of adiposity can be targeted. This is in contrast to other commercially available body contouring devices that can treat larger areas in 1 treatment session. Also, the current clinical benefit is modest, and multiple treatments may be required to achieve the desired clinical outcome. Finally, we are currently still unaware of the long-term side effects of this treatment. As the popularity of the device increases and we move further away from initial approval, rarer side effects may emerge.

Conclusions

The selective targeting of lipid-rich tissue with cold has ushered in a novel methodology with moderate efficacy for noninvasive fat reduction. While cryolipolysis is not a weight-loss tool, it can effectively improve local pockets of adiposity, resulting in an improvement in body contour. The technology is still young, but current clinical experience points to a favorable safety profile with mild transient adverse events.

We have long recognized the utility of cold-based therapies for the nonselective destruction of tissue. In the past, we have relied on modalities using temperatures far exceeding the freezing point of water. Astute attention to clinical cues provided by historical observations led to the evolution of selective targeting of fat. By adapting this strategy to use far warmer temperatures, we can now preferentially target lipid-rich tissue without affecting the surrounding tissue rich with water. Treatment outcomes may be further optimized with additional studies. The knowledge that the target’s susceptibility to injury is in turn dictated by its chemical composition enables us to fine-tune the treatment for different clinical outcomes. With this paradigm in mind, other novel cold-based therapies are theoretically foreseeable. Cryolipolysis has enjoyed the most commercial success in this venue; however, other novel approaches to selectively target tissue are in the pipeline.

References

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.
localized areas of fat and tightening skin, is also being evaluated\textsuperscript{10} 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.\textsuperscript{11,12} Subcutaneous injection of phosphatidylcholine and deoxycholate has also been tested for fat dissolution.\textsuperscript{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.\textsuperscript{14,15} Results from studies exploring the effect of cold on tissue destruction have suggested that temperatures as high as \(1 \text{°C}\) can decrease the viability of adipocytes.\textsuperscript{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 (\(-43.8\) mW/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.
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 tissue massage. Each site was treated for 45 minutes, including a 5-minute period of tissue massage. 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/cm²). 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.
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 treatment. 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 animals (Table 1).
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 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

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

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/cm²) through monitoring of several electronic thermistor sensors on the skin surface. In other situations, cold exposure can result in significant inflammation of the skin, but fat appears to be more susceptible to cold exposure and to ischemia and ischemia-reperfusion injury than other tissues, including the skin.

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.

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

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Posttreatment Day</th>
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<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
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<tr>
<td>A</td>
<td>11.6</td>
</tr>
<tr>
<td>B</td>
<td>-7.7</td>
</tr>
<tr>
<td>C</td>
<td>5.6</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>16.7</td>
</tr>
<tr>
<td>B</td>
<td>25.8</td>
</tr>
<tr>
<td>C</td>
<td>41.2</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>40.0</td>
</tr>
<tr>
<td>B</td>
<td>-2.9</td>
</tr>
<tr>
<td>C</td>
<td>7.7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-22.2</td>
</tr>
<tr>
<td>B</td>
<td>-55.9</td>
</tr>
<tr>
<td>C</td>
<td>-53.7</td>
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</tbody>
</table>

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


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Cryolipolysis for Reduction of Excess Adipose Tissue

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Controlled cold exposure has long been reported to be a cause of panniculitis in cases such as popsicle panniculitis. Cryolipolysis is a new technology that uses cold exposure, or energy extraction, to result in localized panniculitis and modulation of fat. Presently, the Zeltiq cryolipolysis device is FDA cleared for skin cooling, as well as various other indications, but not for lipolysis. There is, however, a pending premarket notification for noninvasive fat layer reduction. Initial animal and human studies have demonstrated significant reductions in the superficial fat layer thickness, ranging from 20% to 80%, following a single cryolipolysis treatment. The decrease in fat thickness occurs gradually over the first 3 months following treatment, and is most pronounced in patients with limited, discrete fat bulges. Erythema of the skin, bruising, and temporary numbness at the treatment site are commonly observed following treatment with the device, though these effects largely resolve in approximately 1 week. To date, there have been no reports of scarring, ulceration, or alterations in blood lipid or liver function profiles. Cryolipolysis is a new, noninvasive treatment option that may be of benefit in the treatment of excess adipose tissue.

Semin Cutan Med Surg 28:244-249 © 2009 Elsevier Inc. All rights reserved.

KEYWORDS cryolipolysis, Zeltiq, non-invasive, body contouring, fat removal, cold panniculitis

Fat treatment and removal is a worldwide, billion-dollar cosmetic industry, and liposuction remains the most common surgical cosmetic procedure performed in the United States.1 Although liposuction is an effective therapeutic option for the removal of fat and can be safely performed on an outpatient basis, it remains an invasive procedure. In recent years, there has been a dramatic trend toward effective, noninvasive procedures. Unfortunately, current noninvasive fat treatments such as Endermologie (LPG Systems, Valence, France), radiofrequency treatment, and lasers have resulted in only modest clinical improvements in the appearance of fat and cellulite.2–8 There is, therefore, a great demand and need for an effective, selective, and noninvasive treatment option for excess adipose tissue.

Cryolipolysis is based on clinical observations that cold exposure, under the proper circumstances, can result in localized panniculitis; this panniculitis ultimately results in the reduction and clearance of adipose tissue. Cold-induced panniculitis was initially described in infants, where it is frequently known as popsicle panniculitis.9,10 However, it has also been observed in adult patients; for example, panniculitis occurring after horseback riding in cold environments is known as equestrian panniculitis.11 Exogenous application of cold, particularly with aggressive cryosurgery, is known to cause epidermal damage as well as damage to the underlying adipose tissue.

Cryolipolysis attempts to use controlled fat cooling, also known as energy extraction, to cause localized panniculitis and fat reduction. By controlling and modulating the cold exposure, it could be possible to selectively damage the adipocytes, while avoiding damage to the overlying epidermis and dermis. This would result in an effective, localized, and noninvasive treatment for excess adipose tissue.

Proposed Pathogenesis

The exact pathogenesis by which cold results in adipose tissue removal is unknown. Case reports of infants suffering from popsicle panniculitis and adults with equestrian panniculitis demonstrate that a perivascular inflammatory infiltr-
trate consisting of histiocytes and lymphocytes develops approximately 24 hours after cold exposure. The inflammatory infiltrate results in a lobular panniculitis. The inflammatory cells cause a rupture of the adipocytes, aggregation of the lipids, and the formation of small cystic spaces. This panniculitis slowly resolves over the next several weeks, ultimately resulting in modulation of the fat without any persistent tissue damage or scarring.

A similar mechanism of action has been proposed for cryolipolysis. In animal models, cold exposure results in inflammation, damage to the fat cells, and ultimately phagocytosis of the adipocytes.12 Immediately after the treatment, no fat damage is observed and the adipocytes are intact. Initial adipocyte damage is noted histologically at day 2, and increases throughout the next month. It is believed that adipocyte apoptosis stimulates the initial inflammatory infiltrate, though the exact mechanism is not fully characterized; pig adipocytes in culture undergo apoptosis and necrosis following exposure to cool temperatures.13 At day 2 after treatment, pig biopsy samples demonstrate localized subcutaneous mixed inflammation, consisting of neutrophils and mononuclear cells, and the adipocytes remain unchanged. Over the next week, the infiltrate becomes denser and an intense lobular panniculitis develops. The inflammation appears to peak at approximately 14 days following treatment when the adipocytes are surrounded by histiocytes, neutrophils, lymphocytes, and other mononuclear cells. During 14-30 days, the inflammatory infiltrate becomes more monocytic, consistent with a phagocytic process. Macrophages begin to envelop and digest the apoptotic adipocytes, thereby facilitating their elimination from the body. As this process occurs, the average size of the adipocytes decrease, a wider range of adipocyte sizes are observed, and the fibrous septae of the fat layer become widened. The actual elimination of the adipocytes from the body occurs slowly over at least the next 90 days. The exact mechanism and pathway by which the phagocytosed adipocytes are eliminated from the body are not fully understood at present. Ultimately, the lobules of fat cells decrease in size, and the fibrous septae constitute a majority of the volume of the subcutaneous layer. Clinically, this corresponds to a decrease in the thickness of the subcutaneous fat layer.12,14

These initial animal studies have helped to shape the likely mechanism of cryolipolysis. However, it should be stressed that the exact mechanism has not been fully elucidated. It is unclear why adipocytes are more sensitive to cold temperature than other cell lines. It is also not fully established why adipocyte apoptosis occurs and how this leads to the observed inflammatory infiltrate. Finally, once the adipocytes are phagocytosed and mobilized, the full mechanism of elimination is not well characterized. The adipocytes are thought to be mobilized via the lymphatic system, but it remains to be determined how they are then eliminated or redistributed throughout the body in response to cryolipolysis. As the technology continues to be developed, future studies will need to further investigate these issues.

Clinical Animal Studies

Manstein et al12 performed the initial exploratory studies of cryolipolysis in Yucatan pigs. In their article, they described the results of 3 different studies: an initial exploratory study, a dosimetry study, and a study of treatment effect on serum lipid levels.

The initial exploratory study used a cold copper applicator, chilled by circulating antifreeze solution. The cooling device was maintained at a constant temperature of −7°C, and was applied to the Yucatan pig for times ranging from 5 to 21 minutes. The highest degree of clinical effect was noted in a treatment area on the buttock; 3.5 months after the single treatment, 80% of the superficial fat layer was removed (40% of total fat layer).

Following the demonstration of efficacy with the copper applicator, a prototype clinical device (Zeltiq Aesthetics Inc., Pleasanton, CA) was developed, which contained a thermoelectric cooling element. This device allowed for the use of variable, present plate temperatures during treatments; the cold temperature was maintained at a constant level via temperature sensors imbedded within the treatment plates. Treatments were performed with this device in either a “flat configuration” with a flat panel cooling the skin or in a “folded configuration” in which the excess tissue was pinched between 2 cooling panels, allowing for cooling on both sides of the tissue. The tissue was exposed to cold ranging from 20°C to −7°C for 10 minutes. All sites treated with cold exposure less than −1°C developed perivascular inflammation, panniculitis, and ultimately fat layer reduction. Fat damage was significantly greater at lower temperatures, and increased over time.

In Manstein’s lipid study, no significant changes in the lipid profiles of the animals were noted immediately or at any time point through 3 months post treatment. There was a temporary decrease in serum triglycerides immediately following the cold exposures, though this was attributed to fasting before and during general anesthesia.

A follow-up animal study was performed by Zelickson et al.14 In this study, 4 pigs were treated with the cryolipolysis device. Three animals underwent a single cryolipolysis treatment, while the fourth pig underwent 7 treatments (90, 60, 30, 14, 7, and 3 days, as well as 30 min before euthanasia) with the cryolipolysis device. About 25%-30% of the total body surface of each animal was treated. Ultrasound assessments demonstrated a 33% reduction in the thickness of the superficial fat layer following cryolipolysis. Pathologic specimens revealed an approximate reduction of 50% in the thickness of the superficial fat layer. Erythema lasting approximately 30 minutes developed in treatment areas. The skin became cool, though not frozen, after treatment. There was no edema, bruising, purpura, or scarring observed in the trial. Lipid panels were performed for each animal at multiple time points; the baseline profile was after a 12 hour fast before treatment, with follow-up lipid profiles performed 1 day, 1 week, and 1, 2, and 3 months after treatment. There were no significant variations in the lipid profiles of the animals throughout the study.
In the above animal studies, the cryolipolysis treatments were well tolerated by the animals. Erythema of the treated areas was common. In the initial animal studies by Manstein et al, whitening, hardening, and freezing of the skin was noted in 30% of the treated areas. Superficial epidermal necrosis was observed in some of the frozen areas, with resulting transient hypopigmentation following re-epithelialization. However, no scarring or ulceration was noted in any of the animal studies.

**Human Clinical Studies**

Following the promising animal studies, the Zeltiq System (Zeltiq Aesthetics Inc, Pleasanton, CA) was developed. This device consists of a control console, with a treatment applicator attached by a cable. A thermal coupling gel is placed on the area to be treated, and the applicator is then applied. Tissue is drawn into the cup-shaped applicator with a moderate vacuum to optimally positioning the tissue between 2 cooling panels; this allows for more efficient cooling of the tissue. A cooling intensity factor (CIF) is then selected by the treating clinician. The CIF is an index value representing the rate of heat flux into or out of tissue opposite the cooling device. Treatment with the cold exposure for up to 60 minutes then begins. The energy extraction rate, or cooling, is controlled by sensors that monitor the heat flux out of the treated areas and is modulated by thermoelectric cooling cells. Following completion of the treatment, the system automatically stops the cold exposure and the clinician releases the vacuum. Depending on the surface area to be treated, multiple applications may be necessary to effectively expose the entire area to cryolipolysis. It is important to note that the Zeltiq device is presently FDA cleared for skin cooling and other various indications. However, the Zeltiq device is not currently FDA cleared for lipolysis, although there is a pending premarket notification for noninvasive fat layer reduction.

A multicenter, prospective, nonrandomized clinical study evaluating the use of cryolipolysis for fat layer reduction of the flanks (ie, love handles) and back (ie, back fat pads) was conducted at 12 sites. Patients underwent cryolipolysis treatment to 1 area, while a symmetric, contralateral area was left untreated to serve as a control for observing clinical efficacy. An interim subgroup analysis of all patients in the “love handle” group, 32 patients, was performed. Clinical efficacy was determined at 4 months post-treatment using visual assessment with digital photography, physician assessment, and subject satisfaction. Most patients had a clinical improvement with a visible contour change, as assessed by physician observation and digital photography (Fig. 1). A subset of 10 patients underwent pre- and post-treatment ultrasound imaging. Of these 10 patients, all had a decrease in the thickness of their fat layer, with an average reduction in thickness of 22.4%. Importantly, regardless of the assessment protocol, the best cosmetic results were achieved in those patients with a modest, discrete fat bulge. The treatment was well tolerated by patients with no adverse events related to the device or treatment.

Kaminer et al, demonstrated that cryolipolysis results in a visible cosmetic improvement in the flank/love handle region. A blinded comparison of preprocedure and 6 month postprocedure photographs was performed on 50 subjects. Three physicians specializing in dermatology, cosmetic surgery, or plastic surgery performed the photographic review. The physicians were able to accurately differentiate between the pre- and post-photographs in 89% of the cases. When the

![Figure 1](image-url)
evaluation was limited to those subjects who maintained their original weight, ±5 lb after the procedure, the physicians were able to accurately differentiate 92% of the cases. This study demonstrates that the improvement following cryolipolysis treatment is clinically apparent on visual inspection, further documenting the usefulness of this technology.

A feasibility study of using cryolipolysis to reduce abdominal fat is currently ongoing. A total of 42 subjects were enrolled in this study. Symmetric abdominal fat bulges, typically to the left and right of the umbilicus, were treated with cryolipolysis. An interim analysis of the subjects' self-assessments indicated that 79% (31 of 39) subjects reported clinical improvement within the first 2-4 months after the procedure (Fig. 2). Further clinical end points, including blinded physician assessments and ultrasound measurements of fat layer thickness, have not been reported as of yet. The interim analysis appears to support that cryolipolysis may be effective for noninvasive contouring of abdominal fat and body sculpting.

Cryolipolysis seems to be an effective treatment option for the reduction of excess adipose tissue, as shown in these clinical studies. It is important to note that the clinical improvements were most pronounced in patients with localized, discrete fat bulges. The technology does not appear to be as effective in patients with significant skin laxity or who are obese. Thus, cryolipolysis is an effective treatment option for the reduction of fat, particularly when the proper patients are selected for treatment.

### Safety Profile

As with any new technology, it is important to establish whether the device results in any significant adverse events. In the previous clinical studies, the device has been well tolerated by the subjects. Patients typically develop erythema of the treatment area, lasting up to a few hours following cryolipolysis. As the device uses a vacuum to increase clinical efficacy, patients may also develop bruising of the treatment area, which may last approximately 1 week. The treated skin also becomes cold and firm following cryolipolysis. In all clinical studies to date, no ulceration or scarring has been reported.

Cryolipolysis has been reported by human subjects to result in a temporary dulling of sensation and numbness in treated areas. To better characterize this phenomenon, Coleman et al performed cryolipolysis on 10 subjects with flank fat bulges. Following cryolipolysis, a 20.4% reduction in the thickness of the fat layer was observed, as assessed by ultrasound. Thus, the patients had achieved an effective cryolipolysis treatment. These subjects underwent neurologic assessment by a board-certified neurologist during the study, including light touch evaluated with a soft tissue, two-point discrimination, temperature sensitivity (cold temperature sense), and pain sensitivity (assessed with a pinprick). Neurologic assessments were performed at baseline and weekly following treatment. One subject underwent skin biopsy for histologic analysis of nerve-fibers. Patients reported numbness in 24 of the 25 treated sites (96% of treated sites), though by 1 week following treatment the numbness had

![Figure 2](image-url) A representative example of clinical improvement following 1 treatment with cryolipolysis for fat layer reduction of the abdomen. The patient underwent a single treatment with cryolipolysis, though 2 applications (1 application to the right and 1 application to the left side) were required to treat the entire abdomen due to the larger surface area. The top pictures show the baseline, while the bottom pictures demonstrate the clinical improvement 4 months after treatment. The patient's weight at the 4 month follow-up day had increased by 3.5 lb from the baseline day. This figure is obtained and used with permission of Ivan Rosales-Berber, MD.
lately resolved. Transient reductions in sensation were reported in 6 of 9 patients (67%), most commonly manifested as reductions in pain sensitivity. However, reductions in light touch, 2 point discrimination, and temperature sensitivity were also reported by a minority of patients. These reductions in neurologic sensation lasted between 1 and 6 weeks, with a mean duration of 3.6 weeks. All reductions in neurologic sensation had resolved by 2 months after the cryolipolysis treatment. No changes were noted in the nerve biopsy 3 months after the cryolipolysis compared with the baseline biopsy. These results indicate that cryolipolysis results in a decrease in sensation of treated areas, but this altered sensation is transient and appears to resolve without any further intervention.

As previously discussed, the exact mechanism of cryolipolysis is not well understood. It is possible that as the fat is destroyed and phagocytosed, the fat could be released into the blood. Many of the clinical studies have therefore analyzed the patient’s lipid profiles and liver function tests following cryolipolysis. In the animal studies by Manstein and Zelickson, no significant changes in the lipid profiles following cryolipolysis were observed. In all human studies to date, no clinically significant alterations in lipid profiles or liver function tests have been observed. Klein et al.,19 reported on 40 patients with bilateral fat bulges on their flanks (ie, love handles) treated with cryolipolysis. The patients were treated on 1 or 2 sites on each flank, depending on the size of the fat bulge, to a maximum of 4 treatment applications. Patients were treated at a CIF of 42 for 30 minutes. Lipid values were obtained, including triglycerides; total cholesterol; and very-low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein (HDL) cholesterol. Additionally, liver-related blood tests, including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, and albumin were obtained before treatment. Follow-up values were determined 1, 4, 8, and 12 weeks after treatment. Triglyceride values were noted to increase slightly in the 12 weeks following cryolipolysis, from a mean of 82.1 to a mean of 93.2; this increase was not statistically significant (P = 0.22) and the mean value remained well below the upper limit of the reference range. There was, however, a statistically significant decrease in HDL cholesterol in the first few weeks following cryolipolysis (P = 0.0296), though the HDL values did return to baseline by 12 weeks. No statistically significant changes from baseline for any of the liver function tests were observed following cryolipolysis.

These initial safety reports support that the Zeltiq cryolipolysis device results in a significant reduction in fat layer thickness with no significant adverse events. During the informed consent process before cryolipolysis treatment, it is important to emphasize known risks including erythema, bruising, and temporary altered sensation. To date, there does not appear to be significant risk of altered lipid profiles or liver function tests associated with cryolipolysis treatment. It remains to be determined whether patients with rare, cold-induced dermatologic conditions, such as cryoglobulinemia, cold urticaria, or paroxysmal cold hemoglobinuria, can be safely treated with cryolipolysis. Patients with a known history of cold-induced disease should probably not be treated with the cryolipolysis device until further data are available.

Discussion

Cryolipolysis is a novel procedure, which uses controlled cold exposure, known as energy extraction, to produce noninvasive, effective, and selective damage to adipocytes. In animal and human clinical studies, cryolipolysis has been shown to result in significant improvement in the clinical appearance of fat. Additionally, reductions in the thickness of the subcutaneous fat layer of up to 50% can occur following a single cryolipolysis treatment. Clinical studies have shown potential efficacy in the treatment of excess back fat, flank fat, and abdominal fat; the potential efficacy of cryolipolysis in other treatment areas and for the treatment of cellulite remains to be determined. In these initial studies, cryolipolysis treatments have been well tolerated by patients with transient, mild adverse events such as erythema and bruising occurring in treated patients. No cases of ulceration, scarring, or significant changes in lipid profiles and liver function tests have been reported following cryolipolysis. Cryolipolysis therefore appears to be a safe and effective treatment option for reduction of excess adipose tissue.

The exact mechanism of cryolipolysis remains to be fully elucidated. It has been shown that cold exposure results in apoptosis of the adipocytes, followed by an inflammatory infiltrate. Ultimately, the inflammatory infiltrate results in phagocytosis and mobilization of the treated adipocytes. The exact mechanism and pathway for this fat elimination are unclear. No significant alterations in blood lipid profiles, other than transient decreases in HDL values, or liver function tests have been observed following cryolipolysis. Further studies to determine the exact mechanism of action for cryolipolysis remains an active area of research.

Although cryolipolysis is a promising new technology, it is important to bear in mind a few potential limitations. In the human clinical studies, results were most visible in patients with discrete, localized fat bulges. Cryolipolysis does not appear to be as effective in obese patients or patients with excess skin laxity. It is unclear whether the device itself is less effective in these patients, or whether the potential improvement associated with cryolipolysis treatment is harder to observe in these patients. Additionally, the improvement following cryolipolysis is not immediate, but rather occurs slowly over the course of 2-3 months. Finally, the currently available data seem to support that cryolipolysis is most effective for localized, discrete fat bulges. Thus, patients seeking large scale fat removal, which can be achieved with liposuction, may not achieve their desired outcomes with cryolipolysis. It is therefore important for physicians to carefully select potential cryolipolysis treatment patients, as well as educate them regarding their expected outcomes and potential limitations.

Cryolipolysis is a new, selective, effective, and noninvasive treatment option for excess adipose tissue. While the device is currently FDA cleared only for skin cooling, a premarket notification application for lipolysis is pending. The device is
particularly appealing given that it is noninvasive, requires not much or no downtime for patients following treatment, and does not require local or regional anesthesia. Ongoing clinical studies will help to determine the full potential and efficacy of this device. Cryolipolysis appears to be a promising new technology for safe, effective, and noninvasive treatment of fat.

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Cryolipolysis™ for Subcutaneous Fat Layer Reduction

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Background and Objective: Cryolipolysis is a unique non-invasive method for the selective reduction of fat cells with controlled, localized cooling. It is important, therefore, to understand the potential efficacy and safety of this new procedure for fat layer reduction.

Materials and Methods: A review of the literature associated with cryolipolysis was performed to evaluate the findings from pre-clinical and clinical studies with respect to the mechanism of action, efficacy, and safety.

Results: Cryolipolysis has demonstrated efficacy in both human and animal studies. Histology findings also confirm the selective reduction of fat in both humans and animals, with evidence of a gradual thinning of the fat layer over a period of two to four months. Importantly, cryolipolysis has not produced any significant adverse side effects in studies to date and any noted effects have been minor and temporary.

Conclusion: Although the mechanism of action for cryolipolysis is not yet completely understood, the efficacy and safety of this non-invasive procedure for fat layer reduction has been demonstrated in the studies available to date. Further studies will assist in identifying the mechanism and elucidate the full potential of this technology to perform safe, non-invasive fat reduction for areas of local fat accumulation.

Key words: cryolipolysis; fat reduction; non-invasive cooling; body contouring

INTRODUCTION

Fat removal and body reshaping, are increasingly popular cosmetic procedures. Currently, liposuction is by far the most common and effective procedure for body contouring. Given the invasive nature of liposuction, and its inherent risks, there has been an ongoing quest for the development of non-invasive forms of body contouring. Various non-invasive techniques have been attempted for body contouring such as laser, ultrasound, radiofrequency, and infrared light, with variable, if any, scientific demonstration of efficacy.

A more recent development in non-invasive lipoplasty has been a novel method of fat layer reduction, termed “cryolipolysis” for the selective destruction of fat cells. This novel non-invasive technology uses controlled cold exposure to effect a gradual reduction of the subcutaneous fat layer using natural thermal diffusion, without damage to other tissues.

BACKGROUND

Numerous studies document that localized inflammation of subcutaneous fat can occur under certain conditions when the tissue is exposed to cold. Such cold-induced inflammation has been described most frequently in infants [1,2], but has also been seen in adults such as females who participate in equestrian activities [3]. Case reports describe the occurrence of a clinically evident inflammation in infants [1,2] as confirmed histologically after apparently minor cold exposure, that is, ice cube application for a few minutes. Histological assessment of the exposed areas in some of these infants demonstrated that perivascular infiltration of histiocytes with a few lymphocytes, most intense at the dermal–subdermal junction with extensions into the adjacent dermis and subcutaneous fat, was initiated about 24 hours after cold exposure. The change became more pronounced through 72 hours with the appearance of additional inflammatory cells in the subcutaneous fat, rupture of some of the adipose tissue cells, and aggregation of the lipids. Slight progression of the inflammatory response continued for three more days with histiocytes, neutrophils, lymphocytes, and other mononuclear cells surrounding the adipose tissue cells [1]. Within a few weeks, the cold panniculitis resolved spontaneously without any persistent tissue damage and there was no evidence of cryoglobulins for any of the infants evaluated. Case reports also have been documented of cold panniculitis in women who have been horseback riding in cold and damp conditions [3]. In these women, the inflammatory reaction in the adipose tissue occurred at the dermal–subcutaneous junction with an infiltrate of lymphocytes and neutrophils, as well as a sparse scattering of mast cells and foamy histiocytes. The infiltrate seemed to extend from...
A perivascular location into adjacent adipose tissue where fat cells were ruptured and small cystic spaces were formed.

PRE-CLINICAL STUDIES

Manstein et al. [4] performed animal experiments to evaluate the potential for selective damage to subcutaneous fat with controlled application of cold to the skin surface. Three complementary pig studies were completed: an initial exploratory study, a dosimetry study, and a safety study to assess the potential impact of such selective damage to subcutaneous fat on lipid levels.

The exploratory study was designed to determine the feasibility of using non-invasive cold exposure to remove subcutaneous fat. A slightly convex, circular copper plate was pressed firmly against the skin surface and cooled by circulating cold antifreeze solution at \(-7^\circ C\) through a heat exchanger chamber attached to the copper plate. The cold exposure was repeated at multiple sites on the pig, with the exposure time varied between 5 and 21 minutes. The pig was observed for 3.5 months for the appearance and persistence of local fat loss. The amount of fat loss at each test site was estimated relative to adjacent unexposed fat at the margins of the test site.

No apparent skin injury was documented in any of the test areas. There was a slight increase in pigmentation at the 1-week follow-up for some of the test sites but there were no hypopigmentation, scarring, or textural changes. On gross observation, selective fat loss was evident by smooth indentation along the surface of the animal of a size and shape similar to that of the cooling device. A reduction of fat in the superficial fat layer was documented at 3.5 months with 80% of the superficial fat layer removed for a total fat loss of 40% from the procedure. Histology further demonstrated a marked reduction in the distance between fat septae.

The dosimetry study was performed on four pigs with a prototype device (Zeltiq™, Aesthetics, Pleasanton, CA) that contained a thermoelectric cooling (TEC) element. A variable, preset plate temperature was maintained constant during each cold exposure by electronic regulation according to temperature sensors embedded within the cooling plate. Test sites were exposed to either a flat configuration with the device applicator pressed firmly against the skin surface or a folded configuration with the skin fold captured between two cooling plates. The cooling temperature ranged from \(-1\) to \(-7^\circ C\) for 10 minutes. The animals were sacrificed at selected time points that ranged from immediately to 28 days after exposure. Test sites and surrounding areas were clinically assessed and photographed. Histological analyses of the test sites were also completed using deep tissue vertical sections (skin, fat, underlying muscle) stained with hematoxylin and eosin to assess the level of fat damage as well as potential damage to the dermis or epidermis.

No apparent injury of either the epidermis or dermis was documented for any of the test sites, at any time period, during the dosimetry study. Adipocytes appeared normal immediately and 1 day after exposure, but inflammation of the subcutaneous fat became evident as localized clusters of mixed neutrophil and mononuclear cell inflammatory infiltrate in a predominately lobular pattern became apparent. The inflammation continued to intensify through 30 days following exposure with evidence of phagocytosis. Lipid-laden mononuclear inflammatory cells became abundant, the average size of the adipocytes appeared reduced, and a wider range of adipocytes sizes was apparent. The degree of the inflammatory response was also dependent on the temperature used. Blinded grading of the extent of inflammation of the subcutaneous fat demonstrated that fat damage was significantly greater at lower temperatures and increased significantly over time when compared to an unexposed control.

The lipid level study included six animals for which a relatively large area of the skin surface (15%) was exposed to cooling with a prototype cooling device that included a flat copper plate cooled by a TEC element. Test sites were exposed to temperatures that ranged between \(-5\) and \(-8^\circ C\) for 10 minutes. Blood samples were obtained after a 12-hour fast prior to treatment, within 1 hour and 1 day, 1 week, and 1, 2, and 3 months post-treatment. The lipid levels over time following cold exposure demonstrated no significant change other than a temporary decrease in serum triglycerides immediately following the cold exposure (attributed to fasting prior to and during general anesthesia).

These pre-clinical animal studies demonstrated that it was possible to non-invasively induce selective, localized damage to subcutaneous fat without epidermal or dermal injury. Selective effects on the subcutaneous fat were evident after exposure to cooling on the skin surface with a range of temperatures and exposure times with both histological assessment and gross observation. Persistent fat reduction without any evidence of damage to the skin or an increase in lipid levels was demonstrated.

The findings from these studies were further supported by additional animal studies performed to assess the ability of cryolipolysis to selectively reduce subcutaneous fat without damage to other tissues or a meaningful change in lipid levels or liver function [5]. Four pigs were treated and survived for 90 days. Three pigs received a single treatment; one pig received multiple treatments staged at 90, 60, 30, 14, 7, and 3 days and immediately prior to euthanasia. Approximately 25–30% of the total body surface area was treated in each animal.

Test sites were exposed to cooling based on the rate of energy extraction, that is milliwatts per centimeter squared (mW/cm²). A numerical value, referred to as the “Cooling Intensity Factor” (CIF), was used to express the rate of heat extraction and, therefore, cooling of the skin. The pigs that received a single treatment were exposed to CIF 24.5 (\(-43.8\) mW/cm²) for 45 minutes with a 5-minute period of tissue massage. The pig that received multiple treatments was treated with CIF 21.5 (\(-36.8\) mW/cm²) for 15 minutes at each site. Treated and adjacent areas were evaluated by standardized flash photography and diagnostic ultrasound after 90 days, and necropsy tissue was collected. Lipid levels were completed (on
MECHANISMS OF ACTION OF CRYOLIPOLYSIS

Histologic analysis at various time periods after cold exposure demonstrates that cryolipolysis results in the death of adipocytes that are subsequently engulfed and digested by macrophages [4,5]. Immediately following treatment, there are no discernible changes in the subcutaneous fat; no inflammatory cells are present and the membranes of the cells are intact. Within 3 days of treatment (Fig. 2A), however, there is evidence that an inflammatory process stimulated by adipocyte apoptosis has begun, as reflected by an influx of inflammatory cells. The inflammation appears to peak at approximately 14 days (Fig. 2B) following treatment as the adipocytes are surrounded by histiocytes, neutrophils, lymphocytes, and other mononuclear cells. Between 14 and 30 days (Fig. 2C) after treatment, phagocytosis of lipids is apparent; macrophages and other phagocytes surround, envelope and digest the contents of dead cells as part of the body’s natural injury recovery response to rid the body of unwanted material. The fat cells become smaller and irregularly shaped as they are slowly digested by the macrophages surrounding them. After this period, the inflammatory response appears to subside and the volume of the fat cells is decreased with apparent thickening of the interlobular septae occurring by 60 days (Fig. 2D). The inflammatory process declines further by 90 days after treatment. The area previously containing fat cells is decreased and the septae constitute a majority of the tissue volume.

It appears that the lipids remain trapped within the subcutaneous tissue until they are digested and cleared by a natural inflammatory process. This resorption takes place over more than 90 days, resulting in a very gradual displacement of the lipids. The histological results are also visible on gross pathology sections at 90 days, showing a clear reduction in fat layer thickness.

The mechanisms governing the death and subsequent elimination of adipocytes are not completely understood. Initial studies have been performed in an attempt to elucidate a potential pathway [6]. Porcine adipocytes were isolated, cultured and exposed to temperatures ranging from −2 to 28°C and 5% CO₂ for 1 hour. Following exposure, the cells were returned to normal culture conditions (5% CO₂ and 37°C) for a recovery period of either 2 or 24 hours. Assays performed to determine the extent of necrotic cell death and apoptotic cell death indicated that adipocytes cooled to −2, 0, and 2°C were all necrotically injured regardless of recovery time, as were most of the adipocytes cooled to 7°C. Adipocytes cooled to temperatures between 14 and 28°C showed no necrotic injury and all showed approximately the same amount of apoptotic injury after

These animal studies also establish the selective, localized effects of cryolipolysis to significantly reduce subcutaneous fat without causing damage to the overlying skin and the lack of effect on serum lipid levels in the animal model.
48 hours of recovery. The results at higher temperatures suggest that the mechanism of action responsible for adipocyte death is based on an event that triggers apoptosis although further studies are needed to determine the exact cause of the apoptotic injury.

Thus, the pre-clinical studies confirmed that the phenomenon of cold-induced subcutaneous fat layer reduction could be replicated in experimental animal models. The mechanism for this process, however, remains unclear. Nonetheless, the dissolution of adipocytes in a gradual fashion over a period of months and the consistency of lipid levels following cold exposure suggests a safe process of metabolism.

CLINICAL STUDIES

Cryolipolysis is performed as an outpatient procedure with the Zeltiq System (Zeltiq™ Aesthetics). The device consists of a control unit with an applicator that is applied to the intended area of treatment. Tissue is drawn into the cup-shaped applicator with a moderate vacuum to position the tissue between two cooling panels. The selected heat extraction rate (cooling) is modulated by TEC elements and controlled by sensors that monitor the heat flux out of the tissue.

Once the desired area of treatment is identified, a coupling gel is applied to the skin surface before placement of the applicator to ensure consistent thermal contact. The applicator is positioned on the treatment area with the use of a moderate vacuum. Once affixed on the treatment area, no further operator intervention is required for the duration of the treatment cycle. Treatment with the cold exposure, which includes a predetermined energy extraction rate (CIF, as described earlier) and cycle duration of up to 60 minutes, is initiated. Just prior to the end of the treatment cycle, an electronic pager summons the clinician to be present at the end of treatment, when the system automatically terminates the cold exposure and the applicator is removed from the patient by the operator’s release of the vacuum. Additional applications sites may be treated to ensure that the entire area of desired reduction is appropriately exposed to cooling.

Clinical evaluation of cryolipolysis demonstrates that the selective reduction of fat, as documented in the early animal studies, is replicated in humans. A multi-center, prospective, non-randomized clinical study performed by Dover et al. [7] evaluated the use of cryolipolysis for fat layer reduction of the flanks (love handles) and back (back fat pads). Pre-programmed treatment profiles were used to control the rate of heat extraction and duration of treat-

Fig. 2. Histological sequence of inflammatory response following treatment at 3 days (A), 14 days (B), 30 days (C), and 60 days (D) after treatment [5].
A contralateral, untreated area was maintained as a control. Based on interim results from 32 subjects, efficacy was assessed by three assessment techniques: ultrasound measurement of fat layer reduction, comparison of pre- and post-treatment photographs, and physician assessment. These assessments confirmed that cryolipolysis results in a visible contour change in a majority of subjects, with the best cosmetic results noted in those patients presenting with modest and discrete fat bulges. Ultrasound measurements taken on a subset of 10 subjects demonstrated a fat layer reduction in 100% of these subjects with an average reduction of 22.4% at 4 months post-treatment. Safety of the cryolipolysis procedure was also demonstrated with these interim results as there were no device-related adverse events reported.

Studies have also been performed to assess whether the cold exposure associated with cryolipolysis is associated with an alteration in local sensory function or nerve fibers, or an elevation in lipid levels or liver functions values. Coleman et al. [8] documented the results of neural assessments and Riopelle et al. [9] assessed lipid levels and liver function tests for 90 days post-treatment.

Ten subjects were treated with a prototype cooling device (Zeltiq™ Aesthetics) during the Coleman study to determine if fat reduction in humans caused by cold exposure is associated with local sensory function or nerve fiber changes. Fat reduction was assessed in 9 of 10 subjects via ultrasound prior to treatment and at follow-up. Sensory function was assessed by neurologist evaluation (n = 9) and nerve staining was completed on tissue obtained with a biopsy from one subject. Treatment resulted in a normalized fat layer reduction of 25.5% at 6 months post-treatment. Transient reduction in sensation occurred in six of the nine subjects assessed by neurologic evaluation. Sensation returned within 7 weeks post-treatment (with a mean of 3.6 weeks). Biopsies also showed no long-term change in nerve fiber structure.

Ten subjects with discrete fat bulges (“love handles”) were treated with the Zeltiq prototype cooling device during the Riopelle study to determine if a cosmetically significant fat layer reduction was associated with a meaningful change in lipid profile and liver function tests. High frequency ultrasound imaging was used to objectively measure fat layer reduction and photographs were taken pre-treatment and at follow-up visits. Lipid profiles and liver function tests were obtained on all subjects prior to treatment and at baseline, 1, 4, 8, and 12 weeks post-treatment, which ensured that they were assessed through the 90 days after treatment normally associated with peak lipid resorption. Pre-treatment and 6-month post-treatment ultrasound images provided objective evidence of fat layer reduction in eight out of ten subjects. No clinically significant changes or abnormal values were identified for either lipid levels or liver function tests over the 90-day follow-up period.

These clinical studies demonstrate that selective cryolipolysis results in reductions in subcutaneous fat without damage to the surrounding tissues. Ultrasound images and photographic reviews demonstrate fat layer reduction, with the greatest cosmetic improvement observed in subjects with modest fat bulges. See Figure 3. Analysis of lipid levels, neurological response, and the lack of device related adverse events demonstrate the safety of cryolipolysis. Further clinical evaluation is required, however, to more fully understand the potential application to other parts of the body and the optimal treatment parameters for each. Additional studies would also be beneficial to assess whether cryolipolysis poses a unique risk to patients with rare conditions such as cryoglobulinemia, paroxysmal cold hemoglobinuria, or cold urticaria.

**DISCUSSION**

Cryolipolysis is a unique non-invasive mechanism for the selective reduction of fat cells. Both human and animal studies confirm previous clinical findings of cold-induced inflammation, as observed in both infants and adults. Histology findings confirm the selective, gradual reduction of fat tissue in both humans and animals. Visual, photographic, and ultrasound evaluations have shown measurable fat layer reduction in clinical studies. Importantly, cryolipolysis has not produced any significant adverse side effects in these studies, no laboratory abnormalities have been noted with lipid levels or liver function values, and any observed effects such as mild discomfort, erythema, bruising, or dysaesthesia have been minor and temporary.

![Fig. 3. Love handle treatment with fat layer reduction at 6 months. Image on left was taken prior to procedure; image on right was taken 6 months after [8].](image-url)
Nonetheless, there are contraindications to cryolipolysis to consider including such cold-induced conditions as cryoglobulinemia, cold urticaria, and paroxysmal cold hemoglobinuria.

Although the mechanism of action for cryolipolysis is not yet completely understood, clinical experience with this device demonstrates that it will be effective for fat layer reduction in confined anatomical areas. At this point, there is no evidence to suggest it can remove fat from large areas on a scale seen with liposuction procedures; however, it is a relatively rapid, non-invasive means of removing localized fat in a safe manner. It requires no pain management, no tumescent or other anesthesia. Moreover, there is no "downtime" associated with this procedure from work or social activities; patients can resume normal activities immediately following the procedure.

Further studies will be needed to more fully characterize the full clinical potential of cryolipolysis and its mechanism of action. Notably, the fat layer reduction provided by cryolipolysis does not approach that of liposuction. Furthermore, it is not a treatment for obesity and patients with significant skin laxity will not appreciate fat layer reduction. Rather, cryolipolysis is best suited for those patients of normal weight with discrete fat bulges. For these patients, cryolipolysis has the potential to provide effective treatment of localized fat accumulation in a safe and gradual manner.

**CONCLUSION**

Cryolipolysis is a unique method of non-invasive, selective reduction of fat that has demonstrated efficacy for gradual thinning of the fat layer in both animal and human clinical studies. It has the potential to provide safe, effective treatment for the reduction of localized fat in patients with areas of modest fat accumulation. Further studies are needed, however, to assess the full clinical potential of this device.

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