Aesth Plast Surg
DOI 10.1007/s00266-008-9286-8

ORIGINAL ARTICLE

Clinical Efficacy of Noninvasive Cryolipolysis and Its Effects on Peripheral Nerves

Sydney R. Coleman · Kulveen Sachdeva · Barbara M. Egbert · Jessica Preciado · John Allison

Received: 23 September 2008 / Accepted: 11 November 2008

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.

S. R. Coleman (✉)
Department of Surgery, NYU School of Medicine, 44 Hudson Street, New York, NY 10013, USA
e-mail: LipoStructure@yahoo.com

K. Sachdeva
San Ramon Regional Medical Center, San Ramon, CA 94583, USA

B. M. Egbert
Department of Dermatology and Pathology, Stanford University Medical Center, Palo Alto, CA 94305, USA

J. Preciado · J. Allison
Zeltiq Aesthetics, Pleasanton, CA 94588, USA

Published online: 19 March 2009

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 powered by direct current and controlled by thermisters 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/cm^2 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/cm^2 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

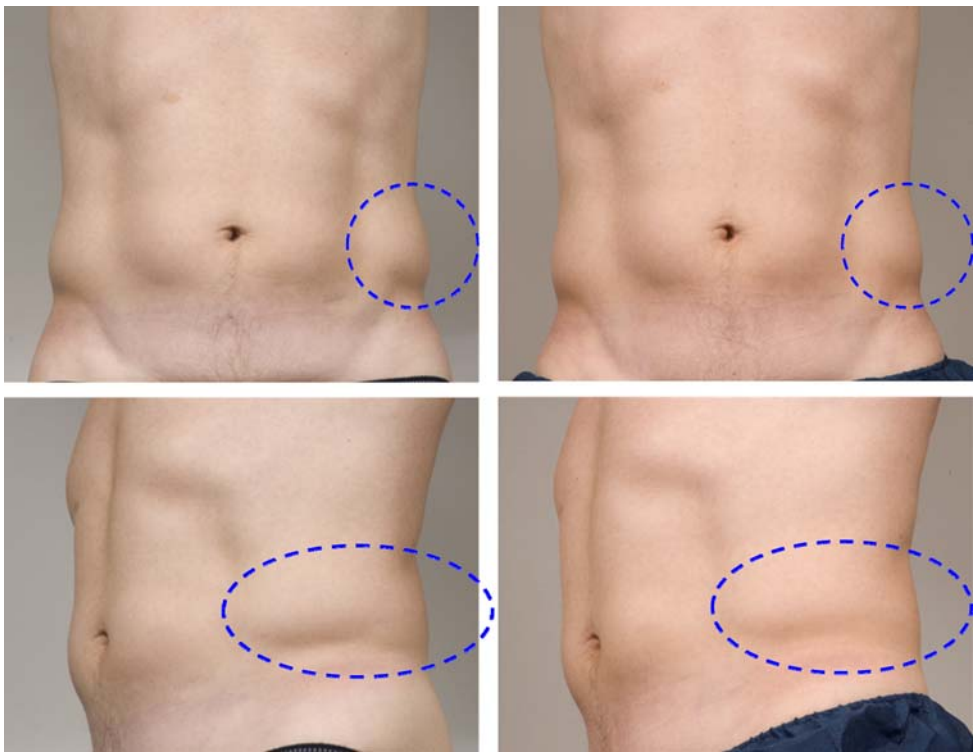

Fig. 1 Front and side views showing reduction in the love handle area (*circled*) of subject LH RIO 012. Pretreatment photographs are on the left, and 6-month posttreatment photographs are on the right. A ring over the treated area indicates the treated area reduced in volume over the follow-up period

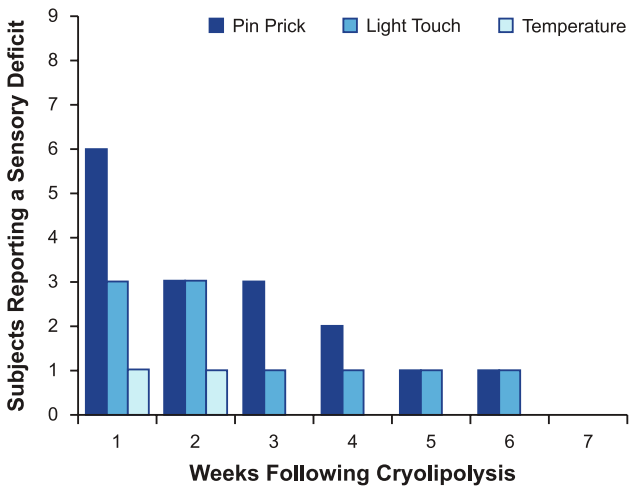

Fig. 2 Number of subjects reporting a sensory deficit to three different stimuli: pin prick (pain), light touch, and temperature (cold). Subjects also were assessed for two-point discrimination, but no subjects reported changes for this stimulus at any of the follow-up visits. As is evident from the graph, all reported sensory deficits returned within 7 weeks of cryolipolysis treatment

and parameters (Nikon D200, Nikon 24- to 120-mm lens, DynaLite strobes). Both treated and untreated matched 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

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 hypoesthesia 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 1 Summary of study group clinical data

Subject[a]	Neurologic assessment	Photographic 6-month follow-up data	Ultrasound 6-month follow-up data
1	✔	✔	✔
2	✔	✔	✔
3	✔		
4	✔		
5	✔	✔	✔
6	✔	✔	✔
7	✔	✔	✔
8	✔	✔	✔
9	✔	✔	

[a] 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 2 Fat layer reduction efficacy resulting from cryolipolysis as measured by ultrasound for 6 subjects with follow-up data at 2 and 6 months

Subject[a]	Weight change (kg) (2 months)	Average fat[b] layer reduction at 2 months (%)	Weight change (kg) (6 months)	Average fat[b] layer reduction at 6 months (%)
1	−3.6	11.5	3.5	10.7
2	−2.2	21.8	−3.6	37.5
5	4.4	26.3	4.5	18.2
6	1.6	21.7	−1.4	27.4
7	2.8	18.9	0.0	29.2
9	−2.8	22.0	0.0	30.1
Mean	0.0	20.4	0.5	25.5

[a] 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

[b] 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

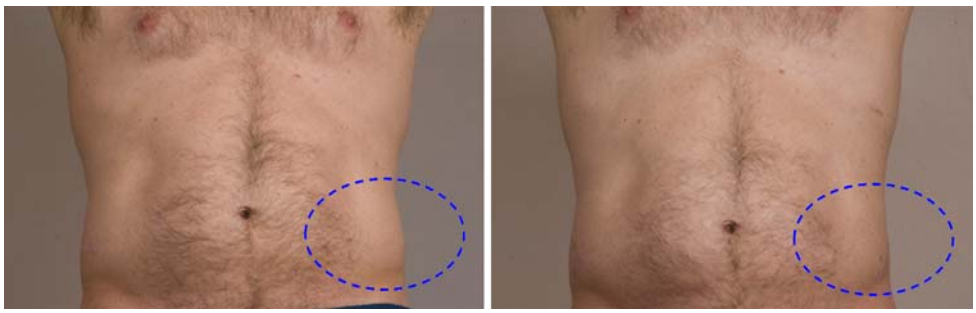

Fig. 3 Front views showing reduction in the love handle area (*circled*) of subject LH OKA 017. Pretreatment photograph is on the left, and 3-month posttreatment photograph is on the right. A ring over the treated area indicates the treated area reduced in volume over the follow-up period

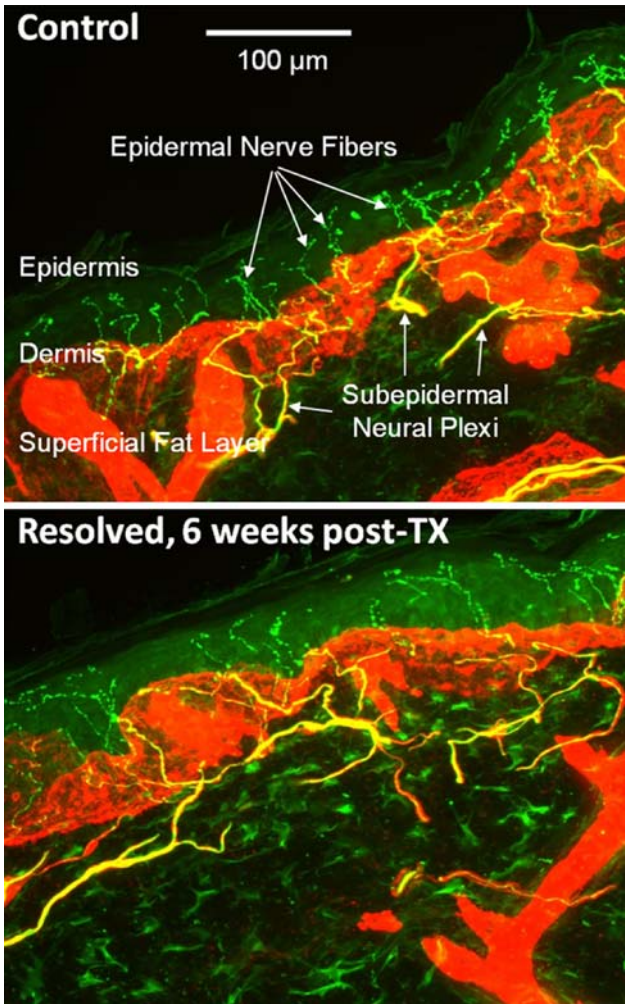

Fig. 4 Confocal microscope images of nerves from the control (*top*) and asymptomatic treated side (*bottom*) approximately 6 weeks after treatment. These photographs show the fluorescent green epidermal nerve fibers that stretch from the basement membrane to the epidermis. The yellow fibers are the subepidermal nerve plexus. The orange staining is capillaries within the dermis. The density of the epidermal nerve fibers per square millimeter of dermis is determined to be equal in both samples, indicating that these nerves, responsible for hot, cold, and pain sensation, do not sustain long-term changes as a result of cryolipolysis

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

1. Manstein D, Laubach H, Watanabe K, Anderson RR (2008) A novel cryotherapy method of non-invasive, selective lipolysis. Lasers Surg Med S20(40):104
2. Preciado J, Allison J (2008) The effect of cold exposure on adipocytes: examining a novel method for the noninvasive removal of fat. Oral presentation at the society for cryobiology annual meeting, 21 July 2008
3. Rispat G, Slaoui M, Weber D, Salemink P, Berthoux C, Shrivastava R (1993) Haematological and plasma biochemical values for healthy Yucatan micropigs. Lab Anim 27:368–373
4. Kennedy WR, Wendelschafer-Crabb G, Polydefkis M, McArthur JC (2005) Pathology and quantitation of cutaneous innervation. In: Dyck PJ, Thomas PK (eds) Peripheral neuropathy. Elsevier, Philadelphia, PA, pp 869–895
5. Kennedy WR, Wendelschafer-Crabb G, Johnson T (1996) Quantitation of epidermal nerves in diabetic neuropathy. Neurology 47:1042–1048
6. Nolano M, Simone DA, Wendelschafer-Crabb G, Johnson T, Hazen E, Kennedy WR (1999) Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. Pain 81:135–145
7. Wiadrowski TP, Marshman G (2001) Subcutaneous fat necrosis of the newborn following hypothermia and complicated by pain and hypercalcaemia. Australas J Dermatol 42:207–210
8. Diamantis S, Bastek T, Groben P, Morrell D (2006) Subcutaneous fat necrosis in a newborn following icebag application for treatment of supraventricular tachycardia. J Perinatol 26:518–520
9. Taylor CA (1949) Survival of rat skin and changes in hair pigmentation following freezing. J Exp Zool 110:77–111
10. Shurtleff D, Gilliatt RW, Thomas JR, Pezeshkpour GH (1993) An assessment of peripheral nerve damage in the rat following non-freezing cold exposure: an electrophysiological and histopathologic examination. Technical report, Jan 1991–Jan 1992. Naval Medical Research Institute: Bethesda, MD. http://stinet.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA264293
11. Irwin MS (1996) Nature and mechanism of peripheral nerve damage in an experimental model of nonfreezing cold injury. Ann R Coll Surg Engl 78:372–379
12. Irwin MS, Sanders R, Green CJ, Terenghi G (1997) Neuropathy in nonfreezing cold injury (trench foot). J R Soc Med 90:433–438
13. Jia J, Pollock M, Jia J (1998) Cold injury to nerves is not due to ischaemia alone. Brain 21:989–1001
14. Iida H, Schmelzer JD, Schmeichel AM, Wang Y, Low PA (2003) Peripheral nerve ischemia: reperfusion injury and fiber regeneration. Exp Neurol 184:997–1002
15. Saray A, Can B, Akbiyik F, Askar I (1999) Ischaemia-reperfusion injury of the peripheral nerve: an experimental study. Microsurgery 19:374–380
16. Schmelzer JD, Zochodne DW, Low PA (1989) Ischemic and reperfusion injury of rat peripheral nerve. Proc Natl Acad Sci U S A 86:1639–1642