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A Double-Blind Randomized Controlled Trial Evaluating the Efficacy and Tolerability of a Topical Body Treatment in Combination With Cryolipolysis Procedures

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ABSTRACT

Background: Non-surgical fat reduction through cold application, cryolipolysis, is an extremely popular procedure. Apoptosis of the fat cell content may take around 3 months to resolve.

Objective: A topical test product was compared to a bland emollient as an adjunct to the cryolipolysis procedure of the upper arms to determine if the product could hasten outcomes in these patients. The product includes a peptide combination thought to stimulate autophagic breakdown of lipid droplets, thus speeding up the apoptotic process seen after cryolipolysis.

Methods: A randomized, double-blind, comparator-controlled study in 11 patients compared the test product to a bland emollient on the upper arms of patients following cryolipolysis. Subjects were followed at 1, 4, 8, 12, and some at 24-weeks post treatment. Assessments were made through subjective and objective photographic analysis of the treated areas comparing changes in both arms.

Results: The test product appeared to speed up the process of contour improvement with results at 8 weeks matching those attained at 12 weeks by the comparator and long-term results at 24 weeks appearing to maintain this advantage. When measured objectively using pixel analysis, 8 and 24-week contour improvement was statistically better than the comparator. Skin laxity was also improved. In additional assessments using 3D volume analysis, cases showed improved reduction of fat tissue on the treated sides.

Conclusion: This pilot study introduces a potential advance in adjuvant topical therapy aiding the outcome of non-invasive fat reduction procedures.

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INTRODUCTION

Non-invasive body contouring has become an extremely popular procedure in recent years. Devices involved utilize 'hot" (radiofrequency/laser) or "cold" (cryolipolysis) technologies to achieve breakdown of fat tissue and elimination of the destroyed fat cells over a period of months. The mechanism of fat breakdown involved in cryolipolysis is thought to be through crystallization of the adipocytes and subsequent damage to the cell membrane, apoptosis, and extracellular leakage of lipid content, although this is still speculative.[1,2] Absorption of the lipid droplet particles following adipose cell apoptosis can take months, delaying the outcome of the procedure and presenting an unknown risk of free fatty acids and triglycerides accumulating in the extracellular matrix prior to digestion.[3] The aim of this study was to ascertain whether the topical application of a formulation purported to improve lipid droplet/debris elimination following cryolipolysis could accelerate this process and produce faster and/or enhanced results.

MATERIALS AND METHODS

A randomized, double-blind, comparator-controlled study was designed to assess the efficacy of a novel topical body treatment with TriHex and additional selected peptides (Alastin TransFORM Body Treatment with TriHex Technology®, Alastin Skincare, Inc., Carlsbad, CA) compared to a bland moisturizer, Cetaphil® lotion, (Galderma Laboratories, Fort Worth, TX) when undergoing cryolipolysis of the upper arm area.

Eleven subjects were randomized to receive the topical body treatment with TriHex on one arm and the comparator (bland moisturizer) on the other arm. Eligible subjects were women between 25 and 65 years of age with clearly visible bilateral subcutaneous arm fat appearing as a distinct bulge of fat in the arm at least 14 cm from the elbow, with soft, pliable tissue of sufficient volume for treatment on both sides. Subjects with previous fat reduction procedures or implants in or near the treatment area, previous surgery in the arms, and any contra-indication to device usage, as determined by the physician relating to existing diseases or drug use were excluded from participating in the study. Subjects were instructed to avoid starting a major diet or exercise program and maintain a constant weight (within 5% of the baseline measure).

Subjects underwent screening, baseline/treatment visit, and follow-up visits at 1, 4, 8, 12, and 24-weeks post treatment.

Treatment involved cryolipolysis of the upper arms using the CoolSculpting System (Allergan, Irvine, CA). Each subject received two -11°C, 35-minute cooling cycles to each arm delivered using the COOLPETITE Advantage™ cups. The cups were placed in two separate positions on each posterior arm (4x 35-minute sessions). Immediately following treatment on each arm, a timed three-minute manual massage was performed.

The topical products were provided in a double-blind fashion with tubes labeled A and B.

Treatment assignment was blinded, arm assignment was randomized. Subjects were instructed to apply one full pump each of Product A to the right upper arm and Product B to the left upper arm twice daily for the entire study duration. This was applied after bathing or showering without manual massage and the area allowed to dry without the use of any dressing or wrapping. The trial was funded by Alastin Skincare Inc., Carlsbad, CA.

Photographic assessments with standardized photography were performed at all visits capturing multiple anterior, posterior (horizontal plane /90 degrees) views in strict standardized positions using Canfield Mirror Software (Canfield Scientific, Parsippany, NJ). In addition, the blinded investigator assessed contour improvement and skin laxity at all visits. Assessments in contour improvement were initially performed by reviewing baseline photos compared to the follow-up visit photos. Canfield objective analysis was subsequently performed as evaluation of photographs was deemed to be too subjective in nature. The Canfield analysis involves a process where 2D images are converted into a 3D space. Landmarks are placed at the top and bottom of the arm and linear distance measurements are then taken from landmark 1 to landmark 2. The same landmark placement at follow up is determined and landmarks 1 to 2 are placed and measured. The delta between baseline measurement and follow up measurement is then calculated. The baseline served as control for each case and changes were assessed comparing baseline with 8-week, 12-week, or 24-week appearances and then comparing these deltas on each side.

In addition, subjects were asked to assess improvement in the shape of their arm at all visits.

RESULTS

Investigator and Subject Contour Assessments

Blinded Investigators recorded the level of improvement by comparing the photos from baseline to follow-up visits. Grades were assigned according to degree of change (none, small, moderate, significant) and within the latter three ranges, these were further subdivided into 3 levels (Table 1A).

Subjects completed the assessment (Table 1B) by comparing their baseline photo to the appearance of their right and left arms at all follow-up visits. Numerical scores were assigned according to category.

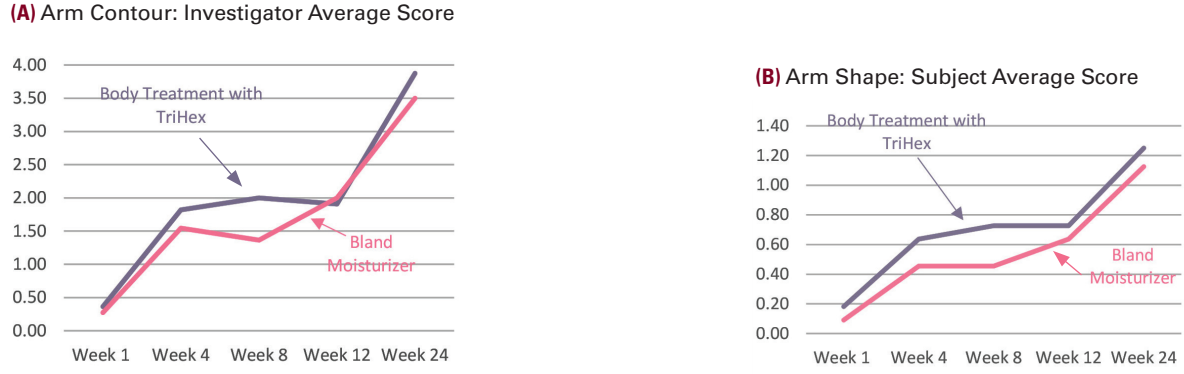
TABLE 1A.

Blinded Investigator Assessment of Contour Improvement				
Overall Contour Improvement	0	1 2 3	4 5 6	7 8 9
Right				
Left				

TABLE 1B.

Subject Assessment of Contour Improvement					
Overall Contour Improvement	Strongly Agree 2	Agree 1	Neutral 0	Disagree -1	Strongly Disagree -2
Right					
Left					

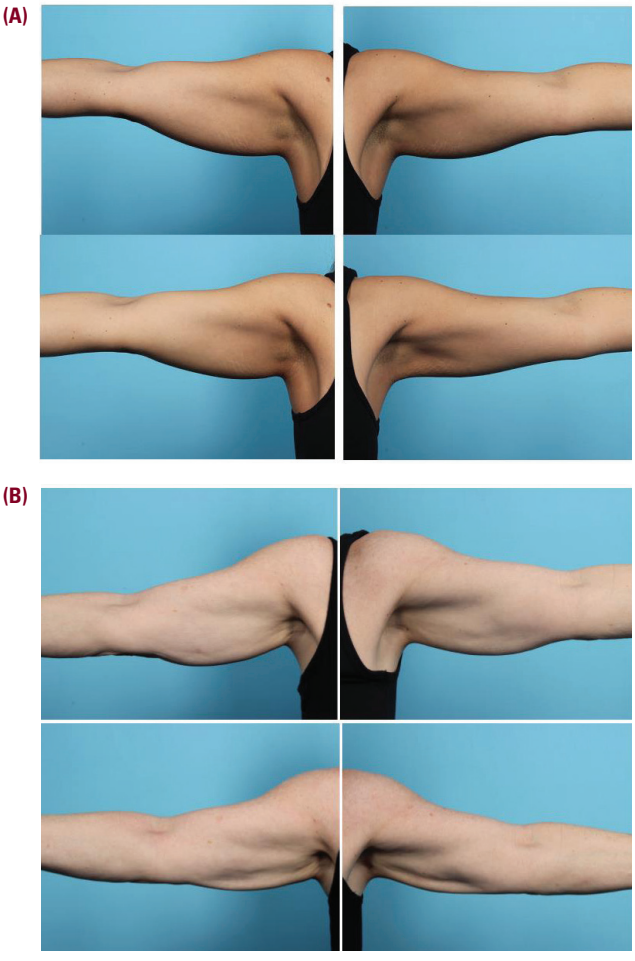
FIGURE 1. (A) Using a contour improvement grading scale, blinded investigators scored the treated body product with TriHex side better than comparator at 8 weeks. This appeared to stabilize at the 12-week assessment but again showed a favorable difference in the body treatment with TriHex group at the final 24-week assessment. **(B)** Subject assessments showed improvements at all time intervals.



Of the 11 subjects assessed, overall contour consistently improved in the body treatment with TriHex arm, with this difference peaking at the 8-week visit. These subjects exhibited faster attainment of contour results equivalent to those obtained by the bland moisturizer group at 12 weeks (Figure 1A). Only at the 12-week assessment was there an inconsistency between subject and investigator assessments. The Investigator average score demonstrated a 'catch-up' of bland moisturizer at 12 weeks, whereas the subject average score did not indicate this. Although the bland moisturizer did make up the difference during the next 4 weeks when examining the final 24-week assessments, the body treatment with TriHex group maintained its apparent advantage over the bland moisturizer (Figure 1B).

In addition, subject assessments reflected the same change in contour with maximum differences between the 2 groups evident at week 8. The body product with TriHex was used on the left arm of both subjects (Figure 2A and B).

FIGURE 2. (A AND B) Representative examples of 2 subjects examining contour changes of upper arms. The upper image of each representing baseline and lower image representing 8-week visit. The body product with TriHex was used on the left arm of both subjects.



Investigator Skin Laxity Assessment

Blinded Investigator skin laxity assessments were completed at the same time periods using a skin laxity grading scale (Table 2), with investigators performing assessments by visually examining the subject during the visit and noting the level of severity. Related to the table, the lower the score, the better the perceived result. In most investigator time point assessments of average subject scores, the body treatment with TriHex treated side showed improved skin tone with less skin laxity compared to control (Figure 3).

FIGURE 3. Investigator time point assessments of average subject scores, the body treatment with TriHex side showed improved skin tone with less skin laxity compared to control (the lower the score, the better the perceived result).

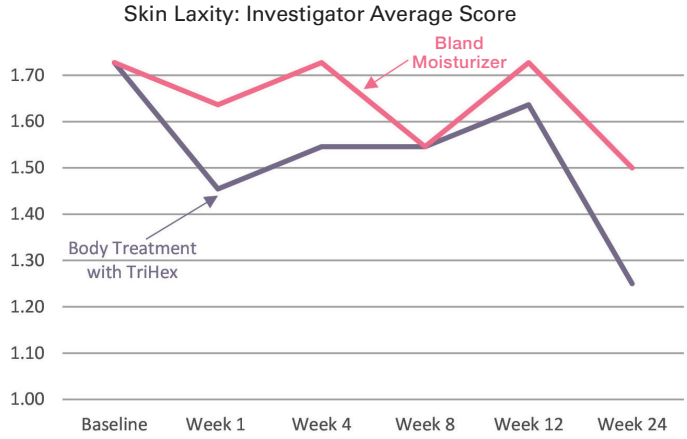


TABLE 2.

Skin Laxity Grading Scale		
Score	**Classification**	**Description**
0	None	No loose skin, toned and firm skin with smooth skin surface texture
1	Mild	Mildly loose skin, somewhat toned with smooth skin surface texture
2	Moderate	Moderately loose skin, no deep tone, few wrinkles and crepiness on skin surface
3	Severe	Very loose skin without underlying tone, multiple wrinkles and crepiness on skin surface, skin distinct from underlying subcutaneous tissue via palpation
4	Extreme	Prominent redundancy of skin without underlying tone, severe wrinkling and crepiness on skin surface

Interestingly, the improvement was most marked at the 1-week visit and improvement over baseline was noted as a subtler change. In addition, at 8 weeks, according to investigators, comparator results appeared to catch up temporarily and then diverge from body treatment with TriHex improved results again at 12 weeks, while little improvement over baseline was noted in the comparator group. At the long term 24-week as-

sessment, investigators noted better improvement in the body treatment with TriHex group.

After thorough assessment of the above results, it was recognized that the subjective nature of assessments severely undermined an accurate outcome analysis. Therefore, photographic assessment was undertaken using Canfield technology, which allowed secondary objective analysis of most photographs to be completed independently by Canfield at the 8, 12, and 24-week timepoints. Thus, an objective analysis was undertaken using the following criteria:

- The process involved the conversion of 2D images into a 3D space. Landmarks are placed at the top and bottom of the arm and linear distance measurements are then taken from landmark 1 to landmark 2. The same landmark placement at follow up is determined and landmarks 1 to 2 are placed and measured. The delta between baseline measurement and follow up measurement is then calculated.
- All photographs assessed needed to have identical positioning, focus, and clarity so that they were comparable. This was determined by Canfield. Two patient photos in the 8-week group and 1 patient photo in the 12 and 24-week groups were deemed to be unusable.
- Patients who showed worsening of outcomes with no contour improvement in both groups were labelled as non-responders to the device. These patients could not be assessed for topical benefit as the device did not appear to cause loss of fat, which restricts the topical agent's efficacy. This applied to 2 patients.
- Thus, the final accurate assessment could be carried out by Canfield on 7 patients at 8 weeks, 8 patients at 12 weeks, and 6 patients at 24 weeks.

Results of Canfield Independent Assessment

Six of seven subjects (86%) assessed had improved results on the body treatment with TriHex side at 8 weeks and this persisted in 6 out of 8 subjects (75%) at 12 weeks. The delta between baseline, 8, and 12 weeks was analyzed and at the 8-week visit, body treatment with TriHex group exhibited a statistically significant difference of over twice the percent reduction from baseline compared to the bland moisturizer group. The 12-week body treatment with TriHex group also showed improved results over comparator, although not statistically significant. At 24 weeks, of the patients who returned for their visit and were suited for analysis, five of six (83%) had improved results on the body treatment with TriHex side and showed a statistically significant improved reduction in contour (Figures 4, 5).

FIGURE 4. Typical example of Canfield analysis. Delta change represents change between baseline and 8, 12, and 24 weeks. Body treatment with TriHex represented in top row.

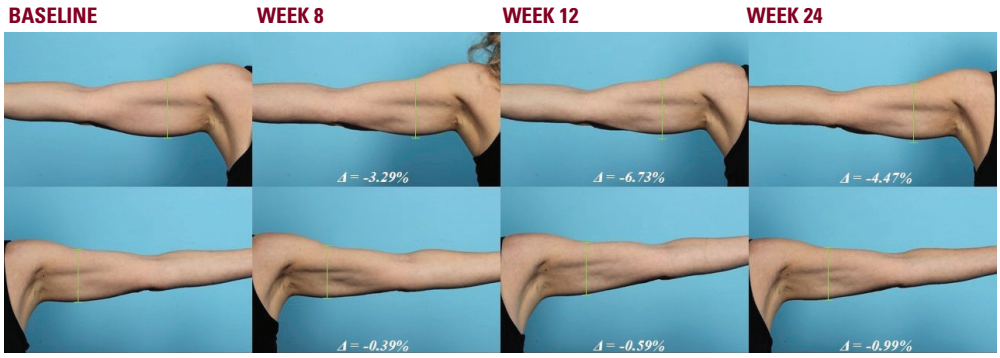
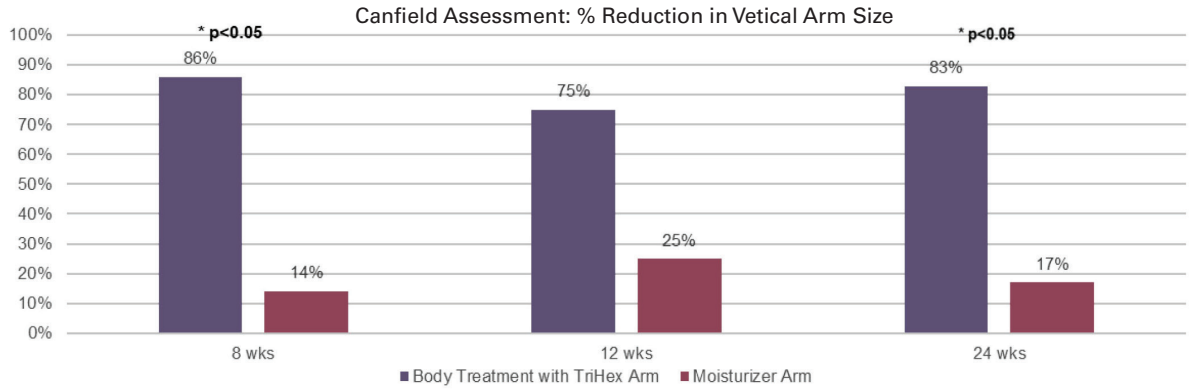


FIGURE 5. Graphic representation of measured changes in contour as calculated by Canfield. Although 12-week results still favored the body treatment with TriHex group, 8 weeks results were the most dramatic showing a more than 2-fold percentage reduction compared to the bland moisturizer group and maintained at 24 weeks.



Statistical Analysis

When subjected to independent statistical analysis, this small cohort demonstrated statistically significant results (*P*=0.0082) at 8 weeks.

TABLE 3A.

Comparison of Percent Reduction in Arm Contour from Baseline to 8 Weeks between Body Treatment With TriHex and Bland Moisturizer

Arm Side	N	Mean	S.D.	95% CI	*P*-value
Body Treatment with TriHex	7	-6.66	5.45	(-11.70, -1.62)	--
Bland Moisturizer	7	-2.08	4.76	(-6.49, -2.32)	--
Paired difference (Body Treatment with TriHex – Bland Moisturizer)	7	-4.58	3.12	(-7.47, -1.69)	.0082

Conclusion

The mean reduction in arm contour measurements from baseline to 8 weeks post treatment was 6.66% for body treatment with TriHex side and -2.08% for bland moisturizer side. The paired difference between body treatment with TriHex and bland moisturizer was -4.58%, which was statistically significant based on a paired t-test (*P*=0.0082). In other words, body treatment with TriHex is associated with a bigger reduction in arm contour than bland moisturizer, with statistical significance.

TABLE 3B.

Comparison of Percent Reduction in Arm Contour from Baseline to 12 Weeks between Body Treatment With TriHex and Bland Moisturizer

Arm Side	N	Mean	SD	95% CI	*P*-value
Body Treatment with TriHex	8	-5.58	5.46	(-10.15, -1.01)	--
Bland Moisturizer	8	-3.48	5.75	(-8.28, -1.33)	--
Paired difference (Body Treatment with TriHex – Bland Moisturizer)	8	-2.10	4.00	(-5.44, -1.24)	.1805

Conclusion

The mean reduction in arm contour measurements from baseline to 12 weeks post treatment was -5.58% for body treatment with TriHex side and -3.48% for bland moisturizer side. The paired difference between body treatment with TriHex and bland moisturizer was -2.10%, which was not statistically significant based on a paired t-test (*P*=0.1805).

TABLE 3C.

Comparison of Percent Reduction in Arm Contour from Baseline to 24 Weeks between Body Treatment With TriHex and Bland Moisturizer

Arm Side	N	Mean	SD	95% CI	*P*-value
Body Treatment with TriHex	6	-6.42	8.75	(-15.60, 2.76)	--
Bland Moisturizer	6	-2.17	6.50	(-8.99, 4.66)	--
Paired difference (Body Treatment with TriHex – Bland Moisturizer)	6	-4.26	3.52	(-7.95, -0.56)	0.0315

When subjected to independent statistical analysis, this small cohort demonstrated statistically significant results (*P*=0.0315) at 24 weeks.

Conclusion

The mean reduction in arm contour measurements from baseline to 24 weeks post treatment was -6.42 for body treatment with TriHex side and -2.17 for bland moisturizer side. The paired difference between body treatment with TriHex side and bland moisturizer was -4.26, which was statistically significant based on a paired t-test (*P*=0.0315).

DISCUSSION

Energy based non-surgical fat reduction is now an extremely popular procedure performed using varying technologies, either 'hot' or 'cold'. Cryolipolysis appears to be the most popular procedure performed in the US for this indication,[2] although a host of new devices or technologies have been introduced to the market in recent years. The common thread among these devices is the damage to fat cell components with delivery of its contents and gradual elimination of these lipid droplets.[4] It would appear that the lipolytic effect of these technologies all precipitate a change or disruption in the cell structure, which release mediators, particularly TNF-α, inducing lipolysis through intracellular signaling cascades, metabolites, and lipid droplet-associated proteins.[4,5]

Macrophages play important roles in the clearance of dead and dying cells, and in particular, of apoptotic cells.[6] Lipid droplets are extremely large in size and challenging for the smaller macrophages to phagocytose. Fortunately, the process of autophagy is a great help in dealing with large intracellular components. Simply put, autophagy is the cell's way of repackaging very large organelles and intracellular bodies (such as lipid droplets) so that macrophages can cope with their digestion.[7] This creates smaller components that can then be digested by macrophages. In recent years, studies have demonstrated that lipid droplets are taken up by autophagy to cope with lipid mobilization and droplet digestion. This process, termed lipophagy, specifically targets lipid droplets and adipose cellular debris for digestion.[7,8]

The novel body treatment with TriHex formulation was developed with active peptides that stimulate autophagy targeting lipid droplet breakdown and macrophage clustering and stimulate elastin and collagen neogenesis thus improving skin tone. The peptides and actives included in this topical preparation improve the autophagic process of lipid droplet absorption (lipophagy), which involves a repackaging of these droplets to smaller sizes so that macrophages can then cope with digestion of these very large particles. Furthermore, liposomal coating of the peptides ensures easy access to the hair follicle from where it enters dermal white adipose tissue en route to the subcutaneous fat. This process including gene validation and in vitro tests

is comprehensively covered in this issue.

This pilot study was designed to assess the ability of the topical body product with TriHex to speed up the process of fat droplet elimination manifesting as a more rapid clinical outcome. Although the numbers are small, the series demonstrate a statistically significant improvement in contour changes at 8 weeks using the test product as opposed to the comparator. Subjective analysis demonstrated that changes in arm contour, both from investigator and subject perspectives, improved positively with the test group showing 8 weeks results equivalent to or greater than the 12-week results of the comparator and then maintaining the advantage from investigators perspective right up to the final 24-week time point. In addition, skin laxity appeared to be improved across all time periods compared to baseline. Further, more accurate objective analysis using Canfield software and measuring the baseline compared to the follow-up to receive a delta, demonstrated significant advantages at 8 and 24 weeks when subjected to statistical analysis.

Although this is a pilot study, it appears that the hastened fat dissolution may be occurring at expected time points with maximal first signs of this evident at 8 weeks. One would then expect a certain amount of remodeling over the following few weeks and it is encouraging to see that long-term assessments at 24 weeks demonstrate an ongoing advantage.

Limitations of the study include the small numbers and the difficulty in relying on subjective assessments. It is also salient to note that the upper arms in many cases have limited fat compartments and overall consistently good results with cryolipolysis may be difficult to achieve. That makes the assessment of an added topical application extra difficult to assess. Objective assessments were therefore added to the protocol and this analysis demonstrated statistical significance at the 8 and 24-week period. These points considered, it is noteworthy that this small study was able to demonstrate this significant difference between the groups tested.

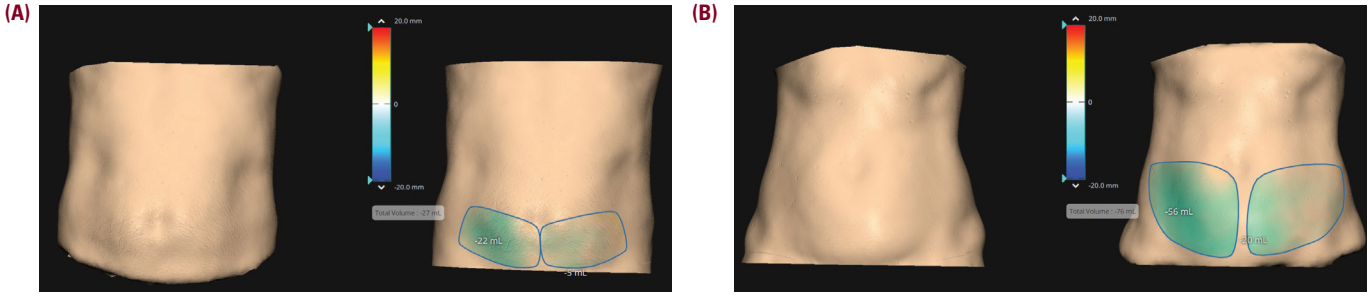
Due to these limitations, a further extension of the study was undertaken, also pilot in nature, to assess if objective volume assessments could improve the predictability and confidence of assessing outcomes. To this end, 3 additional subjects were chosen where procedures were conducted on the abdominal area (2 cryolipolysis, 1 radiofrequency). The body treatment with TriHex was randomized to be used on one side only and all assessments were made using the QuantifiCare LifeViz® Infinity camera and software imaging system (Quantificare Inc., San Francisco, CA). The software displays before/after 3D images to compare volume and contour changes over time. (Figures 6A, B).

This small series provided objective validation of improved fat/volume reduction in all cases on the test side and provided good evidence for objective assessments in these types of studies rather than subjective photo analysis. Results of additional studies using this modality will be reported shortly.

CONCLUSION

Non-surgical fat reduction is an extremely popular procedure. Cryolipolysis is often applied as the technology of choice for upper arm contour improvement. In a study of 11 subjects, a topical product with TriHex technology was compared to a bland emollient as an adjunct to the cryolipolysis procedure. The test product appeared to speed up the process of contour improvement with results at 8 weeks matching those attained at 12 weeks by the comparator, with long term results at 24 weeks appearing to maintain this advantage in contour improvement. When measured objectively using independent additional Canfield 3D space analysis, 8-week contour improvement was significantly better than comparator and maintained at 24-weeks. Skin laxity was also improved. In additional assessments, using objective 3D volume analysis in different anatomic areas, all cases

FIGURE 6. (A) Subject received a CoolSculpting fat reduction procedure on both sides of the lower abdomen. Subject followed-up treatment with a split abdomen regimen that included the Alastin product on the subjects' right side and no topical treatment on the subjects left side (only the procedure treatment). **(B)** Subject received a Vanquish ME™ procedure (Radiofrequency technology) to the abdomen and flanks. Subject followed-up treatment with a split abdomen regimen that included the Alastin product on the subjects' right side and no topical treatment on the subjects left side (only the procedure treatment). Imaging equipment and analysis: The case study photos were taken with the QuantifiCare LifeViz® Infinity camera and software imaging system. 3D photos are displayed in the software to reveal volume and contour changes. Volume changes are measured in mL. The blue color represents volume reduction and red color represents volume increase.



showed impressive reduction of fat tissue on the treated sides. This pilot study and its extensions is extremely encouraging and introduces an exciting potential advancement in an adjuvant topical therapy aiding the outcome of non-invasive procedures.

DISCLOSURES

Alan D. Widgerow is Chief Medical Officer Alastin SkinCare, Carlsbad, CA. Amir Moradi is Consultant and Principal Investigator for Alastin, Galderma, Merz, Lutronic; Principal Investigator and honorarium recipient for SkinMedica and Allergan. Jeanette Poehler has no disclosures to report. Alastin products supplied to Moradi office by Alastin SkinCare Inc., Carlsbad, CA.

REFERENCES

1. Pinto H, Arredondo E, Ricart-Jane D. Evaluation of adipocytic changes after a simil-lipocryolysis stimulus. *Cryo Letters*. 2013;34(1):100-5.
2. Ingargiola MJ, Motakef S, Chung MT, Vasconez HC, Sasaki GH. Cryolipolysis for fat reduction and body contouring: safety and efficacy of current treatment paradigms. *Plast Reconstr Surg*. 2015;135(6):1581-90.
3. Ferraro GA, De Francesco F, Cataldo C, Rossano F, Nicoletti G, D'Andrea F. Synergistic effects of cryolipolysis and shock waves for noninvasive body contouring. *Aesthetic Plast Surg*. 2012;36(3):666-79.
4. Kwon TR, Yoo KH, Oh CT, et al. Improved methods for selective cryolipolysis results in subcutaneous fat layer reduction in a porcine model. *Skin Res Technol*. 2015; 21(2):192-200.
5. Grant RW, Stephens JM. Fat in flames: influence of cytokines and pattern recognition receptors on adipocyte lipolysis. *Am J Physiol Endocrinol Metab*. 2015;309(3):E205-13.
6. Gregory CD, Devitt A. The macrophage and the apoptotic cell: an innate immune interaction viewed simplistically? *Immunology*. 2004;113(1):1-14.
7. Maixner N, Kovsan J, Harman-Boehm I, Bluher M, Bashan N, Rudich A. Autophagy in adipose tissue. *Obes Facts*. 2012;5(5):710-21.
8. Wang CW. Lipid droplets, lipophagy, and beyond. *Biochim Biophys Acta*. 2016;1861(8 Pt B):793-805.

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