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Case Report

Assessing the Efficacy of Cryoadipolysis with the Cooltech® System: Case Study

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Abstract

The purpose of this case study was to assess the efficacy of the Cooltech® system in a cryoadipolysis session for the removal of localized subcutaneous adipose tissue.

Prospective, single-center case study conducted at the Fajardo Clinic in Malaga (Spain). All patients underwent one treatment session in the abdomen and flanks with a Cooltech® cryoadipolysis machine (Cocoon Medical, Spain) and two follow-up visits at 4 and 8 weeks. A substudy was conducted, in which the left side of the abdomen and the right flank were treated. Age, abdominal perimeter and thickness of adipose tissue were the variables analyzed through ultrasound. In the substudy, biopsies were taken from the right flank at baseline and after 4 weeks of treatment.

Eight patients –six women and two men (one included in the substudy)– with a mean age of 44 years (SD 9.17) and a BMI of 26.88 (SD 2.38) participated. Ultrasound results showed a mean decrease of 15.34% (SD 13.32) in the abdomen area and of 11.03% (SD 25.21) and 4.57% (SD 25.68) in the right and left flanks, respectively. Adipose tissue samples taken from the treated area showed no inflammation or necrosis.

Keywords: Cryoadipolysis, cooltech, non-invasive fat removal

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Body contouring is one of the most popular procedures in aesthetic surgery. Cryoadipolysis is a new non-invasive technique that, by the localized application of low temperatures, reduces subcutaneous fat through lysis of adipocytes.[1] This technique poses a smaller risk than invasive procedures like liposuction.[2] Furthermore, other non-invasive technologies have been developed in recent years for the removal of subcutaneous fat. The most widely spread include devices using high-frequency ultrasound, radiofrequency energy or laser light. Just like with cryoadipolysis, adverse effects are minimal and post-operative recovery times are shorter.[3]

Although the mechanism of action used by cryoadipolysis to destroy the adipose tissue is not entirely known, some studies indicate that it might involve an inflammatory process occurring within the adipose tissue when it is exposed to low temperatures but not cold enough to cause damage to other tissues.[3] This procedure causes postponed cell death or apoptosis of the adipocytes exposed for a certain amount time to a temperature below body temperature, but without reaching the water freezing point.[4, 5] Likewise, the areas of the body that are more sensitive to cryoadipolysis are not known either. Several factors might favor this sensitivity, like the degree of vascularization, cellular

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architecture and adipose tissue-specific metabolic activity in that area.6 However, this procedure is indicated for the removal of small or moderate amounts of localized adipose tissue and for the treatment of cellulite.[6] Results are not immediate, being visible after about two months.[7]

One of the cryoadipolysis devices used for body contouring is the Cooltech® system (Cocoon Medical, Spain). This machine creates suction in the area to be treated through an adjustable vacuum applicator that acts by encapsulating the fat tissue in its interior. During the session, the temperature in the area is reduced in a controlled manner until it reaches a minimum temperature of -8ºC.[8] The purpose of this study was to assess the efficacy of one cryoadipolysis session with the Cooltech® system to remove adipose tissue from the abdomen and flanks of non-obese subjects, and observe the effect of this procedure on adipocytes.

Methods

Study Design

Prospective, single-center case study conducted at the Fajardo Clinic in Malaga (Spain). Participants included both men and women over 18 years old that voluntarily agreed to take part in the study. They all underwent one cryoadipolysis session with the Cooltech® machine and the Straight HP applicator (Cocoon® Medical, Spain). Inclusion criteria were patients with localized adiposities in flanks or abdomen. Exclusion criteria were pregnant or nursing women, BMI >35, abdominal hernia, C-section or abdominal scar, acute inflammation, cryoglobulinemia, Raynaud's disease or any other serious disease or reason why the investigator did not consider appropriate to include them.

The study was conducted in compliance with the principles laid down in the current revised version of the Declaration of Helsinki, Good Clinical Practices (GCPs), and all the relevant applicable laws and regulatory requirements for the use of medical devices in Spain. Data collection notebooks did not include any personal information that would enable to identify the participants.

Study Protocol

Subjects were invited to participate in the study and the substudy and, after accepting and confirming they met all inclusion criteria, they were divided in two groups:

Main study: Patients underwent one session of cryoadipolysis in both abdominal areas, on both sides of the umbilicus and in both flanks (right flank [RF] and left flank [LF]) (Fig. 1). Then, they attended two follow-up visits after 4 and 8 weeks of treatment.

Substudy: It was conducted in one patient, who underwent only one treatment session in two asymmetrical areas (the left side of the abdomen and the right flank). With this design, the non-treated area was used as control. After the session, the patient also attended two follow-up visits after 4 and 8 weeks.

Treatment Protocol

The cryoadipolysis treatment with the Cooltech® device did not require any kind of anesthesia. In the main study, the treatment session was performed in four areas: both sides of the abdomen, and right and left flanks; and in the substudy, in two areas: one side of the abdomen and the right flank. The Cooltech® device allows the use of two Straight HP applicators simultaneously, enabling to treat two locations at the same time (Fig. 2). In the main study, all four areas were treated in two hours (60 minutes per area) at a minimum temperature of -8 ºC. In the substudy, where only two areas were treated, the session lasted 60 minutes.

Prior to the procedure, the area to be treated was manually and visually assessed. Then, a cryoprotectant –a cellulose membrane embedded in glycerin, and water (CoolPad)– was placed on the area to protect the epidermis and the dermis.[9] The Cooltech® applicators that best fitted the target area (Straight HP model) were placed over the cellulose

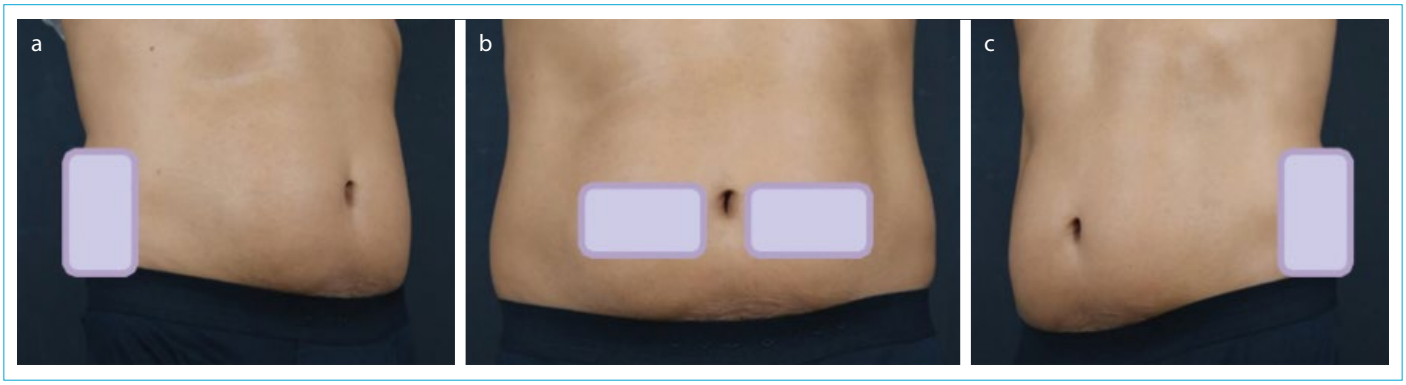

Figure 1. Treatment areas. **(a)** Right flank, **(b)** Abdominal areas, **(c)** Left flank.

Figure 2. Cooltech® machine (Cocoon Medical, Spain). **(a, b)** Applicators, **(c)** screen of the Cooltech device.

membrane, and, in all cases, suction was set at 240 mBar for a simultaneous, 60-minute cold application (-8 ºC) on each of the two treated areas. At the end of this time, applicators were removed without post-treatment massage.

Data Assessment

A clinical history was prepared, in which data about diseases, allergies, medication, surgeries and lifestyle were included. Variables recorded were: sex, age, height, medication, body mass index (BMI), and a study of the body composition was conducted using bioimpedance (Tanita Model BC-420MA), including: weight, fat-free mass (FFM), fat mass (FM), and total water (TW).

Prior to treatment and at the follow-up visits (Weeks 4 and 8), the abdominal perimeter of all participants was measured with measuring tape in the umbilical area and 4 cm above and below it.

An ultrasound was performed with a Toshiba Sonolayer SS11-14UA ultrasound machine and a 3MHz transducer at baseline and after two months of treatment. Adipose tissue thickness of the treated flanks was assessed for potential reductions, and the non-treated area of the umbilicus, at the infraumbilical midline (IUML), was assessed for reference values. In the substudy, an ultrasound of the non-treated flank was also performed.

A 0.5-cm skin punch biopsy of adipose tissue was taken with a 6-mm trocar. Samples of the adipose tissue from the right flank were taken prior to treatment and at 4 weeks. These tissue samples were embedded in paraffin and stained with hematoxylin-eosin 4x, 10x, 20x, 40x.

After two months of treatment, all participants and investigators were surveyed to assess the level of satisfaction with the treatment results. The assessment scale score ranged from 1 to 5, where 1=No Results, 2=Poor Results, 3=Good Results, 4=Very Good Results, 5=Excellent Results. Participants' assessment was subjective. For this assessment to be more objective, investigators answered the survey taking into account perimeter and ultrasound reductions.

Pictures of the treated and control areas were taken. In the substudy, sagittal images were taken to compare the non-treated and treated abdominal areas.

Unless otherwise noted, quantitative variables are described as mean and standard deviation (SD), whereas categorical variables were described as percentages. All estimations were performed with Microsoft Excel.

Results

A total of eight patients –75% of which were women– with a mean age of 44 years (SD 9.17) were included. BMI had a mean of 26.88 (SD 2.38) (range 24.5-30). Seven subjects (one man and six women) with a mean age of 44.28 years (SD 9.86) participated in the main study, and one man aged 42 and a BMI of 27.1 took part in the substudy.

Data Collected for the Medical History: Regarding the diseases recorded, one female patient had osteoporosis and displacement of vertebral disc, and one male patient (from the substudy) had angina and familial hypercholesterolemia. One male patient was allergic to pollen, one female patient suffered from seasonal allergies, and one female patient was allergic to furantoin. Regarding pre-study surgeries, one female patient had a C-section, another underwent tubal ligation, and one male patient had his tonsils removed. Medication reported by participants included calcium, contraceptives, omega and flavonoids; and Cardyl, Adiro and Emconcor by one of the subjects (from the substudy). None of the participants were dieting for weight loss when they were recruited in the study. Two men and one woman played sports regularly.

Pre-Treatment Results (T0):

Body composition using bioimpedance: Participants had a mean weight of 73.44 kg (SD 5.34), a FFM of 49.31 kg (SD 7.14), a FM of 24.13 kg (SD 5.97) and a TW of 34.63 kg (SD 4.87) (Table 1).

Abdominal perimeter values: The mean value of the perimeter at the level of the umbilicus was 96.57 cm (SD 4.85), of the supraumbilical (SU) perimeter was 91.66 cm (SD 5.98) and of the infraumbilical (IU) perimeter was 99.76 cm (SD 5.45) (Table 2).

Fat tissue ultrasound: Fat tissue mean thickness was 4.17 cm (SD 0.94) for the IUML, 2.80 cm (SD 0.97) for the RF, and 2.75 cm (SD 0.97) for the LF (Table 3).

Table 1. Baseline body distribution measured by bioimpedance

	All patients (n=8)		Men (n=2)		Women (n=6)	
	Mean	SD	Mean	SD	Mean	SD
T0						
Weight (kg)	73.44	5.34	77.15	1.77	72.20	5.65
FFM (kg)	49.31	7.14	60.35	3.04	45.63	2.12
FM (kg)	24.13	5.97	16.80	4.81	26.57	4.08
TW (kg)	34.63	4.87	42.10	1.41	32.13	1.74
BMI	26.88	2.38	25.80	1.84	27.23	2.58

Table 2. Abdominal perimeter measurements

	All patients (n=8)		Men (n=2)		Women (n=6)	
	Mean (cm)	SD	Mean (cm)	SD	Mean (cm)	SD
T0 (cm)						
Umbilicus	96.57	4.85	102.00	-	95.67	4.62
SU	91.66	5.98	96.40	5.09	90.08	5.74
IU	99.76	5.45	94.30	5.23	101.58	4.49
T1 (cm)						
Umbilicus	95.70	4.77	100.00	-	94.98	4.80
SU	90.31	4.99	93.75	5.30	89.17	4.79
IU	98.16	5.72	93.00	6.36	99.88	4.85
T2 (cm)						
Umbilicus	94.66	4.94	100	-	93.77	4.76
SU	90.11	5.61	95.50	4.95	87.96	4.56
IU	93.36	5.99	93.00	7.07	98.05	5.24
T1-T0 (cm)						
Umbilicus	-0.87	0.77	-2.00	-	-0.68	0.64
SU	-1.35	1.55	-2.65	0.21	-0.92	1.56
IU	-1.60	1.47	-1.30	1.13	-1.70	1.66
T2-T0 (cm)						
Umbilicus	-1.68	1.71	-1.00	1.80	-1.90	1.85
SU	-1.48	1.80	-0.90	0.14	-1.67	2.08
IU	-2.98	2.01	-1.30	1.84	-3.53	1.86

Results after 4 Weeks of Treatment (T1):

Abdominal perimeter values: The mean value of the perimeter at the level of the umbilicus was 95.70 cm (SD 4.77), of the SU perimeter was 90.31 cm (SD 4.99) and of the IU perimeter was 98.16 cm (SD 5.72). Differences between mean values at baseline and at 4 weeks (T1-T0) were of -0.87 cm (SD 0.77) for the umbilicus, -1.35 cm (SD 1.55) for the SU area, and -1.60 cm (SD 1.47) for the IU area (Table 2).

Results after 8 Weeks of Treatment (T2):

Abdominal perimeter values: The mean value of the perimeter at the level of the umbilicus was 94.66cm (SD 4.94), of the supraumbilical perimeter was 90.11cm (SD 5.61) and of the infraumbilical perimeter was 96.36cm (SD 5.99).

Differences between mean values at baseline and at 8 weeks (T2-T0) were of -1.68 cm (SD 1.71) for the umbilicus, -1.48 cm (SD 1.80) for the supraumbilical area, and -2.98 cm (SD 2.01) for the infraumbilical area (Table 2).

Fat tissue ultrasound: Fat tissue mean thickness was 3.52cm (SD 0.93) for the IUML, 2.38cm (DS 0.79) for the RF, and 2.54 cm (SD 0.96) for the LF. Differences between mean values at baseline and at 8 weeks (T2-T0) were of -0.65 cm (SD 0.63) for the IUML, -0.42 cm (SD 0.58) for the RF, and -0.21 cm (SD 0.63) for the LF. Mean reduction: the IUML reached a mean reduction of 15.34% (SD 13.32), (range of reductions 6.45-35.29%). On the RF, mean reduction was of 11.03% (SD 25.21), (range of reductions 9.37-38.24%) (Table 3).

Treatment assessment survey: The survey answers provided by participants and investigators were a match in 62.5% of cases. The mean score value provided by participants was 3.5 (SD 0.53) and that by investigators was 3.38 (SD 0.74).

Table 3. Subcutaneous adipose tissue thickness measurements through ultrasound

	All patients (n=8)		Men (n=2)		Women (n=6)	
	Mean	SD	Mean	SD	Mean	SD
T0 (cm)						
IUML	4.17	0.94	3.10	-	4.53	0.79
RF	2.80	0.97	1.45	0.35	3.25	0.58
LF	2.75	0.97	1.50	0.42	3.16	0.67
T2 (cm)						
IUML	3.52	0.93	2.80	0.14	3.76	0.96
RF	2.38	0.79	1.55	0.21	2.65	0.70
LF	2.54	0.96	1.60	0.14	2.85	0.90
T2-T0 (cm)						
IUML	-0.65	0.63	-0.30	0.14	-0.77	0.70
RF	-0.42	0.58	0.10	0.57	-0.60	0.51
LF	-0.21	0.63	0.10	0.57	-0.31	0.66
Δ T2-T0 (%)						
IUML	-15.34	13.32	-9.68	4.56	-17.23	15.06
RF	-11.03	25.21	12.01	41.94	-18.70	15.96
LF	-4.57	25.68	12.50	41.25	-10.26	20.67

Substudy

Using sagittal images taken before (T0) and after treatment (T1 and T2), differences were observed between the treated (left) and non-treated (right) abdominal areas (Fig. 3).

Anatomopathological study of the fat tissue biopsy: The skin tissue biopsy taken before the treatment (T0) had normal architecture, and there was no modification of adipocytes (Figs. 4 and 5). After 4 weeks (T1), the microscopic observation of the biopsied tissue showed a decrease in thickness of the adipocyte layer. In the hypodermis, adipose systems were compacted, and cytoplasm was eosinophilic and vacuolated. An image suggesting mature adi-

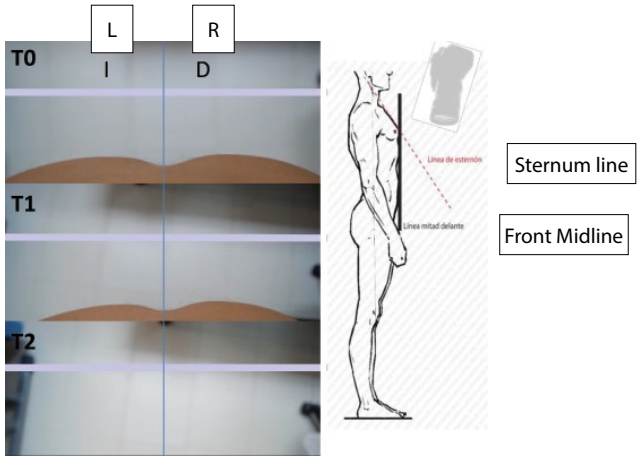

Figure 3. Sagittal image of the treated left side and the non-treated right side of the abdomen (from the substudy).
T0: Baseline; T1: At 4 weeks; T2: At 8 weeks; L: Left; R: Right.

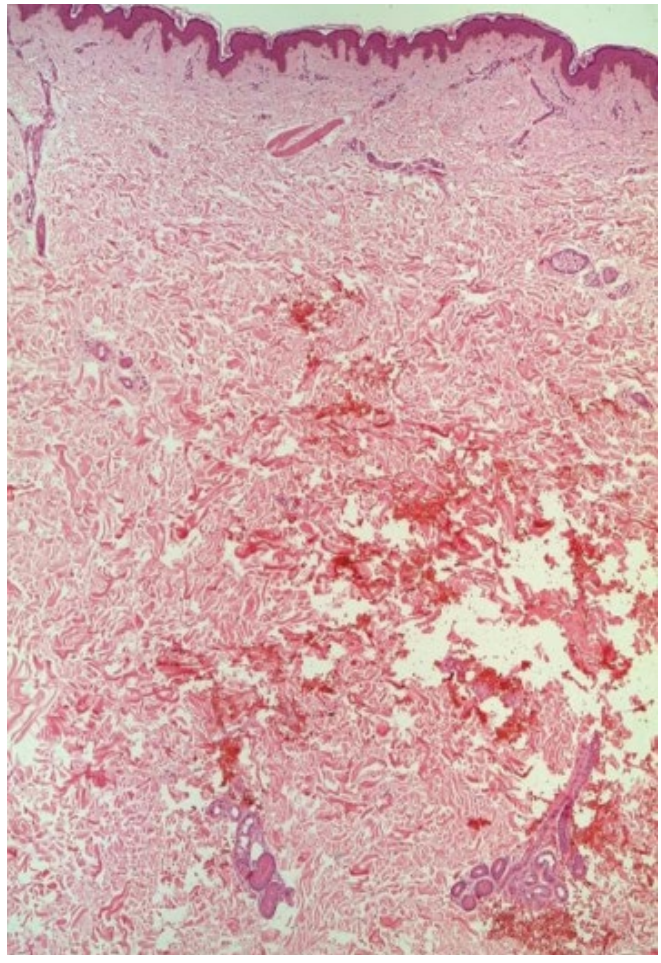

Figure 4. 0.5-cm skin punch taken prior to treatment (T0). Hematoxylin-eosin 4x.

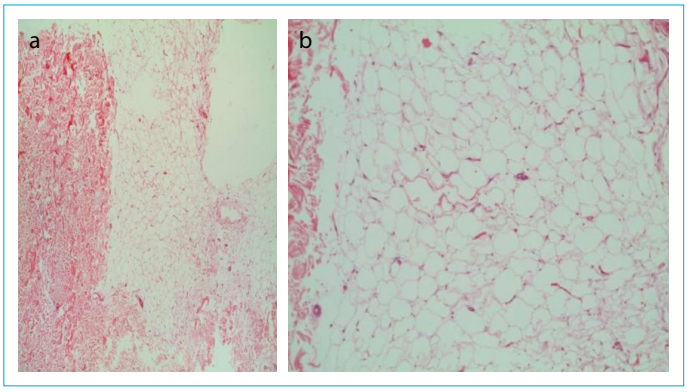

Figure 5. Subcutaneous adipose tissue taken prior to treatment (T0). **(a)** Tissue with normal histology (hematoxylin-eosin 4x), **(b)** Normal adipocytes (hematoxylin-eosin 20x).

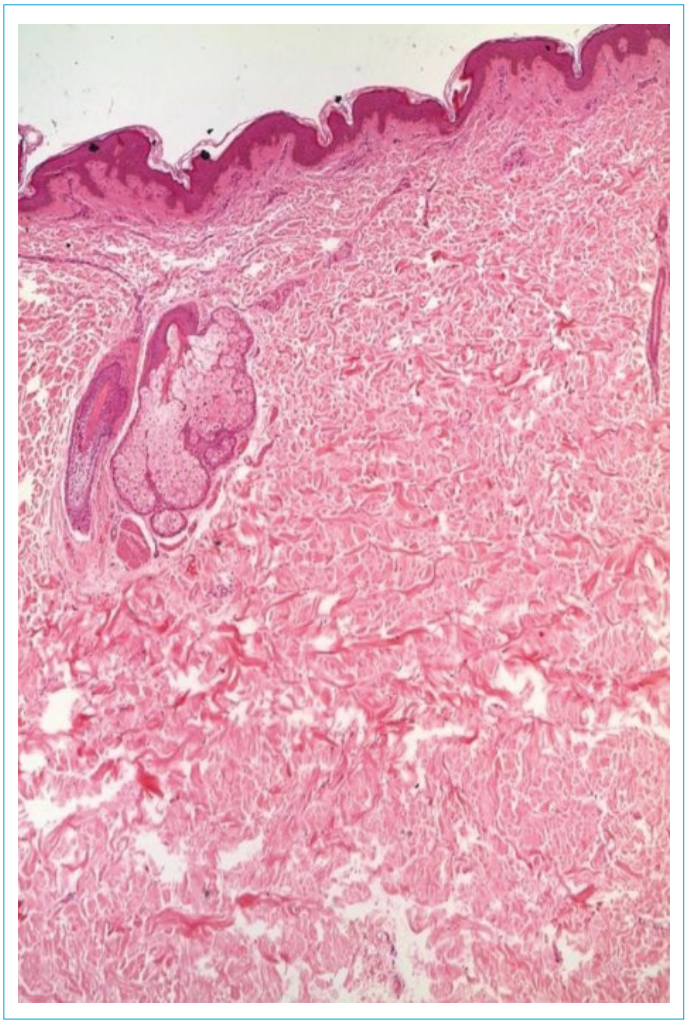

Figure 6. 0.5-cm skin punch taken after 4 weeks of treatment (T1). No damages are observed in the dermis or hypodermis (hematoxylin-eosin 4x).

pocytes (brown fat) or macrophages digesting adipocytes was observed. There was no necrosis or inflammation (Figs. 6 and 7).

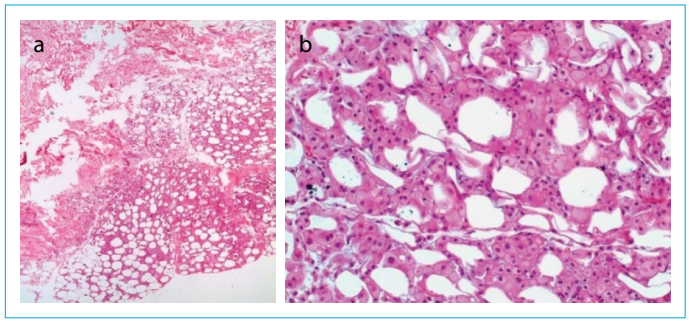

Figure 7. Biopsied adipose tissue taken after 4 weeks of treatment (T1). **(a)** Compacted adipose systems with vacuolated cytoplasm (hematoxylin-eosin 10x) are observed. **(b)** Mature adipocytes (brown fat) and/or macrophages digesting adipocytes (hematoxylin-eosin 40x).

Safety Data

No adverse effects were reported. The only side effects recorded after the treatment were:

Erythema: in all cases.

Edema: in all cases.

Pain: in three cases that subsided within 24 hours.

Parestesia in the treated area: in five cases that lasted one week.

Discussion

Study results showed the efficacy of conducting one session of cryoadipolysis with the Cooltech® device. In the areas treated (abdomen, and left and right flanks), the ultrasound performed after two months of treatment showed a 15.34% (SD 13.32) reduction of adipose tissue thickness in the IUM, 11.03% (SD 25.21) in the RF, and 4.57% (SD 25.68) in the LF (only treated in seven out of eight participants). In similar studies in which the abdominal area was treated, a reduction of 12.6% was obtained without a post-treatment massage, and of 21.0% massaging the treated area for several minutes at the end of the session.[10] In studies in which the flanks were not treated, there was a reduction of 22.4% at 4 months.[11]

Regarding body diameter, a reduction in the perimeter of the treated area was observed. After 8 weeks of treatment, mean reduction of the umbilical and supraumbilical areas was higher in women (Table 2). The same happened with the reduction of subcutaneous adipose tissue thickness assessed through ultrasound. At 8 weeks, a reduction in thickness of the IUML and an increase in the RF and LF were observed in men. In women, a reduction of IUML, RF and LF was observed (Table 3).

Sex-related differences in the reduction of adipose tissue thickness might be explained by men and women's different distribution of body fat.[12] Just like there is a sex-related

difference in the metabolic function of adipose tissue,[13] recent studies have found that, when exposed to low temperatures, adipose tissue response may also be different based on sex.[14] Having this type of information would serve to adapt treatments and provide a more customized care, with better results.

Taking into account thermal profiles that enable the crystallization of fat acids, we are confident that, to get the best results, it is essential to reach a temperature lower than 10 ºC for most of the time during the cryoadipolysis session. The thermal profiles of the most common fatty acids re known and, to reach crystallization and apoptosis, in most cases it is necessary to reach temperatures ≤10 ºC.[15] Therefore, the systems that don't reach these temperatures will not work. To increase the effectiveness of the procedure, it is fundamental to use an applicator causing enough suction to isolate the adipose tissue and keep it as avascular as possible, thus preventing circulation from rising the temperature during the treatment. Furthermore, to ensure the safety of the procedure, it is essential to use crioprotector membranes to protect the skin from thermal burns caused by freezing of the water in the skin.

The physiological mechanisms that cause the removal of fat cells (apoptosis) begin at the end of the treatment session, the results being visible after 3 weeks. Unlike other publications stating that there is inflammation after treatment due to cold exposure [16, 17] or other non-invasive techniques to remove adipose tissue,[18] in the biopsied samples of fat tissue from the study, the destruction of adipocytes occurs without inflammation or necrosis (Figs. 6 and 7). Inflammatory cells usually appear due to traumatic damage or exposure to toxins.[19] This kind of cell destruction leaves a fibrous scar, creates inflammation and deforms the tissue. Another type of cell death is apoptosis, or programmed cell death. This is considered a physiological natural death of damaged cells [19] that does not generate "waste", like fatty acids and glycerol. This form of adipocyte destruction is that observed after treatment with Cooltech®, which makes us think that this technique is more effective than cavitation and less traumatic that focused ultrasound, and that it reaches an optimal temperature to trigger this programmed cell death.

One of the weaknesses of the study is the reduced number of patients, and the high subjective component in the methods used to assess the results. For example, in an ultrasound with just one point measured in mm, different results may be obtained based on the pressure applied on the skin by the transducer. In this study, participants' subcutaneous adipose tissue did not exceed 5 cm thickness in the infraumbilical area and 4 cm in the flanks. The same is true for the perimeter measurement, the satisfaction survey or the pictures. The results from these tests are acceptable, but make us think that they are not the best methods to assess this type of results. The Dual-Energy X-Rray Absorptiometry (DEXA) technique, which can determine the composition of body areas, could be a useful tool to initially assess the areas to treat as well as to conduct efficacy studies.[20] However, subjects' exposure to radiation and the contraindications it may have under certain circumstances must be taken into account.

Patients' level of satisfaction was high, with all eight patients, as well as the investigators, satisfied with the results obtained. The treatment was safe, no adverse effects were reported, and all patients could take up their usual activities at the end of the session.

Conclusion

Cryoadipolysis performed with the Cooltech® machine is a safe, non-invasive technique producing satisfactory results in the removal of localized subcutaneous adipose tissue. Besides, in the biopsied samples of fat tissue from the substudy, the destruction of adipocytes occured without inflammation or necrosis. However, further studies should be conducted, with more patients, a longer follow-up time and a higher number of sessions. Furthermore, it would be interesting to identify those areas that better respond to treatment and see if, based on sex, adipose tissue reacts differently when exposed to low temperatures.

Disclosures

Informed Consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept: C.F.U., L.C.R., J.M.S., M.V.G., G.V.M.; Design: C.F.U., L.C.R., J.M.S.; Supervision: C.F.U., L.C.R., J.M.S., M.V.G., G.V.M.; Material: C.F.U., L.C.R., J.M.S.; Data collection/or processing: C.F.U., L.C.R., J.M.S.; Analysis and/or interpretation: C.F.U., L.C.R., J.M.S., M.V.G., G.V.M.; Literature search: C.F.U., L.C.R., J.M.S., M.V.G., G.V.M.; Critical reviewew: C.F.U., L.C.R., J.M.S., M.V.G., G.V.M.

References

1. Alizadeh Z, Halabchi F, Mazaheri R, Abolhasani M, Tabesh M. Review of the mechanisms and effects of noninvasive body contouring devices on cellulite and subcutaneous fat. Vol. 14, International Journal of Endocrinology and Metabolism. Kowsar Medical Institute; 2016. p. e36727.
2. Meyer PF, Silva RMV da, Oliveira G, Tavares MA da S, Medeiros ML, Andrada CP, et al. Effects of Cryolipolysis on Abdominal Adiposity. Case Rep Dermatol Med 2016;2016:1–7.
3. Mulholland RS, Paul MD, Chalfoun C. Noninvasive Body Con-

touring with Radiofrequency, Ultrasound, Cryolipolysis, and Low-Level Laser Therapy. Vol. 38, Clinics in Plastic Surgery. 2011. p. 503–20.
4. Klein KB, Zelickson B, Riopelle JG, Okamoto E, Bachelor EP, Harry RS, et al. Non-invasive cryolipolysis for subcutaneous fat reduction does not affect serum lipid levels or liver function tests. Lasers Surg Med 2009;41:785–90.
5. Zelickson B, Egbert BM, Preciado J, Allison J, Springer K, Rhoades RW, et al. Cryolipolysis for noninvasive fat cell destruction: Initial results from a pig model. Dermatologic Surg 2009;35:1462–70.
6. 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;135:1581–90.
7. Mulholland R, Stephen, Paul MD, Chalfoun C. Noninvasive Body Contouring with Radiofrequency, Ultrasound, Cryolipolysis, and Low-Level Laser Therapy. Clin Plast Surg 2011;38:503–20.
8. Viera-Mármol G, García P, Villena J. Validation of Cooling and Freezing Dynamics of Cooltech® Using an Experimentally Adjusted Physical Model. SAS J Med 2017.
9. Sánchez Jaime JA. Device for Protecting Skin and Tissues for Aesthetic Cold Treatments [Internet]. 2018 [cited 2018 Aug 21]. Available from: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018060533&recNum=291&office=&queryString=%28ANA%3AES%29&prevFilter=&sortOption=公布日降序&maxRec=34343
10. Boey GE, Wasilenchuk JL. Enhanced clinical outcome with manual massage following cryolipolysis treatment: A 4-month study of safety and efficacy. Lasers Surg Med 2014;46:20–6.
11. Nelson AA, Wasserman D, Avram MM. Cryolipolysis for Reduction of Excess Adipose Tissue. Semin Cutan Med Surg 2009;28:244–9.
12. Wells JCK. Sexual dimorphism of body composition. Vol. 21, Best Practice and Research: Clinical Endocrinology and Metabolism. 2007. p. 415–30.
13. Karastergiou K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissues - The biology of pear shape. Vol. 3, Biology of Sex Differences. BioMed Central; 2012. p. 13.
14. Van Den Beukel JC, Grefhorst A, Hoogduijn MJ, Steenbergen J, Mastroberardino PG, Dor FJMF, et al. Women have more potential to induce browning of perirenal adipose tissue than men. Obesity. 2015;23:1671–9.
15. Manstein D, Laubach H, Watanabe K, Farinelli W, Zurakowski D, Anderson RR. Selective cryolysis: A novel method of non-invasive fat removal. Lasers Surg Med 2008;40:595–604.
16. Dierickx CC, Mazer JM, Sand M, Koenig S, Arigon V. Safety, tolerance, and patient satisfaction with noninvasive cryolipolysis. Dermatologic Surg 2013;39:1209–16.
17. Jalian HR, Avram MM. Cryolipolysis: A Historical Perspective and Current Clinical Practice.
18. Jewell ML, Solish NJ, Desilets CS. Noninvasive body sculpting technologies with an emphasis on high-intensity focused ultrasound. Vol. 35, Aesthetic Plastic Surgery. 2011. p. 901–12.
19. Jordán J. Apoptosis : muerte celular programada. Apoptosis 2003;22:253–75.
20. Wells JCK, Fewtrell MS. Measuring body composition. Arch Dis Child 2006;91:612–7.