

ORIGINAL ARTICLE

Ultrasound assessment of abdominal adipose panniculus in patients treated with a single session of cryolipolysis in a clinical setting

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Abstract

Objective: To assess the effectiveness and safety of cryolipolysis treatment (four-to-six cycles in one session) for reducing abdominal fat.

Methods: Retrospective study conducted on consecutive healthy women who underwent one session of cryolipolysis for abdominal fat reduction. Ultrasound images (USI) were acquired at baseline and at month 6 after treatment. A patient-tailored approach based on adipose tissue depth and architecture was selected.

Results: Thirty women were included. The mean age was 51.4 ± 3.4 years, and 27 (90%) were menopausal. At month 6, the mean abdominal fat layer thickness reduction was 46.6% (41.2%–51.9%). The mean contour was significantly reduced from 84.3, 99.2, 90.6, and 97.1 cm to 81.0, 93.6, 85.8, and 92.2 cm in the infracostal, supraumbilical, umbilical, and ischiopubic regions, respectively; $p < 0.0001$ each. There were no significant changes in weight, body mass index, fat mass, or lean mass throughout the study. After adjusting for smoking, exercise, hypothyroidism, and type of fat, there was a significant reduction in abdominal fat layer thickness (-4.5 ± 0.9 mm, $p < 0.0001$) and contour measurements in infracostal (-3.3 ± 2.4 mm, $p = 0.0317$); supraumbilical (-4.8 ± 2.1 mm, $p = 0.0254$); umbilical (-5.6 ± 2.3 , $p = 0.0161$); and ischiopubic (-4.9 ± 1.8 mm, $p = 0.0080$). The qualitative analysis of the USI suggested the appearance of numerous bands of ordered and structured collagen fibers.

Conclusions: Four-to-six cycles of cryolipolysis, administered in one session, seemed to be a safe and effective procedure for reducing localized fat in the abdominal region in this sample. Additionally, ultrasound images suggested that cryolipolysis was associated with a skin quality improvement and neocollagenesis.

KEYWORDS

abdominal fat, body contouring, cryolipolysis, neocollagenesis, one-treatment session, ultrasounds

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1 | INTRODUCTION

Body contouring is currently among the most common aesthetic surgical procedures worldwide.¹ Liposuction has been the second most commonly performed aesthetic surgical procedure all over the world, second only to breast augmentation, with 1 704 786 procedures performed in 2019.¹

Despite the popularity of this procedure, it is not free of complications, with an incidence of complications that ranged between 8.6% and 20%.^{2,3}

Although liposuction represents an effective and relatively safety procedure for the removal of excess adipose tissue, it is invasive and entails the inherent risks associated with surgery.²⁻⁵ There is, therefore, the need to look for safer techniques, while still being effective.

Different minimally invasive techniques, with variable effectiveness profile, have emerged.⁶ Among them, the practice of body contouring using cryolipolysis has tremendously increased.⁷

The Coolsculpting® (Allergan, an Abbvie company) procedure has received the Food and Drug Administration (FDA) and European Union clearance for treating visible fat bulges in different treatment areas, including submental and submandibular region; flanks; abdomen; outer and inner thighs; bra-bulge; back, underneath the buttocks; and upper arm.⁸

The principle behind cryolipolysis is based on the premise that adipocytes are more susceptible to cooling than other tissues.⁹ Cold temperatures induce apoptosis of the adipocytes, and, thereby, lessens gradually the fat layer.⁹⁻¹² Since the sensitivity of adipose tissue to cold is greater than other tissues, the collateral damage to surrounding tissues is minimal.⁹⁻¹³ From a histological point of view, cryolipolysis induces apoptosis, which is followed by a gradual inflammatory process that promotes slow cell macrophagy.⁹⁻¹³ This inflammatory process begins approximately 3 days after treatment and peaks at day 14 thereafter, approximately. At 14–30 days after cryolipolysis, macrophages and other phagocytes digest the lipid cells as part of the body's natural response to injury and, approximately 4 weeks after treatment, inflammation lessens and the adipocyte volume is decreased.^{12,13}

Cryolipolysis have been identified as a safe and effective treatment in clinical studies.^{12,14-21}

Moreover, McKeown & Payne,²² in a prospective study, published recently, evaluating the effectiveness of an intense CoolSculpting regime (multiple cycles/sessions), found significant improvements in body contouring, with a good safety profile. However, the question of whether a single session of cryolipolysis may significantly improve body contouring has not been fully elucidated.^{22,23}

The purpose of the current study was to assess the effectiveness and safety of four-to-six cycles of cryolipolysis, administered in one session, by using a contoured cup cryolipolysis applicator for reduction of abdominal fat in daily practice.

2 | METHODS

Retrospective and single-center study conducted on consecutive healthy women who underwent cryolipolysis treatment for abdominal fat reduction between December 2018 and June 2019 and had a follow-up of 6 months.

Due to coronavirus disease 2019 (COVID-19) outbreak over the last 1.5 years, the strategies adopted by the different Governments for reducing the risk of infection spreading have dramatically disrupted the provision of health care resulting of deferral of routine aesthetic procedures. Our inclusion period prevented the inclusion of patients from December 2019, since the visit regime could be affected by lockdowns and other preventing measurements.

The study protocol was approved by an independent ethics committee, which waive the need of informed consent for this study. The study was conducted in accordance with the rules of the Declaration of Helsinki and all applicable country-specific regulations governing the conduct of clinical research.

2.1 | Patients

Eligible participants were healthy women aged from 45 to 60 years with visible fat on their abdominal region, body mass index (BMI) ≥ 20 kg/m² and ≤ 30 kg/m² and had at least a follow-up of 6 months. Patients who have undergone previous aesthetic procedures for fat reduction, either surgical or non-surgical, in the treatment region, or any exaggerated or abnormal reaction to cold exposure were excluded.

Before treatment, all the patients underwent a quantitative and qualitative evaluation of their fat distribution, according to their individual metameric distribution. The amount and distribution of fat were analyzed macroscopically by means the portable 3D imaging system LifeViz® Body (QUANTIFICARE SA.).

2.2 | Procedures

2.2.1 | Ultrasounds

The depth and architecture of the different layers of the adipose tissue were determined by ultrasonography. Ultrasound assessment was performed using a linear probe multifrequency mode with probe of 5–13 MHz (SONON 300C; Healerion, Inc.).

Ultrasound images were acquired at baseline and month 6 post-treatment visits by the same device to ensure consistent imaging methods. All the examinations were performed by the same experienced observer, who paid special attention to avoid any pressure on the area (less than 1 newton in all measurements).

Fat layer thickness was measured in mm.

2.2.2 | Cryolipolysis

The treatment was performed with either the contoured cup cryolipolysis applicator CoolAdvantage® or the Cooladvantage plus® (CoolSculpting® Allergan, an Abbvie company).

With the patient in supine position, a single session of cryolipolysis, for 60 min, with a suction pressure of 60 KPa and a temperature of -5°C was applied.

A customized treatment approach was selected according to the depth and architecture of the patient adipose tissue layers. Patients received from 4 to 6 cycles, administered in a single-session, depending on patient's requirements.

With the objective of optimizing the treatment results with a single session, cryolipolysis applicator was placed in such a way that treat the thickest fat region in the best way possible. The treatment strategy is shown in Figure 1.

Patients typically resumed normal activities immediately post-treatment.

2.3 | Study variables

Beside demographic and clinical data (age, smoking, exercise, menopause, hypothyroidism, non-steroidal anti-inflammatory drugs consumption, and type of fat), there were analyzed the following variables: weight, body mass index (BMI), fat mass (FM), lean mass (LM), relationship between FM/LM, fat layer thickness in the central abdominal region (measured by ultrasound), and contour measurements in different anatomic regions using a flexible measuring tape.

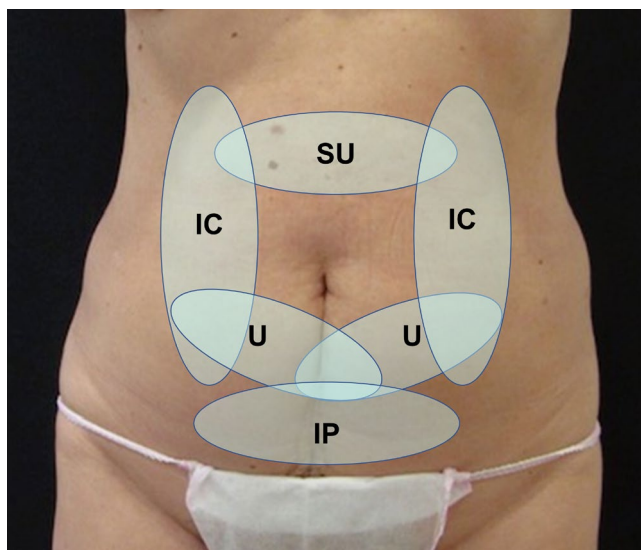


FIGURE 1 Overview of the different treated areas. IC: Infracostal; SU: Supraumbilical; U: Umbilical; IP: Ischiopubic

2.4 | Outcomes

The primary efficacy endpoints were mean abdominal fat layer thickness reduction at month 6 measured with ultrasounds and the mean contour reduction in the infracostal, supraumbilical, umbilical, and ischiopubic regions by using a flexible measuring tape. Secondary endpoint included incidence and severity of adverse events.

2.5 | Statistical analysis

A standard statistical analysis was performed using MedCalc Statistical Software version 20.008 (MedCalc Software Ltd; <https://www.medcalc.org>; 2021).

Before the study was estimated that it was necessary to include a minimum sample of 28 subjects for detecting a difference of 2.0 mm in mean abdominal fat layer reduction at a significance level of 0.01, with a power of 0.99, and assuming a standard deviation of 2 mm.²³ Additionally, this sample had a power of the 99% for detecting a mean difference of 3.5 cm in any of the different contour measurements, assuming a standard deviation of 3.5 cm. The study has an adjusted power of 0.95 ($0.99 \times 0.99 \times 0.99 \times 0.99 \times 0.99$).

Data were evaluated in a masked fashion.

Data are expressed as number (percentage); mean [standard deviation (SD)]; mean [95% confidence interval (95% CI)]; mean [standard error (SE)]; or percentages as appropriate.

Data were tested for normal distribution using a D'Agostino-Pearson test. As data were normally distributed, the two-way paired-sample Student *t* test was used to compare means at baseline and month 6. Due to the high number of tests, Bonferroni correction was used to correct the *p*-value ($\alpha/9$). Statistical significance was accepted for $p < 0.0011$.

In order to assess the impact of the relationship between baseline FM and LM (FM/LM) on the fat layer reduction, FM/LM was divided according to the sample percentiles in \leq percentile 25%; $>$ percentile 25% \leq median; $>$ median \leq percentile 75%; and $>$ percentile 75%.

Changes between baseline and month 6 in abdominal fat layer thickness and contour measurements (infracostal, supraumbilical, umbilical, and ischiopubic) were assessed by the analysis of covariance (ANCOVA) test. "Time" was selected as a factor and smoking, exercise, hypothyroidism, and type of fat as covariates.

Chi-square test and a Fisher's exact test, as appropriate, were used for evaluating qualitative variables.

3 | RESULTS

Among the 90 Screened patients, 30 women, fulfilled the demands of the inclusion/exclusion criteria. Table 1 summarizes the baseline clinical and demographic characteristics of the study population.

The mean (95% CI) age was 51.4 (50.2–52.7) years, 27 (90%) were menopausal, and 14 (46.7%) were taking regular physical exercise. The fat was compact in 19 (63.3%) women. Three (10%) patients

TABLE 1 Baseline demographic and clinical characteristics

Variable	N = 30
Age, years	
Mean (SD)	51.4 (3.4)
95% CI	50.2–52.7
Sex, n (%)	
Female	30 (100.0)
Smoking, n (%)	
Yes	11 (36.7)
Exercise, n (%)	
Yes	14 (46.7)
Menopause, n (%)	
Yes	27 (90.0)
Hypothyroidism, n (%)	
Yes	20 (66.7)
NSAID, n (%)	
Yes	9 (30)
SML, n (%)	
Yes	7 (23.3)
Type of fat, n (%)	
Soft	11 (36.7)
Compact	19 (63.3)
Weight, kg	
Mean (SD)	63.9 (9.0)
95% CI	60.5–67.2
BMI, kg/m ²	
Mean (SD)	24.3 (1.9)
95% CI	23.7–25.1
Fat, kg	
Mean (SD)	19.3 (6.7)
95% CI	16.8–21.8
Lean mass, kg	
Mean (SD)	44.6 (3.8)
95% CI	43.2–46.0
Relation FM/LM, %	
Mean (SD)	42.7 (13.9)
95% CI	37.5–47.9

Abbreviations: BMI, Body mass index; CI, Confidence interval; FM/LM, Fat mass/lean mass; NSAID, Non-steroidal anti-inflammatory drugs; SD, Standard deviation; SML, Scar medium laparotomy.

received 4 cycles/session and 27 (90%) ones received 6 cycles/session.

At month 6, the mean abdominal fat layer thickness reduction was 46.6% (41.2%–51.9%) (Table 2, Figure 2). The mean contour was significantly reduced from 84.3 ± 10.1 cm, 99.2 ± 9.0 cm, 90.6 ± 9.7 cm, and 97.1 ± 8.0 cm to 81.0 ± 9.5 cm, 93.6 ± 9.0 cm, 85.8 ± 8.6 cm, and 92.2 ± 8.4 cm in the infracostal, supraumbilical,

umbilical, and ischiopubic regions, respectively; $p < 0.0001$ each (Table 2, Figure 3). There were no significant changes in weight, BMI, FM, and LM fat mass, and lean mass throughout the study (Table 2).

FM/LM quotient did not show any influence of the mean reduction in fat layer thickness between baseline and month 6 ($p = 0.8081$, one-way ANOVA test) (Table 3).

Even after adjusting for smoking, exercise, hypothyroidism, and type of fat there was a significant reduction in abdominal fat layer thickness (mean [SE], -4.5 [0.9] mm, 95% CI: -6.2 mm to -2.7 mm, $p < .0001$) and contour measurements (Table 4).

The qualitative analysis of the ultrasound images suggested the appearance of numerous bands of ordered and structured collagen fibers (Figure 4).

Clinical results, assessed by means of the 3D imaging system LifeViz[®] Body or by photographs, have shown a significant improvement in the aesthetic results and in the skin quality (Figures 5, 6, and 7).

Regarding safety, beside the mild and limited inflammation or a mild hypoesthesia, which are typically associated with the procedure, no treatment-related adverse events, either mild or serious were reported.

4 | DISCUSSION

The results of this study found a significant reduction of the abdominal fat layer thickness. Additionally, the current study observed a significant reduction of the contour in the infracostal, supraumbilical, umbilical, and ischiopubic areas, respectively.

Our results are in line with those published by McKeow & Payne,²² who evaluated the effectiveness of cryolipolysis (multiple cycles/sessions) in 28 subjects with localized fat in at least one area of their body. They found a significant fat reduction, approximately 40%, in the different treated areas.²² In favor to our study, it should be mentioned that they administered the CoolSculpting treatment over two sessions in some areas, although they did not find significant differences among patients who underwent one session and those who underwent two ones.²²

It is not easy to compare our results with those reported by McKeow & Payne,²² since there were differences in treatment (they administered from 1 to 8 cycles in one or two sessions, while we administered 4-to-6 cycles in one session) and in the outcomes (they measured skinfold thickness, while we evaluated thickness reduction measured with ultrasounds and contour reduction).

Although our results are in accordance with those reported by McKeow & Payne,²² the mean reduction in localized abdominal fat seemed to be substantially greater than those reported in a systematic review of previous studies.²⁴

Hwang et al.²⁵ assessed the effect of a single session of cryolipolysis on visceral and subcutaneous adipose tissue over a period of 12 weeks. The results of this study showed a reduction in visceral adipose tissue, waist circumferences, and the proportion of body fat.

TABLE 2 Overview of the weight, body mass index (BMI), fat mass, loan mass, different contours (Infracostal, Umbilical; Supraumbilical, and ischiopubic), and ultrasound measurements of fatty tissue and their changes from baseline. *p* values were calculated comparing the parameters at month 6 and at baseline (two-way paired sample Student *t* test). *p* values were considered statistically significant if lower than 0.0056 (Bonferroni correction)

Variables	Baseline	Month 6	Difference ^a		
	Mean (SD)	Mean (SD)	Mean (SD)	95% CI	<i>p</i>
Weight, kg	63.9 (9.0)	63.8 (9.0)	-0.1 (1.8)	-0.8 to 0.6	0.7725
BMI, kg/m ²	24.4 (1.9)	24.5 (2.9)	0.1 (2.2)	-0.7 to 0.9	0.7800
Fat mass, kg	19.3 (6.7)	19.0 (6.4)	0.3 (3.5)	-1.1 to 1.6	0.7009
Loan mass, kg	44.6 (3.8)	44.2 (4.4)	-0.4 (2.1)	-1.2 to 0.4	0.2708
FM/LM, %	42.7 (13.9)	42.9 (14.7)	0.2 (10.0)	-3.5 to 4.0	0.8913
Infracostal, cm	84.3 (10.1)	81.0 (9.5)	-3.3 (2.4)	-4.2 to -2.4	<0.0001
Umbilical, cm	99.2 (10.0)	93.6 (9.0)	-5.6 (2.2)	-6.4 to -4.8	<0.0001
Supraumbilical, cm	90.6 (9.7)	85.8 (8.6)	-4.8 (2.1)	-5.6 to -4.0	<0.0001
Ischiopubic, cm	97.1 (8.0)	92.2 (8.4)	-4.9 (3.2)	-6.1 to -3.8	<0.0001
Ultrasounds, mm	9.4 (4.0)	5.0 (2.5)	-4.4 (2.8)	-5.5 to -3.4	<0.0001

Abbreviation: FM/LM: Relationship between fat mass and lean mass.

^aMonth 6 value minus basal value (a negative value means reduction as compared to baseline).

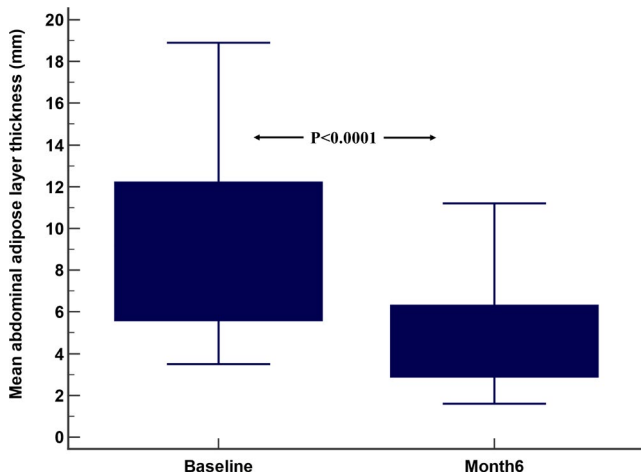


FIGURE 2 Comparison of the mean fat layer thickness measure by ultrasounds at baseline and 6-month after treatment. Mean abdominal fat layer reduction -4.4 mm (95% confidence interval: -5.5 mm to -3.4 mm), *p* < 0.0001. *p* value was calculated by using the two-tailed paired sample Student *t* test

Savacini et al. evaluated the effectiveness of contrast cryolipolysis for subcutaneous-fat reduction.²⁶ They analyzed a sample of 21 healthy subjects who underwent treatment in abdomen and flanks. Mean fat layer reduction in abdomen was 21.6%, with some subjects achieving up to 50.1%. These values were significantly lower than those observed in our study, where the mean abdominal fat layer was reduce by 46.6%, with reductions up to 75.6% (mean difference 25.0%, 95% CI: 17.4 to 32.3, *p* < 0.0001. These data were calculated according to the exposed in the Savacini et al. study).

Kotlus and Mok assessed the effect of cryolipolysis on fifty subjects who underwent one treatment session of cryolipolysis in abdominal region.²⁷ The mean abdomen fat layer reduction of the 25% reported by Kotlus & Mok was significantly lower than that

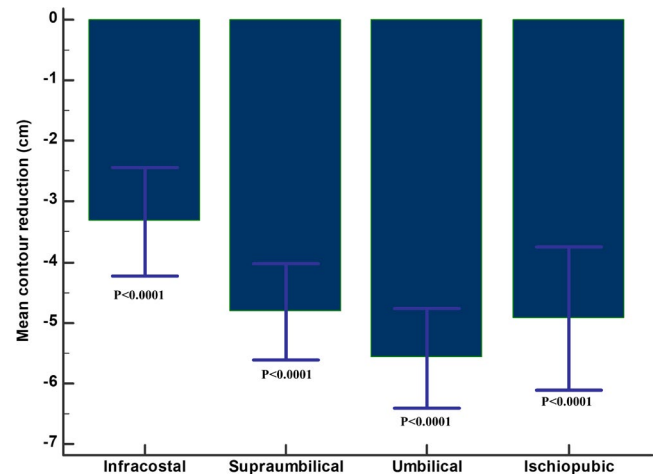


FIGURE 3 Mean contour reduction in infracostal, supraumbilical, umbilical, and ischiopubic regions. *p* values were calculated by using the two-tailed paired sample Student *t* test

observed in our study (mean difference -21.6%, 95% CI: -31.6 to -11.6, *p* < 0.0001. These data were calculated according to the exposed in the Kotlus & Mok study).

Our study did not find any relationship between the baseline FM/LM quotient and the fat layer reduction. It was originally suggested that cryolipolysis would be ideal for fit people with focal dystrophy. Nevertheless, based on our results, cryolipolysis may obtain positive outcomes in a wider morphotype of patients than recommended initially.

During the follow-up period, no significant changes in body weight, BMI, fat mass, or lean mass occurred, which suggested clearly that reduction in fat thickness was due to local treatment.

The qualitative analysis of the ultrasound images has shown the emergence of numerous ordered and structured bands of collagen

TABLE 3 Mean fat layer thickness reduction according to the relationship between baseline fat mass and lean mass (FM/LM)^a. *p* value was calculated by using the Kruskal-Wallis test

FM/LM	Fat layer thickness reduction (%)		
	Mean (SD)	Range	<i>p</i>
Group I (n = 8)	48.3 (17.1)	17.2 to 75.6	0.2378
Group II (n = 7)	47.3 (11.0)	32.6 to 65.2	
Group III (n = 8)	48.5 (16.4)	12.5 to 63.4	
Group IV (n = 7)	41.8 (13.4)	35.0 to 72.2	

Group I: ≤31.97%

Group II: >31.97% to ≤42.27%

Group III: >42.27% to ≤52.17%

Group IV: >52.17%

Abbreviations: SD, Standard deviation; n, Number.

^aFM/LM was divided according to the sample percentiles in ≤percentile 25% (Group I); >percentile 25% ≤ median (Group II); >median ≤ percentile 75% (Group III); and >percentile 75% (Group IV).

TABLE 4 Comparison of adjusted mean change from baseline to month 6 in infracostal, supraumbilical, umbilical, and ischiopubic contours and ultrasound measurement. Statistical significances were assessed using the analysis of covariance (ANCOVA)^a with "Time" as a factor and smoking, exercise, hypothyroidism, and type of fat as covariates

	Difference		
	Mean (SE)	95% CI	<i>p</i>
Infracostal, cm	-3.3 (2.4)	-6.3 to -0.2	0.0317
Supraumbilical, cm	-4.8 (2.1)	-9.0 to -0.6	0.0254
Umbilical, cm	-5.6 (2.3)	-10.1 to -1.1	0.0161
Ischiopubic, cm	-4.9 (1.8)	-8.5 to -1.3	0.0080
Ultrasounds, mm	-4.5 (0.9)	-6.2 to -2.7	<0.0001

^aMonth 6 value minus basal value (a negative value means reduction as compared to baseline).

fibers, which may explain the quality skin improvement observed in our study.

Stevens²⁸ reported that cryolipolysis may be associated with increased skin firmness in patients having flaccidness. Moreover, those patients who experienced an important fat volume reduction did not show skin flaccidity.²⁸

Carruthers et al.²⁹ evaluated the impact of cryolipolysis on the skin texture, laxity, and cellulite. The results of this study suggested a significant improvement in skin texture, laxity, and cellulite after cryolipolysis.²⁹

Although the mechanism through which cryolipolysis induces skin firmness has not been fully elucidated, it may be related to stimulation of collagen production. Carruthers et al proposed that cryolipolysis may stimulate neocollagenesis by stretching of the fibroblasts.²⁹ Most of cryolipolysis treatments are performed using vacuum applicators, which may induce mild stretching to the skin and contribute to neocollagenesis.²⁹

In favor of this hypothesis, Stevens et al.³⁰ evaluated tissue samples of patients who underwent cryolipolysis using both molecular and immunohistochemistry analytical methods. The results of this study found significant induction of molecular and protein markers of Type I collagen, which suggests that neocollagenesis may play a crucial role in reported skin improvement following cryolipolysis.³⁰

Regarding safety, treatment-related adverse events, either mild or severe, were not reported. The presence of scars was not detected after treatment.

However, despite the good safety profile of cryolipolysis, different mild and transient adverse events have been reported in different studies, including pain, erythema, mild swelling, scars, and numbness.¹²⁻²⁰

The main limitation of the current study is its retrospective design. Nevertheless, the strict nature of the inclusion/exclusion criteria and that fact that data have been evaluated in a masked fashion might have at least partially limited these drawbacks. Additionally, this study included only Caucasian women. Appropriate caution is therefore recommended when extending the results to other populations.

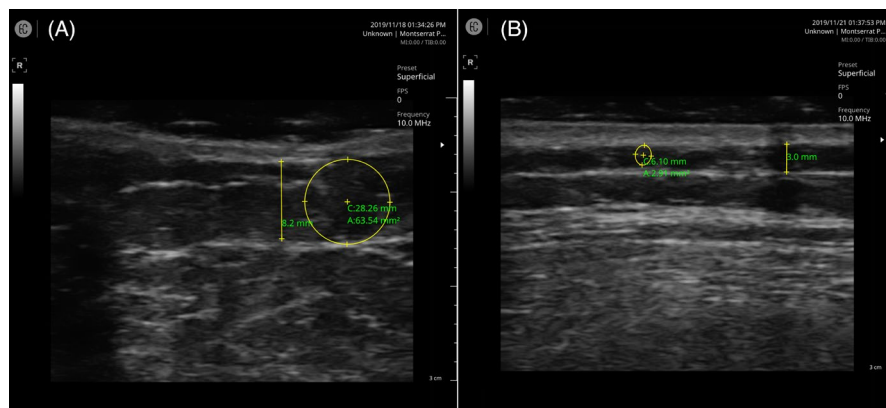


FIGURE 4 Ultrasonographic image of the central abdominal region before treatment (A) and 6 months after treatment (B). Besides the significant reduction in fat layer thickness, an important regeneration of collagen fibers can be observed

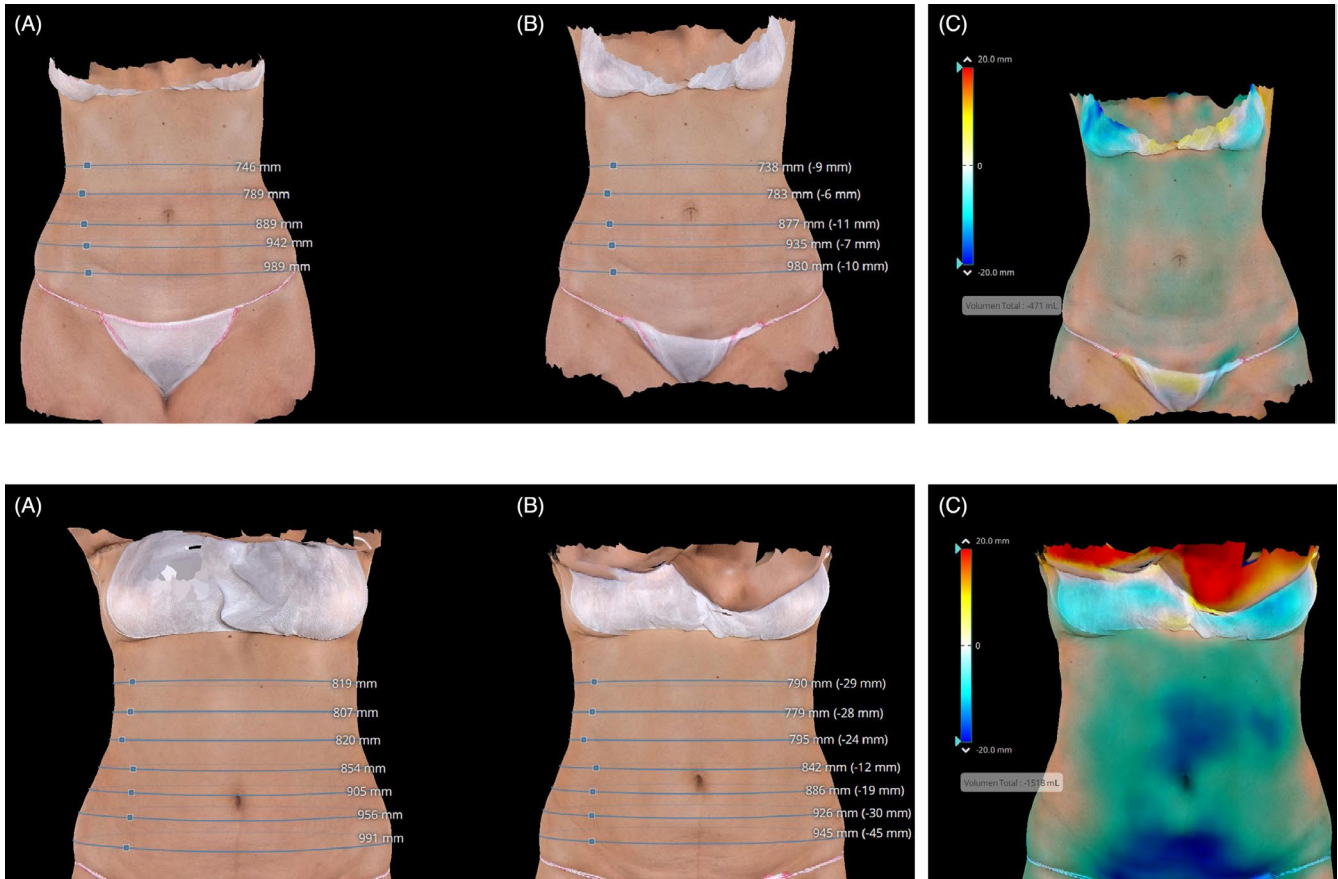


FIGURE 5 Assessment of abdominal fat layer thickness reduction by the 3D imaging system LifeViz® Body (QUANTIFICARE SA.). (A) Pretreatment measurements. (B) Measurements 6 months after treatment. (C) Colorimetric image showing abdominal fat reduction volume in the different areas

FIGURE 6 Image of the abdominal region of a patient before (A) and after treatment (B). It is possible to observe, not only the significant reduction of the abdominal contour but also the objective skin quality improvement in the treated area

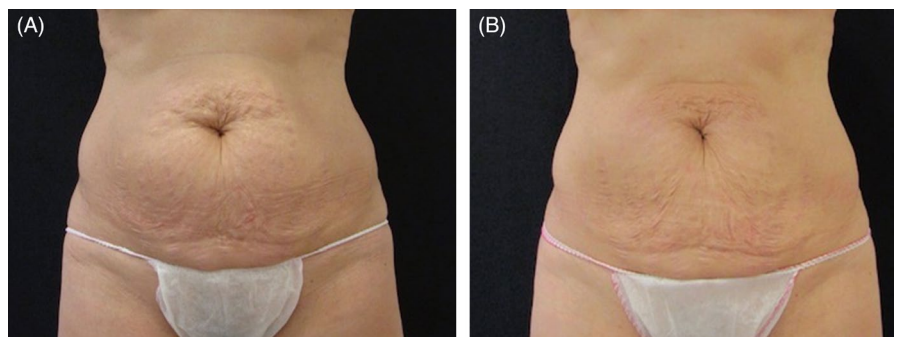
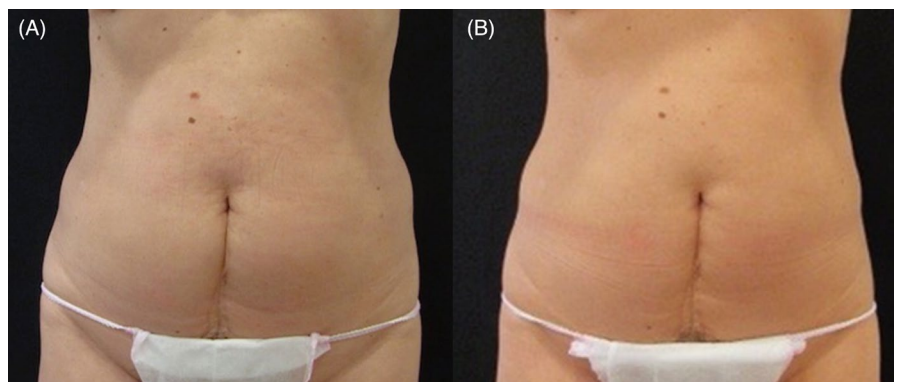


FIGURE 7 Image of the abdominal region of a patient who has previously undergone a cesarean section, before (A) and after cryolipolysis (B). There was a significant reduction in abdominal fat and a significant improvement of the skin quality



5 | CONCLUSIONS

The results of our study suggested that four-to-six cycles of cryolipolysis, administered in one session, was a safe and effective procedure for reducing localized fat in the abdominal region. The treatment strategy used in this study provided a significant fat layer reduction with only one session. Although repeated cryolipolysis treatment has been associated with an additional reduction of the fat thickness layer, the first treatment showed better outcomes.³¹

The lack of relationship between the baseline fat mass and the fat reduction at the end of the study suggested that cryolipolysis may be beneficial in a wider range of patients than initially recommended.

The question of whether the customize cryolipolysis procedure used in this study may positively impact on the results need to be elucidated in further researches. Last but not least, in the current study, cryolipolysis was associated with a skin quality improvement and the emergence of numerous ordered and structured bands of collagen fibers. Nevertheless, further histological studies are needed to confirm and clarify this finding.

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CONFLICT OF INTEREST

Dr. Monserrat Ponga has received research grants to cover the costs of medical writing services and publication fees from Allergan, an AbbVie company.

AUTHOR CONTRIBUTION

All authors met the ICMJE authorship criteria. All authors contributed to the drafting and critical revision of the manuscript, commented on previous versions of the manuscript, and read and approved the final manuscript prior to submission.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The local ethics committee waived the need for written informed consent of the participants for the study.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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