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Skin Tightening and Fat-Layer Reduction after Cryolipolysis Treatment

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ABSTRACT

Introduction: Cryolipolysis is a well-tolerated nonsurgical procedure in which controlled cooling is used to treat localized fat. Investigators have also observed skin tightening as a result of this treatment. However, scholars have not studied the extent to which cryolipolysis influences the skin's biomechanical characteristics.

Objective: This study was intended to provide quantitative measurements of skin tightness after cryolipolysis treatment and to evaluate that treatment's effectiveness in the reduction of localized subcutaneous fat.

Methods: In the study, we treated 21 subjects with mean (SD) age 34 (9) years with cryolipolysis in the abdomen and flanks. We performed evaluated the subjects' anthropometry, took standardized photographs and measured skin firmness. We assessed the subjects' fat layers using skin fold calipers and diagnostic ultrasound. We performed measurements at baseline and followed up at 30, 60 and 90 days. The level of significance was $P<0.05$.

Results: There were no significant differences in body weight or BMI between pretreatment and post treatment. The results of the skin-firmness parameter analysis revealed significant differences between the post treatment measurements at 30, 60 and 90 days, as compared to the pretreatment measures. Cryolipolysis reduced the thickness of the fat layer and thus decreased the waist-circumference, caliper and diagnostic ultrasound measurements.

Conclusion: Although the exact mechanisms through which cryolipolysis affects the skin remain unknown, our cutometer measurements demonstrated that this treatment improved skin tightness in the treated areas and reduced fat-layer thickness.

Keywords: Skin firmness, Cutometer, Fat reduction, Adipocyte, Adipose tissue, Cooling

INTRODUCTION

Cryolipolysis is a popular, well-tolerated nonsurgical procedure in which controlled cooling is used to selectively damage fat cells and consequently induce apoptosis [1-4]. Several research groups have demonstrated that fat cells, as compared to water-rich tissues such as the skin, are more sensitive to cold [1-4]. The cryolipolysis technique is performed using a cup-shaped applicator that induces a vacuum to draw the target area into the applicator and position it between two cooling panels. The vacuum reduces blood flow in the treated area; the constriction of blood vessels accelerates the cooling process [5]. Between the skin and the inner surface of the applicator is a thin membrane of tissue; this tissue is covered with antifreeze lotion to protect the skin and ensure that the opposing applicator plates completely couple [1,6]. The cooling is usually maintained for 45 to 60 min [7]. Clinical researchers have investigated the safety and efficacy of cryolipolysis treatments for subcutaneous fat reduction in several areas of the body, including the abdomen, flanks, inner thighs, outer thighs, arms, chest and sub mental fat [8-14]. Some investigators have even observed an additional, unexpected benefit of cryolipolysis: skin tightening, which leads to improved skin texture and laxity [15,16]. However, we found no studies on the extent to which cryolipolysis influences the skin's biomechanical characteristics. Therefore, the purposes of

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this study were to provide quantitative measurements of skin tightness after cryolipolysis treatment and to evaluate the effectiveness of this treatment in terms of the reduction of localized subcutaneous fat.

MATERIALS AND METHODS

We performed this prospective study (with pre- and post-intervention analysis) by treating subjects with cryolipolysis at the Clinical Laboratory of the Ibramed Center for Education and Advanced Training – CEFAI (Amparo, São Paulo, Brazil). All subjects signed informed-consent forms, and we performed the treatments in accordance with the Declaration of Helsinki. The Research Ethics Committee for Institutions, Teaching and Research approved the study (CAAE: 61499416.5.0000.5490).

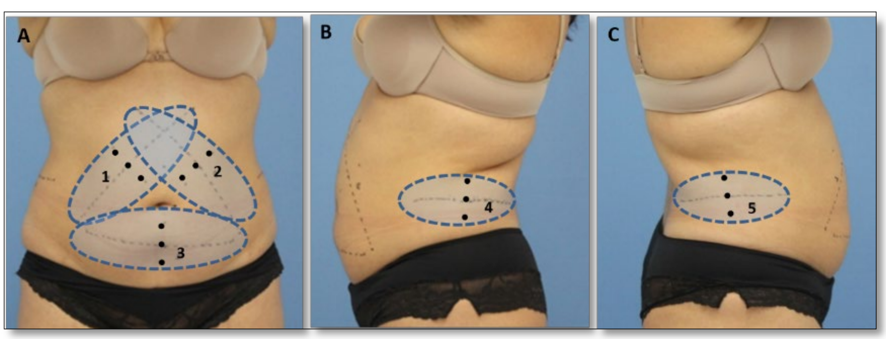
The 21 volunteers who took part in the study had a mean (SD) age of 30 (7) years and an age range of 18 to50 years; 18 (85%) were female. The inclusion criteria were the presence of localized subcutaneous fat in the abdominal and flanks regions and a body mass index (BMI) <30. The exclusion criteria were aesthetic treatment in the treatment areas in the preceding 6 months; current cutaneous diseases in the treatment areas; current systemic diseases; current pregnancy, lactation, or intention to become pregnant; and history of cryoglobulinemia, cold urticaria or paroxysmal cold hemoglobinuria.

We identified, assessed, marked, and treated the treatment sites with a conventional cryolipolysis Polaris device (Ibramed, IndústriaBrasileira de EquipamentosMédicos - Amparo, São Paulo, Brazil). We used a template to draw markings on the patients that would guide the measurements and performed the treatments using medium (~300 cm^3) or large (~700 cm^3) applicators, based on the size of the localized fat areas and the anatomical limitations of the applicators' placement. We exposed each treated to -8°C for 60 min [17]. All participants underwent anthropometric measurements for skin firmness, waist circumference and abdominal fat-layer thickness. A trained physiotherapist took all measurements, and, for each subject, the same physiotherapist performed the evaluations for every visit. The evaluations occurred at pretreatment and at three follow-ups: 30, 60 and 90 days post treatment. We assessed the individuals' heights and weights using a classical mechanical stadiometer (110 CH Model, Welmy, SP, Brazil and measured the circumferences of their abdomens using flexible measuring tape. To evaluate skin firmness, we used a Cutometer MPA 580 (Courage & Khazaka Electronic, Köln, Germany) to probe a 2 mm hole at 500 mbar of suction per second.

We performed the measurements in triplicate and based the analysis on the means of these values. Before each set of measurements, the volunteers spent 20 min in a closed environment at a constant temperature (18°C to 22°C) and controlled relative humidity (55% to 65%). The cutometer generated graphs that depicted immediate deformation or skin extensibility (U_e), delayed distension (U_v), final deformation (U_f), immediate retraction (U_r), total recovery (U_a), and residual deformation at the end of the measuring cycle (R). From these values, we computed two fractions: U_v/U_e, the viscous component of the skin, and U_r/U_f, the biological elasticity [18,19]. We also used the parameter $R_0=U_f$ to refer to skin firmness or dispensability; R_0 is one of the most important parameters [20].

Figure 1 shows the areas that we demarcated for treatment and the points that we used for the cutometry, ultrasound, and caliper evaluations.

Figure 1. Demarcated areas for treatment and points for cutometry and ultrasound measurements.



The treatment areas are shaded and numbered 1-5; the points for the cutometry and ultrasound measurements are marked with dotted lines

We used skin fold calipers (RMC, Amparo, SP, Brazil) to measure the point of greatest thickness within each treatment area. We pulled the folds vertically and measured the thickness 2 cm to the side of the umbilicus.

We performed the diagnostic ultrasound assessments using a linear transducer with frequency of 6 MHz to 18 MHz (MyLab25 Gold; Esaote, Italy) and analyzed the resulting images using quantitative measurements (mm) of the subcutaneous tissue between the anatomic planes (dermis and muscular fascia), at the same points that we demarcated and evaluated using the cutometer. The probe was positioned on the previously demarcated points in the treatment area **(Figure 1)**, with coupling gel and without tissue compression.

We took photographs with a digital camera (Canon EOS Rebel T3i, Canon USA Inc., Melville, NY, USA) at pretreatment and at the final follow-up, 90 days after the treatment.

To avoid bias in relation to the effectiveness of the treatment, we applied a routine standard method for measuring fat reduction. This involved multiple measurement modalities: waist circumference measurements using a measuring tape, as well as fat-layer thickness measurements using skin fold calipers and diagnostic ultrasound [21]. We took the same care with regard to the standardized evaluations of skin firmness, which we performed with a cutometer.

For the statistical analysis, we used Graph Pad Prism 6 (La Jolla, CA, USA). We assessed the normality of the data distribution with the Shapiro-Wilk test. According to these results, we analyzed the differences between the pretreatment and post treatment measurements using either a one-way analysis of variance and Tukey's multiple-comparisons test or the Friedman test and Dunn's multiple-comparisons test. The level of significance for all tests was $P<0.05$.

RESULTS

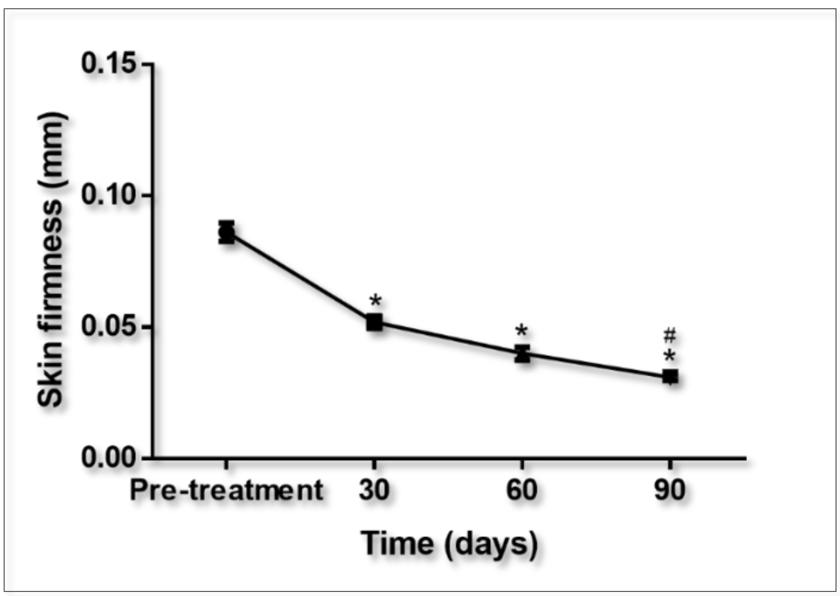
We treated 21 subjects, of whom 18 (85%) were female. The subjects' mean (SD) age was 30 (7) years. The average body weight at pretreatment was almost the same as at post treatment; the BMI slightly declined from pretreatment to post treatment. However, there were no statistically significant differences in pretreatment and post treatment body weight or BMI **(Table 1)**.

Table 1. Clinical characteristics of the study population at pre-treatment and post-treatment (90 days after treatment).

Measure	Pre-treatment, mean (SD)	Post-treatment, mean (SD)	*P* Value
Body weight, kg	66.5 (8.8)	66.2 (9.0)	0.40
BMI, kg/m²	25.0 (2.1)	22.9 (2.1)	0.24

The results of the R_0 parameter analysis for skin firmness (as collected with a cutometer)revealed significant differences between the measurements taken after 30, 60 and 90 days and those taken at pretreatment **(Figure 2)**.

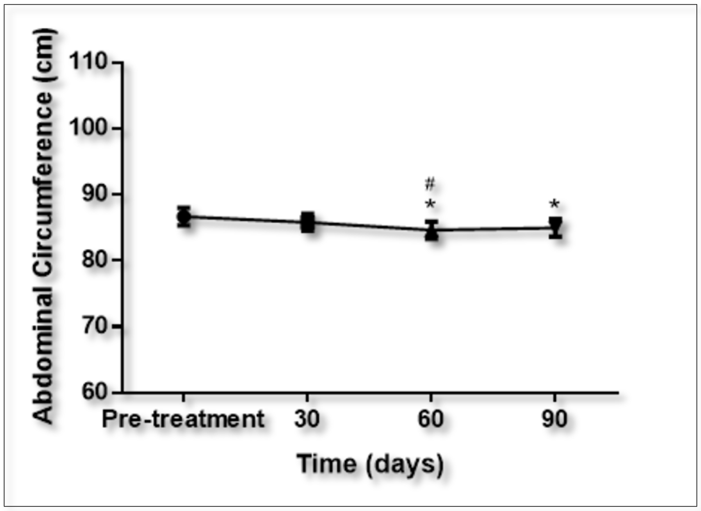
Figure 2. Means and standard errors of the means for abdominal skin firmness at pre-treatment and at 30, 60 and 90 days post-treatment.



An asterisk indicates a significant difference from pretreatment and a pound sign indicates a significant difference from day 30, with significance defined as P<0.05 using a post hoc Dunn's multiple-comparisons test

The waist circumference data are presented in **Figure 3**. There were statistically significant differences between the measurements after 60 and 90 days when compared to pretreatment and also between the measurements after 60 days when compared to those after 30 days.

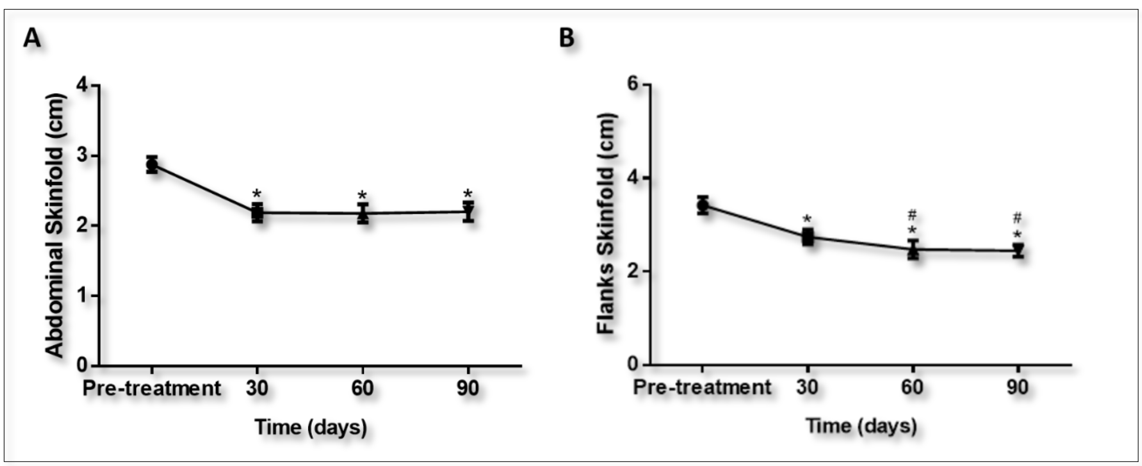
Figure 3. Means and standard errors of the means for abdominal circumference at pre-treatment and at 30, 60 and 90 days post-treatment.



An asterisk indicates a significant difference from pre-treatment and a pound sign indicates a significant difference from day 30, with significance defined as P<0.05 using a post hoc Tukey's multiple-comparisons test

We analyzed the skin fold-caliper data to assess treatment efficacy. There was a statistically significant decrease in skin fold thickness in the treated areas at all follow-up time points when compared to the pretreatment measurements **(Figure 4)**.

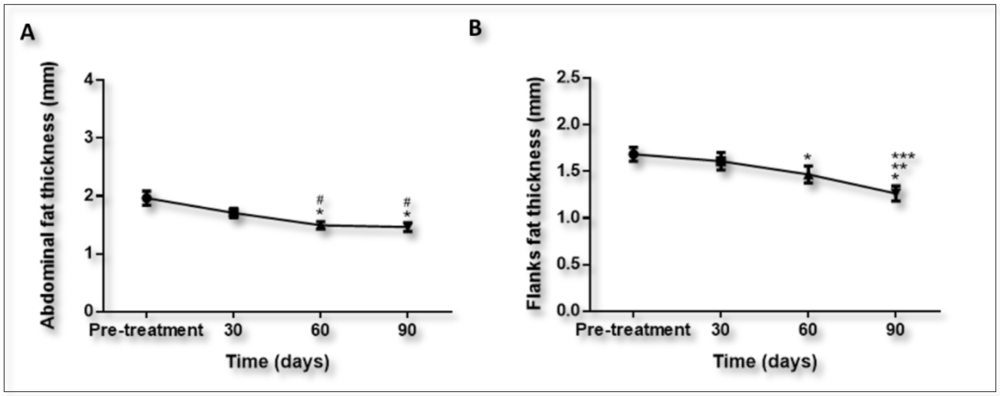
Figure 4. Means and standard errors of the means for the abdominal skinfold and flanks at pre-treatment and at 30, 60 and 90 days post-treatment.



***A**, Abdominal skinfold. **B**, Flank skinfold. An asterisk indicates a significant difference from pretreatment, and a pound sign indicates a significant difference from day 30, with significance defined as P<0.05 using a post hoc Tukey test*

Ultrasound images were analyzed to calculate fat layer reduction. **Figure 5** shows representative ultrasound images captured at pretreatment and at 30, 60 and 90 days after the treatment. Reduction in fat layer was statistically significant in both treated regions: abdomen and flanks.

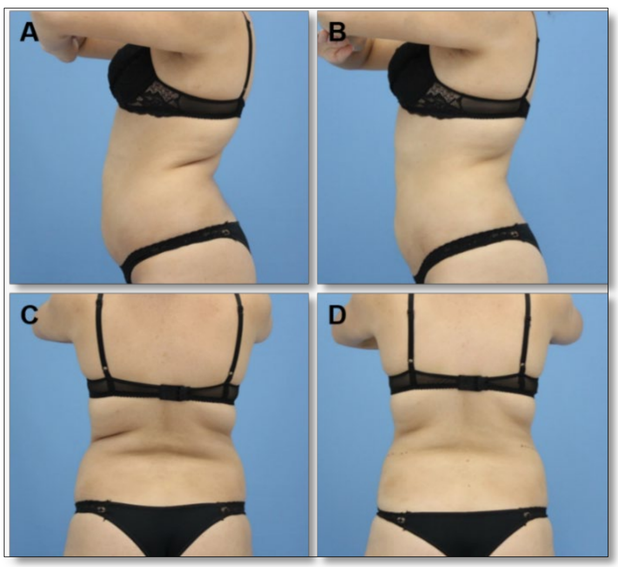
Figure 5. Means and standard errors of the means for abdominal and flank fat thickness using diagnostic ultrasound at pre-treatment and at 30, 60 and 90 days post-treatment.



A, Abdominal fat thickness. B, Flank fat thickness. An asterisk indicates a significant difference from pretreatment and a pound sign indicates a significant difference from day 30, with significance defined as P<0.05 using a post hoc Tukey test

Figure 6 shows the improvement of body contouring 90 days after treatment with cryolipolysis.

Figure 6. Body contouring comparison, pre-treatment and 90 day's post-treatment.



A, Abdominal treatment area at pre-treatment. B, Abdominal treatment area at 90 days post-treatment, with a visible reduction in adipose tissue. C, Flank treatment area at pre-treatment. D, Flank treatment area90 day's post-treatment, with a visible reduction in excess fat and with notable skin accommodation

DISCUSSION

Cryolipolysis is frequently used for localized fat treatment [22]. This treatment is performed with small, medium or large vacuum-pressure applicators, which extract heat from both sides of a skin fold and reduce blood flow by simultaneously compressing the tissue and promoting cold-induced vasoconstriction [6]. In addition to the expected reduction in fat-layer thickness, patients and clinicians have often observed visible skin tightening in cryolipolysis treatment areas [15,16].

Skin is composed of various types of cells in two layers: epidermis and dermis [23]. Subcutaneous adipose tissue is a soft connective tissue that is located under the dermis [24]. Both the skin and the subcutaneous adipose tissue are visco elastic and the toughness of these tissues is determined by their density and by the arrangement of type I collagen [25]. Skin tightening can be achieved through thermal (heat-based) injury to the dermis using radio frequencies; intense pulsed light; fractional, high-potency lasers; or focused ultrasound. These techniques are frequently used in aesthetic procedures [26-31]. Heat shock proteins (HSPs)—which can be induced using a wide variety of stressors (including heat, cold, ultraviolet radiation, oxidative stress, ischemia, cellular-energy depletion, and inflammation)—have a general protective function and enable cellular survival [32-34]. When collagen is heated, its heat-sensitive bonds begin to break down, turning from an organized crystalline structure into a disorganized gel. This process induces the synthesis of both HSP and inflammatory mediators such as tumor necrosis factor-α, interleukin and transforming growth factor-β, which are then released into the injured tissue [25,28,35,36]. Once these mediators are released into the tissue, they trigger are pair cascade by activating fibroblasts and other types of cells [33,37,38]. Researchers have shown that this type of treatment also induces neo collagen formation, thus leading to reduced skin flaccidity [39,40]. Cryolipolysis is an aesthetic procedure that reduces adipose tissue through exposure to cold temperatures and it is generally well-tolerated, with only mild side effects such as bruising, transient neuralgia, erythema and tenderness [41]. The thermal shock of cryolipolysis activates a repair cascade in the skin and promotes skin tightening.

Researchers have described the cutometer as an important and effective tool for objective, noninvasive measurements of biomechanical skin properties; it yields but absolute and relative data [18,42]. Cutometers have been widely used to evaluate human skin's viscoelastic properties using the suction method and cutometer-specific R_0 through R_9 values have been analyzed using instrument software [18]. Researchers have claimed that the R_0 (U_f) parameter is the best way to measure and quantify skin firmness or distensibility [20,43]. The purpose of this study was to evaluate both the extent to which cryolipolysis improved skin tightness through the use of quantitative measurements and the effectiveness of this treatment at reducing localized abdominal fat.

We observed that cryolipolysis treatment significantly improved skin firmness (i.e., by producing lower values of R_0 that indicate greater firmness and lower skin distensibility). We noticed a significant difference in this tissue property after cryolipolysis. The mechanism through which cryolipolysis induces skin firmness is not well-understood, but it seems to result from cold-stimulated collagen production [13]. Although Carruthers et al. [16] suggested that the vacuum suction during cryolipolysis could stimulate neo collagenesis via mechanical stretching of the fibroblasts, in the same study, the authors also observed skin tightening after the use of a plate-surface applicator, which lacks a vacuum.

Indeed, mechanical forces lead to biochemical and molecular responses; in other words, a stretch in the cell membrane would be transmitted via the cytoskeletal network to induce the synthesis of an extracellular matrix from the fibroblasts [44,45]. The process of converting physical forces into biochemical signals and then integrating these signals into cellular responses is referred to as mechano-transduction [46]. Accordingly, when chronically stretched beyond its physiological limits, skin grows; the increased surface area reduces the mechanical load [47]. Similarly, when fibers are subjected to chronic excessive stretching, they may undergo fragmentation, which results in a loss of the ability to return to their original state; this makes the skin more plastic [23,47]. However, this skin laxity is not observed after treatment with cryolipolysis. During this treatment, which usually lasts around 60 min, the skin and adipose tissue deform, cool down and suffer moderate ischemic injury [5]. The crystallization caused by the cooling of the targeted adipocytes induces apoptosis in these cells; although numerous researchers have reported that this cooling does not affect tissues that are rich in water, this cold shock could be sufficient to activate HSP and promote a subclinical inflammatory response. Supporting evidence indicates that lipids form intracellular crystals at around 10°C, as compared to water, which freezes at 0°C [48,49]. Consequently, thermal shock could induce a subclinical inflammatory response and activate the fibrogenic process, which could explain the skin tightening observed in cryolipolysis [5,16,50]. It is important to emphasize that researchers in histologic studies have realized that cryolipolysis treatment causes significant destruction of fat cells due to cold, as well as substitution of the adipocytes with connective tissue, which indicates fibroblast activation [51,52]. In this study, the cutometer measurements indicated improved skin firmness, which may be associated with remodeled skin collagen with improved density **(Figure 2)**. The cutometry results suggest that this effect is probably due to the cold stimulation of the fibroblasts.

In this study, we also used noninvasive methods to measure the adipose-tissue thickness: calipers and ultrasound. These results also demonstrate that non-invasive cryolipolysis is effective at decreasing the thickness of the localized fat layer in the flanks and abdomen. Klein et al. [53] established the safety of multiple same-day treatments (in the abdomen and both flanks); in that study, each subject received treatment on between one and five areas in the same day and each area received only one 60 min cooling cycle at -8°C.

In this study, the subjects did not show significant changes in body weight (P=0.40), as indicated in **Table 1**. However, the cryolipolysis treatment reduced the thickness of their fat layers, as shown in all forms of measurement. Several researchers have used waist-circumference measurements to determine the efficacy of aesthetic procedure sin terms of fat reduction [13,21,54]. With regard to waist circumference, the mean reduction was statistically significant in this study. The other measurements also showed significant reductions in fat (P<0.05). The calipers showed a 23.55% reduction in the abdomen **(Figure 4A)** and a 25.69% reduction in the flanks **(Figure 4B)**. The diagnostic ultrasound showed a reduction of 25.83% in the abdomen **(Figure 5A)** and

19.05% in the flanks **(Figure 5B)**. All these results are based on a comparison of the pretreatment values and the values measured at 90 days post-treatment. Photographs of the clinical results are also shown in **Figure 6**.

No significant complications occurred during the treatment. This study's results corroborated the findings of the systematic review conducted by Ingargiola et al. [22] regarding fat reduction based on caliper and ultrasound measurements. In that review [22], the reductions in the caliper measurements were 14.67% to 28.50% and the reductions in the ultrasound measurements were 10.3% to 25.5%. Although the results from our study shown considerable similarity to those in Ingargiola's review study [22], this comparison has limitations. Notably, the designs of the 19 studies differ from that of our study in terms of treatment time, and there is insufficient information about the temperatures used in the cooling of some studies (variable cooling intensity factor (CIF)/value per mill watt per square centimeter (mW/cm^2)).

Our clinical results suggest that rapid cooling affects not only subcutaneous fat tissue but also skin tissue. This action on the skin is probably due to inflammatory mediators, which initiate the tissue-repair and regeneration pathways.

CONCLUSION

This study's results indicate that cryolipolysis treatment reduces fat-layer thickness and in improves skin tightening. The exact mechanisms behind this effect remain unknown; however, in this study, cutometer measurements demonstrated improved skin tightening in the treated areas. This clinical investigation should encourage researchers to complete further studies so as to better understand the mechanisms by which HSP and/or inflammatory mediators stimulate the skin after cryolipolysis treatment.

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