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Effects of Cryofrequency on Localized Adiposity: Case Study

Patrícia Froes Meyer ([articles.aspx?searchcode=Patr%20Froes+Meyer&searchfield=authors&page=1](#))^{1*}, Laura Vieira Pires Diniz ([articles.aspx?searchcode=Laura+Vieira+Pires+Diniz&searchfield=authors&page=1](#))², Ruth Gaioso Cartaxo Sousa ([articles.aspx?searchcode=Ruth+Gaioso+Cartaxo+Sousa&searchfield=authors&page=1](#))², Júlio Davi Costa Silva ([articles.aspx?searchcode=J%20Davi+Costa+Silva&searchfield=authors&page=1](#))², Eneida de Moraes Carreiro ([articles.aspx?searchcode=Eneida+de+Moraes+Carreiro&searchfield=authors&page=1](#))², Rafaella Rêgo Maia ([articles.aspx?searchcode=Rafaella+R%20aago+Maia&searchfield=authors&page=1](#))³, Ciro Dantas Soares ([articles.aspx?searchcode=Ciro+Dantas+Soares&searchfield=authors&page=1](#))², Fernando Cesar Câmara ([articles.aspx?searchcode=Fernando+Cesar+C%20a2mara&searchfield=authors&page=1](#))³, Rodrigo Marcel Valentim da Silva ([articles.aspx?searchcode=Rodrigo+Marcel+Valentim+da+Silva&searchfield=authors&page=1](#))³

¹University Center of Rio Grande do Norte, UNI-RN, Natal, Brazil ([articles.aspx?searchcode=University+Center+of+Rio+Grande+do+Norte%2c+UNI-RN%2c+Natal%2c+Brazil&searchfield=affs&page=1](#)).

²Universidade de Potiguar (UNP), Natal, Brazil ([articles.aspx?searchcode=Universidade+de+Potiguar+\(UNP\)%2c+Natal%2c+Brazil&searchfield=affs&page=1](#)).

³Federal University of Rio Grande do Norte (UFRN), Natal, Brazil ([articles.aspx?searchcode=Federal+University+of+Rio+Grande+do+Norte+\(UFRN\)%2c+Natal%2c+Brazil&searchfield=affs&page=1](#)).

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Abstract

Cryofrequency is the combination of cryotherapy with radiofrequency, which promotes a “thermal shock” to the tissue, working simultaneously to skin flaccidity and localized adiposity. The efficacy of dermatological and hypodermic cryofrequency treatment was evaluated. This study reports the case of a 45-year-old woman with complaints of flaccidity and fat located in the infra-umbilical region in the preoperative period of abdominoplasty. The evaluation was performed before and after treatment through evaluation protocols, ultrasound examinations and histological findings through optic microscopy, microfocal and immunohistochemical analysis. The treatment was performed in 4 sessions, with a weekly application, using the BHS 156 FULL®—BODY HEALTH BRASIL® apparatus, the parameters were temperature of -10°C, power in 70%, duration of 8 minutes. After the surgical procedure, parts of treated and untreated abdominal tissues were removed for evaluation. In the tests performed, significant destruction of adipocytes was detected, with consequent reduction of fat and greater expression of caspase-3 in the treated tissue, being constated the macrophage increase by CD68 expression. There was improvement in flaccidity, evidenced by the significant increase in the number of fibroblasts and inflammatory cells positive for Ki67, bringing more quantity and better quality to the collagen.

Keywords

Cryofrequency ([articles.aspx?searchcode=Cryofrequency&searchfield=keyword&page=1&skid=0](#)), Localized Adiposity ([articles.aspx?searchcode=+Localized+Adiposity&searchfield=keyword&page=1&skid=0](#)), Skin Flaccidity ([articles.aspx?searchcode=+Skin+Flaccidity&searchfield=keyword&page=1&skid=0](#)), Abdominoplasty ([articles.aspx?searchcode=+Abdominoplasty&searchfield=keyword&page=1&skid=0](#))

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1. Introduction

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Localized adiposity is the accumulation of energy reserves in adipose tissue, determined by caloric intake above that required for body metabolism. This excess is an important factor that can trigger serious health problems such as coronary diseases, hypertension, diabetes, among others, as well as aesthetic issues [1] [2] [3].

Flaccidity is considered an unsightly skin condition, this is related to the decrease of skin fibroblasts, compromising the activity of the collagen and elastin proteins responsible for the support and elasticity [4]. It may be a consequence of physiological aging, due to the gradual loss of skeletal muscle mass, replaced by adipose tissue, and atrophy of subcutaneous adipose tissue, among other changes [5].

There is a great demand for different non-invasive procedures, which seek to minimize aesthetic dysfunctions with minimum risks, among them is cryofrequency. This technology combines cryotherapy with radiofrequency, working simultaneously in the treatment of flaccidity and localized fat. It features a tip that cools only the epidermis at about -10°C , while radiofrequency heat reaches the deeper layers of the skin stimulating the formation of new collagen and elastin fibers and lipolysis [6] [7].

The energy released from this electrode leads to better circulatory and nutrient supply, besides tissue hydration, increased oxygenation, acceleration of the elimination of catabolites, lipolysis, contraction of the connective tissue and allied to this causes the contraction of the collagen fibers, improving simultaneously the changes in the external structure of the skin and allowing greater comfort to the patient during the application [7].

Although it is a novel technology without a fully understood mechanism of action, promising results have been confirmed in studies therefore appearing to be an excellent option for localized adiposity reduction and improvement of sagging without major adverse effects [8] [9]. This case report was carried out with the objective of evaluating the efficacy of the treatment of cryofrequency at the dermal and hypodermic levels, through ultrasound examinations and histological findings.

2. Case Presentation

The research was characterized as a case study of a 45-year-old woman, weighing 59.7 kg, height 1.50 m and BMI of 26.5 (overweight), presenting localized adiposity and flaccidity in the infra-umbilical region, with interest of abdominoplasty surgery (Figure 1). In the inclusion criteria, the participant should have localized abdominal adiposity and sagging, be in a preoperative period of abdominoplasty surgery and be able to understand and sensibility preserved locally. Among the exclusion criteria applied, it could not present contraindications for the use of equipment (cold hypersensitivity, pacemaker, dermatitis, epilepsy, cancer, use of corticosteroids, metal and/or silicone implants in the treated area) and the participant who disagreed with the procedures or did not adapt to the hours and procedures would be discontinued from the research.

Figure 1. Photographic record of the evaluation.

3. Materials and Methods

The research was carried out in the city of Natal, RN, Brazil, in the premises of Vânia Rocha Clinic, and approved (Registered under number 3.237.778) by the Ethics Committee of the Potiguar University (CEP), Natal, RN, Brazil. An ultrasound device (Eco palm Wifi, 10 MHZ, China), a semiprofessional camera (Canon, SX530 HS, Japan), a tape measure (Fiber Glass Tape, China), an adipometer (Sanny, São Paulo, Brazil) a balance (Accumed-Glicomed, Rio de Janeiro, Brazil), a binocular microscope apparatus (Olympus, model CX31, USA), and a cryofrequency apparatus (BHS 156 FULL[®], BODY HEALTH BRASIL[®], Brazil).

3.1. Procedures

Prior to the start of treatment, the volunteer was guided about the procedures and signed the informed consent form. Afterwards, it was submitted to the PAFAL protocol, validated by Meyer *et al.* [10], an instrument used to obtain information such as identification, anamnesis, life habits, physical examination, with measurements and tests such as weight, height, BMI, skinfolds and waist circumference. The skinfolds of the infra-umbilical region were measured three times, verified 2 cm above the iliac crest region, with result based on the mean values obtained in the measurements. The measurement of the abdominal circumference was performed 5 cm above the umbilical scar, in the supra umbilical region and 5 cm below it, in the infraumbilical region.

Subsequently, the volunteer was submitted to ultrasound examination, which was lauded by a specialist physician. The examination was performed in the infra umbilical region, at a central point in the demarcated area of 10 cm², with the volunteer positioned in dorsal decubitus, with the transducer only in contact with the skin, without pressure [11]. This method allowed to evaluate the thickness of the fat layer in centimeters of the infra-umbilical region before the beginning of the procedures and after the proposed treatment.

For the treatment, a 10 cm² area in the left infra-umbilical region was initially demarcated as shown in Figure 2. The right side area also received a marking of the same size and served as control.

The treatment was performed in 4 sessions, with a weekly application of cryofrequency in the left infra-umbilical region, using the BHS 156 FULL® device, of the brand BODY HEALTH BRASIL®. The parameters used were: frequency mix, multipolar + monopolar corporal mode, with temperature of -10°C , power in 70% and duration of 8 minutes. The reevaluation was performed after the final session with the repetition of all procedures performed before the beginning of the protocol.

Twelve days after the sessions of cryofrequency, the patient underwent total abdominoplasty, under epidural anesthesia with sedation. In preparation for surgery, the two areas were marked with gentian violet pencils to be properly identified and cut into the surgical block. The infiltration with 1:3000.00 adrenaline solution was performed under the superimposed technique. Afterwards, the dermolipectomy was performed, starting with the supra-pubic incision, displacement in the supra-aponeurotic plane to the xiphoid appendix, performing plication of the abdominal rectus muscles, removal of excess skin from the lower abdomen, synthesis by planes with nylon and mono. Negative pressure drain was maintained (Portovac 4.8). After the cutaneous flaps (Figure 3) were removed, they were submitted to histological analysis, where they fixed the pieces in 10% Buffered Formalin for 48 hours. They cleaved and processed according to the pathology laboratory process (dehydration, diaphanization, paraffin embedment and inclusion). The resulting paraffin blocks were cut into a 5 μm thick rotating microtome and the blades obtained were stained by Hematoxylin and Eosin (HE) techniques. They were examined in an optical microscope binocular, being blind and tabulated, verifying the amount of adipocytes, collagen fibers, presence of degenerative tissue. Microfocal microscopy was performed to check the type of collagen and also immunohistochemical analysis with the markers caspase-3, macrophage CD68 and Ki67 antigen.

3.2. Data Analysis

Qualitative data were described based on medical reports (descriptive analysis of ultrasound images) and based on the reports of the pathologist (descriptive analysis of histological images and quantification of cells), as well as the quantitative and qualitative analysis of some immunohistochemical markers. Data collection and correlation were presented in tables and figures.

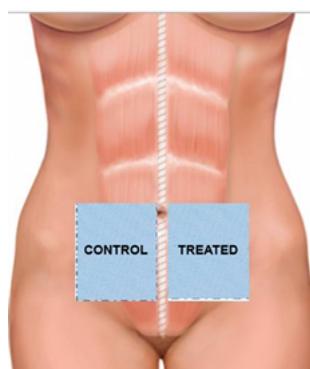


Figure 2. Sampling, control and treated scheme.

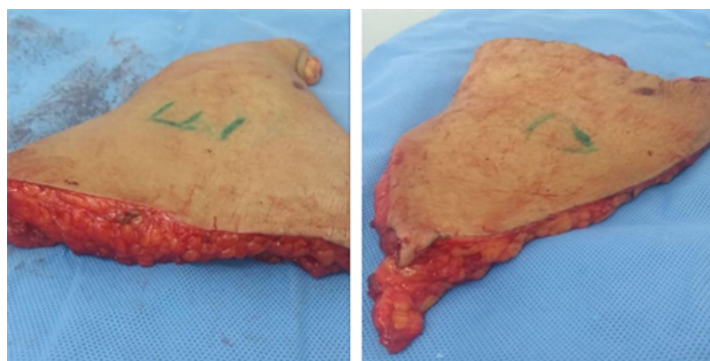


Figure 3. Surgical flaps: left and right.

4. Results

The variables measured before and after application of cryofrequency are described in Table 1. There was an average reduction of 3 cm in the infra-umbilical circumference, being maintained the same value in the supra umbilical region. Weight was observed before treatment was 59.7 kg (BMI 26.5) and after treatment 59.4 kg (BMI 26.4), with a small reduction of 300 g in total body weight. The left skinfold data showed an increase of 1.0 mm in the average obtained, and in the right side of 5.7 mm.

4.1. Ultrasonography Results

Ultrasonography data obtained before and after the last session of application of cryofrequency were recorded in Table 1. The qualitative analysis performed by the physician is shown in Figure 4 and Figure 5. The results obtained with ultrasound demonstrate reduction of the thickness of the fat layer was 0.23 cm after treatment in the left region. The thickness data on the right-side adipose layer varied 0.08 cm.

4.2. Results of Microscopic Analysis

Two tissue samples, corresponding to the treated and untreated areas, were extracted and used for the histological records. In the analysis, it was observed that the untreated side (right) presents an aspect of disorganized collagen, old and with few cells. On the treated (left) side, it is possible to observe, with more detail in the epidermis, a large number of active fibroblasts (blue arrows) and a greater amount of neoformed collagen (red arrow) (Figure 6(a) and Figure 6(b)). In longitudinal microphotography, the treated side presents degenerating adipose tissue and on the control side, the cells present normal characteristics (Figure 6(c) and Figure 6(d)).

The microfocal histological analysis presented results regarding the amount of neoformed collagen, in the treated area it is possible to observe an amount superior to the control side. In addition, a significant amount of Type III (green) collagen was detected on the control side, and a large amount of Type I (red) collagen was detected on the treated side (Figure 6(e) and Figure 6(f)).

Measurement and Weight Assessment	Pretreatment	Post-treatment
Weight (Kg)	59.7	59.4
BMI	26.5	26.4
Plicometry—Right Side (mm)	19.6	25.3
Plicometry—Left Side (mm)	23.3	24.3
Perimetry—Supra Umbilical (cm)	82	82
Perimetry—Infra Umbilical (cm)	90	87
Ultrasonography—Right Side (cm)	1.22	1.14
Ultrasonography—Left Side (cm)	1.21	0.98

Table 1. Anthropometric data of the volunteer.

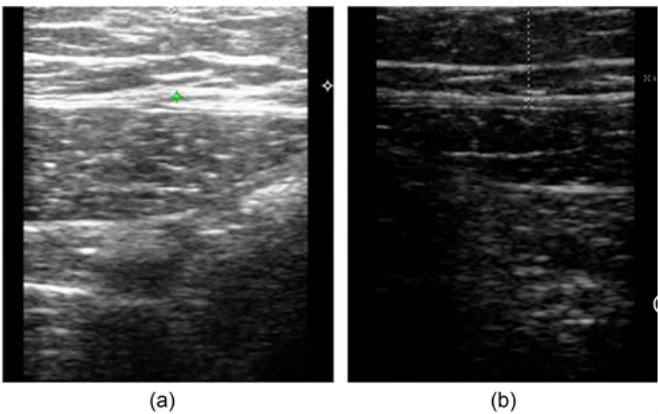


Figure 4. Ultrasonography—Left side: Pretreatment (a) and Post-treatment (b).

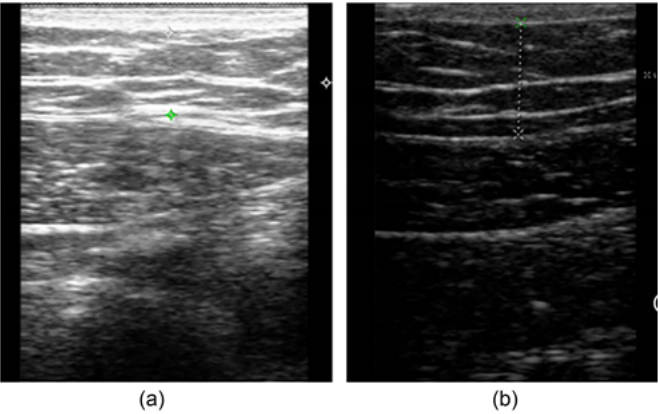


Figure 5. Ultrasonography—Right side: Pretreatment (a) and Post-treatment (b).

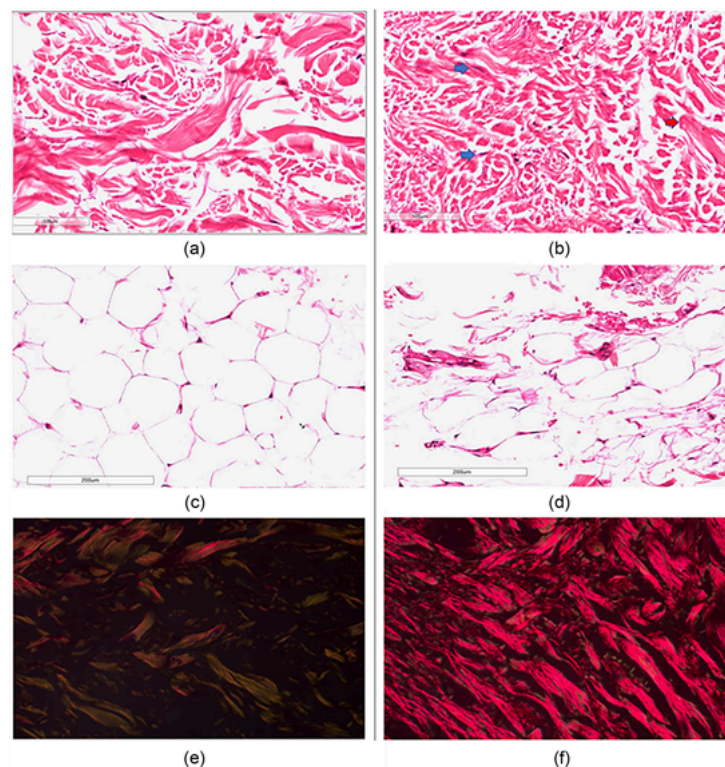


Figure 6. Microscopic analysis of the material collected during abdominoplasty. Longitudinal micrograph HE 100× (a) untreated region and (b) treated region. Longitudinal micrograph HE 200× (c, d) referring to adipose tissue. (e, f) Longitudinal microfocal histology referring to the type of collagen.

4.3. Results of Immunohistochemical Analysis

Immunohistochemical analysis showed greater expression of caspase-3 in the adipose tissue on the treated side, indicating the presence of apoptosis, consequently, a greater degeneration of this tissue ($t \leq 0.05$). The analysis of CD68 expression, the treated side showed an increase in the number of macrophages ($t \leq 0.05$), as demonstrated by the CD68 reaction, which represents an activation of apoptosis and also activation of inflammatory cells, especially macrophages that phagocytose the apoptotic tissue. Analysis of Ki67 expression showed that on the treated side there was a significant increase in the number of fibroblasts and Ki67 positive inflammatory cells ($t = 0.05$), indicating a greater activity in the dermis of the treated area compared to the control side, corroborating with the greater production of type I collagen (Figure 7).

5. Discussion

This study indicated a reduction of 3 cm in the circumference of the infra-umbilical region, a 1.0 mm increase in skinfold measurements and a reduction

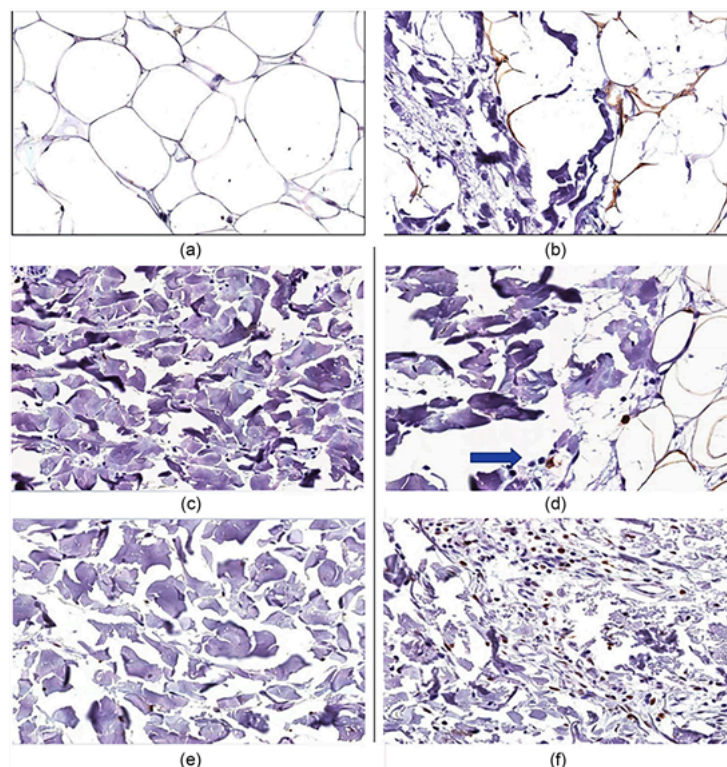


Figure 7. Microscopic analysis of the material collected during abdominoplasty. Expression of Caspase-3 in adipose tissue in (a) untreated region and (b) treated region. CD68-positive macrophages (c, d) in adipose tissue. (e, f) Ki67 positive cells.

of 0.23 cm in the ultrasound images for the treated side, however the weight and BMI did not change in an expressive manner, thus maintaining constant body weight suggests that the reduction of fat layer thickness was due to local treatment [12]. The results reinforce the study by Silva *et al.* [8], in which 30 patients with localized flank fat were submitted to 8 sessions of cryofrequency, showing a reduction in the circumference of the treated area, with a mean decrease of 2.44 mm, and also in the analysis of the ultrasound images that demonstrated a reduction of the fat layer of 0.19 cm at the end of the protocol.

Depending on the intensity of exposure to cold, the treatment induces the degeneration of adipose tissue by two mechanisms: activation of apoptosis and also activation of inflammatory cells, especially macrophages that phagocytose the apoptotic tissue, justifying the decrease of the fat layer found in the results through ultrasonographic evaluation [13]. The study by Meyer *et al.* [14] investigated the effects of cryolipolysis on the elimination of adipocytes through ultrasound imaging, macroscopic and histological analysis. Areas with cell and macrophage infiltrates were found, suggesting an inflammatory condition, with a significant destruction of the adipocytes, with consequent reduction of fat layer.

In the case of cryofrequency, using the cooling associated with the heat of radiofrequency, doubts already exist regarding the real physiological effect in the adipose tissue. The study of Silva [9], used 6 sessions applied to 15 patients and the results found in this study showed reduction of the fat layer through circumference measurements and ultrasound images, noting that cryofrequency (cryotherapy with radiofrequency) was effective in reduction of measures.

Radiofrequency technology has the ability to selectively heat subcutaneous adipose tissue, inducing apoptosis in these cells, and limiting the amount of energy in other regions, minimizing the risk of overheating [15]. The effectiveness of this technology was demonstrated in a study with 35 volunteers who performed 4 radiofrequency sessions. During the treatment, the temperatures reached 46 °C for fat layer and 42 °C on the skin, when analyzing the results an average decrease of 4.93 cm of waist circumference was observed, with mild to moderate erythema reported as a side effect [16]. On the other hand, the use of cryofrequency does not reach high temperatures due to the cooling of the tip, which brings greater comfort during its application.

Through the immunohistochemical analysis, we seek to better understand the process of reduction of adipose tissue by means of the combination found in the cryofrequency. The caspase marker (cysteine-dependent aspartates specific proteases) has the ability to recognize and cleave substrates that have aspartate residues, which is fundamental in the cellular apoptosis process [17]. In this study caspase-3 was found in the tissue treated, signaling and confirming apoptosis, the presence of macrophages that phagocytose degenerating cells, explaining the reduction of measurements by the action of cryofrequency lipolysis.

There have not yet been published studies demonstrating apoptosis and the process of adipose cell degeneration when using cryofrequency, only studies using radiofrequency or cryolipolysis. One study evaluated the radiofrequency results in two women using biopsies. The histological analysis showed that the apoptotic index (pre and 1-hour post) showed an average increase of 487% [18]. The study by Boey and Wasilenchuk [19] in

analyzing the effects of cryolipolysis found that in the histological analysis there was an increase in the inflammatory response, with a peak in 30 days with dense infiltrate of inflammatory cells and reduction in adipocyte size, suggesting that induced apoptosis by cold caused gradual and progressive destruction of fat cells in the treatment areas.

The presence of macrophages has been associated with a greater degeneration of adipose tissue. The CD68 protein, considered a classic marker of human macrophages, works in the elimination of cellular residues, promoting phagocytosis and mediating the recruitment and activation of macrophages in specific targets [20] [21]. The immunohistochemical analysis of this study demonstrated that in the treated side tissue there was an increase in the number of macrophages, as demonstrated by the CD68 reaction, corroborating with studies [22], which demonstrated that between 14 and 30 days after tissue exposure adipose to cold, macrophages begin to envelop and digest adipocytes that have suffered apoptosis, thus facilitating their elimination from the body. Clinically, this corresponds to a decrease in the thickness of the subcutaneous fat layer. Although cryofrequency is not a treatment that exclusively uses the cold, the combined cooling to the radiofrequency heat is present.

Another marker analyzed was a significant increase in the number of fibroblasts and Ki67 positive inflammatory cells, indicating a higher activity in the dermis of the treated region compared to the control side. In treated skins, Ki67 expression in fibroblast nuclei, connective tissue cells and inflammatory cells may indicate increased proliferation of these cells [23].

The human dermis consists primarily of type I collagen, however, changes in morphological and biomechanical properties may lead to sagging and the advancement of age leads to a decrease in the synthesis of this type of collagen and evidence of type III collagen, reducing adhesion between cells and fibers [24]. In the microfocal histological analysis, it was possible to notice that there was a production of type I collagen after the application of cryofrequency, with greater firmness and better quality, consequently improving sagging, while the control side showed a significant amount of Type III collagen.

In this way, the radiofrequency induces the thermal damage to stimulate changes in the collagen conformation and in the production of neocollagenesis. The presence of type I collagen on the treated side indicates an improvement in skin firmness and elasticity, confirming the study by Zelikson *et al.* [25], which through histological analysis demonstrated changes in the areas of collagen fibers, with increased diameter and amount of collagen type I after 8 weeks of treatment with radiofrequency.

Although it is a case study, a limiting factor, this research has its relevance, because it analyzes different methodologies, the evolution of the clinical picture of a patient submitted to treatment with cryofrequency. It is suggested to carry out other studies with a larger number of volunteers. It is necessary to better understand the process of association of cryotherapy and radiofrequency simultaneously for the treatment of adipose tissue, because, in isolation, these physical agents have proven their effectiveness, but together, there is still a shortage of information in the literature.

In conclusion, the technology of cryofrequency can treat both flaccidity and localized adiposity, a fact evidenced by the histological and immunohistochemical findings from this research. The amount of adipose tissue on the treated side was much lower and statistically significant reduction occurred when counting sample cells. A greater amount of collagen on the treated side was perceived, in addition to a higher quality of this collagen.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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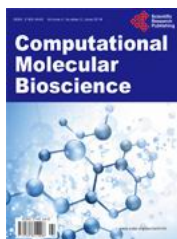
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