CryoLetters 46(1), 41 – 46 (2025)

https://doi.org/10.54680/fr25110110212

EFFECTS OF CRYOLIPOLYSIS ON SUBCUTANEOUS ADIPOSE TISSUE OF ADULT WOMEN: IMMUNOHISTOCHEMICAL ANALYSIS

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Abstract

BACKGROUND: The skin, the largest organ in the human body, is composed of complex layers that include subcutaneous adipose tissue. Understanding the characteristics of this skin structure is essential to optimize therapeutic interventions, such as cryolipolysis, aiming for more effective and personalized results. **OBJECTIVE:** To evaluate the immunohistochemical effects of skin tissue in adult women undergoing cryolipolysis. **MATERIALS AND METHODS:** We carried out an experimental and blind study with immunohistochemical analysis in women with localized abdominal fat, categorized based on the constitution of the skin as flaccid or firm according to the Investigator Assessment Skin Laxity Scoring System scale. Participants were randomized before undergoing the cryolipolysis procedure. Forty-five days after the procedure, they underwent abdominoplasty, with collection of biological material. We evaluated the inflammatory markers EBF-1, TNF-alpha, and CD68, as well as Caspase 3, cleaved Caspase 3, apoptotic BCL2, Ki-67 for fibroblast proliferation, and FIS1 for mitochondrial proliferation. **RESULTS:** Six women were included, divided into two groups; three women with loose and three with firm skin. We observed that after cryolipolysis, the group with flaccid skin showed higher expression of the Casp3, TNF-alpha, BCL2, and FIS1 markers compared to those with firm skin. **CONCLUSION:** Cryolipolysis may act differently according to tissue morphology, suggesting that its apoptotic response is more pronounced in the group with flaccid skin.

Keywords: abdominoplasty; adipocytes; cryotherapies; esthetics; weight loss.

INTRODUCTION

In recent years, aesthetic procedures aimed at body remodeling and the reduction in unwanted focal fat deposits have grown exponentially. Furthermore, women are increasingly looking for treatments that do not involve unwanted surgical complications and that do not result in prolonged downtime (1, 2).

Several technological advances have emerged for the treatment of localized fat reduction, including cryolipolysis, laser, ultrasound, radiofrequency, mesotherapy, and enzyme injections. Among these technologies, cryolipolysis stands out as a non-invasive method that acts directly on subcutaneous adipose tissue. This is performed through the use of an applicator on the target area, set at freezing

temperatures between -5°C and -15°C, for a predefined period. Through controlled cooling, damage to the epidermis and underlying dermis is avoided, promoting a significant reduction in adipose tissue, through induced inflammatory processes and apoptosis signaled by markers such as Casp3, TNF-alpha, BCL2, and FIS1, resulting in an improvement in body contouring (3, 4, 5, 6).

Studies and clinical applications related to cryolipolysis were initially based on observations of cooling-induced panniculitis. In these observations, it was noted that tissues with high lipid content were more susceptible to cold injury than surrounding water-rich tissues (5). From these observations, the first studies on fat reduction through freezing emerged (7). The results of these studies indicate that the application of cryolipolysis in one or more areas of the body results in increased lipid peroxidation (8) and, consequently, localized fat loss (9).

Skin sagging represents a significant aesthetic concern, especially among women. Its manifestation can be aggravated by factors such as smoking, restrictive diets, and weight fluctuations (10). Female vulnerability to dermal sagging is due, in part, to the thinner nature of the dermis, the pregnancy process, which stretches the skin, and the decrease in serum estrogen levels during the climacteric, resulting in less elastic, thinner skin, which is dry and flaccid (10). Accordingly, the Investigator Assessment Skin Laxity Scoring System (IASLSS) scale can be used to classify different skin types, with firm and toned skin having a smooth texture, while sagging skin is characterized by moderate laxity, slight roughness, and superficial creases. This tool helps healthcare professionals to stratify patients for aesthetic interventions, optimizing the application of the technique and positively impacting patients' self-image (11).

Cryolipolysis is widely recognized as a safe and effective procedure, with high levels of satisfaction among patients (9). Despite its increasing adoption in clinical practice and documentation in the literature, significant challenges remain, related to the comprehensive understanding of its mechanism of action and determination of the most appropriate protocol, considering individual skin characteristics. Therefore, the present study aimed to evaluate the immunohistochemical effects of the skin tissue of adult women undergoing cryolipolysis.

MATERIALS AND METHODS

Study design

This experimental and blind study was conducted in a physiotherapy clinic located in Natal, Rio Grande do Norte, Brazil. The sample was obtained through a consecutive approach. The research received approval from the Research Ethics Committee of the Universidade Potiguar, Natal, Rio Grande do Norte, Brazil, under protocol nº 5435.257.

Settings and participants

The study was conducted from October 2021 to June 2022 in a dermatofunctional physiotherapy clinic that serves patients from the private and public sectors. Patients previously recommended for abdominoplasty surgery, were consecutively recruited to participate in a single cryolipolysis session in the right infraumbilical region.

Eligibility criteria

Female participants were included, aged between 20 and 50 years, who had accumulation of fat located in the supra and infra-umbilical region. The selected participants demonstrated the ability to understand the procedure and preserved local sensitivity. Participants were considered ineligible if they presented any of the following conditions: the presence of metabolic disease, inflammatory and/or infectious skin lesions in the area of the body undergoing cryolipolysis, a history of a previous surgical procedure in the same body region subject to the cooling applications, concomitant use of steroidal or non-steroidal anti-inflammatory drugs, the presence of an active inflammatory disease, and a smoking habit.

Allocation

Women previously indicated for abdominoplasty surgery, were recruited sequentially to undergo a single cryolipolysis session.

Blinding

The immunohistochemical analysis of the skin flaps was carried out by an independent evaluator, with no prior knowledge about the submission of skin tissue to cryolipolysis. Codes were assigned to the flaps, and all participant identification information was duly suppressed.

Procedures

Patients eligible for abdominoplasty were previously evaluated at the physical therapy clinic. During this assessment, demographic data, such as sex, age, and race were recorded. In addition, anthropometric measurements were collected, including weight (using the Accumed Glicomed® brand scale, Curitiba, Brazil), height (measured with a Fiber® brand measuring tape, Rio Grande do Sul, Brazil), and the body mass index (BMI). The skin assessment was based on IASLSS. This scoring system for evaluation of skin sagging is classified as follows: [0] toned, firm skin with smooth skin surface texture; [1] slightly smooth, slightly toned skin with smooth skin surface texture; [2] moderately loose, slightly wrinkled skin with creases on the skin surface; [3] very loose without tone, very wrinkled skin with creases, separating the skin from the subcutaneous tissue; [4] prominent redundancy of skin without tone, very wrinkled with creases on the surface of the skin (11).

In the present study, cryolipolysis was performed using the Beauty Shape Duo® device (brand HTM, São Paulo, Brazil). The six participants were divided equally into two groups; one group consisting of three women with firm skin [0], and the other with three women with moderately sagging skin [2]. The application of cryolipolysis was carried out in all participants through a single session in an area measuring 10 x 20 cm, located in the infraumbilical region on the right. During the procedure, the participants were positioned in the supine position, and a 20 cm wide vacuum applicator was used, applying a suction pressure of -30 kPa and a temperature of -11º C to the right infraumbilical region, maintaining these conditions for 30 min, in a controlled manner, without causing tissue freezing. Subsequently, a 5-min period of reperfusion was implemented, accompanied by manual massage with circular movements. Another 30 min of cryolipolysis with suction was then applied (12).

The surgical procedure was scheduled for all participants by the plastic surgeon who was part of the team at the Angiovascular Hospital located in Natal, Rio Grande do Norte. Forty-five days after the cryolipolysis session, the participants underwent classic abdominoplasty, with the procedure conducted under epidural anesthesia with sedation. Infiltration of 1:3000.00 adrenaline solution was performed using the super-moist technique in the operative region. Dermolipectomy began with a suprapubic incision and displacement in the supraaponeurotic plane up to the xiphoid process. During the procedure, the rectus abdominis muscles were plicated, excess skin was removed from the lower abdomen, and the synthesis was performed using nylon and monocryl threads. During the abdominoplasty surgery, skin flaps from the right infraumbilical region were collected and sent for immunohistochemical analysis.

Immunohistochemistry

The markers analyzed included Early B-cell Factor 1 (EBF-1), Caspase-3, Cleaved Caspase-3, Tumor Necrosis Factor-alpha (TNF-alpha), B-Cell Lymphoma 2 (BCL2), Cluster of Differentiation 68 (CD68), Ki-67 Antigen (Ki67), and Fission 1 Protein (FIS1). For the immunohistochemical analysis, tissue samples were fixed, embedded in paraffin, and cut into thin slices. They were then subjected to staining, including a non-primary negative control and a positive control from human skin. Antigen retrieval was performed with heat using Leica Bond Epitope Retrieval Buffer 1 (citrate buffer, pH 6.0) for 20 min. Subsequently, the nonspecific binding of the antibody was blocked with Novolink Protein Block, applied for 10 min. Primary antibodies were applied according to the manufacturer's instructions. Colorimetric detection was conducted using diaminobenzidine substrate (DAB, SK-4100, Vector Laboratories, Burlingame, CA, USA), followed by counterstaining with hematoxylin. A pathologist evaluated the slides under a microscope.

Statistical analysis

Continuous variables are described using mean and standard deviation. Categorical variables are presented as absolute values and percentages. Comparisons between groups were performed using an unpaired t-test. A significance level of $P < 0.05$ was adopted in the statistical tests, with lower results being considered statistically significant. Data analysis was performed using SPSS 17.0 for Windows software (San Diego, CA, USA).

RESULTS

Six women participated in the study, undergoing the skin laxity assessment, followed by the application of cryolipolysis, 45 days before abdominoplasty. The skin in the

abdominal region was evaluated for flaccidity and classified as follows: three women with firm skin (score = 0) and three with moderately flaccid skin (score = 2), according to the IASLSS scale. The demographic characteristics of the groups are presented in Table 1.

The markers analyzed in the skin tissue sample were EBF-1, Caspase-3, Cleaved Caspase-3, TNF-alpha, BCL2, CD68, Ki67, and FIS1. The results are presented in Figure 1. The results showed more pronounced expression of the markers Casp3, TNF-alpha, BCL2, and FIS1 in patients with loose skin [2], compared to those with firm skin.

DISCUSSION

Cryolipolysis is based on the controlled application of low temperatures in specific areas of the body, inducing the freezing of subcutaneous adipose tissue and the crystallization of lipids within adipocytes (3, 9, 11). This crystallization triggers a localized immune response, resulting in inflammation in the subcutaneous adipose tissue and subsequent apoptosis of the adipose cells, without affecting neighboring cells (9, 13, 14). The susceptibility of adipocytes to lower temperatures than other cells contributes to this effect (14, 15). However, changes in adipose tissue are not immediate (15, 16). The apoptotic process triggers an

Table 1. Characteristics of the participants. BMI: body mass index. Variables are reported as mean (± standard deviation); n (%): absolute and relative frequency.

	Group	
	Firm skin n = 3	Sagging skin n = 3
Participant characteristics		
Years of age	37.3 ± 10.7	37.9 ± 6.8
Female, n (%)	3 (100%)	3 (100%)
BMI (kg/m2)	25.2 ± 0.3	26.2 ± 0.2
Ethnicity, caucasian, n (%)	3 (100%)	3 (100%)

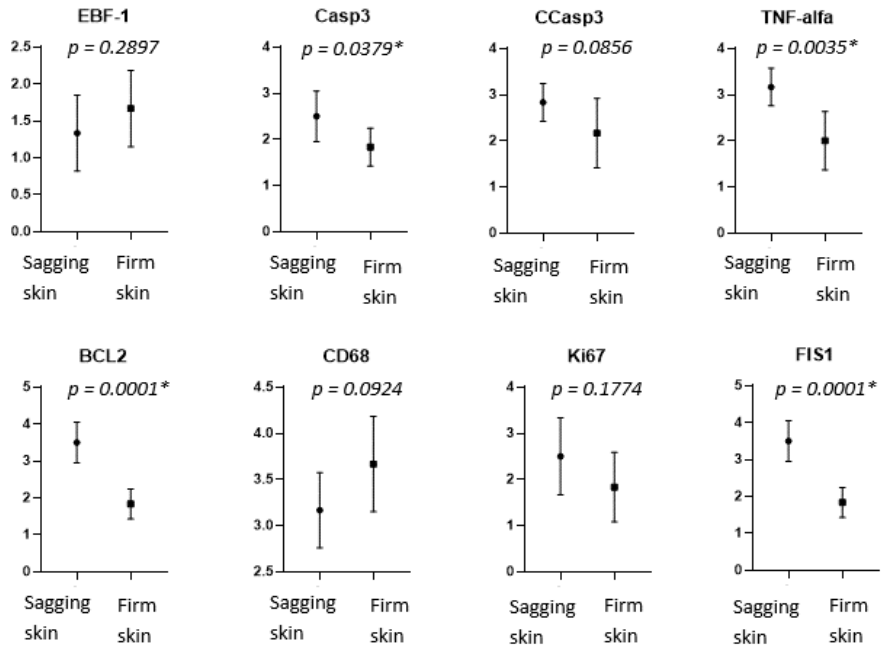

Figure 1. Immunohistochemical markers analyzed. EBF-1, Early B-cell Factor 1; Casp3, Caspase-3; CCasp3, Cleaved Caspase-3; TNF-alpha, Tumor Necrosis Factor-alpha; BCL2, B-Cell Lymphoma 2; CD68, Cluster of Differentiation 68; Ki67, Ki-67 Antigen; FIS1, Fission 1 Protein. Comparisons between groups were performed using an unpaired t-test. A significance level of $P < 0.05$ was adopted in the statistical tests.

inflammatory response that involves the gradual removal of damaged adipocytes over the months following the procedure (3, 17). In the context of the current study, the positive immunohistochemical correlation of markers such as Casp3, TNF-alpha, BCL2, and FIS1 suggests an association between cryolipolysis and the degree of skin flaccidity, contributing to the apoptotic process induced by the procedure.

Casp-3 is a protein resulting from the expression of the CASP3 gene, which employs a cysteine residue as a catalytic nucleophile in its active site to cleave the target protein into an aspartic acid residue (18, 19). Recognized as a crucial mediator of apoptosis, Casp-3 is activated in apoptotic cells via extrinsic (death ligand) and intrinsic (mitochondrial) pathways (19), playing a fundamental role in the apoptosis process of adipocytes exposed to cryolipolysis (9).

On the other hand, TNF-alpha is a pro-inflammatory mediator that plays a role in inflammation, tissue degeneration, and cell survival and proliferation, in addition to coordinating the immune response against pathogens. In the context of cryolipolysis in subcutaneous tissue, TNF-alpha can trigger the formation of complexes IIa and IIb (ripoptosome), resulting in apoptosis. Apoptotic cells remain intact and are readily phagocytosed by macrophages, a process that suppresses the production of inflammatory cytokines (20, 21), especially evident in the early stages (15 to 45 days) (22). In our study, immunohistochemical analysis took place 45 d after cryolipolysis, a period during which inflammatory markers are still detectable, as they tend to decrease in later observations (> 60 d), when more favorable aesthetic results can be observed (22).

Furthermore, BCL-2 and FIS1 family proteins act as key regulators of mitochondrial-mediated apoptosis (23), contributing to the death of adipocytes exposed to cryolipolysis. Thus, it can be inferred that these proteins are directly related to the inflammatory process and cell death triggered by cryolipolysis in subcutaneous tissue, particularly in individuals with loose skin compared to those with firm skin.

In comparative studies between participants undergoing cryolipolysis (experimental group) and those in the control group, significant results were observed in the reduction in localized fat (24, 25, 26, 27). In a prospective study conducted by Dover et al., 32 individuals underwent cryolipolysis treatment for 60 min, among which 84% showed fat reduction according to photographic evaluation (28). After 4 months, 10 participants recorded a 22% reduction in adipose tissue, with no reports of side effects. Our immunohistochemical results are consistent with those findings and the literature. The inflammatory process initially predominates after cryolipolysis, with the reduction in adipose tissue occurring after approximately 60 d (28).

Likewise, Coleman et al., reported reductions of 20.4% and 25.2% in adipose tissue in 10 patients at 2 and 6 months post-treatment, respectively (29), which may be associated with the hypothesis of an initial inflammatory process and subsequent aesthetic results.

A limitation identified in the literature concerns the ability of cryolipolysis to produce a tension effect on the skin, which would be highly desirable in the treatment of areas with sagging skin. According to researchers on the subject, approximately a quarter of cases treated with cryolipolysis that present sagging skin showed measurable skin tightening (22, 30). It is believed that the mechanism by which cryolipolysis can induce skin retraction is through the stimulation of collagen production. The collagen contraction process occurs progressively, with maximum contraction being observed 24 weeks after the procedure. This phenomenon arises from the contraction of different types of collagen in the subcutaneous space, including in the dermis, fascia, septal connective tissue, and reticular fibers. These changes all contribute to general changes in the skin, resulting in the quantified improvements in sagging, skin texture, and cellulite (31).

In summary, cryolipolysis represents a non-invasive and promising approach for reducing localized fat, meeting the growing demand for safe and effective aesthetic procedures, especially among women. The effectiveness of this method is widely supported in the literature, demonstrating significant reductions in fat, with satisfactory aesthetic results. Our immunohistochemical findings corroborate the mechanisms proposed for cryolipolysis, highlighting the activation of apoptotic markers, such as Casp3, TNF-alpha, BCL2, and FIS1, related to the cell death process in subcutaneous adipose tissue. However, a greater expression of these markers was observed in patients with loose skin compared to those with firm skin. Furthermore, the results highlight the prevalence

of the inflammatory process in the initial post-cryolipolysis phases. The originality of the study and its findings, in line with the structure of the skin, indicate the existence of numerous opportunities for advances in this area. However, many challenges remain, including limited understanding of how factors such as metabolism, body composition, and individual skin characteristics influence outcomes. Deeper understanding of these aspects could optimize aesthetic results and expand the role of cryolipolysis in the therapeutic repertoire for body remodeling.

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