CryoLetters 46(5), 321 – 330 (2025)

https://doi.org/10.54680/fr25510110712

EFFECTS OF CRYOLIPOLYSIS BY VACUUM SYSTEM AND BY COOLING PLATES: A COMPARATIVE STUDY

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Abstract

BACKGROUND: Cryolipolysis reduces the thickness of adipose tissue, but are different ways of applying this technology. **OBJECTIVES:** To compare the histological and immunohistochemical differences between the application of suction cryolipolysis versus plate cryolipolysis in adipose tissue**. METHODS:** The sample included two participants with localized fat. One participant received a session of cryolipolysis by vacuum system (-11°C, -30 KPA, two cycles of 30 minutes, and three minutes of manual reperfusion); the other participant received cryolipolysis by cooling plates (-5°C, two cycles of 30 minutes, and three minutes of manual reperfusion). After 60 days, participants underwent abdominoplasty surgeries, and tissue fragments were removed and submitted to biopsy**. RESULTS:** The treated sides showed a significant reduction of localized fat and activation of the adipocyte apoptosis, evidenced by the presence of CASP3. Moreover, this reduction was associated with an increase in COX-2. The total quantity of collagen was greater on the treated sides, without significant differences between application methods. However, type I collagen was predominant in the vacuum system. The presence of FGF2 indicated activation of pathways of fibroblast proliferation, with a neocollagenesis rate approximately 50% higher in the vacuum system. Analysis showed a greater DRP1 expression in the vacuum system, indicating an increase in metabolic rate due to higher inflammation. The differentiation of adipocytes was significantly present in the side treated with the vacuum system, with a PPAR-γ expression 100% higher. **CONCLUSION:** The vacuum system seems to be superior to cooling plates in the reduction of localized fat, neocollagenesis, and fibroblast proliferation.

Keywords: adipose tissue; cryolipolysis; esthetics; immunohistochemistry.

INTRODUCTION

Cryolipolysis is a technique that reduces localized body fat due to the sensitivity of adipocytes to cold when compared to other cells. Cold exposure for 30 to 60 minutes leads to the programmed death (apoptosis) of adipocytes without damage to the integrity of the skin or adjacent tissues. A subsequent inflammatory response also occurs, further damaging the adipocytes not initially affected. At this stage, macrophages are attracted to the region and engulf adipocytes to complete the process of fat metabolization (1, 2, 3).

In clinical practice, two methods are often used for cryolipolysis. The vacuum system uses negative pressure to vacuum the adipose tissue into a cooling chamber, leading to consistent results in the adipocyte apoptosis. However, complications related to the vacuum system have required investigation of new application possibilities, such as the cooling plates (4).

Cryolipolysis by cooling plates uses a cold-conducting plate of copper or aluminum that adjusts to the skin and cools the subcutaneous region with precision. This application method cools the tissue with a slight pressure, minimizing complications caused by the vacuum system, such as bruises and burns. Besides having a simple structure and low manufacturing costs, the cooling plates also allow the treatment of areas previously inaccessible to the vacuum system (5).

Different clinical studies have highlighted the efficacy of cryolipolysis by cooling plates. A significant fat reduction was has been demonstrated through ultrasound analyses and photographic records, with satisfaction reported by most participants (6, 7, 8). According to Meyer et al. (9), cryolipolysis by cooling plates reduced localized fat after two 60-minute sessions at a temperature of -2 °C. The study observed reductions in skinfold thickness and ultrasound measurements of the abdomen and flanks.

Current scientific evidence demonstrates the reduction of localized fat both clinically and histologically after cryolipolysis with both vacuum system and cooling plates. However, differences between methods must be established. Therefore, this study aimed to analyze the differences between cryolipolysis by vacuum system and by cooling plates at the histological and immunohistochemical levels.

MATERIALS AND METHODS

Participants

This case study included two female participants whose main complaint was localized subcutaneous fat in the upper and lower abdomen regions. The study was previously approved by the research ethics committee (no. 6.139.176). Participants signed an informed consent form.

Inclusion criteria comprised the female sex, age between 30 and 40 years of age, weight between 50 and 85 kg, localized abdominal fat, schedule for abdominoplasty, and sedentarism. Participants under other treatments for localized fat, with sensitivity alteration, pregnancy or postpartum, diabetes or metabolic disorders, scars or wounds in the treated region, previous cold allergies, peripheral vascular diseases, or Raynaud's syndrome were excluded. Participants received treatment in 2023.

Procedures

Personal data and medical history were collected following the Protocol of Physical Therapy Evaluation of Located Adiposity (10). Cryolipolysis by the vacuum system and by cooling plates were applied using specific equipment produced by Medical San® and HTM® (São Paulo, Brazil]), respectively.

Sixty days after cryolipolysis, an abdominoplasty surgery was performed and fragments of abdominal tissue were sent to biopsy for histological and immunohistochemical analysis. Slides with the tissue fragments embedded in paraffin were analyzed qualitatively under an optical microscope. We used hematoxylin-eosin staining and analyzed the slides at 40x, 100x, and 400x magnifications. This analysis aimed to examine the presence of adipocyte apoptosis, inflammation, collagen types, fibroblasts, metabolic activity, and the differentiation of white adipose tissue into brown. Analysis observed the markers caspase-3 (CASP3), cyclooxygenase-2 (COX-2), fibroblast growth factor-2 (FGF2), fibroblast growth factor receptor-1 (FGFR1), dynamin-related protein-1 (DRP1), mitofusin-2 (MFN2), and peroxisome proliferator-activated receptor-gamma (PPAR-γ).

Treatment

Participants received cryolipolysis in a single session. Table 1 shows the protocols

followed for application with vacuum system, and Table 2 using the cooling plates. The treatment was administered in supine position and with a protective blanket. Elastic bands were used to maintain skin contact with the plates. Total application time was approximately 66 minutes. The lower right abdomen received cryolipolysis, while the left side served as control. Participants underwent abdominoplasty surgery 60 days after treatment.

Statistical analysis

Slides were prepared with hematoxylin-eosin staining and analyzed at 40x, 100x, and 400x magnifications using the GraphPad Prism (Version 8.0, GraphPad Software, San Diego, California, USA). We used the Mann-Whitney U test to evaluate the differences between treatment types. We adopted $p < 0.05$ for all analyses. The pathologist provided reports containing descriptive analysis of histological images and cell quantification, which were used as qualitative data.

RESULTS

Table 1. Parameters used in cryolipolysis by vacuum system.

Parameter	Treatment
Temperature	-11 ºC
Vacuum pressure	- 30 kPA
Application	30 minutes
Reperfusion	Manual massage
Reapplication	30 min
Applied area	Lower right abdomen (10 x 20 cm)

ºC: degrees Celsius; cm: centimeter; kPA: kilopascal.

Table 2. Parameters used in cryolipolysis by cooling plates.

Parameter	Treatment
Temperature	-5 ºC
Application	30 min
Reperfusion	Manual massage for 3 min
Reapplication	30 min and 3 min of reperfusion
Applied area	Lower right abdomen (10 x 20 cm)

ºC: degrees Celsius.

The presence of CASP3 marker in the fibroadipose tissue was higher in the cryolipolysis groups (Figures 1a and 1b), indicating a greater activation of apoptosis when compared to the control (Figure 1c). The vacuum system induced an apoptosis rate approximately 50% higher than the cooling plates, indicating more efficiency. Furthermore, it also showed a high number of fibroadipose tissue cells compared to the cooling plates ($p = 0.01$).

The increased presence of COX-2 indicated a great inflammatory process in cryolipolisys groups (Figures 2a, 2b, 3a, and 3b) compared to the control (Figures 2c and 3c). The vacuum system presented a COX-2-mediated inflammatory process up to two-fold higher than the cooling plates in fibrous tissue ($p < 0.0001$) and adipose tissue ($p = 0.001$).

In the collagen analysis, both cryolipolysis methods (Figures 4a and 4b) led to greater collagen quantity than the control (Figure 4c). Despite no significant differences in the quantity of collagen between methods, the vacuum system presented a denser and more organized tissue, described as dense connective tissue. Type I collagen was evident after cryolipolysis, despite the method (Figures 5a and 5b). The control showed a predominance of type III collagen (Figure 5c). The presence of type I collagen more prominent after the application of the vacuum system indicates that this method is more effective for neocollagenesis.

The ability to activate pathways of fibroblast proliferation was indicated by the presence of FGF2 in fibroadipose tissue and led to the formation of collagen fibers. We observed higher FGF2 after cryolipolysis, despite the method (Figures 6a and 6b), than in the control (Figure 6c). The vacuum system showed greater expression of FGF2, indicating a neocollagenesis rate approximately 50% higher ($p < 0.0001$) than when cooling plates were used.

The analysis of FGFR1 expression in fibroadipose tissue evidenced a significant difference between cryolipolysis (Figures 7a and 7b) and the control (Figure 7c), but no difference was observed between the methods of application.

The activation of mitochondrial metabolism pathways in fibroadipose tissue was evaluated by mitochondrial fission factor DRP1. Despite the method, cryolipolysis showed significant differences indicating increased metabolic rates

of tissue due to greater inflammation, cell recruitment, and neocollagenesis rate (Figures 8a and 8b) when compared to the control (Figure 8c). The vacuum method showed a significant increase in DRP1 expression compared to the plate method ($p < 0.0001$), with double the metabolic activity.

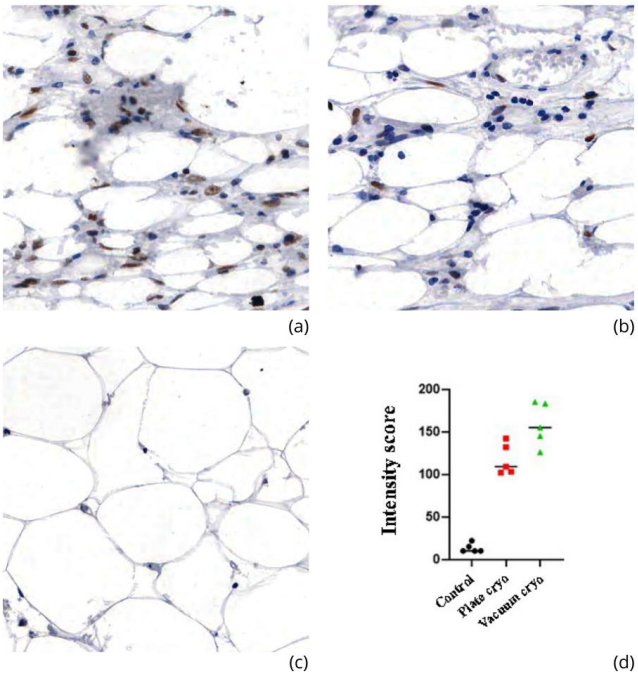

Figure 1. Analysis of the caspase-3 marker in the fibroadipose tissue. (a) Increase when using the plate cryolipolysis method. (b) After the vacuum cryolipolysis method the increase was more expressive. (c) No significant findings in the control. (d) Statistical analysis of the observed groups.

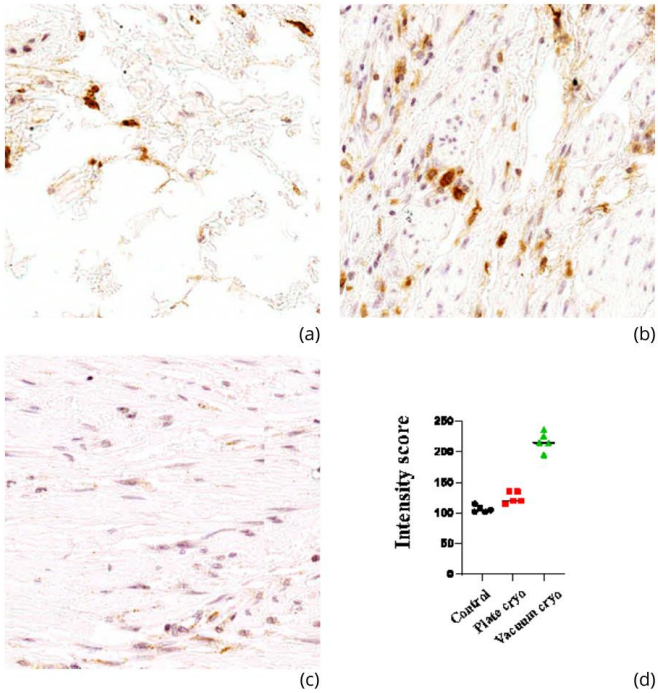

Figure 2. Analysis of the cyclooxygenase-2 marker (COX-2) in the fibrous tissue. Compared to little change in plate cryolipolysis (a), there was more intense presence of the marker in vacuum cryolipolysis (b). (c) No significant findings in the control. (d) Statistical analysis of the observed groups.

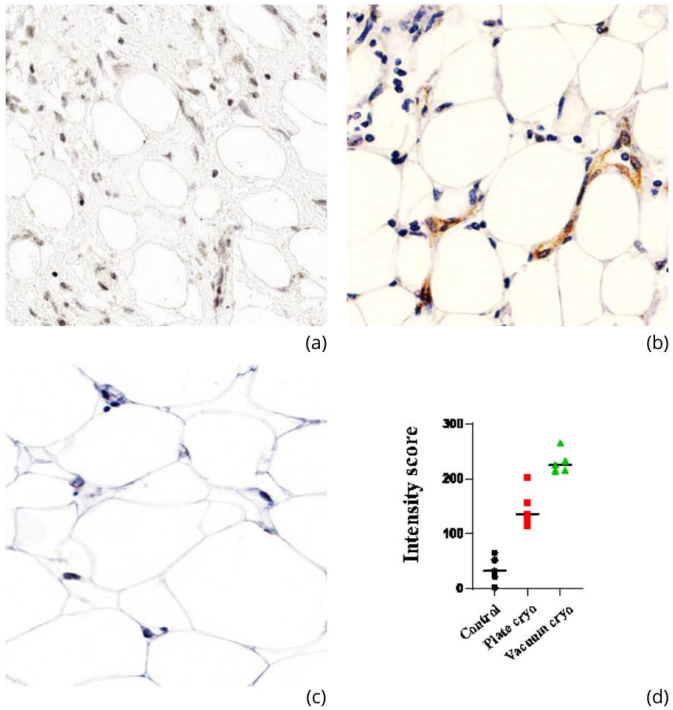

Figure 3. Analysis of the cyclooxygenase-2 marker (COX-2) in the adipose tissue. (a) Increase when using the plate cryolipolysis method. (b) After the vacuum cryolipolysis method the increase in expression was more. (c) No significant findings in the control. (d) Statistical analysis of the observed groups.

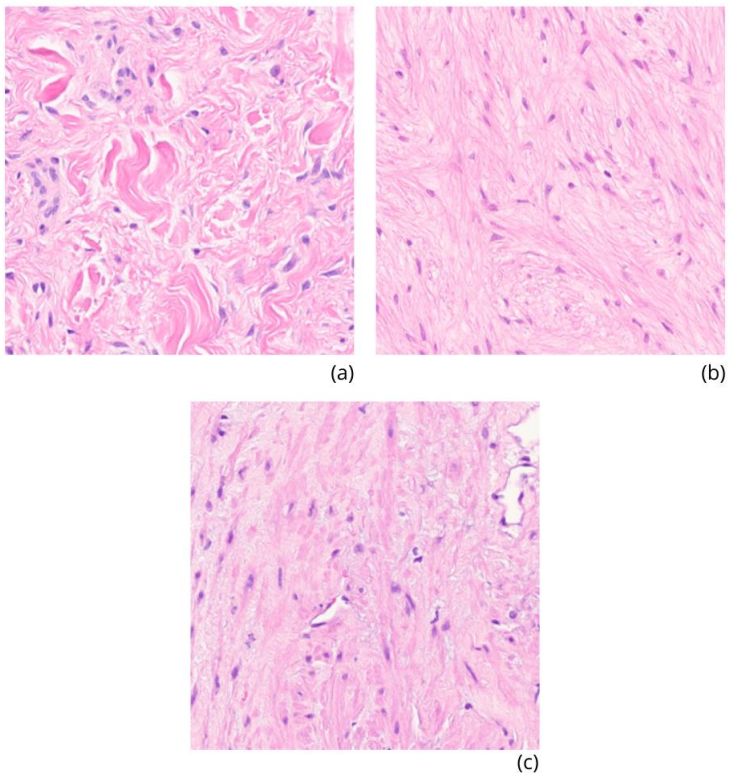

Figure 4. Histological analysis of total collagen quantity and number of fibroblasts. (a) Plate cryolipolysis led to greater collagen quantity, density and more organised tissue, although to a lesser extent than after vacuum cryolipolysis (b). (c) No significant changes in the control.

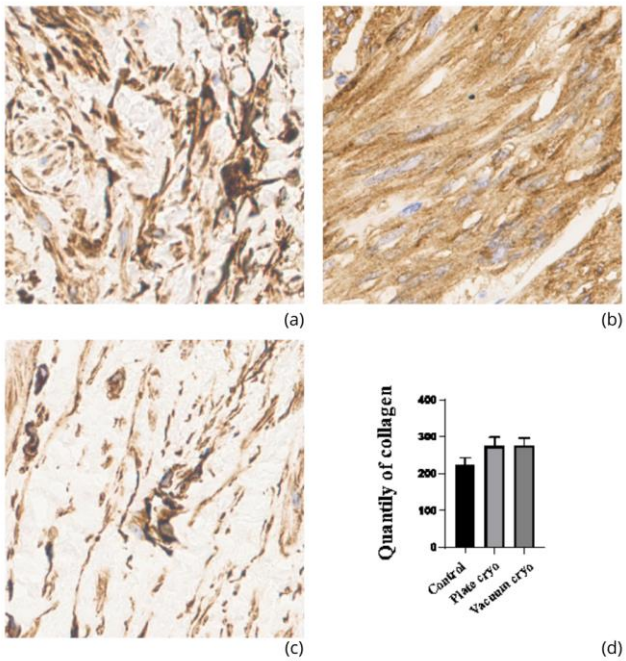

Figure 5. Analysis of type I and III collagen. Type I collagen was more prominent after both plate cryolipolysis (a) and vacuum cryolipolysis (b). (c) No significant findings in the control, with a predominance of type III collagen. (d) Statistical analysis of the observed groups.

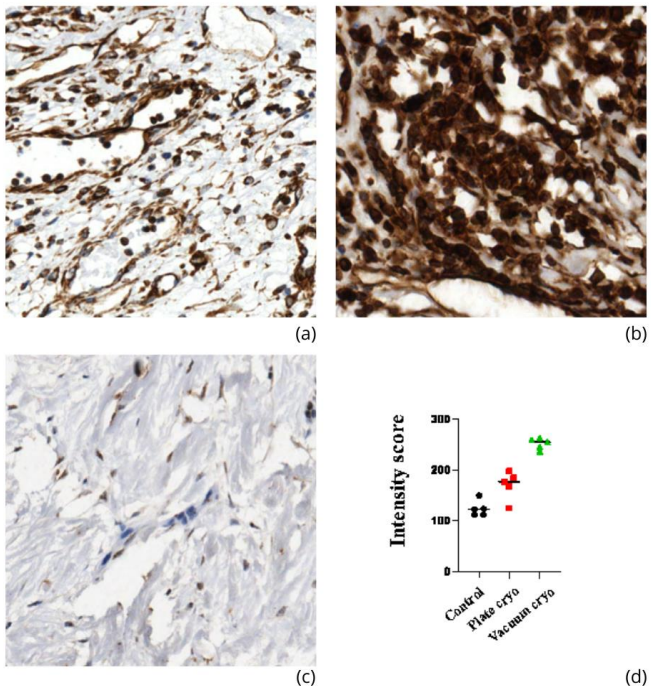

Figure 6. Analysis of fibroblast growth factor-2 in fibroadipose tissue. (a) Increase after using the plate cryolipolysis method. (b) Expression was increased even more after vacuum cryolipolysis. (c) No significant changes in the control. (d) Statistical analysis of the observed groups.

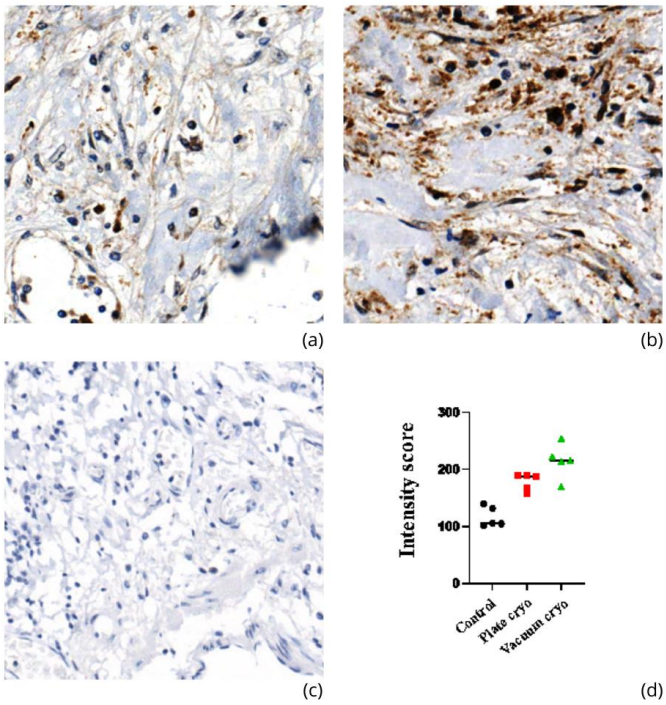

Figure 7. Analysis of fibroblast growth factor receptor-1 (FGFR1) in fibroadipose tissue. (a) Increase after using the plate cryolipolysis method. (b) The increase in expression was even higher after the vacuum cryolipolysis method. (c) No significant changes in the control. (d) Statistical analysis of the observed groups.

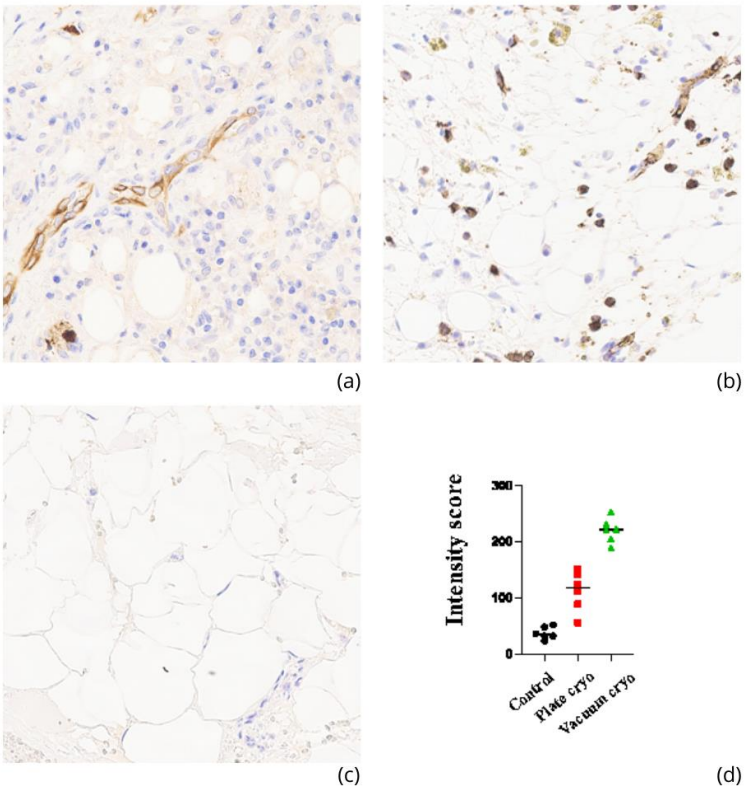

Figure 8. Analysis of the mitochondrial fission factor (DRP1) in fibroadipose tissue. (a) Increased expression after application of the plate cryolipolysis method. (b) Even more expression as a result of vacuum cryolipolysis. (c) No significant changes in the control. (d) Statistical analysis of the observed

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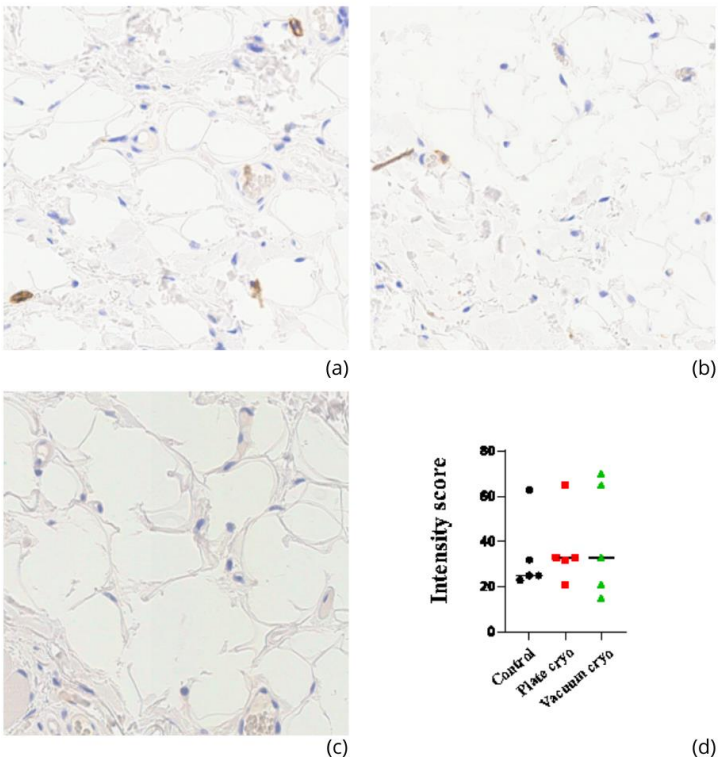

Figure 9. Analysis of the mitofusin-2 marker (MFN2) in fibroadipose tissue. No differences were observed after plate cryolipolysis (a) and vacuum cryolipolysis (b) compared to the control (c). (d) Statistical analysis of the observed groups.

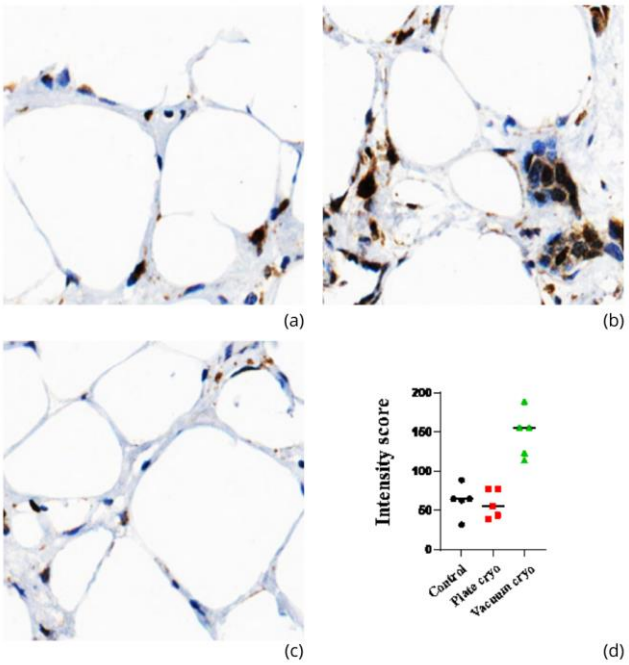

Figure 10. Analysis of the peroxisome proliferator-activated receptor-gamma (PPAR-γ) marker in fibroadipose tissue. Expression after plate cryolipolysis (a) and after vacuum cryolipolysis (b) was the same as or higher than, respectively, the control (c). Statistical analysis of the observed groups.

The analysis of the MFN2 marker in the fibroadipose tissue showed this pathway was not activated during cryolipolysis (Figure 9), and no difference was found between application methods or with the control ($p > 0.05$).

The differentiation of adipocytes is regulated by the expression of genes controlled by PPAR-γ. Cryolipolysis by cooling plates led to activation of necrosis and remodeling of adipose tissue. However, it was not significantly different in terms of PPAR- γ expression compared to the control ($p = 0.9645$). The presence of PPAR-γ expression was significant in the vacuum system, with an expression rate 100% higher than in cooling plates (Figure 10).

DISCUSSION

Choosing the most appropriate method of cryolipolysis must consider potential benefits, side effects, region of application, and individual preferences. Although previous studies (7, 8, 11) have evidenced the efficacy of cryolipolysis for localized fat, the comparison between methods of application, including vacuum system and cooling plates, is scarce. This study presents histological and immunohistochemical results on fibrous and adipose tissues comparing these two methods.

Cooling processes provoke fat reduction due to activation of cellular apoptosis, leading to tissue restructuring (12). Adipocyte apoptosis occurs regardless of the cryolipolysis application method and was confirmed in this study by the presence of the CASP3 marker. However, the vacuum system demonstrated a significantly higher rate of efficient apoptosis and affected a greater number of cells in fibroadipose tissue than the cooling plates method. Clinically, these findings indicate that cryolipolysis by vacuum system leads to a more noticeable reduction of localized fat. Further literature corroborate our findings. For example, Vignoli and Mármol (13) observed an average reduction of 24.6 mm in the adipose tissue with the vacuum system, whereas Choi et al. (14) showed an average reduction of 8.9 mm after cooling plates.

The inflammatory reaction during apoptosis after both methods of cryolipolylisis was confirmed by the presence of COX-2, which aligns with previous published findings (15, 16, 17). Similar to CASP3, the inflammatory process mediated by COX-2 was up to two-fold higher in the vacuum system in fibrous and adipose tissues than in the cooling plates. The vacuum system increases blood perfusion in the tissues, leading to reoxygenation and initiating an inflammatory process. Although the vacuum system leads to a pronounced inflammatory response, studies highlight its safety (18). However, in conditions with ongoing inflammatory processes, including lipedema, choosing a cryolipolysis method that induces a lower inflammatory response might be more effective. For those cases, the cooling plates may be more appropriate.

Higher inflammation and cell recruitment also contributed to cryolipolysis to increase the tissue metabolic activity, as evidenced by DRP1 (19, 20, 21). The vacuum system significantly increased the DRP1 expression, indicating a tissue with a metabolic activity two-fold higher than the baseline control, which is consistent with our previous findings. However, the analysis of MFN2 indicated that this marker of another mitochondrial metabolic pathway was not activated with cryolipolysis, regardless of the application method.

The observation of PPAR-γ expression reinforces the effects of cryolipolysis for the reduction of localized fat. This marker is responsible for the differentiation of white adipose tissue into brown. This marker was significantly present in the tissue after cryolipolysis with the vacuum system, showing an expression rate 100% higher than the one observed after cooling plates. However, previous studies showed no changes in PPAR-γ expression following cryolipolysis (22). Therefore, further investigations with the larger number of participants are necessary.

Besides promoting the reduction of adipocytes, cryolipolysis may also trigger the proliferation of fibroblasts and neocollagenesis, as observed by the expression of FGF2 and FGFR1 in the fibroadipose tissue. The use of the vacuum system showed a significant presence of type I collagen and 50% higher FGF2 expression than what was observed with the cooling plate treatment, indicating this method of application is more effective for neocollagenesis. These results align with the findings of Carruthers et al. (23), in which dermal remodeling occurred with the presence of molecular markers indicative of type I collagen. Therefore, neocollagenesis mediated by fibroblasts appears to help to enhance skin quality following cryolipolysis.

Cryolipolysis is considered a safe and effective procedure widely used in aesthetic

medicine. This study compared two cryolipolysis application methods and observed that the vacuum system demonstrated more consistent results regarding adipose apoptosis, inflammatory process, collagen quantification (especially type I), and lipid metabolism. When compared to the cooling plates method, the vacuum system shows superiority in the reduction of localized fat, neocollagenesis, and fibroblast proliferation. Therefore, the vacuum system may be the preferred method for cryolipolysis in cases with no restrictions. Clinical studies with a greater number of participants are necessary to confirm our results.

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