

## HISTOLOGICAL AND IMMUNOHISTOCHEMICAL EFFECTS OF DYNAMIC PLATE CRYOLIPOLYSIS ON LOCALIZED ABDOMINAL ADIPOSITY: A CASE STUDY WITH MULTIMODAL CELLULAR ANALYSIS

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### Abstract

**BACKGROUND:** Dynamic plate cryolipolysis (DPC) has emerged as a non-invasive alternative technique for treating localized adiposity, particularly in anatomically challenging areas. **OBJECTIVE:** To evaluate the effects of DPC on the cutaneous and subcutaneous tissues of the abdominal region through histological and immunohistochemical analyses. **METHODS:** Four volunteers underwent distinct protocols of DPC (10, 20 or 30 min) or static cryolipolysis (20 min). DPC protocols were performed at -5 °C, followed by 3 min of reperfusion and manual massage. The right infraumbilical region was treated, while the contralateral side served as control. Samples were collected during elective abdominoplasty and analyzed for biomarkers of apoptosis, inflammation, fibroblast activity, collagen remodeling, heat stress response, and metabolic or hormonal modulation. **RESULTS:** DPC induced adipocyte apoptosis, confirmed by detection of caspase-3, COX-2, and macrophage (CD68 and CD163) expression, fibroblast activation (FGF2 and FGFR1), and a predominance of type I collagen deposition. Additionally, modulation of metabolic and hormonal markers was observed, including reduced PPAR- $\gamma$  and aromatase expression, and partial UCP1 expression, suggesting metabolic modulation with potential thermogenic effects. **CONCLUSIONS:** DPC induced significant and controlled cellular responses, supporting its safety and therapeutic potential. Beyond aesthetic applications, these findings point to broader clinical implications. Larger-scale studies are required to validate and expand these findings.

**Keywords:** apoptosis; dynamic cryolipolysis; immunohistochemistry; localized adiposity; tissue remodeling.

## INTRODUCTION

Localized adiposity represents a multifactorial clinical challenge, encompassing aesthetic concerns and relevant metabolic implications. The distribution of adipose tissue is regulated by hormonal, genetic, and behavioral factors, which often complicate therapeutic approaches and render conventional interventions less effective (1, 2). Thus, the development of non-invasive techniques to selectively reduce adipose tissue while preserving adjacent structures has attracted growing interest in aesthetic medicine and dermatofunctional physical therapy (3, 4).

Cryolipolysis has emerged as a promising technique, as it selectively induces adipocyte apoptosis via controlled cooling while preserving the integrity of adjacent tissues (5, 6). Originally developed using vacuum-assisted application, this technique has demonstrated efficacy in reducing subcutaneous fat thickness in multiple body regions. However, its clinical use is still limited in areas with low tissue pliability, hypersensitivity, or anatomical constraints that hinder clamping (e.g., inner thighs, flanks, and back). Moreover, transient adverse effects (e.g., ecchymosis, paresthesia, and localized pain) are frequently reported (7, 8).

The growing demand for body-contouring procedures that minimize recovery periods and complications has driven the exploration of alternatives to vacuum-assisted cryolipolysis. In this context, plate cryolipolysis has emerged as an innovative modality that eliminates suction and mechanical traction, thereby improving adaptability to anatomical variations and reducing vacuum-associated adverse effects (9, 10).

Recently, the dynamic plate cryolipolysis technique (DPC) has been introduced, characterized by alternating cycles of cooling and reperfusion combined with applicator movement. This approach is designed to optimize thermal distribution and promote more homogeneous biological responses. Clinical studies have demonstrated preserved efficacy in reducing localized fat while improving patient tolerability, particularly in anatomically complex body regions (11, 12). However, despite these encouraging clinical outcomes, the cellular mechanisms underlying DPC remain unclear, particularly on its

histological and immunohistochemical alterations (13, 14).

The present study aimed to investigate the effects of DPC on cutaneous and subcutaneous tissues of the abdominal region in women undergoing abdominoplasty. Biomarkers for apoptosis, inflammation, tissue remodeling, and lipid metabolism regulation were assessed to understand the underlying pathophysiological mechanisms activated.

## MATERIAL AND METHODS

### *Study design*

This experimental case, employing a quantitative approach, was conducted to histologically and immunohistochemically evaluate the effects of DPC on the cutaneous and subcutaneous tissues of the abdominal region. The study protocol was approved by the research ethics committee (opinion no. 7101327) according with the ethical principles outlined in Resolution No. 466/2012 of the Brazilian National Health Council.

### *Sample and eligibility criteria*

Four women aged 38 to 44 years, with a body mass index ranging from 22 to 28 kg/m<sup>2</sup> participated in this study. All participants were clinically healthy, presented supra- and infraumbilical abdominal adiposity, and had a surgical indication for elective abdominoplasty.

Inclusion criteria comprised nonsmoking status, absence of metabolic disorders (e.g., diabetes mellitus, dyslipidemia), absence of scars or skin lesions in the treatment area, preserved cold sensitivity, and willingness to complete all study procedures. Exclusion criteria included contraindications to cryotherapy, such as cold allergy, Raynaud's syndrome, peripheral vascular disorders, pregnancy or the puerperal period, recent use of anticoagulants, and use of systemic anti-inflammatory medications.

### *Procedures and intervention*

All participants provided a written informed consent form and underwent an initial assessment based on the previously validated protocol of physical therapy evaluation in localized adiposity (15). Medical history and clinical examination of the abdominal region was conducted.

Subsequently, the therapeutic protocol was performed using calibrated HTM® equipment. Each participant underwent a single session of DPC in supine position. Treatment was applied exclusively to the right infraumbilical region (i.e., experimental area), while the contralateral side served as the untreated control.

Intervention parameters varied according to duration and technique. Participant 1 received DPC for 10 min, with two plate movements every 5 min; participant 2 was treated with DPC for 20 min, with three plate movements every 5 min; participant 3 was treated with DPC for 30 min, with three plate movements every 5 min; and participant 4 received static cryolipolysis for 20 min (without plate replacement). In all cases, the temperature was sustained at -5 °C, followed by 3 min of reperfusion with manual massage immediately after application.

### ***Surgery and collection of biological material***

Fifteen days after the cryolipolysis session, all participants underwent elective abdominoplasty surgery, performed according to a standardized surgical procedure. This procedure consisted of a suprapubic incision, flap dissection to the xiphoid appendix, rectus abdominis muscle plication, removal of excess skin, and layered closure. During surgery, paired samples of cutaneous and subcutaneous tissue were collected from the treated and untreated infraumbilical regions, providing an intra-individual comparison between experimental and control tissue.

### ***Histological and immunohistochemical analyses***

Tissue specimens were immediately fixed in a 10% buffered formaldehyde solution for 48 h, then processed using standard histological techniques, embedded in paraffin, and sectioned at 5 µm of thickness. Sections were stained with hematoxylin-eosin for morphological analysis, focusing on structural integrity, extracellular matrix organization, inflammatory infiltrates, necrosis, and apoptosis features (e.g., chromatin condensation and cytoplasmic retraction).

Indirect immunohistochemistry was performed using specific monoclonal antibodies targeting biomarkers of the biological processes under investigation. Apoptosis was evaluated by caspase-3 and cleaved caspase-3 expression. Inflammation was assessed through COX-2,

TNF- $\alpha$ , CD68, and CD163 expression, allowing characterization of inflammatory mediators and macrophage activity. Fibroblast activation and tissue remodeling were investigated through the FGF<sub>2</sub> expression, FGFR1 expression, and type I and type III collagen deposition. Heat stress response was examined through HSP60, HSP70, and HSP90 expression. Lastly, metabolic modulation and the potential induction of white-to-brown adipose tissue conversion were investigated by uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), and aromatase CYP19A1 expression.

Slides were examined under light microscopy at 400x magnification and assessed using a semi-quantitative scoring system of staining intensity and distribution across randomly selected fields.

### ***Statistical analysis***

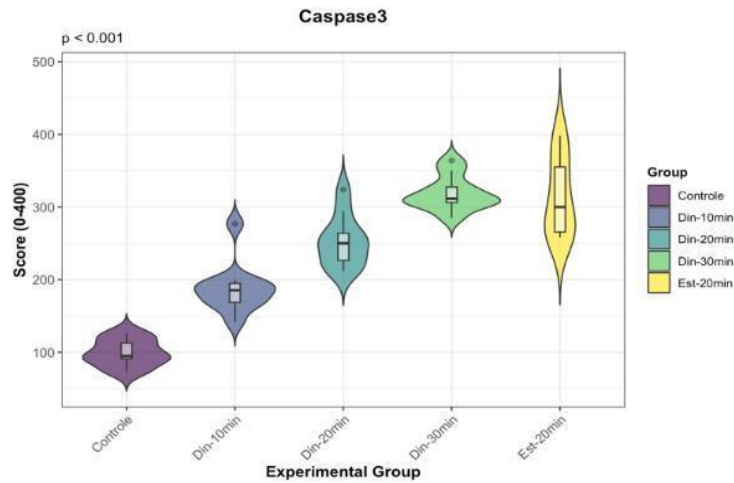
Data were analyzed using SPSS software version 17.0 for Windows. Descriptive statistics calculated included mean, standard deviation, and relative frequency. Comparisons between control and treatment groups were performed using non-parametric tests (Mann-Whitney or Wilcoxon). Statistical significance was set at  $p < 0.05$  with 95% confidence interval.

## **RESULTS**

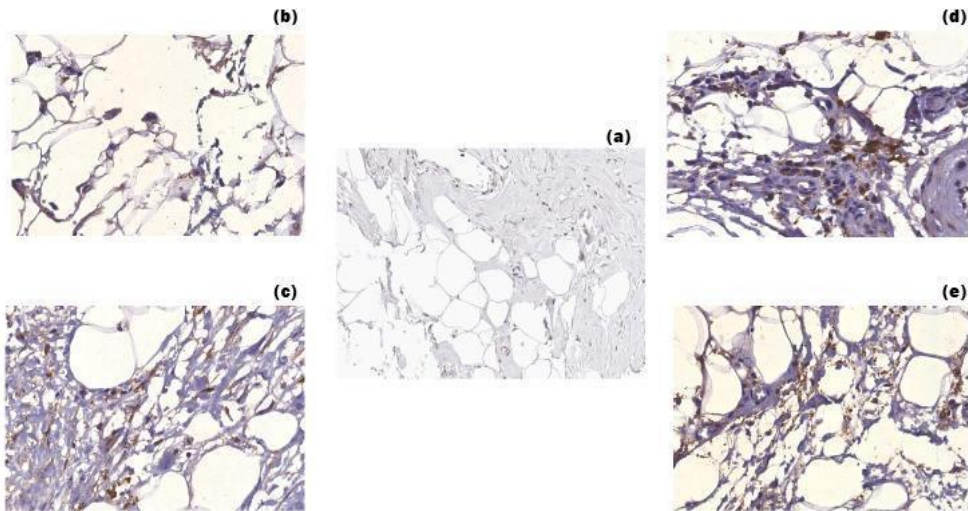
The analysis of tissues treated with DPC revealed morphological and molecular alterations related to a therapeutic response to selective cooling. Findings are presented according to functional categories, based on the histological and immunohistochemical markers.

### ***Evidence of apoptosis***

Apoptosis was confirmed by the detection of caspase-3 and cleaved caspase-3 in tissues exposed to dynamic plate cryolipolysis, evidenced by dark brown staining predominantly in adipocytes within the treated area ( $p < 0.001$ ), as shown in Figures 1 and 2. Caspase-3 expression was significantly increased in the experimental group (right infraumbilical region) compared with the control group (left region), supporting the activation of apoptotic pathways by dynamic plate cryolipolysis.



**Figure 1.** Caspase-3 immunostaining in adipose tissue treated with dynamic plate cryolipolysis, showing apoptotic adipocytes. Expression predominated in static and 30-min dynamic protocols ( $p < 0.001$ ).



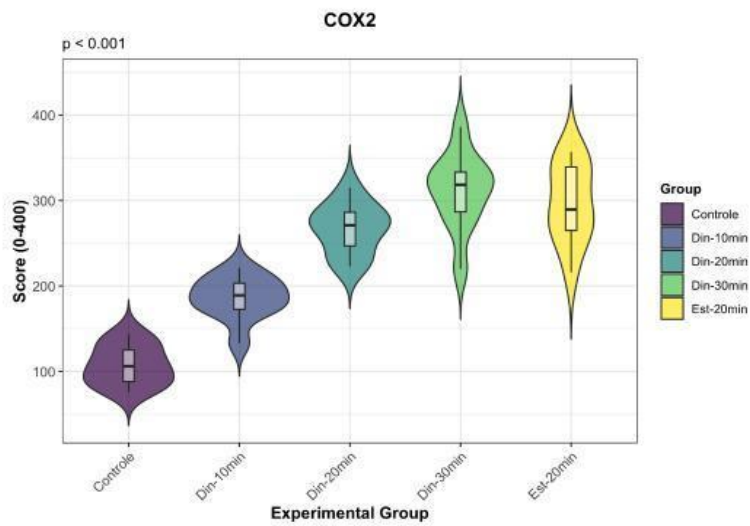
**Figure 2.** Immunostaining for cleaved caspase-3 in adipose tissue after cryolipolysis, confirming apoptosis pathway activation. (a) control, (b) dynamic cryolipolysis (10 min), (c) dynamic cryolipolysis (20 min), (d) dynamic cryolipolysis (30 min), (e) static cryolipolysis (20 min).

Nonetheless, the greatest staining intensity was observed in the static cryolipolysis-treated group.

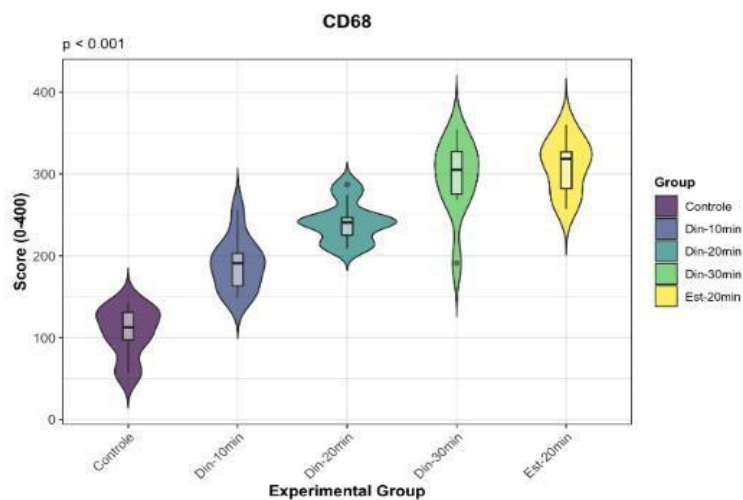
### ***Inflammatory activity***

COX-2 immunostaining (Fig. 3), a marker of inflammation induced by external stimuli, was significantly increased in the treated groups ( $p < 0.001$ ), confirming that cryolipolysis activated inflammatory responses. Static cryolipolysis and prolonged (30 min) dynamic plate protocols showed increased COX-2 expression. Similarly, CD68 and CD163, biomarkers of macrophages infiltration, were detected in cryolipolysis-treated

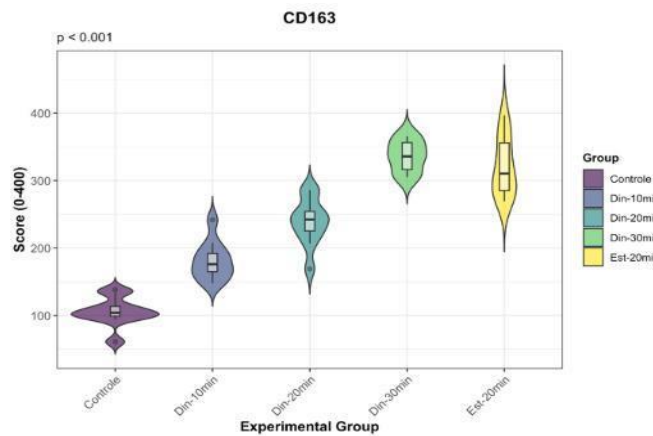
groups, suggesting an inflammatory infiltrate consistent with cell degradation and clearance ( $p < 0.001$ , Figs 4 and 5). Although DPC presented increased expression of these biomarkers compared with control group, static cryolipolysis elicited the greatest responses.



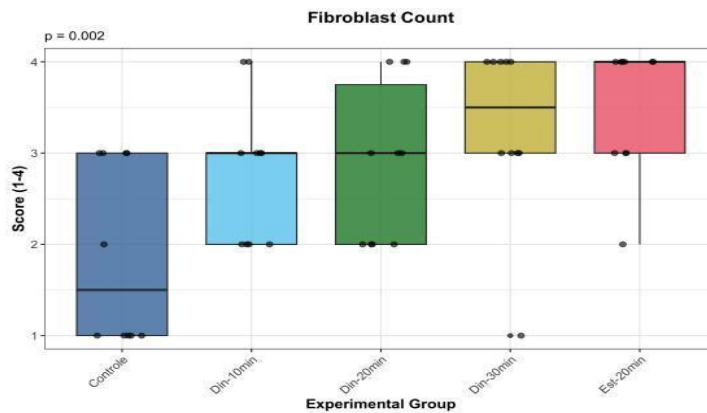
**Figure 3.** COX-2 expression in treated tissues, demonstrating a local inflammatory response to cryolipolysis, with predominance in the static and dynamic 30-min protocols ( $p < 0.001$ ).



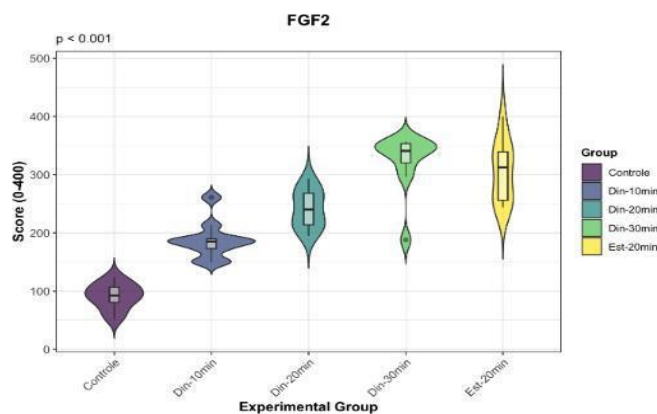
**Figure 4.** CD68 immunostaining indicating macrophage infiltration in abdominal fat tissue samples treated with cryolipolysis ( $p < 0.001$ ).



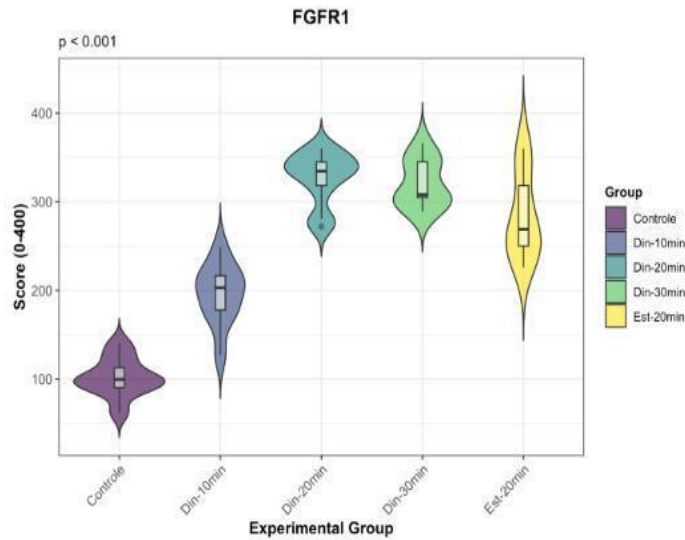
**Figure 5.** CD163 immunostaining confirming macrophages infiltration in abdominal fat tissue samples treated with cryolipolysis, which was predominant in static 20-min and dynamic 30-min protocols ( $p < 0.001$ ). .



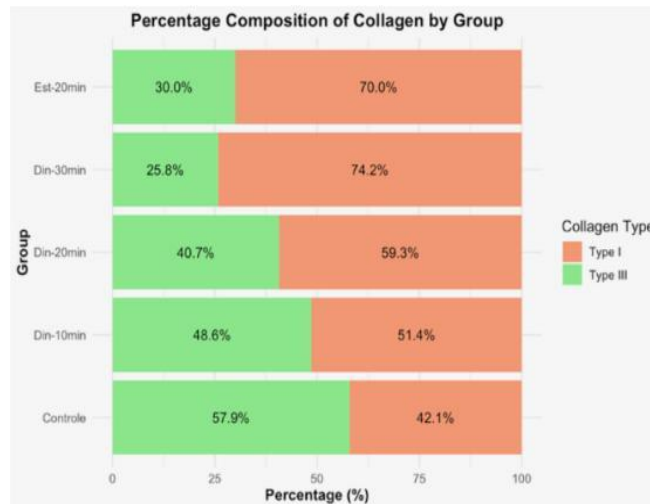
**Figure 6.** Increased fibroblast density in the treated areas, as shown by hematoxylin-eosin staining, was predominant in both the static 20-min and dynamic 30-min protocols ( $p = 0.002$ ).



**Figure 7.** Positive immunostaining for FGF2, indicating fibroblast activation after cryolipolysis, was predominant in the static 20-min and dynamic 30-min protocols ( $p < 0.001$ ).



**Figure 8.** Expression of FGFR1, associated with fibroblastic stimulation and tissue remodeling, was predominant in the static 20-min and dynamic 20- and 30-min protocols ( $p < 0.001$ ).



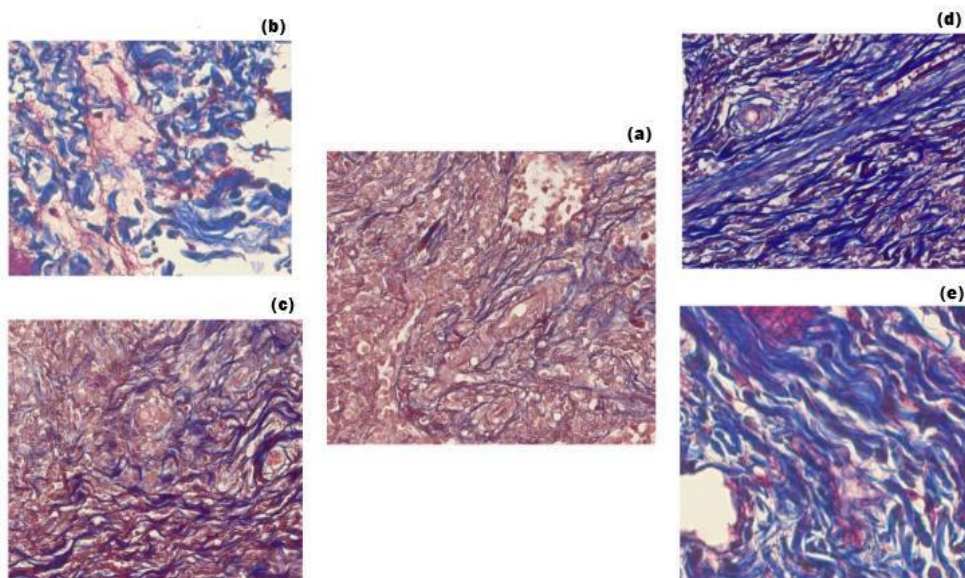
**Figure 9.** Predominance of type I collagen in treated tissues, confirming extracellular matrix reorganization across all protocols.

### ***Tissue remodeling and collagen synthesis***

Increased fibroblast density was found particularly in static 20-min and prolonged (30-min) dynamic cryolipolysis (Fig. 6), in parallel with observed increased expression of FGF2 (Fig. 7) and FGFR1 (Fig. 8). Collagen analysis revealed a predominance of type I over type III collagen (Figure 9), especially in static 20-min cryolipolysis and prolonged (30-min) dynamic protocols, suggesting extracellular matrix reorganization with

deposition of mature, denser collagen, consistent with tissue repair (Fig. 10).





**Figure 10.** Type III collagen expression at a lower intensity compared with type I, consistent with tissue remodeling. (a) control, (b) dynamic, 10 min, (c) dynamic, 20 min, (d) dynamic, 30 min, and (e) static.

### ***Heat stress response***

Stress-response pathways were also activated. Expressions of heat shock proteins HSP60, HSP70, and HSP90 were dose-dependently increased in treated groups compared with control group (panels A, B, and C of Fig. 11, respectively), supporting the induction of protective and reparative mechanisms underlying cryolipolysis.

### ***Metabolic modulation and thermogenesis***

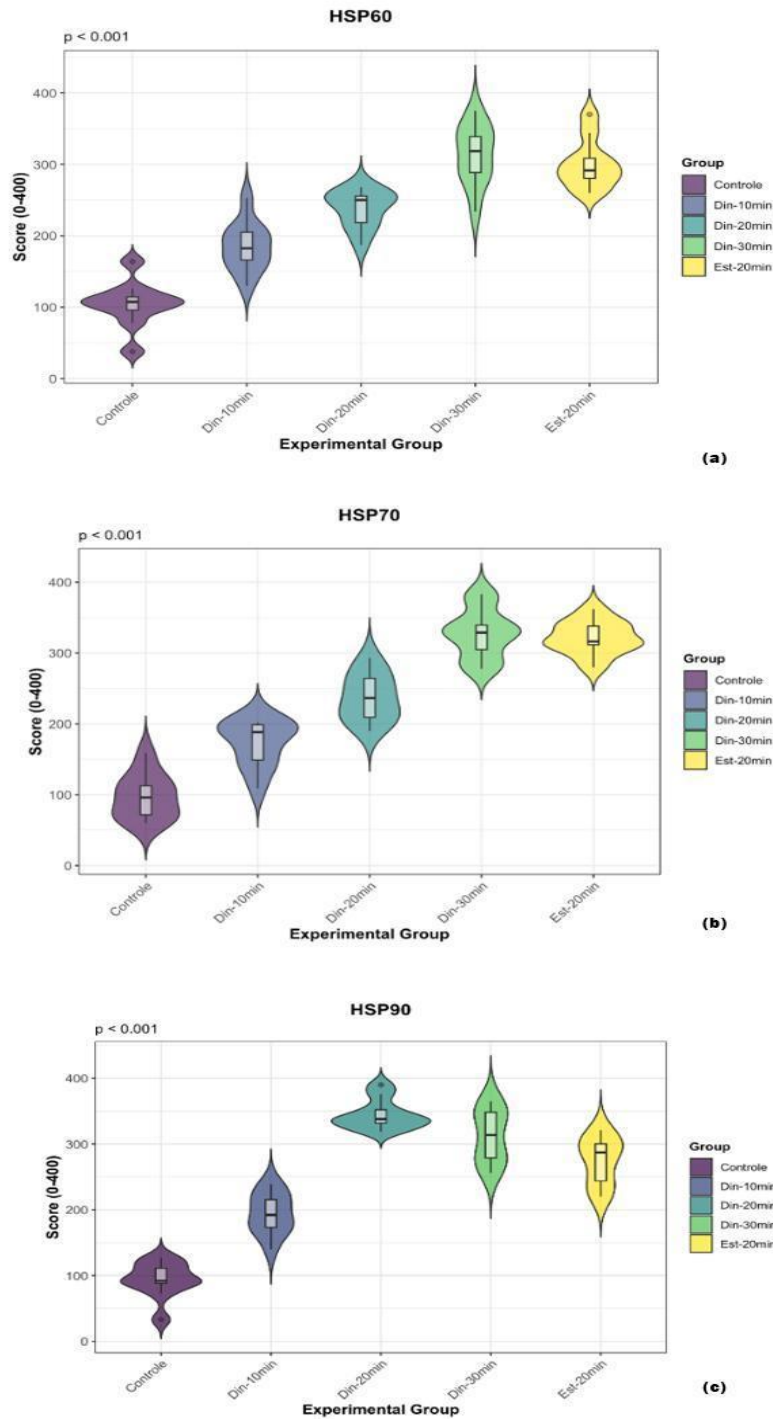
Although UCP1 expression, a marker of thermogenesis and adipose tissue browning, was not significantly increased in low-dose DPC compared with the control group, marked increases were observed in static 20-min cryolipolysis and prolonged (30-min) dynamic protocols (Figs 12 and 13). Conversely, PPAR- $\gamma$  expression, a central regulator of adipocyte differentiation and lipid homeostasis, was consistently reduced across treated samples (Figs 14 and 15), suggesting suppression of adipogenesis. A similar reduction was observed for aromatase CYP19A1 in dynamic plate cryolipolysis-treated groups (Fig. 16). This is a key enzyme in androgens- to- estrogens conversion and increasingly implicated in adipose tissue dysfunction, including lipedema.

## **DISCUSSION**

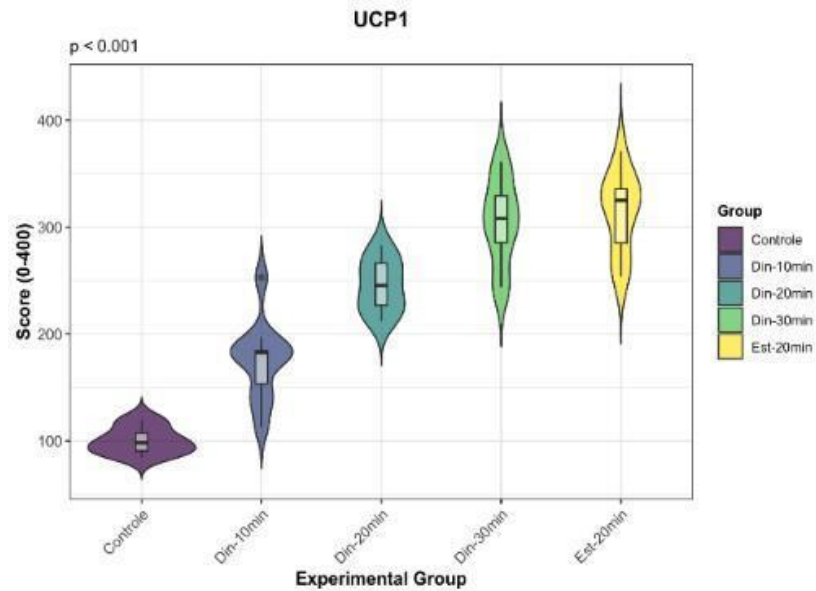
The intricate cellular mechanisms elicited by DPC underscore the sophistication of this innovative technique. Beyond simple fat tissue cooling, DPC induced a multifactorial response involving controlled inflammation, tissue remodeling, and metabolic modulation.

The activation of apoptotic pathways was confirmed by the expression of caspase-3 and cleaved caspase-3, indicating that cryolipolysis induced programmed adipocyte death without compromising the structural integrity of superficial skin layers, thereby supporting its safety (1). Furthermore, the dose-dependent effects observed in dynamic plate protocols suggest that the treatment period is a critical determinant of apoptotic outcomes, consistent with previous reports. Notably, longer sessions can produce increased apoptosis (12), reinforcing application period as a key therapeutic variable.

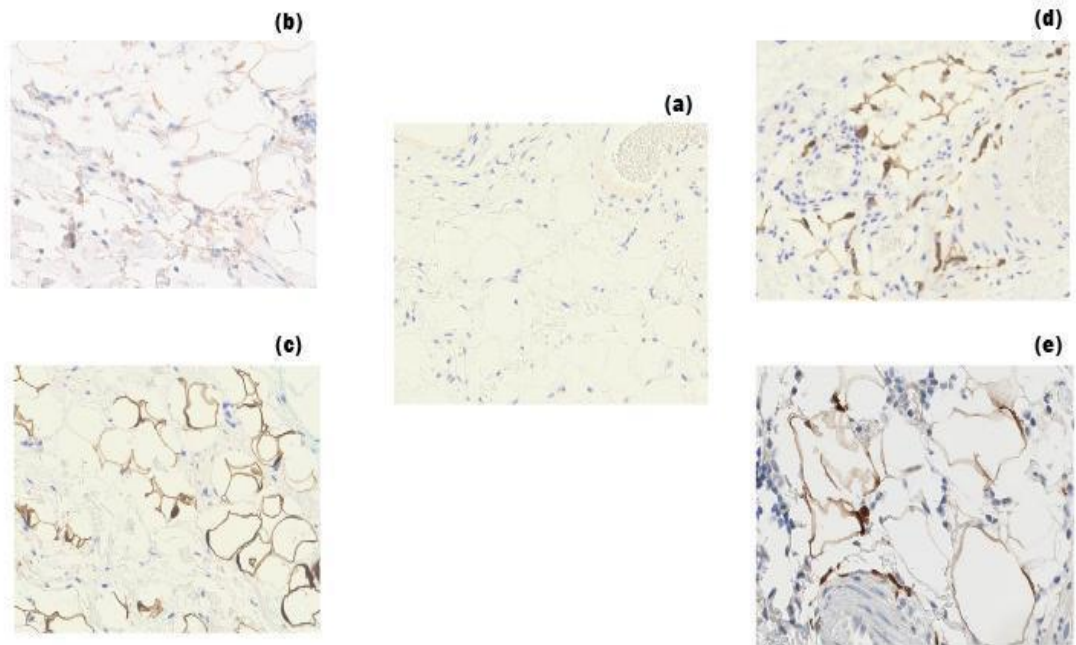




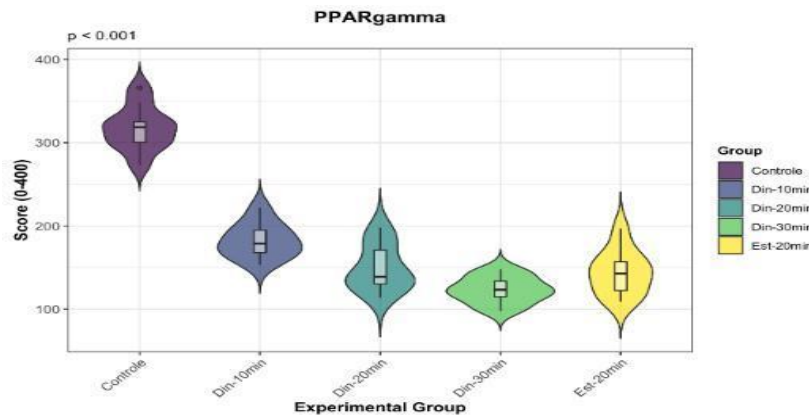
**Figure 11.** Heat shock proteins expression in adipose tissue treated with dynamic or static plate cryolipolysis, indicating adaptive response to cryolipolysis-induced stress. HSP60, HSP70, and HSP90 expression are shown in panels (a), (b) and (c), respectively ( $p < 0.001$ ).



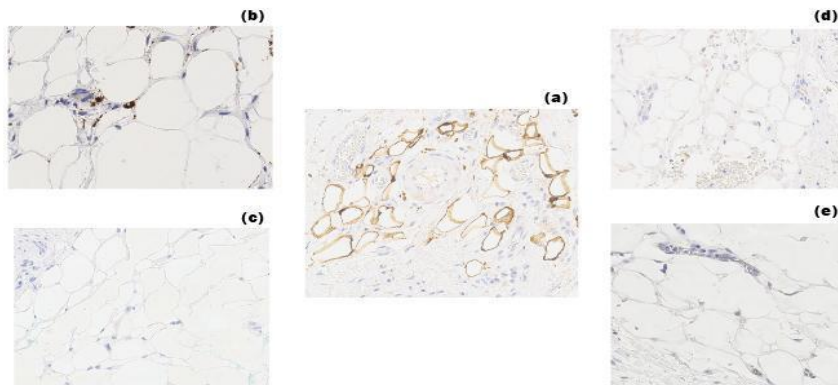
**Figure 12.** UCP1 expression in static and dynamic plate cryolipolysis-treated groups, indicating partial activation of brown adipocytes with increased expression in static 20-min and dynamic, 30-min protocols ( $p < 0.001$ ).



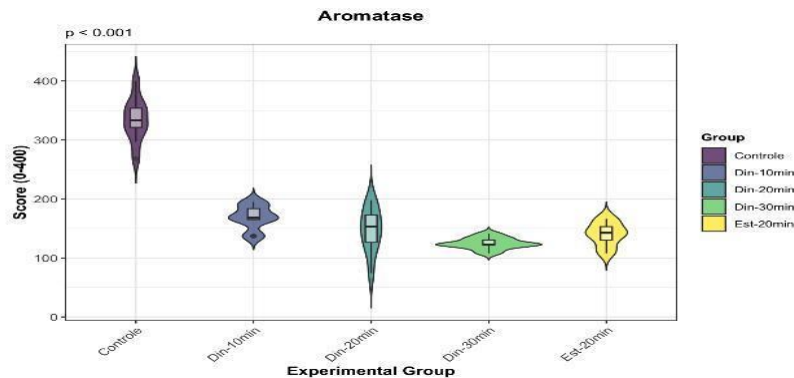
**Figure 13.** Increased UCP1 expression in static and prolonged dynamic plate cryolipolysis, reflecting enhanced thermogenesis. (a) control, (b) dynamic, 10 min, (c) dynamic, 20 min, (d) dynamic plate cryolipolysis, 30 min, (e) static cryolipolysis.



**Figure 14.** Reduction in PPAR- $\gamma$  expression in treated tissue, suggesting suppressed adipogenesis ( $p < 0.001$ ).



**Figure 15.** Immunostaining for PPAR- $\gamma$  comparing the control and treated areas, with visible downregulation post-cryolipolysis. (a) control, (b) dynamic, 10 min, (c) dynamic, 20 min, (d) dynamic, 30 min, (e) static.



**Figure 16.** Reduced aromatase (CYP19A1) expression after dynamic plate cryolipolysis, suggesting hormonal modulation ( $p < 0.001$ ).

Detection of COX-2 and CD68 and CD163 (macrophage infiltration biomarkers) expressions indicated the induction of a controlled inflammatory milieu, which is considered

beneficial in regenerative protocols. A mild and regulated inflammatory response contributes to the apoptosis-resolution-remodeling cascade, and its regulation directly influences clinical outcomes (3).

In this study, the strongest expression of inflammatory biomarkers was observed in static and prolonged dynamic protocols (20 and 30 min), suggesting that the period and plate immobility may intensify cell responses to cold.

The extracellular matrix was another relevant mechanism identified underlying the dynamic plate cryolipolysis. Increased expression of FGF2 and FGFR1 was observed at doses that also promoted predominant type I collagen deposition, indicating mature tissue reorganization. These findings corroborate previous clinical reports which demonstrate improved dermal firmness after cryolipolysis. Conventional cryolipolysis induced collagen remodeling and enhanced dermal firmness in patients treated with static cryolipolysis (11), which was also promoted by the dynamic plate protocol. In addition, heat shock proteins (HSP60, HSP70, and HSP90) were increasingly expressed in all treated groups, particularly in those treated for longer periods. Heat shock proteins play cytoprotective roles and contribute to cellular homeostasis in response to external stress. Their upregulation may reflect adaptive responses within physiological limits, as previously reported in studies investigating the effects of cryotherapy on cell function (7).

At the metabolic level, reduced PPAR- $\gamma$  expression indicated suppression of adipogenic pathways, potentially limiting the recruitment of new adipocytes to treated areas. This effect may be clinically relevant for patients prone to localized fat recurrence. Concurrently, increased UCP1 expression, particularly after longer treatment durations, suggests partial induction of thermogenesis and the browning of white adipose tissue. These findings broaden the perspective on the metabolic potential of cryolipolysis, especially for patients resistant to conventional fat-reduction strategies.

Reduced aromatase (CYP19A1) expression was also observed following dynamic plate cryolipolysis. This enzyme mediates the conversion of androgens to estrogens and has been implicated in estrogen-dependent adiposity and lipedema. Together with the modulation of thermogenic biomarkers, these findings reinforce that DPC promotes multifactorial processes beyond fat reduction (14).

These findings support DPC as a safe, versatile, and adaptable technique, particularly suitable for anatomically challenging regions in patients intolerant to vacuum-based devices. Plate mobility is suggested to enhance homogeneous thermal distribution, reduce the risk of localized burns, and improve anatomical adaptation, thereby broadening clinical applicability.

## CONCLUSION

DPC promoted adipocyte apoptosis associated with a mild, regulated inflammatory response. Additionally, it stimulated fibroblast activity and extracellular matrix remodeling, with predominant type I collagen deposition, particularly after static and longer periods of dynamic plate cryolipolysis. Metabolic and hormonal modulation, reflected by reduced PPAR- $\gamma$  and aromatase expression, further suggests potential benefits in the management of adipose tissue disorders (e.g., lipedema).

Overall, DPC was demonstrated to be a safe and feasible technique presenting dose-dependent effects and distinct advantages over vacuum-based cryolipolysis in difficult-to-treat areas. Despite the limitation of a small sample size, the present findings reinforce its therapeutic potential. Future studies with larger cohorts and extended clinical follow-up are warranted to validate these results and to elucidate long-term outcomes.

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