Received: 17 April 2023 | Revised: 24 July 2023 | Accepted: 6 September 2023

DOI: 10.1111/jocd.16002

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Innovation of the protocol for the application of cryolipolysis: Effects and mechanisms of action

Christiane Rodrigues Tofoli Palauro[1] | Patrícia Froes Meyer PhD[2] | Ciro Dantas Soares PhD[2] | Eneida de Morais Carreiro MD[2] | Flávio de Paiva Dumaresq[3] | Fernando Cesar Camara de Oliveira[4] | Ana Laura Martins de Andrade[5] | Priscila da S P S Daumas PhD[6]

[1]Centro Universitário de Vila Velha, Vila Velha, Brazil

[2]International Research Group, Natal, Brazil

[3]Universidade Estadual de Ciências da Saúde de Alagoas, Maceio, Brazil

[4]Universidade Federal do Rio Grande do Norte, Natal, Brazil

[5]Universidade Brasil, Sao Paulo, Brazil

[6]Universidade Estácio de Sá, Rio de Janeiro, Brazil

Correspondence
Ana Laura Martins de Andrade, Universidade Brasil, Sao Paulo, Brazil.
Email: anandrade90@yahoo.com.br

Abstract

Background: In recent years, aesthetic procedures aiming at body remodeling and have grown exponentially. Cryolipolysis (CLL) has stood out as a noninvasive resource that acts directly on the subcutaneous adipose tissue promoting a significant reduction of adipose tissue through of cooling that could lead to the crystallization cytoplasmic lipids, loss of cellular integrity, apoptosis/necrosis of adipocytes, and local inflammation, producing selective loss of adipose tissue. Thus, the objective of the present study was to evaluate the effects of a specific technique of CLL application on the inflammatory reactions of the target tissue in different post-application times.

Methods: This is a randomized, blind clinical study that evaluated the tissue sample of six patients after 45, 60, and 90 days of an innovative protocol for the application of CLL, with samples collected through abdominoplasty surgeries. The samples were evaluated by immunohistochemical analyses of several markers.

Results: A significantly greater increase in fibroblasts was observed at 45 days and greater phagocytic action at 60 days.

Regarding the apoptosis process, the expression of caspase 3 and cleaved caspase 3 markers varied at different times, with cleaved caspase 3 being higher at 45 and 90 days after CLL application.

Conclusion: The protocol of the CLL presented in this study was able to induce inflammatory responses in addition to confirming the selective apoptotic action at the different times studied.

KEYWORDS
adipose tissue, apoptosis, cryolipolysis, inflammation

Abbreviations: CLL, Cryolipolysis; FDA, Federal Drug Administration; IHC, Immunohistochemistry; TCL, Informed Consent Form.

1 | INTRODUCTION

In recent years, aesthetic procedures aiming at body remodeling and reduction of unwanted focal fat deposits have grown exponentially. Patients have been increasingly seeking treatments without undesirable surgical risks that do not promote downtime.[1,2] Thus, Cryolipolysis (CLL) has stood out as a noninvasive resource that acts directly on the subcutaneous adipose tissue through controlled cooling without causing damage to the epidermis and underlying dermis, promoting a significant reduction of adipose tissue and improving body contour.[3,4]

Studies and clinical applications involving CLL were initially based on observations of cold-induced panniculitis, where it was noticed that lipid-rich tissues were more susceptible to cold injury than surrounding water-rich tissues.[5] Thus, in early 2007 the first studies emerged for fat reduction with freezing, using a resource called CLL.[6] The Federal Drug Administration (FDA) first cleared the clinical use of CLL in 2010 for localized reduction in the flanks and abdomen, and in 2014 it was also cleared for the thigh region. In the following years, new areas of application areas as submentonian, arms, back and lower buttocks.[7]

CLL is considered a safe and effective procedure with high patient satisfaction rates. It uses an applicator on the target area, set at a cooling temperature ranging from −5 to −15°C for a predefined period. It is noteworthy that although the temperature is already preestablished in the literature, the application forms vary in both clinical practice and scientific studies, making it difficult to understand the mechanisms of action. The resource targets subcutaneous adipocytes, aiming to induce apoptosis without damaging other adjacent tissues.[3,4,8]

Although several clinical and experimental studies confirm that CLL can reduce subcutaneous adipose tissue,[9–11] the mechanisms of action are not fully clarified. Two hypotheses are extensively discussed in the literature, the first being based on the reduction of subcutaneous adipose tissue without apoptosis through energy expenditure using lipid oxidation and thermogenesis after the application of cooling.[12,13] The second hypothesis, currently being discussed as the most accepted, is based on the theory that cooling could lead to the crystallization cytoplasmic lipids, loss of cellular integrity, apoptosis/necrosis of adipocytes, and local inflammation, producing selective loss of adipose tissue.[8,10,14]

Thus, despite its extensive and growing use in clinical practice, the literature on the mechanisms of action and application of CLL is still scarce. Considering the above, the objective of the present study was to evaluate the effects of a specific technique of CLL application on the inflammatory reactions of the target tissue in different post-application times.

2 | MATERIALS AND METHODS

This randomized clinical study was carried out from October 2021 to June 2022 in a population of patients who sought intervention to reduce subcutaneous fat located in the supra and infraumbilical region, with an indication for abdominoplasty. Six participants were selected based on the following inclusion/exclusion criteria.

The established inclusion criteria were female gender, age between 20 and 50 years, localized accumulation in the supra and infraumbilical region with capacity for understanding and preserved local sensitivity. The exclusion criteria were individuals with metabolic diseases; inflammatory and/or infectious skin lesions in the area where CLL was used; previous surgical procedure in the body region where CLL was used; use of steroidal or nonsteroidal anti-inflammatory drugs; active inflammatory disease; smoker.

All participants were informed about the study's objectives and signed an informed consent form (TCL) about the procedure, including authorizations part of the tissues resulting from the surgery to perform a microscopic examination.

The abdominoplasty operation was scheduled for all participating individuals, with a plastic surgeon at the Hospital Angiovascular, located at Av. Rodrigues Alves, 861—Tirol, Natal—RN, 45, 60 or 90 days after the CLL session.

2.1 | Proceedings

The CLL equipment used was the Beauty ShapeTM, from HTMTM, being applied with the patient in dorsal decubitus, with a single session with the following protocol: Temperature of −11°C; large size vacuum applicator (20-cm wide) with suction pressure of −30 kPa; 30 min of application in the infraumbilical region always on the right, while the left side was kept as control (untreated), followed by reperfusion with manual massage (circular movements) for 5 min. In sequence, again 30 min of CLL application, still on the right side, but in the central area relative to the place where the applicator did not reach freezing in the first 30 min.

Patients underwent abdominoplasty surgery 45 days (two patients), 60 days (two patients), and 90 days (two patients), respectively, after the CLL session. Abdominal wall biopsies were collected simultaneously with the removal of the surgical flap of cutaneous and subcutaneous human skin from the two abdomen regions: right infraumbilical, which received the intervention with CLL and left infraumbilical, without intervention. These biopsies were approximately 5 mm in diameter and included skin and subcutaneous tissue.

2.2 | Immunohistochemistry

To detect the inflammatory profile promoted by CLL, IHC analyses were performed using the samples obtained from the abdominoplasty surgery, using the markers described in Table 1.

Casp 3, CCasp 3 and CD68 markers were analyzed the three periods after CLL, whereas EBF-1, BCL-2 and TNF-α markers were analyzed only at the end of 90 days.

Tissue samples were stained with a non-primary negative control and a human skin positive control. Heat-induced antigenic retrieval

was performed with Leica Bond Epitope Retrieval Buffer 1 (citrate buffer, pH 6.0) for 20 min. Nonspecific antibody binding was blocked with Novolink Protein Block for 10 min. The primary antibodies (Table 1) were applied following the manufacturer's recommendations. Colourimetric detection was performed with diaminobenzidine substrate (DAB, SK-4100, Vector Laboratories) and hematoxylin.

ImageJ software was used for quantitative analysis of marker expression through the percentage of pixels in the area marked with the plug-in Color Deconvolution with Methyl Greem DAB.[15] Three images of each slide were captured at 20x magnification. The arithmetic means of the pixel values of the samples were used for the statistical analysis.

TABLE 1 Details of the antibodies used.

Antibody 1°	Antibody Brand	Dilution
Anti-Caspase 3	Santa Cruz Biotechnology	1:500
Anti-EBF-1*	Santa Cruz Biotechnology	1:3000
Cleaved Anti-Caspase 3	Santa Cruz Biotechnology	1:300
Anti-BCL-2	Santa Cruz Biotechnology	1:50
Anti-TNF-α**	Santa Cruz Biotechnology	1:100
Anti-CD68	Santa Cruz Biotechnology	1:50
Anti-perilipin	Santa Cruz Biotechnology	1:1000

Note: * Early B-Cell Factor 1; ** Tumor Necrosis Factor.

2.3 | Statistical analysis

The results were analyzed using the SPSS 17.0 Software. Results were expressed as mean ± standard deviation. Statistical analysis was performed using a standard two-tailed paired *t*-test for means.

3 | RESULTS

All six participants completed the study between October 2021 and June 2022. Notably, all were female, aged between 25 and 45 years. In the biopsies performed in the untreated areas (left side), it was observed that the skin epithelium was completely intact.

Regarding the IHC analyses with the 45-day biopsies (Figures 1-3), it was observed that for the Caspase 3, Cleaved Caspase 3 and CD68 markers, the region that received the CLL intervention (right side) showed statistically greater expression significant ($p < 0.05$; $p < 0.001$; $p < 0.001$) for all markers when compared to the control region (left side).

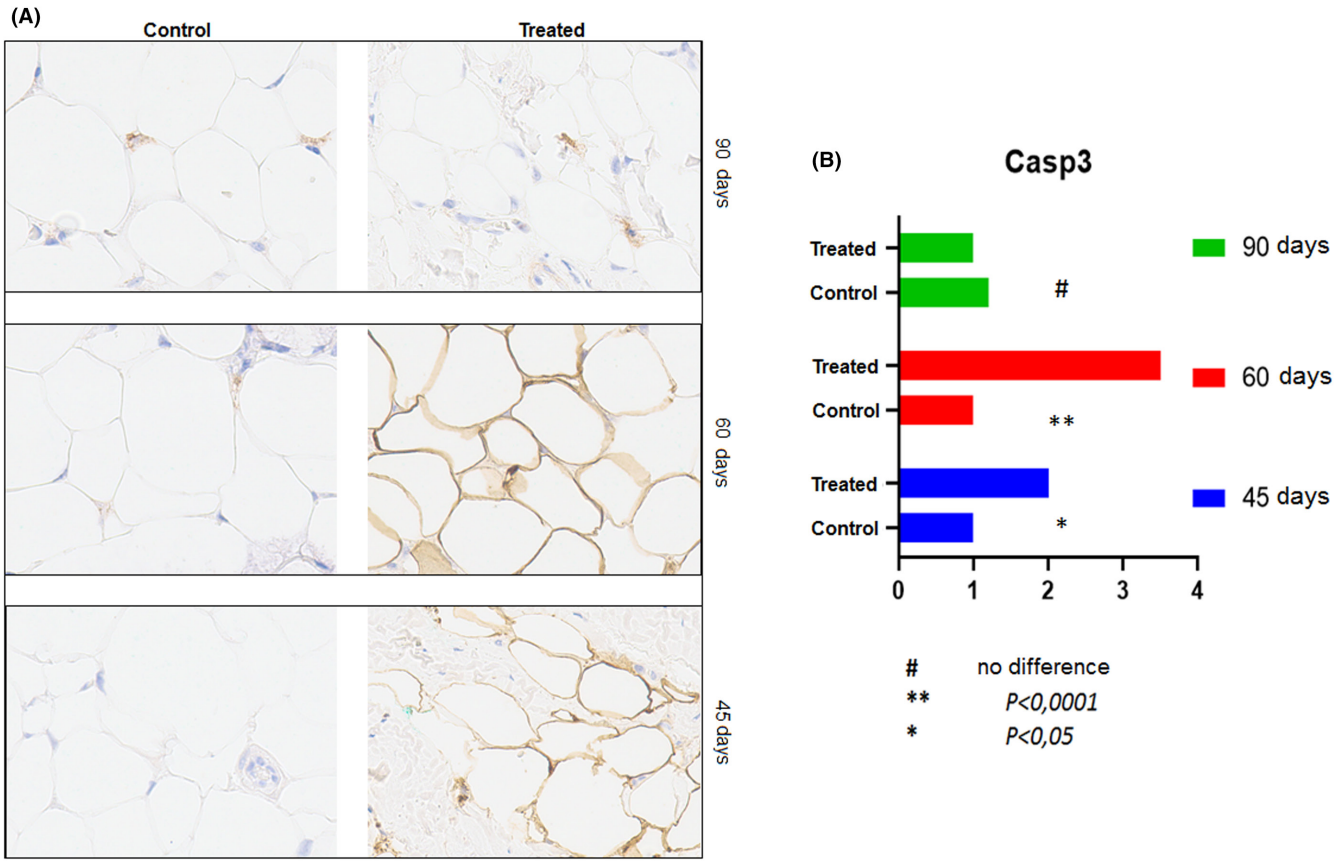


FIGURE 1 (A) Representative samples of the control and treated regions analyzed by immunohistochemistry for Caspase 3 in the three times studied. (B) Quantification of Caspase 3 in control and treated samples at different times.

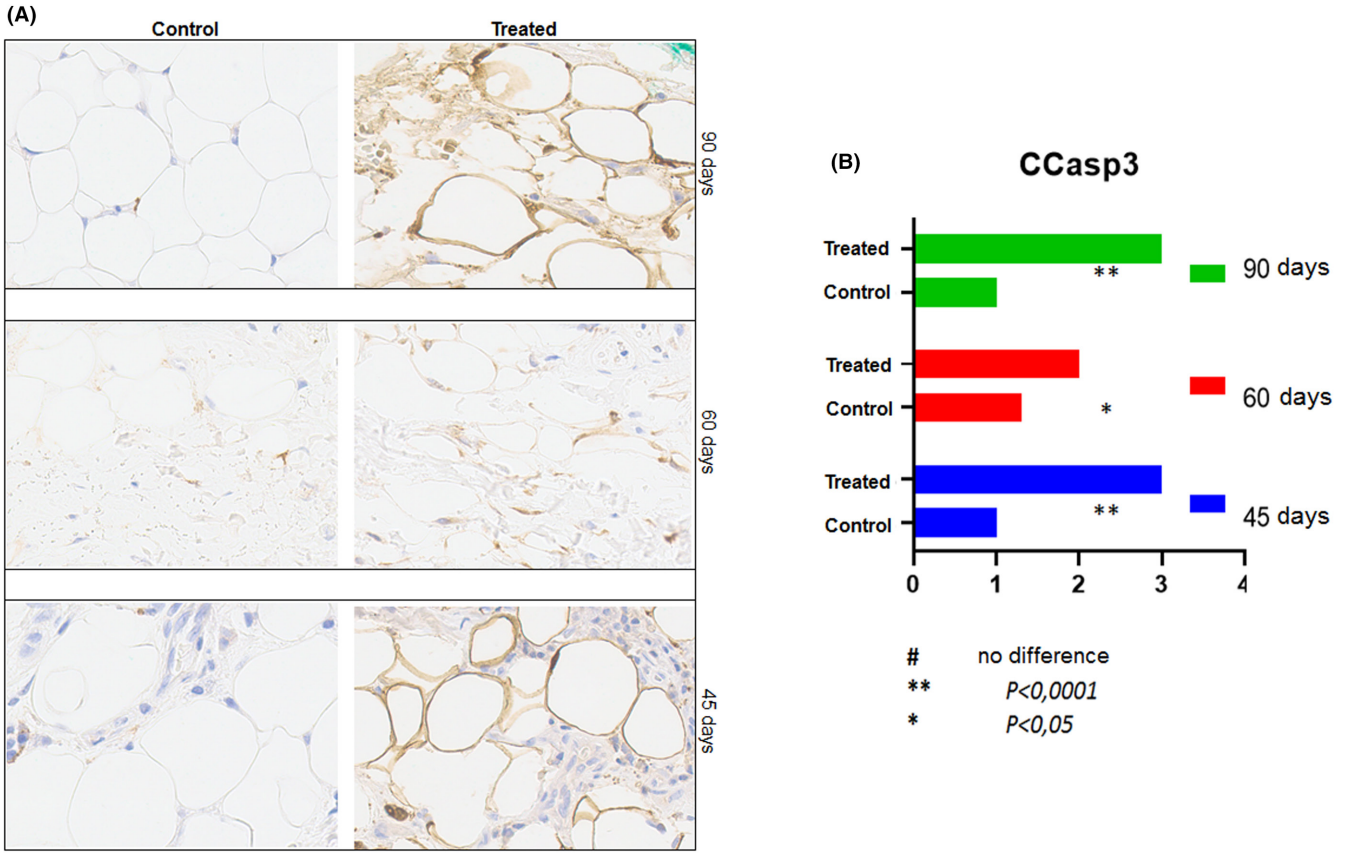


FIGURE 2 (A) Representative samples of the control and treated regions analyzed by immunohistochemistry for CCaspase 3 in the three studied times. (B) Quantification of CCaspase 3 in control and treated samples at different times.

For biopsies collected in 60 days (Figure 2), the same markers (Caspase 3, cleaved Caspase 3 and CD68) also showed higher immunoexpression in the treated region (right side) when compared to the control region ($p < 0.001$; $p < 0.05$; $p < 0.001$) Figure 3.

For the samples collected after 90 days (Figures 2 and 3), the markers of cleaved Caspase 3 and CD68 for the treated region (right side) still showed an increase in immunoexpression when compared to the control region ($p < 0.001$), however for Caspase 2, this increase was not observed, with no statistical difference between regions.

The EBF-1 marker stands out as an adipocyte inflammatory marker.[16] Through the IHC analysis, it was observed that both regions, treated and not treated with CLL, did not present a statistically significant difference ($p = 0.599$) for this marker, as observed in Figure 4. The same can be seen in Figure 5 for the perilipin marker, in which the IHC analysis showed no statistical difference between the treated and control regions ($p = 0.4$) (Figure 5).

The BLC-2 protein is discussed as a cell marker that primarily determines whether a cell has apoptotic features.[17] Through the analysis of IHC, the area treated with CLL and the untreated one, presented similar results, with no statistical difference ($p = 0.73$), as observed in Figure 6.

Also, the IHC analysis verified the TNF-α marker because it is a group of cytokines capable of causing cell death (apoptosis).[18] Thus, it was observed that the treated region showed statistically more significant immunoexpression than the control region ($p < 0.05$), as shown in Figure 7.

In Figure 8, it is possible to observe the behavior of all IHC markers after 90 days of CLL application. Statistical difference was observed in the IHC analysis for CCasp3, CD68 and TNF-alpha markers.

4 | DISCUSSION

Although clinical practice and the literature are concise in stating that CLL can reduce subcutaneous adipose tissue and even promote an improvement in the appearance of the skin, the mechanisms of action are still discussed in the literature, mainly regarding the inflammatory reactions that would be produced by the resource, culminating in apoptosis of the fatty cells.[9,10,19]

Thus, the present study sought to evaluate several inflammatory markers at different times after a single application of CLL to determine clearer pathways of action of the resource, in addition to proposing a safe application form that produces the results expected by patients.

CLL application protocols regarding the number of sessions needed per region and session time are frequently discussed in the literature.[3,20] Studies demonstrate that for abdominal regions, 3–5 sessions are necessary.[20] However, the session application time

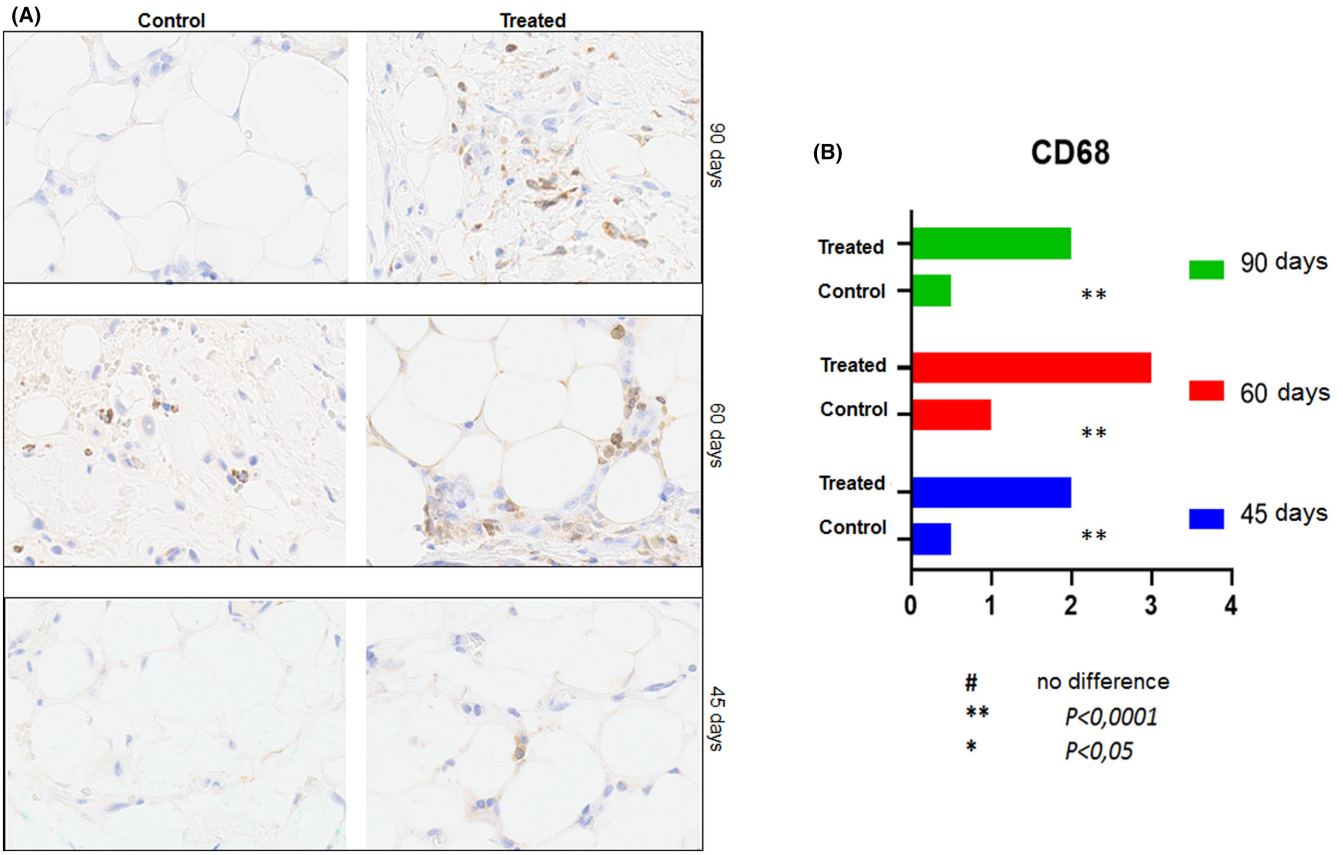


FIGURE 3 (A) Representative samples of the control and treated regions analyzed by immunohistochemistry for CD68 in the three studied times. (B) CD68 quantification of control and treated samples at different times.

varies from 45 to 60 minutes.[21,22] Our study sought to present a new application protocol in an attempt to reduce the number of necessary sessions promoting the expected inflammatory process, since the second 30-min application cycle, after manual reperfusion, reaches the central area, which is normally not achieved. With this protocol, in a single session, it was observed through the IHC analyses that the entire area that received the application developed the expected inflammatory and apoptotic process.

The selection of study time was based on the findings of Stevens et al., 2015, who observed that the full effect of CLL can be observed 3 onths after application.[23] In addition, some studies argue that the peak of inflammatory tissue reactions occurs around 4 weeks or more.[10]

Avram et al., 2009[24] published one of the first reviews on the possible mechanisms of action involved in the treatment with CLL, discussing that after applying the resource, the target adipocytes crystallized suffered apoptosis, being then eliminated by engulfment of macrophages after approximately three months. Our study corroborates these findings since the IHC results for Casp3, CCasp 3, and CD68 showed that the treated region had a higher expression of these markers related to the presence of apoptosis, degeneration, and cell phagocytosis at the different times evaluated.

Da Costa et al., 2022[8] carried out a study that addressed the evaluation of some blood inflammatory markers in women who underwent a CLL session. The results of the study demonstrated that such markers had not shown differences. However, it is worth mentioning that it was a systemic evaluation since the sample collection was blood, unlike our study, which evaluated the local effects after applying the resource. Our study, despite showing no difference for all the inflammatory markers studied, demonstrated that after 45 days, all participants had inflammatory reactions followed by apoptosis.

One of the most discussed mechanisms in the literature is related to the phenomenon of apoptosis resulting from the application of CLL. Pugliese et al., 2020, presented a study that evaluated a single CLL session at 15, 45, and 60 days after application. The authors also evaluated the resource locally and stated through their analyses that the adipose cells underwent and remained in the process of apoptosis at all times studied. They also point out that this phenomenon could last even longer. These findings corroborate our study since the apoptosis markers (Casp3, CCasp3, and TNF-α) evaluated by the IHC were more expressed in the treated region at the different times evaluated compared to the control region.

Another analyzed marker that deserves to be highlighted in our study is CD68. In adipose tissue, the presence of this protein signals cell degeneration, and phagocytosis.[25] Pugliese et al., 2020, highlight that the presence of macrophages and other inflammatory cells may be the logical consequence of the need to "clean up" the effects

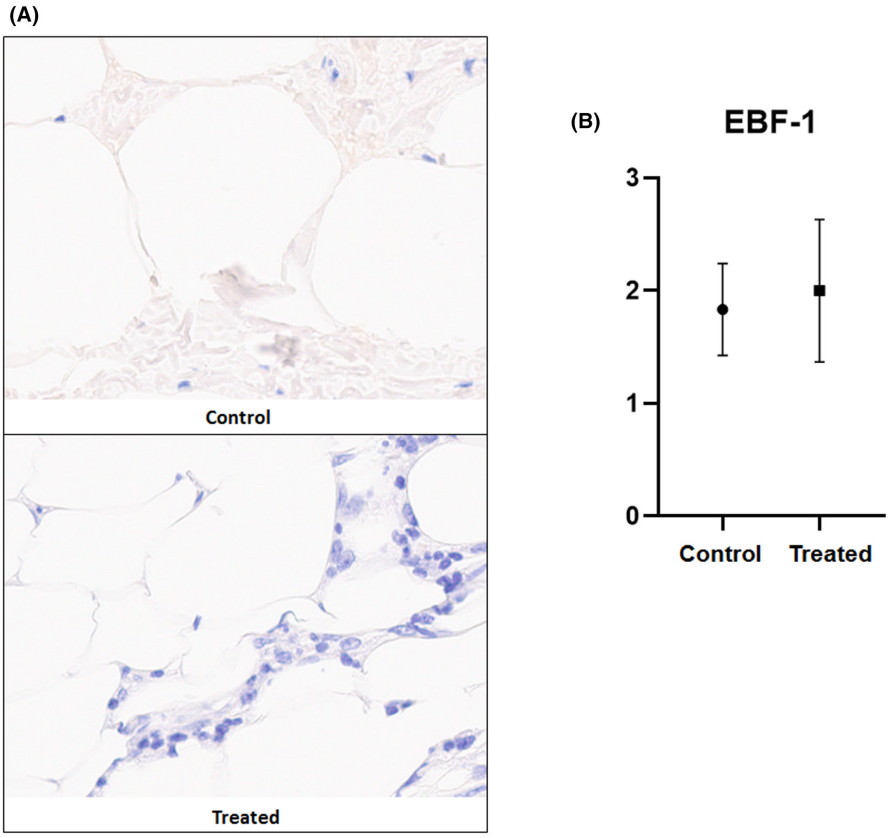


FIGURE 4 (A) Representative samples of control (upper row) and treated (lower row) of the six individuals analyzed by immunohistochemistry for EBF-1. (B) EBF-1 quantification of control and treated samples (*$p = 0.599$).

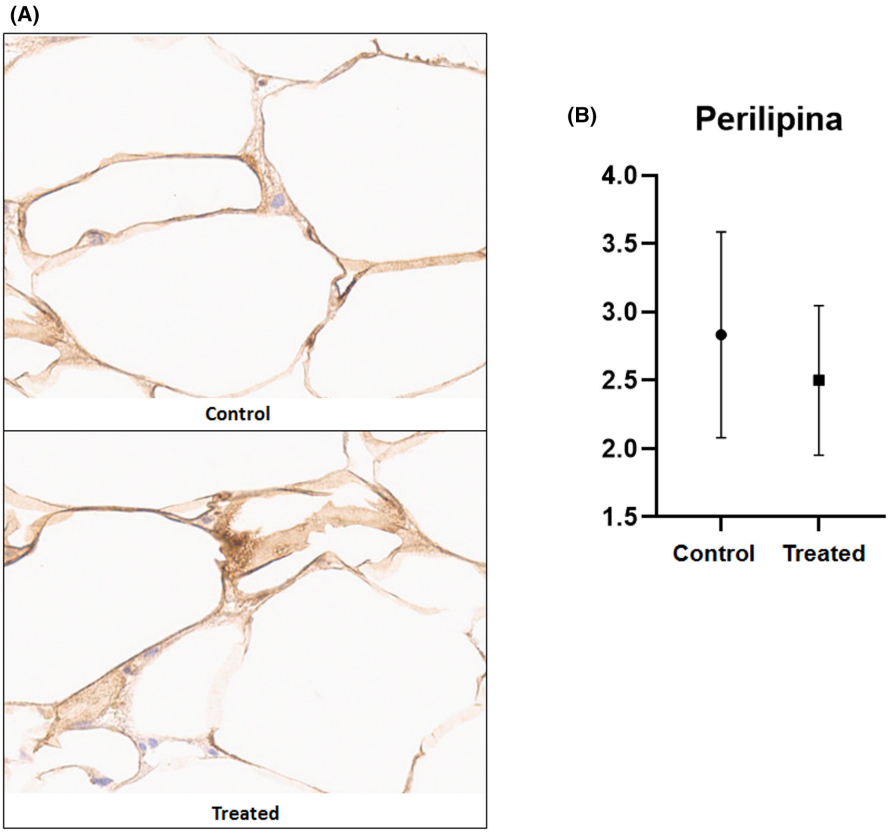


FIGURE 5 (A) Representative samples of the control (upper row) and treated (lower row) of the six individuals analyzed by immunohistochemistry for perilipin. (B) Perilipin quantification of control and treated samples (*$p = 0.4$).

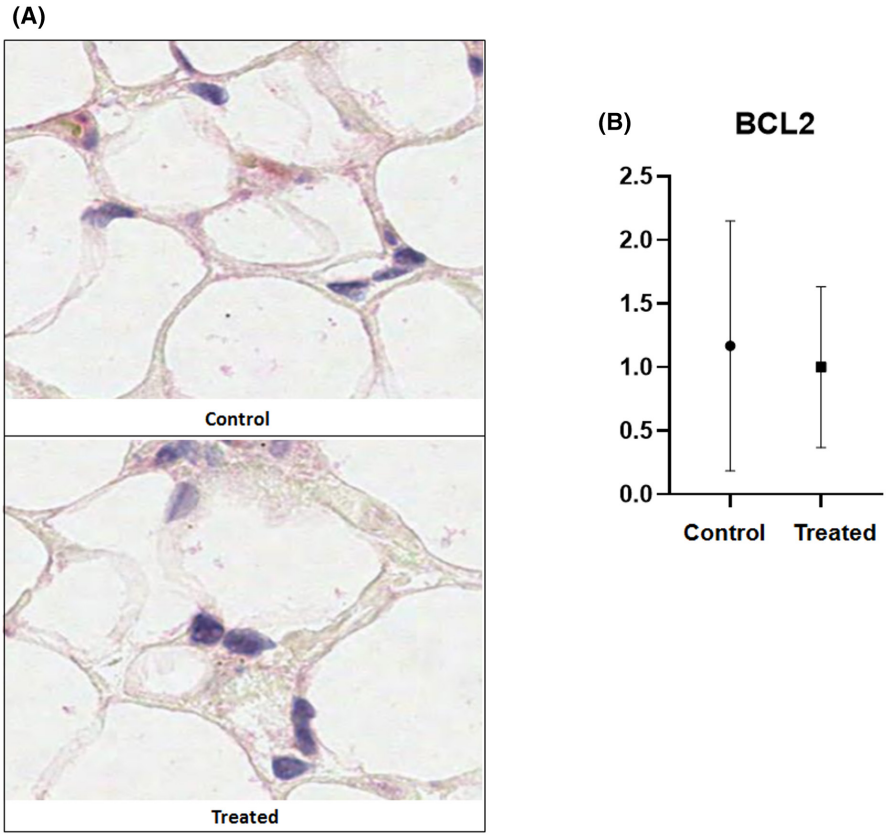


FIGURE 6 (A) Representative samples of the control (upper row) and treated (lower row) of the six individuals analyzed by immunohistochemistry for BCL2. (B) BCL2 quantification of control and treated samples (*$p = 0.73$).

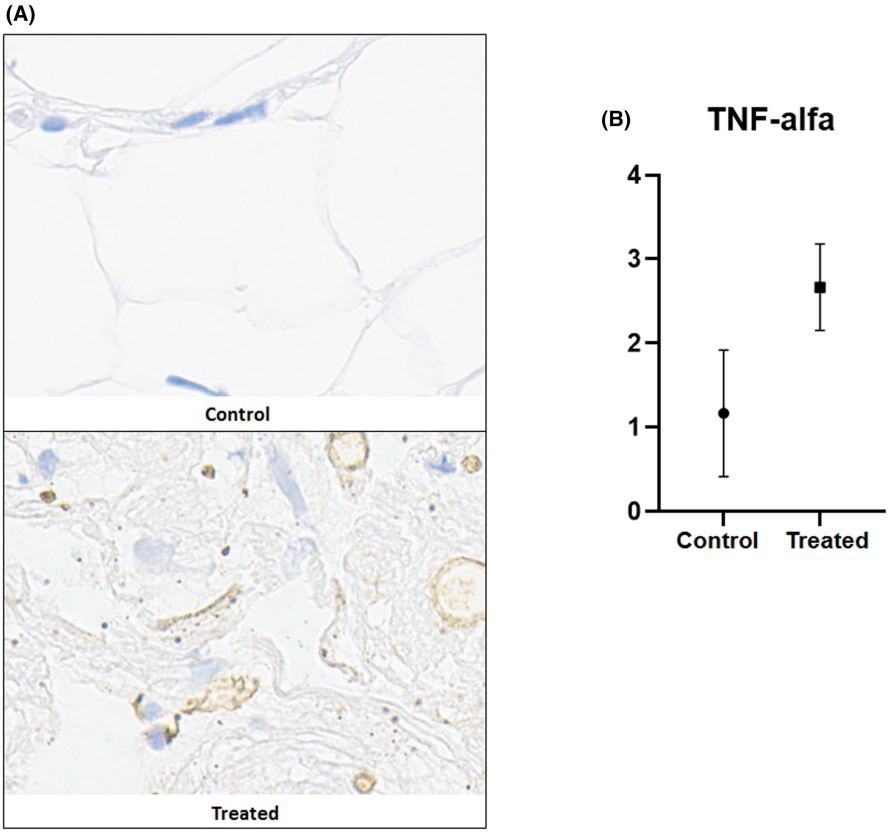
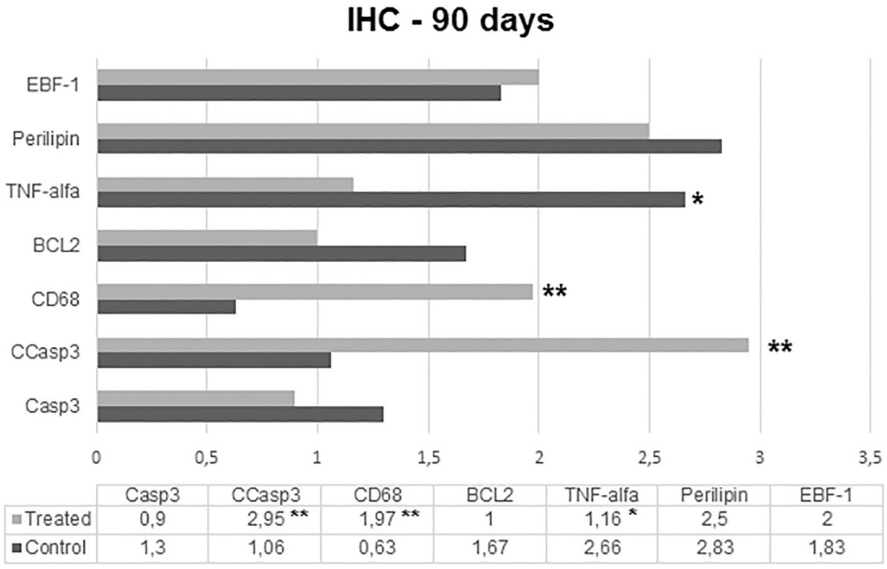


FIGURE 7 (A) Representative samples of the control (upper row) and treated (lower row) of the six individuals analyzed by immunohistochemistry for TNF-α. (B) TNF-α quantification of control and treated samples (*$p < 0.05$).

of cell death from adipocytes, corroborating our study that demonstrates greater expression of CD68 in the treated region, at all times when cell apoptosis is also observed.

The EBF-1 marker represents inflammation in the adipose tissue when expressed in IHC analyses. Our study showed no statistical difference between the treated and control regions for EBF-1. Still,



	Casp3	CCasp3	CD68	BCL2	TNF-alfa	Perilipin	EBF-1
Treated	0,9	2,95 **	1,97 **	1	1,16 *	2,5	2
Control	1,3	1,06	0,63	1,67	2,66	2,83	1,83

FIGURE 8 Mean of immunoexpression of EBF-1, Perilipin, TNF-alpha, BCL2, CD68, CCasp3, Casp3 markers. Asterisk indicates statistical difference (*$p < 0.05$; **$p < 0.0001$).

our analysis was performed only on participants aged 90 days, so it is expected that local inflammation will be under control by this time.

The findings of this study solidify the hypotheses that the mechanisms of action of CLL involve inflammatory reactions followed by apoptosis in the tissues applied. However, the number of participants is still limited, which may also have influenced the results of the IHC of EBF-1, Perilipin, and BCL-2, moreover, these markers were analyzed only in patients collected after 90 days, in which possibly the inflammatory and apoptotic actions expressed by these markers would have already normalized. Thus, it is stands out that these results were obtained through a new application protocol that sought to reach completely the target area which, which is not often done since the central area is not usually reached in most protocols.

We emphasize that the study has some limitations: a larger number of individuals must be investigated to confirm the findings of this study; some markers (BCL-2, Perilipin, and EBF-1) were analyzed only in patient samples after 90 days. Thus, it is believed that new studies with the same scientific rigor should be carried out to reinforce and clarify the effects and mechanisms of CLL.

5 | CONCLUSION

The protocol presented in this study was able to induce inflammatory responses in addition to confirming the selective apoptotic action of cold on target cells at the different times studied, confirming the effects of CLL on subcutaneous adipose tissue. Notably, the present study is one of the pioneers in analyzing specific markers for adipose tissue in response to the application of CLL.

AUTHORS CONTRIBUTIONS

Palouro CRT, Soares CD and Carreiro EM designed the research study. Dumaresq FP, Daumas FP and Oliveira FCC contributed essential reagents or tools. Palouro CRT and Soares CD analyzed the data. Andrade AL and Meyer PF wrote the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This randomized clinical study was approved by the Ithe Ethics and Research Committee of the Universidade Potiguar (protocol no. 5435.257). All patients provided informed consent for the collection of data and treatment records.

ORCID

Patrícia Froes Meyer https://orcid.org/0000-0001-8530-8183
Ciro Dantas Soares https://orcid.org/0000-0002-6861-6640
Ana Laura Martins de Andrade https://orcid.org/0000-0003-1137-7180

REFERENCES

1. Kilmer SL, Cox SE, Zelickson BD, et al. Feasibility study of electromagnetic muscle stimulation and Cryolipolysis for abdominal contouring. *Dermatologic Surg*. 2020;46(1):S14-S21.
2. The American Society for Aesthetic Plastic Surgery's Cosmetic Surgery National Data Bank. Statistics 2018. *Aesthetic Surg J*. 2019;39(Suppl_4):1-27. Available from: https://pubmed.ncbi.nlm.nih.gov/31226205/
3. Few J, Saltz R, Beaty M, et al. Cryolipolysis: clinical best practices and other nonclinical considerations. *Aesthetic Surg J*. 2020;2(2):1-11.
4. Ingargiola MJ, Motakef S, Chung MT, Vasconez HC, Sasaki GH. Cryolipolysis for fat reduction and body contouring: safety and efficacy of current treatment paradigms. *Plast Reconstr Surg*. 2015;135(6):1581-1590. Available from: https://pubmed.ncbi.nlm.nih.gov/26017594/.
5. Arora R, Nandhagopal T, Raza S. Popsicle panniculitis. *N Engl J Med*. 1970;282(17):966-967.
6. Manstein D, Laubach H, Watanabe K, Farinelli W, Zurakowski D, Anderson RR. Selective cryolysis: a novel method of

noninvasive fat removal. Lasers surg med [internet]. *Lasers Surg Med*. 2008;40(9):595-604. Available from: https://pubmed.ncbi.nlm.nih.gov/18951424/

7. US Food and Drug Administration 2023. Available from: http://www.accessdata.fda.gov/cdrh_docs/pdf13/K133212.pdf.
8. Costa ADS, Oliveira AS d SS, Brito AK d S, et al. Cryolipolysis on more than one body area increases lipid peroxidation without changing lipid profile and inflammatory markers. *Biology (Basel)*. 2022;11(12):1-14.
9. Savacini MB, Bueno DT, Molina ACS, et al. Effectiveness and safety of contrast Cryolipolysis for subcutaneous-fat reduction. *Dermatol Res Pract*. 2018;2018:5276528.
10. Pugliese D, Melfa F, Guarino E, et al. Histopathological features of tissue alterations induced by Cryolipolysis on human adipose tissue. *Aesthetic Surg J*. 2020;40(7):1-6. Available from: https://academic.oup.com/asj/article/40/7/761/5728570
11. Jeong SY, Kwon TR, Seok J, Park KY, Kim BJ. Noninvasive tumescent cryolipolysis using a new 4D handpiece: a comparative study with a porcine model. *Skin Res Technol*. 2017;23(1):79-87.
12. Loap S, Lathe R. Mechanism underlying tissue cryotherapy to combat obesity/overweight: triggering thermogenesis. *J Obes [Internet] Hindawi Limited*. 2018;2018. Available from: /pmc/articles/PMC5954866/:1-10.
13. Van Der Lans AAJJ, Hoeks J, Brans B, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J clin invest [internet]. *J Clin Invest*. 2013;123(8):3395-3403. Available from: https://pubmed.ncbi.nlm.nih.gov/23867626/
14. Grant Stevens W, Pietrzak LK, Spring MA. Broad overview of a clinical and commercial experience with CoolSculpting. *Aesthetic Surg J [Internet]. Aesthet Surg J*. 2013;33(6):835-846. Available from: https://pubmed.ncbi.nlm.nih.gov/23858510/
15. Andrade ALM, Brassolatti P, Luna GF, et al. Effect of photobiomodulation associated with cell therapy in the process of cutaneous regeneration in third degree burns in rats. *J Tissue Eng Regen Med*. 2020:673-683.Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/term.3028.
16. Griffin MJ. Nipping adipocyte inflammation in the bud. *Immunometabolism*. 2021;3(2):1-16.
17. Ivanovska I, Galonek HL, Hildeman DA, Hardwick JM. Regulation of cell death in the lymphoid system by Bcl-2 family proteins. Acta Haematol [internet]. *Acta Haematol*. 2004;111(1–2):42-55. Available from: https://pubmed.ncbi.nlm.nih.gov/14646344/
18. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol*. 2016;12(1):49-62.
19. Abboud S, Hachem JP. Heat shock lipolysis: radiofrequency combined with Cryolipolysis for the reduction of localized subcutaneous fat. *Dermatol Res Pract [Internet] Hindawi Limited*. 2020;2020. Available from: /pmc/articles/PMC7029295/:1-5.
20. Altmann J, Burns AJ, Kilmer SL, et al. Global expert opinion on Cryolipolysis treatment recommendations and considerations: a modified Delphi study. *Aesthetic Surg J*. 2022:1-12.
21. McKeown DJ, Payne J. Significant improvement in body contour with multiple cycles of CoolSculpting: results of a prospective study. *Dermatol Ther*. 2021;34(2):1-7. Available from: /pmc/articles/PMC8047906/.
22. Harper M, Lassetter J. CoolSculpting or Cryolipolysis: a guide for primary care practitioners. *Student Work*. 2019;15(9):666-670. Available from: https://scholarsarchive.byu.edu/studentpub/265
23. Stevens WG, Bachelor EP. Cryolipolysis conformable-surface applicator for nonsurgical fat reduction in lateral thighs. *Aesthetic Surg J [Internet]. Aesthet Surg J*. 2015;35(1):66-71. Available from: https://pubmed.ncbi.nlm.nih.gov/25568236/
24. Avram MM, Harry RS. CryolipolysisTM for subcutaneous fat layer reduction. *Lasers Surg Med*. 2009;41(10):703-708. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/lsm.20864.
25. Moreno-Navarrete JM, Ortega F, Gómez-Serrano M, et al. The MRC1/CD68 ratio is positively Associated with adipose tissue lipogenesis and with muscle mitochondrial gene expression in humans. *PLoS One [Internet] PLOS*. 2013;8(8) Available from: /pmc/articles/PMC3741275/:e70810.

How to cite this article: Palauro CRT, Meyer PF, Soares CD, et al. Innovation of the protocol for the application of cryolipolysis: Effects and mechanisms of action. *J Cosmet Dermatol*. 2023;00:1-9. doi:10.1111/jocd.16002