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Cryolipolysis in a Multimodal Approach to Adipose Tissue Reduction: A Case Series with Metabolic-Muscular Preparation and Ultrasound Monitoring

Ávila, R. R. B.[1]; Ávila, L. R. B.[2]; Ruiz-Silva, C.[3]; Nalim, V. A. S.[4]
[1]FACOP Colleges; Ávila Institute of Education; physiotherapist; postgraduate degrees in Dermatofunctional Physiotherapy, Higher Education Teaching, and Aesthetic and Orthomolecular Nutrition, Brazil.
[2]FACOP Colleges; Ávila Institute of Education; Biomedicine, Brazil.
[3] (PhD, MSc), Physiotherapist, Aesthetician and Cosmetologist, Dentistry Student; College of International Medicine and Aesthetics Harold Gillies (USA), Faculdade CTA (Brazil)
[4]Ávila Institute of Education; aesthetic biomedical scientist, Brazil.

Abstract
Cryolipolysis promotes controlled cooling of subcutaneous adipose tissue and triggers cellular responses that contribute to the reduction of the adipose layer. This paper describes a case series of two women with resistant localized adiposity who underwent a multimodal treatment plan centered on cryolipolysis. Preparation included two weekly sessions of ultrasonic hydrolipoclasy and four sessions of neuromuscular electrical stimulation combined, on the same day, with intramuscular L-arginine administration. Five days after the second hydrolipoclasy session, plate-based cryolipolysis was performed at −5°C for 40 or 50 minutes, depending on the thickness of the adipose fold. Forty-eight hours later, eight additional sessions of electrical stimulation and L-arginine were initiated, administered twice weekly. Adipose tissue thickness was measured via ultrasound at a standardized anatomical point two days before the start of treatment and 45 days after cryolipolysis. Reductions observed were from 19.4 mm to 9.1 mm in Case 1 (53.1%) and from 32.1 mm to 23.2 mm in Case 2 (27.7%). No adverse effects were observed. These findings document the clinical progression of the protocol; however, due to the descriptive study design, it is not possible to attribute the outcome to specific components or to demonstrate synergy. Controlled studies are needed to evaluate the contribution of the proposed combined therapies.
Keywords: *cryolipolysis; localized adiposity; low-frequency ultrasound; neuromuscular electrical stimulation; L-arginine.*

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I. INTRODUCTION

Subcutaneous adipose tissue does not constitute a uniform compartment. Its organization into superficial and deep layers, separated by fascial planes, involves anatomical, vascular, and metabolic differences. The deep layer features larger, less defined lobules and may exhibit slower lipid mobilization—a relevant characteristic when assessing resistant localized adiposity. This distinction does not imply a lack of response to cryolipolysis; rather, it indicates that treatment planning must take into account the architecture and thickness of the tissue to be treated (CUNHA; CUNHA; MACHADO, 2014).

Cryolipolysis is a non-invasive body contouring modality based on the susceptibility of adipocytes to cooling. Histological and immunohistochemical studies describe changes in cellular integrity, inflammatory response, caspase-3 expression, and phagocytosis over the ensuing weeks. Increases in fibroblasts and markers associated with dermal remodeling, as well as elevated heat shock proteins linked to cutaneous adaptation and extracellular matrix organization, have also been observed; however, these responses are protocol-dependent and should not be automatically extrapolated across different devices and temperatures (STEVENS et al., 2022; STEVENS et al., 2023). Cryolipolysis induces a biological environment characterized by intense thermal stress, controlled reperfusion, and adipose tissue reorganization. Nevertheless, cold-initiated cellular events remain active for several days post-procedure—a period marked by intense metabolic activity related to adipocyte apoptosis, intracellular lipid clearance, and extracellular matrix remodeling. In the protocols developed by the authors, this physiological interval presents a therapeutic opportunity for the application of microcurrents, aimed not only at stimulating local circulation but also at modulating bioenergetic mechanisms involved in post-cryolipolysis cellular adaptation (RUIZ-SILVA; MOLEIRO; RUIZ-SILVA, 2024).

Cryolipolysis promotes the activation of the PERK protein—a key cellular stress sensor—triggering a molecular cascade that involves the phosphorylation of O-GlcNAc transferase (OGT). Activation of this axis promotes the phosphorylation of the TOM70 protein at serine 94, thereby increasing the import of the MIC19 protein into the mitochondria. This sequence of events contributes to the reorganization of the MICOS complex, which is responsible for mitochondrial cristae architecture and the maintenance of respiratory chain efficiency. The authors propose that this reorganization represents a key biological mechanism following cryolipolysis (RUIZ-SILVA; MOLEIRO; RUIZ-SILVA, 2024).

The reorganization of mitochondrial cristae is an event of significant physiological importance, given that these structures harbor the enzyme complexes responsible for oxidative phosphorylation. In the studies presented, the improvement in mitochondrial architecture is interpreted as a mechanism capable of enhancing the efficiency of β-oxidation of fatty acids released during adipocyte apoptosis. Consequently, the energy derived from these lipids could be utilized more efficiently by the adipose tissue cells themselves and by other metabolically active tissues, facilitating adaptation to the new metabolic state following weight loss (RUIZ-SILVA; MOLEIRO; RUIZ-SILVA, 2024).

Based on this therapeutic core, a proposal for a local and systemic preparation was developedemic-muscular. Regarding the local component, saline-solution hydrolipoclasy followed by low-frequency ultrasound was used prior to cryolipolysis. Ex vivo models have demonstrated that saline infiltration facilitates ultrasonic propagation and amplifies changes in adipocyte architecture. Under experimental conditions, DNA fragmentation, a reduction in procaspase-9, and an increase in active caspase-3—findings consistent with the activation of apoptotic pathways—were observed; while these results establish biological plausibility, they do not constitute clinical proof that cryolipolysis is potentiated by this method (PALUMBO et al., 2011; PUGLIESE et al., 2013). In a previous clinical series, Ávila et al. (2025) combined FES and low-frequency ultrasound (preceded by saline infiltration) in six women, reporting improved body contouring and no adverse events during follow-up. That study provides a clinical reference directly relevant to the development of the current proposal, although it did not evaluate the inclusion of cryolipolysis or isolate the specific effect of each intervention.

Regarding the muscular component, functional electrical stimulation (FES) was employed to induce contractions combined with movement and progressive loading. Frequency, intensity, movement, and load were treated as four interdependent clinical variables. Literature indicates that frequency and intensity influence evoked force and fatigue, and that neuromuscular stimulation can induce adaptations when combined with resistance exercise (DREIBATI et al., 2010; NARVAEZ et al., 2025). Muscle contraction is also linked to endocrine signaling via myokines; however, responses vary, and exercise studies do not, in themselves, demonstrate that this specific FES protocol increased levels of irisin or other specific myokines (BETTARIGA et al., 2024).

L-arginine was administered on the same day as the electrical stimulation. In rats, dietary supplementation with L-arginine increased nitric oxide signaling, PGC-1α, UCP1, and beige adipocyte markers compared to control and cold-exposed groups. These data support a mechanistic hypothesis but do not validate a human intramuscular dosage, a temporal association with FES, or synergy with cryolipolysis (KALEZIC et al., 2022). Thus, the objective was to describe the protocol and ultrasound progression of two cases, without converting biologically plausible associations into proven causal relationships.

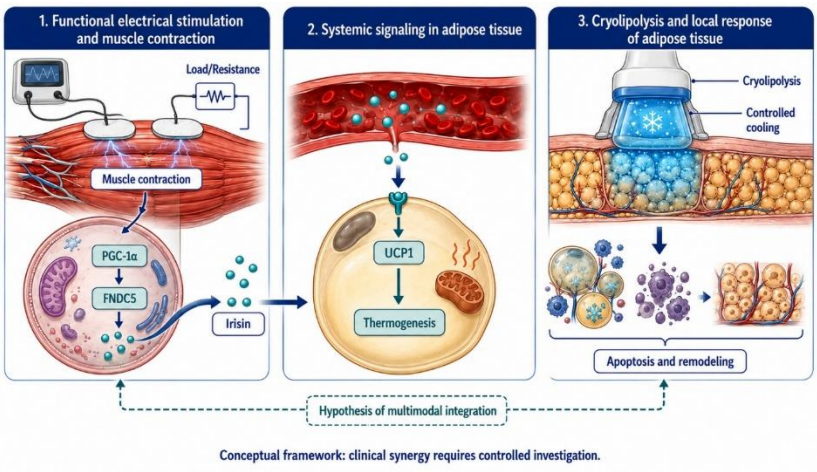

Figure 1. Conceptual diagram of the proposed multimodal integration. Functional electrical stimulation induces muscle contraction and may mobilize the PGC-1α/FNDC5/irisin pathway, which is linked to thermogenic signaling and UCP1; in parallel, cryolipolysis promotes controlled cooling and a local adipose tissue response.

The dashed line represents a hypothesis of integration, the clinical synergy of which requires controlled investigation. Illustration prepared by the authors.

II. METHODOLOGY

This is a descriptive case series involving two women with localized abdominal adiposity, identified as Patient 1 (40 years old) and Patient 2 (37 years old). Both authorized the use of their clinical data, photographs, and ultrasound images for scientific purposes. There was no control group, randomization, or histological assessment of the patients; therefore, this study documents the overall response to the treatment plan rather than comparing the isolated efficacy of the interventions.

Abdominal ultrasound was performed along the right umbilical line, 7 cm horizontally from the umbilicus. The initial examination took place two days before the first hydrolipoclasy session, and the final examination occurred 45 days after cryolipolysis. Distance, positioning, and anatomical landmarks were replicated in both assessments.

Local preparation involved two hydrolipoclasy sessions spaced seven days apart. An 18G × 70 mm cannula was used to distribute 10 mL of sterile 0.9% saline solution into each 10 cm² area. Subsequently, low-frequency ultrasound (40 kHz, 2.5 W/cm² intensity) was applied for seven minutes per 10 cm² effective radiating area (ERA). The second session took place five days prior to the cryolipolysis procedure.

Systemic-muscular preparation consisted of four Functional Electrical Stimulation (FES) sessions prior to cryolipolysis. In each session, the quadriceps, hamstrings, abdominal muscles, and dorsal musculature were treated individually. Parameters included a frequency of 30 Hz and a pulse width of 450 μs, with four contraction periods per muscle group: 60 seconds of "ON" time and 90 seconds of "OFF" time between periods. This corresponded to approximately 8 minutes and 30 seconds per group and a total of 34 minutes for the four groups, excluding transition times. During the first two periods, movement was performed without external load; starting from the third period, an individualized load was added based on contraction intensity, capacity, and movement quality. Current intensity was also individually adjusted to achieve visible and functional contraction while maintaining tolerability and proper execution.

On each day of FES, intramuscular L-arginine was administered at a dose of 1,000 mg/2 mL. The same timing sequence was maintained in the post-cryolipolysis phase. This arrangement is part of the described clinical protocol and should not be interpreted as a combination validated by a specific trial.

Plate-based cryolipolysis was programmed to −5°C using an antifreeze membrane. Areas with an adipose tissue fold exceeding 3 cm were treated for 50 minutes; areas with lesser thickness were treated for 40 minutes. For Patient 1, the plates were distributed across the abdomen and lateral regions; for Patient 2, they covered the abdomen, lateral regions, and dorsal region. The equipment brand and model were omitted, while the parameters necessary to describe the procedure were retained.

Forty-eight hours after cryolipolysis, both patients began eight additional FES sessions combined with intramuscular L-arginine on the same day, at a frequency of two sessions per week. Initial and final ultrasound measurements and the occurrence of adverse effects were recorded. Percentage change was calculated as: [(initial thickness − final thickness) / initial thickness] × 100.

III. RESULTS

Case	Cryo Regions	Initial (mm)	Final (mm)	Reduction
1	Abdomen and flanks	19.4	9.1	10.3 mm (53.1%)
2	Abdomen, flanks and dorsum	32.1	23.2	8.9 mm (27.7%)

Table 1. Ultrasound progression at the standardized point. Source: author's clinical records.

CASE DOCUMENTATION

In Patient 1, ultrasound-measured thickness decreased from 19.4 to 9.1 mm. In Patient 2, it decreased from 32.1 to 23.2 mm. Photographs illustrate body contour, but differences in clothing, lighting, and positioning limit metric comparisons; therefore, standardized ultrasound was adopted as the primary objective record. No adverse effects were observed during follow-up.

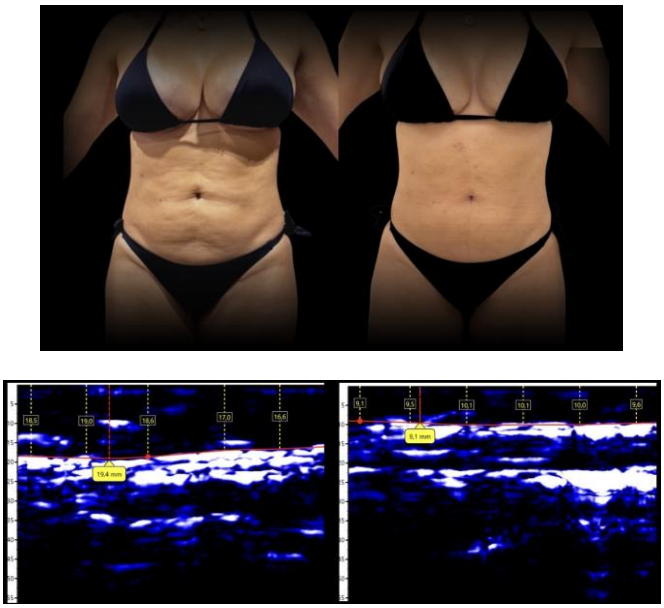

Figure 2. Patient 1: photographic and ultrasound documentation before and after the protocol (19.4 and 9.1 mm). Images from the author's clinical records, published with the patient's authorization.

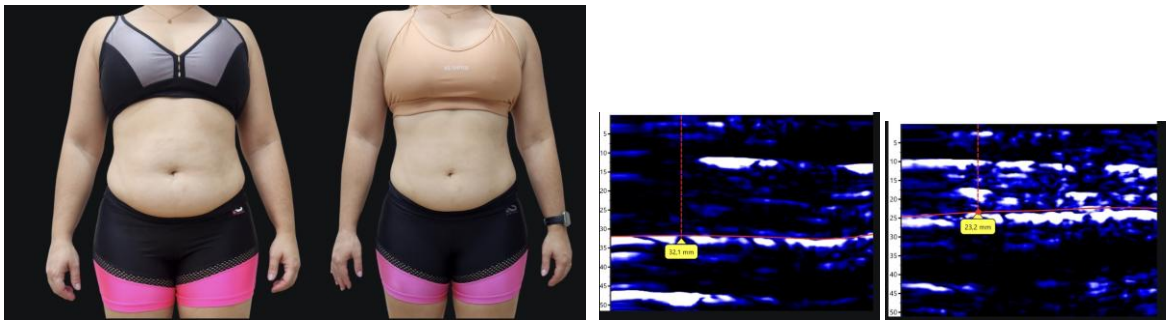

Figure 3. Patient 2: photographic and ultrasound documentation before and after the protocol (32.1 and 23.2 mm). Images from the author's clinical records, published with the patient's authorization.

IV. DISCUSSION

Both cases showed a reduction in ultrasound-measured thickness at the standardized point, with no adverse events observed. The greater percentage reduction in Case 1 should not be interpreted as indicating the protocol's superiority for a specific phenotype, as two observations do not allow for controlling variables such as initial thickness, adipose distribution, habits, anatomical variations, or other individual factors.

Cryolipolysis remained the central element of the treatment plan. Another relevant aspect described by Ruiz-Silva et al. concerns the possible functional conversion of white adipose tissue into a metabolically more active phenotype. Activation of the PERK–OGT–TOM70–MIC19 axis is thought to be associated with increased UCP1 expression and the formation of beige adipocytes, which are characterized by higher mitochondrial density and greater thermogenic capacity. Under the proposed hypothesis, this adaptation represents not merely a response to cold but a mechanism of metabolic remodeling capable of enhancing the oxidative capacity of the treated adipose tissue (Ruiz-Silva, Moleiro, & Ruiz-Silva, 2024).

This latter finding suggests that cryolipolysis influences the metabolic profile and differentiation of adipose tissue, though it does not describe the early kinetics of UCP1 during the initial days.

The cutaneous response also broadens the understanding of the procedure. Stevens et al. (2022) observed increases in type I procollagen, TGF-β, and HSP47—findings linked to fibroblast activity and collagen synthesis. Subsequently, in a study of 11 women evaluated between three days and five weeks post-cryolipolysis, Stevens et al. (2023) identified a 1.32-fold increase in HSP70 expression in the epidermis and a 1.92-fold increase in the dermis compared to untreated areas. HSP70 plays a role in protein protection and refolding following thermal stress and may contribute to extracellular matrix remodeling and the organization of structural elements, including collagen. Collectively, these studies support the view that cryolipolysis not only produces an adipocyte response but also triggers dermal adaptation and tissue remodeling.

Hydrolipoclasia was positioned as a local preparatory step due to its ability to distribute a liquid medium and enhance the mechanical effects of low-frequency ultrasound. Studies by Palumbo et al. (2011) and Pugliese et al. (2013) demonstrated adipocyte alterations in *ex vivo* human tissue—including in deep layers—that were amplified following saline infiltration. In a clinical context, a study by Ávila et al. (2025) documented a therapeutic regimen combining FES, movement, and hydrolipoclasia with 40 kHz ultrasound at 2.5 W/cm²; this served as a direct precursor to the multimodal approach expanded upon here with the addition of cryolipolysis. However, the laboratory and clinical conditions, timeframes, and study designs do not allow for the conclusion that hydrolipoclasia induced a specific amount of apoptosis beforehand or that it potentiated the effects of cryolipolysis.

FES was used to induce contractions in four major muscle groups and was combined with movement and loading starting in the third period. A frequency of 30 Hz was selected to balance contraction generation with fatigue control; comparative studies indicate that while higher frequencies may increase initial force, they also accelerate the decline in force (DREIBATI et al., 2010). The combination of stimulation and resistance exercise is supported by evidence regarding strength and muscle mass gains—albeit with a modest additive effect—though this research was conducted outside the context of cryolipolysis (NARVAEZ et al., 2025).

UCP1 expression is particularly significant as it characterizes mitochondrial thermogenic capacity. Unlike the classic brown adipocyte, the beige adipocyte exhibits high plasticity: under basal conditions, it may display low UCP1 levels and behavior similar to the energy-storage function of white adipose tissue; ...in response to thermogenic stimuli, it can increase UCP1, uncoupled respiration, and substrate utilization, becoming functionally similar to brown adipose tissue (WU et al., 2012). Therefore, it is not enough to consider only the formation or presence of beige cells; it is important to discuss which signals favor the maintenance of a metabolically active phenotype.

The temporal dimension of this response was demonstrated by Jankovic et al. (2015) in the retroperitoneal white adipose tissue of rats subjected to 45 days of continuous cold exposure. UCP1 mRNA levels peaked on the first day, while protein content remained elevated from day 1 to day 21, with the most pronounced expression observed on days 3 and 7. By day 45, UCP1 levels had returned to near-control values, and only a minority of adipocytes retained a brown-like phenotype. Thus, even under prolonged exposure, the initial thermogenic recruitment was intense but predominantly transient (JANKOVIC et al., 2015).

Although this experiment involved cold acclimation in rats rather than a clinical cryolipolysis session, it provides the temporal basis that guided the post-treatment protocol. The resumption of FES and L-arginine 48 The timing—hours after cryolipolysis—coincides with the start of the window during which UCP1 induction was most intense in the experimental model. The aim was to introduce metabolic stimuli during this initial phase and sustain them for four weeks, a period when thermogenic expression might otherwise progressively decline without appropriate maintenance. This interpretation does not rely on the claim that UCP1 levels are already reduced by the third day; rather, it views the initial days as an opportunity to support a response that tends to lose intensity over time.

Based on this rationale, FES was resumed 48 hours after cryolipolysis to induce repetitive muscle contractions and maintain a metabolic-muscular stimulus. Contraction transforms the muscle into a metabolically active organ that secretes myokines; notably, irisin has been linked to the activation of the thermogenic program and increased UCP1 levels in experimental models. However, exercise literature indicates variable myokine responses depending on the type and dose of the stimulus (BETTARIGA et al., 2024). Thus, in the proposed protocol, electrical stimulation was not used merely for local strengthening but as a tool to induce contraction in large muscle groups, thereby creating a physiological environment potentially conducive to maintaining an oxidative and thermogenic profile.

L-arginine, administered on the same day as FES, formed the second component of this maintenance strategy. In rats, L-arginine has been shown to enhance nitric oxide signaling, PGC-1α and UCP1 expression, and markers of beige adipocytes within white adipose tissue (KALEZIC et al., 2022). The clinical hypothesis, therefore, rests on a convergence of effects: cryolipolysis initiates adipocyte remodeling; muscle contraction provides a metabolic stimulus and potential myokine signaling; and L-arginine supplies substrate for the nitric oxide pathway associated with the thermogenic program. The aim of this post-cryolipolysis combination is to promote UCP1 expression and functional maintenance, seeking to ensure that tissue with "beige" characteristics retains an oxidative and thermogenic profile rather than a storage-oriented one. Since UCP1, irisin, and nitric oxide levels were not measured in the patients, this interpretation represents the mechanism proposed for the protocol and constitutes a hypothesis to be tested in comparative studies.

Key limitations include the small number of cases, the absence of a control group, the simultaneous combination of interventions, the lack of anthropometric measurements and lifestyle monitoring, and the inability to isolate the specific contribution of each modality. Furthermore, levels of UCP1, PGC-1α, irisin, caspases, collagen, or myokines were not measured in the patients. Thus, while these mechanisms explain the working hypothesis, they do not represent measured outcomes of this case series.

V. CONCLUSION

This case series demonstrated an ultrasound-confirmed reduction in adipose tissue thickness in two patients who underwent cryolipolysis as part of a multimodal treatment plan. This plan included local preparation via ultrasonic hydrolipoclasy and systemic-muscular preparation/maintenance using Functional Electrical Stimulation (FES) combined—on the same day—with intramuscular L-arginine. The protocol was carried out without observed adverse effects. The results are clinical and descriptive; they support the feasibility of this therapeutic approach but do not prove potentiation, synergy, or specific molecular mechanisms. Comparative trials involving larger sample sizes, standardized outcomes, and groups designed to isolate each component are required to test the hypothesis.

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