Comparative Study of Intradermotherapy With Pressurized Injection System and Needles

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Abstract
Intradermotherapy allows for treatment of different aesthetic conditions. Currently, it can be applied in its conventional form using needles or using the pressurized technique. The sample consisted of 35 women with adiposity located in the abdominal region. The volunteers were randomly assigned to 3 subgroups: G1 (n = 12) who were subject to the pressurized technique and the conventional technique with needles using only saline substance, with 6 volunteers in each application mode; G2 (n = 9), who received pressurized application using Concept Ti Liporedutor (lipolytic substance); and G3 (n = 14), who were treated with the needle injection technique, with the same substance used in G2. All groups received 4 treatment sessions with 2-week intervals between them. The analysis of the fat layer conducted 90 days after the initial application demonstrated a significant reduction in measurements in the treated groups, and also when compared with the control group, both for ultrasound, perimetry, and pliometry (only right side) data. Despite the effectiveness of the 2 application techniques, the pressurized method showed superior results. Hyperemia and skin marks were among the adverse reactions reported by the groups, but they showed quick resolution. It is noteworthy that most of the volunteers in the treated groups evaluated the results positively and were satisfied with the treatment. The intradermotherapy protocol with lipolytic substance significantly reduced the fat layer, with more evident results when using the pressurized application method.

Keywords
mesotherapy, localized fat, lipolysis

Introduction
Intradermotherapy, also known as Mesotherapy, is intradermal injections of diluted pharmacological substances that are given directly into the region to be treated. It was initially performed in the 1950s and has since become a treatment option for various conditions, including aesthetic dysfunctions, such as localized fat, wrinkles and expression lines, sagging, and cellulite, and results can be obtained by combining different substances.1 The mechanism that leads to the reduction of localized adiposity is related to the type of substance used and can be based on the activation of lipolysis or accidental cell death, with the latter being known as “ablative,” and involving cell swelling and coagulation of the cytoplasm.2,3

Currently, intradermotherapy can be applied in its conventional form using needles or using the pressurized technique. Traditionally, multiple intradermal or subcutaneous injections are applied using very fine gauge needles, directly on or near the affected sites. The pressurized intradermotherapy method, on the contrary, uses a needle-free technology, which aims to release the therapeutic substance in the skin or subcutaneous tissue using forces, gas pressures, and mechanical shock.
Some studies carried out indicate the effectiveness of the technique for treatment of localized adiposity without major adverse effects in both application methods when used separately. However, there is little information on how the techniques relate and how their effects compare when the same substance is used in the treatment of localized fat. In view of these gaps, it is necessary to conduct a study that confronts the 2 forms of application. Therefore, the objective of this study is to compare the effects of intradermotherapy with 2 forms of application mode; G2, formed by 9 volunteers, who received pressurized application using lipolytic substance; and G3, with 14 volunteers, who were treated with the needle injection technique, using the same substance as G2.

Materials and Methods

This is a randomized clinical trial. The research was approved by the Ethics Committee of Universidade Potiguar (code: 3.199.475) and was conducted in accordance with the recommendations of the Consolidated Standards of Reporting Trials—CONSORT (CONSORT TRANSPARENT REPORTING OF TRIALS, 2010). All volunteers signed an informed consent form before the study started. The groups’ allocation sequence was followed according to a list generated by the Software Research Randomizer™, and the volunteers were allocated according to the sequence in which they were evaluated.

Participants

The sample was composed of 35 women, aged 25 to 50 years, who had subcutaneous adiposity located on the supra and infraumbilical region, had body mass index (BMI) between 18.5 and 29.99 (Normal to Overweight), were sedentary, and were not using anti-inflammatory drugs up to 1 week before the beginning of the study. Prior to the study, we verified if any of the volunteers was allergic to the substances used.

Among the exclusion criteria applied were: the volunteer had to have an adipose layer thickness of 1 to 4 cm and could not be under food restriction (diets, nutritional re-education, or the like). Also, the participant who did not agree with the procedures, or did not adapt to the schedules and techniques, were excluded from the study.

The volunteers were randomly assigned to 3 subgroups: G1, with 12 volunteers, who were subject to the pressurized and needle injection techniques using only saline “therapeutic substance” (control group), with 6 volunteers in each application mode; G2, formed by 9 volunteers, who received pressurized application using lipolytic substance; and G3, with 14 volunteers, who were treated with the needle injection technique, using the same substance as G2.

Evaluation Procedures

All participants underwent anthropometric and ultrasound measurements that were performed pretreatment, 45 and 90 days after the start of applications (after 02 and 04 sessions, respectively). The equipment used included an ultrasound device (Eco Palm Wi-fi, 10MHZ, China), a semi-professional camera (Canon, SX530 HS, Japan), a measuring tape (Fiber Glass Tape, China), an adipometer (Sanny, São Paulo, Brazil), a scale (Accumed-Glicomed, Rio de Janeiro, Brazil), pressurized injection equipment (Comfort In™), injection equipment (4 mm 32G Lebel needles), Smart Gr™ brand, and the lipolytic substance (Mezzo Dermocosméticos™) composed of caffeine, carnivorous plant, Actigym™, and Lipoxyn™.

The instruments for data collection in this research were the Protocol for Physiotherapeutic Assessment in Localized Adiposity (PAFAL), an instrument used to obtain information such as identification, anamnesis, lifestyle, physical examination with measurements, and tests such as weight, height, BMI, skin folds, and waist circumference. The measurement of abdominal circumference was performed on the supra and infraumbilical regions, 5 cm above and below the umbilical scar. Skin folds were measured 3 times on the left and right lateral regions of the umbilical scar, 4 cm below, and the result was based on the average of the values obtained in the measurements. The volunteers’ body weight was also checked during treatment.

The photos were registered in orthostatism with anterior and lateral views (right and left), and the volunteer was asked to perform a 90° arm flexion during the photo. The photos were taken using a tripod and a neutral colored background for standardization. Subsequently, the volunteers underwent an ultrasound examination performed in the infra and supraumbilical regions, at 6 different points of analysis: 3 points located 4 cm above the umbilical scar, and 3 points located 4 cm below it. The distance between the points was approximately 5 cm, with the volunteer positioned in the supine position. This method allowed us to evaluate the thickness of the fat layer of the abdominal region, in a standardized manner and in centimeters, before the beginning of the procedures and after the proposed treatment.

Two days before the beginning of the procedures, the volunteers were asked to come to the treatment site for the predictive test of allergy to the substance. For this, they received an injection of 0.3 mL of the lipolytic active intradermally in the posterior region of the arm. The possible episodes of allergic reaction or irritation to the product could be identified by prolonged hyperemia, excessive pain and itching, and persistent edema. However, no volunteer had such reactions, with them reporting only that the region was sore after the test.

Treatment Protocol

The volunteers received 4 treatment sessions with 2-week intervals between them. During the treatment, all volunteers...
were placed in the supine position and the applicator injected 0.2 mL of the active substance or saline into the points of the supra and infraumbilical region, as shown in Figure 1, totaling 25 points with distance of 2 cm between them.

In the G1 group, a protocol was used to control the study using saline as a “therapeutic substance”: the 12 volunteers were equally divided, with the saline injected with the pressured application in one half, while in the other we used the traditional system with needles. The other groups received the application of the lipolytic substance, with G2 (n = 9) with pressured intradermtherapy, and G3 (n = 14) with intradermtherapy with needles. For all volunteers, before the beginning of the procedures, the abdominal region received antiseptic cleaning with gauze soaked in 70% alcohol, then the demarcation was performed, and immediately followed by the application of the active substance or saline.

The reassessments were performed 45 and 90 days after the beginning of the applications (respectively, after 02 and 04 sessions) with the repetition of all the procedures performed before the beginning of the treatment. Upon completion of the established protocol, the volunteers answered the patient satisfaction analysis questionnaires by Segot-Chicq et al.10 and the Global Aesthetic Improvement Scale (GAIS) by Narins et al.11 Through these questionnaires, we were able to classify the responses to treatments, allowing for comparison at different times after the therapeutic intervention.

Statistical Analysis

Analyses of statistical data were performed using the Statistical Package for the Social Sciences (SPSS) software version 22.0 for Windows. Ultrasound images and anthropometric measurements were analyzed to calculate the reduction of the fat layer, comparing the averages obtained before and after treatment, using the paired t-test for intra-group analysis, and the independent t-test for analysis between groups. In all statistical analyses, a significance level of 95% was assigned, with P < .05.

Results

The evaluation data (Table 1) indicated a small variation in the weight of the volunteers, although they did not show statistically significant differences (P > .05). The analysis of the supraumbilical perimeter data showed significant reduction when G2 was compared with the control group (P = .03); and when the comparison between the treated groups (P = .02) was performed, the average reduction in the G2 was 3.2 cm, while in G3 the average reduction was 3.1 cm. Similarly, the infraumbilical perimeter showed significant reduction when comparing G2 with the control group (P = .04) and in the comparison between the treated groups (P = .03), with average reduction of 3.5 cm for G2 and 3.9 cm for G3. The plicometry values, performed on both sides of the abdomen, showed significant reduction for the right side when comparing G2 to the control group (P = .01), as well as for G3 to the control group (P = .01). For the left side, although there was a reduction in the treated groups, this was not significant. No significant differences were observed in the perimeter values when comparing the groups treated with the lipolytic substance.

The values of the fat layer evaluated by ultrasound showed significant reduction in different groups. For the right side, the comparison between the groups treated with the lipolytic substance showed significant reduction (P = .01), with G2

Table 1. Volunteers Anthropometric Data.

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting weight</td>
<td>60.68 kg</td>
<td>67.27 kg</td>
<td>73.30 kg</td>
</tr>
<tr>
<td>Final weight</td>
<td>61.03 kg</td>
<td>64.25 kg</td>
<td>72.90 kg</td>
</tr>
<tr>
<td>Initial perimetry (S)</td>
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<td>78.6 cm</td>
<td>84.3 cm</td>
</tr>
<tr>
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<td>75.4 cm</td>
<td>81.2 cm</td>
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<tr>
<td>P value</td>
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<td>.02**</td>
<td></td>
</tr>
<tr>
<td>Initial perimetry (Lo)</td>
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<td>95.8 cm</td>
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<tr>
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<td>93.7 cm</td>
<td>85.3 cm</td>
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</tr>
<tr>
<td>P value</td>
<td>.04**</td>
<td>.03**</td>
<td></td>
</tr>
<tr>
<td>Initial plicometry (R)</td>
<td>2.93 cm</td>
<td>3.36 cm</td>
<td>3.36 cm</td>
</tr>
<tr>
<td>Final plicometry (R)</td>
<td>4.97 cm</td>
<td>2.05 cm</td>
<td>3.10 cm</td>
</tr>
<tr>
<td>P value</td>
<td>.01*</td>
<td>.01†</td>
<td></td>
</tr>
<tr>
<td>Initial plicometry (L)</td>
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<td>3.35 cm</td>
<td>3.57 cm</td>
</tr>
<tr>
<td>Final plicometry (L)</td>
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<td>3.07 cm</td>
<td>3.34 cm</td>
</tr>
<tr>
<td>P value</td>
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<td>.02†</td>
<td></td>
</tr>
<tr>
<td>Initial US (R)</td>
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<td>1.99 cm</td>
</tr>
<tr>
<td>Final US (R)</td>
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</tr>
<tr>
<td>P value</td>
<td>.01**</td>
<td>.02†</td>
<td></td>
</tr>
<tr>
<td>Initial US (L)</td>
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<tr>
<td>Final US (L)</td>
<td>1.96 cm</td>
<td>1.67 cm</td>
<td>1.64 cm</td>
</tr>
<tr>
<td>P value</td>
<td>.01*</td>
<td>.02†</td>
<td></td>
</tr>
</tbody>
</table>

Note. S = superior; Lo = lower; R = right; L = left; US = ultrasonography.

P value: *Comparison between intradermtherapy groups with needle and control group. †Comparison between the pressured intradermtherapy groups and the control group. **Comparison between the pressured intradermtherapy and intradermtherapy with a needle groups.
showing average reduction of 0.97 cm, while in G3 the average reduction was of 0.23 cm. Also, it was observed that this reduction was significant when comparing the G3 group to the control group ($P = .02$). For the left side, differences were also found in G2 in comparison to the control group ($P = .01$), as well as in the comparison of G3 to the control group ($P = .02$). For this side, the reduction of the fat layer in the G2 was on average 0.5 cm, whereas in G3 the reduction was 0.34 cm. The results of the treated groups were superior to that of the control group, and when comparing the techniques with the use of the lipolytic substance, the greatest reduction in the fat layer occurred when using the pressurized method.

Regarding ultrasound, below are 2 cases of volunteers who showed differences in both groups (pressurized method in Figure 2 and injectable method in Figure 3).

Regarding the adverse reactions reported by the volunteers (Table 2), hyperemia was observed on the treated area, with most of the control group noting the redness at some point, and only 10% of the group not identifying this reaction. In the pressurized intradermotherapy group, most of the volunteers were divided on whether they noticed the hyperemia at some point in the sessions or did not observe it (40%). For the group that used the application of the lipolytic substance with needles (G2), the vast majority did not report redness on the application area, with only 20% of this group noticing this reaction.

The photographic analysis showed the following physical changes in each group (Figures 4, 5, 6 and 7).

Despite rapid resolution, there was variation in the duration of the hyperemia. The volunteers in the control group experienced this reaction for a longer period, with the hyperemia lasting more than 3 hours in about 60% of them. For G2, the variation was quite heterogeneous, but 40% of the volunteers indicated that the duration was longer than 3 hours. The G3 group showed the fastest resolution of this reaction, with it disappearing right after the session or, at most, within 1 hour after the application.

Another adverse reaction observed were marks on the skin, which were present in all sessions in all groups studied, being observed in 90% of the control group, 70% of the G2 group, and 50% of the G3 group, respectively. When asked about pain after the applications, there was no significant difference between the volunteers’ opinions.

On the topic of global aesthetics improvement (Figure 8), most of the G2 group evaluated their response to treatment positively: about 50% of them felt that they had a “better” aspect compared with the initial moment, while 30% of them presented “unchanged” results. In the G3 group, the improvement in aesthetics was perceived as “much better” by half of the volunteers and only 10% presented “unchanged” results. None of the groups reported “worse” results at the end of the treatment.

When evaluating treatment satisfaction, 100% of the control group were not satisfied, while 60% of the volunteers in the pressurized intradermotherapy group were satisfied and 40% were not. The needle intradermotherapy group showed greatest satisfaction with the results, with 90% of
the volunteers having a positive opinion, and only 10% dissatisfied with the results obtained.

Discussion

The intradermotherapy technique is an alternative for a less invasive approach in the treatment of aesthetic dysfunctions, being considered effective in reducing the fat layer through the application of biocompatible substances that promote lipolysis. However, currently there are a large number of protocols or formulas used for treatment in this therapy, which makes comparative analysis of the results in its different forms of use difficult.\textsuperscript{12,13} In view of the gaps observed in the literature, this study compared 2 methods of application using the same formulation in all volunteers in the treatment groups.

The data evaluated 90 days after the initial application demonstrated a significant reduction in plicometry, perimetry, and ultrasound measurements when comparing the groups treated to the control group, and also in the analysis between the groups that used the lipolytic substance, showing greater reduction in the fat layer after 4 applications of intradermotherapy with both methods of application. Corroborating with Maia et al\textsuperscript{7} who analyzed the effects of pressurized intradermotherapy in the treatment of localized abdominal fat in 30 women, using the same substance as in this study. After 4 biweekly sessions, the results indicated a decrease in the fat layer analyzed through perimetry, plicometry, and ultrasound, when compared with the group that was treated only with saline solution.

The effectiveness of the traditional application method has also been elucidated in several studies, such as that by Song et al\textsuperscript{14} who used intradermotherapy application by the conventional needle method in 25 patients with fat located in the thigh region in 9 weekly sessions and noticed that there was a reduction in circumference on the treated area, although without significant change in weight and BMI throughout the study. Corroborating this study, where there was a small but not significant variation in the weight of the volunteers.
(P > .05), this suggests that the results found are related only to local treatment.

In this study, both methods of applying the therapy using the lipolytic substance showed better results than the control group, which received only the saline solution. However, when comparing the results of perimetry and ultrasonography only of the treated groups, we observed that the pressurized application method showed better results than the conventional application with needles, and this may have occurred due to the way the active substance was delivered. When needle application is used, the substance tends to be retained at the point where it was injected, with its dispersion reduced when compared with the pressurized application.

The pressurized application manages to deliver the doses in a standardized way, thus obtaining faster physiological responses thanks to the tissue spreading of the active substance, therefore avoiding undesirable effects caused by the manual technique. Another advantage reported is less painful applications because skin tension is kept stable while the substance is applied.15,16

The use of intradermotherapy to reduce the fat layer is based on the activation of lipolysis in adipose cells. For this, there are at least 3 general mechanisms that can amplify the effect, they are inhibition of phosphodiesterase or the adenosine receptor and activation of the β-adrenergic receptor or inhibition of the α-2 receptor, which will present varied

Figure 4. Group G1—Volunteer who received only saline solution using the conventional technique: (A) pretreatment and (B) after 90 days.

Figure 5. Group G1—Volunteer who received only saline solution using the pressurized technique: (A) pretreatment and (B) after 90 days.
results according to the place where it is applied and the chosen substance, with isoproterenol, aminophylline, caffeine, and yohimbine being usually used, alone or in combination. In addition to lipolytic stimulation, Intradermotherapy can be performed by the “ablative” type, described in the literature as the use of an “ionic detergent” that causes non-specific lysis in the adipose cell wall, where pharmacological substances such as deoxycholate sodium or phosphatidylcholine are used, although there is no standard protocol for this use.\textsuperscript{13,17,18}

More recent analyses have allowed for the development of different theories that try to explain the rupture of these cells when the “ablative” type is used. Among them is the mechanism of accidental cell death, also called by the term “oncosis,” due to cell injury and death being accompanied by cell swelling, bleeding, and increased membrane permeability caused by the failure of the plasma membrane ion pumps. This process is usually seen after cellular oxygen deprivation events or due to environmental toxicity, leading to a rapid decrease in intracellular ATP. This consequently leads to the destruction of the adipose cell, which will be replaced by scar tissue. Initially, it was assumed that the apoptosis mechanism would be responsible for the lipolysis process after the application of the substances; however, there is little scientific evidence that this mechanism has an important clinical effect in the process.\textsuperscript{5,13,19}
In this study, the combination of the substances used promoted the reduction of the fat layer by activating lipolysis. The product used is composed of caffeine, ADIPOTRAP™ (active substance derived from Sundew or Drosera Ramentacea), ACTIGYM™ (considered a marine active—Plankton extract), and LIPOXYN™ (tripeptide 41), which act in different ways to promote measurement reduction. However, the literature reports some disadvantages to this type of treatment, with the most commonly observed being the duration of the effects, which are usually transient and last only as long as the lipolytic threshold is reduced. Thus, when the injections stop, the fat tends to return to the normal distribution for these patients, although it is not yet clear how long this would take.

The use of these substances in clinical practice can lead to a reaction of hyperemia around each injection site, erythema in the treatment region, sometimes accompanied by itching and a burning sensation or transient pain that last around 15 to 30 minutes. It is noteworthy that between applications there may also be swelling in the area, especially during the first 24 to 48 hours after injection, although these reactions are considered expected after therapy. For Vega-López et al., intradermotherapy interventions should be performed analyzing the clinical context of patients, their particular characteristics or hypersensitivity, always taking into account the risk/benefit, to prevent the occurrence of unwanted reactions.

In this study, the presence of hyperemia was observed among the adverse reactions; however, there were fewer occurrences in the groups that used the lipolytic substance, especially in the volunteers that received the application by needles. This fact can be justified by the combination of the different active principles of the substances used, because caffeine can act as an anti-inflammatory, depending on the dose and concentration used. Brothers et al. demonstrated that treatment with caffeine attenuated inflammation induced by lipopolysaccharides (LPS) in neuroinflammatory models. Allied to this is also the presence of the active derivative of Sundew or Drosera Ramentacea, commercialized by the term ADIPOTRAP™ and Actigym™, considered a marine active substance (Plankton extract), which have different activities including anti-inflammatory action. It is noteworthy that a part of the group treated with pressurized application also observed hyperemia on the area; this may be due to the greater mechanical trauma generated during the application, which consequently promotes a greater inflammatory response.

Other adverse reactions reported in the literature include hyperpigmentation, persistence of irregular body contours, lower than expected fat reduction, localized infections, painful scars, and subcutaneous nodules in the injection area. The volunteers from all groups in this study observed the presence of marks on the skin in all sessions, which were related to the formation of bruises, due to the mechanical trauma caused by the application methods. These, however, presented spontaneous and relatively quick resolution, considering the individual characteristics of each volunteer. According to Duncan and Rotunda (2011), the hyperpigmentation caused by intradermotherapy is related to hemosiderin deposition and, in several cases, its presence is only temporary.
The intradermotherapy method has shown favorable results in the reduction of measurements. There is also a good receptivity and satisfaction of patients with this therapy, either when it is applied by the traditional technique, or by the pressurized technique, obtaining high approval rates in the questionnaires applied after the treatment. In this study, it was found that most of the volunteers in the groups treated with the lipolytic substance were satisfied with the treatment and indicated that the effects were positive, with the results most cited by them being: “better,” “much better,” and “a lot better.” Yet, a small portion of these groups was not satisfied with the results obtained, indicating that they were “unchanged,” although there was no report that the results were “worse” compared with the initial moment.

The use of the placebo methodology is essential for the correct analysis of the supposed effects of a therapy, and the technique is simulated in a similar way to the treated group. Therefore, the application of injectable saline solution was used to replace the lipolytic substance and we observed that, in addition to there being no reduction in the variables analyzed, 100% of the volunteers in this group were not satisfied with the results obtained. Therefore, we highlight that the positive influence normally observed by the placebo effect did not have any effect on the applied methodology. Nonetheless, in the literature, there are some cases in which the use of this methodology showed a positive interference in the results, as shown in the study by Rzany et al., who treated 363 patients with submental fat using injections with deoxycholic acid (ATX-101). The results were significantly better in the treated groups in relation to the control group, although a substantial placebo effect was also observed in this group, exceeding about 30% in some variables analyzed, mainly in the results analyzed by self-perception.

Conclusions

Intradermotherapy, with pressurized or conventional application with needles using the lipolytic substance, showed favorable results in reducing localized adiposity, causing a decrease in the fat layer analyzed, which was more evident after 90 days of the initial application, when compared only to the use of saline solution. However, in spite of promoting greater mechanical trauma and greater inflammatory process during applications, pressurized intradermotherapy associated with lipolytic substance showed better results in reducing measurements in the abdominal region in the perimeter and ultrasound conditions evaluated in this study, compared with the application of intradermotherapy with needles.

The limitations were presented by the impossibility of equitable distribution of volunteers in the proposed groups, which may have influenced the analysis of the variables. In addition, the results of this study were monitored for up to 15 days after the last application of the therapy, which made it impossible to know the duration of the effects found. Therefore, it is recommended that new studies are conducted with longer follow-up of the results, for further clarification on their behavior over time.

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