e-ISSN 3050-8460
Journal of Dermatology Research

ATHENAEUM SCIENTIFIC PUBLISHERS

Research Article

Clinical Effects of Ozone Therapy on Localized Adiposity: A Therapeutic Approach

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Citation: Lucas GCG, et al. Clinical Effects of Ozone Therapy on Localized Adiposity: A Therapeutic Approach. J Dermatol Res. 2026;7(2):1-8.

https://doi.org/10.46889/JDR.2026.7201

Received Date: 07-04-2026

Accepted Date: 18-05-2026

Published Date: 25-05-2026

Abstract

Localized adiposity represents an increasing demand in aesthetic clinical practice. Among the available therapeutic approaches, surgical procedures and minimally invasive techniques stand out. Ozone therapy has been investigated due to its potential to modulate oxidative stress in a dose-dependent manner. However, there is still no consensus regarding the optimal concentration for the reduction of subcutaneous adipose tissue.

The aim of this study was to compare three ozone concentrations (20, 30 and 40 µg/mL) in reducing abdominal adipose tissue thickness in adult men. This was a randomized clinical trial comparing different concentrations. Thirty healthy participants were allocated into three groups (GI: 20 µg/mL; GII: 30 µg/mL; GIII: 40 µg/mL).

The intervention consisted of eight subdermal sessions performed over four weeks, with standardized applications at 24 abdominal points spaced 5 cm apart. Evaluations were conducted before treatment and at 15, 30 and 60 days after its completion, including anthropometric measurements, perimeter measurements, bioimpedance analysis and ultrasonography to assess adipose tissue thickness.

A significant reduction in adipose tissue thickness over time was observed in all groups ($p < 0.05$), with an earlier response in GIII treated with 40 µg/mL. These findings suggest that ozone therapy exhibited a concentration-dependent effect on the rate of reduction of abdominal adipose tissue thickness.

Keywords: Ozone; O3 Therapeutic; Localized Adiposity; Body Fat Reduction

Introduction

Localized adiposity is associated with adipocyte dysfunction, as these cells are responsible for energy storage in the form of triglycerides. Under conditions of metabolic imbalance, adipocytes may undergo hypertrophy and/or hyperplasia, resulting in fat accumulation, frequently observed in regions such as the thighs, abdomen, gluteal area and arms. Functional alterations in the enzyme lipoprotein lipase may contribute to impaired lipid mobilization; however, when energy expenditure is lower than caloric intake, adipose accumulation becomes even more pronounced. This process is related to factors such as dietary habits, physical activity, genetic predisposition, endocrine alterations, sex and age [1-3].

Data from the Brazilian Ministry of Health indicate that approximately 51% of the Brazilian population is overweight, comprising 54% men and 48% women [4]. In addition to metabolic implications, excess adipose tissue may impact psychosocial aspects, including self-esteem and body image, factors directly associated with contemporary aesthetic standards [5]. Furthermore, in

men, localized fat is more compact with differences in the arrangement of the septa and is also associated with visceral fat, which increases cardiovascular risks. Therapeutic approaches for the treatment of localized surgical procedures include surgical procedures such as liposuction, which are considered effective but invasive and associated with risks including infection, edema, pain, deep vein thrombosis, pulmonary embolism and anesthetic complications [6]. Alternatively, minimally invasive or noninvasive techniques have been widely used, including radiofrequency, cryolipolysis, low-level laser therapy, ultrasound, electrostimulation and manual therapies, with the aim of inducing lipolysis or cellular apoptosis [5-7].

In this context, ozone therapy has been investigated as a potential therapeutic approach due to its ability to modulate the balance between oxidant and antioxidant systems. Ozone, generated from medical oxygen through controlled electrical discharge, exhibits affinity for polyunsaturated fatty acids present in cell membranes, inducing the formation of reactive oxygen species and Lipid Peroxidation Products (LPPs) [8-11]. The magnitude of the oxidative stress generated is dependent on the concentration applied and may activate distinct biochemical pathways, such as nuclear factor erythroid 2–related factor 2 (Nrf2), associated with antioxidant modulation and nuclear factor kappa B (NF-κB), associated with inflammatory responses [9-13]. Thus, while high levels of oxidative stress may result in cellular damage, moderate levels may trigger biological responses with potential therapeutic effects.

Despite the molecular mechanisms described in the literature, there is still a lack of clinical evidence establishing objective parameters regarding the optimal ozone concentration for the reduction of localized fat, as well as the magnitude and timing of the therapeutic response. The absence of standardized protocols limits the consolidation of this technique in aesthetic clinical practice. However, due to the high incidence of overweight individuals, with a higher prevalence in men, this therapeutic approach may be an interesting strategy to reduce the accumulation of localized fat, directly impacting the quality of life of individuals.

Therefore, the present study aimed to compare the effects of three different ozone concentrations (20, 30 and 40 μg/mL) on the reduction of abdominal adipose tissue thickness in male individuals, as well as to analyze the temporal response to treatment over a 60-day follow-up period.

Methodology

Study Design and Setting

A randomized clinical trial with three parallel arms was conducted to compare different ozone concentrations. The study was carried out at the Clinical Research Institute of UNIFAE (IPECLIN), located in São João da Boa Vista, São Paulo, Brazil. The research protocol was approved by the Research Ethics Committee under approval number 4.962.021.

Participants

Participants were screened at the Clinical Research Institute of UNIFAE (IPECLIN), located in São João da Boa Vista, São Paulo, Brazil. All individuals were informed about the objectives, risks, benefits and methodology of the study and were free to decide whether or not to participate without any coercion or modification to their usual treatment, as stated in the Informed Consent Form (ICF). A total of 30 healthy male participants presenting complaints of localized adipose tissue accumulation in the abdominal region were included in the study. Participant randomization was performed using simple randomization, characterizing a simple randomization process. Each participant was randomly allocated to one of the study groups through manual randomization, using previously identified slips of paper placed in an opaque container, ensuring unpredictability in the allocation. The procedure was conducted by an independent researcher, guaranteeing the concealment of the allocation sequence and minimizing selection biases. Therefore, the number of participants was determined based on operational feasibility and the exploratory nature of the investigation. The following exclusion criteria were established: pregnancy; Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency; age below 18 years or above 65 years; previous facial or body plastic surgery in the treatment area; use of home-care dermocosmetics or undergoing other dermatological procedures; presence of hemophilia or bleeding disorders; diagnosis of diabetes mellitus or uncontrolled metabolic disorders; having undergone aesthetic treatment in the target area within the previous three months; presence of skin lesions such as dermatitis or dermatoses; malignant lesions on or near the treatment area; presence of a cardiac pacemaker or other implanted electronic device; refusal to sign the informed consent form; absence from two consecutive treatment sessions; or initiation of anti-inflammatory treatment or use of dermocosmetics during the study period. Participants were instructed to maintain their usual lifestyle habits, including dietary

patterns and level of physical activity. No dietary interventions or behavioral modifications were implemented during the follow-up period.

Clinical and Experimental Procedure

To ensure the safety of all individuals involved in the research, the treatment areas were previously disinfected with 5% chlorhexidine and the professionals used the necessary biosafety measures (such as gloves, protective eyewear, sterile needles and syringes) to prevent potential contamination risks.

Experimental Groups

Participants were instructed to maintain their habitual physical activity and dietary patterns throughout the intervention period. After the evaluation of the previously listed criteria, the participants were randomized into three distinct groups:

- *Intervention Group I (GI):* Participants received ozone therapy at a concentration of 20 μg/mL, with a volume of 5 mL per treatment point. A distance of 5 cm was maintained between treatment points
- *Intervention Group II (GII):* Participants received ozone therapy at a concentration of 30 μg/mL, with a volume of 5 mL per treatment point. A distance of 5 cm was maintained between treatment points
- *Intervention Group III (GIII):* Participants received ozone therapy at a concentration of 40 μg/mL, with a volume of 5 mL per treatment point. A distance of 5 cm was maintained between treatment points

Application Method

For ozone application, the Ozion equipment, developed and manufactured by the Brazilian medical equipment industry – IBRAMED, was used. Ozone generation is controlled by an internal system of the equipment. Thus, medical oxygen passes through an electrical discharge generator, which promotes the generation of O_3, according to the flow necessary for the desired concentration, in an inversely proportional ratio, that is, the higher the concentration, the lower the oxygen flow.

For clinical application, ozone therapy was administered subcutaneously in the abdominal region, with standardized distribution of application points along the umbilical line, including one row positioned 5 cm above and another 5 cm below this reference. The application area was laterally delimited up to the region corresponding to the femoroacetabular joint, forming three horizontal lines and four vertical lines of points, with uniform spacing of 5 cm between them.

The application technique followed principles commonly used in infiltration protocols employed in carboxytherapy, using a sterile syringe and a 30G ½ needle. Infiltrations were performed with needle insertion at a 90° angle relative to the skin surface. The treatment was performed twice a week for four consecutive weeks, totaling eight sessions.

Data Collection

Assessments

Participants underwent the following assessments: medical history of the treatment area, abdominal circumference measurement, skinfold thickness measurement (adipometry), bioimpedance analysis, standardized photographic documentation, ultrasonographic measurement of adipose tissue thickness and administration of pain and satisfaction questionnaires. Ultrasonographic measurement of adipose tissue thickness was considered the primary outcome of the study. The remaining variables were considered secondary outcomes. Data collection was performed in the morning by the same previously trained evaluator, who was blinded to participant allocation, at the following time points: before the start of treatment and at 15, 30 and 60 days after completion of the sessions. Ultrasonographic measurements were obtained using standardized anatomical landmarks and acquisition procedures in all evaluations. In addition, the reviewers were blinded to previous images, patient identification and the temporal sequence of image acquisition, minimizing potential assessment bias. Although formal intra- and inter-observer reliability analyses were not performed, the use of a single trained evaluator and standardized measurement procedures was adopted to reduce measurement variability. For standardization of the measurements, two anatomical points were defined on each hemiabdomen: (1) a superior point, located 5 cm lateral to the linea alba and 5 cm above the umbilical scar; (2) an inferior point, located 5 cm lateral to the linea alba and 5 cm below the umbilical scar. Measurements were performed bilaterally (right and left sides).

Medical History of the Treatment Area

A standardized medical history assessment was conducted, including personal information, lifestyle habits, medication use, dietary pattern, inspection of the treatment area, history of previous aesthetic procedures and skin characteristics, in order to verify the eligibility criteria of the study.

Abdominal Circumference

Abdominal circumference was measured using an inelastic measuring tape positioned at the level of the umbilical scar, with the participant in the orthostatic position and breathing normally.

Bioimpedance

Bioimpedance analysis was performed to determine body weight and body composition (lean mass, fat mass and total body water) using an InBody® device. Height was measured with a stadiometer for insertion into the device's software. The participant remained in an orthostatic position, with feet placed on the platform electrodes and upper limbs extended while maintaining contact with the device's hand electrodes.

Photographic Analysis

Photographic documentation was performed with the participant positioned 70 cm from the wall and 1.5 m from the camera, standing upright and looking toward the horizon. Images were captured using a smartphone camera (iPhone 16) mounted on a tripod at 1.05 m from the floor, centered relative to the participant, with standardized zoom. Lighting conditions were kept constant, using a central white light source and a blue background.

Ultrasonography

The thickness of the subcutaneous adipose tissue layer was measured by ultrasonography using a linear transducer (6–18 MHz) (MyLab™ 25 Gold, ESAOTE, Italy) and VPAN® software. The participant was positioned in the supine position and images were obtained at the previously defined anatomical points, considering both the upper and lower abdomen. The reviewers were blinded to the previous data and patient analysis, ensuring that the reviewers did not have access to the subjects' identification or the temporal sequence of the images.

Patient Tolerance to Treatment

Pain perception in the treated area was evaluated using a subjective 0–10 visual analog scale, where: 0 = no pain or warmth, 1–4 = mild pain or warmth, 5–7 = moderate pain or warmth and 8–10 = intense pain or warmth.

Patient Satisfaction Assessment

Participant satisfaction regarding treatment results and comfort in the treated area was assessed using a subjective 1–5 scale, where: 1 = very dissatisfied/very uncomfortable, 2 = dissatisfied/uncomfortable, 3 = no difference/no opinion, 4 = satisfied/comfortable, 5 = very satisfied/very comfortable.

Adverse Events

Participants were monitored for the occurrence of adverse events throughout the entire study period. Local erythema and hematoma were considered expected events resulting from the application protocol. The presence of persistent severe pain, marked local inflammation or cutaneous lesions was classified as a serious adverse event.

Results

The results of this study demonstrated a reduction in localized fat in all treated groups. The mean age among the groups, presented in Table 1, did not show substantial variation, particularly between groups GI and GIII. Regarding the level of physical activity, the majority of participants reported being physically active. However, group GI showed greater homogeneity, with 100% of participants reporting daily physical activity. The predominant type of activity across all groups was resistance training.

Group	N	Age (years), mean ± SD	Physical activity (%)	Frequency	Main activities
GI	7	37.0 ± 10.03	100	Daily	Resistance training, handball, running
GII	6	27.5 ± 5.21	67	3–6 times/week	Resistance training
GIII	10	34.0 ± 6.91	90	2–6 times/week	Resistance training, CrossFit, functional training, swimming
SD: Standard Deviation. Note: Participants were classified as physically active when reporting engagement in structured physical exercise at least 2 times per week, in accordance with general physical activity recommendations for health. Participants were classified as physically active when meeting the minimum recommendations of at least 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity physical activity per week.					

Table 1: Sample characterization according to age and physical activity level.

Bioimpedance

In the bioimpedance assessment, only the fat mass variable showed statistically significant changes. In group GII, a significant reduction was observed when comparing baseline with 15 days ($p = 0.014$), as well as between baseline and 30 days ($p = 0.018$). However, at 60 days, the values returned close to baseline, indicating a non-sustained effect over the follow-up period. In group GIII, a significant reduction was observed between baseline and 15 days ($p = 0.009$) and between baseline and 30 days ($p = 0.027$), without maintenance of statistical significance at 60 days. In group GI, no statistically significant differences were observed at any of the evaluated time points. The remaining variables (body weight, BMI, lean mass and total body water) did not show significant differences over time ($p > 0.05$) (Table 2).

Group	Variable	Before (Mean ± SD)	15 days	30 days	60 days	Significant comparisons ($p < 0.05$)
GI (20 μg/mL)	Fat (kg)	16.57 ± 2.08	16.61 ± 2.50	16.86 ± 2.41	16.96 ± 2.80	None ($p > 0.05$)
GII (30 μg/mL)	Fat (kg)	20.10 ± 2.56	19.08 ± 3.32	19.10 ± 3.44	20.62 ± 3.08	Before vs 15d ($p = 0.014$) Before vs 30d ($p = 0.018$) 15d vs 60d ($p = 0.021$)
GIII (40 μg/mL)	Fat (kg)	33.13 ± 7.99	30.81 ± 9.37	32.39 ± 10.33	32.80 ± 9.48	Before vs 15d ($p = 0.009$) Before vs 30d ($p = 0.027$)

Table 2: Mean ± SD: Average value ± standard deviation. p-value: Probability that the difference occurred by chance. $p < 0.05$: Statistically significant difference. Highlighted rows: Only Fat mass in GII and GIII showed significant changes (temporary reductions at 15–30 days). Other variables: No significant differences detected.

The analysis demonstrated a significant time effect, indicating a progressive reduction in the mean thickness of adipose tissue throughout the follow-up period. A significant Time × Group interaction was also observed, indicating that the magnitude and rate of response differed according to the concentration used. Group GIII showed a significant reduction as early as 15 days after treatment, whereas groups GI and GII demonstrated more gradual reductions over the evaluated period. Nonparametric sensitivity analyses (Friedman test and paired Wilcoxon test) confirmed these findings, showing significant differences between baseline and the 60-day evaluation in all groups. Regarding global body composition variables, a transient reduction in fat mass and abdominal circumference was observed, with greater impact at 15 and 30 days after treatment. However, total body weight and lean mass remained stable throughout the follow-up period.

Adipometry

In the adipometry analysis, group GIII showed significantly greater mean reductions across all abdominal regions, with p-values < 0.05 and moderate to large effect sizes, whereas GI did not show relevant differences. GII demonstrated intermediate reductions, although these were less consistent (Table 3).

Group	n	Baseline (mean ± SD)	15 days (mean ± SD)	30 days (mean ± SD)	60 days (mean ± SD)	p-value*
GI	7	3.31 ± 0.58	3.29 ± 0.69	3.14 ± 0.58	3.36 ± 0.75	>0.05
GII	6	3.17 ± 0.58	3.08 ± 0.85	3.25 ± 1.18	2.97 ± 0.62	<0.05
GIII	10	4.38 ± 0.76	4.52 ± 0.58	4.34 ± 0.96	3.68 ± 0.64	<0.05
SD: Standard Deviation. *p-value: Estimated based on temporal trend described; ideal calculation via repeated measures ANOVA.						

Table 3: Adipometry outcomes (abdominal superior right region) over time according to treatment group.

The GI group demonstrated minimal variation in mean adipometric values over time, accompanied by relatively high standard deviations, indicating considerable intra-group heterogeneity and lack of a consistent treatment effect. In the GII group, a modest reduction was observed at 60 days; however, the increase in standard deviation at the 30-day time point (SD = 1.18) suggests substantial inter-individual variability in response. In contrast, the GIII group exhibited the greatest absolute reduction in adipometric measurements (from 4.38 to 3.68), with moderate standard deviations, reflecting a more homogeneous and clinically meaningful response. Collectively, these findings support a dose-dependent effect, with higher concentrations associated with more consistent and pronounced reductions.

Ultrasound Imaging

The mixed ANOVA analysis demonstrated a significant Time effect ($p < 0.001$), indicating a progressive reduction in the mean thickness of abdominal adipose tissue throughout the follow-up period. A significant Time × Group interaction was also observed ($p < 0.01$), indicating that the trajectory of reduction differed according to the ozone concentration used. The GIII group showed a statistically significant reduction as early as 15 days after completion of treatment, whereas the GI and GII groups demonstrated progressive reductions, with statistical significance observed at later time points, particularly between 30 and 60 days. Nonparametric sensitivity analyses (Friedman and paired Wilcoxon tests) confirmed these findings, showing significant differences between baseline and the 60-day evaluation in all groups. The graphical representation of the mean trajectories (Fig. 1) demonstrates a distinct response pattern among the groups, with an earlier reduction in GIII, an intermediate response in GII and a more gradual reduction in GI, with the decrease in adipose thickness maintained until the end of the follow-up period. The analysis of the magnitude of reduction showed that the GI group presented the greatest final percentage reduction in adipose thickness, whereas the GIII group demonstrated the greatest absolute reduction and the earliest response to treatment. The GII group presented intermediate values in both magnitude and speed of response. These findings indicate differences in the response dynamics among the evaluated concentrations, with variations in both the rate and magnitude of reduction throughout the follow-up period.

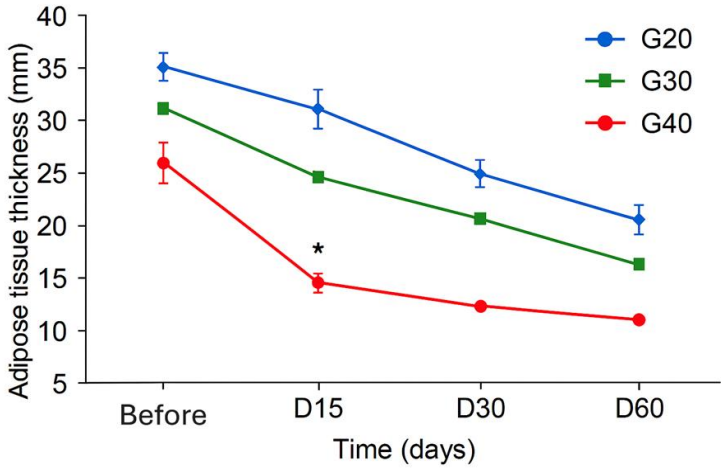

Figure 1: Trajectory of adipose tissue thickness over time in response to ozone therapy at different concentrations. Mean values ± 95% confidence intervals for each group (GI = 20 μg/mL, GII = 30 μg/mL, GIII = 40 μg/mL) across four time points: baseline (Before), Day 15 (D15), Day 30 (D30) and Day 60 (D60). The GIII group showed a rapid and statistically significant reduction in adipose tissue thickness by Day 15 ($p < 0.05$), with stabilization thereafter. GII exhibited a moderate decline, more pronounced between Day 30 and Day 60, while GI demonstrated a gradual and continuous reduction. Asterisks (*) indicate significant differences compared to baseline ($p < 0.05$).

Adverse Events

No serious adverse events were recorded during the study period. However, erythema, hematoma and localized pain were observed; these were considered transient and resolved shortly after completion of the treatment protocol, with no significant sequelae.

Patient Satisfaction Assessment

Most patients reported being satisfied with the treatment protocol as well as with the outcomes achieved, which were classified as grade 4 on the evaluation scale. However, two patients reported no perceived change in localized fat reduction following the treatment.

Discussion

Minimally invasive therapies aimed at reducing localized fat have been widely investigated as alternatives to surgical procedures, as they are associated with shorter recovery times and a lower risk of complications. In this context, ozone therapy has increasingly been incorporated into clinical practice in different areas of medicine, with a history of use in inflammatory processes and tissue regeneration. In the present study, a significant reduction in abdominal adipose tissue thickness was observed across all concentrations evaluated over the 60-day follow-up period. However, the dynamics of the response differed among the groups. The concentration of 40 μg/mL promoted an earlier reduction, with statistically significant differences already observed at 15 days, whereas the 20 and 30 μg/mL concentrations showed a more gradual reduction throughout the evaluation period.

From a biological perspective, the mechanisms of action of ozone are related to the modulation of the balance between oxidant and antioxidant systems. Its interaction with polyunsaturated fatty acids present in cell membranes leads to the formation of Reactive Oxygen Species (ROS) and lipid peroxidation products, which may act as modulators of cellular signaling pathways. The magnitude of this response depends on the concentration used and may result either in an adaptive antioxidant stimulus or in more pronounced oxidative stress. Although the GI presented the greatest final percentage reduction, the GIII demonstrated the greatest absolute reduction and a faster temporal response. These findings suggest that concentration may influence the rate of therapeutic response, even though the final percentage magnitude of reduction was not linearly proportional to the dose.

Conclusion

It is important to highlight that, although the literature suggests that higher levels of oxidative stress may induce cellular apoptosis in certain contexts, the present study did not directly evaluate histological or molecular markers of apoptosis. Therefore, any mechanistic inference should be interpreted with caution, being limited to the hypothesis that oxidative modulation may be involved in the observed reduction. It should also be considered that the observed reductions in adipose tissue thickness may not necessarily represent true adipocyte loss. Part of the findings may reflect transient physiological alterations, including local fluid redistribution, tissue hydration changes, inflammatory responses or inherent variability of ultrasonographic and anthropometric measurements. Since the present study did not include histological analysis, metabolic biomarkers or advanced imaging methods capable of confirming adipocyte reduction, the results should be interpreted cautiously. Therefore, these findings may represent short-term tissue changes rather than definitive structural reduction of adipose tissue. Experimental studies, such as that of Cisterna, et al., demonstrated that low ozone concentrations may activate antioxidant pathways in *in-vitro* adipocyte models, suggesting a possible cytoprotective effect under certain conditions. The divergence between experimental and clinical findings may be related to differences between *in-vitro* models and the *in-vivo* tissue environment, which involves more complex metabolic, inflammatory and structural factors. Despite the promising results, this study presents important limitations. The absence of a placebo control group prevents the exclusive attribution of the observed effect to the intervention. In addition, it was not possible to fully control participants' dietary habits and physical activity levels throughout the follow-up period. The relatively small sample size also limits the generalization of the findings. Therefore, future studies including a larger number of participants, the inclusion of a control group and the evaluation of biological markers may contribute to a better understanding of the mechanisms involved and to the consolidation of evidence-based clinical protocols.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding Statement
This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Acknowledgement
The authors have no acknowledgments to declare.

Data Availability Statement
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement
The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations, and therefore was exempt.

Informed Consent Statement
Informed consent was obtained from all participants included in the study.

Authors' Contributions
All authors contributed equally to this paper.

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