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# Subcutaneous Infiltration of Carbon Dioxide (Carboxytherapy) for Abdominal Fat Reduction: A Randomized Clinical Trial

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**Capsule Summary:**

- Patients have shown an increasing preference for more non-invasive fat reduction options.
- Five weeks after study initiation and one week after the fifth treatment session, carboxytherapy reduced abdominal fat more than sham treatment, but there was no difference at 28 weeks.
- This study showed only a transient benefit for carboxytherapy.

**ABSTRACT**

**Background:** Non-invasive fat removal is preferred because of decreased downtime and lower perceived risk. It is important to seek new non-invasive fat removal treatments that are both safe and efficacious.

**Objective:** To assess the extent to which carboxytherapy, the insufflation of carbon dioxide gas into subcutaneous fat, results in reduction of fat volume.

**Methods:** Randomized, sham-controlled, split-body study. Adults (BMI 22-29) were randomized to receive five weekly infusions of 1000 cc $CO_2$ to one side of the abdomen, and five sham treatments to the contralateral side. Primary outcome measures were ultrasound measurement of fat layer thickness, as well as total circumference before and after treatment.

**Results:** Sixteen participants completed the study. Ultrasound measurement indicated less fat volume on the sides treated with carboxytherapy one week after the last treatment, ($p=0.011$), but was not maintained at 28 weeks. Total circumference decreased nominally but not significantly at Week 5 compared to baseline ($p=0.0697$). Participant body weights did not change over the entire course of the study ($p=1.00$)

**Limitations:** Limitations included modest sample size and some sources of error in circumference and fat layer measurements.

**Conclusion:** Carboxytherapy provides a transient decrease in subcutaneous fat that may not persist. Treatment is well-tolerated.

**Key words:**

Carboxytherapy, fat reduction, noninvasive fat reduction, subcutaneous fat, fat volume

**Trial Registration and ID:** Clinicaltrials.gov NCT00974415

https://clinicaltrials.gov/ct2/show/NCT00974415

## INTRODUCTION

Non-invasive fat reduction has become increasingly sought-after by patients. Major benefits of a non-invasive approach are diminished downtime, avoidance of scarring, and perceived safety. Current technologies routinely used for non-invasive fat reduction include cryolipolysis, high intensity ultrasound, radiofrequency, chemical adipocytolysis, and laser-assisted fat reduction. A less well-established technology is subcutaneous infiltration of carbon dioxide, or carboxytherapy.[1-16]

Carboxytherapy has been performed primarily outside the US, with a small body of clinical studies suggesting it may provide a lasting improvement in abdominal contour.[1-6] The mechanism of action of carboxytherapy is not well understood. It is believed that injection of carbon dioxide induces changes in the microcirculation, including capillary transcutaneous oxygen tension ($tcPO_2$), with this damaging lipocytes through an oxidative effect.[5] There is direct histological evidence of adipocytolysis following carboxytherapy.[5] However, there are no randomized controlled trials for carboxytherapy efficacy and the benefit over time. The purpose of this study was to assess the effectiveness of carboxytherapy for fat reduction in a randomized, controlled trial, and to determine if any observed benefits persisted for six months.

## METHODS

Trial Design: This was a single center, balanced [1:1] randomization, double-blind, sham-controlled, split-body study conducted in the US. There were no changes after study commencement. The study was approved by the Northwestern University IRB, and registered

with ClinicalTrials.gov (NCT00974415). Reporting is in accordance with CONSORT 2010 and CONSORT-NPT for non-pharmacologic treatment interventions.

Participants: Eligible participants in good general health, at least 18 years old, and with body mass index (BMI) 22-29, who agreed to maintain their weight within five pounds of screening weight, while avoiding changes in diet or exercise. Participants were excluded if they: were pregnant or lactating; had received or were about to undergo other procedures in the treatment area; had changed diet or exercise regiments in the preceding six weeks, or had taken diet pills within six months; had a history of asthma, chronic obstructive pulmonary disease, or hematologic disorders; had active skin disease or infection in the treatment area; or were allergic to lidocaine.

Participants were recruited through announcements posted at Northwestern University and Northwestern Medicine facilities, other local Chicago areas that provided permission, and the Internet. The setting was the Dermatology Clinic of the Northwestern Medical Group in Chicago, IL. All participants provided written informed consent.

Interventions

*Microtattooing*: Two microtattoos (SteriTatt® ink) were placed on each flank. Each pair of microtattoos were, respectively, on the anterior and posterior flanks, horizontally aligned with the umbilicus and spinal vertebrae, 6 inches apart, with each tattoo three inches from the lateral extent of the flank. The skin was anesthetized with <1 cc of 1% lidocaine with 1:100,000 epinephrine before microtattoo placement.

*Ultrasound Imaging*: Controlled ultrasound images were obtained from the flanks using tattoo marks as reference. An ultrasound imaging system (GE LogiQ P6 Ultrasound System, GE Healthcare, Wauwatosa, WI) was used, in combination with a linear matrix array (6 – 15 MHz), with a 50 mm field of view. Care was taken to acquire the same image planes at baseline, and each of the follow up visits. For standardization of all image data, machine settings were kept unchanged, following the initial optimization of image quality at the beginning of the study.

*Carboxytherapy Treatment:* The flank assigned to the experimental arm was cleansed with topical chlorhexidine gluconate 4%. The needle insertion site, at the edge of each flank, midway between the two ipsilateral microtattoos, was anesthetized with <1 cc of 1% lidocaine with 1:100,000 epinephrine placed intradermally. One thousand mL of carbon dioxide were slowly insufflated by a tube-pump apparatus (Carbomed unit, CarbossiUSA, Italy) at a rate of 50 mL/min with a 26-gauge, 1.5 inch needle into the mid-to deep subcutaneous tissue. The personnel administering the $CO_2$ massaged the area with the other hand as the gas was insufflated.

*Sham Treatment:* On the contralateral flank, chlorhexidine was applied, anesthetic was injected, and the needle insertion site determined exactly as in the carboxytherapy treatment arm. As in the active treatment arm, the needle was inserted into the mid- to deep subcutis, but this was not connected to the tube-pump apparatus. The pump unit was turned on but the $CO_2$ was flowing into the room rather than through the needle. The investigator massaged the area where the needle was inserted.

*Standardization of Interventions and Adherence to Intervention Protocols:* Experimental and sham interventions were standardized by: (1) pre-study training on carboxytherapy treatment procedures during a pilot study of five patients (unpublished); (2) use of the same study team (N.V., W.D.) for all injection procedures. Adherence to injection protocols was enhanced and assessed by (1) development of a detailed standard operating procedure manual; and (2) live monitoring of the delivery of interventions by an investigator responsible for ensuring correct procedure but uninvolved with hands-on procedures (D.S., A.G.).

Objectives: The purpose of this study was to assess whether carboxytherapy can reduce the volume of subcutaneous fat.

Outcomes

*Primary Outcome Measures:* There were two co-primary outcome measures: (1) the thickness of the subcutaneous fat layer of each flank, as measured by ultrasound evaluation between the two microtattoos using a plastic cut-out template aligned with the microtattoos that contained slots for placement of the ultrasound tip to allow precise localization (slots were: superior, middle, inferior, anterior and posterior on the flank); and (2) total circumference, measured by a non-compressible, non-stretchable filament along the hypothetical line connecting the midpoint of the navel and the midpoint of the skin overlying the corresponding spinal vertebrae, while passing through the two ipsilateral flank microtattoos. Ultrasound images were obtained by a technician specifically trained in the use of skin ultrasound technique (D.S., A.G.), and images were subsequently interpreted by a research scientist expert in skin and superficial tissue ultrasound (I.M.)

*Secondary Outcome Measures:* There were three secondary outcome measures: (1) skin elasticity on each side, measured using a dermal torque meter (Dia-stron Ltd., Andover, UK); (2) participant reported pain on a 10-point VAS; and (3) participant satisfaction. Body weight was measured as a potential confounder.

*Intervention, Measurement and Assessment Schedule:* At the screening visit, those who met inclusion criteria and provided written informed consent, were randomized; microtattoos were placed; weight was recorded; and circumference, fat layer thickness, and elasticity of each flank were measured. Thereafter, five paired active and sham treatments were delivered to each participant once per week (i.e., Weeks 0-4). Weight and circumferences were measured prior to each treatment and at each follow-up visit (Weeks 5, 16, and 28). Ultrasound images were obtained before and after treatments one, three, and five (Weeks 0,2,4), and at each follow-up visit. Skin elasticity was measured at each follow-up visit. Participant pain scores were elicited after each treatment, and participant satisfaction at each follow-up visit. Standard photographs (not an outcome measure) were obtained at all visits. There were no changes to outcomes after the trial was commenced.

Randomization

*Sequence Generation and Type:* Random number generating software was used. *De facto* blocking occurred since in this paired, split-body design, each participant received both treatments, one per flank, with the side not being assigned active carboxytherapy receiving sham treatment.

*Allocation Concealment Mechanism:* Each pair of numbers (each of which was a zero or one) produced by the randomization process was recorded on a notecard and sealed in an opaque,

sequentially numbered envelope. The first number in each pair signified whether the active carboxytherapy treatment was to be delivered to the right flank (zero), or the left flank (once). The second number determined whether the active treatment would be delivered first (zero) or following the sham treatment (one). The sequence was concealed until interventions were assigned, with envelopes secured in a locked cabinet.

*Implementation:* The allocation sequence was generated by (N.V.), participants were enrolled by (D.S.), and participants were assigned to their groups by (I.A.).

*Blinding (masking):* Participants as well as those assessing the outcomes were were blinded as to group assignment. Those administering interventions were not blinded.

Participants were masked by the application of opaque and occlusive metal goggles prior to the start of interventional procedures. The two interventions were similar in terms of the: (1) positioning of the body; (2) anatomic site (flank) treated; (3) method of marking and microtattooing; (4) preprocedure skin prep and anesthetic injection; (5) length and caliber of the needle inserted, and its depth; (5) procedure duration, including the duration of needle insertion into the subcutis; (6) conversation, instructions, and information provided by the team; and (7) ambient sounds, including the sound of the hissing gas and of the operating pump. The principal difference between interventions was the inability to completely mask participant discomfort associated with influx of gas into the subcutis on the active treatment side.

Masking of those assessing the outcomes was accomplished by censoring written information about treatment assignment.

Statistical Methods:

*Sample Size:* A sample of 16 patients will provide 80% power to detect mean differences of 1.5 for paired *t*-tests in fat thickness assuming a standard deviation of change is 2. There were no interim analyses or stopping rules. All treatments were at a single center by the same team who were expert in the study procedures; this clustering by team and center likely reduced power, and was addressed by adjustment in the sample size.

Paired *t*-tests were used to determine if there were differences of subcutaneous fat thickness at baseline vs. Week 5 and Week 28 for each treatment, if there were any treatment differences at Week 5 and Week 28, and in total circumference measurements at baseline vs. Week 5 and Week 28. Repeated measures ANOVA was used to determine treatment differences in pain and skin elasticity over time. One-way ANOVA was used to determine if there was a change in weight over time.

## RESULTS

Data were collected from January 2014 through December 2014. A total of 23 patients were enrolled in the study, four withdrew and three were lost to follow-up. Sixteen patients completed the study per protocol and were analyzed. Two out of the four subjects who withdrew complained of discomfort associated with carboxytherapy, but no serious adverse events were observed or reported. Demographic data for all participants completing the study per protocol is summarized in Table 1. Results and data from the ultrasound measurements

are presented in Table 2. Results of flank circumference, weight, pain, and dermal torque skin elasticity are presented in Table 3.

Ultrasound measurements of subcutaneous fat thickness indicated significantly less fat in the flanks treated with carboxytherapy versus sham one week after the last treatment (Week 5) (anterior location, p=0.004; across all locations, p=0.011) (Table 2). Representative ultrasound images acquired at baseline, Week 5, and Week 28 for a carboxy treated side as well as a sham treated side are shown in Figures 1 (A – F).

Total circumference decreased nominally but not significantly at Week 5 compared to baseline (p=0.0697) and from baseline compared to Week 28 (p=0.612) (Table 3). Weight did not significantly change over the study (p=1.00) (Table 3). Carboxytherapy was significantly more painful than the sham procedure (p<0.0001), and this did not change over time (Table 3). Skin elasticity was not significantly different between the two treatments over time (p=0.585) (Table 3).

Of patients completing the study, three (18.75%) preferred the sham procedure, three (18.75%) preferred the carbon dioxide procedure, nine (56.25%) preferred having both procedures, and one (6.25%) preferred neither procedure.

**DISCUSSION**

Insufflation of carbon dioxide gas into the subcutaneous fat, known as carboxytherapy, appears to cause a transient decrease in the thickness of the fat layer. In this study, the treated flanks experienced a fat layer decrease greater than the sham treated sides at one week after

five carboxytherapy treatments. This difference was not maintained at six months suggesting that the treatment stimulated a temporary metabolic process that reduced the size of adipocytes without inducing cell death, or adipocytolysis. Participant reported preferences were similar for both sides, as were skin elasticity measures. However, carboxytherapy was associated with greater discomfort.

Notably, efforts were made to ensure that the procedures were considered to be maximally therapeutic. We employed the usual practice of multiple (five) treatments. Additionally, each carboxytherapy treatment was high, 1L. The insufflator model used was designed for carboxytherapy and previously used in a clinical trial. Finally, we first conducted a pilot study, in the same setting and staff, to establish safety and to train investigators in carboxytherapy. This was particularly important since it has been shown that, inadequate staff proficiency with novel interventions can result in technique errors leading to under-detection of effectiveness.

While carboxytherapy is generally regarded as safe, at least one case of artifactual emphysema has been reported,[10] and we wished to minimize this risk through low pressure injections. Risk of infection, previously reported[12], was also minimized using a single injection point per flank rather than many injection sites 1-2 cm apart.[1]

Carboxytherapy has been previously studied and found to be quite effective by several investigators for fat reduction, [1-16] as well as for a range of other indications,[6] including peripheral arterial occlusive disease, stable angina pectoris, migraine headaches, scars,[13] skin laxity,[4,9,16] and cellulite.[14] Our findings differ from those previous investigations[5,7] in that we did not detect an abdominal circumference difference of about 2 cm, rather a non-

significant difference of about 1 cm at best, one week after the last treatment, and in the context of 1.5 lbs mean weight loss. Similarly, unlike prior studies,[9,11] we did not see an improvement in skin elasticity, which may have been due to scar tissue formation after multiple needle insertions. Importantly, we did use multiple treatments of high volume carbon dioxide injection using a standard device so it is clear that our results are not a consequence of undertreatment or non-standard treatment.

The reasons for the disparity may be our randomized control design (the first in this area of investigation) was superior at eliminating unknown confounders. All other studies have been case series or uncontrolled cohort studies in which all subjects received active therapy.[14-16] Additionally, our high resolution ultrasound and our microtattoo measurement protocol allowed reproducible measurements that may have minimized measurement error, as shown in previous studies.[17-19] We did not rely on imprecise girth circumference or skin caliper measurements, which have been shown to be less reliable and reproducible. Unlike prior studies, which have generally assessed outcomes immediately after or one week after the last treatment,[14-16] we provided a long-term follow-up, suggesting for the first time that carboxytherapy may provide only transient fat reduction.

Limitations of our study include modest sample size, which precluded subgroup analyses of particular ages, genders, skin types or abdominal morphologies. However, we did include both sexes of various ages and ethnicities. Additionally, we could not eliminate all sources of error in circumference and fat layer measurements.

In summary, insufflation of carbon dioxide into the subcutaneous fat does not induce a durable decrease in fat layer thickness or abdominal circumference. Reduction in fat layer

did appear to occur short-term, immediately following a series of treatments. Fortunately, significant adverse events were not seen. Additional research is warranted to better describe the potential utility of carboxytherapy for body contouring through exploration of other treatment approaches.

337 **REFERENCES**

338

339 1: Costa CS, Otoch JP, Seelaender MC, das Neves RX, Martinez CA, Margarido NF.
340 Cytometric evaluation of abdominal subcutaneous adipocytes after percutaneous $CO_2$
341 infiltration. Rev Col Bras Cir. 2011 Jan-Feb;38(1):15-23. English, Portuguese.
342 PubMed PMID: 21537738.

343

344

345 2: Ferraro GA, De Francesco F, Cataldo C, Rossano F, Nicoletti G, D'Andrea F.
346 Synergistic effects of cryolipolysis and shock waves for noninvasive body
347 contouring. Aesthetic Plast Surg. 2012 Jun;36(3):666-79. doi:
348 10.1007/s00266-011-9832-7. Epub 2011 Nov 1. PubMed PMID: 22042359.

349

350

351 3: Brandi C, Campana M, Russo F, Brafa A, Nisi G, Grimaldi L, D'Aniello C. Carbon
352 dioxide: maybe not the only one but an efficient and secure gas for treating
353 local adiposities. Aesthetic Plast Surg. 2012 Feb;36(1):218-9. doi:
354 10.1007/s00266-011-9758-0. PubMed PMID: 21656063.

355

356

357 4: Brandi C, D'Aniello C, Grimaldi L, Caiazzo E, Stanghellini E. Carbon dioxide
358 therapy: effects on skin irregularity and its use as a complement to liposuction.
359 Aesthetic Plast Surg. 2004 Jul-Aug;28(4):222-5. PubMed PMID: 15599534.

360

361

5: Brandi C, D'Aniello C, Grimaldi L, Bosi B, Dei I, Lattarulo P, Alessandrini C. Carbon dioxide therapy in the treatment of localized adiposities: clinical study and histopathological correlations. Aesthetic Plast Surg. 2001 May-Jun;25(3):170-4. PubMed PMID: 11426306.

6: Brockow T, Hausner T, Dillner A, Resch KL. Clinical evidence of subcutaneous CO2 insufflations: a systematic review. J Altern Complement Med. 2000 Oct;6(5):391-403. Review. PubMed PMID: 11059501.

7: Lee GS. Carbon dioxide therapy in the treatment of cellulite: an audit of clinical practice. Aesthetic Plast Surg. 2010 Apr;34(2):239-43. doi: 10.1007/s00266-009-9459-0. Epub 2010 Jan 29. PubMed PMID: 20111965; PubMed Central PMCID: PMC2847160.

8: Koutná N. [Carboxytherapy--a new non-invasive method in aesthetic medicine]. Cas Lek Cesk. 2006;145(11):841-3. Review. Czech. PubMed PMID: 17168416.

9: Pinheiro NM, Crema VO, Millan BM, Carvalho FA, Mendonça AC. Comparison of the effects of carboxytherapy and radiofrequency on skin rejuvenation. J Cosmet Laser Ther. 2015 Jun;17(3):156-61. doi: 10.3109/14764172.2014.1003238. Epub 2015 Jan 30. PubMed PMID: 25549818.

10: Calonge WM, Lesbros-Pantoflickova D, Hodina M, Elias B. Massive subcutaneous emphysema after carbon dioxide mesotherapy. Aesthetic Plast Surg. 2013 Feb;37(1):194-7. doi: 10.1007/s00266-012-0006-z. Epub 2013 Jan 8. PubMed PMID: 23296768.

11: Paolo F, Nefer F, Paola P, Nicolò S. Periorbital area rejuvenation using carbon dioxide therapy. J Cosmet Dermatol. 2012 Sep;11(3):223-8. doi: 10.1111/j.1473-2165.2012.00633.x. PubMed PMID: 22938007.

12: Couderc C, Carbonne A, Thiolet JM, Brossier F, Savey A, Bernet C, Ortmans C, Lecadet-Morin C, Coudière I, Aggoune M, Astagneau P, Coignard B, Cambau E. [Non-tuberculous mycobacterial infections related to esthetic care in France, 2001-2010]. Med Mal Infect. 2011 Jul;41(7):379-83. doi: 10.1016/j.medmal.2011.02.007. Epub 2011 Mar 25. French. PubMed PMID: 21440389.

13: Nach R, Zandifar H, Gupta R, Hamilton JS. Subcutaneous carboxytherapy injection for aesthetic improvement of scars. Ear Nose Throat J. 2010 Feb;89(2):64-6. PubMed PMID: 20155672.

14: Pianez LR, Custódio FS, Guidi RM, de Freitas JN, Sant'Ana E. Effectiveness of

carboxytherapy in the treatment of cellulite in healthy women: a pilot study. Clin Cosmet Investig Dermatol. 2016 Aug 22;9:183-90. doi: 10.2147/CCID.S102503. eCollection 2016. PubMed PMID: 27578994; PubMed Central PMCID: PMC5001663.

15: Lee GS. Quality survey on efficacy of carboxytherapy for localized lipolysis. J Cosmet Dermatol. 2016 Dec;15(4):484-492. doi: 10.1111/jocd.12242. Epub 2016 Jun 21. PubMed PMID: 27325211.

16: Pinheiro NM, Crema VO, Millan BM, Carvalho FA, Mendonça AC. Comparison of the effects of carboxytherapy and radiofrequency on skin rejuvenation. J Cosmet Laser Ther. 2015 Jun;17(3):156-61. doi: 10.3109/14764172.2014.1003238. Epub 2015 Jan 30. PubMed PMID: 25549818.

17: Dover J, Burns J, Coleman S, Fitzpatrick R, Gardner J, Goldberg D, Geronemus R, Kilmer S, Mayoral F, Tanzi E, Weiss R, Zelickson B. A Prospective Clinical Study of Non-invasive Cryolypolysis for Subcutaneous Fat Layer Reduction—Interim Report of Available Subject Data. Laser Surg Med 2009;S21:45

18: Adjadj L, SidAhmed-Mezi M, Mondoloni M, Meningaud JP, Hersant B. Assessment of the Efficacy of Cryolipolysis on Saddlebags: A Prospective Study of 53 Patients. Plast Reconstr Surg. 2017 Jul;140(1):50-57. doi: 10.1097/PRS.0000000000003433. PubMed PMID: 28654588.

19: Zelickson B, Egbert BM, Preciado J, Allison J, Springer K, Rhoades RW, Manstein D. Cryolipolysis for noninvasive fat cell destruction: initial results from a pig model. Dermatol Surg. 2009 Oct;35(10):1462-70. doi: 10.1111/j.1524-4725.2009.01259.x. Epub 2009 Jul 13. PubMed PMID: 19614940.

443 **Figure Legends**

444

445 **Figure 1. Representative Ultrasound Images**

446 Ultrasound images from the anterior section of the carboxytherapy injection are presented
447 from a representative patient. A-C images are taken from baseline, week 5, and week 28
448 respectively of the carboxytherapy treatment and shows a reduction of fat thickness at week
449 5, but not at week 28. D-F images are taken from baseline, week 5, and week 28 respectively
450 of the sham treatment and shows no reduction of the fat thickness.

451

452 **Tables**
453
454 Table 1. Patient Demographics

| | | |
|---|---|---|
| Gender, No. (%) | Female | 10 (62.5) |
| | Male | 6 (37.5) |
| Age, Mean (SD) | | 50 (9.94) |
| Race, No. (%) | White/Caucasian | 10 (62.5) |
| | Black/African American | 5 (31.25) |
| | Other | 1 (6.25) |
| Skin Type, No. (%) | I | 3 (18.75) |
| | II | 3 (18.75) |
| | III | 4 (25) |
| | IV | 4 (25) |
| | V | 0 (0) |
| | VI | 2 (12.5) |
| Weight (lbs), Mean (SD) | | 169.4 (22.53) |
| Height (inches), Mean (SD) | | 66.81 (4.16) |
| BMI, Mean (SD) | | 27.3 (3.27) |

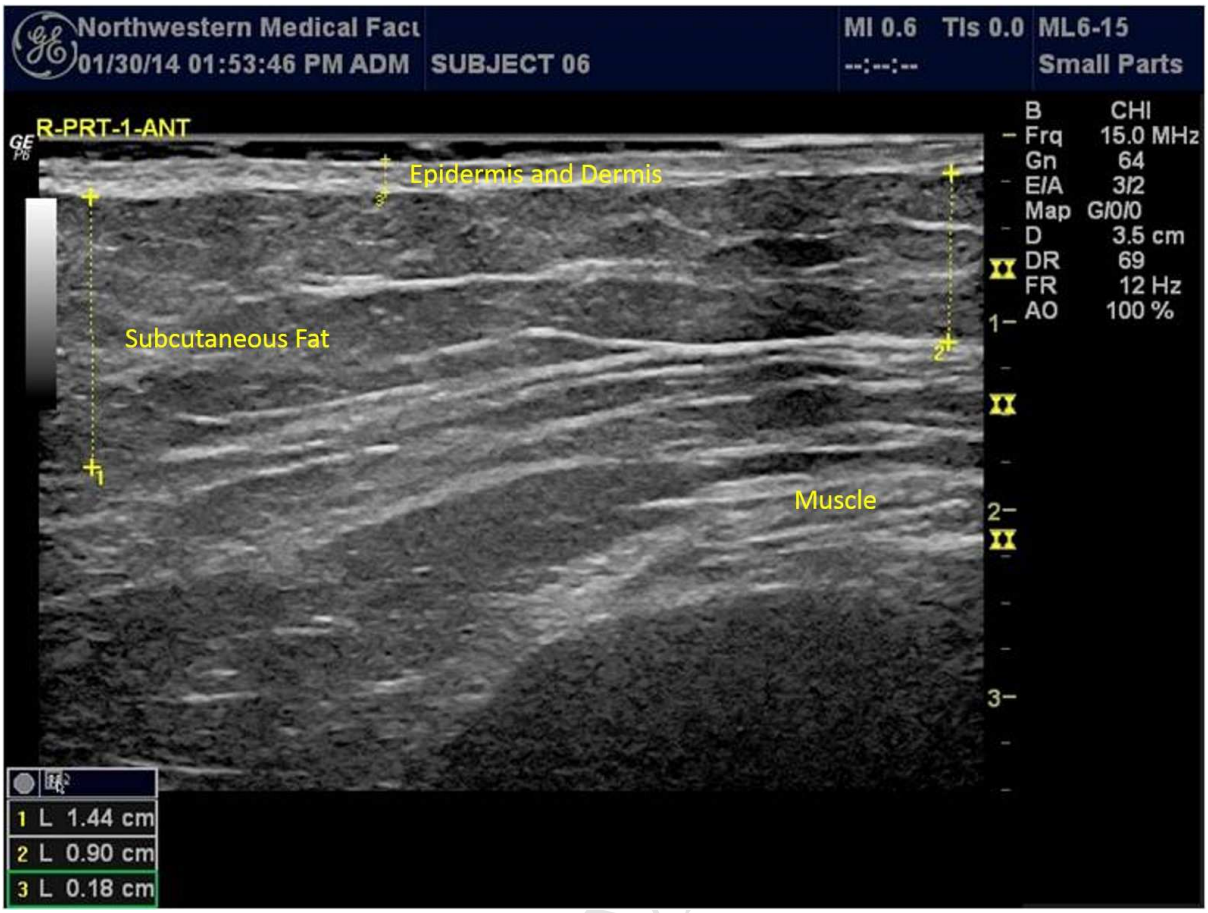
Table 2. Ultrasound Measurements of Fat Layer Thickness for Carboxytherapy vs. Sham, Over Time

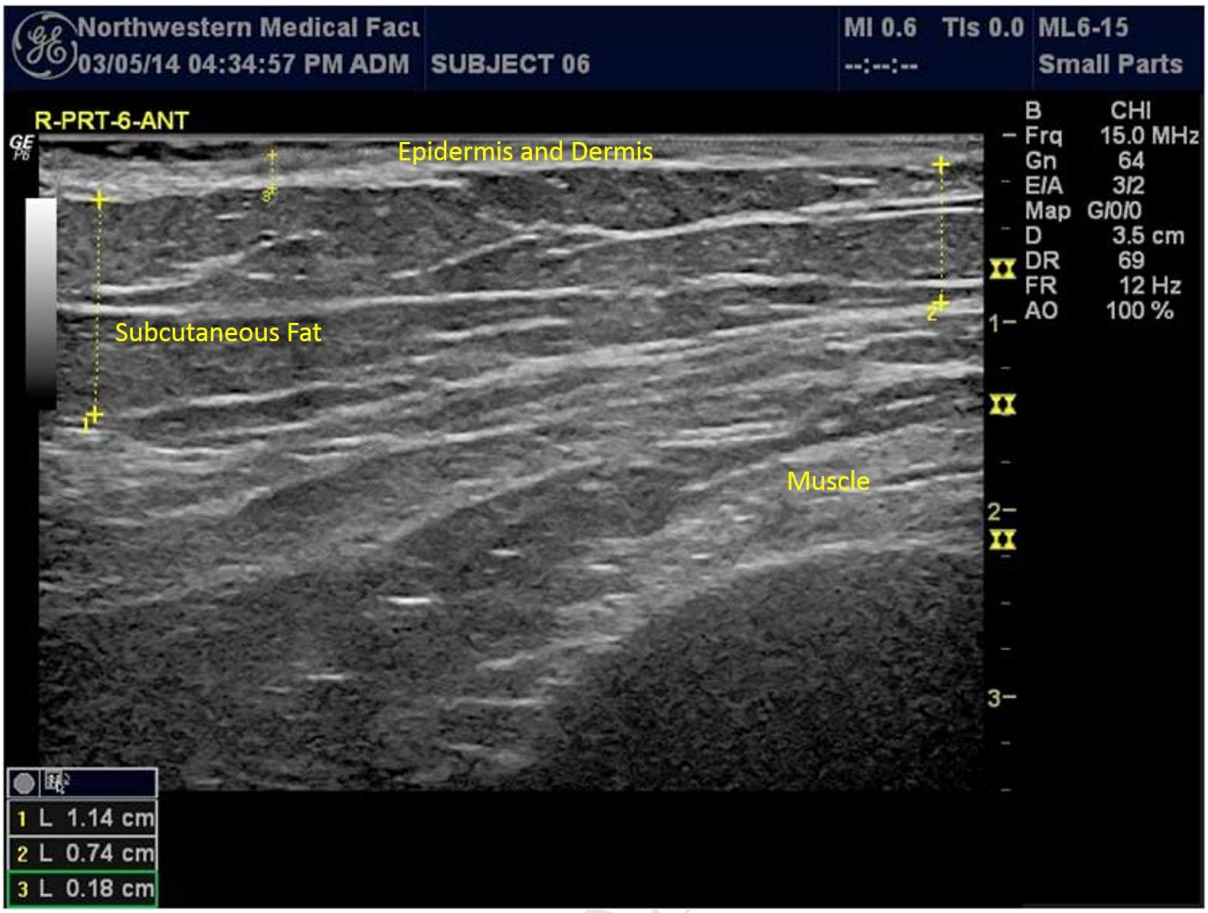
| | **Subcutaneous fat thickness (cm) at week 5, Mean (SD)** | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | Superior | Middle | Inferior | Anterior | Posterior | All Locations |
| Carboxytherapy | 1.12 (0.32) | 0.98 (0.24) | 0.82 (0.33) | 0.74 (0.23) | 1.16 (0.29) | 0.97 (0.33) |
| Sham | 1.16 (0.35) | 1.11 (0.36) | 0.92 (0.24) | 0.85 (0.22) | 1.22 (0.33) | 1.05 (0.34) |
| **p-value** | 0.708 | 0.171 | 0.243 | 0.004 | 0.337 | 0.011 |
| | **Subcutaneous thickness (cm) at week 28, Mean (SD)** | | | | | |
| **Treatment** | Superior | Middle | Inferior | Anterior | Posterior | All Locations |
| Carboxytherapy | 1.16 (0.26) | 1.12 (0.38) | 0.93 (0.34) | 0.92 (0.35) | 1.31 (0.41) | 1.09 (0.38) |
| Sham | 1.17 (0.46) | 1.14 (0.36) | 1.07 (0.34) | 0.93 (0.32) | 1.35 (0.36) | 1.13 (0.40) |
| **p-value** | 0.983 | 0.76 | 0.144 | 0.8 | 0.615 | 0.265 |

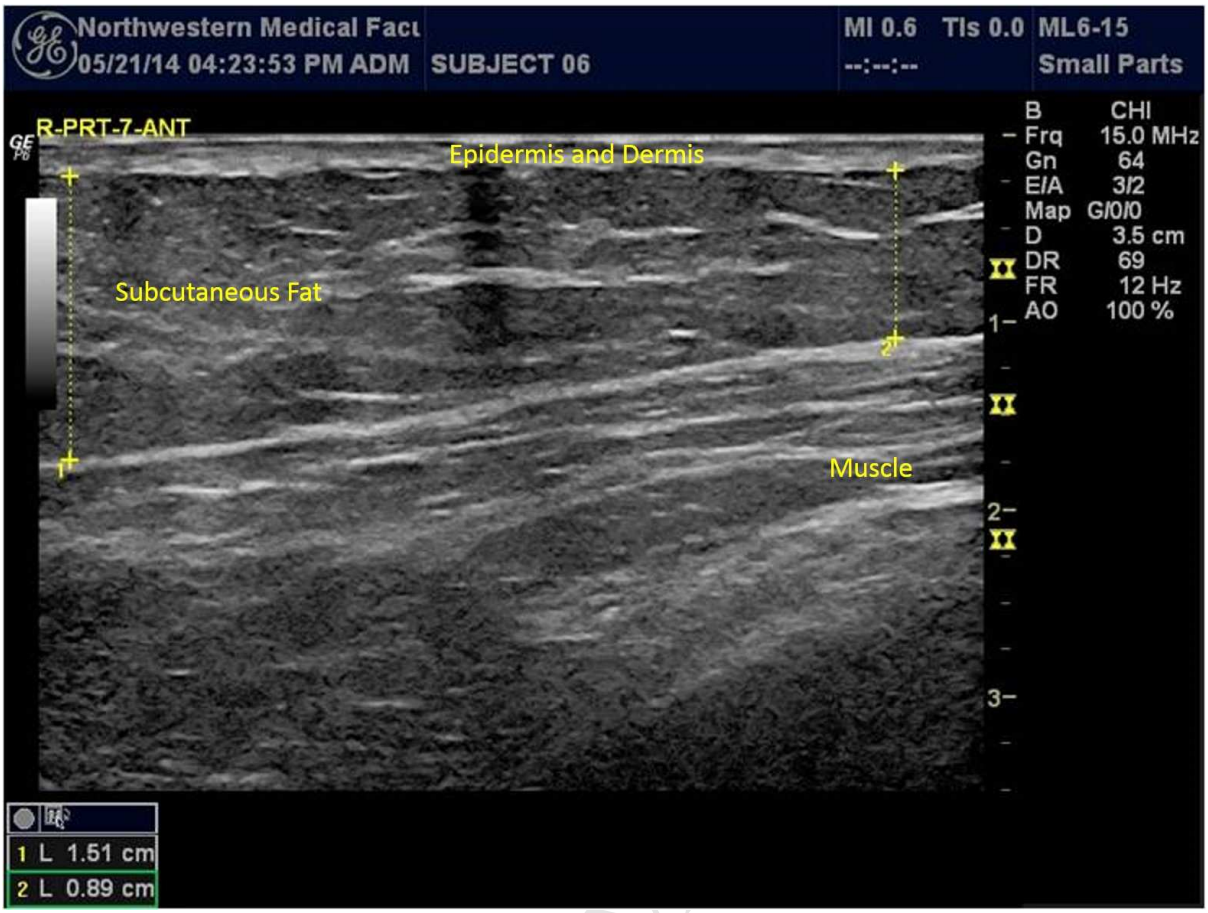
Table 3. Weight, Total Circumference, Pain (VAS) Scores and Skin Elasticity Measurements

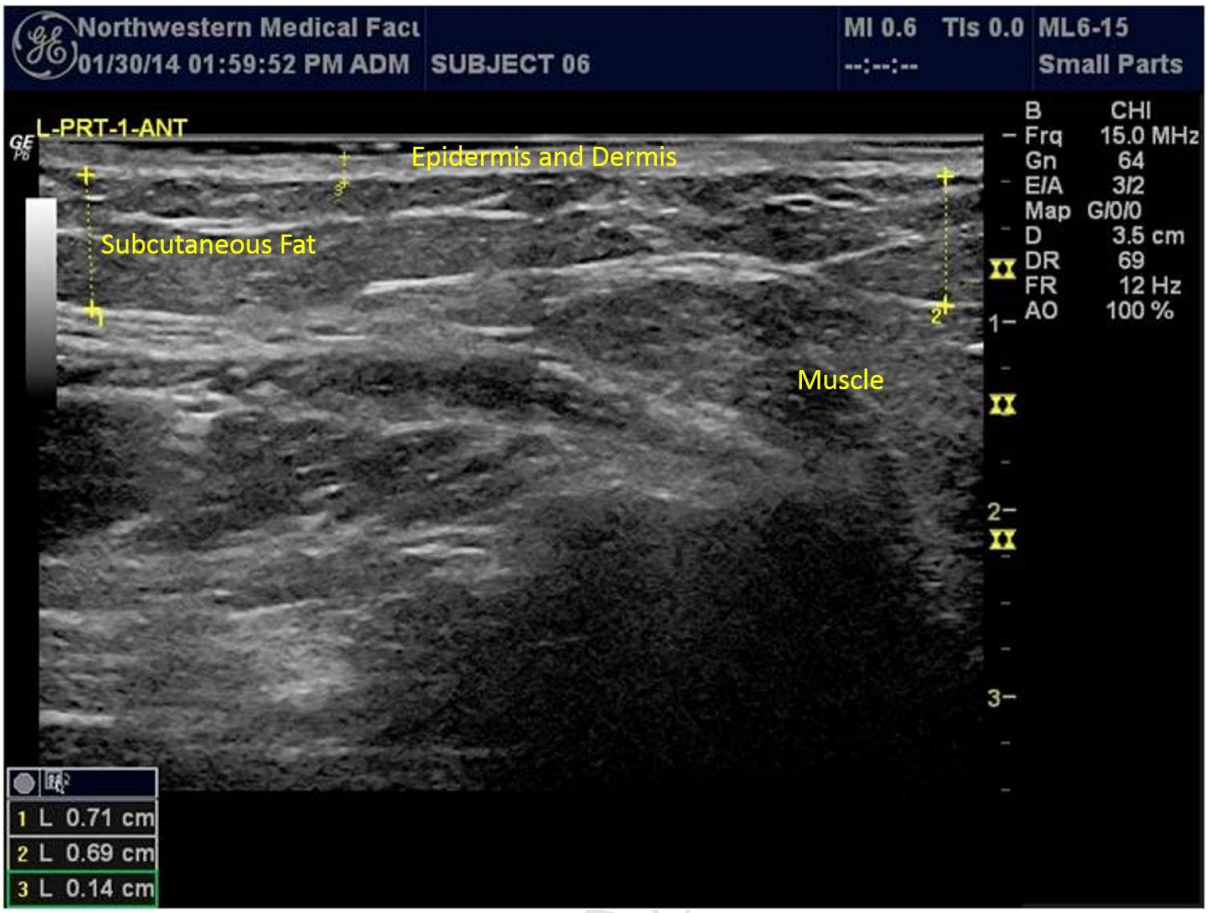
| **Outcome** | **Baseline** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **Week 5** | **Week 16** | **Week 28** | **p-value** | **p-value** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Weight (lbs), Mean (SD)** | 169.43 (23.27) | 168.95 (23.96) | 168.14 (23.34) | 168.10 (24.00) | 168.55 (24.15) | 167.99 (23.75) | 167.92 (22.70) | 166.86 (19.35) | 1.00 | |
| **Total Circumference (cm), Mean (SD)** | 95.53 (14.11) | 95.43 (13.90) | 94.82 (14.35) | 93.45 (15.83) | 94.80 (14.17) | 94.42 (14.26) | 98.22 (11.74) | 97.56 (9.71) | 0.0697* | 0.612¥ |
| | **Treatment** | **Baseline** | **Week 1** | **Week 2** | **Week 3** | **Week 4** | **p-value** | | | |
| | Carboxy | 3.01 (2.75) | 2.78 (2.33) | 3.34 (3.17) | 2.77 (2.85) | 2.07 (2.70) | <0.0001 | | | |
| **Pain Scores, Mean (SD)** | Sham | 0.39 (0.53) | 0.39 (0.59) | 0.54 (0.93) | 0.25 (0.31) | 0.51 (0.86) | | | | |
| | **Treatment** | **Baseline** | **Week 5** | **Week 16** | **Week 28** | **p-value Treatment** | | | | |
| | Carboxy | 0.69 (0.35) | 0.55 (0.14) | 0.54 (0.15) | 0.50 (0.14) | | | | | |
| **Net Skin Elasticity Ur/Ue Ratio±, Mean (SD)** | Sham | 0.71 (0.20) | 0.58 (0.20) | 0.55 (0.16) | 0.51 (0.17) | 0.585 | | | | |

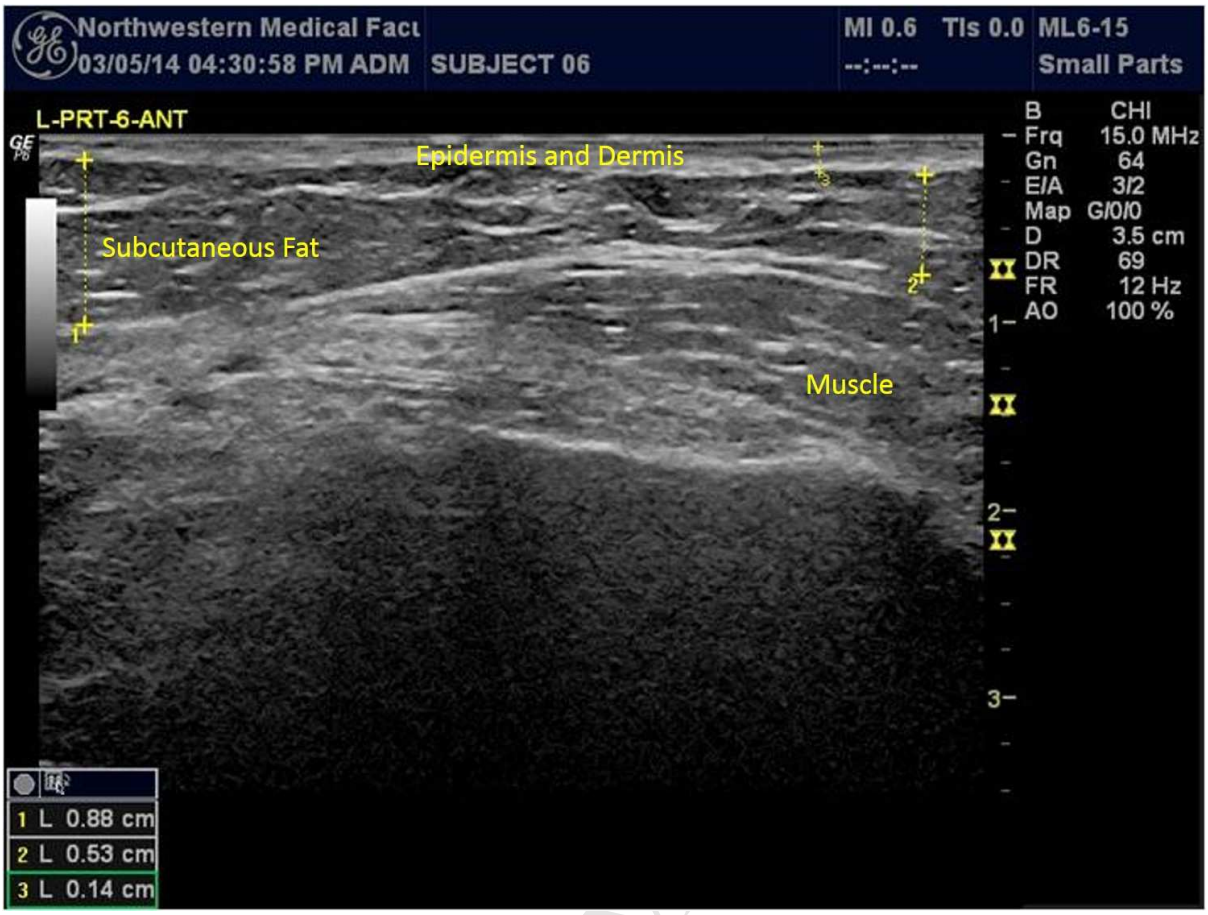
*Paired t-test comparing Baseline to Week 5
¥Paired t-test comparing Baseline to Week 28
±Ur=Immediate retraction of skin; Ue=Immediate extensibility; Ur/Ue=Net skin elasticity in units of radians

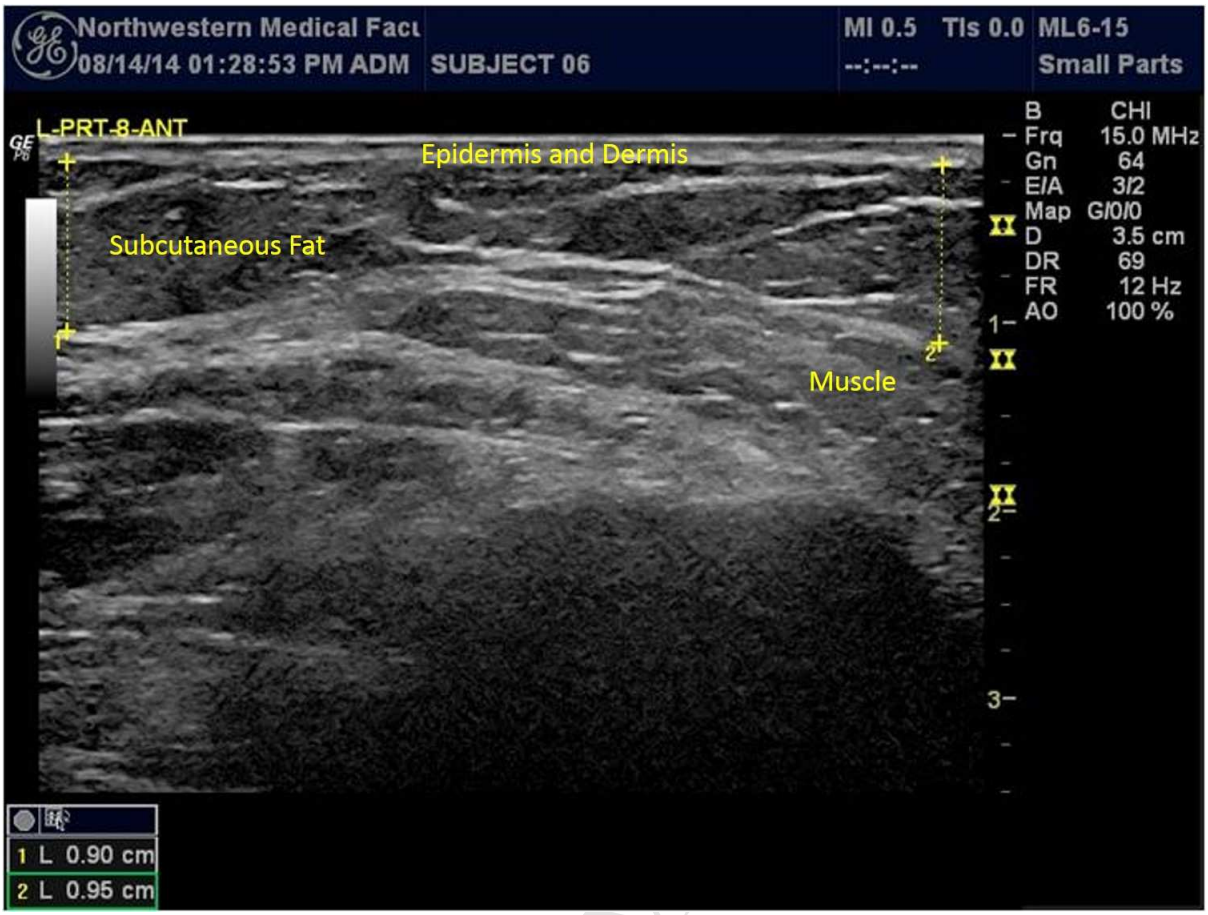












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# Study of the efficacy of carboxytherapy in alopecia

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## Summary

**Background:** Management of alopecia areata (AA) and androgenetic alopecia (AGA) is often challenging. The use of carboxytherapy may be a novel therapeutic option for such cases.

**Objective:** To evaluate the clinical efficacy and safety of carboxytherapy in alopecia areata and androgenetic alopecia.

**Patients and methods:** This study was conducted on 80 patients with alopecia divided into two groups; Group I included 40 AA patients (Group IA received carboxytherapy and Group IB control received placebo), and Group II included 40 AGA patients (Group IIA received carboxytherapy and Group IIB control received placebo), and followed up monthly for 3 months. They were evaluated clinically (by assessment of Severity of Alopecia Tool (SALT) score in group I, and Sinclair scale and Norwood-Hamilton scale in group II), by dermoscopy and digital dermoscopy at each visit.

**Results:** Group IA patients showed significant clinical improvement in SALT score and dermoscopic improvement after carboxytherapy and at the end of follow-up period with significant reduction in dystrophic hair, black dots, yellow dots, and tapered hair coinciding with significant emergence of regrowing hair. Group IIA patients showed significant clinical and dermoscopic improvement after carboxytherapy with significant increase in hair density measured by digital dermoscopy. However, regression of these results was observed during the follow-up period but was still significantly better than before treatment. There were statistically significant improvements in clinical score, global assessments, dermoscopic, and digital dermoscopic findings in both group IA and group IIA received carboxytherapy in comparison with group IB and group IIB received placebo injections, respectively.

**Conclusion:** Carboxytherapy seems to be a promising therapeutic option for patchy AA and could be helpful as an adjuvant therapy of AGA but more than 6 sessions are required and adjuvants are recommended for maintenance of the results.

KEYWORDS
alopecia areata, androgenetic alopecia, carboxytherapy

## 1 | INTRODUCTION

Carboxytherapy refers to the intradermal and/or subcutaneous microinjections of sterile, medical-grade $CO_2$ gas for therapeutic purpose. It induces a state of relative hypercapnia and decreases local pH, which elicits a strong vasodilator response, ultimately increasing blood flow to the injected site.[1] Carboxytherapy is not a novel technique. It is developed in France in 1932, when it was found that bathing in $CO_2$ rich pools speed up wound healing. By the 1950s, the technique was used by cardiologists to treat patients with

  

peripheral arterial occlusive diseases. The term "carboxytherapy" was coined by Luigi Parassoni in 1995, during the XVI national meeting of Italian Society of Esthetical Medicine.[1,2] Then, it has increased in popularity in the field of esthetics particularly for lipolysis and dermal rejuvenation. It is considered a safe, minimally invasive, clinically proven method to rejuvenate, restore, and recondition the skin.[3,4]

Alopecia Areata (AA) and androgenetic alopecia (AGA) are common causes of hair loss that significantly impact the patient's quality of life.[5,6] Their management is often challenging, and numerous therapeutic options have been proposed ranging from topical applications, oral preparations, to injectable treatments and even surgical interventions. However, despite the variety of options available, alopecia is a complex condition to treat and no one product or procedure offers a perfect solution.[7]

The pathogenesis of hair loss in AA or AGA is complex and caused by a combination of factors, including diminished vascular support to the affected scalp tissue.[8-11] As carboxytherapy improves circulation at the injection site,[1] it seemed interesting to evaluate its efficacy and safety in the treatment of AA and AGA.

## 2 | PATIENTS AND METHODS

This study was conducted on 80 patients presented with alopecia. They were divided into two main groups; Group I included 40 AA patients and Group II included 40 AGA patients. All were selected from the Outpatient Clinic of Dermatology and Venereology Department, Tanta University Hospitals. Ethical approval was obtained from Ethical committee before the commencement of the study. Inclusion criteria were patients with early localized scalp AA (≤3 patches and ≤50% scalp involvement, duration not more than 6 months) or mild-to-moderate AGA (ranging from Norwood–Hamilton type I to V for males and Sinclair scale grade 1 to 4 for females), patients who did not receive any medication for at least 1 month before starting the study and those who agreed to join the study and signed written consent. Exclusion criteria were patients with any other dermatological, systemic, or autoimmune disease, pregnant, and lactating females and those with unrealistic expectation.

All patients included in the study were subjected to complete history taking, thorough general and dermatological examinations, and routine laboratory investigations.

### 2.1 | Therapeutic regimen

After taking written informed consent, patients were treated as follows:

- Group I AA patients (40 patients) were randomized into 2 subgroups; Group IA (20 patients) received intradermal injection of $CO_2$ gas (carboxytherapy) and Group IB (20 patients) received intradermal injection of distilled water to serve as control. In total, 6 sessions were given for each patient (once/week) for 6 successive weeks or until improvement, which was first.
- Group II AGA patients (40 patients) were randomized into 2 subgroups; Group IIA (20 patients) received intradermal injection of $CO_2$ gas (carboxytherapy) and Group IIB (20 patients) received intradermal injection of distilled water to serve as control. In total, 6 sessions were given for each patient (once/2 weeks) for 6 successive weeks.
- All were followed up monthly for 3 months after last session.
- No other treatment modalities were allowed as a combination in any group of patients throughout the study.

### 2.2 | Injection technique

Under complete aseptic technique, Groups IA & IIA treated by $CO_2$ gas injections using carboxytherapy apparatus (CONCERTO, BFP Electronique Pôle Technologique de Vimenet 48 100 MONTRODAT – France). Waiting few seconds was recommended until full gas flow obtained. The injection was performed slowly, intradermal using sterile disposable 30 G needle, at an angle 15° with depth adjusted at 2 mm and flow rate of 1 cc/s. The gas volume per injection spot was 2 mL. Group IB & IIB received intradermal injections of distilled water to serve as control groups. The injection was done slowly, intradermal using insulin syringe. The water volume per injection spot was 2 mL.

- Group I (AA patients): The injection point was somewhere in the center of small alopecic patches. While, for larger patches, several points were injected.
- Group II (AGA patients): The injections were in seven points according to Kotuna et al[2] (Figure 1).

### 2.3 | Evaluation of therapy

All patients were assessed at baseline (before treatment), 1 week after last session and at the end of follow-up period as follows:

- Clinical assessment:

  Group I (AA patients): The extent of scalp hair loss among them was assessed according to Severity of Alopecia Tool (SALT) score (S0 = no hair loss; S1 < 25% hair loss; S2 = 25%-49% hair loss; S3 = 50%-74% hair loss; S4 = 75%-99% hair loss; and S5 = 100% hair loss). Hair regrowth was calculated as follows: {(SALT score at base line-SALT score at follow up)/SALT score at base line} × 100.[12]

  Group B (AGA patients): A generally applicable classification for the extent of AGA does not exist. The pattern of scalp hair loss among AGA patients was assessed using; Sinclair scale for female pattern hair loss (FPHL) and Norwood-Hamilton classification for male pattern hair loss (MPHL).[13] However, the classification for pattern distribution of AGA is often not suitable to reflect the course of the disease. Therefore, the evaluation and follow-up of hair regrowth was substantiated by assessment of standardized global photographs.

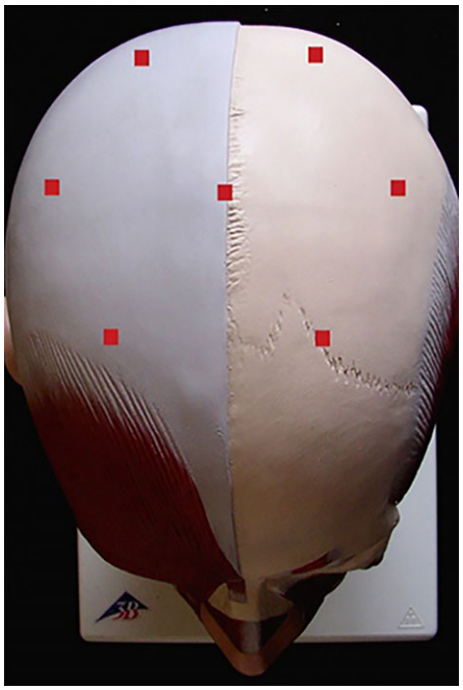

**FIGURE 1** Seven injection points for androgenetic alopecia patients[2]

- Global assessment for hair regrowth: Digital colored photographs of the lesion were taken using 18.2 mega pixels digital camera (SONY CYBERSHOT DSC-WX300). Digital image analysis of standardized global photographs was performed to determine the percentage of improvement as follows:
  - A0: No improvement or further hair loss.
  - A1 (Fair response): <25% improvement
  - A2 (Good response): 25%-50% improvement
  - A3 (Very good response): >50%-75% improvement
  - A4 (Excellent response): >75% improvement
- Dermoscopic examination of the lesion (Dermlite II Pro HR; 3Gen LLC, San Juan Capistrano, California, USA) and digital imaging for the dermoscopic patterns of the alopecia were taken. Group A (AA patients) were evaluated for the presence of dystrophic hair, black dots, tapered hair (the most specific findings which correlate with disease activity),[14,15] yellow dots (the most sensitive finding which correlate with disease severity),[14,16] and upright regrowing hair. Group B (AGA patients) were evaluated for the presence of hair diameter diversity >20% (a diagnostic criterion reflecting follicle miniaturization),[17] dominance of single hair per follicular unit (unlike normal follicles which bear up to 4 terminal hairs),[14] peripilar sign (reflecting perifollicular inflammation),[14,17] yellow dots, and upright regrowing hair.
- Digital dermoscopic examination of the lesion (Dlite STR CA_USA) to evaluate both hair density and hair shaft width.
- Safety assessment: The patients were instructed to report any complains or side effects of the treatment.

## 2.4 | Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp).[18] Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum) and mean and standard deviation (mean ± SD) and median. Comparison between different groups regarding categorical variables was tested using chi-square test. Wilcoxon signed ranks test used for abnormally quantitative variables to compare between two periods. Significance of the obtained results was judged at $P$ value ≤.05*.

# 3 | RESULTS

The clinical characteristics of the studied groups are summarized in Table 1.

## 3.1 | Assessment of clinical efficacy

### 3.1.1 | Group I (AA patients)

- Clinical score: Group IA patients showed statistically significant clinical improvement in SALT score after last session of carboxytherapy and at the end of follow-up period in comparison with that before treatment with $P$ value <.05. On the other hand, Group IB patients showed no significant difference of SALT score after last session of placebo injection and at the end of follow-up period in comparison with that before treatment. Furthermore, statistically significant improvements in SALT score were observed in Group IA patients received carboxytherapy in comparison with group IB received placebo both after last session and at the end of follow-up periods with $P$ value <.05, (Table 2).
- Global assessment: Regarding Group IA patients; after 6 sessions of carboxytherapy, only two patients of Group IA (10%) showed no hair regrowth (A0) and none of them reported worsening of the condition. The remaining 18 patients (90%) reported variable degrees of clinical improvement with hair regrowth as illustrated in Table 3. At the end of follow-up period, four patients (20%) reported no hair regrowth (A0); two of them reported no response to carboxytherapy at all from the start, while the other two reported gradual loss of the regrowing hair after cessation of carboxytherapy sessions but not worsened than that before treatment. However, the remaining 16 patients (80%) reported variable degrees of improvement with hair regrowth as illustrated in Table 3. There was statistically significant clinical improvement of the AA patients at the end of follow-up period in comparison with that after treatment with $P$ value <.05, (Figure 2, Table 3). On the other hand, Group IB patients showed no clinical improvement in 75% of patients after last session of placebo injection and in 70% at the end of follow-up period. There was statistically significant clinical improvement evaluated by global

**TABLE 1** Distribution of the studied groups according to demographic data

| | Group I (AA patients) | | Group II (AGA patients) | |
|---|---|---|---|---|
| | Group IA (n = 20) | Group IB (n = 20) | Group IIA (n = 20) | Group IIB (n = 20) |
| Sex | | | | |
| Male | 12 (60%) | 11 (55%) | 14 (70%) | 13 (65%) |
| Female | 8 (40%) | 9 (50%) | 6 (30%) | 7 (35%) |
| Age/y | 22-38 | 22-33 | 20-38 | 20-38 |
| | 27.9 ± 5 | 27.2 ± 3.9 | 27.1 ± 5.2 | 27.9 ± 5.5 |
| Duration/mo | 0.8 (0.3-2) | 0.6 (0.3-2) | 3 (0.5-6) | 2.4 (0.6-6) |
| Clinical score | 9 (4.5–30) | 12.5 (5.4-30) | 3 (1-5) | 3 (2-5) |

Qualitative data were described using number and percent and was compared using chi-square test, while normally quantitative data were expressed in mean ± SD and were compared using Student's *t* test, abnormally distributed data were expressed in median (Min.-Max.) and were compared using Mann-Whitney test.
AA, Alopecia areata; AGA, Androgenetic alopecia.

**TABLE 2** Distribution of AA patients according to clinical assessment by SALT score before, after treatment sessions and at the end of follow-up period

| | | | | Sig. bet. periods | | |
|---|---|---|---|---|---|---|
| SALT score | Before treatment sessions | After treatment sessions | At the end of follow-up period | Before vs after | Before vs follow-up | After vs follow-up |
| Group I (AA patient) | | | | | | |
| Group IA | 9 (4.5-30) | 8.2 (2-30) | 5.7 (0-29.7) | <0.001* | <0.001* | 0.011* |
| Group IB | 12.5 (5.4-30) | 11.9 (8-35.4) | 16 (0-40) | 0.092 | 0.152 | 0.093 |
| U(*P*) | 0.965 | 0.043* | 0.040* | | | |

Abnormally distributed data were expressed in median (Min.-Max.) and were compared Friedman test, Sig. bet. periods was done using Wilcoxon signed ranks test.
U, *P*: U and *P* values for Mann-Whitney test for comparing between Group IA & Group IB.
AA, alopecia areata; SALT, Severity of Alopecia Tool Score.
*Statistically significant at $P \leq .05$.

assessment in Group IA patients received carboxytherapy in comparison with group IB received placebo both after last session and at the end of follow-up periods with *P* values < .05, (Table 3).

- Dermoscopic evaluation of AA lesions in both group IA and IB before treatment revealed that dystrophic hair and black dots were the most common findings, followed by yellow dots and tapered hair in the same order. Regarding group IA, significant reduction in dystrophic hair, black dots, yellow dots, and tapered hair together with significant emergence of upright regrowing hair were observed after carboxytherapy, and at the end of follow-up period, indicating significant improvement, (Figure 2, Table 4). On the other hand, no significant reduction of dermoscopic findings was observed in group IB in spite of significant emergence of regrowing hair (Table 4). In the majority of patients in both groups, the regrowing hair after treatment was initially white in color and became pigmented at the end of the follow-up period.
- Digital dermoscopic examination of group IA detected improvement in the median of hair density (and not in hair shaft width) after carboxytherapy in comparison with that before treatment but was statistically insignificant with *P* value >.05. While at the end of follow-up period, there were statistically significant improvements in both hair density and hair shaft width in comparison with that before and after treatment with *P* values < 0.05, (Figure 2, Table 5). On the other hand, group IB patients showed no significant difference in the median of hair density or hair shaft width after placebo injections or at the end of follow-up period in comparison with that before treatment (Table 5).

### 3.1.2 | Group II (AGA patients)

- Global assessment: Group IIA patients reported variable degrees of clinical improvement after carboxytherapy in 100% of patients as illustrated in Table 3 with 50% of them showed very good response (A3) and 30% excellent response (A4). While, at the end of follow-up period, 30% of patients showed very good response (A3) and only 10% showed excellent response (A4). Significant clinical improvement was observed in group IIA patients after

**TABLE 3** Distribution of the studied groups according to global assessment after treatment sessions and at the end of follow-up period

| | Group IA | | Group IB | | | |
|---|---|---|---|---|---|---|
| **Global assessment** | **After treatment sessions** | **At the end of follow-up** | **After treatment sessions** | **At the end of follow-up** | $P_1$ | $P_2$ |
| Group I (AA patients) | | | | | | |
| A0 | 2 (10%) | 4 (20%) | 15 (75%) | 14 (70%) | <.001* | .005* |
| A1 | 10 (50%) | 2 (10%) | 3 (15%) | 2 (5%) | | |
| A2 | 4 (20%) | 2 (10%) | 2 (10%) | 2 (10%) | | |
| A3 | 4 (20%) | 7 (35%) | 0 (0%) | 1 (5%) | | |
| A4 | 0 (0%) | 5 (25%) | 0 (0%) | 1 (5%) | | |
| *P* | .012* | | 1.000 | | | |
| | **Group IIA** | | **Group IIB** | | | |
| | **After treatment sessions** | **At the end of follow-up** | **After treatment sessions** | **At the end of follow-up** | $P_1$ | $P_2$ |
| Group II (AGA patients) | | | | | | |
| A0 | 0 (0%) | 0 (0%) | 20 (100%) | 20 (100%) | <.001* | <.001* |
| A1 | 0 (0%) | 4 (20%) | 0 (0%) | 0 (0%) | | |
| A2 | 4 (20%) | 8 (40%) | 0 (0%) | 0 (0%) | | |
| A3 | 10 (50%) | 6 (30%) | 0 (0%) | 0 (0%) | | |
| A4 | 6 (30%) | 2 (10%) | 0 (0%) | 0 (0%) | | |
| *P* | .043* | | 1.000 | | | |

Qualitative data were described using number and percent and were compared using chi-square test or Monte Carlo test.
*P*: *P* value for comparing between after treatment sessions and at the end of follow-up in each group.
$P_1$: *P* value for comparing between group A and group B after treatment.
$P_2$: *P* value for comparing between group A and group B at follow-up.
AA, Alopecia areata; AGA, Androgenetic alopecia; A0, No improvement; A1, <25% improvement; A2, 25%-49% improvement; A3, 50%-75% improvement; A4, >75% improvement.
*Statistically significant at $P \leq .05$.

carboxytherapy in comparison with that before treatment. However, during the follow-up period, most patients reported significant clinical regression (gradual falling of the regrowing hair) after cessation of carboxytherapy sessions in comparison with that after treatment and continued till the end of follow-up period but was still statistically significantly better than that before treatment (Figures 3 and 4, Table 3). On the other hand, no improvement reported at all in any patient in group IIB patients received placebo injections (Table 3).

- Dermoscopic examination of group II AGA patients before treatment revealed that hair diameter diversity >20% and dominance of single hair per follicular unit were the most common findings observed in all patients (100%), followed by peripilar sign, and yellow dots in the same order. Group IIA patients reported statistically significant improvement of dermoscopic finding after carboxytherapy and at the end of follow-up period, with significant reduction of hair diameter diversity and significant emergence of regrowing hair (Figures 3 and 4, Table 4). On the other hand, no significant difference detected in dermoscopic findings in group IIB received placebo whether after treatment or at the end of follow-up period (Table 4).
- Digital dermoscopic examination of group IIA patients after carboxytherapy detected statistically significant increase in the median of hair density with no significant change in the median of hair shaft width. At the end of follow-up period, statistically significant decrease in the median of hair density was observed in comparison with that after treatment indicating regression of the regrowing hair density but was still statistically significantly better than that before treatment. However, statistically significant increase in the median of hair shaft width was observed at the end of follow-up period in comparison with that before and after treatment (Figure 3 and 4, Table 5). On the other hand, no significant difference detected in hair density or hair shaft width in group IIB patients whether after placebo injection or at the end of follow-up period (Table 5).

## 3.2 | Safety assessment

All the studied patients in both groups tolerated the procedure well. The reported side effects of carboxytherapy in group IA and IIA were minimal and transient in the form of minor pain, burning sensation, erythema, and edema at site of injection that disappeared spontaneously within 10-15 minutes. No history of hematoma or ecchymosis was reported. However, 2 AA patients and eight AGA patients reported headache that resolved spontaneously in <24 hours. Females were less tolerable to carboxytherapy than males. Generally, patients with AGA complained headache more than patients with AA.

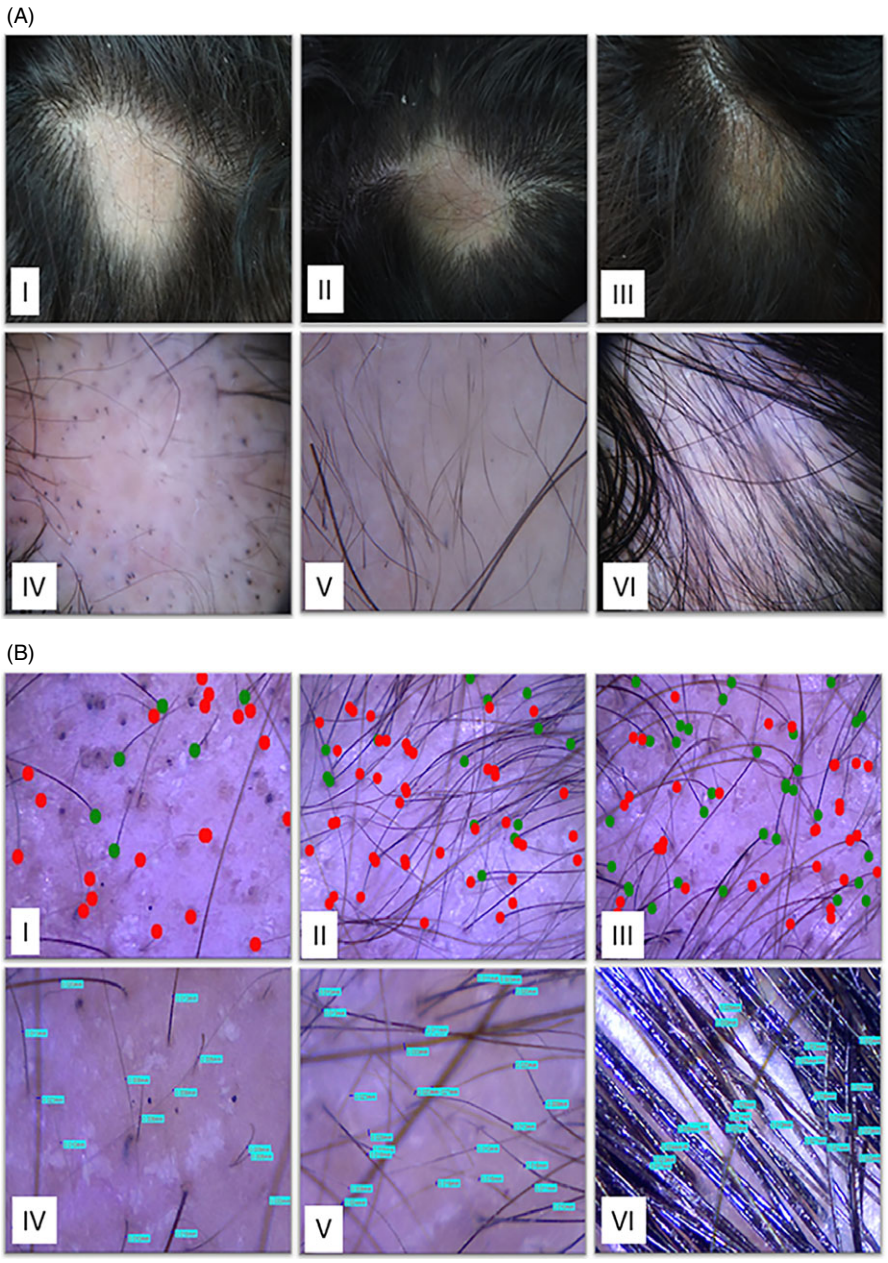


**FIGURE 2** A, A 22-year-old male patient with single patchy alopecia areata on the scalp of 2 wk duration (I) before treatment, (II) after 6 sessions of carboxytherapy showed fair response (A1), and (III) 3 mo after the last session of carboxytherapy showed very good response (A3). Dermoscopic picture; (IV) before treatment showed black dots and dystrophic hair, (V) after carboxytherapy showed reduction of black dots and dystrophic hair with appearance of regrowing hair, and (VI) at the end of follow-up period showed significant improvement. B, Digital dermoscopic examination showed that; average hair density (I) before treatment was 52.1/cm$^2$, (II) after 6 sessions of carboxytherapy was 188.8/cm$^2$, and (III) 3 mo after the last session of carboxytherapy was 343/cm$^2$. While, average hair shaft width; (IV) before treatment was 0.010 mm, (V) after carboxytherapy was 0.015 mm, and (VI) at the end of follow-up period was 0.038 mm

## 4 | DISCUSSION

The current work evaluated the clinical efficacy and safety of carboxytherapy in two common types of alopecia: AA and AGA. They received 6 sessions of intradermal injections of $CO_2$ gas in group IA and IIA and distilled water in group IB and IIB, weekly in AA patients and every 2 weeks in AGA patients, and followed up monthly up to 3 months. They were evaluated clinically and by dermoscopy and digital dermoscopy 1 week after the last session and at the end of follow-up period.

In the present study, clinical assessment of AA patients by SALT score after carboxytherapy revealed statistically significant clinical improvement in comparison with that before treatment and progressed gradually till reach its maximum at the end of follow-up period. Only two patients not improved after treatment. None of them reported worsening of the condition. These results coincide with

**TABLE 4** Distribution of the studied groups according to dermoscopic findings before, after treatment sessions and at the end of follow-up period

| Dermoscopic findings | Group IA | | | | Group IB | | | |
|---|---|---|---|---|---|---|---|---|
| | Before treatment sessions | After treatment sessions | At the end of follow-up | *P* | Before t reatment sessions | After treatment sessions | At the end of follow-up | *P* |
| Group I (AA patients) | | | | | | | | |
| Dystrophic hair | 18 (90%) | 6 (30%) | 6 (30%) | **<.001*** | 18 (90%) | 15 (75%) | 14 (70%) | **.385** |
| Black dots | 18 (90%) | 14 (70%) | 4 (20%) | **<.001*** | 16 (80%) | 15 (75%) | 15 (75%) | **1.000** |
| Yellow dots | 16 (80%) | 14 (70%) | 12 (60%) | **.029*** | 17 (85%) | 16 (80%) | 16 (80%) | **1.000** |
| Tapered hair | 14 (70%) | 3 (15.0%) | 2 (10%) | **<.001*** | 16 (80%) | 15 (75%) | 14 (70%) | **.930** |
| Regrowing hair | 0 (0%) | 18 (90%) | 16 (80%) | **<.001*** | 0 (0%) | 5 (25%) | 6 (30%) | **.028*** |
| | **Group IIA** | | | | **Group IIB** | | | |
| | **Before treatment sessions** | **After treatment sessions** | **At the end of follow-up** | ***P*** | **Before treatment sessions** | **After treatment sessions** | **At the end of follow-up** | ***P*** |
| Group II (AGA patients) | | | | | | | | |
| Hair diameter diversity >20% | 20 (100%) | 20 (100%) | 12 (60%) | **<.001*** | 20 (100%) | 20 (100%) | 20 (100%) | **1.000** |
| Single hair/ follicular unit | 20 (100%) | 20 (100%) | 20 (100%) | **1.000** | 20 (100%) | 20 (100%) | 20 (100%) | **1.000** |
| Peripilar sign | 8 (40%) | 7 (35%) | 4 (20%) | **.367** | 11 (55%) | 11 (55%) | 11 (55%) | **1.000** |
| Yellow dots | 4 (20%) | 4 (20%) | 4 (20%) | **1.000** | 6 (30%) | 6 (30%) | 6 (30%) | **1.000** |
| Regrowing hair | 0 (0%) | 16 (80%) | 14 (70%) | **<.001*** | 0 (0%) | 0 (0%) | 0 (0%) | **1.000** |

Qualitative data were described using number and percent and were compared using chi-square test or Monte Carlo test.
AA, Alopecia areata; AGA, Androgenetic alopecia.
*Statistically significant at $P \leq .05$.

**TABLE 5** Distribution of the studied groups according to digital dermoscopic findings before, after treatment sessions and at the end of follow-up period

| Digital dermoscopic findings | Before treatment sessions | After treatment sessions | At the end of follow-up | *P* | Sig. bet. periods | | |
|---|---|---|---|---|---|---|---|
| | | | | | Before vs After | Before vs Follow-up | After vs Follow-up |
| Group I (AA patients) | | | | | | | |
| Group IA | | | | | | | |
| Density | 48.5 (9.8-150.2) | 83 (17.5-188.8) | 120.8 (16.3-400) | <.001* | 0.073 | <0.001* | 0.006* |
| Width | 0.02 (0.01-0.03) | 0.02 (0.01-0.05) | 0.04 (0.03-0.11) | <.001* | 0.107 | <0.001* | <0.001* |
| Group IB | | | | | | | |
| Density | 37.9 (8.7-80.6) | 42 (11.5-150.2) | 43 (25.1-390.6) | .497 | 0.285 | 0.139 | 0.114 |
| Width | 0.02 (0.01-0.03) | 0.01 (0.01-0.03) | 0.02 (0.01-0.09) | .695 | 0.121 | 0.593 | 0.307 |
| Group II (AGA patients) | | | | | | | |
| Group IIA | | | | | | | |
| Density | 205.1 (52.1-302.7) | 266.6 (104-335.3) | 210 (107.4-345.1) | <.001* | <0.001* | 0.018* | 0.044* |
| Width | 0.02 (0.01-0.12) | 0.02 (0.01-0.09) | 0.05 (0.03-0.10) | .001* | 0.948 | 0.043* | 0.002* |
| Group IIB | | | | | | | |
| Density | 222.6 (49.5-306.7) | 219.6 (43.3-296.5) | 214.4 (34.3-265.1) | .794 | 0.074 | 0.173 | 0.385 |
| Width | 0.02 (0.01-0.10) | 0.02 (0.01-0.09) | 0.02 (0.01-0.07) | .165 | 0.165 | 0.340 | 0.713 |

Abnormally distributed data were expressed in median (Min.-Max.) and were compared Friedman test, Sig. bet. periods was done using Wilcoxon signed ranks test.
AA, Alopecia areata; AGA, Androgenetic alopecia.
*Statistically significant at $P \leq .05$.

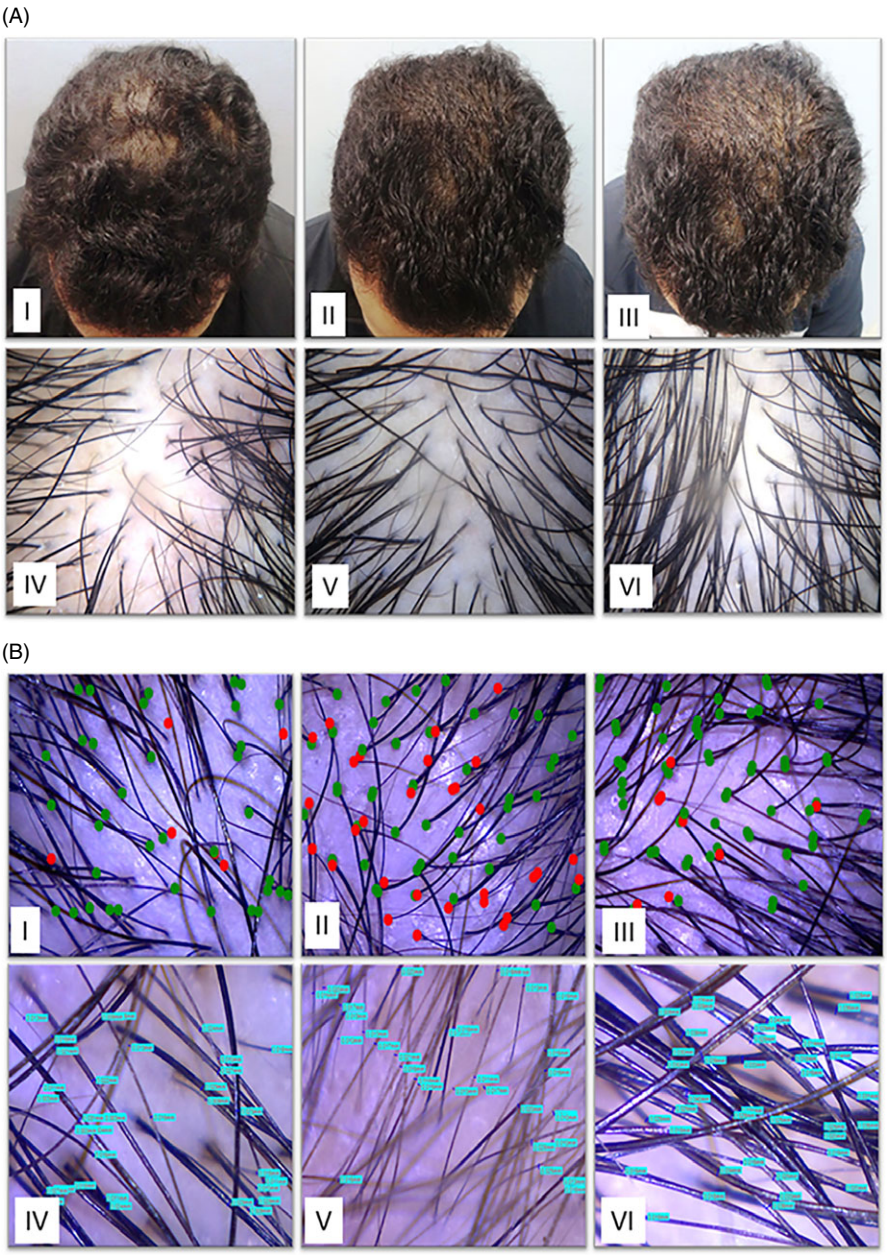


**FIGURE 3** A, A 27-year-old male patient with MPHL of 6 mo duration. (I) before treatment, (II) after 6 sessions of carboxytherapy showed very good response (A3), and (III) 3 mo after the last session of carboxytherapy showed clinical regression (A2). Dermoscopic picture (IV) before treatment showed hair diameter diversity >20%, dominance of single hair per follicular unit and presence of peripillar sign. (V) after 6 sessions of carboxytherapy, and (VI) at the end of follow-up period showed reduction of hair diameter diversity. B, Digital dermoscopic examination showed that; average hair density (I) before treatment was 136.7/cm$^2$, (II) after 6 sessions of carboxytherapy was 247.4/cm$^2$, and (III) 3 mo after the last session of carboxytherapy was 221.4/cm$^2$. While average hair shaft width; (IV) before treatment 0.020 mm, (V) after 6 sessions was 0.017 mm, and (VI) at the end of follow-up period was 0.038 mm

King S and King M[7] in their case study on a patient with a single localized AA patch who received four carboxytherapy sessions on weekly bases without any other treatment, and they reported regrowth of hair after 3 weeks. In the current study, the clinical improvement in AA patients was confirmed by dermoscopic examination which detected significant improvement of dermoscopic findings in comparison with that before treatment, in the form of regression of dystrophic hair, black dots, and tapered hair indicating regression of disease activity.[14,15] In addition, significant reduction of yellow dots was observed indicating regression of disease severity,[14,16] together with significant increase of upright regrowing hair at the end of follow-up period indicating significant improvement.

Furthermore, digital dermoscopic evaluation of AA after carboxytherapy revealed improvement in the median of hair density (and not in hair shaft width) in comparison with that before treatment but they were statistically insignificant. While at the end of follow-up

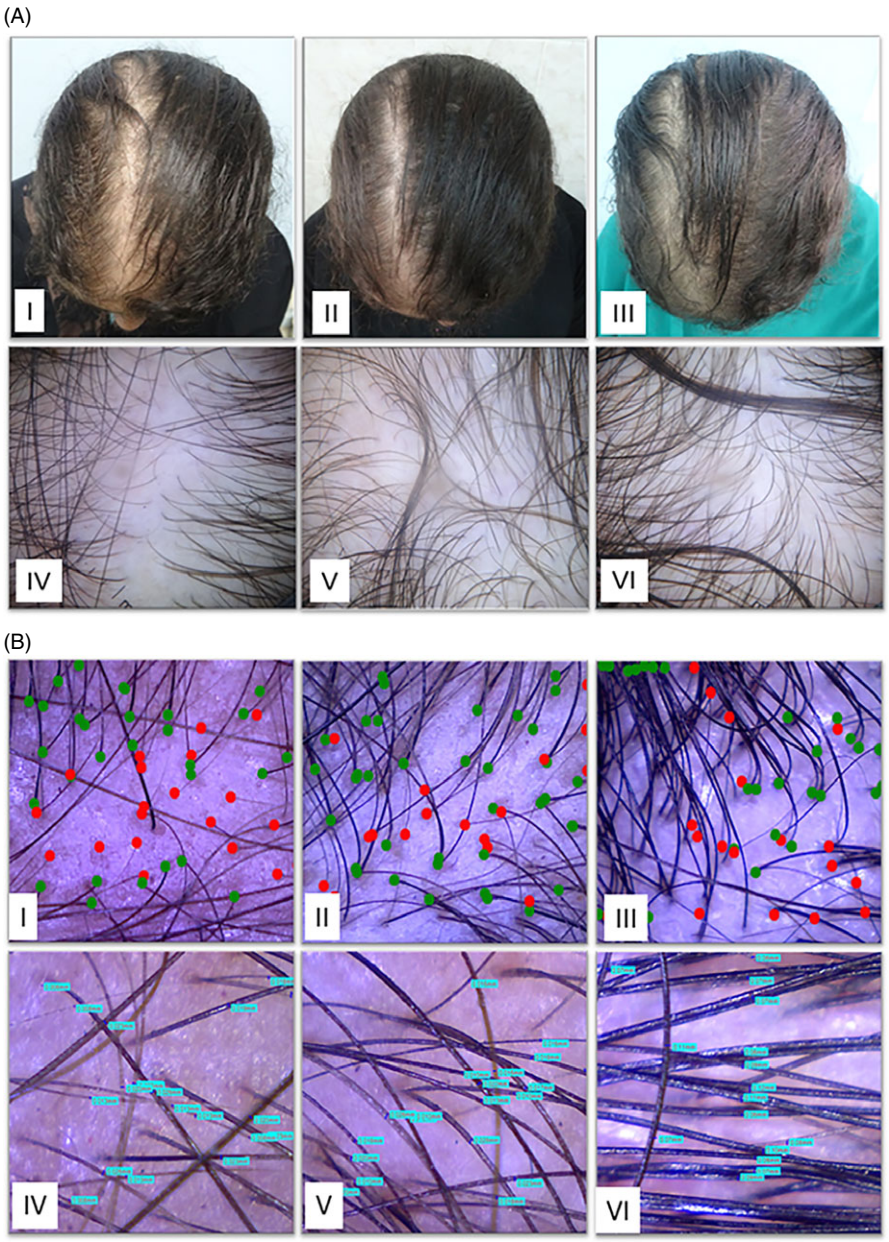


**FIGURE 4** A, A 33-year-old female patient with FPHL of 5-mo duration. (I) before treatment, (II) after 6 sessions of carboxytherapy showed excellent response (A4), and (III) 3 mo after the last session of carboxytherapy showed clinical regression (A2). Dermoscopic picture (IV) before treatment showed hair diameter diversity >20%, and dominance of single hair per follicular unit, (V) after carboxytherapy, and (VI) at the end of follow-up period showed reduction of hair diameter diversity. B, Digital dermoscopic examination showed that; average hair density (I) before treatment was 188.8/cm$^2$, (II) after 6 sessions of carboxytherapy was 236.2/cm$^2$, and (III) 3 mo after the last session of carboxytherapy was 212.8/cm$^2$. While average hair shaft width (IV) before treatment was 0.038 mm, (V) after carboxytherapy was 0.026 mm, and (VI) at the end of follow-up period was 0.053 mm

period, there was statistically significant improvement in both hair density and hair shaft width in comparison with that before and after treatment. From our point of view, measures of digital dermoscopy after treatment were not reliable as the regrowing white hair cannot be easily detected by it, thus providing inaccurate readings. While at the end of follow-up period, the regrowing hair became pigmented and therefore significant improvement could be detected.

In the current study, there were statistically significant improvements in clinical score, global assessments, dermoscopic, and digital dermoscopic findings in group IA patients with AA who received carboxytherapy in comparison with group IB received placebo injections allowing us to suggest that it could be an effective therapeutic option for patchy AA.

Regarding group II AGA patients in the current study, statistically significant clinical improvement was observed after carboxytherapy in comparison with that before treatment in the form of stoppage of further hair loss and induction of hair regrowth with 50% of patients showing very good response and 30% excellent response. These

results coincide with King S and King M[7] who evaluated carboxytherapy in two male patients with AGA, and they observed regrowth of hair in areas where it had previously disappeared and the hair became thicker. However, the current work detected gradual loss of the regrowing hair at the end of follow-up period but still better than that before treatment and only 30% of the studied patients reported very good response (A3) and 10% reported excellent response (A4). We confirmed our results by dermoscopic evaluation that revealed significant emergence of regrowing hair in AGA patients after carboxytherapy in comparison with that before treatment and significant reduction of hair diameter diversity at the end of follow-up period.

Furthermore, digital dermoscopic evaluation of AGA after last session of carboxytherapy revealed statistically significant improvement in hair density only and not in hair shaft width. This finding could be explained by the observation that the regrowing hair was initially a thin fine hair. However, at the end of follow-up period, significant reduction in the median of hair density was observed, but still significantly better than that before treatment in spite of significant improvement of hair shaft width in comparison with that before and after treatment. These results denoting that the therapeutic effect of carboxytherapy was not continued after cessation of sessions allowing us to recommend more sessions and combination with other therapeutic modalities as adjuvants for maintenance of hair regrowth.

In the current work, there was statistically significant clinical improvement in group IIA patients with AGA received carboxytherapy in comparison with group IIB received placebo injections as evaluated by global assessments, dermoscopic, and digital dermoscopic findings suggesting that carboxytherapy could be helpful as therapeutic option for AGA.

The reported side effects of carboxytherapy in this study in all groups were minimal and transient. Females were less tolerable to carboxytherapy than males. Patients with AGA complained headache more than patients with AA mostly due to injecting a larger amount of $CO_2$ per session with more compressive effect of the $CO_2$ gas when injected in addition to its direct vasodilator effect.

The clinical efficacy of carboxytherapy in management of alopecia observed in this study could be explained by the fact that carboxytherapy improves circulation at the injection site.[1] The injected $CO_2$ gas creates a relative state of hypercapnea that rapidly compensated by vasodilatation increasing the blood flow which in turn improve oxygenation and nutrients supply to the injection site. In addition, carboxytherapy promotes the Bohr Effect, which results in a shift in the oxygen–dissociation curve and a greater release of oxygen from hemoglobin at the cellular level.[1,2] Also, it increases levels of endothelial progenitor cells and various growth factors, including vascular endothelial growth factors (VEGF-A, B, C, and D)[2] that are important in the regulation of vascular endothelial cell proliferation through specific receptors leading to neoangiogenesis.[19] VEGF not only could be responsible for new capillary formation but also for the maintenance of a proper transendothelial exchange and stimuli between hair follicle keratinocytes and the capillary plexus, thus representing a further regulatory mechanism in stimulation of hair growth.[20]

The dermal papilla of the normal hair follicle (the presumptive location of follicular stem cells) presents a well-developed vascularization, thus providing optimal growth conditions.[21,22] It has been hypothesized that the reduction of VEGF production by alopecic hair follicles may diminish vascular support to affected scalp tissue, with subsequent inflammatory changes. This hypothesis is supported by the finding that loss of capillaries is an early change in alopecia and revascularization precedes hair regrowth.[8,23] As carboxytherapy stimulates release of VEGF with subsequent neoangiogenesis and improvement of oxygenation and nutrient supply at the injection site,[1] it could be a promising therapeutic option for different types of alopecia.

Regression of improvement noticed in our study during the follow-up period after cessation of sessions of CO2 injection particularly in AGA possibly explained by the fact that carboxytherapy has no direct effect on the main pathogenesis of these conditions as AA is an autoimmune disease[24] and AGA is an androgen-dependent disorder.[25] Therefore, carboxytherapy cannot be considered as a curative therapy for AA or AGA and could be regarded as a valuable adjuvant treatment for alopecia by improving vascularization of alopecic HFs with subsequent induction of hair regrowth. Therefore, increasing the number of sessions in combination with other therapeutic modalities is recommended for better and more long-lasting effect.

## 5 | CONCLUSION

Carboxytherapy is a cost-effective, well-tolerated, safe, and minimally invasive technique. It is a nonsurgical, very simple clinic procedure for an experienced dermatologist. Considering its excellent safety profile and clinical efficacy in our study in comparison with placebo, it seems to be a promising therapeutic option for patchy AA and could be helpful as an initial therapy of AGA but more than 6 sessions are required and adjuvants are recommended for maintenance of the results. Although carboxytherapy has theoretical scientific basis to support its use in hair restoration, clinical evidence is still weak. Therefore, further studies on larger scale population and comparative studies as a monotherapy vs combinations with other therapeutic modalities are recommended.

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### CONFLICTS OF INTEREST

None.

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## REFERENCES

1. Varlaro V, Manzo G, Mugnaini F, et al. Carboxytherapy: effects on microcirculation and its use in the treatment of severe lymphedema. *Acta Phlebol*. 2007;8:79-91.
2. Koutná N. Carboxytherapy in aesthetic medicine. In: Prendergast PM, Shiffman MA, eds. *Aesthetic Medicine: Art and Techniques*. New York, NY: Springer-Verlag Berlin Heidelberg; 2011:547-576.
3. Pianez LR, Custódio FS, Guidi RM, de Freitas JN, Sant'Ana E. Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study. *Clin Cosmet Investig Dermatol*. 2016;9:183-190.
4. Ferreira JC, Haddad A, Tavares SA. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. *J Drugs Dermatol*. 2008;7:201-206.
5. Fabbrocini G, Panariello L, De Vita V, et al. Quality of life in alopecia aerata: a disease specific questionnaire. *J Eur Acad Dermatol Venereol*. 2013;27:e276-e281.
6. Zhang M, Zhang N. Quality of life assessment in patients with alopecia areata and androgenetic alopecia in the People's Republic of China. *Patient Prefer Adherence*. 2017;11:151-155.
7. King S, King M. Using carboxytherapy as an adjuvant aesthetic treatment for patients with alopecia. *J Aesthetic Nurs*. 2013;2:434-438.
8. Goldman CK, Tsai JC, Soroceanu L, Gillespie GY. Loss of vascular endothelial growth factor in human alopecia hair follicles. *J Invest Dermatol*. 1995;104(5 Suppl):18S-20S.
9. Gerkowicz A, Krasowska D, Pietrzak A, et al. Video capillaroscopic alterations in alopecia areata. *Biomed Res Int*. 2013;2013:160203.
10. Hernandez BA. Is androgenic alopecia a result of endocrine effects on the vasculature? *Med Hypotheses*. 2004;62:438-441.
11. Li C, Li W, Lu Z. Vascular endothelial growth factor and its receptor may be one molecular mechanism for androgenetic alopecia. *Med Hypotheses*. 2009;72:366-367.
12. Olsen E. Investigative guidelines for alopecia areata. *Dermatol Ther*. 2011;24:311-319.
13. Kaliyadan F, Nambiar A, Vijayaraghavan S. Androgenetic alopecia: an update. *Indian J Dermatol Venereol Leprol*. 2013;79:613-625.
14. Jain N, Doshi B, Khopkar U. Trichoscopy in alopecias: diagnosis simplified. *Int J Trichology*. 2013;5940:170-178.
15. Kharkar V. Overview of Trichoscopy. In: Khopkar US ed. *Dermoscopy and Trichoscopy in Diseases of the Brown Skin*, 1st edn. New Delhi, India: Jaypee, 2012:169-181.
16. Rudnicka L, Olszewska M, Rakowska A, Slowinska M. Trichoscopy update. *J Dermatol Case Rep*. 2011;5:82-88.
17. Hu R, Xu F, Han Y, et al. Tricoscopic findings of androgenetic alopecia and their association with disease severity. *J Dermatol*. 2015;42:602-607.
18. Kirkpatrick LA, Feeney BC. *A simple Guide to IBM SPSS Statistics for Version 20.0*. Student ed. Belmont, CA: Wadsworth, Cengage Learning; 2013.
19. Ferrara N. VEGF-A: a critical regulator of blood vessel growth. *Eur Cytokine Netw*. 2009;20:158-163.
20. Simonetti O, Lucarini G, Bernardini ML, Simoncini C, Biagini G, Offidani A. Expression of vascular endothelial growth factor, apoptosis inhibitors (survivin and p16) and CCL27 in alopecia areata before and after diphencyprone treatment: an immunohistochemical study. *Br J Dermatol*. 2004;150:940-948.
21. Kozlowska U, Blume-Peytavi U, Kodelja V, et al. Expression of vascular endothelial growth factor (VEGF) in various compartments of the human hair follicle. *Arch Dermatol Res*. 1998;290:661-668.
22. Li W, Man XY, Li CM, et al. VEGF induces proliferation of human hair follicle dermal papilla cells through VEGFR-2-mediated activation of ERK. *Exp Cell Res*. 2012;318:1633-1640.
23. Yano K, Brown LF, Detmar M. Control of hair growth and follicle size by VEGF-mediated angiogenesis. *J Clin Invest*. 2001;107:409-417.
24. Pratt CH, King LE Jr, Messenger AG, Christiano AM, Sundberg JP. Alopecia areata. *Nat Rev Dis Primers*. 2017;3:17011.
25. Lolli F, Pallotti F, Rossi A, et al. Androgenetic alopecia: a review. *Endocrine*. 2017;57:9-17.

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# HBO and gas embolism

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*Gas embolism, which occurs with the entry of gas into the circulatory system from the vein, artery or both, is a potentially serious even fatal condition. The two main causes of gas embolism are iatrogenic and diving. The site of entry and the signs and symptoms distinguish between arterial and venous embolism. The entering gas may be air, but may also be $CO_2$ or other gases, especially in iatrogenic embolism. Supportive care is the primary therapy for venous gas embolism, while hyperbaric oxygen therapy in addition to supportive care is the first line of treatment for arterial gas embolism. In this article, we will review the pathophysiology, etiology, diagnosis and treatment of gas embolism.* [Neurol Res 2007; **29**: 142–145]

*Keywords: Gas embolism; hyperbaric oxygen therapy*

## INTRODUCTION

Gas embolism is the entry of gas into the circulatory system. It can be venous, arterial or both. It is a potentially serious, even fatal condition, particularly when the arterial system is involved. The two main causes of gas embolism are iatrogenic and diving. Iatrogenic gas embolism occurs with direct entry of gas into the vascular structure during medical procedures. Diving embolism occurs when the diver holds his breath during ascent: the air within his lungs expands, creating a higher intrapulmonary pressure that can cause alveolar rupture and entry of air into the pulmonary capillaries. The site of entry and the signs and symptoms distinguish between the arterial and venous embolism. The entering gas may be air, but may also be $CO_2$ or other gases, especially in iatrogenic embolism. Supportive care is the primary therapy for venous gas embolism, while hyperbaric oxygen therapy in addition to supportive care is the first line of treatment for arterial gas embolism. In this article, we will review the pathophysiology, etiology, diagnosis and treatment of gas embolism.

## VENOUS GAS EMBOLISM

Gas in the venous system is transported to the lungs through the pulmonary arteries. Usually, filtration by the pulmonary vessels protects against the gas bubble reaching the systemic and coronary circulation[1]. However, when the volume of gas bubbles or their velocity within the pulmonary arteries is great enough, some gas emboli may transgress the pulmonary capillaries and enter the arterial circulation via the pulmonary veins[2,3]. Animal studies have shown that the cover depends on the dose (amount of gas) and injection speed as well as the pressure gradient. Gas bubbles in the venous system can also remain trapped in the pulmonary capillary bed. This can lead to decreased gas exchange[4], cardiac arrhythmias[5], pulmonary hypertension[6] and cardiac failure[7]. A large volume of gas bubbles can increase pulmonary arterial pressure, which in turn can strain right ventricular outflow decreasing pulmonary venous return. With decreased left ventricular preload, cardiac output will be compromised, causing arrhythmias and cardiac failure[8]. Venous gas emboli occur most often during manipulation of central venous catheters[9], but can also occur during neurosurgical operations performed with the patient in a sitting position[10], barotrauma associated with mechanical ventilation[11] and laparoscopic procedures[12]. Venous gas embolism is assessed by clinical findings and when suspected, it is vital to prevent further entry of gas into the vasculature. Supplemental 100% oxygen is recommended to increase the gas gradient and increase nitrogen diffusion out of the bubble, thus decreasing the bubble size[13]. Pouring sterile fluid in the field will prevent further gas entry. Fluid resuscitation for volume expansion will also prevent the further entry of gas into the venous circulation by elevating the venous pressure. Increasing the preload may also reduce right to left shunting in the heart, and thus reduce the possibility of paradoxical embolism or heart failure.

## PARADOXICAL EMBOLISM

Paradoxical embolism occurs when gas that has entered the venous system migrates to the arterial system and causes obstruction of end arteries[14]. Right to left shunting through a patent foramen ovale and pulmonary capillary filtration overload can cause paradoxical emboli[15]. The treatment is the same as for an arterial

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gas embolism. Any venous gas embolism has the potential to become an arterial gas embolism.

## ARTERIAL GAS EMBOLISM

Arterial gas embolism occurs with the entry of gas into the pulmonary veins or to the arteries of the systemic circulation. It can be direct, as with injection of air into the artery during radiologic interventions or cardiac bypass, or paradoxical. The mechanism of injury is two-fold[16]. First, ischemia is induced by direct occlusion from a gas thrombosis. Then the bubbles adhere to vessel walls and the endothelium is damaged, triggering a cascade of inflammatory responses creating further injury[17,18]. The obstruction of functional end arteries caused by the gas bubbles can occur in any organ, but is the most serious if it is in the coronary or cerebral circulation. Coronary arterial gas embolism may present with ischemic and depressed myocardial symptoms such as chest pain and shortness of breath possibly evolving into cardiac failure or arrest[7]. The cerebral circulation receives ~20% of cardiac output and because arterial gas would distribute according to blood flow, it is the most susceptible to gas embolism. When gas bubbles contact the endothelium of the blood–brain barrier, it leads to the activation and adhesion of polymorphonuclear leukocytes in the damaged area, causing brain swelling and inflammation[19]. The symptoms of cerebral arterial gas embolism often present as a stroke and the severity is determined by the quantity of the gas and area of embolism[20]. Neurological symptoms and signs such as motor weakness, paralysis, headaches, dizziness, nausea, convulsions, loss of consciousness and coma may occur. Loss of consciousness due to acute ischemia is often followed by an awake phase as bubbles clear the circulation. Loss of consciousness often recurs with the onset of ischemia-reperfusion injury and bubble-induced endothelial injury. Unrecognized intraoperative gas embolism may present as a delayed recovery from general anesthesia[21].

## DIAGNOSIS

Gas embolism is primarily diagnosed by history (Table 1). The clinician should suspect the diagnosis whenever characteristic symptoms follow any invasive procedures carrying great risks for gas embolism such as manipulation of central venous catheters[9], cardiac surgery with cardiopulmonary bypass[22,23], craniotomy performed in a sitting position[10], laparoscopic procedures, Cesarean section[24] or hip replacements[25]. All these procedures involve incision of a vascular bed and a hydrostatic gradient favoring the intravascular entry of gas. During such procedures, a sudden loss of consciousness or hemodynamic collapse may indicate arterial gas embolism. Transthoracic and transesophageal echocardiography can visualize intravenous or intracardiac bubbles directly[26,27]. During anesthesia, a sudden increase in the pulmonary artery pressure or a decrease in end-tidal $CO_2$ may be indicative of a venous gas embolism of the pulmonary circulation[28]. CT and MRI may sometimes be useful to distinguish between cerebral infarct and intracerebral bleeding (*Figure 1*)[21].

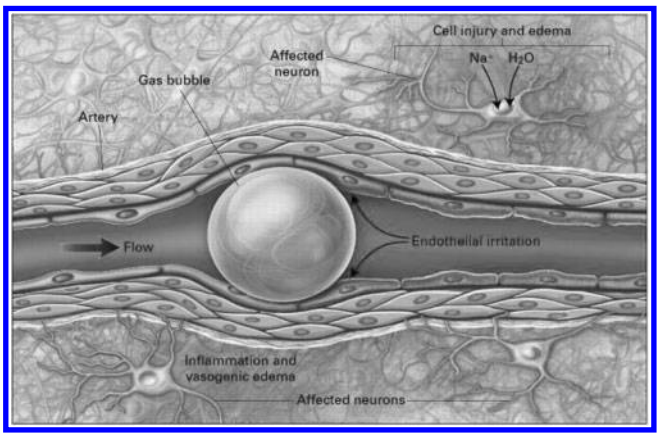


**Figure 1:** Bubble obstructing end-arterial flow in cerebral vessel causing distal ischemia[21]

## TREATMENT

The treatment for venous embolism is supportive. Treatment for arterial embolism includes both supportive treatment and hyperbaric oxygen therapy. The initial treatment is aimed at reversing the embolic event and protecting vital functions. Oxygen administration at the highest possible concentration is important not only to treat hypoxia and hypoxemia but also to establish a diffusion gradient that favors the egress of gas from the

**Table 1:** Causes and treatment for gas embolism

| | Venous gas embolism | Paradoxical embolism | Arterial gas embolism |
|---|---|---|---|
| Cause | Central line manipulation<br>Neurosurgical operations in sitting position<br>Barotrauma<br>Laparoscopic procedures | Right to left shunting | Paradoxical embolism<br>Injection of air during radiologic interventions<br>Surgery (CABG, sitting craniotomy laparoscopy/video assisted surgery)<br>Lung biopsy<br>Central line manipulation<br>Hemodialysis<br>Others |
| Treatment | 100% oxygen | Hyperbaric oxygen therapy | Hyperbaric oxygen therapy |

CABG=coronary artery bypass graft.

bubbles. Endotracheal intubation may be necessary in some patients to maintain adequate oxygenation and ventilation[21]. Patients should be placed in a flat supine position. The head down position is not favored because the buoyancy of the bubbles is not sufficient to counteract blood flow and the position may aggravate cerebral edema[16]. Blood pressure should be aimed at maintaining normal end-organ perfusion. Systemic hypertension following bubble entrapment in the cerebral circulation is commonplace and may be beneficial in promoting bubble redistribution through the arterioles to the capillaries and into the veins. However, prolonged hypertension may lead to increased intracranial pressure compromising neurological outcome[21]. Hypotension should also be avoided because it will keep the bubble entrapped and decrease cerebral blood flow to below normal levels. Normovolemia should be maintained to achieve optimal microcirculation and seems more important than maintaining a normal hematocrit. Colloid solutions are preferable to crystalloids in preventing cerebral edema[29].

### Hyperbaric oxygen therapy

Hyperbaric oxygen therapy is the primary treatment for arterial gas embolism. In the acute phase, it decreases the intravascular bubble size by increasing the ambient pressure[30]. Hyperoxia also produces a gradient that increases the rate at which nitrogen or other gases diffuse out of the gas phase (bubble) into solution[31]. Moreover, it facilitates oxygenation of ischemic tissue. Another important benefit of hyperbaric oxygen therapy, particularly in the context of ischemia-reperfusion injury, is the inhibition of neutrophil attachment to blood vessels[32]. Cell surface molecules called '$\beta$2 integrins' mediate irreversible adherence of activated neutrophils[33]. Hyperbaric oxygen inhibits membrane guanylate cyclase, which in turn inhibits $\beta$2 integrin adherence[32]. Treatment with hyperbaric oxygen thus decreases the deleterious effects of cerebral gas embolism on intracranial pressure and brain metabolism. The clinical outcome of patients with cerebral gas embolism is related to the time to treatment. However, the critical time interval after injury within which hyperbaric oxygen may still be effective is not certain and has been variably reported to be 3–48 hours[34,35]. Because the occurrence of a gas embolism is unpredictable and infrequent, it is difficult to plan a prospective randomized trial to prove its use. Still, hyperbaric oxygen therapy is considered as the first-line treatment in arterial gas embolism.

### Adjuvant therapy

Although corticosteroid therapy has been advocated to treat cerebral edema after gas embolism, more recent studies have shown that it increases ischemic injury because of vessel occlusion and it is no longer generally recommended[21]. Anticoagulant therapy using heparin may be beneficial but is controversial because despite its effect against platelet aggregation, it has the risk of hemorrhage into the infarcted tissue[21]. Lidocaine has been shown to preserve neuroelectric function, reduce infarct size, preserve cerebral blood flow, reduce cerebral edema and reduce intracranial pressure in animal models of cerebral arterial gas embolism[36]. Its use in humans is unproven, but it seems reasonable to use given the benefits seen in animal models and a low risk. Continuous intravenous lidocaine administration may improve cerebral function but overdose may cause central nervous system depression, convulsions and bradyarrhythmias, so it should be used with caution and appropriate monitoring. Finally, cerebral gas embolism may cause generalized seizures that are unresponsive to benzodiazepines and the use of barbiturates is recommended in these cases. Barbiturates have the advantage of reducing cerebral oxygen consumption and intracranial pressure, and of releasing endogenous catecholamines resulting in cerebral protection after ischemia[37]. Interactions between blood-borne macromolecules adsorbed to the bubble surface and the endothelial surface lead to the development of an adhesion force causing embolism bubbles to lodge in the vasculature. New strategies to treat gas embolism will depend on knowledge of the interaction between bubble interface and vascular endothelium. If adhesion forces are smaller than the forces tending to advance the bubble downstream, this would speed up the course of bubbles through the vasculature. One possible means of decreasing the adhesion force would be the use of surfactants. Studies have suggested that the administration of surfactants prevent or reduce gas bubble adhesion to the endothelium. There are currently no clinically approved drugs available to treat gas embolism, but the use of surfactants may be a target based therapeutic approach for the future treatment of gas embolism related injury[38,39]. This would also be beneficial in high-risk situations such as cardiac patients.

## CONCLUSIONS

Gas embolism is a risk in all areas of clinical care and can cause serious morbidity and even death. Steps to reduce the entry of gas bubbles into the vascular structures must be a routine part of all procedures in which gas embolism is a risk. When gas embolism occurs, rapid detection and treatment are essential. Along with cardiopulmonary stabilization, administration of 100% oxygen and ventilatory support, hyperbaric oxygen therapy is the treatment of choice for arterial gas embolism.

## REFERENCES


1 Butler BD, Hills BA. The lung as a filter for microbubbles. *J Appl Physiol* 1979; **47** (3): 537–543

2 Butler BD, Robinson R, Sutton T, *et al.* Cardiovascular pressures with venous gas embolism and decompression. *Aviat Space Environ Med* 1995; **66** (5): 408–414

3 Butler BD, Hills BA. Transpulmonary passage of venous air emboli. *J Appl Physiol* 1985; **59** (2): 543–547

4 Bove AA, Hallenbeck JM, Elliott DH. Circulatory responses to venous air embolism and decompression sickness in dogs. *Undersea Biomed Res* 1974; **1** (3): 207–220

5 Van Blankenstein JH, Slager CJ, Schuurbiers JC, *et al.* Heart function after injection of small air bubbles in coronary artery of pigs. *J Appl Physiol* 1993; **75** (3): 1201–1207

6 Evans DE, Kobrine AI, Weathersby PK, *et al.* Cardiovascular effects of cerebral air embolism. *Stroke* 1981; **12** (3): 338–344A

7 Durant TM, Oppenheimer MJ, Webster MR, *et al.* Arterial air embolism. *Am Heart J* 1949; **38**: 481–500

8 Durant TM, Long J, Oppenheimer MJ. Pulmonary (venous) air embolism. *Am Heart J* 1947; **33**: 269–281

9 Halliday P, Anderson DN, Davidson AI, e*t al.* Management of cerebral air embolism secondary to a disconnected central venous catheter. *Br J Surg* 1994; **81** (1): 71

10 Porter JM, Pidgeon C, Cunningham AJ. The sitting position in neurosurgery: A critical appraisal. *Br J Anaesth* 1999; **82** (1): 117–128

11 Marini JJ, Culver BH. Systemic gas embolism complicating mechanical ventilation in the adult respiratory distress syndrome. *Ann Intern Med* 1989; **110** (9): 699–703

12 McGrath BJ, Zimmerman JE, Williams JF, *et al.* Carbon dioxide embolism treated with hyperbaric oxygen. *Can J Anaesth* 1989; **36** (5): 586–589

13 Van Liew HD, Conkin J, Burkard ME. The oxygen window and decompression bubbles: Estimates and significance. *Aviat Space Environ Med* 1993; **64** (9 Pt 1): 859–865

14 Black M, Calvin J, Chan KL, *et al.* Paradoxic air embolism in the absence of an intracardiac defect. *Chest* 1991; **99** (3): 754–755

15 Fraker TD, Jr, Harris PJ, Behar VS, *et al.* Detection and exclusion of interatrial shunts by two-dimensional echocardiography and peripheral venous injection. *Circulation* 1979; **59** (2): 379–384

16 Moon RE, de Lisle Dear G, Stolp BW. Treatment of decompression illness and latrogenic gas embolism. *Respir Care Clin N Am* 1999; **5** (1): 93–135

17 Levin LL, Stewart GJ, Lynch PR, *et al.* Blood and blood vessel wall changes induced by decompression sickness in dogs. *J Appl Physiol* 1981; **50** (5): 944–949

18 Nossum V, Koteng S, Brubakk AO. Endothelial damage by bubbles in the pulmonary artery of the pig. *Undersea Hyperb Med* 1999; **26** (1): 1–8

19 Mitchell S, Gorman D. The pathophysiology of cerebral arterial gas embolism. *J Extra Corpor Technol* 2002; **34** (1): 18–23

20 Orebaugh SL. Venous air embolism: Clinical and experimental considerations. *Crit Care Med* 1992; **20** (8): 1169–1177

21 Muth CM, Shank ES. Gas embolism. *N Engl J Med* 2000; **342** (7): 476–482

22 Armon C, Deschamps C, Adkinson C, *et al.* Hyperbaric treatment of cerebral air embolism sustained during an open-heart surgical procedure. *Mayo Clin Proc* 1991; **66** (6): 565–571

23 Stump DA, Rogers AT, Hammon JW, *et al.* Cerebral emboli and cognitive outcome after cardiac surgery. J *Cardiothorac Vasc Anesth* 1996; **10** (1): 113–118

24 Weissman A, Kol S, Peretz BA. Gas embolism in obstetrics and gynecology. A review. *J Reprod Med* 1996; **41** (2): 103–111

25 Evans RD, Palazzo MG, Ackers JW. Air embolism during total hip replacement: Comparison of two surgical techniques. *Br J Anaesth* 1989; **62** (3): 243–247

26 Mammoto T, Hayashi Y, Ohnishi Y, *et al.* Incidence of venous and paradoxical air embolism in neurosurgical patients in the sitting position: Detection by transesophageal echocardiography. *Acta Anaesthesiol Scand* 1998; **42** (6): 643–647

27 Boussuges A, Molenat F, Carturan D, *et al.* Venous gas embolism: Detection with pulsed Doppler guided by two-dimensional echocardiography. *Acta Anaesthesiol Scand* 1999; **43** (3): 328–332

28 Bedford RF, Marshall WK, Butler A, *et al.* Cardiac catheters for diagnosis and treatment of venous air embolism: A prospective study in man. *J Neurosurg* 1981; **55** (4): 610–614

29 Reasoner DK, Ryu KH, Hindman BJ, *et al.* Marked hemodilution increases neurologic injury after focal cerebral ischemia in rabbits. *Anesth Analg* 1996; **82** (1): 61–67

30 Leitch DR, Greenbaum LJ, Jr, Hallenbeck JM. Cerebral arterial air embolism: I. Is there benefit in beginning HBO treatment at 6 bar? *Undersea Biomed Res* 1984; **11** (3): 221–235

31 Moon RE. Bubbles in the brain: What to do for arterial gas embolism? *Crit Care Med* 2005; **33** (4): 909–910

32 Thom SR. Effects of hyperoxia on neutrophil adhesion. *Undersea Hyperb Med* 2004; **31** (1): 123–131

33 Granger DN, Kubes P. The microcirculation and inflammation: Modulation of leukocyte-endothelial cell adhesion. *J Leukoc Biol* 1994; **55** (5): 662–675

34 Baskin SE, Wozniak RF. Hyperbaric oxygenation in the treatment of hemodialysis-associated air embolism. *N Engl J Med* 1975; **293** (4): 184–185

35 Wherrett CG, Mehran RJ, Beaulieu MA. Cerebral arterial gas embolism following diagnostic bronchoscopy: Delayed treatment with hyperbaric oxygen. *Can J Anaesth* 2002; **49** (1): 96–99

36 Mitchell SJ, Pellett O, Gorman DF. Cerebral protection by lidocaine during cardiac operations. *Ann Thorac Surg* 1999; **67** (4): 1117–1124

37 van Hulst RA, Klein J, Lachmann B. Gas embolism: Pathophysiology and treatment. *Clin Physiol Funct Imaging* 2003; **23** (5): 237–246

38 Suzuki A, Eckmann DM. Embolism bubble adhesion force in excised perfused microvessels. *Anesthesiology* 2003; **99** (2): 400–408

39 Suzuki A, Armstead SC, Eckmann DM. Surfactant reduction in embolism bubble adhesion and endothelial damage. *Anesthesiology* 2004; **101** (1): 97–103

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ORIGINAL CONTRIBUTION

# Effects of carboxytherapy on skin laxity

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## Abstract

**Background:** Carboxytherapy is capable of inducing tissue repair which results in an increase in elastic and collagen fibers.

**Objectives:** To evaluate the effects of carboxytherapy upon human skin collagen and elastic fiber synthesis.

**Methods:** Case study of nine volunteers who received a single application of carboxytherapy in the left infraumbilical region, with infusion rate of 100 mL/min and 0.6 mL/kg weight over an area of 25 $cm^2$. After 60 days on average, the skin was collected for histological analysis and stained with picrosirius red for collagen and Verhoeff for elastic fibers. The percentage of fibers found was marked by the Image J® program and recorded in a Microsoft Excel spreadsheet. Statistical analysis was performed using SPSS (version 20), with a significance level of 95%.

**Results:** An increase in the collagen and elastic fibers sample was observed in the treated group. Morphometrically, a significant increase in the percentage of collagen in the Carboxytherapy group (41.44 ± 4.50%) was observed compared with the control group (37.44 ± 3.87%) with $P = .04$; for elastic fibers, the percentage showed no significant difference between the control group (10.55 ± 4.33%) and the carboxytherapy group (10.44 ± 3.71%).

**Conclusions:** Carboxytherapy with the parameters used in this study was able to stimulate collagen and elastic fiber synthesis, with significant differences in the morphometry for collagen fibers.

KEYWORDS

carbon dioxide, carboxytherapy, collagen, skin aging, skin laxity

## 1 | INTRODUCTION

Carboxytherapy or carbon dioxide therapy refers to transcutaneous administration of $CO_2$ for therapeutic purposes.[1] Carboxytherapy is an old technique that was first developed in France in 1932, when it was discovered that wound healing was accelerated after bathing in $CO_2$-enriched pools. The technique was used by cardiologists in the 1950s to treat patients with peripheral arterial occlusive pathologies. The term "carboxytherapy" was created in 1995 by Luigi Parassoni during the XVI National Meeting of the Italian Society of Aesthetic Medicine.[1-3]

The controlled $CO_2$ infusion is considered a safe, nonsurgical procedure consisting of applying medicinal carbon dioxide to the skin and adipose tissue.[4] Therapeutic indications for $CO_2$ include vascular, cardiac, metabolic and rheumatic pathologies, cellulitis, chronic wounds, localized adiposities, migraine, stretch marks, wrinkles, unaesthetic scars, and skin laxity.[2,5-7]

  

In the tissue, water and $CO_2$ molecules react and result in carbonic acid molecules that reduce tissue pH. The lower the pH, the weaker the bonding between hemoglobin and oxygen, leading to hemoglobin oxygen release. The lower the pH, the weaker the binding between hemoglobin and oxygen, leading to hemoglobin oxygen release and microcirculation vasodilation along with increased peripheral blood flow.[8] In addition, intradermal $CO_2$ injections cause an increase in remodeling collagen.[9]

Subcutaneous injection of carbon dioxide results in increased thickness of the skin and capillary blood circulation. Furthermore, it induces an intense dermic collagen change, a new collagen synthesis, and a compact and organized collagen arrangement.[9,10] Carboxytherapy also improves cutaneous circulation, which is reflected by the overall improvement of skin function.[11]

This study aims to evaluate the effects of carboxytherapy upon the collagen and elastic fiber synthesis in abdominal skin.

## 2 | METHODS

### 2.1 | Study design

Quasi-experimental case series study with blinding of the evaluator and convenience sample.

We selected 10 women in good general health condition who were awaiting abdominoplasty at the Clinical Hospital of the Federal University of Triângulo Mineiro (UFTM) from August 2017 to February 2018. It refers to a convenience sample, in which only these volunteers were invited to participate in the study by the cosmetic surgery team. The ones excluded from the study were as follows: underaged; women with sensory and cognitive impairment; with signs of infection, circulatory disorders, neoplasia or any other condition in which carboxytherapy was contraindicated.

After reading and agreeing to participate in the study, the volunteers signed the free and informed consent form. Next, an initial evaluation was performed with anthropometric (weight, height, and body mass index-BMI) and personal data collection, as well as the cutaneous phototype following Fitzpatrick scale.

The project was approved by CEP-UFTM under no. 1362.

### 2.2 | Procedures

The volunteers received a single application of carboxytherapy (Carbtek® device, at infusion rate of 100 mL/min and 0.6 mL/kg—volunteer's weight), at a single point in a 25 $cm^2$ area on the left side of the infraumbilical region.

The skin was collected during the surgical procedure, about 60 days after the application and the fragment was sent to histological analysis. The skin of the contralateral region of the flap removed by the surgeon, far from the area that received the treatment, was used as a control.

### 2.3 | Histological and histomorphometric evaluation

The skin samples were collected inside the operating room, kept in 10% formaldehyde and then sent to the general pathology service of the Federal University of Triângulo Mineiro for histological processing. These were separated into two groups: control group and carboxytherapy treatment group. After fixation, the samples were dehydrated in ethanol, diaphanized in xylol, and embedded in paraffin. Histological sections of 5 μm thick were obtained in microtome and placed on precoated polylysine slides. Histological sections of 5 μm thickness were obtained in microtome and placed on precoated polylysine slides. Histological sections were stained with picrosirius red to evaluate collagen and Verhoeff fibers for elastic fibers.

The slides were viewed with a 20× objective. The images were captured with a common light z-microscope system and analyzed by the Axionvision Automatic Image Analyzer System. Thus, the area to be quantified was captured and photographed through a camera (AxioCam ICc 5) connected to the microscope and the computer to digitize the images that were saved in TIFF format.

The percentage of collagen fibers and elastic fibers found in each area was calculated by the Image J® program and was later recorded in a Microsoft Excel spreadsheet.

### 2.4 | Statistical analysis

The data were submitted to statistical analysis using the SPSS software (version 20) and were also submitted to the Shapiro-Wilk normality test. Next, the paired t test was used due to the normality of the data. Significant difference was considered when $P < .05$.

## 3 | RESULTS

The final sample consisted of nine volunteers with a mean age of 45.3 ± 10.5 years, BMI (mean 25.45 ± 2.02) and predominance of phototype III with 66.33%, followed by phototype V with 22.33% and only one volunteer with Phototype II (11.33%).

In this study, five volunteers with skin phototype III showed significant improvement in collagen fibers. The first volunteer presented an average of 33.39% of collagen fibers, and later in the treatment, the average was 45.41%; the second one, the improvement was from 38.45% to 43.77%, a third one from 36.72% to 41.37%, a fourth one from 40.29% to 44.18%, and a fifth one, with a slight improvement, from 42.65% to 44%. One volunteer with skin phototype II initially presented 38.71% of collagen fibers and after the application presented 43.63%. Two volunteers with skin phototype V also had a significant improvement with an initial average from 34.19% and 30.96% to 45.58 and 36.51%, respectively. Out of the nine volunteers, only one with skin phototype III did not respond well to carboxytherapy treatment.

Histological findings demonstrated an increase in collagen synthesis (Figure 1) and elastic fibers (Figure 2) in the treated

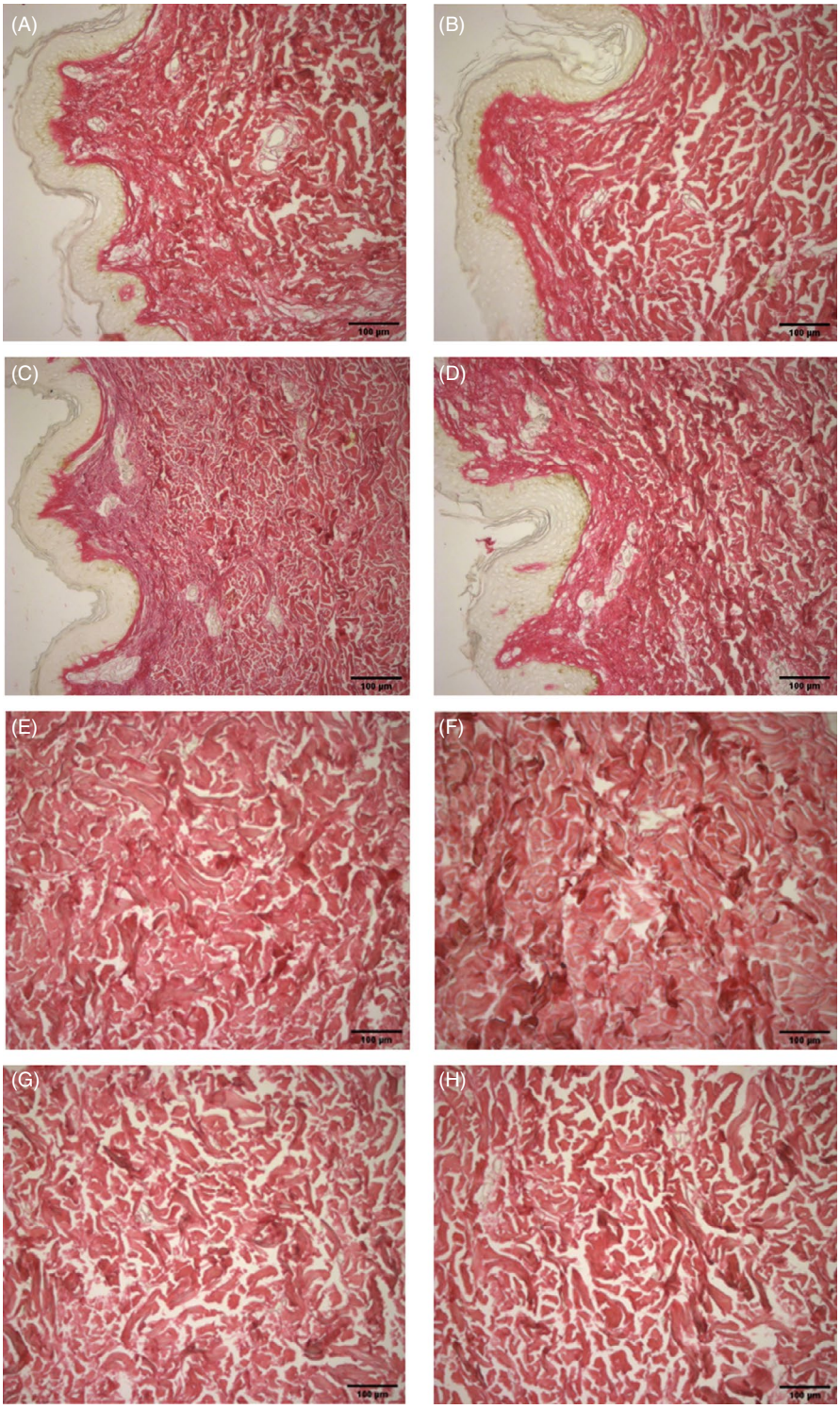


**FIGURE 1** Effect of carboxytherapy treatment on picrosirius-stained human skin fibers. Skin of 32-year-old woman: (A) control; (B) collected after 61 d of treatment with carboxytherapy (CA). Skin of 51-year-old woman: (C) control; (D) collected 66 d after CA treatment. Skin of a 57-year-old woman: (E) control; (F) collected 55 d after CA treatment. Skin of 32-year-old woman: (G) control; (H) collected 61 d after CA treatment. Bar = 100 μm

groups compared with the control group. The morphometry showed a significant increase in the percentage of collagen in the group treated with Carboxytherapy (41.44 ± 4.50%) compared to the control (37.44 ± 3.87%) with $P = .04$ (Figure 3A). The morphometric analysis of the percentage of elastic fibers showed no significant difference between the control group (10.55 ± 4.33%) and the one treated with carboxytherapy (10.44 ± 3.71%) (Figure 3B).

## 4 | DISCUSSION

The results showed stimulation of collagen and elastic fiber synthesis after a single session of carboxytherapy, with significant results for collagen morphometry.

No studies on morphometric evaluation after carboxytherapy application were found, except for histological analysis, in which the authors also found an increase in collagen.[9,12,13]

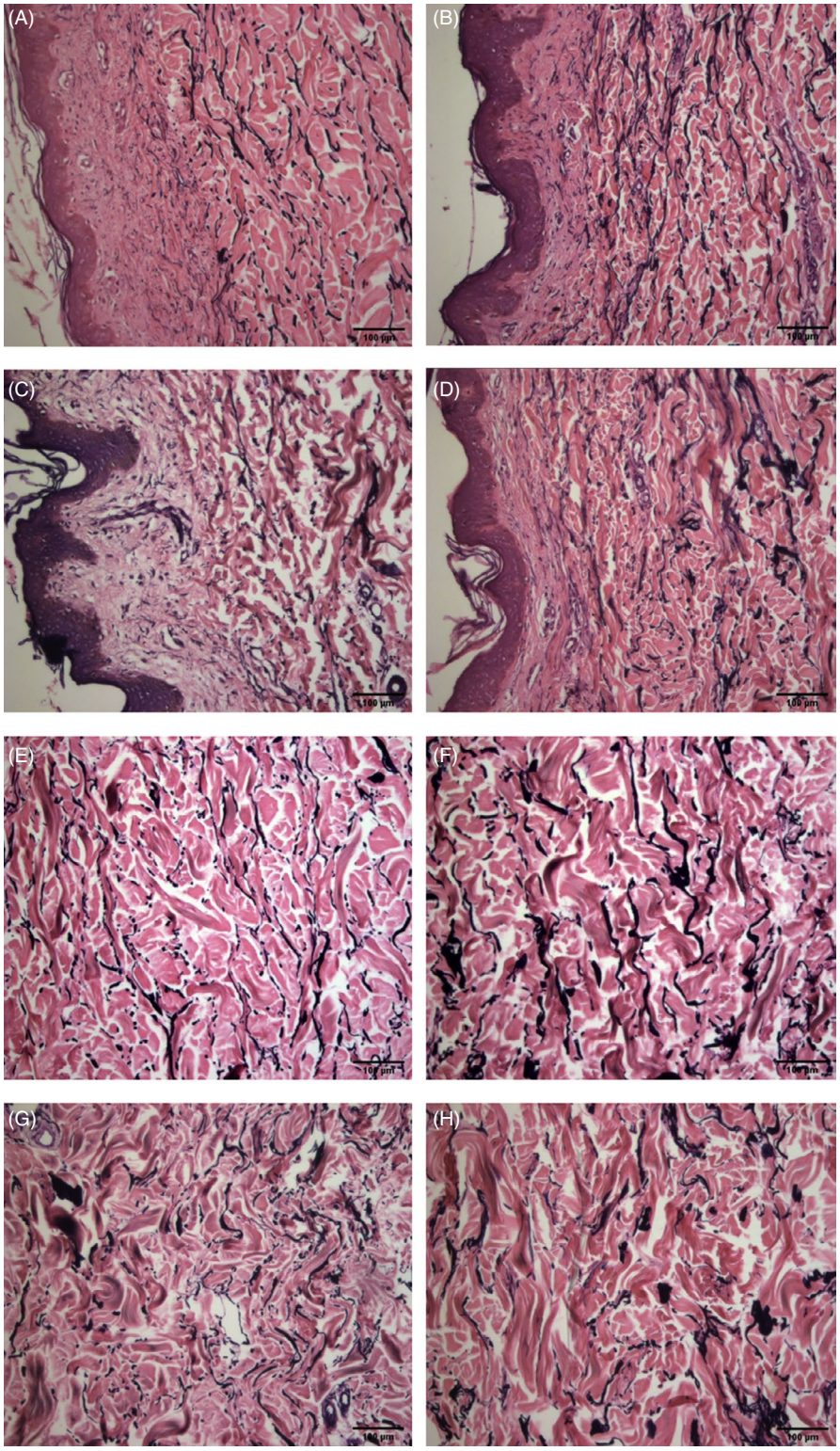


**FIGURE 2** Effect of carboxytherapy treatment on Verhoff-stained human skin elastic fibers. Skin of 35-year-old woman: (A) control; (B) collected 49 d after treatment with carboxytherapy (CA). Skin of 52-year-old woman: (C) control; (D) collected 62 d after CA treatment. Skin of a 57-year-old woman: (E) control; (F) collected 55 d after CA treatment. Skin of 58-year-old woman: (G) control; (H) collected 55 d after CA treatment. Bar = 100 μm

In Ferreira, Haddad, and Tavares's study,[9] carboxytherapy was used in a single session, at a flow rate of 20 mL/min in Wistar rats with intradermal injections on the right side and subcutaneous injections on the left side. The material was collected 6 days after the session and histological analysis demonstrated an intense renewal of collagen. The authors did not mention the amount of gas injected and the number of points.

Pinheiro et al[12] used a rate of 40 mL/min also in a single session at only one point with a total infusion of 0.6 mL/kg; the needle was inserted into the papillary dermis at an angle of approximately 30 degrees, and they observed a discrete improvement in the synthesis of collagen and elastic fibers, inferior to the one obtained with radiofrequency, which was also used in the study. This was a series study of cases with material collection for analysis between zero and 120 days after application.

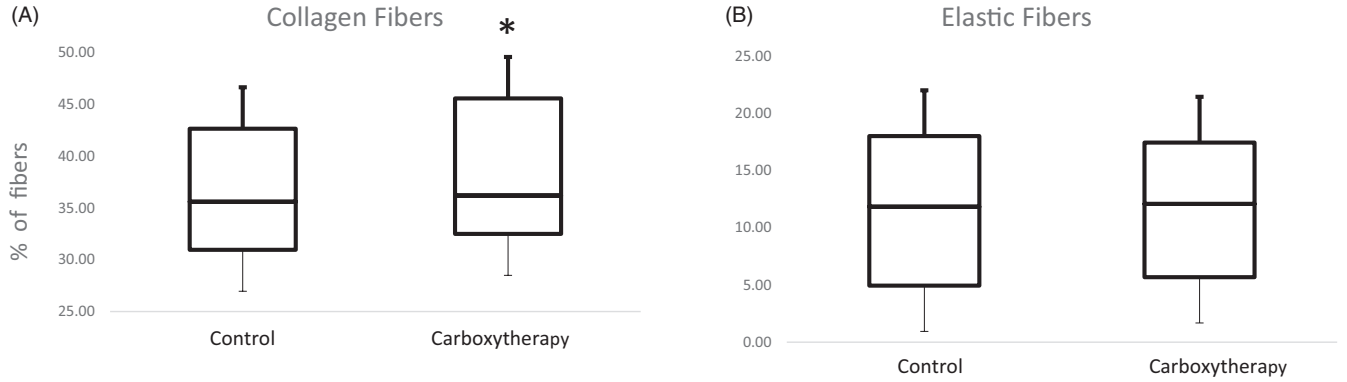


**FIGURE 3** Morphometric analysis of collagen fibers (A) and elastic fibers (B)

In the comparative study between carboxytherapy, platelet-rich plasma (PRP), and radio frequency of Ahmed and Mostafa,[13] carbon dioxide was administered with a 30G needle at flow rate of 50 mL min to provide 3 mL of $CO_2$ per mark in the stretch mark treatment, with an average time of 5-9 minutes per session. Carbon dioxide was administered in the surface dermis in five sessions with an interval of 1 week. Three months after the end of treatment, the material was collected and histological analysis demonstrated a sharp increase in collagen fiber deposition with carboxytherapy.

Brandi et al[14] studied the effect of $CO_2$ therapy on skin laxity in the thigh and knee region of 42 patients and the role that this treatment can play as a complement to liposuction. The authors divided the volunteers into groups A (treated with liposuction), B (liposuction and carboxytherapy), and C (treated only with carboxytherapy). Two weekly subcutaneous applications of $CO_2$ were performed for ten consecutive weeks, with a flow rate of 100 mL/min and the total amount of $CO_2$ was 300 mL per limb. Elasticity studies were conducted at the beginning and end of treatment (2 months after surgery). Although the study presents superior results with carboxytherapy associated with liposuction, carbon dioxide therapy alone also presented a satisfactory result with a relative increase in the mean values of skin elasticity at 55.5%.

Nisi et al[15] compared the efficacy, tolerability, and duration of the effect of hyaluronic acid filling and the use of carbon dioxide therapy associated with hyaluronic acid in the aesthetic correction of nasolabial folds. Forty healthy female patients participated in the study who were randomly distributed in groups A and B. All volunteers received an injection of reticulated hyaluronic gel for cosmetic correction of the nasolabial folds. Group B received the same conditions of group A associated with ten subcutaneous injections of carbon dioxide every 3 days for 1 month before the filling and 7 days after the filling. The implants were performed by the same surgeon with the same technique. In this study, no parameters of administration of carboxytherapy in the treated region were mentioned. The results were evaluated by two blind cosmetic surgeons 1 week after implantation and follow-up doctor appointments at 4 and 6 months after the treatment. Patients treated with hyaluronic acid along with carbon dioxide maintained a satisfactory aesthetic result, while those treated only with hyaluronic acid showed, in almost all patients, a return to the pretreatment aspect.

In the study by Brandi et al,[16] carboxytherapy was associated with mesotherapy and hyaluronic acid for biorevitalization and rejuvenation of the face skin. The ones who participated in the study were volunteers aged at between 30 and 70, who received carboxytherapy after mesotherapy and hyaluronic acid injection in different procedures. The flow rate was 15-20 mL/min, and volume was between 5 and 15 mL per area. This treatment had the following sequence: For group 1, it was repeated once a week for 4 weeks, and then once a month for 2 months; for group 2: once a month for 3 months, and one maintenance every 4 months; and for group 3: once a month for 3 months and one maintenance every 4 months. The authors considered favorable the combination of methods with satisfactory results in facial rejuvenation and high degree of patient satisfaction.

In this study, we evaluated the changes in collagen and elastic fiber synthesis after 60 days—on average—in a single carboxytherapy application. This period between the session and the collection of the material has to be considered, because it demonstrates that the effects are not only immediate, but the carboxytherapy is able to stimulate the remodeling and synthesis of collagen and elastic fibers, besides the fact that it was a single session, making the resource quite promising, since multiple sessions are clinically performed with weekly intervals.

No differences related to age and skin phototype of the volunteers regarding collagen increase were observed, although some authors suggest that the younger, the better the results to treatments that stimulate collagen synthesis.[17,18] In this study, this connection was not observed, including the volunteer who presented the largest increase in the percentage of collagen, and who was the oldest volunteer in the sample (58 years). This correlation was not observed by other authors either.[9,12] There seem to be individual characteristics, which determine the response to collagen stimulation, and these are not directly related to the skin phototype nor age.

Carboxytherapy parameters are quite divergent in the literature and many authors do not mention the parameters used,

therefore making it difficult to suggest a more effective procedure. In this study, we elected to use a single session with an infusion rate of 100 mL/min and 0.6 mL/kg—volunteer's weight—in a single point restricted to an area of 25 $cm^2$, with full gas insertion between 40 and 50 mL. Parameters with lower infusion rate showed little significant improvement in a previous study,[12] for this reason, a higher infusion rate was chosen to evaluate the results, which in this study had better results, probably due to the barotrauma, which caused a greater stimulation of collagen synthesis when it was high.

Tissue trauma caused by gas infusion induces an inflammatory process that leads to tissue regeneration with macrophage migration, fibroblast proliferation, and endothelial cells that stimulate neovascularization and extracellular matrix remodeling.[19] Higher rate gas infusions may provide better results because, as mentioned above, they cause serious trauma, inducing an exacerbated inflammatory process with migration and proliferation of fibroblasts to the region, resulting in greater collagen synthesis.

In addition, carboxytherapy improves skin blood circulation levels, which causes general improvement of the skin and its functions when compared to other treatments which also improve skin elasticity.[11] Studies have also demonstrated the emergence of new capillary vessels after treatment with carboxytherapy.[1,14]

Histological analysis shows an evident increase in elastic fibers after a single session (application) of carboxytherapy. However, in the morphometric analysis, there was no significant difference. By analyzing each volunteer individually, it was observed that the response was quite varied, some with significant increase in elastic fibers which ranged from 21% to 79% (four volunteers), others with <10% increase (two volunteers) and three volunteers who showed no improvement. However, when these data are statistically analyzed, they show no difference compared to the control group. This study corroborates Ahmed and Mostafa study[13] who also observed an increase in the amount of longer elastic fibers, observing as well that these fibers were thicker and evenly disposed after intradermal $CO_2$ injection. However, these authors did not perform morphometric analysis.

As a limitation of this study, we can mention the reduction in volunteers due to the schedule of cosmetic surgery, which restricts the number of abdominoplasties each year. In addition, performing a single session also restricts the response to treatment. However, the protocol was defined this way to prevent the difference in number of sessions between volunteers, as it is common for unforeseen events to occur which make it impossible for individuals to attend sessions.

There is no consensus in the literature related to the parameters described, which also limits the evidence-based practice, since different studies have different parameters regarding the volume used, infusion rate, and application technique. Another important point to be mentioned is regarding physical characteristics such as age, gender, skin phototype, and BMI. These peculiarities are infrequently mentioned in studies making it difficult to develop an individualized protocol.

## 5 | CONCLUSION

Carboxytherapy, with the parameters used in this study, was able to stimulate collagen and elastic fiber synthesis, with significant differences in morphometry for collagen fibers.

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## REFERENCES

1. Brandi C, D'Aniello C, Grimaldi L, et al. Carbon dioxide therapy in the treatment of localized adiposities: clinical study and histopathological correlations. *Aesthetic Plast Surg*. 2001;25(3):170-174.
2. Doghaim NN, El-Tatawy RA, Neinaa YMEH, Abd El-samd MM. Study of the efficacy of carboxytherapy in alopecia. *J Cosmet Dermatol*. 2018;17(6):1275-1285.
3. Varlaro V, Manzo G, Mugnaini F, Bisacci CFP. Carboxytherapy: effects on microcirculation and its use in the treatment of severe lymphedema. *Acta Phlebol [Internet]*. 2007;8(8):1-13.
4. Abramo AC, Teixeira TT. Carboinsuflação em úlceras crônicas dos membros inferiores. *Rev Bras Cir Plástica*. 2011;26(2):205-210.
5. Durães EFR, Durães LdC, Carneiro FP, Lino Júnior RdS, Sousa JB. The effect of carbon dioxide therapy on composite graft survival. *Acta Cir Bras*. 2013;28(8):589-593.
6. Ferreira LM, da Silva EK, Jaimovich CA, et al. Carboxiterapia: buscando evidência para aplicação em cirurgia plástica e dermatologia. *Rev Bras Cir Plástica*. 2012;27(3):350-351.
7. Paolo F, Nefer F, Paola P, Nicolò S. Periorbital area rejuvenation using carbon dioxide therapy. *J Cosmet Dermatol*. 2012;11(3):223-228.
8. Moftah NH, El Khayyat MAM, Ragai MH, Alaa H. Carboxytherapy versus skin microneedling in treatment of atrophic postacne scars: a comparative clinical, histopathological, and histometrical study. *Dermatologic Surg*. 2018;44(10):1332-1341.
9. Ferreira JCT, Haddad A, Tavares SAN. Increase in collagen turnover induced by intradermal infection of carbon dioxide in rats. *J Drugs Dermatol*. 2008;7:201-206.
10. Chin SH, Burm JS, Kim YW. Selective dermal rejuvenation using intradermal injection of carbon dioxide and hyaluronic acid for facial wrinkles. *Ann Plast Surg*. 2013;70(6):628-631.
11. Kołodziejczak A, Podgórna K, Rotsztejn H. Is carboxytherapy a good alternative method in the removal of various skin defects? *Dermatol Ther*. 2018;31(5):1-5.
12. Pinheiro NM, Crema VO, Millan BM, Carvalho FA, Mendonça AC. Comparison of the effects of carboxytherapy and radiofrequency on skin rejuvenation. *J Cosmet Laser Ther*. 2015;17(3):156-161.
13. Ahmed NA, Mostafa OM. Comparative study between: Carboxytherapy, platelet-rich plasma, and tripolar radiofrequency, their efficacy and tolerability in striae distensae. *J Cosmet Dermatol*. 2019;18(3):788-797.
14. Brandi C, D'Aniello C, Grimaldi L, Caiazzo E, Stanghellini E. Carbon dioxide therapy: Effects on skin irregularity and its use as a complement to liposuction. *Aesthetic Plast Surg*. 2004;28(4):222-225.
15. Nisi G, Cuomo R, Brandi C, Grimaldi L, Sisti A, D'Aniello C. Carbon dioxide therapy and hyaluronic acid for cosmetic correction of the nasolabial folds. *J Cosmet Dermatol*. 2016;15(2):169-175.
16. Brandi C, Cuomo R, Nisi G, Grimaldi L, D'Aniello C. Face rejuvenation: a new combinated protocol for biorevitalization. *Acta Biomed*. 2018;89(3):400-405.

17. Tay YK, Kwok C. A novel radiofrequency device for the treatment of rhytides and lax skin: a pilot study. *J Cosmet Laser Ther*. 2009;11(1):25-28.
18. Alster TS, Tanzi E, Ruiz-Esparza J. Improvement of neck and cheek laxity with a nonablative radiofrequency device: a lifting experience. *Dermatol Surg*. 2004;30(4 I):503-507.
19. Pianez LR, Custódio FS, Guidi RM, de Freitas JN, Sant'Ana E. Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study. *Clin Cosmet Investig Dermatol*. 2016;9:183-190.

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Clinical, Cosmetic and Investigational Dermatology

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ORIGINAL RESEARCH

# Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study

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**Background:** Carbon dioxide therapy, better known as carboxytherapy, relates to percutaneous infusion of medical carbon dioxide with therapeutic approaches, and its use in the treatment of localized fat has demonstrated good results. Gynoid lipodystrophy, also known as cellulite, affects 80%–90% of women after puberty, especially in the buttocks and thighs. Its etiology is complex and involves multifactorial aspects. Its treatment and evaluation require the use of new technologies (more effective and low-cost approaches). The objective was to investigate the effectiveness of carboxytherapy in the treatment of cellulite in the areas of buttocks and posterior thigh.

**Patients and methods:** Ten women, 29±6.1 years, were selected and all of them received eight treatment sessions, with an interval of 7 days between sessions. Standardized digital photographs were used to assess the severity of cellulite, and panoramic images were collected by ultrasound diagnosis. The evaluations were performed before the first treatment (baseline) and 7 days after the last treatment session of carboxytherapy.

**Results:** After the treatment, there was a significant reduction ($P$=0.0025) of the cellulite from degree III to degree II, and this improvement had correlation with the improvement in the organization of the fibrous lines and the disposal of adipose tissue lines of the treated regions observed through the panoramic ultrasound images diagnosis.

**Conclusion:** Carboxytherapy is an effective technique of treatment of cellulite in the buttocks region and posterior thighs of healthy women.

**Keywords:** carbon dioxide, cellulite, localized fat, gynoid lipodystrophy, skin, panoramic ultrasound

## Introduction

Cellulite refers to a change that gives the skin a wavy and irregular appearance, and it affects 80%–90% of women after puberty. Numerous treatments have been proposed such as balanced diet, physical activity, massage, topical products, radiofrequency, therapeutic ultrasound, and light emitting diode therapy, among others.[1] Carbon dioxide ($CO_2$) therapy, commonly known as carboxytherapy, refers to the administration of $CO_2$ with therapeutic proposals. The technique originated in France in 1932, and originally the treatment was carried out percutaneously (through the skin) by the so-called heated carbonated water baths or the application of water-saturated $CO_2$ directly to the skin of patients. The technique was used for arteriopathy and ulcer treatments.[2,3] The results encouraged further studies, leading to the expansion of indications of new treatments. After the development of new technologies, the application was no longer topical and involved passing the $CO_2$ to be

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infused directly into the subcutaneous tissue, ensuring faster and better results. Sequential studies described the effectiveness of the carboxytherapy treatment of localized adiposities; demonstrated measurable reductions in circumference regions of the abdomen, thigh, and/or knee; and showed histological evidence of the effect of gas leakage, showing its possible lipolytic effects.[3,4] Ferreira et al[5] described the increase in collagen remodeling induced by intradermal injections of $CO_2$. In another study, Abramo et al[6] showed that after the controlled infusion of $CO_2$, vasodilation of the microcirculation skin was observed, accompanied by an increase of peripheral blood flow and an increase in skin temperature at the injection site (on average 3.48°C). Cellulite affects, especially, the buttocks and thighs; its etiology is multifactorial and involves complex issues, and its treatment and evaluation require the use of new methodologies.[7–11] The lipolysis caused by carboxytherapy seems to be caused by temperature increase and local blood flow and have been demonstrated in previous studies; however, clinical studies are still required, with good analysis techniques that prioritize their effects on cellulite.[2–6]

This study aimed to verify the effectiveness of controlled infusion of $CO_2$ in the treatment of cellulite in gluteal and posterior thigh bilaterally.

## Materials and methods

This study began with a sample of 12 candidates and finished with ten of them with an average age of 29±6.1 years and body mass index (BMI) of 25.5±3.3 kg/m$^2$. Inclusion criteria were healthy women, aged 20–35 years, BMI <29.9 kg/m$^2$, with regular menstrual cycle, and presence of cellulite of gluteal and posterior thigh bilaterally as classified in degrees of severity II and III as in the current classification of cellulite[12].

This study was approved by the Research Ethics Committee: União das Instituições de Serviço, Ensino e Pesquisa – UNISEPE (CAAE: 02242112.9.0000.5490) and all subjects signed informed consent forms. The treatment was performed in the Clinical Laboratory of the Center for Education and Advanced Training CEFAI (Amparo, SP, Brazil). Volunteers were excluded if they were in aesthetic treatment; had had some kind of treatment in the gluteal region and thighs for a period leading up to 6 months before the start of this study; if they were pregnant or had had a recent pregnancy (<6 months); if they had cardiovascular problems, metabolic disorders, respiratory disorders, immunosuppression, kidney and liver failure, and skin lesions at the treatment site; or if they had diabetes mellitus. In assessing weight and height, the volunteers wore only underwear without shoes.

A classical mechanical stadiometer (model 110 CH; Welmy, SP, Brazil) was used. The BMI was evaluated by applying the formula BMI = weight in kilograms divided by the square of the height in meters (kg/m$^2$).[13–15] Body fat percentage of each volunteer was measured with Biodynamics (Model 310E; TBW, SP, Brazil).

All volunteers received directions about the treatment steps and procedures to be performed. The treatment protocol consisted of eight sessions with an interval of 7 days. The evaluations were performed before the first treatment (baseline) and 7 days after the last treatment session. The total time between the baseline and the posttreatment reevaluation was ~2.5 months. After analysis of the area, ten points were selected for the infusion of $CO_2$ (four in the gluteal area and six in the posterior thigh) as shown in Figure 1A. The treated areas were inspected and constantly monitored during all sessions. With a proper pen, points equidistant from each other were marked, 12 cm apart from each other, in places where the infiltration of $CO_2$ was held. The antisepsis was performed with alcoholic chlorhexidine at 0.5%. At each selected points, 80 mL of gas with a flow rate of 80 mL/min was infused. The needle was positioned at 45° (inferior angle), and a subcutaneous puncture was made with a depth of ~10 mm (Figure 1B).

After the application of the gas, the skin of the treated area was inspected. The equipment used in this study was ARES Carboxytherapy (IBRAMED, Indústria Brasileira de Equipamentos Médicos EIRELI, ANVISA 10360310032), and the medicinal $CO_2$ used was from White Martins Gases Industriais Ltda (São Paulo, Brazil). Cellulite grades were determined by clinical inspection of the patient's skin, and standardized digital photographs with a digital camera (Canon EOS Rebel T3i, Canon USA Inc., Melville, NY, USA) were taken at baseline and 7 days after the proposed treatment. All patients were photographed in standing positions in three views: rear view, right side, and left side. The focus of the image was in the gluteal cleft with a focal length of 1 m, and the muscles of the photographed areas were relaxed. Seven days after the last $CO_2$ infiltration, new photographs were taken. The images were offered to independent evaluators together with the criteria for the verification aspect of the severity of cellulite. The stage of severity of cellulite according to what is generally accepted was used. Nürnberger and Müller describe different grades or stages of severity based on clinical presentation from 0 (zero) to III (three); 0= there is no alteration of the skin surface (visible without changes); I= the skin of the affected area is smooth while the subject is standing or lying, but the alterations to the skin surface can be seen by pinching the skin or with muscle contraction (visible changes with skin clamping or muscle contraction); II= the orange peel aspect of the skin or mattress appearance is evident when standing, without the use of any manipulation of the skin pinching or

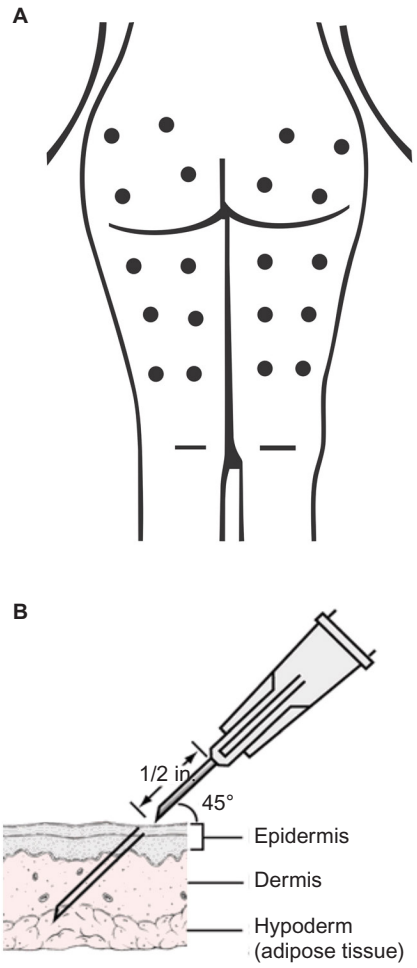


**Figure 1** (**A**) Points of $CO_2$ infusion; (**B**) position and angulation of needle placement for infusion, depth of ~10 mm (30 G × 1/2 inch size needle).

muscle contraction (visible without manipulation); and III= the alterations described in grade or stage II, are present together with raised areas and nodules (visible changes associated with nodules).[12] To minimize trends, reviews of the photographs were partially blind, ie, the evaluators received them in pairs without identifying which one represented the subjects before or after treatment. All the volunteers participating in the study underwent diagnostic ultrasound that was performed before and after the proposed treatment. All the volunteers underwent diagnostic ultrasound examination using a linear transducer (frequency 6–18 MHz) (MyLab™25 Gold; Esaote, Italy), and VPan software (Esaote, Italy) was used for the construction of panoramic images. All panoramic images were taken in the standing position. The probe was slipped at a slow and regular speed in the distal/proximal direction along the areas. Panoramic images were analyzed qualitatively considering the hyperechoic areas. Bright echoes represent highly reflective structures (white = dermis and fibrotic septa) and hypoechoic areas represent sparse echoes, reflection, or intermediate transmission (gray = adipose tissue and skeletal muscle).

The paired Student's *t*-test was used for statistical analysis, with an alpha level of 5% ($P<0.05$) being considered significant.

## Results

The volunteers were numbered 1–10, and evaluations were performed at baseline and 7 days after the last treatment session. Table 1 shows the anthropometric values before and after the infusion of $CO_2$. There were no significant changes in

**Table 1** Mean and standard deviation of the average of our patient population (age, body weight, BMI, and body fat percentage) at baseline and after treatment (7 days after the last carboxytherapy session).

| Volunteer N=10 | | Weight (kg) | | BMI (kg/m²) | | Body fat (%) | |
|---|---|---|---|---|---|---|---|
| | Age (years) | Baseline | After treatment | Baseline | After treatment | Baseline | After treatment |
| 1 | 32 | 74.8 | 76.7 | 27.1 | 27.8 | 13.5 | 25.4 |
| 2 | 31 | 63.5 | 64.0 | 23.8 | 23.9 | 13.7 | 20.2 |
| 3 | 29 | 73.5 | 74.0 | 27.7 | 27.9 | 27.1 | 27.6 |
| 4 | 36 | 85.5 | 87.5 | 29.6 | 30.3 | 30.8 | 23.5 |
| 5 | 23 | 60.0 | 63.0 | 20.3 | 21.3 | 18.0 | 18.3 |
| 6 | 21 | 64.5 | 63.0 | 23.1 | 22.6 | 21.9 | 18.7 |
| 7 | 27 | 65.5 | 67.0 | 26.6 | 27.2 | 24.7 | 21.5 |
| 8 | 33 | 62.5 | 65.5 | 25.0 | 26.2 | 15.4 | 17.4 |
| 9 | 38 | 64.5 | 65.7 | 28.1 | 27.7 | 28.7 | 28.3 |
| 10 | 20 | 56.0 | 53.8 | 21.2 | 20.5 | 17.1 | 16.0 |
| **Mean ± SD** | 29±5.9 | 67.0±8.1 | 68.0±9.3 | 25.3±2.9 | 25.5±3.3 | 21.1±09 | 21.7±4.3 |
| *P*>0.05 | | *P*=0.10 | | *P*=0.20 | | *P*=0.73 | |

**Notes:** Classification weight by BMI: <18.5 (underweight), 18.5–24.9 (normal range), 25.0–29.9 (overweight), 30.0–34.9 (obesity class I), 35.0–39.9 (obesity class II), and ≥40.0 (obesity class III).[13–15]

**Abbreviations:** BMI, body mass index; SD, standard deviation.

anthropometric measures of weight ($P$=0.10), BMI ($P$=0.20), and body fat percentage ($P$=0.73), at baseline and after the treatment.

Figure 2 shows a statistically significant reduction ($P$=0.0025) in the aspect of cellulite; the images were handed over to three independent evaluators, and assessments of the photographs were partially blind according to generally accepted classification of cellulite.

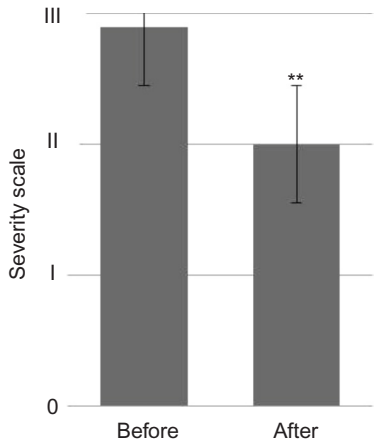


**Figure 2** Cellulite degree evaluation according to generally accepted classification of cellulite, baseline (before) and 7 days after the last carboxytherapy session.
**Note:** **$P$=0.0025.

Figure 3 shows comparative photographic register of the aspect of cellulite at baseline and 7 days after the last carboxytherapy session.

In the panoramic image analysis performed by the ultrasound diagnosis, reduction of adipose tissue lodged between the skin and the muscles of the treated regions was verified, as seen in Figures 4 and 5. Qualitative analysis describes morphological improvement with respect to the subcutaneous tissue, fibrotic septa, and aspects of the dermis-related cellulite. The morphological improvement with respect to the subcutaneous tissue, fibrotic septa, and associated dermis aspect of the cellulite can also be seen.

## Discussion

Cellulite is a multifactorial disorder and has a high prevalence in young adult women and despite no morbidity causes great dissatisfaction and negative influence on quality of life.[1] The pathophysiology of cellulite was described as an aesthetically unpleasant disorder for most women after adolescence. It is a complex problem that includes changes involving the microcirculation, lymphatic system, extracellular matrix, and adipocytes. It affects certain body areas with more emphasis, such as thighs and buttocks.[8] Studies also describe

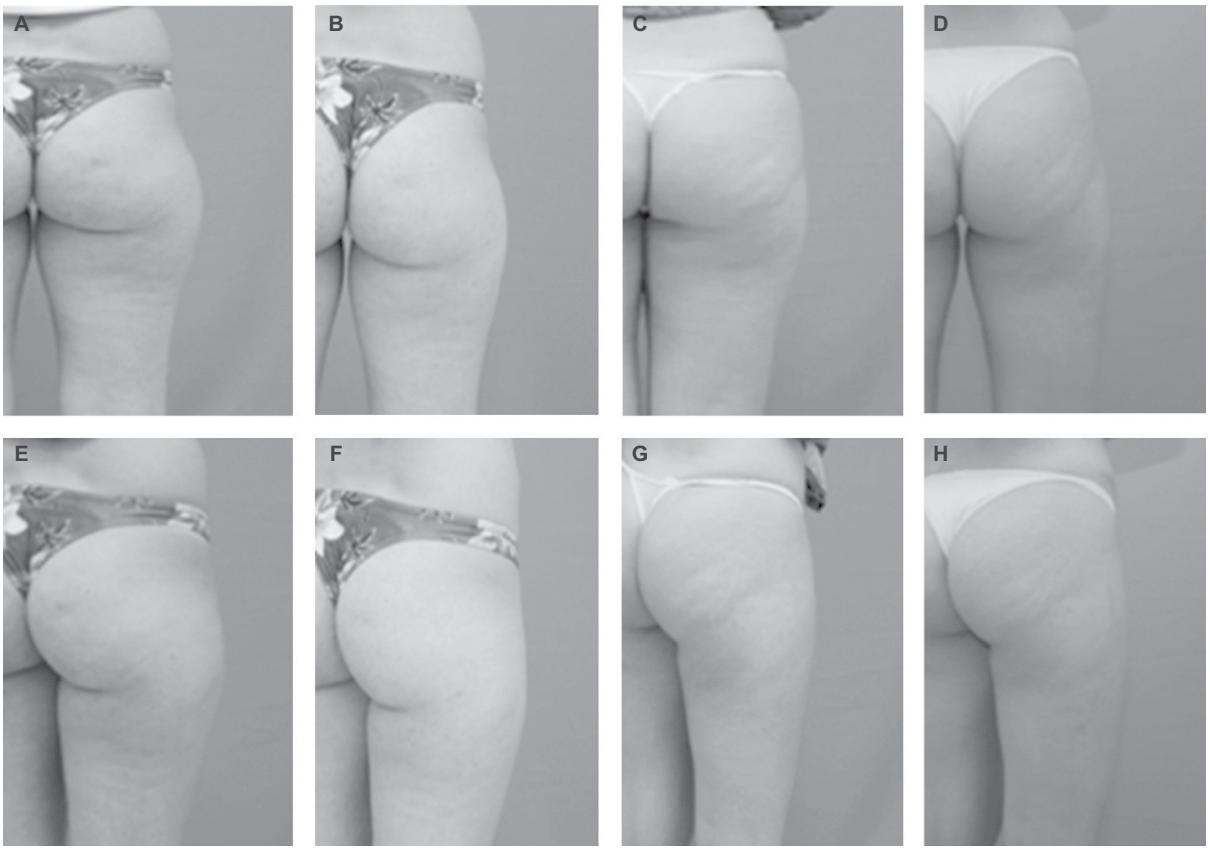


**Figure 3** Comparative photographic register of the aspect of cellulite. Notes: (**A**, **C**) Rear view of gluteus and posterior right thigh at baseline; (**B**, **D**) rear view of right gluteus 7 days after the last carboxytherapy session; (**E**, **G**) right side view at baseline; and (**F**, **H**) rear view of the right gluteus after the last session of carboxytherapy.

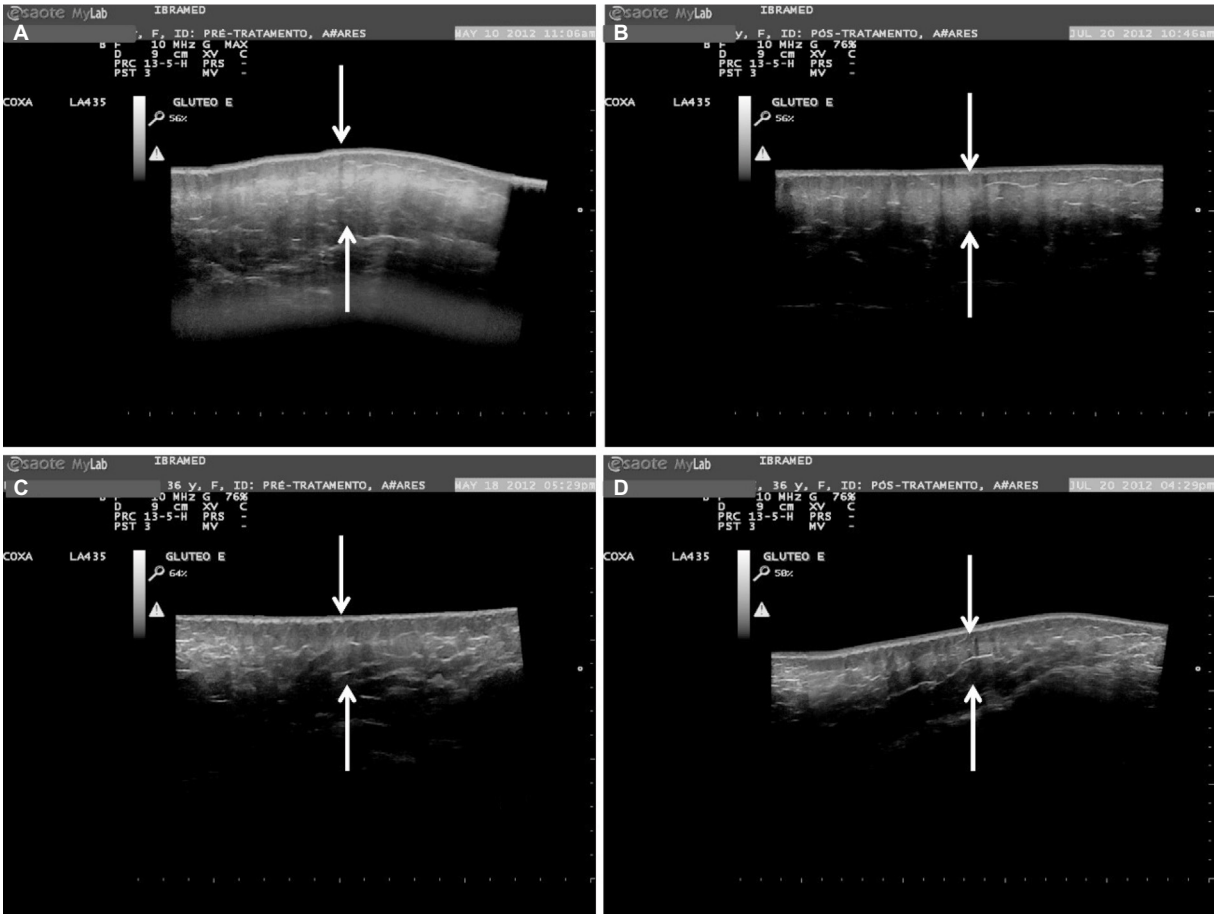


**Figure 4** Comparative panoramic images of gluteal region.
**Notes:** (**A**, **C**) Before treatment with carboxytherapy; (**B**, **D**) 7 days after the last session. Note the hyperechoic areas: bright echoes, highly reflective structures (white = dermis and fibrotic septa) and hypoechoic areas: sparse echoes, reflection, or intermediate transmission (gray = adipose tissue and skeletal muscle). The arrows indicate the areas compared and the decrease of the thickness of the fibrotic septa after treatment.

the pathophysiology involving a hyperactivity of fibroblasts stimulated by estrogen, which increases the synthesis of glycosaminoglycans and collagen that increase interstitial osmotic pressure and water retention. A decrease in the capillary osmotic pressure in relation to the interstitial osmotic pressure generates edema and reduction in drainage with subsequent hyperpolymerization and formation of micronodules and fibrosclerotic collagen. Symptoms include edematous adiposity, incipient cellulite, or changes in the skin relief measured by the clinical severity.[9,16,17] Adipose tissue can be divided anatomically into two layers by a layer of fibrous tissue called Camper's fascia.[8] The areolar layer has fibrous septa arranged in a peculiar vertical architecture that connect the dermis filled by large globular adipocytes; below, the lamellar layer septa has more horizontal axis and the fat lobes are flattened. Adipocytes are specialized cells in the storage of fats.[8–11,16–19] In 2004, it was demonstrated for the first time that the hypodermic infiltration of $CO_2$ as an alternative measure to be associated with liposuction procedures was effective in treating localized adiposity or skin irregularities, resulting from the surgical procedure. Since then, the method has been used with increasing frequency in the treatment of different forms of lipodystrophies, as well as in aesthetic medicine. In this area, its main indications are in fighting cellulite in localized fat and sagging.[20] Other studies show that controlled administration of medical $CO_2$ into the subcutaneous tissue induces hypercapnia and decreases local pH, which elicits a strong vasodilator response through the relaxation of the pre-arteriolar smooth muscle on the site.[2,6,25] Histological features in the repair process showed the proliferation of newly formed small blood vessels and fibroblasts.[21] The tissue stretching during infusion induces a subclinical inflammation, which triggers the repair and tissue regeneration processes that induce the activation of macrophages, fibroblasts, and endothelial cells that stimulate neovascularization and remodeling of the extracellular matrix. Among the diseases that may need carboxytherapy are peripheral artery disease and microangiopathy, psoriasis, varicose, and diabetic ulcers. This technique has also been frequently used for the treatment of cosmetic changes such

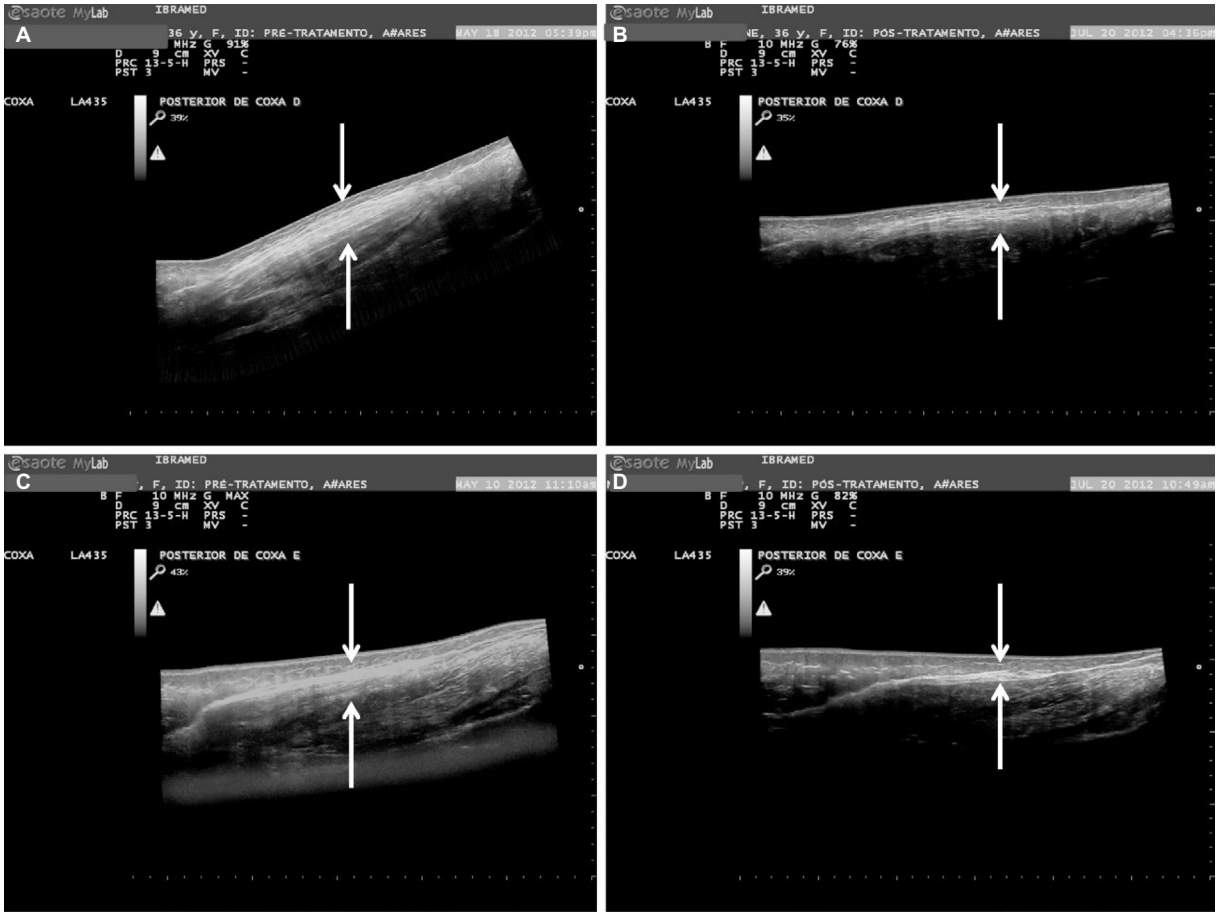


**Figure 5** Comparative panoramic images of the posterior thigh.
**Notes:** (**A**, **C**) Before treatment with carboxytherapy; (**B**, **D**) 7 days after the last session. Note the hyperechoic areas: bright echoes, highly reflective structures (white = dermis and fibrotic septa) and hypoechoic areas: sparse echoes, reflection, or intermediate transmission (gray = adipose tissue and skeletal muscle). The arrows indicate the areas compared and the decrease of the thickness of the fibrotic septa after treatment.

as localized fat, cellulite, facial skin rejuvenation, alopecia, dark circles, and striae.[2,4,20,22–26] In a study by Abramo et al,[6] after the infusion of $CO_2$, there was an average temperature increase of 3.48°C in the skin of the treated site. In addition, skin biopsies before and after treatment were evaluated by histology, and the authors observed that the diameter of the vessels increased by 3.24 times after treatment. Brandi et al[4] described the effectiveness of carboxytherapy in the treatment of localized fat through measurable reductions in the circumference regions of the abdomen, thigh, and/or knee and showed histological findings of the effects of $CO_2$ gas infiltration on the subcutaneous adipose tissue and their possible lipolytic effects. Corroborating these findings, a standardized study involved 15 volunteers subjected to carboxytherapy sessions on the anterior wall of the abdomen for 3 consecutive weeks. Two sessions per week at intervals of 2–3 days between each session were made, with infused fixed volume of 250 mL of $CO_2$ per 100 $cm^2$ of treated surface. Tissue biopsies were collected before and after treatment and analyzed by flow cytometry, which showed a significant reduction in the number and change in the morphology of the adipocytes in the treated area.

The objective of the study was to verify the effectiveness of carboxytherapy in the treatment of cellulite. During the treatment period, there were no significant changes in the body weight or BMI, as shown in Table 2. Photographic images captured in a standardized manner were analyzed by three independent evaluators and were partially blind. The visual inspection of photographic records of the areas treated with carboxytherapy showed statistically significant ($P$=0.0025) changes regarding the aspect of improvement of the degree of severity, as shown in Figure 2. All volunteers showed improvement of skin appearance after carboxytherapy sessions, suggesting decreased tensile forces on the skin and possible redistribution of vertical forces (vector forces) in the septum (Figure 3). With regard to the safety of the carboxytherapy treatment, important studies report that the use of $CO_2$ for contrast angiography attests to the safety of this gas and have shown that it is not likely to promote clots. $CO_2$ can be used with intravascular bolus injections of up

to 100 mL and continuous flows between 20 and 30 mL/s without adverse reactions.[27] In this study, 80 sessions with carboxytherapy were performed and no volunteer had any sort of significant adverse effects, but reported only mild transient discomfort, tolerable during treatment. One volunteer had two small bruises that resolved spontaneously, which suggests that carboxytherapy can be a safe technique. The treatment was tolerable for all the patients.

Noninvasive assessment techniques have been used to evaluate the cellulite and its posttreatment results, among which stand out the magnetic resonance imaging (MRI) and diagnostic ultrasound. Researchers, using microimages of the MRI, correlate the anatomy of the subcutaneous tissue to the typical changes in the cellulite at different degrees of severity;[28] other researchers also used MRI to study the cellulite and its treatment with lymphatic drainage.[29] However, the development of software that allows the use of panoramic images captured by diagnostic ultrasound is more inexpensive. Del Pino et al[19] used this method to observe the results of the treatment of cellulite with nonablative radiofrequency. In this study, panoramic images taken by diagnostic ultrasound were used to evaluate the results of the treatment of cellulite with carboxytherapy. These images allowed the registration of large anatomical areas and the access of the subdermal structures, especially fibrotic regions of septa. All images were collected and evaluated, and the comparative differences can be clearly seen in Figures 4 and 5. The analysis of the structural aspect of cellulite allowed the observation of a significant improvement in the organization of fibrous lines observable by hyperechoic area (whitish structures of fibrotic septa) compared to pictures taken before treatment, also noting hypoechoic areas of adipose tissue. These results support the findings described by Lee[25] in a compilation of clinical outcomes over 4 years (2004–2008) in 110 patients using carboxytherapy in the treatment of localized fat and cellulite; despite the methodological limitations of this study, the author concluded that carboxytherapy is a technique that demonstrates effectiveness for treating both localized fat and cellulite. The degrees of reduction of cellulite and possible reshaping of septa in this study likely occurred by the action of $CO_2$ on the microcirculation and tissue perfusion, described in the subcutaneous tissue. Studies showed that local application of $CO_2$ can promote improvement of peripheral circulation, increasing tissue perfusion and oxygen partial pressure by reflex vasodilation, and stimulate the neoangiogenesis.[12] In the study, after the treatment of carboxytherapy, there was a significant reduction ($P$=0.0025) of the cellulite from degree III to degree II, and this improvement showed correlation with the improvement in the organization of fibrous lines and the disposal of adipose tissue lines of treated regions observed through the panoramic ultrasound image diagnosis. Another factor that may contribute to the effectiveness of carboxytherapy in the treatment of cellulite is the decrease in the density and shape of adipocytes in the treated area as demonstrated in the study by Costa et al,[24] since the accumulation of localized fat could contribute to increased local tissue compression, favoring the traction of fibrotic beams and herniation of adipocytes in the dermis causing the appearance of the orange peel skin depressions, a characteristic feature of cellulite disorder.

## Conclusion

This pilot study showed that carboxytherapy may be used to improve the degree of severity of cellulite in the subcutaneous tissue at the buttocks and posterior thighs of healthy women. Further studies with a larger number of patients will be needed to more fully characterize the full clinical potential of carboxytherapy and its mechanism of action.

## Acknowledgment

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## Disclosure

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## References

1. Schonvvetter B, Soares JLM, Bagatin E. Longitudinal evaluation of manual lymphatic drainage for the treatment of gynoid lipodystrophy. *An Bras Dermatol*. 2014;89(5):712–718.
2. Varlaro V, Manzo G, Mugnaini F, et al. Carboxytherapy: effects on microcirculation and its use in the treatment of severe lymphedema. *Blood*. 2007;8:1–13.
3. Corassa JM, Pereira FLC, Penha MR, Corassa MP. Uso da carboxiterapia no tratament o de distúrbios vasculares : resultados preliminares [Use of carboxitherapy in the treatment of vascular disorders: preliminary results]. *Revista de Angiologia e Cirurgia Vascular*. 2006; 8. Portuguese.
4. Brandi C, D'Aniello C, Grimaldi L, et al. Carbon dioxide therapy in the treatment of localized adiposities: clinical study and histopathological correlations. *Aesthetic Plast Surg*. 2001;25(3):170–174.
5. Ferreira JCT, Haddad A, Tavares SAN. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. *J Drugs Dermatol*. 2008;7(3):201–206.
6. Abramo AC, Oliveira TT, Ledo-Silva MC, Oliveira EL. Elevação da temperatura cutânea após a infusão controlada de dióxido de carbono [Increase of the skin temperature after infusion-controlled carbon dioxide]. *Rev Bras Cir Plástica*. 2009;24(3):257–261. Portuguese.
7. Sant'Ana EMC, Marqueti RDC, Leite VL. Revisão fibro edema gelóide (celulite): fisiopalogia e tratamento com endermologia [Gynoid lipodistrophy: pathophysiology and treatment with endermologie. A review]. *Fisioterapia Especialidades*. 2007;1:30–35. Portuguese.
8. Rawlings AV. Cellulite and its treatment. *Int J Cosmet Sci*. 2006;28(3): 175–190.

9. Rossi ABR, Vergnanini AL. Cellulite: a review. *J Eur Acad Dermatol Venereol*. 2000;14(4):251–262.
10. Querleux B, Cornillon C, Jolivet O, Bittoun J. Anatomy and physiology of subcutaneous adipose tissue by in vivo magnetic resonance imaging and spectroscopy: relationships with sex and presence of cellulite. *Skin Res Technol*. 2002;8(2):118–124.
11. Rosenbaum M, Prieto V, Hellmer J, et al. An exploratory investigation of the morphology and biochemistry of cellulite. *Plast Reconstr Surg*. 1998;101(7):1934–1939.
12. Hexsel D, Mazzuco R. Cellulite. In: Tosti A, Hexsel, D, editors. *Update in Cosmetic Dermatology*. Mumbai: Springer-Verlag Berlin Heidelberg; 2013:21–32.
13. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. *British Medical Bulletin*. 1997;53(2):238–252.
14. Anuurad E, Shiwaku K, Nogi A, et al. The new BMI criteria for Asians by the regional office for the Western Pacific Region of WHO are suitable for screening of overweight to prevent metabolic syndrome in elder Japanese workers. *J Occup Health*. 2003;45:335–343.
15. James PT, Leach R, Shayeghi M. The worldwide obesity epidemic. *Obesity Research*. 2001;9(4):228S–233S.
16. Terranova F, Berardesca E, Maibach H. Cellulite: nature and aetiopathogenesis. *Int J Cosmet Sci*. 2006;28(3):157–167.
17. Khan MH, Victor F, Rao B, Sadick NS. Treatment of cellulite. Part I. Pathophysiology. *J Am Acad Dermatol*. 2010;62(3):361–370.
18. De Lorenzi DRS, Basso E, Fagundes PDO, Saciloto B. Prevalência de sobrepeso e obesidade no climatério. *Rev Bras Ginecol e Obs*. 2005;27(8):479–484.
19. Del Pino ME, Rosado RH, Azuela A, et al. Effect of controlled volumetric tissue heating with radiofrequency on cellulite and the subcutaneous tissue of the buttocks and thighs. *J Drugs Dermatol*. 2006;5(8):714–722.
20. Paolo F, Nefer F, Paola P, Nicolò S. Periorbital area rejuvenation using carbon dioxide therapy. *J Cosmet Dermatol*. 2012;11(3):223–228.
21. Maia-Figueiró TL, Odashiro AN, Menezes GP, et al. Semi-quantitative histological analysis of the effect of intense pulsed light (IPL) and carbon dioxide ($CO_2$) intradermic injection on fibroblast and collagen proliferation in the skin of Wistar rats. *J Cosmet Dermatol Sci Appl*. 2012;02(03):164–173.
22. Irie H, Tatsumi T, Takamiya M, et al. Carbon dioxide-rich water bathing enhances collateral blood flow in ischemic hindlimb via mobilization of endothelial progenitor cells and activation of NO-cGMP system. *Circulation*. 2005;111(12):1523–1529.
23. Piazzolla LP, Louzada LL, Scoralick FM, Martins ME, de Sousa JB. Preliminary experience with carbon dioxide therapy in the treatment of pressure ulcers in a bedridden elderly patient. *J Am Geriatr Soc*. 2012;60(2):378–379.
24. Costa CS, Otoch JP, Seelaender MCL, et al. Avaliação citométrica dos adipócitos localizados no tecido subcutâneo da parede anterior do abdome após infiltração percutânea de $CO_2$ [Cytometric evaluation of abdominal subcutaneous adipocytes after percutaneous $CO_2$ infiltration]. *Rev Col Bras Cir*. 2011;38(1):15–23. Portuguese.
25. Lee GSK. Carbon dioxide therapy in the treatment of cellulite: an audit of clinical practice. *Aesthetic Plast Surg*. 2010;34(2):239–243.
26. Brandi C, Grimaldi L, Nisi G, et al. The role of carbon dioxide therapy in the treatment of chronic wounds. *In Vivo*. 2010;24(2):223–226.
27. Simão JR, Guillaumon AT. Estudo angiográfico de fístula arteriovenosa utilizando gás carbônico como meio de contrante [Arteriovenous fistula angiography using carbon dioxide as contrast medium]. *Radiol Bras*. 2004;37(6):397–403. Portuguese.
28. Mirrashed F, Sharp JC, Krause V, Morgan J, Tomanek B. Pilot study of dermal and subcutaneous fat structures by MRI in individuals who differ gender, BMI, and cellulite grading. *Ski Res Technol*. 2004;10(3):161–168.
29. Meyer PF, Martins NM, Martins FM, Monteiro RA, de Mendonça KMPP. Effects of lymphatic drainage on cellulitis assessed by magnetic resonance. *Brazilian Arch Biol Technol*. 2008;51(Special issue):221–224.

# Carboxytherapy for Striae Distensae : A Promising Modality

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# Carboxytherapy for Striae Distensae: A Promising Modality

## Abstract

***Background:*** Striae distensae (SD) is a very common skin problem. Although a lot of treatment modalities have been proposed, few of them are effective. Recently Carbon dioxide therapy (CDT) or Carboxytherapy was used in many indications of cosmetic dermatology such as SD.

***Objectives:*** To objectively evaluate the use and effectiveness of CDT for treatment of SD.

***Patients and methods:*** Twenty patients were subjected to 8 sessions of CDT injection at 2-week intervals using carboxy-gun. Patients were photographed and skin specimens were obtained from the treated area before and after 4 months of treatment. Using a computerized 3D camera, skin topography was objectively analyzed before and after treatment. Evaluation of collagen and elastic fibers by special histopathological staining, in addition to histometric analysis, were also done to evaluate treatment efficacy.

***Results:*** Clinically, SD was statistically significantly improved after CDT injection compared to baseline (mean percentage of improvement of length and width, 59.8±15.9; $p<0.05$). Meanwhile, the improvement observed by the 3D camera correlated to the clinical improvement. Histometric analysis showed an increase in epidermal thickness ($p<0.0001$) in association with re-appearance of rete ridges following treatment. Histochemical evaluation of changes in elastic and collagen

fibers after treatment showed better organization of curled and fragmented elastic fibers , which was accompanied by an increase in collagen content that became denser, arranged in bundles and parallel to the epidermis.

***Conclusions:*** CDT is an effective, promising and simple minimally invasive procedure for improving SD with few side effects and low downtime.

Keywords:

Striae distensae (SD), Stretch marks, Carbon dioxide therapy (CDT), Carboxytherapy, Collagen, Elastic fibers

## Introduction

Striae distensae (SD) (stretch marks, striae atrophicans, striae gravidarum) are popular skin condition, that may create a remarkable psychological load for patients ***(1)***. Since firstly described in 1889, SD has presented a considerable challenge regarding its evaluation and treatment ***(2, 3).*** Despite the fact that SD is commonly seen by both patients and physicians , the frequency of SD mentioned in publications differs greatly, ranging from 11 to 88%. Simultaneously, anatomical affected areas vary, with sites mostly involved including the abdomen, breasts, thighs and buttocks. Two clinically different variants of SD were reported; striae rubrae (the early erythematous or violaceous lesions) and striae albae (the wrinkled, hypopigmented, atrophic scar-like marks) ***(4).***

The term striae gravidarum describes SD occurring with pregnancy, frequently after the $24^{th}$ week ***(5, 6).*** SD has also been reported in association with Cushing's disease, as well as oral and topical steroids. Other crucial etiological speculations have been suggested including insufficient skin development (especially elastic and collagen fibers), mechanical stretching of the skin as well as endocrinal imbalance ***(3, 7).*** Frequency and anatomical areas involved differ in relation to gender and age. In adolescents, nearly 40% of males and 70% of females suffer from SD. In adolescent males the lower back and knees are commonly involved, while in females' thighs and calves are usually affected. Striae gravidarum commonly affect the breast and abdomen ***(4, 8).***

Histologically, SD are scars that show an atrophic flat epidermis with attenuated rete ridges (RR), fraying, splitting with horizontal organization of collagen bundles, dilatation of blood vessels, as well as clumping and fragmentation of elastic fibers ***(9, 10).*** Deficiency of fibrillin was reported to have a crucial role in SD histogenesis ***(11).*** Many types of minimally invasive procedures are at hand for management of SD including; skin needling, microdermabrasion, chemical peels, mesotherapy, platelet-rich plasma, stem cell therapy, soft tissue fillers, non-ablative light-based rejuvenation, fractional photo-thermolysis, radiofrequency as well as carboxytherapy ***(12-17).***

Carbon dioxide therapy (CDT) or carboxytherapy refers to subcutaneous or transcutaneous administration of $CO_2$ to produce therapeutic actions, which enhance

microcirculation and tissue oxygenation ***(18, 19).*** $CO_2$ is an odorless, colorless gas that if injected intradermally or subcutaneously, diffuses at the skin microcirculatory level. Subsequently, the body will seek to restore what it considers an imbalance of oxygen/ $CO_2$ levels, improving blood flow to supply oxygen and nutrients to the skin and vessels. This eventually improves the skin appearance**.** One of the mechanisms of action of CDT might be due to lysis of adipocytes resulting in release of triglycerides into intercellular spaces, with subsequent improvement of skin elasticity ***(5, 20).*** In addition, the vasodilatation produced by $CO_2$ gas permits the accumulation of inflammatory response and improves the healing process resulting in an increase of collagen formation and reorganization as well as ultimate improvement in skin texture and tone ***(20-22).***

The present study aims to objectively evaluate the use and effectiveness of CDT in the management of SD.

## Patients and methods

The present study involved 20 females (12 with striae alba and 8 with striae rubra) of Fitzpatrick skin type III-IV visiting the dermatology outpatient clinic for treatment of SD. Patients' ages ranged from 18 to 39 years (27 ± 6.4, mean ± SD). Patients received a total of 4 months of treatment (8 sessions at 2-week intervals). Treatment and study details were fully described to patients, and all signed an informed consent. The study was approved by the Ethical Committee, and the Committee for Postgraduate Studies and Research of the University.

Full clinical evaluation was performed. Patients with some skin diseases such as active cutaneous infections, recurrent herpes activation, connective tissue diseases, or keloidal tendency and with hematologic disorders were excluded from the study. Meantime, patients receiving anticoagulant therapy, oral isotretinoin, or using any treatment for SD, such as microneedling, laser or light therapy, within one year of study initiation were also excluded. Pregnant or nursing

women, patients on carbonic anhydrase inhibitors or with systemic diseases such as severe anemia, diabetes or any systemic disease (respiratory, cardiac or liver disorder) were also excluded from participation in this study.

**Device and treatment protocol**

Skin was sterilized with povidone iodine followed by 70% ethyl alcohol; then medical $CO_2$ was injected intradermally using carboxy-gun, connected to $CO_2$ cylinder (Concerto Carboxy and Meso-gun, 602-0845, Lyion Cosmo Trade, France); which permitted sterile administration of medical $CO_2$ gas. The gas was injected directly into the SD with an angle of $15^o$, while the bevel border is up. Bulging caused by injection of the gas, could be seen and felt, which guarantees the injection of the whole area. Erythema as well as tissue distension were used as a clinical endpoint of $CO_2$ infusion. The carboxy-gun parameters were adjusted according to device manufacturing as follows: needle gauge 32 , 3 mm depth and 15 cc of the gas that could be increased up to 45 cc in large areas of SD. Then, gentle massage was performed. Before starting injection, patients were informed about the resulting emphysema; which will resolve within 5 minutes. Skin stabilizer of carboxy-gun allowed controlling the depth of injection, and care was taken with every needle's movement to keep the skin stretched and the maneuver is consistent.

For patients with multiple closely related SD lesions, injection was done to the whole area (not to each individual lesion). We noticed that 15 cc of carbon dioxide gas can spread intradermally and diffuse to cover an area of about 2 to 3 cm in diameter. Minimal distance between the injection sites was about 4 cm, this allow the gas to cover the affected area completely.

**Assessment of therapeutic response**

**Clinical evaluation**

The clinical changes and improvement in SD regarding width, length, number, texture, color mismatch and skin atrophy were assessed by the patients, 2 physicians and 2 independent observers, before treatment and after 4 months of starting treatment. This assessment was based on a five-point scale (none = 0%, mild = 1 - 25%, moderate = 26 - 50%, good = 51 - 75% and very good = 76 - 100%).

**3D skin analysis**

Skin topography was evaluated in site of interest (SD) in vivo using Antera 3D skin imaging device before the 1st treatment and 4 months post treatment. Antera 3D camera (Antera3D®, Miravex Limited, Dublin, Ireland) is a novel imaging tool for analysis and assessment of skin health relying on an advanced optical technology. It permits the user to evaluate the skin in 2 and 3 dimensions along with presenting a multispectral evaluation of the dermis and epidermis ***(23)***. It permits dermatologists to accurately analyze; indentations, texture, scars, skin color and redness as well as pigmentation in a seamless manner.

Software assisted image comparisons reveal before-and-after treatments efficacy and allow progress follow-up. What one should do is to determine the site you wish to evaluate in the "before" image and select the "ancor" icon on the toolbar of the "after" image. Automatically, the software will find the corresponding site and register the 2 images to one another. Advantages of this camera are ultimate 3D view which evaluate the skin texture (roughness) and indentations (indentation index), spot-on automatic matching, compare “before and after” images, generate a report, and it can save a data archive of your treatments.

**Histological evaluation and histometry**

Skin specimens using 4 mm punch biopsies were obtained from SD before treatment (baseline) and 4 months after the start of treatment (post treatment). Tissues were fixed in 10% buffered formalin, embedded in paraffin and sectioned into 5 µm-thick sections. Specimens were stained with Haematoxylin and Eosin (H & E), Orcein (for demonstration of elastic fibers) and Masson trichrome (for dermal collagen). All histological evaluations were carried out under light microscope (Accu-Scope #3025 Five Headed (A 3025-5)-OLYMPUS), with a built-in camera (Olympus, digital camera E-330 SLR, Japan).

The histometric analysis was performed on H&E stained sections using a computer - based software (analySIS®Five by Olympus Soft Imaging Solutions GmbH, Johann-Krane-Weg 39, D-48149 Münster, Germany). The epidermal thickness was measured as the mean distance between the outermost surface of the epidermis, excluding the stratum corneum, and to the dermal/epidermal junction. Five measurements were calculated for each section.

**Statistical analysis**

Data were tabulated and analyzed using the Software Package for Statistical Science (SPSS) (Version 16; Chicago, IL, USA). Statistical analysis was performed using dependent (paired) t test. Correlations between clinical results and the improvement observed by 3D camera were studied using Pearson's test to assess the correlation coefficient (r value) and its significance. Data were expressed as mean value ± standard deviation. Significance was expressed in terms of *P* value, which was significant when it was $\leq 0.05$ and highly significant when $\leq 0.001$.

## Results

**Clinical evaluation**

All 20 patients completed the study. Clinical evaluation of patients revealed significant ($p<0.05$) clinical improvement in SD after CDT **(Table 1, Fig. 1)**. A very good and good clinical improvement of striae rubra was noticed, while good and moderate improvement could be seen in striae alba. Apart from temporary erythema, hotness and bulging of the treated site that showed complete resolution, within one hour, no other potential adverse effects or complications were encountered.

Antera 3D Camera Skin Analysis showed improvement in skin indentation index (Large, medium and small) and skin texture (Large, medium and small) after 4 months of treatment of SD ($p<0.05$) **(Table 2, Figs 2 and 3)**. A significant positive correlation ($r= 0.32$, $p = 0.04$) in improvement of textural changes was observed when comparing clinical data to the results obtained by 3D camera.

**Histological Evaluation**

**Epidermal changes and Histometric analysis**

Before treatment, SD showed thin and flattened epidermis in most skin biopsies, with attenuation of RR. Interestingly in response to treatment, there was overall morphological and architectural improvement of the epidermis with development of RR (marked undulations of the dermoepidermal junction). Meanwhile, compared to the baseline, histometric evaluation of H&E stained sections showed a significant increase in the mean epidermal thickness from 44.2 ± 6.7 µm to 65 ± 11.4 µm at the end of treatment ($p < 0.0001$) **(Fig 4-A)**.

**Dermal changes**

Before treatment, disorganized collagen bundles (detected by Masson Trichrome stain) were observed in all specimens, with marked decrease in dermal elastic fibers (detected by Orcien stain), that appeared fragmented, thick and curled. This was followed by new formation of collagen bundles that became more compact, dense and better organized, accompanied by marked increase in elastic fibers in response to treatment, showing normal linear microfibrillar pattern **(Fig 4-B and C)**.

## Discussion

CDT refers to the transdermal/percutaneous administration of $CO_2$ to get therapeutic benefits ***(24, 25).*** Typically, therapeutic actions of $CO_2$ gas in the tissue include; vasodilatation, improvement of blood supply locally, normalization of the microcirculation, neogenesis of capillaries, and enhancement of local metabolism resulting in improvement of ulcers ***(5, 25, 26).***

In cosmetic medicine, CDT has been commonly used for treatment of localized fat deposition, cellulite, hair loss, dark peri-ocular haloes, and most recently to reduce abdominal fat ***(27, 28)*** and facial rejuvenation ***(23)***. Since CDT may result in surprising impressive

improvement, even after just 1 session , in some patients with aging, scars, stretch marks, or cellulite. Hence, it was supposed that many other factors might play an important role in creating such improvement. These factors include; mechanical undermining by the gas flow ***(5, 25),*** mechanical tension on the cells (especially in rejuvenation) and pressure (particularly in management of cellulite or adiposities) that result from relatively strong gas flow during $CO_2$ infusion ***(25),*** as well as mild influence of transient acidosis ***(25, 29, 30).***

As far as we know, the current study is the first to objectively evaluate the efficacy of CDT, as a minimally invasive procedure, in the management of SD. Minimally invasive procedures are frequently used for skin rejuvenation, tightening and scar remodeling. They enhance dermal extracellular matrix (ECM) proteins leaving the epidermis mostly intact, therefore, decreasing side effects and reducing down-time. The target of most minimally invasive rejuvenation modalities is to induce selective dermal insult leading to wound healing response while the epidermis stays relatively intact ***(13).***

SD is a relatively common complaint in aesthetic dermatology. Clinically, striae are linear or fusiform lesions that vary in length and width depending on the anatomical area and condition in which they develop. However, there are only few conclusive studies regarding effective treatments for SD ***(31, 32).***

The present study was conducted on 20 patients seeking for treatment of SD. At the end of intradermal administration of CDT (3mm depth), patients documented temporary erythema, hotness and bulging of the treated site that showed complete resolution within one hour. There was mild tolerable pain during injection in all patients. This is consistent with ***Nach et al. (2010) (20).*** Furthermore, there were no other side effects detected during and/or after treatment. The present study showed a strikingly significant clinical improvement in SD, especially striae rubra, after CDT injection. A very good and good clinical improvement in striae rubra was noticed, while good and moderate clinical improvement could be observed in mature striae alba.

Clinical improvement in length, width, texture, as well as pigment changes, was strikingly observed after treatment with CDT. Meanwhile, the percent of improvement observed by 3D camera were significantly correlated to the clinical findings **(P=0.04)**.

Best we can tell, there were no previous studies evaluating the histopathological effect of CDT on SD. However, ***Brandi et al. (2001)*** reported the effect of CDT injection on the fatty tissue on histological level. They observed adipolysis resulting from fracturing of fatty tissue with liberation of triglycerides in the intercellular spaces. These changes however, did not damage connective tissue spaces with vascular structures and nerves. It was also noticed that improvement of cutaneous elasticity following CDT measured by cutometer was as high as 55.5% ***(18)***.

Collagen fibers, the chief structural and most ample ECM component of the dermis (nearly 80% of the skin dry weight) are in charge of its tensile characteristics, and providing the skin with its protective function against external insults ***(33, 34).*** At the same time, elastic fibers, another component constituting ~ 2-4% of ECM proteins in sun protected skin, create an interconnecting network which offers elasticity and normal resilience to the normal skin ***(34).***

Intradermal injection of $CO_2$ enhances local blood supply resulting in increase in tissue oxygenation, and when injected superficially in the skin, it activates fibroblasts with subsequent collagen and elastin formation. These processes contribute to skin retraction resulting in skin rejuvenation and sagging reduction ***(35)***. Moreover, CDT may result in collagen remodeling ***(36)***. The current study demonstrated a remarkable increase in collagen remodeling after 4 month of treatment with CDT, which agrees with previous reports ***(24,35).*** In the present work, histological evaluation in addition to histometric and Antera 3D objective analysis confirmed and gave further evidence to the significant clinical improvement attained.

In conclusion, CDT is a promising minimally invasive method for SD management as it is an easy, safe, simple and effective modality for collagen induction and better reorganization of elastic fibers. It showed marked improvement in management of SD, with minimal downtime. Larger-scale studies with a longer follow-up period are necessary to verify such results and to standardize the used protocols.

## References

1. Manuskiatti W, Boonthaweeyuwat E, Varothai S. Treatment of striae distensae with a TriPollar radiofrequency device: a pilot study. J Dermatolog Treat. 2009; 20: 359-64.

2. Aldahan AS, Shah VV, Mlacker S, et al. Laser and Light Treatments for Striae Distensae: A Comprehensive Review of the Literature. Am J Clin Dermatol. 2016; 17: 239-56.

3. Draelos ZD, Gold MH, Kaur M, et al. Evaluation of an onion extract, Centella asiatica, and hyaluronic acid cream in the appearance of striae rubra. Skinmed. 2010; 8: 80-6.

4. Ud-Din S, McGeorge D, Bayat A. Topical management of striae distensae (stretch marks): prevention and therapy of striae rubrae and albae. J Eur Acad Dermatol Venereol. 2016; 30: 211-22.

5. Lee GS. Carbon dioxide therapy in the treatment of cellulite: an audit of clinical practice. Aesthetic Plast Surg. 2010; 34: 239-43.

6. Suh DH, Chang KY, Son HC, et al. Radiofrequency and 585-nm pulsed dye laser treatment of striae distensae: a report of 37 Asian patients. Dermatol Surg. 2007; 33: 29-34.

7. de Angelis F, Kolesnikova L, Renato F, et al. Fractional nonablative 1540-nm laser treatment of striae distensae in Fitzpatrick skin types II to IV: clinical and histological results. Aesthet Surg J. 2011; 31: 411-9.

8. Ostovari N, Saadat N, Nasiri S, et al. The 308-nm excimer laser in the darkening of the white lines of striae alba. J Dermatolog Treat. 2010; 21: 229-31.

9. Hague A, Bayat A. Therapeutic targets in the management of striae distensae: A systematic review. J Am Acad Dermatol. 2017; 77: 559-68 e18.

10. Mazzarello V, Farace F, Ena P, et al. A superficial texture analysis of 70% glycolic acid topical therapy and striae distensae. Plast Reconstr Surg. 2012; 129: 589e-90e.

11. Shokeir H, El Bedewi A, Sayed S, et al. Efficacy of pulsed dye laser versus intense pulsed light in the treatment of striae distensae. Dermatol Surg. 2014; 40: 632-40.

12. El Taieb MA, Ibrahim AK. Fractional CO2 Laser Versus Intense Pulsed Light in Treating Striae Distensae. Indian J Dermatol. 2016; 61: 174-80.

13. El-Domyati M, Medhat W. Minimally invasive facial rejuvenation: current concepts and future expectations. Expert Rev Dermatol. 2013; 8: 565-80.

14. Elsaie ML, Hussein MS, Tawfik AA, et al. Comparison of the effectiveness of two fluences using long-pulsed Nd:YAG laser in the treatment of striae distensae. Histological and morphometric evaluation. Lasers Med Sci. 2016; 31: 1845-53.

15. Hodeib AA, Hassan GFR, Ragab MNM, et al. Clinical and immunohistochemical comparative study of the efficacy of carboxytherapy vs platelet-rich plasma in treatment of stretch marks. J Cosmet Dermatol. 2018; 17: 1008-15.

16. Khater MH, Khattab FM, Abdelhaleem MR. Treatment of striae distensae with needling therapy versus CO2 fractional laser. J Cosmet Laser Ther. 2016; 18: 75-9.

17. Pongsrihadulchai N, Chalermchai T, Ophaswongse S, et al. An efficacy and safety of nanofractional radiofrequency for the treatment of striae alba. J Cosmet Dermatol. 2017; 16: 84-90.

18. Brandi C, D'Aniello C, Grimaldi L, et al. Carbon dioxide therapy in the treatment of localized adiposities: clinical study and histopathological correlations. Aesthetic Plast Surg. 2001; 25: 170-4.

19. Brandi C, Grimaldi L, Nisi G, et al. The role of carbon dioxide therapy in the treatment of chronic wounds. In Vivo. 2010; 24: 223-6.

20. Nach R, Zandifar H, Gupta R, et al. Subcutaneous carboxytherapy injection for aesthetic improvement of scars. Ear Nose Throat J. 2010; 89: 64-6.

21. Liang J, Kang D, Wang Y, et al. Carbonate ion-enriched hot spring water promotes skin wound healing in nude rats. PLoS One. 2015; 10: e0117106.

22. Liang X, Boppart SA. Biomechanical properties of in vivo human skin from dynamic optical coherence elastography. IEEE Trans Biomed Eng. 2010; 57: 953-9.

23. El-Domyati M, El-Din WH, Medhat W, et al. The Use of Carboxytherapy Alone or in Combination With Fractional CO 2 Laser for Facial Rejuvenation: A Split-Face Comparative Study. J Cosmet Dermatol. 2020 Jul; 19(7):1648-1655.

24. Ferreira JC, Haddad A, Tavares SA. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. J Drugs Dermatol. 2008; 7: 201-6.

25. Koutna N. [Carboxytherapy--a new non-invasive method in aesthetic medicine]. Cas Lek Cesk. 2006; 145: 841-3.

26. Toriyama T, Kumada Y, Matsubara T, et al. Effect of artificial carbon dioxide foot bathing on critical limb ischemia (Fontaine IV) in peripheral arterial disease patients. Int Angiol. 2002; 21: 367-73.

27. Alam M, Sadhwani D, Geisler A, et al. Subcutaneous infiltration of carbon dioxide (carboxytherapy) for abdominal fat reduction: A randomized clinical trial. J Am Acad Dermatol. 2018; 79: 320-26.

28. Alam M, Voravutinon N, Reynolds KA, et al. Safety of Subcutaneous Infiltration of Carbon Dioxide (Carboxytherapy) for Abdominal Fat Reduction: A Pilot Study. Dermatol Surg. 2019 Jul; 11.

29. Guerra RR, Kriazhev L, Hernandez-Blazquez FJ, et al. Progranulin is a stress-response factor in fibroblasts subjected to hypoxia and acidosis. Growth Factors. 2007; 25: 280-5.

30. D'Arcangelo D, Facchiano F, Barlucchi L, et al. Acidosis inhibits endothelial cell apoptosis and function and induces basic fibroblast growth factor and vascular endothelial growth factor expression. Circ Res. 2000; 86: 312-18.

31. El-Domyati MB, Attia SK, Saleh FY, et al. Trichloroacetic acid peeling versus dermabrasion: a histometric, immunohistochemical, and ultrastructural comparison. Dermatol Surg. 2004; 30: 179-88.

32. Rolfe H, Wurm E, Gilmore S. An investigation of striae distensae using reflectance confocal microscopy. Australas J Dermatol. 2012; 53: 181-5.

33. Bernstein EF, Lee J, Brown DB, et al. Glycolic acid treatment increases type I collagen mRNA and hyaluronic acid content of human skin. Dermatol Surg. 2001; 27: 429-33.

34. Uitto J. Understanding premature skin aging. N Engl J Med. 1997; 337: 1463-5.

35. Maia-Figueiró TL, Odashiro AN, Menezes GPd, et al. Semi-quantitative histological analysis of the effect of intense pulsed light (IPL) and carbon dioxide ($CO_2$) intradermic injection on fibroblast and collagen proliferation in the skin of Wistar rats. J Cosmet Dermatol. 2012; 2:164-73.

36. Pinheiro NM, Crema VO, Millan BM, et al. Comparison of the effects of carboxytherapy and radiofrequency on skin rejuvenation. J Cosmet Laser Ther. 2015; 17: 156-61.

**Table 1.** Clinical improvement in stria distensae after carboxytherapy

| Improvement | Length and width | Textural changes | Pigment changes |
|---|---|---|---|
| **Very good** | 3 cases (15%), | 10 cases (50%) | 3 cases (15%) |
| **Good** | 10 cases (50%) | 4 cases (20%) | 8 cases (40%) |
| **Moderate** | 6 cases (30%) | 6 cases (30%) | 7 cases (35%) |
| **Mild** | 1 case (5%) | | 2 cases (10%) |
| **Total** | 20 cases (100%) | 20 cases (100%) | 20 cases (100%) |
| **% of clinical improvement** | 59.8±15.9 | 66.8±21.7 | 54±19.2 |
| **P value** | 0.03* | 0.01* | 0.04* |

*Significant value.

**Table 2.** Percent of improvement in striae distensae as shown by 3D camera.

| Striae (n=20) | Percent of improvement (%) | P value |
|---|---|---|
| **Small indentations** | 61.7±22.3 | 0.02* |
| **Moderate indentations** | 63.4±18.5 | 0.01* |

| | | |
|---|---|---|
| **Large indentations** | 60.5±17.8 | 0.02* |
| **Small textural changes** | 61.5±19.7 | 0.02* |
| **Moderate textural changes** | 55.1±19.5 | 0.04* |
| **Large textural changes** | 63.7±25.1 | 0.01* |

*Significant value.



## Figure Legends

**Figure 1.Clinical evaluation in response to carboxytherapy injection.**

**[A-C]** Three patients with SD at baseline and 4 months post treatment showing a very good (A and B) and good (C) improvement.

**Figure 2.Images of Antera 3D Camera for striae distensae indentations.**

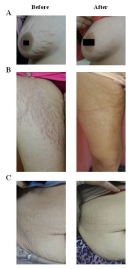
Improvement in small **[A]**, and large **[B]** striae indentations (shaded area) after 4 months of carboxytherapy, and bar chart of percent of changes in SD indentations showing significant improvement after treatment.

**Figure 3.Images of Antera 3D Camera for striae distensae texture.**

Improvement in textural changes (roughness) of striae (small **[A]**, and large **[B]**) after 4 months of carboxytherapy (shaded area) and bar chart of percent of changes in texture of SD showing significant improvement after treatment.

**Figure 4.Histologic evaluation of striae distensae before and after treatment.**

**[A]** Histometric evaluation showing increased thickness of the epidermis associated with development of rete ridges in SD patients after treatment. **[B]** Disorganized collagen bundles is evident before treatment, which was followed by enhancement of dermal collagen that appears interlaced, dense and well organized after 4 months of CDT. **[C]** Elastic fibers appears fragmented, thick and curled before treatment, then improved to normally appearing elastic microfibrils after treatment **(H&E [A] , Masson trichrome [B],and Orcien [C] stains , x 400)** .

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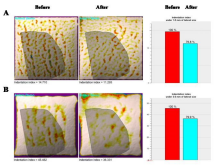


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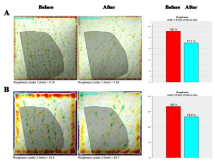

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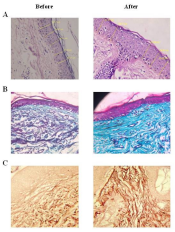


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# Carbon Dioxide Embolism

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## Continuing Education Activity

Laparoscopic surgery has gained increasing popularity in clinical practice. As part of laparoscopic surgeries, gas insufflation is usually adopted to increase operative space and visualization for surgeons. The abdomen is the most common location for these laparoscopic interventions, particularly in general and gynecologic surgeries. Carbon dioxide (CO2) is the most commonly used gas for insufflation during laparoscopic surgery because it is colorless, inexpensive, nonflammable, and has higher blood solubility than air, which reduces the risk of complications if venous embolism occurs. The use of CO2 gas for insufflation presents some risk. Among the most common complications associated with CO2 insufflation is CO2 embolism. Although CO2 microembolism commonly occurs during laparoscopy, clinically significant emboli are rare and potentially fatal. The clinical sign of CO2 embolism depends on embolized gas volume and ranges from asymptomatic to cardiovascular collapse or even death. This activity reviews the epidemiology, pathophysiology, clinical presentation, treatment, and prevention of CO2 embolisms and the medical team's role in managing this condition.

**Objectives:**

- Describe the pathophysiology of carbon dioxide embolism.
- Outline the presentation of a patient with carbon dioxide embolism.
- Summarize the treatment options for carbon dioxide embolism.
- Discuss the role of the medical team in managing patients with a carbon dioxide embolism.

Access free multiple choice questions on this topic.

## Introduction

Laparoscopic surgery has gained increasing popularity in clinical practice. As part of laparoscopic surgeries, gas insufflation is usually adopted to increase operative space and visualization for surgeons. The abdomen is the most common location for these laparoscopic interventions, particularly in areas such as gastrointestinal and gynecologic surgeries. Carbon dioxide (CO2) is the most commonly used gas for insufflation during laparoscopic surgery because it is colorless, inexpensive, non-flammable, and has higher blood solubility than air, which reduces the risk of complications if venous embolism occurs.[1]

The use of CO2 gas for insufflation presents some risk. Among the most common complications associated with CO2 insufflation is CO2 embolism. Although CO2 microembolism commonly occurs during laparoscopy, clinically significant emboli are rare and potentially fatal. The clinical sign of CO2 embolism depends on the volume of embolized gas and ranges from asymptomatic to cardiovascular collapse or even death. This article reviews the epidemiology, pathophysiology, clinical presentation, treatment, and prevention of CO2 embolisms.

## Etiology

CO2 embolism may occur during insufflation of the abdomen for laparoscopic surgeries. This usually occurs due to the accidental placement of the Veress needle into an organ or large vessel.[2] After negative aspiration, the insertion of the Veress needle and subsequent CO2 insufflation are both techniques performed without visual guidance. Later onset embolism may be associated with injured vessels that allow CO2 to enter the circulation.[3]

## Epidemiology

The incidence of CO2 embolism is very rare. A recent meta-analysis reported an occurrence of 7 in 489335 laparoscopic surgeries (0.001%).[4] However, when transesophageal echocardiography (TEE) was used during laparoscopic surgery to monitor for CO2 embolism, the incidence of any grade of gas embolism during laparoscopic surgeries varied widely. The incidence of CO2 embolism varied between 6.25% and 100%.[5][6] Despite these variations in incidence, clinically significant CO2 embolism remains fatal, with mortality as high as 28%.[7]

## Pathophysiology

Two possible mechanisms can explain the pathophysiology of CO2 embolism:

1) CO2 embolism can occur from the accidental intravascular injection of CO2, which may arise from the inappropriate placement of the Veress needle within the intravascular space. A similar mechanism is possible with trocar insertion.

2) CO2 embolism can also result from gas entering injured vessels, abdominal wall, or operative sites. This proposed mechanism results in less profound clinical change and may explain late-onset CO2 embolism.

The volume of gas entrained affects clinical presentation. In a study on cardiopulmonary responses to experimental venous CO2 embolism in pigs, researchers found a mortality of 60% at a continuous intravenous CO2 infusion rate of 1.2 mL/kg/min.[8] When converted for a 60 kg person, this equates to a rate of 72 mL/min and represents approximately 5% of the volume of CO2 that could be infused into a vein by a Veress needle in one minute at a low-flow rate.[8]

Gas in the venous circulation may obstruct pulmonary circulation and subsequently cause cardiac symptoms, including cardiovascular collapse and neurological sequelae.[2] It is associated with hypotension, increased central venous pressure (CVP), increased pulmonary arterial pressure (PAP), and hypoxemia.[9] In patients with patent foramen ovale, paradoxical arterial embolism may be possible and can result in transient or permanent neurological deficits.

## History and Physical

CO2 embolism may be small, asymptomatic, transient, and self-resolving. Signs of gas embolism include systemic hypotension, tachypnea, dyspnea, cyanosis, tachycardia or bradycardia, arrhythmia, asystole, or “mill-wheel” splashing auscultatory murmur.[6] Paradoxical embolism may be associated with altered mental status, focal neurological deficits, or loss of consciousness.

## Evaluation

Transesophageal (TEE) is the most sensitive method for detecting subclinical intravenous CO2 as small as 0.1mL/kg.[10] The TEE transgastric view has been shown to identify CO2 embolism optimally.[11][12][13]

The transesophageal Doppler is a highly sensitive but less expensive alternative to TEEs.[13] The precordial Doppler may also be used but has a high false-negative rate associated with the positioning of the probe.[6] Standard intraoperative noninvasive monitors can aid in detecting CO2 embolism, albeit with less sensitivity. Five-lead ECG may show right ventricular strain as

indicated by widened QRS complex, right bundle branch block, right axis deviation. Sudden decrease or loss of end-tidal CO2 suggests a drastic decrease in cardiac output due to gas embolism. Continuous pulmonary arterial pressure can be used to evaluate for gas embolism.[6]

## Treatment / Management

Management of a suspected CO2 embolism begins with desufflation of the abdomen.[14] Surgeons should be informed immediately and stop insufflation when there is clinical suspicion for CO2 embolism. Note that hemorrhage is possible when the intraabdominal pressure is reduced since the embolism may have been due to a vascular injury. The Durant or Trendelenburg position is used to direct the gas bubble into the right ventricle apex and away from the pulmonary artery.[2][15]

Ventilation with 100% oxygen could be used to wash out CO2, reduce ventilation-perfusion mismatch, and improve hypoxemia.[16] Hyperventilation is also used to help eliminate CO2. While the placement of multi-orifice central venous catheter may be a consideration in surgeries with a high-risk of air embolism (e.g., specific neurosurgical cases) to perform an aspiration, placement of a central venous catheter in an unanticipated case of CO2 embolism would be more beneficial for potential vasopressor administration, although aspiration could be attempted.[2]

Also, hyperbaric oxygen may be used to reduce bubble size in patients experiencing neurologic deficits. Supportive treatment with fluid, vasopressors and cardiopulmonary bypass may be necessary for patients with severe cardiovascular collapse.[6]

## Differential Diagnosis

- Air embolism
- Pulmonary embolism
- Pneumothorax
- Bronchospasm
- Pulmonary edema
- Hypovolemia
- Cardiogenic shock
- Myocardial infarction
- Septic shock
- Electromechanical dissociation
- Cerebral hypoperfusion
- Stroke
- Other embolisms (e.g., amniotic fluid, fat)

## Prognosis

Prognosis varies depending on the size of the embolism and severity of clinical presentation.

## Complications

- Cardiac arrest
- Neurological sequelae (e.g. motor deficits, cognitive deficits, seizures)

- Death

## Deterrence and Patient Education

Prevention of CO2 embolism targets potential methods of gas entry into circulation during laparoscopic surgery. Correct positioning of the Veress needle should be verified with a negative aspiration of blood before insufflation with low flow rate and low-pressure setting, or alternative modes of entry and pneumoperitoneum creation should be utilized.[14] Low insufflation pressure during laparoscopic surgery may diminish the pathophysiological changes. After proper placement of the trocars, patients should be placed in the Trendelenburg position.[2] Positive end-expiratory pressure (PEEP) of 5 cm H2O may be used intraoperatively to decrease atelectasis caused by pneumoperitoneum.[17]

## Enhancing Healthcare Team Outcomes

Minimally invasive laparoscopic procedures have increased in popularity and, in many cases, have superseded traditionally open surgical procedures. Patients should be assessed via preoperative medical evaluation to determine cardiopulmonary risks and anticipate possible complications. [Level 5] Additionally, it is important to foster a “speak up” culture where all interprofessional team members feel comfortable communicating potential concerns related to patient safety.[18] [Level 5]

An alternative to using the Veress needle technique is to apply the Hasson technique for establishing pneumoperitoneum.[4] In a systematic review, gas embolism was 0.001% (7/489000 cases) with the Veress needle, while no embolisms were reported in 12444 cases using the Hasson technique.[4] [Level 1]

Further, reducing the insufflation pressure can reduce the risk of CO2 embolism. In a randomized trial of 498 patients undergoing endoscopic saphenous vein harvesting, the incidence of CO2 embolisms was significantly higher in the high insufflation pressure group (15 mg Hg CO2) compared to that of the low insufflation group (12 mg Hg CO2).[11] [Level 2]

The healthcare team, including clinicians and nurses, must coordinate to prevent CO2 embolism by preventing gas entry into circulation during laparoscopic surgery. Correct positioning of the Veress needle should be verified with a negative aspiration of blood before insufflation with low flow rate and low-pressure setting, or alternative modes of entry and pneumoperitoneum creation should be utilized. Operative specialty trained nurses assisting in surgery can identify issues and call the surgeon's attention to them. Perioperative nurses monitor patients and provide feedback to the team. [Level 5]

## Review Questions

- Access free multiple choice questions on this topic.
- Comment on this article.

## References

1. Naude GP, Bongard FS. Helium insufflation in laparoscopic surgery. Endosc Surg Allied Technol. 1995 Aug;3(4):183-6. [PubMed: 8846034]
2. Gutt CN, Oniu T, Mehrabi A, Schemmer P, Kashfi A, Kraus T, Büchler MW. Circulatory and respiratory complications of carbon dioxide insufflation. Dig Surg. 2004;21(2):95-105. [PubMed: 15010588]
3. McDonald NJ, Lavelle P, Gallacher WN, Harpin RP. Use of the oxygen cost of breathing as an index of weaning ability from mechanical ventilation. Intensive Care Med. 1988;14(1):50-4. [PubMed: 3343430]
4.

Bonjer HJ, Hazebroek EJ, Kazemier G, Giuffrida MC, Meijer WS, Lange JF. Open versus closed establishment of pneumoperitoneum in laparoscopic surgery. Br J Surg. 1997 May;84(5):599-602. [PubMed: 9171741]

5. Fahy BG, Hasnain JU, Flowers JL, Plotkin JS, Odonkor P, Ferguson MK. Transesophageal echocardiographic detection of gas embolism and cardiac valvular dysfunction during laparoscopic nephrectomy. Anesth Analg. 1999 Mar;88(3):500-4. [PubMed: 10071994]
6. Kim CS, Kim JY, Kwon JY, Choi SH, Na S, An J, Kim KJ. Venous air embolism during total laparoscopic hysterectomy: comparison to total abdominal hysterectomy. Anesthesiology. 2009 Jul;111(1):50-4. [PubMed: 19512874]
7. Cottin V, Delafosse B, Viale JP. Gas embolism during laparoscopy: a report of seven cases in patients with previous abdominal surgical history. Surg Endosc. 1996 Feb;10(2):166-9. [PubMed: 8932621]
8. Mayer KL, Ho HS, Mathiesen KA, Wolfe BM. Cardiopulmonary responses to experimental venous carbon dioxide embolism. Surg Endosc. 1998 Aug;12(8):1025-30. [PubMed: 9685535]
9. Yau P, Watson DI, Lafullarde T, Jamieson GG. Experimental study of effect of embolism of different laparoscopy insufflation gases. J Laparoendosc Adv Surg Tech A. 2000 Aug;10(4):211-6. [PubMed: 10997844]
10. Couture P, Boudreault D, Derouin M, Allard M, Lepage Y, Girard D, Blaise G. Venous carbon dioxide embolism in pigs: an evaluation of end-tidal carbon dioxide, transesophageal echocardiography, pulmonary artery pressure, and precordial auscultation as monitoring modalities. Anesth Analg. 1994 Nov;79(5):867-73. [PubMed: 7978402]
11. Chiu KM, Lin TY, Wang MJ, Chu SH. Reduction of carbon dioxide embolism for endoscopic saphenous vein harvesting. Ann Thorac Surg. 2006 May;81(5):1697-9. [PubMed: 16631658]
12. Lin TY, Chiu KM, Wang MJ, Chu SH. Carbon dioxide embolism during endoscopic saphenous vein harvesting in coronary artery bypass surgery. J Thorac Cardiovasc Surg. 2003 Dec;126(6):2011-5. [PubMed: 14688720]
13. Mann C, Boccara G, Fabre JM, Grevy V, Colson P. The detection of carbon dioxide embolism during laparoscopy in pigs: a comparison of transesophageal Doppler and end-tidal carbon dioxide monitoring. Acta Anaesthesiol Scand. 1997 Feb;41(2):281-6. [PubMed: 9062614]
14. Daugherty A, Woodward B. Carbachol and dibutyryl cyclic GMP on the vulnerability to ventricular fibrillation in rat isolated hearts. Br J Pharmacol. 1985 Jul;85(3):621-7. [PMC free article: PMC1916527] [PubMed: 2992667]
15. Fong J, Gadalla F, Druzin M. Venous emboli occurring caesarean section: the effect of patient position. Can J Anaesth. 1991 Mar;38(2):191-5. [PubMed: 2021988]
16. Nichols SL, Tompkins BM, Henderson PA. Probable carbon dioxide embolism during laparoscopy; case report. Wis Med J. 1981 Mar;80(3):27-9. [PubMed: 6456601]
17. Kim JY, Shin CS, Kim HS, Jung WS, Kwak HJ. Positive end-expiratory pressure in pressure-controlled ventilation improves ventilatory and oxygenation parameters during laparoscopic cholecystectomy. Surg Endosc. 2010 May;24(5):1099-103. [PubMed: 19915912]
18. Schwappach D, Sendlhofer G. Speaking Up about Patient Safety in Perioperative Care: Differences between Academic and Nonacademic Hospitals in Austria and Switzerland. J Invest Surg. 2020 Sep;33(8):730-738. [PubMed: 30644786]



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