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ORIGINAL ARTICLE

A pilot study evaluating the anti-aging benefits of a CO_2-emitting facial mask

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Abstract

Background: Since 1936, injectable carboxytherapy has been used for the treatment of circulatory issues and lack of tissue trophism. In the last 25 years, it has been applied to aesthetic issues, especially those related to the signs and symptoms of skin aging. Presently, carboxytherapy is available as a combination of transcutaneous gels that produce CO_2 with benefit for atrophic skin.

Objective: The objective of this study was to investigate the efficacy and safety of a topical carboxy mask on facial photoaging after short term use of 4 weeks and long term use of 10 weeks.

Methods: The short term study was conducted for 14 days after 3 times weekly application of the facial mask for 1 h followed by a regression phase with evaluations at days 21 and 28. 11 healthy female subjects age 45–75 years were enrolled. Subjects applied the facial mask for 45 min, 3 times per week during the 2-week treatment period. The long term study was conducted for 10 weeks on 35 subjects 35–65 years with mild to moderate facial photoaging of Fitzpatrick skin types I–VI. Subjects underwent photography, elasticity, hydration, and VAS questionnaire assessments.

Results: The short term 4 week study demonstrated improvement in laser-Doppler measured blood flow and skin hydration. The long term 10 week study demonstrated improvement in firmness (16%, $p = 0.001$), sagging (9%, $p = 0.023$), and overall skin appearance (12%, $p = 0.002$). These findings were supported by the retraction time decrease at week 10 (−10%, $p = 0.05$).

Summary: The combination of two gels produced the liberation of CO_2, which improved short term skin hydration after 4 weeks of use and improved longer term skin elasticity after 10 weeks of use.

KEYWORDS
anti-aging mask, carboxy mask, face mask

1 | INTRODUCTION

Carboxytherapy is a medical technique utilizing the injection of sterile carbon dioxide (CO_2) into the subdermal tissue as a method for the management of multiple disorders. Carboxytherapy has been used for skin rejuvenation, atrophic scars, striae distensae, cellulite-fibrolipodystrophy adhesions after liposuction, and certain types of alopecia. Carboxytherapy originally referred to the subcutaneous injection of CO_2 to improve the microcirculation and thus promote wound-healing. [1] Table 1 summarizes the physiologic effects of CO_2.

Carboxytherapy has been used in Argentina and France to treat peripheral arteriopathies, especially obliterative arteriopathy of the lower limb, and diabetic ulcers. When CO_2 is injected subcutaneously, it immediately diffuses at the cutaneous and muscular microcirculatory levels, increasing microcirculatory vasodilatation, and improving blood flow through a direct action on arteriole smooth muscle cells.[2] Via the Bohr effect, it also increases the tissue pO2 at the injection site.[3,4] The Bohr effect refers to a decrease in the affinity of hemoglobin for oxygen due to an increase in CO_2, which means that extra oxygen is available to other tissues.[2,5] There is also a subsequent stimulation of fibroblasts and an increase in the quality of the extracellular matrix.[6,7]

Carboxytherapy for antiaging purposes is a safe minimally invasive procedure that may be administered as intradermal and/or subcutaneous microinjections of sterile purified carbon dioxide into different parts of the body for rejuvenation purposes.[8–10] Since 1932, the injection of carbon dioxide has therapeutically been used in balneotherapy predominantly for treating articular conditions, altered blood circulation of the lower limbs in ischemic diseases, cutaneous conditions in diabetic patients, systemic vascular diseases, and cardiac diseases.[11] These uses are based on the vasodilation effect induced. In a clinical trial by Nassar et al. on 60 patients with facial wrinkles, the efficacy of radiofrequency, intense pulsed light, and carboxytherapy was assessed and compared in facial rejuvenation. Patients received four sessions of each of these treatments with 3- to 4-week intervals, and the subjects were followed up 3 months after the last treatment. This study revealed that intense pulsed light was the most effective in facial rejuvenation, followed by carboxytherapy and radiofrequency, respectively. The results were confirmed by immunohistochemical assessment of matrix metalloproteinase-1 (MMP1).[13]

Further work by Oliveira et al., investigated the efficacy of carboxytherapy in managing skin laxity through histological assessment of collagen and elastin fibers in nine subjects who received treatment on the left side of infraumbilical region. After 60 days of treatment, the results demonstrated elastic fiber synthesis and improvement in collagen fiber morphology.[14] Medrano et al. evaluated the efficacy and subject satisfaction of carboxytherapy in the acceleration of wound healing after facial nano fractional radiofrequency treatment in nine subjects with photoaging. The study showed an improvement in photodamage, pigmentation, and wrinkles with significant improvement of erythema, dryness, edema, crusting, and healing after radiofrequency at 24 h.[15] Thus, carboxytherapy has a history of safety and efficacy for a variety of medical uses.

TABLE 1 The Physiotherapeutic action of CO_2.

- Increased flexibility and decreased firmness of collagen fibers at pH ≤6.5
- Reduction of divalent calcium ions
- Decreased tonus of arteries and capillaries leading to vasodilatation
- Increased blood flow to the injected site
- Decreased blood pressure
- Improved tissue drainage due to increased tissue perfusion and lymph circulation
- Release of local growth factors resulting in promotion of angiogenesis, lipolysis, and skin regeneration
- Dissociation of carbonic acid to H^+ and HCO_3 and subsequent formation of Ca $(HCO_3)_2$, $NaHCO_3$, and $KHCO_3$ Eventual reactional increase of pH (alkaline state) resulting in an analgesic and spasmolytic effect
- Improved activity of nerve endings resulting in improved trophicity of the treated body site activated oxidation of fats in the fatty tissue

2 | TRANSDERMAL CARBOXYTHERAPY

Carboxytherapy can also be administered transdermally through a new treatment option that provides carbon dioxide (CO_2) through the skin's superficial layers. CO_2 promotes tissue oxygenation through the Bohr effect inducing vasodilation. Several prior studies have examined the benefits of transdermal CO_2. Sakai et al. reported transcutaneous CO_2 was beneficial for therapeutic purposes via an increase in blood flow and microcirculation as evaluated by laser Doppler providing evidence of the Bohr effect in vivo.[16] Leibaschoff and co-workers used video capillaroscopy to evaluate the effect of a CO_2 transdermal gel and found improved microcirculation comparable to the improvement in microcirculation observed after subcutaneous CO_2 injection.[17]

This research examined the use of CO_2 transdermal, which is a new technology based on mixing two packets of ingredients. One packet contains butylene glycol, magnesium carbonate, hydrolyzed collagen, ceramides, botanical antioxidants (passiflora edulis fruit extract, pueraria thunbergiana root extract, chlorella vulgaris extract, aloe barbadensis leaf extract), and calcium carbonate while the second packet contains gluconolactone, squalene, butylene glycol, and water. The gels are mixed with a plastic tongue depressor type applicator in a bowl and applied as to the entire face, including eyelids, for 45 min. The mixing of the two packets initiates a chemical reaction resulting in the release of CO_2 on the skin surface, some of which may enter the skin. Magnesium carbonate is an inorganic salt that reacts with aqueous acids to release CO_2 and water. The gluconolactone may have a mild exfoliating effect on the skin surface. The increase in skin carbon dioxide release may increase skin blood flow, exposing the skin to increased oxygen levels, while the mask itself induces moisturization.[17] This creates an environment suitable for skin barrier repair. The application of the combination of gels is peeled or washed away with water after the treatment has been completed.

The carboxy mask was examined for both 4 week short term benefits and 10 week longer term benefits in two studies.

2.1 | Short Term 4 Week Study (Biometrix)

2.1.1 | Method

The short term study was conducted for 28 days after one application of the facial mask for 1 h at baseline (CO2 Lift, Lumisque Skincare). 16 healthy female subjects with mild to moderate photoaging age 45–75 years without skin disease who were not pregnant or lactating were enrolled. The subjects were asked to use their own self-selected skincare products that they had used without problem for 14 days prior to study enrollment. Subject also were not allowed to get more than 1 h of sun exposure daily and had to refrain from wax epilation of the face 14 days prior to study entry and facial procedures, such as chemical peels, within 30 days of study initiation.

Subjects completed an IRB approved consent form and were provided with study instructions and a daily diary, in which to record times of test material use and any comments. Subjects were directed to use the test material two to three times per week, in the evening on the face.

Subjects applied the facial mask for 1 h two to three times per week during the 2-week treatment period. Subjects underwent Visia photography CR 4.3 (Canfield Scientific), corneometer measurements, and subject assessments (dryness, skin tone evenness, fine lines, and wrinkles, scaling, and dark circles) on a visual analog scale (VAS) from 1 to 10 at baseline, 1 h post-application, 4 days, 8 days, 14 days during the product application phase. Following a discontinuation of the mask treatments for a regression phase, subjects returned at Days 21 and 28.

2.1.2 | Results

12 of 16 subjects successfully completed the 28-day study. Corneometry demonstrated immediate and cumulative facial moisturization. The baseline corneometery reading of 43.58 increased to 50.92 immediately after removal of the facial mask ($p < 0.001$). Continued statistically significant ($p < 0.001$) increases occurred at Day 4 (75.85), day 8 (83.50), and day 14 (87.65). Continued improvement occurred during the regression phase on day 21 (83.50) and day 28 (95.93) after the mask had been discontinued ($p < 0.001$).

The statistically significant decreases in laser Doppler measurements at Days 21 and 28 and regression phase visits indicated that cessation of use of the CO2 facemask decreased blood flow. This may suggest that the CO_2 mask helps to maintain healthy blood flow. This is likely a transient effect, and blood flow will return to normal when the mask is discontinued.

2.2 | Long Term 10-Week Study (Dermatology Consulting Services, PLLC, High Point, NC)

2.2.1 | Method

The long term study was carried out for 10 weeks on 35 healthy female subjects age 35–65 years with mild to moderate facial photoaging of Fitzpatrick skin types I–VI who signed an IRB approved consent (Allendale IRB). Subjects agreed to use the same cleanser, SPF30 sunscreen, and colored cosmetics for the duration of the study. Subjects who were pregnant or lactating were not enrolled. Subjects applied the carboxy mask to the face after cleansing for 10 weeks using the following schedule: 3 times per week for Week 1, once weekly for Week 2, once weekly for Week 3, once weekly for Week 4, no treatment Week 5, no treatment Week 6, once weekly for Week 7, no treatment for Weeks 8, 9, and 10 (CO2 Lift, Lumisque Skincare).

Subjects were seen at Baseline, Week 4, and Week 10. The dermatologist investigator and subjects assessed efficacy in terms of fine lines, wrinkles, luminosity, radiance, skin roughness (tactile), skin roughness (visual), erythema, pigmentation, even skin tone, firmness, facial sagging, and overall appearance on a 5-point ordinal scale (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe). The investigator assessed tolerability in terms of dryness, peeling, erythema, and edema while the subjects assessed tolerability in terms of dryness, peeling, stinging, and itching on the same 5-point scale.

Photographs were taken at baseline, week 4, and week 10 with the Visia CR 4.3 (Canfield Scientific) using standard lighting 1 of the central, right, and left face. Noninvasive assessments consisting of erythema/melanin measurements with the dermaspectrophotometer, hydration measurements with the corneometer, and elasticity measurements with the elastometer were conducted at baseline, week 4, and week 10 to assess skin barrier function and performance.

Along with descriptive statistics (means, standard deviations and percentages), investigator ordinal nonparametric data was analyzed using Wilcoxon signed rank test for paired comparison at different time points. The noninvasive parametric data was analyzed using a paired t-test. Change was considered significant at the alpha level of 0.05.

2.2.2 | RESULTS

34/35 female subjects successfully completed the 10-week study. One subject withdrew consent for personal reasons unrelated to the study product. The enrolled subjects included 8 African Americans, 1 Hispanic, and 26 Caucasians. All Fitzpatrick skin types were represented. The investigator noted no tolerability issues. No changes were noted in erythema or pigmentation with the dermaspectrophotometer indicating no irritation from post-inflammatory erythema (PIE) or post-inflammatory hyperpigmentation (PIH).

Elasticity measurements were also obtained to assess skin firmness. The retraction time was the length of time in milliseconds (ms) required for the skin to snap back to its original conformation after distention by a vacuum pump. The retraction time decreased in a statistically significant manner at week 4 (−12%, $p=0.004$) and week 10 (−10%, $p=0.05$). This means that the skin exhibited better elastic properties. The elastic modulus, also known as Young's modulus, is measured in megapascals (MPa) and is a measure of how ductile the skin is when stretched. The elastic modulus decreased indicating an improvement in the elasticity of the skin at weeks 4 (−26%) and 10 (−28) ($p<0.001$). This data indicates that the skin elasticity improved with the face mask resulting in better skin firmness and less sagging.

There was a statistically significant investigator assessed improvement in luminosity, radiance, tactile roughness, visual roughness, and erythema at week 4 after using the face mask ($p<0.001$). This improvement continued into week 10. At week 10, there was a 36% improvement in luminosity, a 33% improvement in radiance, a 35% improvement in tactile roughness, and a 38% improvement in visual roughness ($p<0.001$). In addition, improvement was seen in firmness (16%, $p=0.001$), sagging (9%, $p=0.023$), and overall skin appearance (12%, $p=0.002$) (Figure 1). The improvement in firmness and sagging was supported by the noninvasive elasticity measurements.

The subjects assessed statistically significant improvement in all evaluated criteria both at week 4 and week 10. There was highly statistically significant improvement in lines (24%), wrinkles (20%), luminosity (39%), radiance (42%), tactile roughness (43%), visual roughness (44%), erythema (26%), pigmentation (27%), skin tone (29%), firmness (30%), sagging (30%), and overall appearance (34%) after 10 weeks of use ($p<0.001$) (Figure 2). Representative subject before and after photos are presented in Figure 3.

3 | DISCUSSION

The CO_2 combination of gels that produce CO_2 is a topical variation on a well-known procedure called carboxytherapy, a term coined in

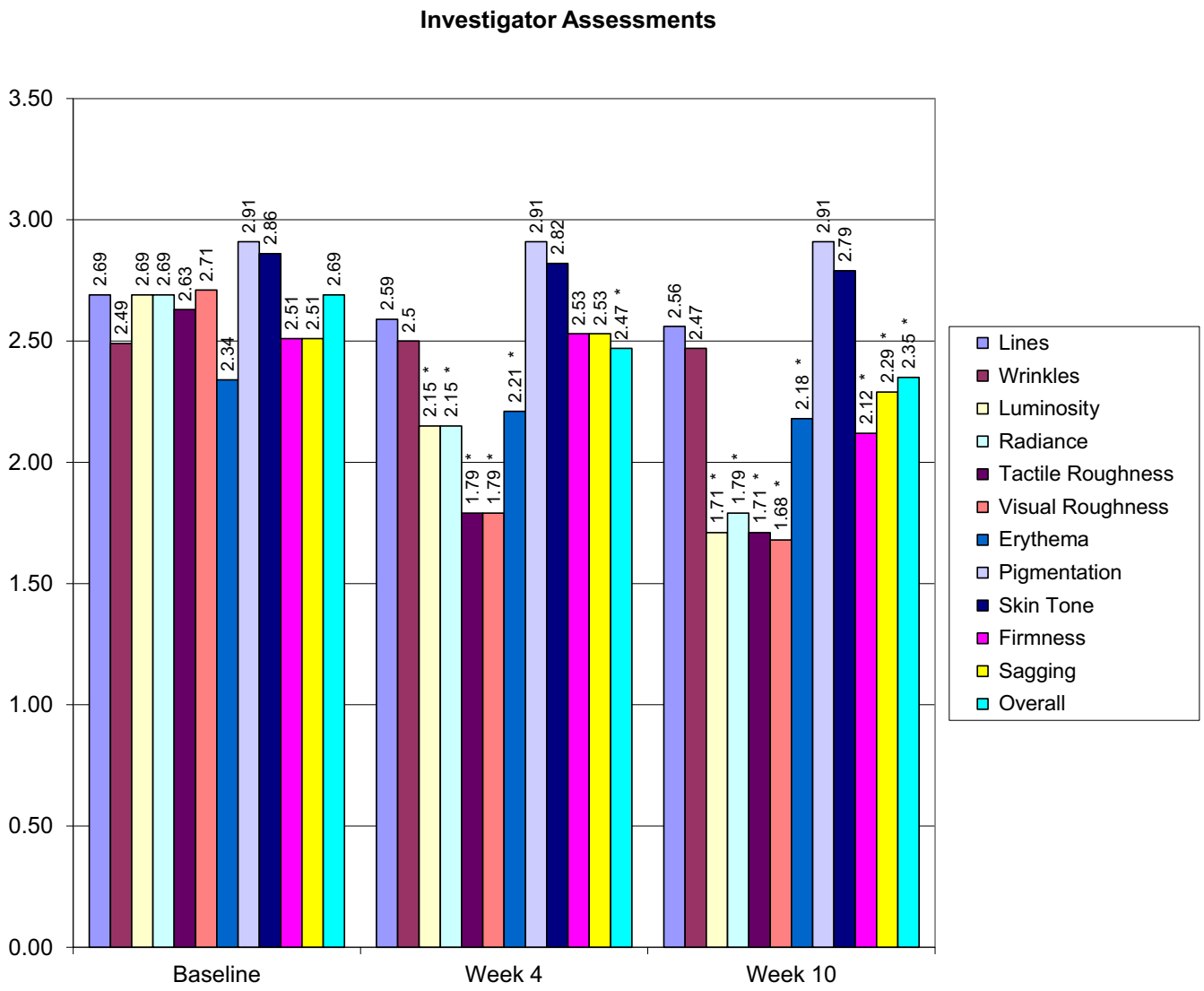

FIGURE 1 Investigator assessments. Sustained improvement in facial appearance was noted by the dermatologist investigator.

1995.[14] Carboxytherapy involves the exposure of the skin and subcutaneous tissue to carbon dioxide, a colorless and odorless gas, through injection.[18] The technique was developed in France in 1932 with the original treatments carried out in heated carbonated water baths or with water-saturated CO_2 applied directly to the skin. The treatments progressed to the transcutaneous or subcutaneous infusion of CO_2. Currently, carboxytherapy has been studied for aesthetic dermatology indications of skin rejuvenation, atrophic scars, striae distensae, cellulite, and alopecia. However, carboxytherapy has been used for the treatment of vasculopathies, lower limb ischemia, diabetic ulcers, Raynaud syndrome, and chronic venous ins ufficiency.[9,10,12,19–25]

In the skin, the CO_2 reacts with water producing carbonic acid that reduces the skin pH. This lower pH weakens the bonding of O_2 to hemoglobin leading to hemoglobin oxygen release and microcirculation vasodilatation with increased peripheral blood flow. CO_2 causes a decrease in oxygen consumption and shift in the oxygen disassociation curve, known as the Bohr effect, which decreases the affinity of hemoglobin toward oxygen resulting in the release of oxygen to the tissues. Injectable CO_2 has been demonstrated to increase collagen remodeling and synthesis. Carboxytherapy also lyses adipocytes resulting in the release of triglycerides into the intercellular spaces with an improvement in skin elasticity as demonstrated in striae distensae treatment.[22] This improvement in skin elasticity was seen in the longer term 10 week study.

The infusion of CO_2 into the subcutaneous tissue is a painful process with some side effects, thus the use of a topical combinations of gels that releases CO_2 percutaneously is more appealing for aesthetic indications. This research examined the cutaneous effects of such a mask used short term for 14 days and longer term for 10 weeks. No adverse events occurred, and the tolerability was excellent as assessed by the dermatologist investigator and the subjects. The most interesting results revolved around the improvement in skin elasticity, which may have been due to the collagen remodeling induced by CO_2. The vasodilation induced by the mask may also have released local growth factors, including vascular endothelial growth factor

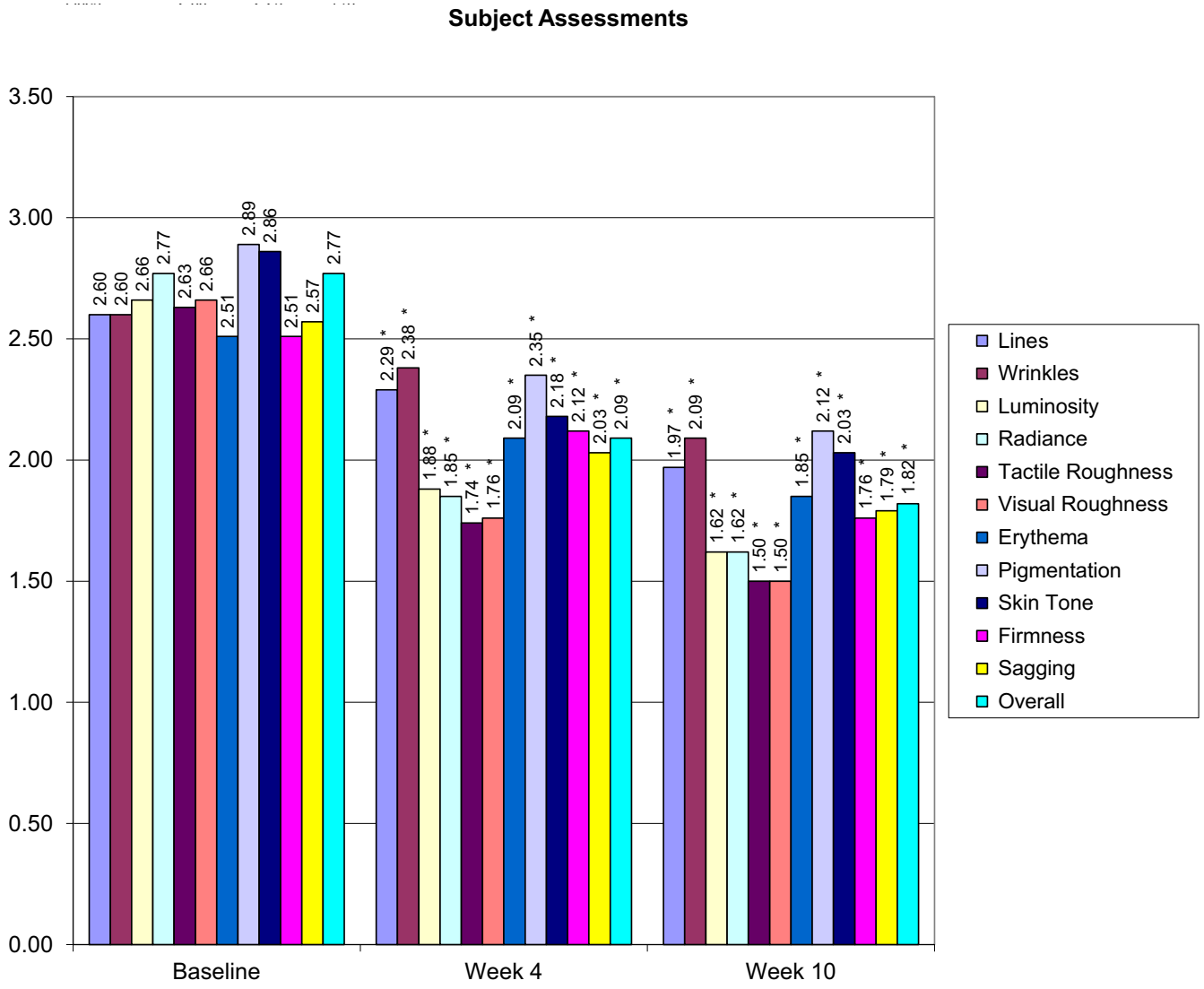

FIGURE 2 Subject assessments. The subjects noted continuous improvement in facial parameters.

FIGURE 3 Subject images. Representative before and after 10 weeks of mask use images. (A) before and 3B after in a Fitzpatrick skin type II subject. Figures 3C before and 3D after in a Fitzpatrick skin type III subject.

(VEGF), improving circulation. These effects may have contributed to the statistically significant improvement in the dermatologist investigator's assessment of overall facial appearance in subjects using the study product for 10 weeks as compared to baseline.

Mask administered carboxytherapy can be conducted by the patient in the privacy of their own home. There is no need for medical supervision, as demonstrated by the studies conducted. The mask can be easily mixed to release CO_2, put on the face, removed, and then disposed. No needles or source of CO_2 is required. Limited skill on the part of the user is necessary and the mask can be applied whenever desire to improve facial appearance.

The limitations of the studies are the small sample size and the fact that the treatment evaluations were not continued on beyond 10 weeks.

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This study was approved by the Allendale IRB, Old Lyme, CT.

CONFLICT OF INTEREST STATEMENT

ZDD received a research grant from Lumisque to conduct the research detailed in this manuscript.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was IRB approved by Allendale IRB and all subjects signed consent prior to the conduct of any research activities.

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REFERENCES

1. Colin C, Lagneaux D, Lecomte J. Sur l'action vasodi-latatrice du dioxyde de carbone injecte sous forme gazeuse dans le tegument del'homme. *Presse Thermale Climatique*. 1979;116(4):255-258. (in French).
2. Finzgar M, Melik Z, Cankar K. Effect of transcutaneous application of gaseous carbon dioxide on cutaneous microcirculation. *Clin Hemorheol Microcirc*. 2015;60(4):423-435. doi:10.3233/CH-141898
3. Savin E, Bailliart O, Bonin P, Bedu M, Coudert J. Vasomotor effects of transcutaneous CO2 in stage II peripheral occlusive arterial disease. *Angiology*. 1995;46(9):786-791.
4. Fabry R, Monnet P, Schmidt J, et al. Clinical and microcirculatory effects of transcutaneous CO_2 therapy in intermittent claudication. Randomized double-blind clinical trial with a parallel design. *Vasa*. 2009;38(3):213-224.
5. Sinozić T, Kovacević J. Carboxytherapy–support-ive therapy in chronic wound treatment. *Acta Med Croatica*. 2013;67(Suppl 1):137-141.
6. Ferreira JC, Haddad A, Tavares SA. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. *J Drugs Dermatol*. 2008;7(3):201-206.
7. Khiat L, Leibaschoff GH. Clinical prospective study on the use of subcutaneous carboxytherapy in the treatment of diabetic foot ulcer. *Surg Technol Int*. 2018;1(32):81-90.
8. Kroumpouzos G, Arora G, Kassir M, et al. Carboxytherapy in dermatology. *Clin Dermatol*. 2022;40(3):305-309.
9. Doghaim NN, El-Tatawy RA, Neinaa YME, Abd El-Samd MM. Study of the efficacy of carboxytherapy in alopecia. *J Cosmet Dermatol*. 2018;17(6):1275-1285.
10. Kołodziejczak A, Podgórna K, Rotsztejn H. Is carboxytherapy a good alternative method in the removal of various skin defects? *Dermatol Ther*. 2018;31(5):e12699.
11. Zelenková H. Carboxytherapy non-invasive method in dermatology, aesthetic dermatology and some other branches of medicine. *Acta Sci Med Sci*. 2019;3(5):42-48.
12. Bagherani N, Smoller BR, Tavoosidana G, Ghanadan A, Wollina U, Lotti T. An overview of the role of carboxytherapy in facial rejuvenation. *J Cosmet Dermatol*. 2023. doi:10.1111/jocd.15741. Epub ahead of print.
13. Nassar S, Assem M, Mohamed D, Hassan G. The efficacy of radiofrequency, intense pulsed light and carboxytherapy in facial rejuvenation. *J Cosmet Laser Ther*. 2020;22(6–8):256-264.
14. Oliveria SMD, Rocha LB, da Cunha MTR, Cintra MMM, Pinheiro NM, Mendonca AC. Effects of carboxytherapy on skin laxity. *J Cosmet Dermatol*. 2020;19:3007-3013.
15. Medrano K, Arruda S, Oza N, Sadick N. Carboxy therapy mask as post nanofractional radiofequency treatment for improvement of facial skin quality and photoaging. *J Drug Dermatol*. 2021;20(4):461-465.
16. Sakai Y, Miwa M, Oe K, et al. A novel system for transcutaneous application of carbon dioxide causing an "artificial Bohr effect" in the human body. *PLoS One*. 2011;6(9):e24137.
17. Leibaschoff GH, Coll L, Roberts WE. A prospective clinical and instrumental study on the effects of a transcutaneous cosmeceutical gel that is claimed to produce CO2. *Surg Technol Int*. 2018;1(32):33-45.
18. 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(2):320-326.
19. Brandi C, Campana M, Russo F, et al. Carbon dioxide: maybe not be the only one but an efficient and secure gas for treating local adiposities. *Aesthetic Plast Surg*. 2012;36(1):218-219.
20. 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.
21. 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.
22. El-Domyati M, El-Din WH, Medhat W, Ibrahim MR, Khaled Y. Carboxytheapy for striae distensae: a promising modality. *J Cosmet Dermatol*. 2021;20:546-553.
23. Podgorna K, Kolodziejczak A, Rotsztejn H. Cutometric assessment of elasicity of skin with striae distensae following carboxytherapy. *J Cosmetic Dermatol*. 2018;17:1170-1174.
24. 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.
25. Eldsouky F, Ebrahim HM. Evaluation and efficacy of carbon dioxide therapy (carboxytherapy) versus mesolipolysis in the treatment of cellulite. *J Cosmet Laser Ther*. 2018;20(5):307-312.

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