Annexure A

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ORIGINAL RESEARCH REPORT

Comparison of the effects of carboxytherapy and radiofrequency on skin rejuvenation

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Abstract

Background: Carboxytherapy (CA) refers to the cutaneous and subcutaneous administration of CO_2 for therapeutic purposes. Radiofrequency (RF) is a method that uses electric current for heating layers of the skin. Both techniques are indicated for the treatment of skin laxity. *Objective*: The aim of this study was to compare the effects of CA and RF on human skin. *Methods:* After eight patients underwent abdominoplasty, each of them received a single treatment of CA and a single treatment of RF on the right and left infra-umbilical regions, respectively. In the infra-umbilical region, CA was performed on the right and RF was performed on the left side. Untreated skin was used as a control. The sample collection period lasted 120 days. CA was administered at a velocity of 40 mL/min, and the total quantity of CO_2 infused was approximately 20 mL. RF was carried out at a temperature higher than 40°C on the epidermis for 5 min. *Results:* CA and RF led to collagen remodeling; however, this result was more evident and lasted longer with RF. With CA an increase in elastic fibers was observed, whereas with RF no alteration was observed. *Conclusion*: Our results suggest that RF is more efficient than CA in stimulating collagen synthesis.

Key Words: *carbon dioxide, carboxytherapy, radiofrequency, rejuvenation, skin aging*

Introduction

The demand for rejuvenating procedures is growing at a rate proportional to the aging population. Aging skin is characterized by wrinkles, atrophy of epidermis and dermis, rough texture, mottled pigmentation, telangiectasias, and laxity (1). With the development of new technologies, procedures that once required surgery are being replaced by minimally invasive techniques (2), such as carboxytherapy (CA) and radiofrequency (RF).

The CA, which uses carbon dioxide (CO_2) subcutaneously, is safe, with low cost and no toxicity or side effects. Therapeutic indications of CO_2 therapy include vascular, cardiac, metabolic, and rheumatic diseases; cellulite; chronic wounds; localized adiposities; and migraine headaches (3). Controlled infusion of CO_2 in the tissue produces an acute inflammatory response characterized by the dilation of the peripheral blood vessels and stimulation of cutaneous microcirculation, which increases local blood flow (4). It also induces an increase in tissue oxygen partial pressure, due to an increase in local oxygen availability caused by its release from hemoglobin related to reduction in pH (Bohr effect) and a hypercapnia-induced capillary blood flow (3).

Heat is generated by the RF due to resistance, called impedance, natural response of the tissue to the movement of the electrons in RF (Ohm's law). The impedance produces heat according to the amount of current (amps) and time (seg). Thus, heat is produced when the tissue impedance converts the electric current into thermal energy (5). In addition, the RF energy produces an electrical current instead of a light source, so it does not damage tissue or

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epidermal melanin (6). The main effect of RF treatments is the synthesis of collagen and elastin (7). In the dermis and in the subcutaneous tissue, RF induces neocollagenesis (8). This study compared the effects of CA and RF on human skin.

Materials and methods

The Research Ethics Committee of Federal University of Triângulo Mineiro—UFTM, approved this study. Eight patients who underwent abdominoplasty received a single procedure of CA and RF treatments in a single session at the Clinical Hospital of the UFTM. Patient characteristics, including age, Fitzpatrick type, Glogau scale, and time of sample collection are shown in Table I. The mean age of the patients was 37 ± 4.7 years.

Untreated skin was used as control. In an area of 49 cm^2 (7×7 cm) in the infra-umbilical region, CA was performed on the right side and RF was performed on the left side. CA was administered using the beveled end of a 30G *1/2* needle introduced into the skin in an angle of approximately 30°C and delivered at a velocity of 40 mL/min. The total quantity of CO_2 infused was approximately 20 mL (0.3–0.6 mL/kg of patient's body weight) encompassing the entire delineated area. For the RF treatment, the epidermal temperature was controlled using an infrared thermometer monitored to reach 40°C and treatment time was 5 min after reaching this goal temperature. Sample collection varied from 0 to 120 days after application. The samples were then fixed, cut in paraffin (5 μm), and stained with Picrosirius red and Weigert's hematoxylin. Images were acquired using a Zeiss Axiophot® microscope and processed using Adobe Photoshop®.

Results

Histologically, heterogeneity was observed among samples of untreated control skin (Figure 1 A, D, G, J, and M). Peculiarities about each individual were found, even among individuals of the same age group. In patients 1, 2, 3, and 8, aged 26, 38, 41, and 38 years with skin phototypes II, III, V, and II, respectively, changes due to skin aging were more evident and classified as skin aging of degree III based on Glogau's classification of photoaging. As for patients 4, 5, 6, and 7, aged 36, 38, 40, and 39 years with skin phototypes V, III, VI, and V respectively, we observed improvement in skin quality according to Glogau's classification.

Comparisons were made before and after treatment, between the same patient's treated skin and control skin and among those who presented at similar intervals of sample collection (Table I).

Histologically, remodeling of collagen fibers was observed in both treatments with some peculiarities. RF induced collagen remodeling in majority of the patients and in different periods of sample collection, from 4 to 120 days after a single session (Figure 1 F, I, L, and O), except on the same day of the treatment (Figure 1 C), with better results in longer periods, from 61 to 120 days (Figure 1L and O, respectively).

As for CA, the changes were more subtle. In the early periods of sample collection (same day and four days after a single treatment), these changes were insignificant (Figure 1 B and E, respectively). In the other periods, there was an increase, which was more evident at 44 and 120 days after a single CA treatment (Figure 1 H and N, respectively).

Regarding the amount of elastic fibers, a significant increase was observed after CA (Figure 2 B, E, H, K, and N), which did not occur after the RF treatment (Figure 2 C, F, I, L, and O).

Table I. Clinical features of patients.

Patients	Skin aging degree*	Age (years)	Skin phototype**	Time of sample collection (days)***
4	II	36	V	0
5	II	38	III	44
7	II	39	V	120
6	II	40	VI	61
10	III	26	II	4
2	III	38	III	20
8	III	38	II	117
3	III	41	V	28

*According to Glogau.
**Skin phototype by Fitzpatrick classification.
***Time interval between the therapy session and collection of the sample.

Discussion

The histological findings in this study demonstrated the positive effects of both CA and RF on human skin, particularly on alterations caused by dermal aging. Both techniques induced collagen remodeling, each with its peculiarities. When collection was done on the same day of the treatment (patient 4), neither CA nor RF showed significant effects, probably because the time was insufficient for the stimulus to trigger a response. Four days after the procedure, RF stimulated discreet collagen remodeling in patient 1, whereas CA did not stimulate any significant effects.

Collagen remodeling was observed throughout the other periods of collection in both CA and RF, and the most significant effects were seen at 44 and 120 days after CA in patients 5 and 7 (38 and 39 years, respectively) and at 61 and 120 days after RF in patients 6 and 7 (40 and 39 years, respectively). These patients presented skin aging of degree II according to Glogau, showing that both techniques are more effective in moderate and early skin aging degrees.

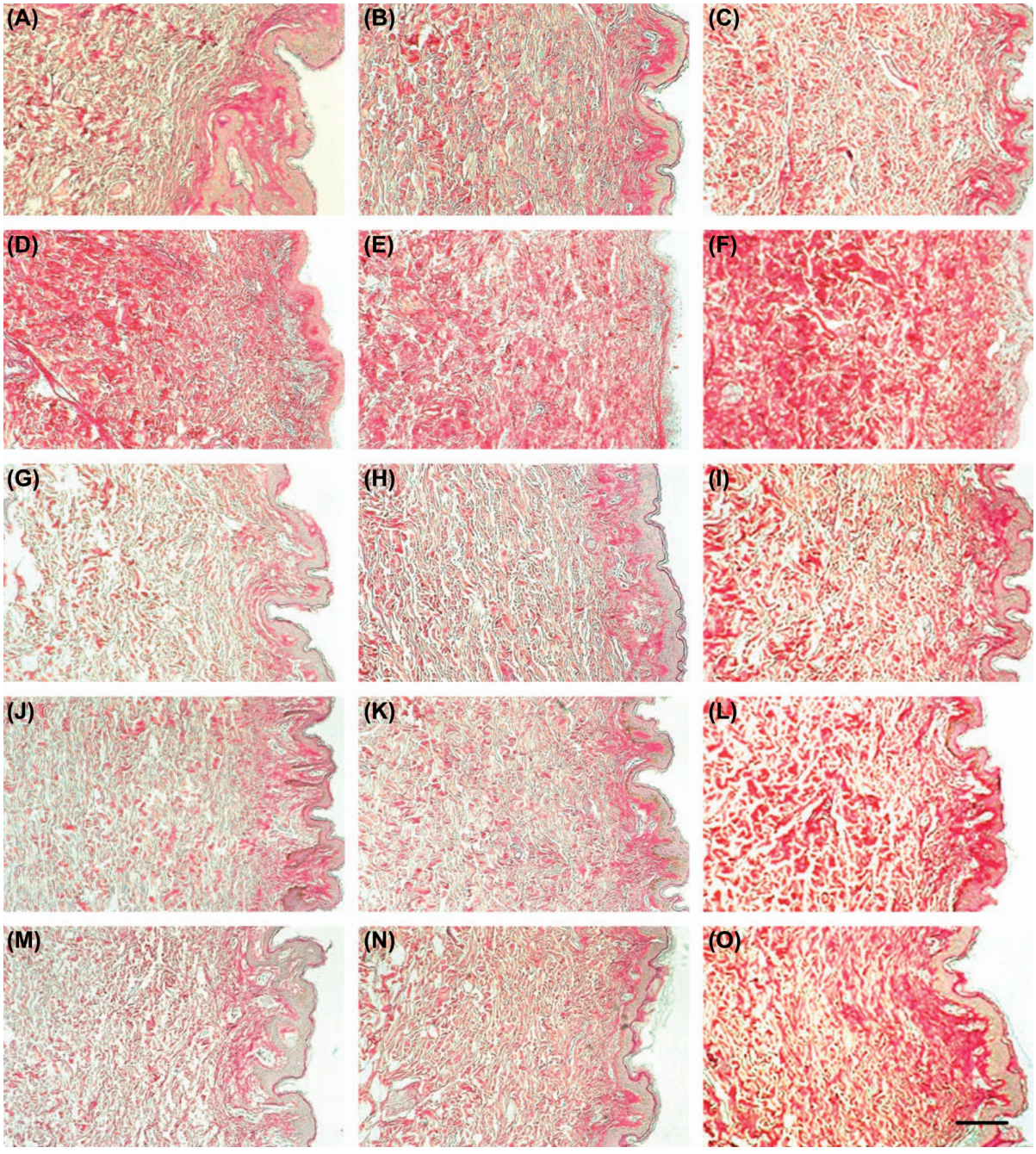

Figure 1. Comparison of the effect of the treatment with CA and RF on collagen fibers in human skin. Picrosirius staining. Collagen fibers are seen in red. 36-year-old woman's skin—(A) control; collected on the same day, after treatment with (B) CA and (C) RF. 26-year-old woman's skin—(D) control; collected 4 days after treatment with (E) CA and (F) RF. 38-year-old woman's skin—(G) control; collected 44 days after treatment with (H) CA and (I) RF. 40-year-old woman's skin—(J) control; collected 61 days after treatment with (K) CA and (L) RF. 39-year-old woman's skin—(M) control; collected 120 days after treatment with (N) CA and (O) RF. Bar = 100 μm.

It is interesting to observe that although patients 2 and 8 had the same age as patient 5 and had similar skin phototypes (III, III, and II, respectively), the results were not similar after the treatments, probably because patient 5 presented at lower skin aging degree.

CA is used for the treatment of skin irregularities, since its mechanical action produces an inflammatory process with subsequent migration of fibroblasts to the area, stimulating the synthesis of collagen and other connective tissue molecules, which improves skin laxity (9). Similar to CA, RF is also indicated for the treatment of skin laxity. However, the process is different. While in CA the tissue needs to be damaged in order to stimulate a repair process, RF initiates tissue repair process.

The response of an organism to a stimulus is directly associated with its age. As skin ages, collagen is gradually replaced by non-reducible crosslinks (10). Younger patients appear to respond better to

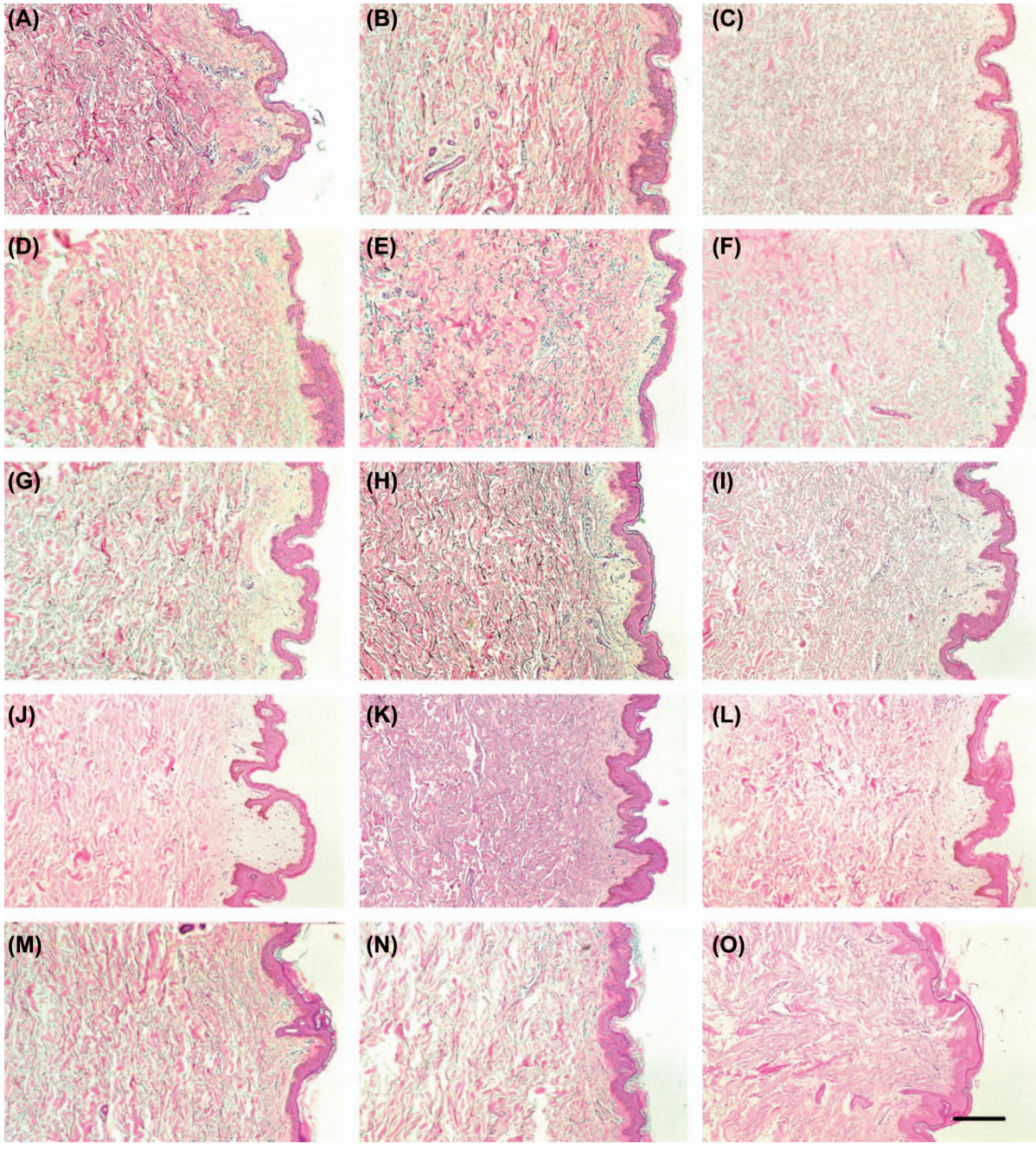

Figure 2. Comparison of the effect of the treatment with CA and RF on elastic fibers in human skin. Weigert's hematoxylin staining. Elastic fibers are seen in black. 36-year-old woman's skin—(A) control; collected on the same day, after treatment with (B) CA and (C) RF. 26-year-old woman's skin—(D) control; collected 4 days after treatment with (E) CA and (F) RF. 38-year-old woman's skin—(G) control; collected 44 days after treatment with (H) CA and (I) RF. 40-year-old woman's skin—(J) control; collected 61 days after treatment with (K) CA and (L) RF. 39-year-old woman's skin—(M) control; collected 120 days after treatment with (N) CA and (O) RF. Bar = 100 μm.

the treatment when compared with patients over 45 years (11–14). In this study, the best results were observed in skin with a moderate degree of photoaging (degree II according to Glogau).

Aging degree is likely to be the main determinant of the results obtained in both treatments, since patient 7 (39 years, degree II according to Glogau, and Fitzpatrick type II) exhibited better results than patient 8 (38 years, degree III according to Glogau, and Fitzpatrick type III) with similar sample collection times (120 and 117 days, respectively). We observed in this study that results after treatment with either CA or RF depend on several factors: patients' individual characteristics, degree of photoaging, skin phototype, and time of sample collection.

There are reports about the importance of the patient's physical demography, such as age, weight, and skin type, since chronological age is not the only factor responsible for the characteristics of aging (9). In this study, we found characteristics of aging which

were not directly related to the patients' chronological age, for example, patient 7 (39 years and phototypeV), whose control skin was thicker when compared with that of patient 1 (26 years and phototype II). In this case, the difference may be related to these patients' different skin phototypes and also to individual factors. It reinforces the necessity to evaluate the patient's physical demography before deciding on skin rejuvenation treatments.

It is important to highlight that, except for the same day of collection, in all patients and in all periods of sample collection, RF presented better results when compared with CA. Studies demonstrate that after a single RF session there is an increase in collagen level and improvement in skin quality; these responses continue to occur. These changes are evident at three weeks after treatment and up to six months after the treatment (7,8,11). Another study treated 15 patients with four RF sessions at 1–3 weeks' intervals. It was observed that 58% of them showed improvement three months after the treatments and 48% of them showed improvement up to six months after the treatments (8,10,11,14–17). This should be considered when defining the treatment protocol. Other authors found better results ten weeks after a single session of RF when compared with 28, 14, and 2 days after the procedure (18). These findings corroborate the present study, in which we observed the effects of RF continuing after the treatment, showing better long-term responses. Consequently, short-term sessions of this treatment are not necessary.

As for the amount of elastic fibers, an increase was observed after the CA treatment and it was more evident in patients 5 (38 years) and 7 (39 years), 44 and 120 days after a single session, respectively, probably due to the same factors that induced a greater increase in collagen level in these two volunteers.

Some authors demonstrated the effects of CA upon skin elasticity through histological analysis of thigh and abdominal skin in patients undergoing twice-weekly sessions over the course of three consecutive weeks. An increase of 55% was found in the mean values of elasticity in the skin treated with CA (9). In our study, an increase of elastic fibers synthesis was observed as a result of CA.

Interestingly, elastic fiber synthesis was not stimulated by RF in these patients. This is different from results obtained by other authors who observed an increase in neo-collagenase, particularly after 10 weeks, and also a significant increase in elastin level, compared with untreated skin (18).

Conclusion

Both CA and RF proved to be promising techniques for the treatment of skin laxity. CA proved to be effective in the treatment of skin aging for a short period and remodeling elastic and collagen fibers, while RF showed long-lasting effects after a single session.

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