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Research

Effects of Bipolar Radiofrequency on Collagen Synthesis from Patients with Brachial Ptosis

Laura C. Cala Uribe, MD*
Mauricio E. Perez Pachon, MD†
Andreina Zannin Ferrero, MD‡
Constanza Neri Morales, MD‡
Juliana Silva Gutierrez, MD‡
Angela D. Manrique Cruz, MD‡
María F. Maza, MD‡
Edward J. Acero Mondragon, Msc, PhD§
Luis G. Celis Regalado, BS, Msc‡

Background: Radiofrequency is frequently used for skin rejuvenation, localized fat elimination and cellulite treatment. It prompts the expression of thermal shock proteins that lead to dermal thickening as a result of collagen synthesis. The authors report a histological and clinical analysis of the arm subdermal changes before and after bipolar radiofrequency treatment plus liposuction to determine their benefits for arm contouring.

Methods: Inclusion criteria included patients with stage 1, 2a, and 2b brachial ptosis (Duncan classification) and upper limb fat deposits who were considered candidates for third-generation ultrasound-assisted liposculpture plus radiofrequency-assisted lipolysis/skin tightening. Arm subdermal tissue samples (5 mm^3) were analyzed before and after the intervention. We used 10% formaldehyde for tissue fixation and stained each sample with hematoxylin/eosin, Masson trichrome, and antibody markers against the cell cycle Ki-67 protein.

Results: We analyzed a total of 12 biopsies from six patients who meet the inclusion/exclusion criteria. Histological findings with hematoxylin/eosin revealed hyperplastic and metaplastic changes with focal distribution within the papillary and reticular dermis. Masson trichrome staining showed an increase of the characteristic basophilia of thin type-I and type-III collagen fibers. In contrast, molecular analysis reported an increase in fibroblast activity mediated by the activation of the heat shock protein HSP47.

Conclusion: Radiofrequency may be a great alternative to improve skin retraction in patients with mild to moderate brachial dermatochalasis through the activation of HSP47 heat shock protein and the production of type-I and type-III collagen. *(Plast Reconstr Surg Glob Open 2023; 11:e4924; doi: 10.1097/GOX.0000000000004924; Published online 13 April 2023.)*

BACKGROUND

Modern plastic surgery currently considers multiple anatomic and aesthetic concepts that allow the surgeon to provide a wide range of outcomes that fit a particular population.[1] In terms of liposuction, multiple innovations and modifications have been added to the original technique to improve results and reduce scarring and morbidity to the patient.[2,3] Arm liposuction has been one of those of special interest among women, as aging and posterior sagging due to fat deposits are a common concern. Hoyos et al[4,5] changed the perspective of traditional liposuction into a new standard of high-definition liposuction (HDL), where different planes and layers for carving end up in an improved aesthetic concept that follows the natural underlying anatomy of the individual. However, in certain cases for which HDL is not enough, introduction of new technologies such as radiofrequency (RF) comes at hand to enhance the aesthetic outcome. Nowadays, multiple medical specialties other than aesthetic and reconstructive plastic surgery, such as dermatology, ophthalmology, gynecology, and otorhinolaryngology, have taken advantage of RF due to its particular benefit of a minimally invasive technology and, above all, a great safety profile.[6]

*From the *Private Practice, Bogotá, Colombia; †Total Definer, Rochester, Minn.; ‡The Cell Therapy and Metabolism Research Group at Universidad De La Sabana School of Medicine, Bogotá, Colombia; and §PROSEIM Group, Department of Morphophysiology at Universidad de La Sabana, Bogotá, Colombia.*

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RF is an electromagnetic energy source that produces an electric current at different frequencies, which provokes the movement of charged particles within a specific tissue. This subsequently generates a rising temperature depending on the target tissue.[7,8] By the time heat reaches collagen fibers, hydrogen bonds are broken; then, fibers are shortened, and greater tension is achieved. Moreover, fibroblasts are stimulated to reorganize collagen fibers into new ones with greater tensile strength.[7,9] The sum of RF effect will end up stimulating neo-collagen formation and generating localized lipolysis.[10] This process brings up direct effects over the fibroseptal network situated in the subcutaneous space[11]: modifies different molecular pathways of collagen type 1, type 3 synthesis, and heat shock protein (HSP) expression. These influence both skin retraction and the healing process.[12]

HSPs are usually expressed after a variable number of conditions such as heat, toxins, hypoxia, ultraviolet radiation, infections, and tumors. Their role in cell repair and protection is fundamental after physiologic/environmental stress, which also aids in the organization of extracellular stress.[2,13,14] This results in thermal stress due to RF that stimulate their response (increased expression) to damage, by creating new collagen fibers and a fresh epidermal surface. *HSP27*, *HSP47,* and *HSP70* are the ones that have demonstrated a strong association with collagen fiber regeneration.[15,16] All these molecular effects have made RF one of the most widely used systems in the world. It offers great clinical outcomes: (1) optimal soft tissue contraction through thermal coagulation (> 35% of tissue contraction after 12 months),[17] with high efficacy among patients with excessive skin laxity and high body mass index; (2) an opportunity to perform ambulatory procedures under local anesthesia with minimum scarring. These allow the patient to return to their daily activities almost immediately[18] (Table 1). We are reporting the changes of subdermal tissue caused by radiofrequency-assisted lipocoagulation (BodyTite; INMODE aesthetics 2022, Lake Forest, Calif.) in patients with mild (I), moderate (IIa), and moderate-to-severe (IIb) brachial ptosis.[19] We will analyze histological sections and photographs before and after treatment, with the aim to provide statements about clinical outcomes.

Table 1. HSPs and Their Effects on Skin Tissue

HSP	Effects
HSP27	• New collagen formation • Prevents programmed cell death (apoptosis) • Collagen matrix contraction
HSP47	• New collagen formation • Procollagen production • Collagen-assemble protein
HSP60	• Mitochondrial signaling processes
HSP70	• New collagen formation • Prevents programmed cell death (apoptosis)
HSP90	• Intracellular signaling molecules

Takeaways

Question: Does bipolar radiofrequency have any effects on collagen synthesis?

Findings: We carried out a clinical study along with an anatomopathological analysis of patients with dermatochalasis who underwent liposculpture plus bipolar radiofrequency treatment of the arm contour. Before and after biopsies (total: 12) from six patients showed dramatic hyperplastic and metaplastic changes with focal distribution within the papillary and reticular dermis and also an increase in fibroblast activity after radiofrequency treatment.

Meaning: Radiofrequency is an effective technology to improve skin retraction in patients with mild-to-moderate brachial dermatochalasis.

MATERIALS AND METHODS

We enrolled patients with stages I, IIa, and IIb brachial ptosis according to Duncan classification. All patients underwent HDL of the arm in addition to radiofrequency-assisted skin retraction. Inclusion and exclusion criteria are portrayed in Table 2. Arm liposuction technique was part of HDL, which involves a three-step approach: (1) infiltration (tumescent solution: 1000 ml of saline + 1 ml of 1:1000 epinephrine + lidocaine 10 mg/kg); (2) fat emulsification through third-generation ultrasound (VASER; Bausch Health Companies Inc, Bothell, Wash.); and (3) power-assisted liposculpture by using MicroAire Liposuction System (MicroAire Surgical Instruments, LLC., Charlottesville, Va.). Then, a trained scrub nurse used a #15 blade to cut out a 5-mm^3 full-skin-thick arm biopsy at 2 cm above the axillary fold over the posterior axillary line. Afterward, bipolar RF therapy (BodyTite) was applied over the posterior arm at a frequency of 1 mHz (cut-off temperatures: 40ºC and 70ºC for the external and internal electrodes, respectively). Probe is moved in a fanning-motion, and there were no more than two passes over the same area (cut-off temperature is reached). After that, the scrub nurse cut out a second biopsy just next to the prior one. IV medications used include antibiotic prophylaxis with cefazolin (2 gr IV, 60 minutes before incision) or clindamycin 600 mg IV (if allergic to beta-lactams), dexamethasone 8 mg, metoclopramide 10 mg, and ranitidine 50 mg. A professional photographer took records before surgery and during follow-up appointments at 2 days, and at 1, 3, 6, and 12 months. Both patients and surgeon were asked to answer a nonstandardized survey 3 to 6 months after surgery during postoperative appointments, to rate the results.

Biopsy Preservation

A circulating nurse took both samples and stored them in 10% formaldehyde (preservation) within a transport container of biologic specimens at room temperature. Each sample was taken to the laboratory technician, who ensured the proper fixation after 12 hours (to allow the tissues and cell structures to stabilize). Then, samples were dehydrated through a 20% alcohol-based battery

Table 2. Inclusion and Exclusion Criteria

Inclusion Criteria
• Ages between 18 and 65 years • ASA I and ASA II patients • BMI <30 kg/m^2 • Patients who undergo brachial liposculpture at the moment of the intervention • Mild-to-moderate brachial dermatochalasis, including stages 1, 2a, 2b
Exclusion Criteria
• Underage • Pregnant or breast-feeding women • History of skin cancer or premalignant lesions • Users of pacemakers, internal defibrillators, active electrical implants in any part of the body • Plates, metal pins, silicone implants, or injected chemical substance • Cardiac disorders, epilepsy, uncontrolled hypertension, diabetes mellitus, thyroid dysfunction, liver, or kidney disease • Hemorrhagic coagulopathies or use of anticoagulants • Immunosuppressive diseases such as AIDS and HIV or use of immunosuppressive drugs • Any active condition in the treatment area, such as sores, psoriasis, eczema and rashes, skin disorders, keloids, abnormal wound healing, and very dry and fragile skin • Use of isotretinoin (Accutane, Roaccutane) in the 6 months before treatment • Laser treatment or chemicals on the treated area within 3-6 months before surgery (hyaluronic acid, fractional CO_2 laser, micro needling, Morpheus, or other technologies) • Allergies • Mental disorders such as body dysmorphic disorder (BDD) • Any condition that the practitioner believes to be unsafe for the patient • Severe dermatochalasis in patients requiring brachioplasty • Patients with previous interventions on the arms (liposculpture, biopolymers)

(1 hour). Finally, samples were embedded in paraffin to form a block, which was subject to micrometer cuts for staining. Processing time per sample was ≈24–48 hours. All histological sections were stained with hematoxylin/eosin (HE), Masson trichrome (MT), and immunohistochemistry with antibodies against the Ki-67 cell cycle protein (specific marker of cell proliferation that is induced by the fibroblastic activity secondary to the heat shock protein HSP47).

Biopsy Analysis

A blinded pathologist analyzed all pre- and posttreatment samples by (1) looking for differences between pre- and posttreatment samples in terms of skin thickness and collagen synthesis with HE staining; (2) measuring the distribution of collagen patterns within the dermis after TM staining; (3) determining changes/similarities after immunohistochemistry (increased expression of Ki-67 protein).

Outcomes Analysis

We ran a morphometric analysis at the laboratory to compare the percentage density of type-I collagen fibers in perilesional reticular dermis from samples with versus without radiofrequency intervention. Criteria for dermal collagen digital image evaluation was carried out through *ImageJ* (JAVA Digital Image processing program – Public Domain, BSD-2. Developed by the National Institutes of Health, USA) as suggested by Brianezi et al[20] and Chavoshnejad et al.[21] In addition, we compared the preoperative versus 3-month postoperative photographs to assess the amount of skin retraction by photographic analysis. Patients posed in a standard position at a plain black background for photo shooting. A professional photographer made five measurements of the right and left arms from three random patients using the FIJI tool (2023 GitHub, Inc.)[22] and ran the statistical analysis in JAMOVI software[23,24] by paired *t* test and Wilcoxon signed-rank test.

Ethical Considerations

Our work follows resolutions 8430 of 1993 and 2378 of 2008 of the Ministry of Health and Social Protection of Colombia. Each patient was informed of the purpose, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the authors, the anticipated benefits and potential risks of the study and the discomfort it may entail, poststudy provisions, and outcomes according to the Declaration of Helsinki (Fortaleza,

Table 3. Patient Demographics

	Average (Range)	n = 6 (100%)
Age (y)	32 (29–54)	
Weight (kg)	68.1 (54–78)	
Height (m)	1.63 (1.5–1.68)	
BMI (kg/m^2)	25.8 (23.5–28.2)	
Gender		
Men		0 (0%)
Women		6 (100%)
Ethnicity		
African American		0 (0%)
White		4 (67%)
Asian		0
Hispanic		2 (33%)
Smoking history		
Before surgery		0
No history		6 (100%)
Brachial ptosis		
Stage 1		2 (33%)
Stage 2a		3 (50%)
Stage 2b		1 (17%)

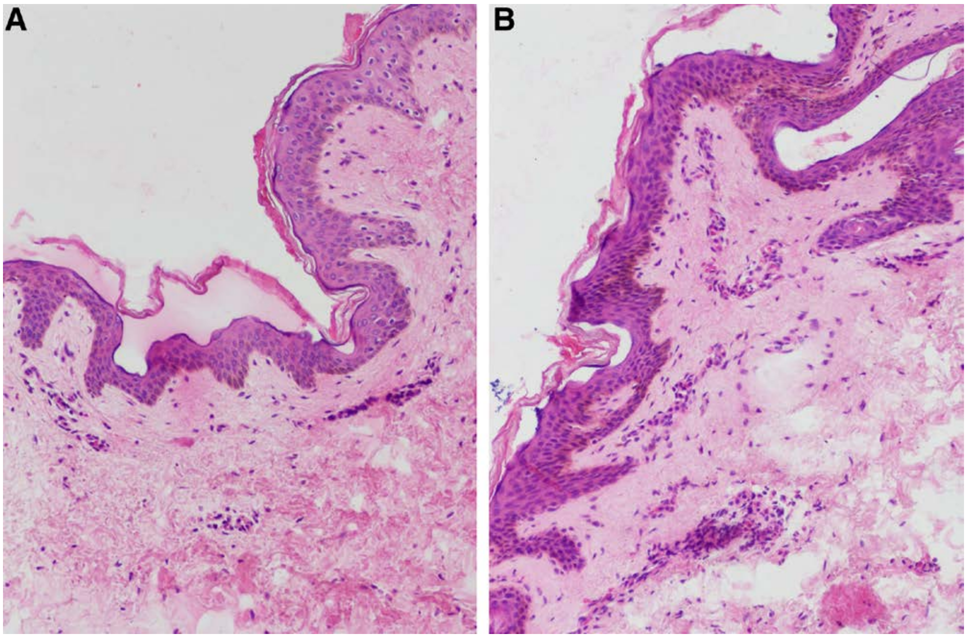

Fig. 1. HE-stained biopsy from a 36-year-old female patient with no history of previous liposuction (10× Magnification). The pre-RF skin biopsy picture (A) shows the papillary dermis with a histological constitution of lax areolar subepidermic connective tissue, whereas the post-RF one (B) shows a metaplastic-to-focal change of the subepidermal papillary dermis towards an irregular dense connective tissue.

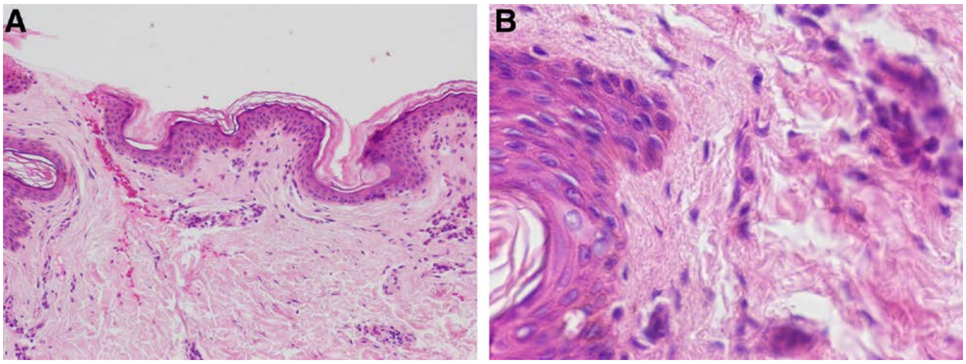

Fig. 2. HE-stained biopsy from a 42-year-old female patient with no history of liposuction, who underwent VASER-assisted Arm HDL plus RF treatment for skin laxity. Both the 5×- (A) and the 40× magnification (B) photographs portray the papillary dermis with a significant increase of fibrous and fibroblastic infiltration.

2013). They were also informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. A freely given informed consent was signed for each patient participating in our report; hence, each patient had the autonomy to decide whether to undergo fat grafting of certain muscles or not. Patients were informed about the nature of the procedure, the potential risks and benefits specific from HDL, and RF-based skin retraction.

RESULTS

A total of 12 samples were analyzed from six patients who were enrolled in our study. Demographic data are included in Table 3. A natural and slim shape of the arms was achieved in all patients. No fat grafting was done over the arm muscles. Five patients underwent buttocks augmentation through autologous fat grafting as part of HDL. No major or minor complications were reported. Both the main surgeon (L.C.) and all patients were satisfied with aesthetic postoperative results. One patient had temporary numbness sensation over the posterior forearm, which lasted for 2 weeks and solved entirely by physical means. Follow-up period ranged from 2 to 12 months (average = 6 months).

HE-stained samples revealed hyperplastic and metaplastic changes of focal distribution in the papillary dermis and an evident transition from lax areolar connective

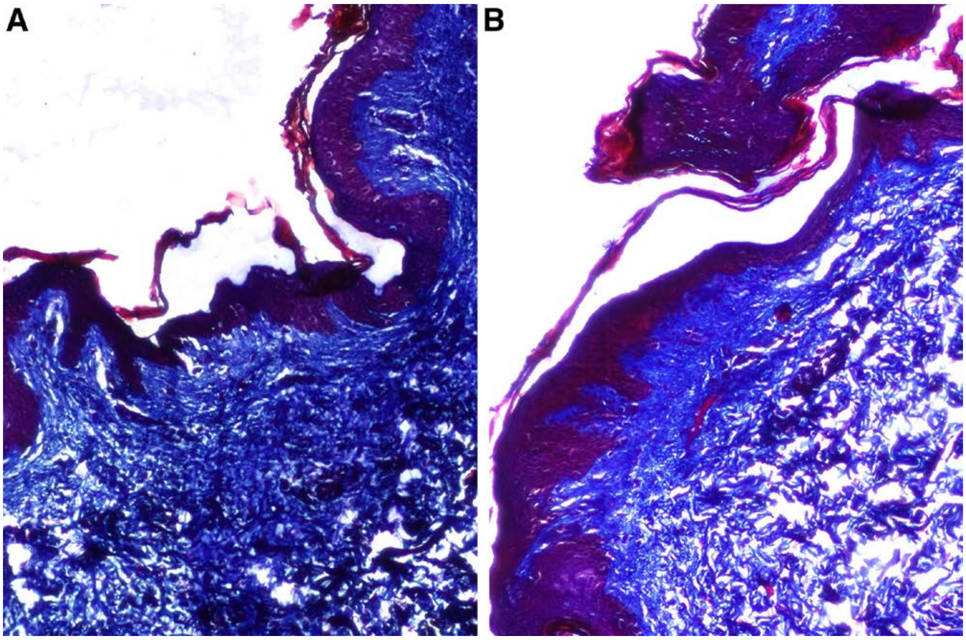

Fig. 3. TM-stained sample from a 36-year-old woman who underwent HDL (10× magnification). Picture from skin without RF intervention (A) shows the papillary dermis with histological constitution of lax areolar connective tissue represented as low density of type-III collagen fibers (thin fibers at the basophilic subepidermis). The picture from the skin after RF intervention (B) shows the papillary dermis with histological constitution of lax areolar connective tissue, but with an increased density of type-III collagen fibers.

tissue to irregular dense tissue, both of which are considered due to the increase of collagen synthesis after RF treatment of the posterior arm (Figs. 1 and 2). In a similar fashion, MT staining showed not only an increase in the tissue basophilic take up, which is characteristic of thin type-III collagen fiber production, but also an organized-pattern distribution of the perilesional type-I collagen within the reticular dermis. These changes denote an amplified synthesis of collagen and an overall increase of tissue thickness after RF treatment (Fig. 3). On the other hand, immunohistochemistry revealed a positive labeling for epidermal keratinocytes of the stratum basale (subepidermal fibroblasts) by using antibodies against the Ki-67 protein (Fig. 4), which demonstrates that cell proliferation was induced by the fibroblastic activity generated by the heat shock protein HSP47, secondary to RF treatment.

Regarding tissue analysis (Fig. 5), Wilcoxon test revealed an average percentage density of type-I collagen

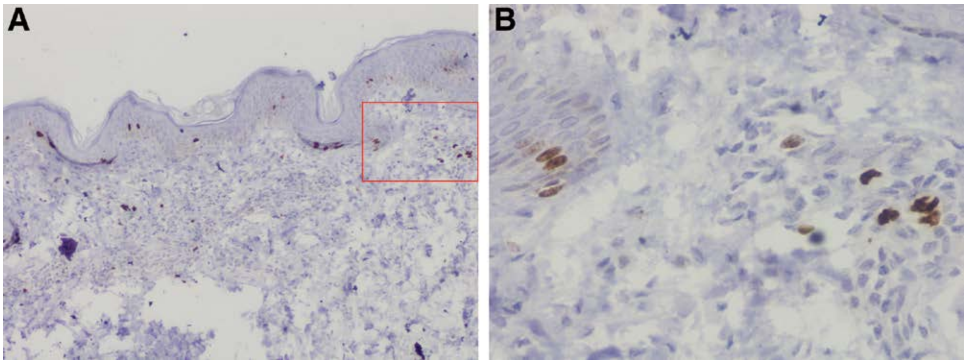

Fig. 4. Immunohistochemical detection of Ki-67 protein from the arm biopsy of a 54-year-old woman who underwent VASER HDL + radiofrequency treatment. The macro view at 5× magnification (A) shows a positive immunolabeling for both epidermal keratinocytes of stratum Basale (brown color) and subepidermal fibroblasts (purple color). The 40× magnification (B) shows the change in expression associated with an increased fibroblastic activity of collagen synthesis due to increased demand. To note, Ki-67 cell cycle protein is co-expressed in tissue reparation due to the increase in fibroblast activity that is mediated by the activation of HSP70.

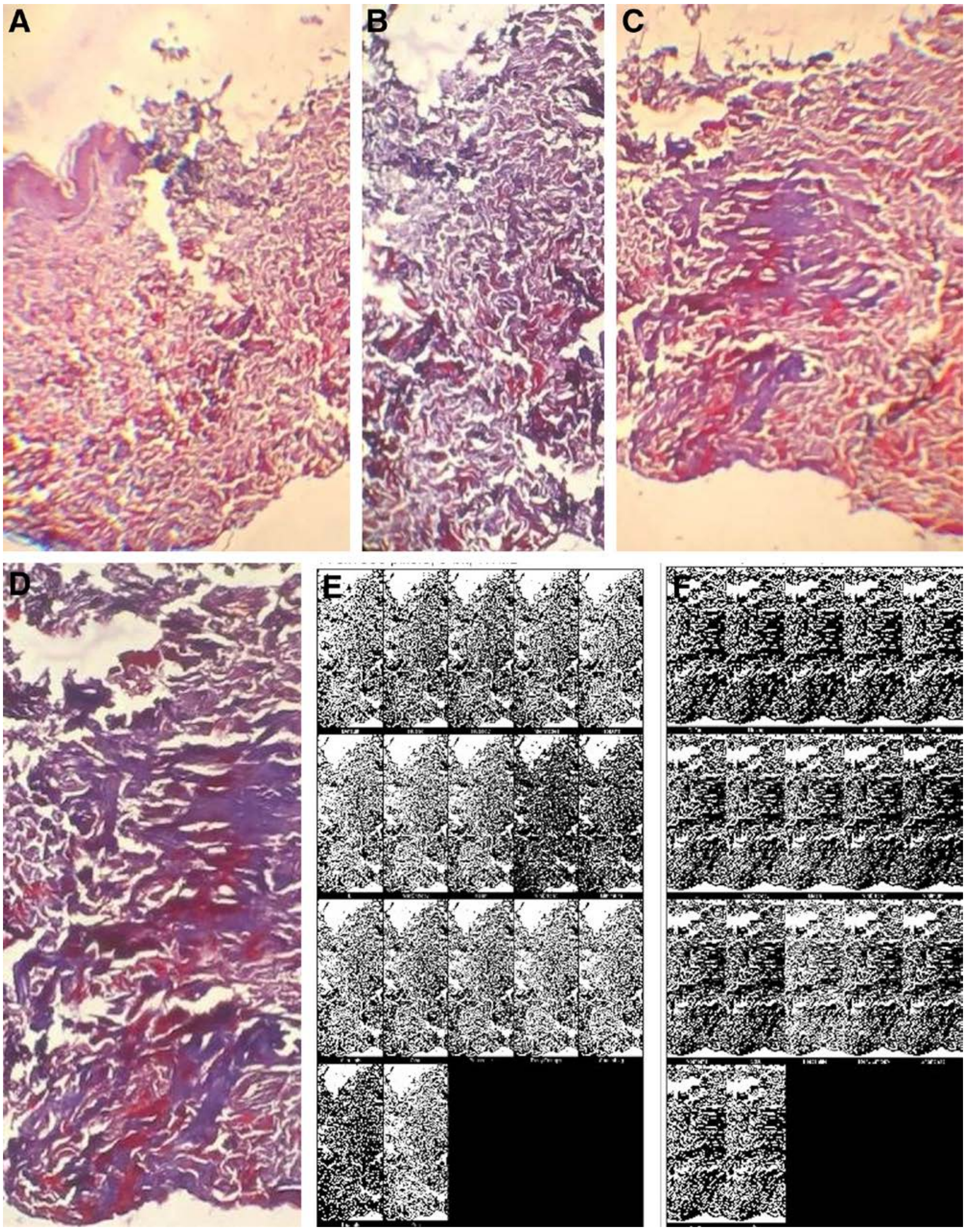

Fig. 5. Dermal type-I collagen percentage density analysis through ImageJ. MT-stained biopsy from a 36-year-old female patient who underwent Arm HDL. Notice the usual appearance of the reticular dermis before RF (A, B), compared with the distribution of new type-I collagen fibers at the perilesional reticular dermis after RF treatment (C, D). Photomicrographs are at 5×- (A, C) and 10× Magnification (B, D). Black and white sequential images before (E) and after (F) radiofrequency therapy portray how the images are analyzed by ImageJ.

fibers of 74.75% versus 58.25% for RF treated versus non-RF-treated samples, respectively ($P < 0.05$) (Table 4). (**See figure, Supplemental Digital Content 1,** which shows the percentage density of type-I collagen fibers in biopsies before vs. after RF treatment. **http://links.lww.com/PRSGO/C487**.) Similarly, the photographic analysis (Fig. 6) showed a statistically significant difference between the skin area before versus after radiofrequency therapy ($P < 0.001$) (see CI specific for each measure and other additional data in Table 5.)

DISCUSSION

Arm liposuction has been challenging due to the anatomical configuration of the subdermal tissues and the variable distribution of adipose tissue among its different facets (Fig. 7). (**See figure, Supplemental Digital Content 2**, which shows a 49-year-old female patient with moderate to severe brachial dermatochalasis who underwent RF treatment of the arm in addition to HDL. A new rejuvenated arm is seen in the 3-month postoperative photograph (B), while the preoperative photograph (A) shows

Table 4. Percentage Density of Type-I Collagen Fibers in Biopsies before versus after RF Treatment (See also SDC 1)

	Collagen Fiber Density %	
	Before RF	After RF
Sample 1	62	74
Sample 2	55	77
Sample 3	60	76
Sample 4	62	80
Sample 5	55	63
Sample 6	51	68
Sample 7	62	79
Sample 8	59	81
Average	58.25	74.75

W-value = 0; mean difference = −18.75; sum of positive ranks = 0; sum of negative ranks = 36
Z-value = −2.5205; Dif. significance: $P < 0.05$.

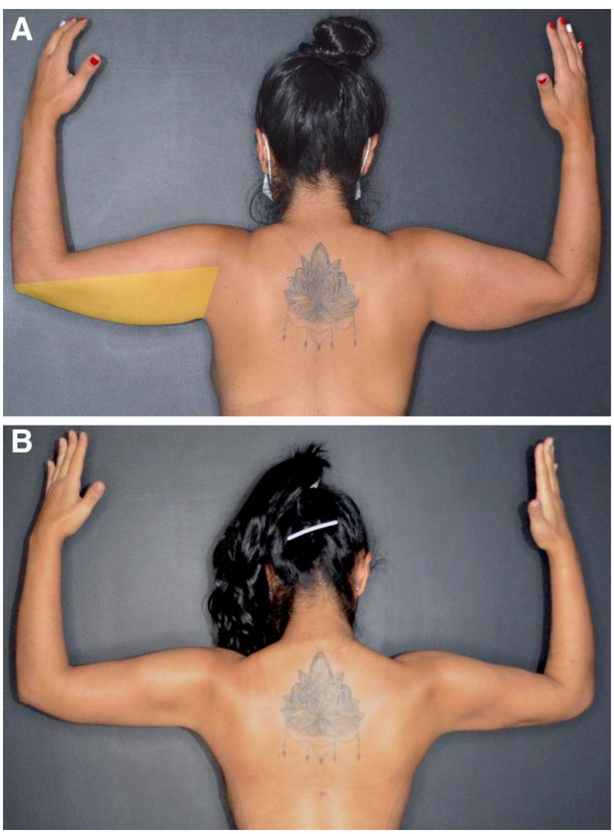

Fig. 6. Photographic analysis of skin area below a nonvariable anatomic line between the elbow and the shoulder joint. Patients were asked to pose with 90-degree shoulder abduction, 90-degree elbow flexion and arm external rotation. We set the most distant point of the elbow and the shoulder joint depression as the anatomic references. An example of the measurement is shown in photographs before (A) and after (B) radiofrequency.

severe skin sagging and adipose deposits. **http://links.lww.com/PRSGO/C488**.) (**See figure, Supplemental Digital Content 3,** which shows a 32-year-old female patient with moderate to severe brachial dermatochalasis who underwent RF treatment of the arm in addition to HDL. **http://links.lww.com/PRSGO/C489**.) HDL and traditional liposuction can be considered effective when skin laxity is minimum to mild, but for those with mild to moderate skin laxity and other special conditions (eg, poor skin quality, Fitzpatrick phototype I–III, age >40 years), new strategies have been recently proposed as more reliable and effective.[2,19] Clinically, RF has been shown to improve 50% skin laxity and 35% average skin contraction in patients undergoing RF therapy in addition to conventional liposuction,[25] whereas VASER-assisted lipoplasty and suction-assisted lipoplasty have shown only 15%–16% and 10%–11% of skin retraction 6 months after surgery,[26] respectively. In addition, Gasperoni and Salgarello reported that gradual liposuction with a progressive increase in cannula diameter over areas with variable adipose thickness avoid skin irregularities after lipoplasty.[27] Consequently, the combined use of VASER-assisted arm liposuction and RF therapy would not only improve the skin tightening and retraction effect in almost 60%–80% but will also provide a better aesthetic and functional result due to the three-dimensional nature of HDL itself.[15,28–30] To note, such effects of tensile strength and improved skin contraction depend on the time of RF exposure, the highest temperature reached, age, and hydration state of the patient.[9] Our study compares the effects of VASER alone on skin retraction through HDL of the arm versus those after the addition of RF treatment by analyzing skin tissue samples from patients who underwent such procedures. We found a statistically significant difference ($P < 0.05$) of the percentage of collagen type 1 fibers between samples that were subject to RF and those that were not. This finding was also supported by the results reported by a blinded pathologist who did not know what the purpose of the study was and was able to analyze the biopsies with different magnifications and stains. (**See figure, Supplemental Digital Content 4,** which shows full-skin thickness biopsy of a 29-year-old female patient who underwent arm HDL plus RF therapy. **http://links.lww.com/PRSGO/C490**.) (**See figure, Supplemental Digital Content 5,** which shows full-skin thickness biopsy of a 29-year-old female patient who underwent arm HDL plus RF therapy. **http://links.lww.com/PRSGO/C491**.) A summary of the histological findings before and after radiofrequency treatment are shown in Table 6. The role of RF to induce HSP47 expression and subsequently neocollagenesis goes in line with former studies reported by several authors,[12,31–34] in addition to the increase in both collagen production and the two to 15 times expression of genes related to skin collagen differentiation reported by Louis et al.[35] Furthermore, Xu G et al[36] confirmed the matrix structural changes and the new collagen fiber formation to last until 6 months after the procedure due to a steady expression of HSP47. In addition to the histopathological changes, we are able to demonstrate the skin retraction improvement after RF treatment through a photographic analysis, which further supports its clinical benefits.

Our article explains how the stimulus of HSP47 expression by RF-generated heat resulted in an increased production of epidermal keratinocytes at the stratum basale (subepidermal fibroblasts) from the samples marked with Ki-67 protein antibodies. In effect, the molecular analysis

Table 5. Photographic Analysis of Preoperative and Postoperative Measurements of the Area below the Anatomic Line between the Elbow and the Shoulder Joint

Descriptive Data	Patient	RA Pre	RA Post	LA Pre	LA Post
N	7	5	5	5	5
	8	5	5	5	5
	9	5	5	5	5
Mean	7	142,292	124,519	154,033	131123
	8	131,197	118,446	139,114	117,810
	9	137,205	105,675	156,543	120,260
Median	7	139,366	125,866	154,166	130,452
	8	130,668	118,102	139,062	116967
	9	136,358	105,139	156299	119801
Standard deviation	7	6710	3432	2544	1258
	8	2445	2133	4950	1834
	9	1842	1226	1559	1746
Minimum	7	13,6146	118,572	,	130130
	8	128,287	115,602	133,834	116,214
	9	135,115	104,235	154,457	118,315
Maximum	7	149,940	127,332	157,474	133,110
	8	134,255	121,491	144,998	120,384
	9	139,434	106,964	158,815	122987

Paired Samples t Test								95% Confidence Interval	
			Statistic	Difference	*P*	Mean Difference	SD Difference	Lower	Upper
RA pre	RA post	Student *t*	8.98	14	<0.001	20,684	2303	15,744	25624
		Wilcoxon *W*	120		<0.001	21,014	2303	14,449	26,006
LA pre	LA post	Student *t*	13.54	14	<0.001	26,832	1982	22,581	31,083
		Wilcoxon *W*	120		<0.001	27,010	1982	21,939	30,688

We analyzed the difference in the measures between preoperative (pre) and postoperative (post) photographs from three patients on both the right (RA) and the left arms (LA). *Note*: H_a μ Measure 1 - Measure 2 ≠ 0.

of biopsies exposed to RF therapy showed an increase in the expression of heat shock protein translation when analyzing RNA samples, compared with that from nonexposed biopsies. (**See figure, Supplemental Digital Content 6**, which shows RNA analysis from six biopsies of patients who underwent arm HDL. **http://links.lww.com/PRSGO/C492**.) To our knowledge this is the first clinical and histopathological study that reports the benefits of RF and VASER on skin retraction and tightening with improved aesthetic outcomes. On the other hand, there are additional pathways involved in neocollagenesis by HSP47 expression that support the remodeling effect on the extracellular matrix (ECM), increased collagen type I, and fibronectin gene expression, which will prompt TGF-β activation and intracellular SMAD 2/3 signaling, thus greater fibroblast proliferation[37–39] (Fig. 8).

Limitations

We found it difficult to enroll individuals without prior history of liposuction, and therefore the reduced number of patients. Although clinical changes are usually more evident after 3 months of RF therapy, patients did not agree to a new cut/scar in their body. This resulted in the posttreatment immediate biopsy instead of a deferred one.

CONCLUSIONS

The combination of HDL and radiofrequency ensures an optimal aesthetic result in patients who undergo arm lipoplasty due to an increase in collagen synthesis, promoting a greater contraction of the skin. RF could be considered as a great alternative to avoid more invasive procedures such as transverse and/or vertical brachioplasty in patients with mild and moderate-to-severe brachial ptosis.

Laura C. Cala Uribe, MD
Carrera 15 #83 - 33
Dhara Clinic - Suite 202
Bogotá,
Colombia
E-mail: plasticsurgeon@drlauracala.com
Instagram: @drlauracala

DISCLOSURE

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PATIENT CONSENT

The patients provided written consent for the use of their images.

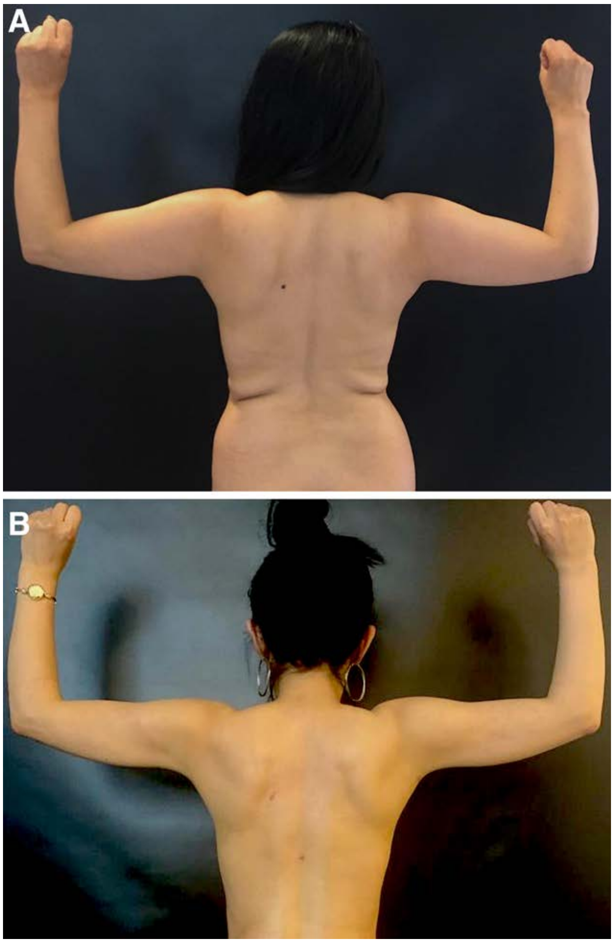

Fig. 7. A 32-year-old female patient with moderate brachial dermatochalasis who underwent RF treatment of the arm in addition to HDL. Note the preoperative mild-to-moderate skin ptosis from the posterior arm (A), which is further accentuated by adipose deposits. The 6-month postoperative photograph (B) shows a new slim contour and a youthful appearance of the arm with a soft muscular definition but without skin sagging.

Table 6. Histologic and Immunohistochemistry Main Findings of Brachial Samples before and after RF Intervention

Coloration	Before RF	After RF
HE	Papillary dermis with histological constitution of lax areolar connective tissue	Focal metaplastic change of the subepidermal papillary dermis to irregular dense connective tissue
MT	Papillary dermis with lax areolar connective tissue represented in low density of type-III collagen fibers	Papillary dermis with lax areolar connective tissue, representing increased density of thin type-III collagen fibers in the subepidermal region
IHC	Papillary dermis with increased fibrocyte and fibroblastic infiltrate	Positive immunolabeling for both epidermal keratinocytes of the stratum Basale and subepidermal fibroblasts

HE, hematoxylin-eosin; IHC, immunohistochemistry; MT, Masson trichrome.

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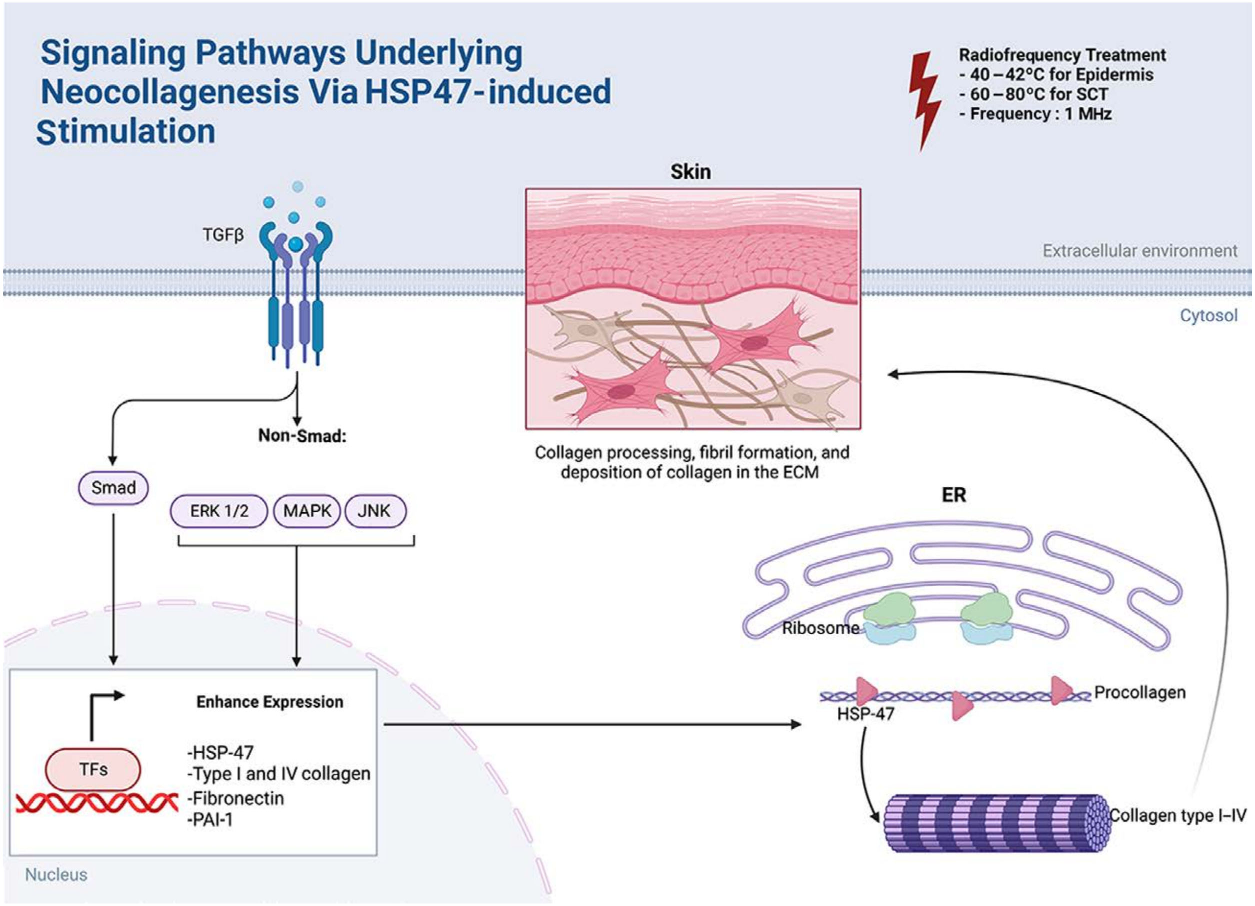

Fig. 8. Additional pathways involved in neocollagenesis by HSP47 expression. This protein interacts during procollagen assembly, folding and transport from the ER (endoplasmic reticulum) to the Golgi apparatus. Additionally, RF heating effect activates SMAD 2/3 intracellular signaling pathways as an expected effect after the activation of TGF-β, which further increases the expression of HSP47, hence increased ECM production by dermal fibroblasts.[33,34] Note: Other TGF-β intracellular pathways as ERK-1/2, MAP-K, and JN-K, which not only induce HSP47 expression, but also fibronectin, collagen type I, and type II genes are all associated with ECM remodeling.[33,34]

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