Abstract

Background: Regardless of diet and exercise, genetics plays an important part in creating puckering skin or dimples, which are difficult to hide at any age. The demand for a nonsurgical, noninvasive treatment of cellulite has inspired some manufacturers to invest in a new age of sophisticated devices and treatment therapies to repair the skin and improve contours. Although many of these new choices have demonstrated a smoothing effect (following a multitude of treatments), the objective documentation has in most cases been limited to biopsies, circumference measurements, and photographic evidence.

Hypothesis: We believe that the application of noninvasive high-energy radiofrequency (RF) to the skin of the thigh and buttocks heats the subcutaneous adipose tissue, causing collagen fibers to contract. The resulting impact to the subcutaneous tissue and collagen is expected to improve the skin's external architecture. Given that the subcutaneous tissue and adipose tissue are difficult to evaluate through histological methods, this investigation seeks to demonstrate the changes that occur when applying two treatments of high-energy RF on the subcutaneous tissue of thighs and buttocks utilizing real time ultrasound image scanning.

Materials and Methods: Twenty-six healthy female patients (ages 18 to 50) with visible bilateral cellulite (grade 1 to 3) on either the buttocks and/or thighs received 2 treatment sessions (15 days apart) of unipolar RF using the Accent RF System (Alma Lasers Inc). The system utilizes a unipolar RF applicator that is electrically cooled to aid in patient comfort during the treatment. Appropriate energy was set and the treatment was delivered in 3 passes of 30 seconds each. Evaluation of the thickness of the subcutaneous tissue on buttocks and thighs took place before the first treatment, second treatment, and 15 days following the second treatment with a with real-time scanning image ultrasound (Philips Medical Systems). Clinical improvement was objectively evaluated through comparative pre- and post-treatment measurements of the distance between the stratum corneum to the Camper's fascia and from the stratum corneum to the muscle. The study also evaluated the structure and changes of the collagen (thickening and realignment of septae) resulting from 2 treatments of RF. Photography was used to document contour and superficial changes.

Results: From the measurements of the distance between the stratum corneum to the Camper's fascia and from the stratum corneum to the muscle we were able to demonstrate that 68% of the patients presented a contraction of the volume of approximately 20%.

Conclusions: Based on the demonstrated results with real-time ultrasound scanning, we have observed that 2 RF treatments on the subcutaneous tissue of the buttocks and thighs provide a volumetric contraction effect in the majority of patients. This validates the primary hypothesis of our protocol and establishes that the RF energy works on the connective tissue of the subcutaneous adipose tissue. This effect should be the same on any other body part.
opinion regarding the optimal therapeutic algorithm, the mechanism of action, the final improvement clinical reports, or the long-term follow-up of these thermally modified tissues. However, there appears to be agreement concerning the basic science of thermal modification of connective tissue, as well as several clinical implications associated with its use.

When the collagen is heated, the bonds that are sensitive to heat begin to break. In a transition process, the protein transforms from a highly organized crystalline structure to a disorganized gel (denaturalization). Collagen contraction occurs through an unfolding of the triple helix when crossed intermolecular unions, which are sensitive to heat, are destroyed and the tension of residual crossed intermolecular unions stabilizes to the heat. Collagen denaturalization is usually present at 65°C. The behavior of the heat-induced connective tissue and the amount of tissue contraction depends on various factors, including the highest reached temperature (peak temperature), the RF exposure time, and the mechanical stress applied to the tissue during the heating process. Thermal properties of tissue can also vary depending on species, age, pH, the electrolyte concentration in the environment, the concentration and orientation of collagen fibers, and the tissue’s hydration levels.

The selective electrothermolysis produced by RF is highly effective in creating a thermal effect on biologic tissues. Unlike optic energy that depends on chromophore concentration of the skin in order to achieve a selective thermal destruction of target tissue, RF depends on the electrical properties of tissues. The RF technology may help to increase the disruption of adipose tissue, which at the same time helps to move and eliminate the fat deposits in a noninvasive way without having fat necrosis.

The Accent equipment consists of a base system that generates RF technology (40.68 MHz) delivered through one of 2 handpiece applicators to induce controlled volumetric tissue heating (CVTH). The individual applicators provide a functional delivery of energy to different depths. The first handpiece delivers bipolar energy and has a penetration between 2 and 6 mm into the tissue to stimulate dermal structural changes. The second handpiece delivers unipolar energy with a penetration of 20 mm that is designed to reach the subcutaneous adipose tissue.

In this therapeutic modality of high-frequency RF, the energy waves work on a molecular level oscillating at high speed and causing a displacement of charged particles. This results in the rotation of water molecules which will dissipate energy depending on the electrical conductivity of tissue. Both modes (bipolar and unipolar) deliver energy to the tissue through an electrode tip that is cooled to prevent epidermal heating and provide additional comfort to the patient. When heating deep tissues therapeutically, local blood circulation improves, helping the zones affected by edema to promote the drainage and replenishment of the retained fluids and the catabolic products.

RF systems have demonstrated good results correcting irregularities of the cutaneous surface with an efficacy comparable to lasers. RF has the advantage of faster recovery time and not being influenced by competing chromophores in the cutaneous surface. Therefore, any skin type can be treated. Although this study did not focus on other areas of the body, there are reports of studies of the RF device applied to the face where there was an improvement of skin tightening with visible results from the first week following treatment, but more evident 3 months post-treatment without any complications. Continuing improvement following the discontinuation of treatment has been seen in this investigation as well. The prior reports have been validated largely through photographic evidence. To date, it is the authors’ experience that real-time ultrasound imaging has never been used to document or observe the effect of volumetric RF on the subcutaneous adipose tissue.

Subcutaneous Adipose Tissue
Anatomic and physiologic studies on the adipose tissue have been focused on in vivo studies of individual adipocytes or in vivo studies with functional and minimally invasive methods.

Traditionally, the subcutaneous adipose tissue has been considered insulation and a source of stored energy. More recently, there has been greater interest in the distribution and composition of the adipose tissue in relation to health and morbidity. The actual concepts of the adipose tissue’s anatomy are derived from the histological studies of Nürnberg and Müller, who analyzed samples of healthy men and women’s adipose tissue and of women with cellulite. They reported indentations into the deep adipose tissue through the dermis on women, but not in men. They also described modifications on the fibrous septae architecture oriented perpendicular to the cutaneous surface on women and in a crisscross pattern on men.

Histological Characteristics of Subcutaneous Adipose Tissue
The histology of subcutaneous adipose tissue has been thoroughly investigated by Piérard et al who made studies on corpses of the macroscopic and microscopic characteristics of the skin, thighs, and buttocks of men and women without alterations and on women with cellulite. The macroscopic examination of the specimens of full thickness proved the complexity of the 3-dimensional net formed by the fibrous bands, which are born from the hypodermis. Piérard felt that there were no continuous layers of connective tissue that may be called septae between the lobules of adipose tissue in women with cellulite, even though the microscopic examination of thigh skin in men shows a dermal-hypodermic leveled interface without any clinical signs of cellulite. In contrast, the dermal-hypodermic interface of women’s thigh skin (even without cellulite) demonstrates that the adipose lobules have a granular aspect, which protrudes into the dermis. The lobules rise as valleys and hills under the dermal surface. In some cases, the sweat glands are trapped in these fat lobules. There is no correlation between the extent of this finding and the clinical type and severity of cellulite. A more undulated dermis-hypo-
dermic interface on women, which corresponds to the fibrous bands observed in the macroscopic studies on corpses, has been confirmed using high resolution ultrasound images.

Recently, the architecture of the fibrous septae net has been visualized through a 3-dimensinal MRI as well as with a high-resolution ultrasound (Figure 1). The Camper’s fascia can clearly be observed as a thin flat structure more or less parallel to the cutaneous surface. Other septae were detected as thin structures oriented like pillars in 3 directions: perpendicular, parallel, and with a 45° angle. In women with cellulite, there are a higher percentage of perpendicular fibers in comparison with women (and men) that do not have cellulite. As for the fibers in other directions, women with cellulite have a lower percentage of parallel septae to the skin and a higher percentage of angled septae. Furthermore, an MRI study on adipose tissue comparing young and mature women found a higher content of water within the dermis in the older group. A larger amount of free water between the dermis has been related to collagen architecture degradation during the aging process, leaving less interaction sites between water and macromolecules (Figures 1).

Skin aging is a process that can be classified into 2 groups: intrinsic aging and photaging. These are considered different processes with the first caused by the passage of time and the second due to continuous exposure to the ultraviolet rays from the sun. In both types of aging, the most dramatic histological changes are found in the dermis. Collagen alterations, the main skin component, have been identified as the cause of the changes observed.

The dermis contains mainly collagen type I (85% to 90%) and less collagen type III (10% to 15%). The dermal fibroblasts synthesize the individual chains of polypeptide procollagen I and II, precursors of collagen type II and type III that are formerly polymerized in the carboxylic rings and amino terminals to form the triple helixes. Skin that is not normally exposed to the sun’s ultraviolet radiation, such as the thighs and buttocks, mainly goes through the intrinsic aging process. In a study about collagen metabolism in the aging process, it was observed that in the areas not exposed to sun, the synthesis of collagen diminishes as the aging process goes on, maintaining a negative balance between synthesis and collagen degradation. Since the buttocks and thighs undergo a lesser degree of photaging, they are ideal anatomical areas to observe the effect of RF energy on the chronologically aged collagen in the adipose tissue.

Cellulite
Cellulite is a type of lipodystrophy considered by many to be an aesthetic disorder in which the alteration is a morphological constitutional disposition with no significant histological or biological alterations of the adipose tissue. It affects females almost exclusively, and appears around puberty. Approximately 90% of the female population have some degree of cellulite. It is common to confuse cellulite appearance with obesity, even though it is a different condition. Obesity is a generalized condition in which the adipocytes increase in number and size. Cellulite is localized to specific sites with characteristic structural changes (lipodystrophy).

Cellulite is mainly located on the lateral aspects of the thighs and buttocks and is highly related to hormonal changes in females. Cellulite differs from the fat on the abdominal wall, which is more dependent on metabolism and diet, and is more easily removed. The skin with cellulite is rough to the touch. When it is pinched, it has the appearance of orange skin, and is often associated with a painful sensation.

Cellulite Pathogenesis
In the gynoid zones (thighs, hips, and buttocks), women have adipocytes 5 times greater than in other body zones. The cutaneous microcirculation has certain special characteristics that deposit more fat and retain more interstitial fluids. The fat is kept in the adipocytes that are found between the skin and muscles and divided by fibrous tissue bands. These fibrous bands give the adipose tissue a wall-like aspect between the skin and muscles which slow down the lymphatic drainage.

Ultrasound in the Study Cellulite
It is complicated to study the RF thermal effect on the subcutaneous tissue in large areas like the thighs and buttocks. A biopsy may cause trauma to the tissue, which would modify the next sample by leaving scar tissue that would alter the histological morphology of the study zone. It is technically difficult to take the whole thickness of healthy adipose tissue without causing a deformity during extraction or processing. It was determined that in vivo observation in real time with noninvasive methods, like the ultrasound, would allow us to register changes on large anatomical zones, quantify them, and keep the records of what could happen when heating the tissue with RF. The Real Time Scanning Compound Image (RTSCI) ultrasound has a great variety of
medical applications including blood vessels, musculoskeletal system, gynecological and abdominal exams, and so forth. However, its use for the study of skin and subcutaneous tissue is not well known.9

We will assume that applying RF energy, which creates heat through water molecule rotation and tissue impedance, will move the trapped interstitial fluids. We believe that this must trigger a contraction of the collagen fibers and improve the skin’s contour and texture.

**Hypothesis**

High-energy RF application in a unipolar mode heats the subcutaneous adipose tissue, which will cause contraction of collagen fibers, simultaneously improving the alterations of the skin’s external architecture. Given that the subcutaneous tissue and adipose tissue are difficult to evaluate through histological methods, we wish to observe through real-time ultrasound the changes produced by the volumetric RF heating on both tissues.

H1: Radiofrequency, when emitting energy at a high energy, contributes to the connective tissue contraction; therefore, with a more compact subcutaneous adipose tissue, skin tightening will be observed.

H2: Radiofrequency, when raising temperature locally, helps to eliminate the excess liquid in the subcutaneous adipose tissue, which will contribute to a more compressed subcutaneous adipose tissue.

H0: No changes on the architecture of subcutaneous tissue were observed after the RF treatments.

HA: The RTSCI is useful to evaluate the changes caused by the RF in the subcutaneous tissue.

**Materials and Methods**

The study included 26 healthy, voluntary women between 18 and 50 years old. All volunteers signed an informed consent and a photograph authorization. The high-energy RF source used was the Accent RF system (Alma Lasers Inc, Ceasaria, Israel; Fort Lauderdale, FL) in the unipolar mode, which emits RF energy from the tip, allowing the energy to penetrate up to 2 cm in the tissue. The applied energy can be 100 to 200 watts, designed to produce heat in the dermis and the hypodermis. A real-time scanning image ultrasound (Philips Medical Systems, The Netherlands) with a multi-frequency linear transducer for soft tissue (2-5 MHz), was used to evaluate the thickness of the subcutaneous tissue on the buttocks and thighs.

Inclusion Criteria

- Adult women between 18 and 50 years old
- Without liposuction or previous surgery on thighs and buttocks
- With normal weight, or not exceeding more than 20% of the anthropometric scales for age and size

Exclusion Criteria

- Pregnancy
- Pacemakers or any other electronic implant
- Skin cancer
- Viral or bacterial infections
- Epilepsy
- Autoimmune disease
- Isotretinoin treatment in the last 12 months
- Scleroderma
- Extensive radiotherapy treatments

**Treatment Methodology**

1) The RF treatment was delivered in 2 sessions 15 days apart.

2) The evaluations with ultrasound were made before the first treatment and 15 days after the second treatment. The ultrasound was performed on the right lateral aspect of the thigh and buttock with the patient standing. On the buttock the ultrasound was done on the area of greatest deformity. On the thigh, it was taken on the lateral aspect at the level of the great femoral trochanter (frequently the zone of most fat accumulation known as the “saddlebags”).

The following measurements were taken during the ultrasound:

a. The distance between the deep dermis and the first line of fibrous tissue (Camper’s fascia), on the larger distance and on the shorter one.

b. The distance from the deep dermis to the muscle.

c. A longitudinal panoramic take: on the buttocks from the medial point to the gluteal fold on the thigh; and on the thigh, from the great trochanter to the lower third. The first evaluation was used as a treatment control. The quantity, thickness, and quality of the observed fibrous bands were registered and described as continued straight lines, discontinued straight, continuous undulated, and discontinuous undulated. The measurements were always taken on the same anatomical location.

3) Before starting the treatment, the patients filled out the following registers: clinical history, informed consent, photograph authorization.

4) Before each session, the following data were recorded: weight, size, circumference of waist, abdomen, hips, and thighs as well as photographs.

5) Photographs were taken in an ex profeso photographic stand of the posterior, right lateral and left lateral views.

6) The zones to be treated were marked with the patient lying down. The marks were grids of 7 x 7 cm made with a skin marker and numbered on top of the whole area.
affected with cellulite, excluding the bony prominences and the thighs’ medial aspect.

7) The initial parameters were 150 W x 30 sec = 4,500 joules = 91 J/cm² with 3 consecutive passes on each area during each session. Parameters were modified to a higher or lower energy depending on the patient’s tolerance to heat or until the temperature rose to between 39°C and 41°C on each treated zone. This was a variable on each patient depending on the amount of fat, thickness of the skin, and the anatomical site.

8) Mineral oil was used to slide the hand piece over the treating area and to avoid friction on the skin.

9) During the treatment, the electrode’s tip was kept perpendicular and in full contact with the skin. The electrode was also maintained in constant movement for up to 30 seconds or until the desired temperature was achieved (±40°C), and before the patient complained of any pain.

10) Patients filled out a treatment survey form.

11) A specially designed temperature register form was filled out with the following information:
   a) Chosen parameters for the treatment and record of changes.
   b) Anatomical schemes of the treated zones were marked with numbers and correlated with each of the anatomical treated sites.
   c) Applied energy and duration of each application.
   d) Basal temperature and peak temperature of each square were measured with an infrared thermometer.
   e) Any adverse effects.

12) The duration of each treatment was of approximately 1.5 hours.

Results

Independent Variable
A register was made of the peak temperatures reached during the RF application. The patients had different tolerances to heat and the peak temperatures ranged between 36°C and 40°C.

Quantitative Dependent Variables
Ultrasounds were studied and measurements of the initial and final ultrasound were recorded and divided into the thighs and buttocks:

1) Thighs: A measurement of the dermal thickness from the dermal-epidermal union up to the limit of the Camper’s fascia in 2 different points, the most superficial and the deepest. For purposes of this study, we took the average of the 2 distances. The measurements of the thickness of the dermis to the Camper’s fascia proved that there was a shortening of this space in 18 patients (72%), with an average reduction of 2.64 mm ±3.00. This equals a percentage of the distance contraction of 27%, with a maximum reduction of 74% and a minimum of 1.4% (Table 1). The measurements of the thickness of the dermis to the muscle demonstrated that there was a shortening of the distance between them in 15 patients (68%), with an average reduction of 5.40 mm ±4.1. This means a percentage of the distance contraction of 16%, with a maximum reduction of 57% and a minimum of 0% (Table 2). The percentage of shrinking of the thickness is larger on the distance from the dermis to the Camper’s fascia. (Figure 2)

2) Buttocks: The measurements of the thickness of the dermis to the Camper’s fascia demonstrated a reduction between them in 16 patients (64%), with an average reduction of 1.8 mm ±1.54. This is a mean reduction of 27%, with a maximum reduction of 65% and a minimum of 5.4% (Table 3). The measurements of the thickness of the dermis to the muscle indicated a reduction between them in 17 patients (68%), with an average reduction of 4.8 mm ±4.7. This is a mean reduction of 15%, with a maximum reduction of 41% and a minimum of 1% (Table 4).

Figure 2a. Pretreatment Ultrasound of Subcutaneous Tissue.

Figure 2b. Ultrasound of Subcutaneous Tissue 15 Days after Second Treatment.
Table 1. Thigh Measurement of Dermis to Camper’s Fascia.

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Table 2. Thigh Measurement of Dermis to Muscle.

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Table 3. Buttocks Measurement of Dermis to Camper’s Fascia.

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Table 4. Buttocks Dermis to Muscle.

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Difference = difference between the first and last session measurements. Increase = those patients who increased measurements between the first and the last session. Reduction = those patients who reduced measurements between the first and the last session; Avg = average; Count = the number of patients of the sample the total and the number of the different categories of the sample. Min. = minimum. Max = maximum; 1stdv. = 1 standard deviation.
Radiofrequency on Cellulite and the Subcutaneous Tissue of the Buttocks and Thighs

To evaluate if there was a correlation between the applied temperatures and the amount of tissue contraction obtained from the ultrasounds, a statistical regression was applied and revealed that the results on the thigh were better than on the buttocks. (Table 5).

**Qualitative Dependent Variables**

Qualitative dependent variables were defined by the description of echogenesis of the adipose tissue. Regarding the morphological improvement achieved with the Accent RF treatment, we found that the improvement of the fibrous bands went from curved to straight and from discontinuous to continuous, and therefore established a grading of 5 as the highest digit for the most disorganized fibers and 1 for the most organized. We observed that 50% of the patients obtained morphological improvement. On the thigh, the improvement averaged 1.7 digits (scale 5 to 1) and on the buttocks it averaged 1.3 digits.

When analyzing the changes in the Camper’s fascia between the first session and 45 days later, we repeatedly observed on the ultrasound a noticeable organization of the fibrous lines, as well as an increase of the fibrous tissue in 53% of the cases, and an increase of the thickness of the fibers in 57% of the cases (Figure 5).

<table>
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<tr>
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<td>Standard Error</td>
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<td>Observations</td>
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**Figure 3a.** Pretreatment Ultrasound of Subcutaneous Tissue.

**Figure 3b.** Ultrasound of Subcutaneous Tissue 15 Days after Second Treatment.

**Figure 4a.** Pretreatment Ultrasound of Subcutaneous Tissue.

**Figure 4b.** Ultrasound of Subcutaneous Tissue 15 days after Second Treatment.
Photographs were not considered in the protocol to observe the effect of the RF; nevertheless they are essential to evaluate the efficacy of the treatment by itself, without it being the central objective of this protocol (Figures 6a-h).

Adverse Effects
During the treatment, small blisters appeared in some areas in 2 patients, and ecchymosis on the thighs of 3 patients the day following treatment. All of the above adverse effects were resolved and without complication.

Patient Degree of Satisfaction
Patient satisfaction was evaluated by their perception of the benefits from the treatment on the body contour, the way their clothes fit, and skin texture. Most of them were satisfied with the results. The most satisfied group were the women who had the most accentuated defects.
Discussion
We believe that the RF treatment on the subcutaneous adipose tissue of the thighs and buttocks produces volumetric contraction on the body contour, which validates the primary hypothesis of the protocol that the RF energy acts on the connective tissue of the subcutaneous adipose tissue. However, we were not able to demonstrate, with the available data and size of the study group, a direct correlation between temperature and connective tissue contraction.

We believe that the ultrasound is an adequate diagnostic method to evaluate the connective tissue's characteristics and observe the effects of volumetric heating with RF for the treatment of alterations in deep tissues (subcutaneous tissue). The RF effect over the connective tissue was evident in the ultrasounds where a visible compression of the entire thickness of the dermis to the muscle could be appreciated but with better results on the thigh. The observed changes reflect an increased echodensity of the structures that correspond to the connective tissue, shown as an increase in the amount of fibers and a thickening of the existent fibers. This allows us to assume that the RF, as reported in the literature, impacts the collagen. This study also allows us to endorse the usefulness of the RF unipolar system on the treatment of defects on the body contour, like cellulite and skin laxity. We propose that this effect can be repeated on any other body area.

The RF is a safe treatment with minimal complications and no downtime for the patient. This report is written 6 months after having finished the treatments. The effects on the body contour are still present in the majority of patients (some were lost to follow-up). This study was not designed for the treatment of cellulite because it would involve too many factors. Nonetheless, high-energy RF is a useful modality for treatment of cellulite because it would involve too many factors. Hopefully, this small promising study should encourage further investigation of this technology. We will perform a follow-up of the patients to evaluate them for the long term.

References