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Effect of capacitive radiofrequency on adipose tissue of the abdominal region

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Abstract

Objectives: To evaluate histological changes in the adipose tissue after capacitive radiofrequency (RF) stimulation (thermotherapy).

Methods: This is a case series study (n=5). The volunteers received in the right infraumbilical abdominal region a single application of capacitive RF (BTL-6000 TR-Therapy Pro®), with a frequency of 480 to 520KHz, power of 150 W for 10 minutes (2 minutes per head area, 50mm in diameter). The epidermal temperature was above 40°C, and, the left side was used as control. After 30 days, an average, tissue sample was collected for histological analysis and stained with hematoxylin and eosin. Statistical analysis was performed using the SPSS program (version 20), with a significance level of 95%, and the adipocyte count was performed using Image J®. This study was registered with the XXXX Ethics Committee n. 3,461,688, Jul/12/19 and, the number of clinical trial registration was NCT04182542.

Results: The number of adipocytes on the treated side was 41.2 ± 2.0 per field and, in the control side was 36.6 ± 2.0 per field, with a significant difference (p=0.009), because of the size of adipocytes being smaller on the side treated.

Conclusion: Radiofrequency can lead to morphological changes in adipocytes in the abdominal region with an important reduction in their size.

Keywords: Radio Waves; Radiofrequency Therapy; Lipolysis; Adipose Tissue.

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1. Introduction

Non-invasive fat reduction is a rapidly expanding field of cosmetic rejuvenation, as many patients are unwilling to suffer the downtime and scarring associated with traditional invasive cosmetic procedures. The radiofrequency (RF) resource has proven to be safe and effective for the non-invasive reduction of fat, as well as skin enhancement and improvement of skin flaccidity [1].

The term tecar therapy is one of the types of RF that comes from CPT (Capacitive Power Transfer), having as its main effect the production of heat as it dissipates through the body. Some benefits expected after treatment with the use of this technology are: improved skin appearance due to the increase in the quantity of nutrients and oxygen, in addition to the reduction of the subcutaneous tissue in the abdomen[2].

There are controversies regarding the fat mobilization mechanism. Some authors, when observing human skin cultures at the end of a series of RF treatments, found an important effect on subcutaneous adipocytes. The authors analysis resulted in altered morphology and increased expression of apoptosis markers, suggesting that irreversible electroporation-induced apoptosis is the mechanism of action, however, the authors associated RF with shock waves, and this may have caused cellular rupture, which would not happen with the use of RF alone. Adipocyte apoptosis results in the release of triglycerides from disintegrated cell membranes, but in a delayed and gradual way, allowing for a slow and safe removal through the interstitial space and fluid transport systems, lymphatics and other metabolic functions, without risk of necrosis or inflammatory changes[1].

Other authors report that the increase in skin and subcutaneous temperature caused by RF has some physiological effects, such as increased cellular metabolism, increased vasodilation and the amount of blood, and the activation of branches of the autonomic nervous system releasing adrenaline and noradrenaline[3-6]. These effects culminate in the better efficiency of lipolysis, which is a biochemical process where we have the hydrolysis of lipids.

RF for lipolysis purpose has been clinically used, however few studies have demonstrated its effects on adipose tissue, therefore the aim of this study was to evaluate the histological changes in adipose tissue when stimulated by capacitive radiofrequency therapy (BTL-6000 TR-Therapy Pro®) from the abdominal region.

2. Methodology

2.1. Study Design

Case series study, quasi-experimental, interventional, cross-sectional, with convenience sample and blinding of the evaluators.

We selected five women in good general health who were waiting for abdominoplasty at the Hospital das Clínicas do Triângulo Mineiro from October 2018 to February 2019. It refers to a convenience sample, in which only these volunteers were invited to participate in the research.

The following non-inclusion criteria were used: women with sensory and cognitive deficit, under the age of 18, having metallic implant at the site of application, with pacemakers, with signs of infection, circulatory disorders, neoplasia or any other condition that contraindicates the use of RF.

After being informed about the treatment, they signed the Free and Informed Consent Form. Then, an initial evaluation was performed where personal data, anthropometric measurements (weight, height and body mass index – BMI) were collected, as well as the cutaneous phototype following the Fitzpatrick scale.

The project was approved by CEP-UFTM (Ethics Committee) under no. 3,461,688 on 07/12/2019 and registered on the clinical trial platform under n. NCT04182542.

2.2. **Procedures**

The volunteers received radiofrequency (BTL-6000 TR-Therapy Pro® device) in a single session, with a frequency of 480 to 520KHz, power of 150 W, capacitive mode and epidermal temperature above 40°C, in the right abdominal region, with 10 minutes of application (2 minutes per applicator area, 50mm in diameter) and the left region was used as a control. Such parameters were selected due to the depth of RF action with lower frequencies, since these frequencies have greater effects on deep tissues, such as in the case of adipose tissue[1].

Approximately 30 days after application, subcutaneous tissue was collected during surgery, and the collected parts – control and treated ones– were sent for histological analysis.

2.3. Sample processing and slide mounting

The fragments were collected in the operating room during the abdominoplasty procedure, the sampling location on both sides as well as their depth were similar to ensure symmetry of the samples, then they were set in Metacarn (60% methanol, chloroform 30%, acetic acid 10%) for 2 hours at room temperature (TA). The slides were made by the general pathology department of UFTM. The samples were dehydrated in ethanol, diaphanized in xylol and included in paraffin. Histological sections of 5 µm thick were obtained in microtome (Jung) and positioned on slides pre-covered with polylysine. Histological sections were cordoned with hematoxylin and eosin (HE). The tissue general morphology was analyzed including the amount and size of adipocytes.

2.4. Microscopic and Morphometric Analysis

The slides stained by HE were visualized with a 20x lens. The images were captured by a common light microscope and analyzed by the Axion Vision Automatic Image Analyzer System.

Thus, the field to be quantified was captured, photographed by a camera connected to the microscope and to a computer for image scanning. The images were saved in TIFF format. A single slide from the control and treated side of each volunteer was used for analysis, and 15 fields from each of these slides were photographed, from which the average value of adipocytes per field was obtained.

After the fields were saved, the Image J® program was used to improve them, using the lighting reference so that they could all have the same quality.

The analysis of the adipocytes amount was performed by the Image J® program and recorded in a Microsoft Excel spreadsheet.

2.5. Statistical analysis

The variable evaluated was the number of adipocytes. First, the normality test was performed with Shapiro Wilk, through the data parametric behavior, the paired t-test was performed. The differences observed were considered significant when the rejection probability of null hypothesis was less than 0.05. For this analysis, the SPSS program (version 20) was used.

3. Result

The final sample consisted of five female volunteers with a mean age of 39.4 ± 10.3 years, mean body mass index (BMI) of 25.9 ± 2.9 Kg/m2, from these, one volunteer with phototype III, two with phototype IV and two with phototype V.

3.1. Morphological and morphometric analysis of adipocytes

Adipocytes were evaluated by HE staining. The comparations were made between the control side and the side treated with RF. Morphologically, in the treated side sample, a decrease in the adipocytes size can be observed compared to the control group (Fig 1). Due to the smaller size of adipocytes on the RF-treated side, morphometry showed a higher number of adipocytes on this side (41.2 ± 1.9 adipocytes per field), in relation to the control side (36.6 ± 1.7 adipocytes per field), as observed in Figure 2, with a statistically significant difference (p=0.009).

The average number of adipocytes/field for each volunteer did not show a relevant variation, as it can be seen in table 1.

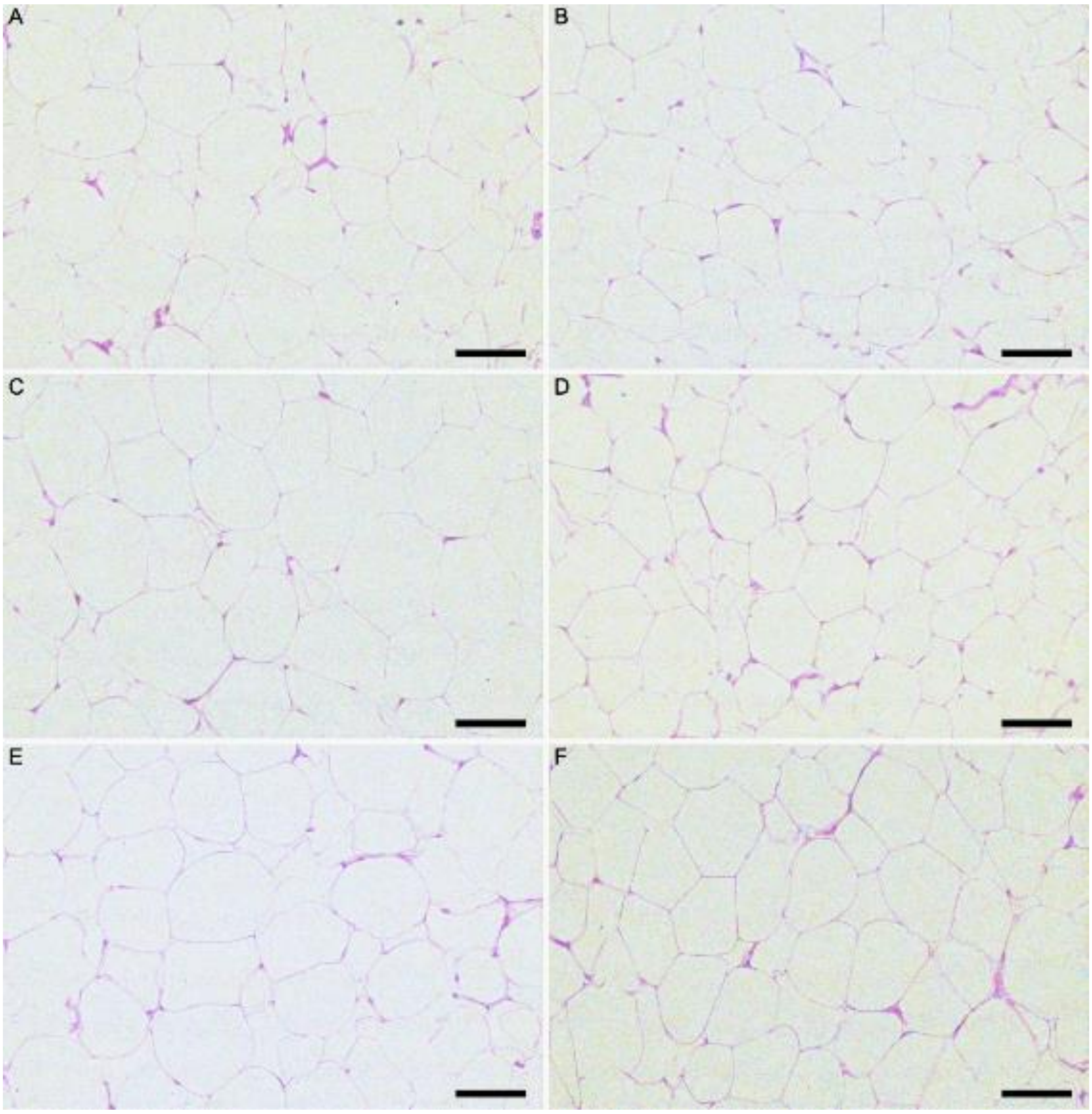

Figure 1 Morphological analysis of adipose tissue. Adipocytes with decreased volume compared to the control side (A, C and E) are observed on the treated side with capacitive RF (B, D and F). No inflammatory process and necrosis (100 µm bar) are observed.

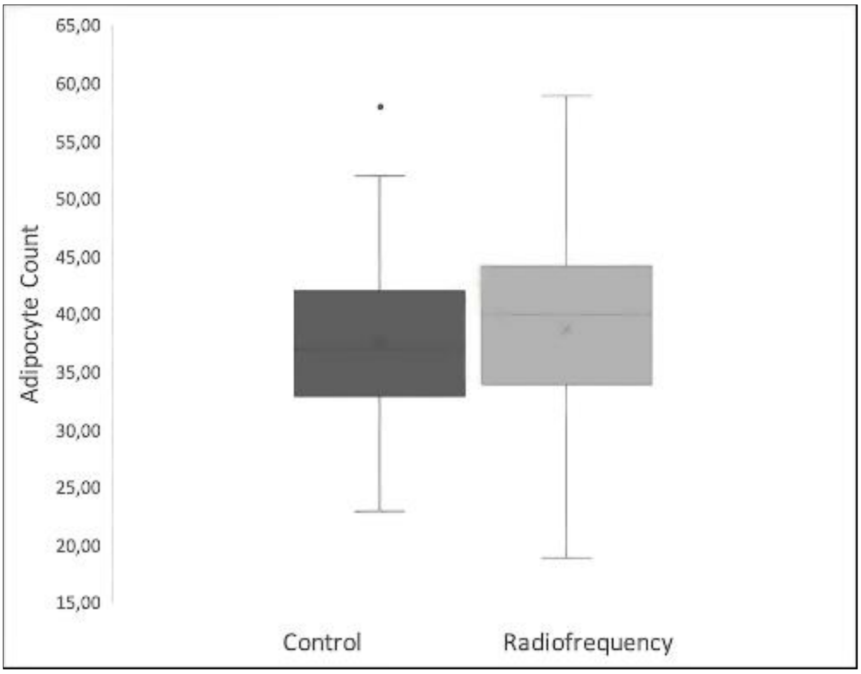

Figure 2 Morphometry of adipocytes. A higher number of adipocytes was observed on the treated side (radiofrequency) compared to the control, a statistically significant difference (p=0.009).

Table 1 Mean number of adipocytes per volunteer on the treated and untreated sides, overall mean and SD.

Volunteer	**Treated side mean**	**Untreated side mean**
1	42.8	38.8
2	38.7	36.6
3	41.4	34
4	40	36.7
5	43.1	37.7
General mean	41.2	36.6
Standard Deviation (SD)	1.9	1.7

In the analyzed fields in both groups, no alterations were found in the adjacencies of adipose tissue, such as inflammatory infiltrate and necrotic tissue.

4. Discussion

The results of this study showed a morphological and morphometric change of adipocytes in the abdominal region after a single session of capacitive RF (tecar therapy), showing a decrease in the size of adipocytes on the treated side compared to the control side, which was confirmed by morphometry, in which the treated side showed a greater number of adipocytes per field, since, with reduced size, a greater number of adipocytes per field is counted.

Costa et al (2009)[7] also used tecar therapy to treat localized adiposity and they observed a reduction in measurements through anthropometry (perimetry, plicometry, and ultrasonography), because the objective was the clinical evaluation of the thermotherapy; hence, they used 12 sessions of capacitive RF. Unlike our study, whose objective was to evaluate the histological response of adipocytes to the stimulus of capacitive RF, for this reason we performed only one session of RF, which was able to demonstrate histologically a reduction in the size of adipocytes.

When comparing the findings of Costa et al (2009)[7] regarding the study of localized adiposity reduction with the findings of our study of adipocyte size reduction histologically, we can indicate a beneficial effect of capacitive RF on adipose tissue, with promising results of this resource both clinically and histologically.

The mechanism of adipose tissue reduction must be better understood, especially to evaluate the pathway used by the studied resource. For Pinto et al (2016)[8] the terms used to define the reduction of adipose tissue are: lipolysis and adipocytolysis. Lipolysis is a natural, molecular, and reversible metabolic chemical process. When specific receptors are stimulated, a cascade pathway is activated, in which various intermediate enzymes are phosphorylated and undergo changes until they eventually stimulate any lipase. Lipases acting on triglycerides (TAGs) release fatty acids and glycerol. Adipocytolysis is a predominantly physical, cellular, and irreversible process, where the destruction of adipocytes occurs; in this case there is no destruction of their lipids, but rather of the cell. The authors also classify adipocytolysis in two groups: clastic, when there is rupture of the cell membrane, as occurs in hydrolipoclasy, carboxitherapy, and ultracavitation, among others; and phagic, where the adipocyte is not destroyed directly, but through physical principles that cause structural changes in several elements of the adipocyte, inducing the apoptotic response. In this case, we can mention what occurs in cryolipolysis. The difference between them is that in clastic adipocytolysis, there is an inflammatory process, and in phagic, that does not happen.

For clastic procedures, it is important for the blood study lipids to be evaluated individually, because there is the possibility of an increase of these lipids in the bloodstream and liver, as suggested by the study of Meyer e cols (2020)[9], in which it was observed hepatic steatosis in rabbits 48h after treatment with ultracavitation (UCV), with normalization after 96h. It is suggested that resources using classical processes be preceded by the evaluation of blood lipids, and further studies are needed to evaluate the involvement of blood vessels in these procedures.

Although it is not clear how adipose tissue reduction occurs after the use of capacitive RF, both hypotheses (lipolysis and apoptosis) are suggested. In this study, no inflammatory infiltrate or disruption of the adipocyte cell membrane was observed in the histological analysis, so we believe that the pathway for adipocyte volume reduction was either lipolysis or hydrolysis of TAGs.

There are few articles in the literature that have histologically analyzed adipose tissue after the use of capacitive RF, thus making comparison with this study difficult. Most of the studies have evaluated the results clinically, though anthropometry, and in all these studies the authors observed a reduction in adipose tissue[10-12].

It is known that the results of RF treatment for lipolysis depend on the parameters used in each equipment, as well as the number of sessions and each person's individual characteristics.

Franco et al (2010)[12] confirmed that at high frequencies of electromagnetic waves, energy is rapidly transferred across the surface, attenuating the wave as it is dispersed. At low frequencies, the energy penetration is deeper. In the present study, the capacitive RF device (BTL-6000 TR-Therapy Pro®) with a frequency of 520KHz was used, therefore, of greater penetration, being more suitable for fat mobilization.

A limitation found was the lack of information regarding the frequency of the equipment used in the studies, which makes it difficult to compare the results. In the studies that mentioned frequency, there is a trend towards better results with lower frequencies[7,13,14], similar to the results found in our study. Some studies used RF associated with suction[1,11], while others used selective field RF and high frequency contactless (27.12 MHz)[10,15,16,17,18] associated or not with diet and physical activity[18], with time of 30 min.[16,18] and 45 min[10,15,17].

The type of electrode also interferes with the results. In low frequency RF, we have capacitive and resistive electrodes. The capacitive electrode has a polyamide coating that acts as a dielectric medium, isolating its metal body from the skin surface, forming a capacitor with the tissues, generating faster heating but of shorter duration. The resistive electrode is not coated and, therefore, transfers the RF energy directly through the body and into the neutral plate, its heating is slower, but of longer duration[14].

In the study of Kumaran and Watson (2015)[14], low frequency RF (448Kz) was used with capacitive electrode (tecar therapy) showing faster temperature gains than resistive electrodes, however RF with resistive electrode had better temperature retention 45 minutes after intervention (60.3% resistive electrode and 15.5% capacitive electrode), the faster heating and heat degradation associated with capacitive electrode may be indicative of its slightly shallow penetration. In the resistive electrode, the greater heat retention and the fact that there is no sharp drop in temperature post-intervention suggest that there is greater energy penetration. In this study, we used a capacitive electrode, and although it has less ability to retain heat for longer, it seems that the rapid temperature rise was sufficient to stimulate metabolism and perform hydrolysis of TAGs.

The application mode, monopolar, bipolar or multipolar also determines the depth of RF penetration. In monopolar RF we have active and passive electrodes. The active electrode causes a localized thermal effect on the tissue, generating tissue stimulation, while the passive electrode consists of a conductive plate to close the current circuit, returning energy to the patient[19,20]. In bipolar/multipolar, the depth of propagation of the electric current is limited by the distance between the two (or more) electrodes on the same head, set at a certain distance. Both are in contact with the treated skin, because the greater the distance between the bipolar terminals, the greater the depth of energy penetration[3,21]. In this study we used the monopolar mode, consequently with a deeper range, which may have contributed to obtaining the results found in the adipose tissue.

The number of sessions also interferes in the results obtained, and the greater the number of applications, the better the results. Most studies performed treatment protocols with sessions between 4 and 10[1,11,17,18]. From these, only two performed histological analysis[1,11], however, the equipment used associated RF with suction, whose results cannot be compared to our study. No histological studies were found with low frequency RF and capacitive electrode (tecar therapy) in humans, only one study in an animal[20], where they also observed a decrease in adipocytes, thus making it difficult to compare our studies with others. Since the aim of this study was to evaluate histological changes in adipose tissue after stimulation by capacitive RF (tecar therapy), a single session was proposed, whose results showed a decrease in adipocyte size. It is likely that a greater number of sessions will be able to show even higher histological results, justifying the need for further studies that histologically evaluate adipose tissue.

Another factor that may influence the reduction of adipose tissue due to RF would be the increase in temperature. There are two hypotheses to explain this effect, one would be the increase in metabolic activity of adipocytes, leading to a non-destructive reduction in the cell volume of these adipocytes by inducing hydrolysis of TAGs[7,22] and the other would be the apoptosis of adipocytes[15,16].

The studies by Moradi and Palm (2015)[15] and Weiss et al (2013)[16] demonstrated that adipocytes heated to 42-45.8°C and maintained at this temperature for at least 15 minutes reach the apoptosis threshold, leading to mild membrane denaturation and lipid release. Thus, increasing and maintaining the temperature of adipocytes above the apoptotic threshold induces apoptosis and reduces the number of adipocytes, resulting in excessive fat volume reduction. It is worth noting that the equipment and parameters used in these studies were different, in the study of Moradi and Palm (2015)[15] used selective RF without contact, with high frequency (27.12MHz), with time between 30[16] and 45 minutes[15] of application, in which this temperature was maintained for the entire period in the entire area, this in itself already differs from the application with contact electrodes, allowing a temperature rise kept for a prolonged period.

In our study, a temperature was kept on the skin above 40ºC, varying between 40 and 41°C, for 10 minutes, 2 minutes per head area. This heating was not enough for apoptosis to be achieved, and this was observed in histology, where no ruptured adipocyte membranes were observed. This finding is in line with what the authors Moradi and Palm (2015)[15] and Weiss et al (2013)[16] state, stating the need to maintain the temperature for more than 15 minutes to reach the apoptotic threshold.

There are a lot of types of RF equipment, as well as the parameters used in different treatments. Studies evaluating the performance of RF in different tissues due to these parameters are of utmost importance so that the target tissues are reached without damaging the adjacent tissues. More recently, the technology of equipment using selective non-contact RF has shown great results in the reduction of adipose tissue[10,15,16,17,18], as well as equipment that combines RF with other technologies in the same equipment[1,11]. We emphasize the importance of knowing the parameters of the RF equipment in order to obtain the best results for the target tissue.

The small sample size of this study could be considered a limiting factor; however, the sample was conditioned to the number of plastic surgeries performed in the teaching hospital during the study period, which was a convenience sample. Studies with a larger sample size and histological evaluation using tecar therapy are necessary for a better scientific basis.

There is little scientific evidence on the effects of RF on adipose tissue and many articles have low methodological quality (Vale, et al)[23], reinforcing the need for more studies with high methodological criteria.

5. Conclusion

Capacitive RF (tecar therapy) with the methodology used in this study caused morphological and morphometric changes in subcutaneous adipose tissue, with decreased adipocyte size, and may be a promising resource in the treatment of localized adiposity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

We declare authorship and absence of conflict of interests of the article entitled Effect of capacitive radiofrequency on adipose tissue in the abdominal region, approval by CEP-UFTM and completion of the free and informed consent form by all volunteers in this study.

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