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STUDY PROTOCOL COVER PAGE:

Official Study Title: Lipolysis of Visceral Reserve Fat Using Tecar Therapy: Anthropometric, Biochemical and NMR Imaging Study.

NCT NUMBER: 06377358

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BRIEF DESCRIPTION OF THE PROJECT

The burden of obesity has increased globally in recent decades and its association with insulin resistance and related cardio-metabolic problems. Radio frequency (RF) is a form of high-frequency electromagnetic energy that works by heating tissues. Its action aims to increase cellular metabolism. Its action aims to increase cellular metabolism. Frequencies around 1 MHz are used, which are more effective in the cell, but with high power, adjustable temperature and treatment area greater than 200 cm2, which mechanically destroys the adipose cells, without damaging the skin, blood, vessels, nerves or connective tissues.

The lymphatic system eliminates lidis, reducing tissue volume. Magnetic resonance imaging (MRI) has similar accuracy to CT scans, but with no exposure to ionizing radiation. The MRI method for quantifying abdominal adiposity is effective. Chronic inflammation that lasts a long time is characterized by the presence of lymphocytes, macrophages, proliferation of blood vessels and connective tissue, it is considered a feature of metabolic syndrome, characterized by secretion of inflammatory adipokines usually from adipose tissue, leptin, interleukin (IL-6), tumor necrosis factor α (TNF-α), monocyte chemoattractant protein 1 (MCP-1) and resistin. RNA markers involved in apoptotic processes revealed that tissue exposed to RF treatment demonstrated a pro-apoptotic reaction. Therefore, we propose to apply through four channels and four electrodes of 200 cm2 a powerful RF energy raising the temperature and thus reducing visceral fat, evaluating the biochemical parameters of inflammation of the adipocyte.

MAIN OBJECTIVE: To understand the effect of high-power resistive capacitive RF on the decrease of body fat and changes in biochemical markers linked to inflammation.

METHODOLOGY: at the inclusion visit, the patient will be given the information sheet (Annex 1) and, if they agree, they will be asked to sign the informed consent. We aim to declare a difference of 2 points or more as significant, taking the SD from published research that gives around 8.0 with a confidence level of 95%, a power of 80% and a correlation coefficient of 0.70 between the values of abdominal circumference before and after treatment. Noted by Duarte et al. in 2015. We have obtained by EPIDATA that 15 pairs are needed.

INCLUSION CRITERIA: Men and women aged 20 to 60 years. With Waist to Hip Index Greater than: 0.84 normal for women, 0.94 normal for men. Body Mass Index defined as: Overweight: Between 25-29.9 or Obesity: 30 or higher. Measurement of Visceral Fat (GV) by Inbody greater than 9 which is what is considered Normal. The GV value will be measured using the MRI method in addition to the MRI.

RADIOFREQUENCY PROCEDURE: Each patient will have 4 active, automatic plates placed on the abdomen located two on the right side of the midline and two on the left side. Energy will be applied for 50 minutes, controlling the temperature. Subsequently, 10 minutes of manual capacitive and resistive electrodes will be applied to the abdomen afterwards. 10 minutes of lymphatic drainage by placing one active plate in the foot and the other in the lumbo-dorsal area.

For the determination of biochemical parameters of inflammation and adipokines, the blood will be drawn in coordination with the Echevarne Accredited Clinical Laboratory in Barcelona. Its processing will be carried out at the Biomedical Research Institute of Malaga (IBIMA) Lipids and Arteriosclerosis Research Unit of University of Málaga. IL-6, TNF-alpha, leptin, human adiponectin, human resistin, human ultrasensitive C-reactive protein will be determined by ELISA and IMMUNOASSAY methods simultaneously. A Kolmogorov-Smirnov test will be applied, if the data behaves normally, parametric tests will be applied. If not, non-parametric tests will be performed.

PROJECT

INTRODUCTION

The burden of obesity has increased globally in recent decades and its association with insulin resistance and related cardio-metabolic problems has negatively affected our ability to reduce population morbidity and mortality.

Traditionally, adipose tissue in the visceral fat deposition has been considered one of the main culprits in the development of insulin resistance. However, there is growing evidence supporting the role of subcutaneous trunk and abdominal adipose tissue in the development of insulin resistance, and more and more evidence supports the role of adipose tissue function in the development of metabolic complications independent of adipose tissue volume or distribution. Radiofrequency is an inexpensive, virtually side-effect-free, well-tolerated method that reduces belly fat.

RADIOFREQUENCY AND FAT CELLS.

Radio frequency (RF) is a form of high-frequency electromagnetic energy that works by heating tissues. Its action in deeper tissues (subcutaneous layer) aims to increase cellular metabolism (van der Lugt et al 2009, Prumpa et al 2015). When applied to tissues, RF generates oscillating magnetic fields that move electrically charged particles, producing heat in the tissues, and the amount of heat produced depends on the resistance (bioimpedance) of the target tissue (Alexiades-Armenakas et al 2008, Kaplan and Gat 2009). Electrical energy is converted into thermal energy. There is evidence of a transient RF effect on autonomic homeostasis with no known negative effects. This autonomic response to RF is reflected in a thermoregulatory vasomotor mechanism, in changes in chemoreceptor activity, and even in fluctuations in the renin-angiotensin system, responses related to the control of energy metabolism. The elevation of tissue temperature appears to be sufficient to activate the sympathetic branch of the Autonomic Nervous System, leading to the release of catecholamines (adrenaline and noradrenaline), which are the trigger to activate lipolysis (Pumprla et al 2015, Belenky et al 2012, Ryden and Arner 2017). Lipolysis is the reversible biochemical process where triglyceride catabolism is stored. This process culminates in the generation of non-esterified fatty acids and glycerol. Fatty acids released into the bloodstream can be used as a substrate to produce energy (Pinto 2016).

The available literature on this topic still lacks information that would allow us to assume whether the effects of RF are reversible or not, and this process may depend on the temperature used and the exposure time. At the same time, tissue heating may also be related to vasodilation and increased perfusion and local oxygenation that promote oxidation and lipid turnover that will culminate in a decrease in adipose cell volume (Mulholland et al 2011). Considering that the processes enhance each other, improving blood perfusion and hormone flow could make lipolysis more efficient.

Ex vivo human skin cultures after completion of the RF treatment series showed a profound effect on subcutaneous adipocytes. Adipocyte cells were observed to have altered morphology and increased expression of the apoptosis marker, APAF-1, suggesting that RIE-induced apoptosis is the mechanism of action. Adipocyte apoptosis results in the release of triglycerides from disintegrated cell membranes, but in a delayed and gradual manner, allowing for slow and safe elimination through the interstitial space, and subsequent lipid transport systems, lymphatic systems, and other metabolic functions. There was no evidence of necrosis or inflammatory changes observed in adipocytes after treatments with this new RF device. While IRE has been shown to be effective in inducing cellular apoptosis in other tissues, to our knowledge, this is the first documentation of IRE in adipose tissue with RF treatments.

It is known that adipose tissue, apart from serving as an energy storage site, has an endocrine function by secreting multiple proteins known as adipokines.

Obesity causes an increase in adipose tissue and an increased infiltration of inflammatory cells into that tissue. The expansion of TA is associated with an altered pattern of fat tissue cytokines with a predominance of pro-inflammatory cytokines (TNF-α, IL-6 among others) and a decrease in anti-inflammatory cytokines, resulting in the development of a chronic low-intensity inflammatory state.

The chronic low-intensity inflammatory state causes a state of insulin resistance at the level of muscle and liver tissue due to lipid accumulation in these tissues and organs, resulting in the pathological condition known as non-alcoholic fatty liver.

RADIOFREQUENCY AND LIPOLYSIS

Adipocytolysis is a term used to describe the phenomena caused by non-surgical techniques (cytolytic methods) for the reduction of localized fat, in which lipids could be broken or solubilized through the partial or total rupture of adipocytes, destroying their plasma membrane (Pereira et al. 2017).

Treatments combining non-thermal forms of focused ultrasound and radiofrequency treatment have been performed for body contouring in the abdominal region and it has been concluded that it is effective and can show positive results for at least one year if patients maintain body weight (Chang et al. 2016).

Frequencies around 1 MHz are used, which are the ones that act most effectively on the cell, but with a high power, adjustable temperature and a treatment area greater than 200 cm2, which allows it to mechanically destroy the adipose cells, with greater effectiveness in the subcutaneous adipose tissue without damaging the skin. blood, vessels, nerves, or connective tissues. Lipids are gradually eliminated through the lymphatic system, reducing tissue volume. Previous studies have shown that single or multiple treatments with these devices are effective, safe, and comfortable for the abdomen, flanks, and thighs. The average girth reduction of three treatment regimens ranges from 2.9 to 3.95 cm (Fritz et al. 2018, Hart 2015).

This procedure has been used quite effectively in aesthetics to reduce subcutaneous fat. A recent study highlights the efficacy of this RF treatment modality in primarily tightening the skin and reducing fat. (Mazzoni et al) Another recent study has pointed to the use of 1 MHz monopolar RF in body contouring, for fat reduction and tightening of the lower face. On the other hand, he has pointed out that this treatment can maintain a fixed therapeutic temperature and is very effective in reducing fat and tightening the lower part of the face (Sugawara et al. 2017).

BIOCHEMICAL MARKERS

Inflammation is an orderly sequence of events designed to maintain homeostasis of organs and tissues. Timely release of mediators and expression of receptors are essential to complete the sequence of events and restore tissues to their original condition [1]. In addition, inflammation is a protective tissue response to injury or tissue destruction that serves to destroy or dilute both the damaging agent and the injured tissues. There are two types of inflammation; The first is an acute inflammation that lasts a short time and is characterized by edema and leukocyte migration, and the second is a chronic inflammation that lasts a long time and is characterized by the presence of lymphocytes and macrophages and the proliferation of blood vessels and connective tissue. This is considered a characteristic feature of metabolic syndrome, characterized by the secretion of inflammatory adipokines usually from adipose tissue, such as leptin, interleukin (IL-6), tumor necrosis factor α (TNF-α), monocyte chemoattractant protein. 1 (MCP-1) and resistin. Obesity, which is a feature of metabolic syndrome, was associated with chronic inflammation in obese subjects. Several inflammatory indicators are linked to obesity and shed light on the associated health complications. Inflammatory indicators include IL-6 and C-reactive protein (CRP) as inflammatory markers and adiponectin as an anti-inflammatory marker (Ellulu et al 2017). In summary, when adipose tissue increases more than expected, a cytokine imbalance appears that increases the release of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, resistin, and leptin, while adiponectin, which is anti-inflammatory, decreases. This imbalance results in chronic, low-grade inflammation. Hence the importance of measuring these parameters to assess the anti-inflammatory effect of the treatment.

Sustained inflammation is considered a major risk factor for developing many diseases, including cardiovascular disease, metabolic syndrome, diabetes, and cancer. As a risk factor, obesity predisposes to a pro-inflammatory state through the increase of inflammatory mediators IL-6 and TNF-α, and reduced levels of adiponectin, which has a totally anti-inflammatory function. According to Badawi et al. 2010, TNF-α is overexpressed in overweight status, while IL-6 is more related to obesity status influencing the liver to synthesize and secrete CRP, which is a feature of systemic inflammation. This state is associated with reduced levels of adiponectin, which is important for improving insulin sensitivity, reducing metabolic abnormalities, and adjusting energy expenditure. In addition, the inflammatory state followed by vascular and endothelial dysfunction is characterized by a decrease in nitric oxide and an increase in reactive oxygen species leading to oxidative stress. Both states of oxidative stress and inflammation initiate atherosclerosis, hypertension, alteration of metabolic markers, and thus major adverse cardiovascular events. Hence the importance of studying these markers before and after treatment.

RF treatment induces an increase in the apoptotic index in adipocytes 1 hour after RF treatment. This is accompanied by a maximum temperature of 45°C in the grease layer. Skin surface temperatures remain substantially lower than fat temperatures. (McDaniel & Lozanova 2015). The application of RF has shown greater increases in TNF-α Pro-Apoptotic Markers. Analysis of RNA markers involved in apoptotic processes revealed that tissue exposed to RF treatment demonstrated a pro-apoptotic reaction. Several studies have documented the role of TNF-α in adipocyte apoptosis (Weiss & Bernardy 2019).

VISCERAL FAT MEASUREMENT BY IMAGE

While computed tomography (CT) is the most commonly used imaging modality for measuring abdominal fat, magnetic resonance imaging (MRI) has similar accuracy (Klopfenstein et al 2012). An advantage of MRI is the absence of exposure to ionizing radiation, a limitation that restricts the use of CT in children and adolescents. In addition, the MRI method for quantifying abdominal adiposity is effective, allowing imaging within 5 minutes. Magnetic resonance imaging can provide reliable and good quality images for visceral and subcutaneous quantification (Eloi et al 2017).

With the scanning parameters employed, fat appears as a loud signal against a muted background of other tissues and noise. The images were segmented and analyzed using a software program that employed knowledge-based image processing to label voxels as fatty and non-fat components. The image processing and/or analysis procedure employed a contour tracking algorithm to isolate individual structures from binary images produced by thresholding. The threshold required to identify voxels associated with grease components was automatically calculated from gray intensity histogram analysis and background noise calculation.
The adipose tissue volumes (cm3) of each compartment were calculated by adding the relevant voxel counts and multiplying by the voxel dimensions in cubic centimeters. The volume of adipose tissue for the entire abdomen was calculated by multiplying the volumes of adipose tissue from each slice by the sum of the thickness of the slice (5 mm) and the distance between slices. This analysis provides a direct measurement of adipose tissue volume. Adipose tissue in grams is calculated from the following formula:

Fat mass (g) = Adipose tissue volume cm3 x 0.66 g/cm3

For all of the above, we propose to apply through four RF channels and four 200 cm2 electrodes a powerful energy raising the temperature and thus reducing visceral fat by evaluating the biochemical parameters of adipocyte inflammation.

OBJECTIVES

MAIN OBJECTIVE.

1. To understand the effect of high-power resistive capacitive radiofrequency on the decrease of body fat and changes in biochemical markers linked to inflammation.

SIDE OBJECTIVES.

2. To assess the role of radiofrequency in reducing abdominal fat.
3. To assess and compare the decrease in abdominal circumference measured by anthropometric tape measure, Body Mass Index, Waist-to-Hip Ratio and skinfold before and after RF treatment.
4. Evaluate changes in Human IL-6, Human TNF-alpha, Human-Leptin, Human-Adiponectin, Human-Resistin, C-reactive protein values before and after RF sessions. Evaluar y comparar el impacto de la calidad de vida antes y después del tratamiento con radiofrecuencia.
5. To assess and compare the impact of quality of life before and after radiofrequency treatment.

METHODOLOGY

At the inclusion visit, the patient will be given the information sheet (Annex 1) and, if they agree, they will be asked to sign the informed consent. Once included in the study, they will be randomly assigned to one of the two study groups in order of consecutive inclusion.

The project will be presented to the Ethics Committee of the Autonomous University of Barcelona UAB.

SAMPLE SIZE CALCULATION

The aim of this study was to assess the difference between a decrease in the diameter of the abdominal circumference in a group of women, before and after radiofrequency treatment. The aim is to be able to declare a difference of 2 points or more as significant, taking the SD from published research that gives around 8.0 with a confidence level of 95%, a power of 80% and a correlation coefficient of 0.70 between the values of abdominal circumference before and after treatment. These are taken from the article published by Duarte et al. in 2015 (Duarte et al, 2015), and the following results are obtained according to EPIDATA: 15 pairs of data will then be taken: Before and after the application of RF.

[1] Sample size. Comparison for paired means.

Data:

Mean differences to be detected: 6.442

Standard Deviation of the differences: 8.221

Level of confidence: 95.0%

Results:

Stat. Power (%)	Number of Pairs
80.0	15

Inclusion criteria:
- Men and women from 20 to 60 years old
- With Waist-Hip Index. Greater than: 0.84 normal for women. 94 normal for men.
- Body Mass Index that is defined as: Overweight: Between 25-29.9. Obesity: 30 or higher.
- Measurement of Visceral Fat per Inbody greater than 9, which is what is considered Normal.
- Signed informed consent (Annex 1).

Exclusion Criteria:
- Contraindication to the use of radiofrequency: pregnancy, metal prostheses, active infection.
- Patients with a history of cancer, chemotherapy and radiotherapy treatments.
- Not having diabetes or metabolic disease.

MEASUREMENTS

Body Mass Index, Waist-t-Hip ratio and visceral fat:

Body Mass Index is calculated as follows: Body Weight (Kg) ÷ (Height in cm)2

Waist-to-Hip Ratio: According to World Health Organization guidelines, the waist should be measured at the midpoint between the last palpable rib and the top of the iliac crest (upper edge of the pelvis). The hip should be measured at the point of maximum circumference, i.e. the point where the buttocks reach maximum width. Both measures should be taken immediately after the air in the lungs is exhaled. It is important to keep your feet together and your arms in a normal position next to your body. It is advisable to take each measure twice and repeat it if different results are obtained (WHO 2008). The caliper takes the thickness of the abdominal wall at 5 cm in an oblique line towards the navel on the right and left side.

The visceral fat value: will be measured using, in addition to the MRI method, an Inbody electrical impedance equipment, in the first and last session.

TREATMENT

Each patient will have 4 active, automatic plates placed on the abdomen, two on the right side of the midline and two on the left side. Energy will be applied for 50 minutes, controlling the temperature. Subsequently, 15 minutes of Capacitive and Resistive manual electrodes will be applied to the abdomen, simultaneously, 20 minutes of Lymphatic Drainage placing one active plate in the foot and the other in the lumbo-dorsal area. You will be informed that you will only feel comfortable warmth. Five sessions will be applied from Monday to Friday resting Saturday and Sunday, for 2 weeks. Total 10 sessions.

DETERMINATION OF BIOCHEMICAL PARAMETERS

For the determination of biochemical parameters of inflammation and adipokines. In coordination with the Echevarne Laboratory Accredited Clinical Laboratory of Barcelona. Blood will be drawn from the patient, the sample is divided into two tubes, one with a yellow cap, which will be left to rest for 30 minutes to obtain serum. The other part will be placed in purple tubes with EDTA. Then, tubes are centrifuged at 4°C for plasma/serum extraction. 4 serum milliliters are placed in 6 tubes of Eppendorf. 300 plasma microliters/tube per are placed in 4 Eppendorf tubes.

All tubes will be frozen until processed at the Biomedical Research Institute of Malaga (IBIMA) Lipid and Arteriosclerosis Research Unit. Medicine at the University of Malaga Andalusia, Spain. IL-6, TNF-alpha, leptin, human adiponectin, human resistin, human ultrasensitive C-reactive protein will be determined. By ELISA and IMMUNOASSAY methods simultaneously.
Samples will be stored by the laboratory at -70°C until the test days. Withdrawn by us and transferred to the University of Malaga for processing using the following tests:

- Human IL-6 QuantiGlo ELISA Kit
- Human TNF-alpha QuantiGlo ELISA Kit
- QuantiGlo Immunoassay Control Set 732 for Human TNF-alpha
- QuantiGlo Immunoassay Control Set 732 for Human IL-6
- Human-Leptin EIA 96 tests
- Human-Adiponectin EIA 96 tests
- Human-Resistin EIA 96 tests
- Ultrasensitive C-Reactive Protein (Human)

Statistical analysis and expected results

The statistical analysis of the results will be performed using the Software Package for Social Sciences (SPSS 24.0.).

A Kolmogorov-Smirnov test will be applied, if the data behaves normally, parametric tests will be applied. If not, non-parametric tests will be performed.

The differences between proportions will be analyzed using Fischer's exact test. The differences between the medians will be assessed using the Student's t-test for paired samples and independent samples. To determine the correlation between two quantitative variables, Pearson's correlation coefficient will be used.

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ANNEX:

STUDY PROTOCOL

The study will be carried out to 20 candidates who want to participate free of charge in exchange for their testimony and their commitment to the study.

The treatment will be carried out with the only technology on the market from Capernergy Medical, with its C-400 equipment, approved and authorized by the Ministry of Health, with its own national and international patents.

+ info: http://capenergy.com/es/product-detail/

The treatment will consist of the application of diathermy, providing the effects of tecartherapy, which are perceived as endogenous heat on the treated area.

The treatment will be individual and exclusive, with maximum discretion, in a quiet and relaxed environment.

The duration of the treatment will be 5 weeks, where 10 sessions will be carried out, 2 per week.

The cost of the treatment is valued at 1000 euros. Participants agree to start and end the study. In case of abandonment, they must reimburse the cost of the treatment.

The cost of the tests carried out will not be passed on to the patients, it is valued at 212 euros.

On the part of the professionals of INNEOTERAPIA, the treatment protocol established in the study will be applied.

Candidates are required to meet a number of criteria:

- Candidates are required to meet a number of criteria:
- Body mass index > 25.0
- CC value > 80cm for women and > 94cm for men
- Non-pregnant people, metal prostheses, suffering from bacterial infection, with a history of oncological, with chemotherapy or radiotherapy treatments.
- Commit to following a diet and physical exercise pattern for the duration of the treatment, of which they will be informed on the first day of assessment.

Objective of the study:

- Main
  - Decrease in body fat and biochemical markers.
- Secondary
  - Reduction in abdominal fat.
  - Decrease in abdominal circumference, body mass index.
  - Changes in glycemia, cholesterol and triglycerides.
  - Evaluate and compare the impact of quality of life.

The contribution of the testimony will consist of:

- Signature of informed consent.
- Signature of acceptance of participation in the study.

- Internal data of the study (specialized physiotherapist) will be completed.
- Each treatment will be graphically documented with photographs of the abdominal area.
- The discretion of the person will be maintained since at no time will he or she be identified by name.
- A video summary of the treatment will be recorded with the oral comments and image of each patient recounting their experience.

At the documentary level, the following is required:

- Informed Consent
- Clinical Case Participation Information Document (Protocol Fact Sheet).
- Determination of the protocol to be followed.
- Completion of the Data Notebook (one for each patient according to ISO format).
- Preparation of a brief report with conclusions.
- Accumulation of various photographic material (before/after), videos of the treatment and final testimony of satisfaction.

Continuity of treatments:

All candidates who subsequently want to continue the treatment after the 10 sessions will be able to do so according to the conditions as ordinary patients of INNEO applying the rates that are agreed.

Place of processing:

INNEOTERAPIA Physiotherapy Clinic
Carrer de Bordeus, 2 Barcelona
Phone: 687452613
E-mail: fisio@capenergy.com

Requirements:

- Wear comfortable clothes to the session.
- Be punctual at the appointed time.

Participant:____
First Appointment:
Day: Time:
Declaration of Conformity and Participation:
I understand the terms set forth in this protocol, for the treatment that will be carried out during 10 sessions with tecartherapy.
Sign: _________________________

National Identification Number: __________________________
The maximum temperature reached and the exposure time would be relevant factors to understand the response of the tissues to the increase in temperature. Goldberg, Fazeli and Berlin used a protocol with temperatures of 40-42 °C in the epidermis, not to mention the time spent at this temperature (Golberg et al 2008). Van Der Lugt et al in 2009, observed lysis of the adipocyte membrane after RF application. The authors used equipment with variable frequency (0.6-2.4 MHz) appropriate to the temperature of the tissues. Moving in a frequency range is advantageous, as tissues have different impedances, this decreases with increasing temperature, and by adjusting the frequency, a certain temperature can be maintained in a target tissue longer or with greater control, depending on the depth (van der Lugt et al, 2009). That is, according to van Der Lugt et, a temperature in the epidermis of 42 to 44 °C is equivalent to a temperature of 45 to 48 °C at 20 mm of depth. This difference is due to the low water content of adipocytes which increase their impedance. The authors report that at these temperatures, protein denaturation and thus adipocyte membrane lysis occur (van der Lugt et al 2009). Cell death increases with temperature exposure (increase in temperature and duration of exposure), signaling that an exposure to 3-minute cell phone at a temperature of 45°C reaches 40% cell death (Franco et Al 2010). Radiofrequency thermal stimulation of adipose tissue is thought to result in heat-mediated stimulation of adipocyte metabolism and increased lipase-mediated activity of enzymatic degradation of triglycerides into free fatty acids and glycerol that adds to the induction of fat cell apoptosis (Weiss 2013).