Efficacy of Low-Frequency, High-Intensity Ultrasound for Reduction in Subdermal Adipose Layers

Device Investigated: Ultimate Contour Body Sculpting Device

Indication Studied: Application of ultrasound for non-invasive waist circumference reduction.

A randomized, blinded comparison of waist circumference reduction of an active test group vs. a placebo control in adults.

Sponsor: CAO Group, Inc.
Development Phase: Performance Validation

Protocol ID: 005-00036-8
NCT ID: Not Yet Assigned

Anticipated Initiation Date: 15 December 2019

Anticipated Completion Date: 1 May 2020

Document Date: 10 October 2019

SYNOPSIS

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Study Title: Efficacy of Low-Frequency, High-Intensity Ultrasound for Reduction in Subdermal Adipose Layers
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Objectives

The purpose of this study is to evaluate principally the effectiveness, and secondarily the safety, of external application of lower frequency ultrasound to the waist/abdominal region of adults to achieve a reduction of adipose cells/tissues in the subcutaneous region. The application of higher frequency (200kHz to 3MHz) ultrasound for this purpose has previously been demonstrated and such devices have received clearance for treatment in the United States. The question remains whether application of ultrasound at a lower frequency (35kHz to 45kHz) can achieve comparable results without introducing any new or elevated risks to the patient. This study seeks to answer this question.

Methodology

Samples of the test device (Ultimate Contour Mini by CAO Group, West Jordan, Utah, U.S.A.) will be provided for the test. The devices consist of a touch screen interface whereby the operator may select the intensity of the delivered energy and the duration of treatment. The device features a single output cable to which is attached the ultrasound handpiece assembly. For the purposes of this test, the duration of treatment will be set at 30 minutes, and the intensity set at Level 4 (the maximum the device is capable of).

Application of the ultrasound energy consists of applying a treatment gel or liquid to the patient's skin (to eliminate air gaps between the applicator and the skin) and gently kneading the skin in circular motions, slowly progressing across the surface of the treatment area, making methodical passes until the entire treatment area has been exposed, then returning to the origin point and repeating this application approach.

Following treatment, the patient will be asked regarding the extent of discomfort experienced during the ultrasound application, and assessment will be made by a blinded, independent evaluator whether a number of visible skin conditions are observed or experienced by the patient.

Prior to the first treatment, the patient's weight and height will be measured by the blinded, independent evaluator, and the patient's waist circumference measured using a constant-tension flexible tape measure. Each patient will be treated a total of three (3) times, with a gap of 6-8 days between each treatment. Prior to each treatment, the patient will be weighed. Following the final treatment, the patient will be advised of support mechanisms (healthy diet, exercise, and so forth) to support a successful outcome. At 1, 4, and 12 weeks after the last treatment, the patient will be recalled for a final measurement and review of any outstanding adverse events. Study success shall be determined by achieving at least 1 inch of waist reduction between the initial measurement and the 12-week follow-up.

Inclusion/Exclusion Criteria

Inclusion in the study consists of the following criteria:

- Age equal to or above 18.
- Body Mass Index ≥ 25.

Exclusion from the study consists of the following criteria:

- Age equal to or below 17.
- Body Mass Index < 25.
- Open sores, wounds, or otherwise compromised skin in the treatment area
- History of keloid formation, hypertrophic scarring, or abnormal/delayed wound healing.
- Known or suspected pregnancy, or active nursing.
- General systemic conditions of arteriosclerosis, anemia, aortic aneurysm, or hypertension.
- Liver conditions such as hyperlipidemia, hepatitis, liver disease, or abnormal liver function
- Diabetes or blood-glucose sensitivity
- Any prior invasive cosmetic surgery to the waist or abdominal area, such as liposuction.
- Hernias or diastasis recti within the treatment area.
- Concurrent, or within the last 6 months, participation in any clinical trial for another device or drug.
- Existing bacterial or viral infections (influenza, rhinovirus, hepatits, pneumonia, tuberculosis, and the like)
- Presence of acne vulgaris, herpes zoster, psoriasis vulgaris, or similar skin conditions in the treatment area.
- Any type of cosmetic treatment to the target area within the last 6 months.
- Implanted active medical device anywhere in the subject, or metallic or polymeric implants in the vicinity of the treatment area.
- Currently undergoing, or recently underwent, chemotherapy or radiation treatment.
- Per the investigator's discretion, any physical or mental condition which may compromise the patient's safety or welfare.
- Failure to complete the study as outlined.

Statistical Methods

Single factor ANOVA analysis will be used to determine if the extent of circumference reduction in the test group is statistically significant over changes in the control group. Additional correlation assessment will be performed to identify any potential relationships between patient data sets, and single-factor ANOVA assessment applied to those correlations as applicable. Missing or incomplete data shall be populated using least-favorable data from participants who did complete the study.

Recruitment

Recruiting shall consist of about 50 patients, with a target of at least 40 patients completing the study. Should patients drop out of the protocol such that 37 patients are not achieved at the end of the study, additional patients shall be recruited to achieve the target number.

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LIST OF ABBREVIATIONS AND TERMS

BMI - Body Mass Index: A calculation incorporating a patient's height and weight to provide a comparative value on the extent of obesity of the patient.

RF - Radio Frequency. In the context of this device, this is radio frequency emissions intended by the product. This feature of the device will not be included in this study.

US - Ultrasound

INVESTIGATORS AND ENVIRONMENT OF TREATMENT

All testing conducted under this investigation will be performed at a single site, an existing dermatology practice located in Midvale, Utah. This setting is representative of the medical office environment under which the device would be used in the market. Consistent with this setting, patients will be scheduled for treatment and upon arrival have the procedure performed, along with appropriate pre-treatment and post-treatment assessment and instructions, and then allowed to depart. The principal investigator for this study has no contractual or financial relationship with the sponsor or with the specification developer. Additional research staff, in the persons of medical staff and assistants already present at the study site, shall assist with the performance of this study. Treatment and measurement activities shall be conducted in separate rooms, and shall be performed by different staff. Assessment shall be performed by licensed physicians.

INTRODUCTION

The purpose of this study is to evaluate principally the effectiveness, and secondarily the safety, of external application of lower frequency ultrasound to the waist/abdominal region of overweight adults to achieve a reduction of adipose cells/tissues in the subcutaneous region. The application of higher frequency (200kHz to 3MHz) ultrasound for this purpose has been previously demonstrated and such devices have received clearance for treatment. The question remains whether application of ultrasound at a lower frequency (35kHz to 45kHz) can achieve comparable results without introducing any new or elevated risks to the patient. This study seeks to answer this question. Some studies have been published that touch on this question to some extent, but these studies do not take into account variables such as instructions for a healthy lifestyle, or the operation of a device at the intensity levels designed into the Ultimate Contour device.[1-5]

The proposed clinical mechanism of operation for this device is as follows: High intensity ultrasound energy is applied externally to the outside skin surface. A sonically conductive medium such as an ultrasound-conducting gel or fluid is applied to the skin surface to help avoid air pockets or gaps between the transducer surface and the skin. The ultrasound energy passes through the skin and into the subcutaneous adipose layer, where sonic cavitation occurs. This cavitation disrupts and ruptures cell membranes of the adipose cells. The liberated contents of the adipose cells are then cleared away naturally via the lymphatic system. It should be noted that the purpose of this study is not to verify this mechanism of action, but to validate overall results via assessment of factors that may occur as a result of this theory.

The device in question, presently branded as the Ultimate Contour Mini, is at the stage of development where functional demonstration devices have been constructed and assembled. The design at present is representative of the final released configuration to the extent that third-party safety testing and certification has been conducted on the device and the device demonstrated to be safe relative to the design and constructional requirements of IEC 60601-1 (3rd Edition) for safety of electrical and electronic medical devices and systems, as well as IEC 60601-1-2 (3rd Edition) for electromagnetic compliance safety and performance of electrical and electronic medical devices and systems.

The study has a fixed endpoint of three (3) treatment sessions and three follow-up visits per patient, to the extent that a sufficient quantity of patients have completed the study. Given that a gap of 6-8 days will exist between treatments, the scheduling of patients will be staggered as necessary to best utilize investigator and equipment resources, with the overall course of the study treatments is not expected to exceed 6 weeks of total study time. The follow-up visits are mandated to occur at intervals of one, four, and twelve (12) weeks from the date of final treatment. Once all patient treatments are completed, analysis of the data shall be performed by the sponsor (and reviewed by an independent statistical resource) to determine the extent of success in achieving the primary and secondary objectives.

STUDY OBJECTIVES

PRIMARY - The primary objective of this study is to determine if the application of lower frequency (35kHz to 45kHz) ultrasound energy to the waist/abdominal region of the human body is capable of effecting a reduction in the amount of subcutaneous adipose cells and/or tissue to an amount of at least 1 inch and to a statistically significant level. Determination of the reduction in this adipose tissue is achieved via measurement of the body circumference that includes the abdominal area that was treated, and comparing measurements prior to the initial treatment with measurements after the entire treatment regime is concluded. Per the proposed clinical mechanism of action, the disrupted tissue is cleared by the lymphatic system which facilitates excretion or elimination of the disrupted tissue contents. Having been eliminated, the patient should exhibit a reduction in waist circumference. For this objective to be successfully achieved, a reduction in waist circumference of at least one inch must be demonstrated at a point of 12 weeks after the last of three treatment sessions has been applied.

SECONDARY - The secondary objective of this study is to assess the safety of this device. Assessment is made by having the patient rate the treatment experience and report any symptoms, side effects, or associated abnormal conditions. Additional assessment is made by the investigators to qualitatively confirm if visually detectable abnormal conditions (example: edema) are presented following the treatment. Success is determined by whether the patients indicate that any discomfort experienced during the treatment, and following the treatment that are directly attributable to the treatment, along with an absence of side-effects or abnormal conditions directly attributable to the treatment, create a risk/benefit condition where the benefit of treatment is regarded to outweigh the risks and temporary discomfort. Quantitatively, the benefit is regarded to outweigh the risk when: 1) No patients report a pain rating of 4 or higher on the Stanford Pain Scale, 2) No patients exhibit any side effects or abnormal conditions at the conclusion of the final treatment, and 3) No patient who was included in the Test group reports that the treatment regimen was unsuccessful.

INVESTIGATIONAL PLAN

Device description and settings

The Ultimate Contour device is a portable device that consists of a single central unit which houses all of the device electronics. The device features a fold up touchscreen that shows the device's operating condition and allows the operator to select the device's functional parameters. A single external, detachable power cord connects the device to an ordinary electrical outlet. The device contains an internal power supply that is auto switching to the input voltage and input frequency (100-240VAC, 50-60Hz). The device includes a single, detachable output cable. The operator attaches the ultrasound treatment handpiece to the device. The device is designed to allow application of RF energy via separate specifically designed handpieces, but the application of RF energy is not within the scope of this study. The device is capable of sensing if a handpiece is attached and to identify which handpiece is attached, in order to prevent any inappropriate power from reaching the handpiece or prevent the device from being configured in an inappropriate manner.

After attachment of the handpiece, the operator selects which treatment type (US or RF) is to be done. The device then presents only two parameters for operator to adjust: the duration of the treatment, and the relative intensity of the applied energy. The duration can be selected from 10, 20, 30, or 40 minutes duration. The intensity is selected from a comparative rating Level of "1, 2, 3, or 4", with Level 4 being the maximum energy level the device can output and Levels 1 through 3 being at staggered levels less than the maximum. Once the settings are selected, the operator places the device into a Ready state. At this point, the handpiece is placed in contact with the skin and the operator presses a button on the screen to Activate the handpiece. The unit applies energy to the handpiece at the selected intensity Level and the unit begins to count down from the selected time duration. Generally, the operator allows the device to operate until the selected time elapses, at which point the device automatically returns to the Ready state and beeps to inform the operator the cycle is complete. The operator may also stop the treatment at any time, either by pressing the stop button on the screen or activating a separate emergency stop button located on the device. During treatment, the device monitors the temperature of the handpiece (and by contact, the temperature of the skin) via temperature sensors located in the handpiece and in direct contact with external metal surfaces of the handpiece that contact the skin. If excessive temperature is measured, the device halts operation of the handpiece. For the purposes of this study, the device will be set at a treatment time of 30 minutes and an intensity level of 4.

Adjunctive activities associated with the treatment will also be performed. These included conversation with the patient about what to expect during the individual treatments and overall outcomes of the entire regimen, as well as instructions to the patient at the conclusion of the study regarding care and proper lifestyle habits. All of these adjunctive activities are described in the operator's manual for the device, and the clinicians will be instructed on the presence of these consultative activities in the manual and the execution of these activities for the purposes of this study.

Patient population

The population for this study shall consist of adults age 18 and over. Recruiting efforts shall focus on obtaining a reasonably even number of male and female patients and to include patients exhibiting a body mass index (BMI) of 25 or higher. No concern or focus will be given regarding the ethnicity of the patients, although a wide range of ethnicities would be beneficial. Patients will be evaluated relative to the inclusion and exclusion criteria indicated below. A recruitment target of a total of 50 patients is established. During recruitment, each candidate will be assigned a test subject number for identification purposes.

Study blinding

Blinding for this study shall primarily be at the level of the study investigators, whereby the investigator performing the treatment shall not be the same as the investigator who makes the pre-treatment assessment, who is not the same as the clinician performing the post-treatment assessment of the patient for immediate adverse effects. In other words, at any given time while a patient is present at the study site, there will be a minimum of three clinicians present at the site, and each respective clinician shall be assigned to one of the three activities (pre-assessment, treatment, or post-assessment) for the duration of that day's activities. Patient forms shall be designed such that dedicated forms are provided for each of these three activities, and the forms of any one particular activity (for example, the pre-treatment assessment) are not provided to or made available during the other activities of the patient's visit (ex. provided during the post-treatment assessment).

Study configuration

This study is designed as a single-arm study. All eligible candidates shall be enrolled to undergo treatment by the device according to the procedure and protocol that are discussed herein. This approach is consistent with other studies that have been done with similar devices where the primary objective of waist circumference reduction was also intended.

Sequence and duration of study period

Patients will be scheduled according to each one's availability for the first of three treatment sessions. After each particular treatment, the patient will be scheduled for the next visit, with it being preferred that subsequent treatments occurring between 6-8 days into the future of the present treatment. Each patient is to receive 3 treatment sessions, with each session featuring the same device settings and conditions of application relative to which study group the patient is assigned to. Overall, the study treatments are anticipated to take about 6 weeks to accomplish. The patient is recalled 1 week, 4 weeks, and 12 weeks after the last treatment for follow-up evaluation.

Measurement tools and methods

CIRCUMEFERENCE

The patient's circumference will be measured via use of a Gulick II (Model: 67020) tape measure with constant-tension feature that is factory-calibrated by the manufacturer. The process of obtaining a measurement proceeds as follows:

1. The area of the body is exposed (in this case lifting up the shirt to expose the abdominal region).
2. The patient is informed to stand straight and to breathe normally, but to focus on steady shallow breaths.
3. The clinician applies the tape measure by locating the top of the hip bone (iliac crest) and positioning the tape just above this bony landmark, just where one finger can fit between the iliac crest and the lowest rib.
4. The clinician ensures that the tape measure is positioned horizontally, parallel to the floor.
5. Measurement is taken at the end of normal expiration.
6. At a signal from the clinician the patient is to pause at the conclusion of an exhale, at which point the clinician pulls the tape until the constant-tension feature is enabled. The clinician then observes the measurement value and documents this value in the treatment record.

PREGNANCY

Women who enroll in the study shall be administered an over-the-counter pregnancy strip test to determine if the candidate is pregnant. The test strip to be used in the study is branded as the "Pregnancy hCG Test Strip", distributed by Pregmate located in Ft. Lauderdale, Florida. This product is demonstrated with a detection threshold of 25 IU/L and an accuracy level of 98%. This product features a control indicator to demonstrate validity of the test strip, and a results indicator – if the second indicator appears, this is a results of "pregnant". If the indicator does not appear, this is a results of "not-pregnant". Results of the test must be indicated on the

Inclusion/Exclusion with discussion of rationale; address both sexes and race allowance vs. availability

Inclusion in the study consisted of the following criteria:

- Age equal to or above 18.
- Body Mass Index ≥ 25.

Exclusion from the study consisted of the following criteria:

- Age equal to or below 17.
- Body Mass Index < 25.
- Open sores, wounds, or otherwise compromised skin in the treatment area
- History of keloid formation, hypertrophic scarring, or abnormal/delayed wound healing.
- Known or suspected pregnancy, or active nursing.
- General systemic conditions of arteriosclerosis or heart disease, anemia, aortic aneurysm, or hypertension.
- Liver conditions such as hyperlipidemia, hepatitis, liver disease, or abnormal liver function
- Diabetes or blood-glucose sensitivity
- Any prior invasive cosmetic surgery to the waist or abdominal area, such as liposuction.
- Hernias or diastasis recti within the treatment area.
- Concurrent, or within the last 6 months, participation in any clinical trial for another device or drug.
- Existing bacterial or viral infections (influenza, rhinovirus, hepatits, pneumonia, tuberculosis, and the like)
- Presence of acne vulgaris, herpes zoster, psoriasis vulgaris, or similar skin conditions in the treatment area.
- Any type of cosmetic treatment to the target area within the last 6 months.
- Implanted active medical device anywhere in the subject, or metallic or polymeric implants in the vicinity of the treatment area.
- Currently undergoing, or recently underwent, chemotherapy or radiation treatment.
- Per the investigator's discretion, any physical or mental condition which may compromise the patient's safety or welfare.
- Failure to complete the study as outlined.

The primary rationale for the exclusion criteria that were identified is to ensure that any existing health conditions that may be adversely impacted by the use of this device are not aggravated or made worse. Additionally, exclusion of women who are pregnant or nursing is viewed as an appropriate precaution to prevent any possible complications to the fetus or nursing child that cannot presently be anticipated. For women who enroll for the study, a urine-based pregnancy test (Pregnancy hCG Test Strips, by Pregmate) shall be administered to determine if the subject is pregnant.

A secondary rationale for these exclusions is that the conditions and situations listed in the exclusions may contribute to a change in weight, skin condition, or the patient's response to the ultrasound energy which may obscure the resultant data and make it more difficult to objectively assess if the study objectives have been met. The exclusion of persons age 17 and under primarily is a matter of responsible consent and the prospective patient being capable of making an informed and rationale decision regarding the possible risks and benefits of the treatment, as well as being in a legally permissible status to elect a medical procedure on their own behalf.

The study will be conducted at a single existing medical clinic: Just the Right Curves, 7525 Union Park Ave., Midvale, UT 84047, with recruiting efforts being made in the immediate vicinity of this location.

During selection, the patient's gender, age, height, and ethnicity will be documented. The patient shall be provided with information regarding the treatment and the risks and benefits associated with the treatment, and provided with and asked to sign the informed consent. A copy of the informed consent is then provided to the patient.

Details of the treatment protocol

STUDY STAFF

- Principal Investigator - Licensed medical doctor. Discuss patient adverse interactions, and directly observe patient adverse interactions following treatment; Supervise overall execution of the study protocol, including study documentation;
- Treatment Clinician - Directly trained by sponsor in the use and application of the device. Licensed medical doctor. Responsible for application of the device to the patient. This staff shall not be permitted to serve as Assessment staff.
- Assessment Clinician - Directly trained by sponsor in the use and application of the measurement tool to be used for this study. Licensed medical doctor. Responsible for measuring the patient's waist circumference and documenting such in the patient record. This staff shall not be permitted to serve as Treatment staff.
- Clerical Staff - Directly trained by the sponsor on the study protocol and the use and management of forms associated with this study. Minimum of a high school diploma. Responsible for receiving patients at arrival and scheduling next visits, and for providing and collecting study records from each of the clinicians. May also assist with organization of study records.

RECRUITMENT/SCREENING (total time of activity, 2-3 weeks):

- Advertisement shall be made in social media channels regarding the study. This method is thought to be appropriate to bring the study to the attention to prospective participants located in the vicinity close to the study site.
- An appointment is made with a prospective patient, where the candidate is evaluated according to the inclusion and exclusion criteria, and medical history and medications. The purpose and protocol of this study are discussed, any candidate questions or concerns are addressed, and the consent form is presented. The candidate may sign the consent form at this time, or arrange for time to further review their participation. Women participating in the study who are of child-bearing age or capable of becoming pregnant shall take a pregnancy test. Patient Information, Medical History, and Medication forms are presented for the patient to populate.

- Recruitment continues until 50 included candidates are identified and consent obtained on those candidates.
- A candidate is accepted to participate after the following are confirmed by the principal investigator:
    - The Informed Consent Form is signed
    - The Patient Information Form is completed and the patient is not excluded due to any of the indicated criteria
    - The Medical History and Medication forms are populated and reviewed to ensure the candidate does not meet any of the exclusion criteria or other circumstances that may put the candidate at elevated risk by participating.

TRAINING (concurrent with recruitment)

- The sponsor shall review the education and experience of personnel proposed as study staff.
- The principal investigator and staff shall be trained by the sponsor regarding the proper use of the device and the details of the study protocol.

TREATMENT (total time of activity, 14-18 weeks - 4-6 weeks for the 1st, 2nd, and 3rd treatment applications, and 12-14 weeks following the 3rd treatment for follow-up visit):

Individual Treatment Session (approximately 70 minutes)
Prior to the Treatment (about 10 minutes):

- The patient is scheduled to arrive at the study site to undergo a treatment.
- The date of the treatment and the time of arrival will be documented in the treatment session record.
- During the first treatment only: The patient is given the Q-Pre Survey to fill out.
- The assigned pre-treatment investigator measures the waist circumference of the patient and documents this in the Pre-Treatment record, with these measurements being made by the blinded assessment clinician as described in the Measurement Tools and Methods section of this protocol.
- Prior to Treatments 2 and 3, the patient will be asked for the study diary, and the pre-treatment investigator shall review the diary and the patient's previous post-treatment Anticipated Effects and Adverse Event forms and discusses with the patient the items that are recorded about any abnormal body functions or observations that may be relevant to the treatment. If any are indicated, these will be documented in the patient record. Additional record shall be made by the investigator as to whether past events and observations have been resolved or are still unresolved.
- The patient is moved to the treatment area.

Application of the Treatment (approximately 45 minutes):

- The treatment clinician prepares the patient for treatment by displacing clothing from the treatment area, which is the abdominal area from the bottom of the sternum to the iliac crest.
- The patient lies down on their back on the treatment table.
- The treatment clinician turns on the Ultimate Contour Mini device and sets it to the indicated parameters for this study: Time Setting of 30 minutes; Intensity Setting of 4.
- The treatment oil/fluid, a massage oil with a cottonseed or grapeseed oil base, is applied to the patient's skin over the area intended for exposure.

- The treatment clinician applies the treatment handpiece to the patient's skin in the intended treatment area.
- The treatment clinician presses the Activation button on the Ultimate Contour screen to begin energy emissions from the handpiece.
- The treatment clinician begins moving the handpiece along the skin with slight pressure to the handpiece to maintain contact with the skin, moving in circular, orbital motions approximately twice the diameter of the handpiece diameter, and progressively advancing in a linear direction from one edge of the treatment zone to the other in an overall back-and-forth approach.
- The treatment clinician continues application of the handpiece until either the proscribed 30 minutes has expired, or the patient indicates to halt the treatment due to discomfort.
- Once energy emissions cease, the handpiece is removed from the skin, and the treatment oil is cleaned off from the skin.
- The patient replaces clothing and is moved back to the assessment room.

Post-Treatment Assessment (approximately 15 minutes):

- The Post-Treatment assessing investigator observes the treated area for any redness, edema, swelling, or other indications specifically called for in the Anticipated Effects treatment record. The patient is asked for any unexpected sensations, for example sensations of high heat or of a "pins and needles" tingling effect in the tissue. These anticipated effects are documented in the treatment session record. Un-anticipated effects are recorded in the Adverse Event form.
- The patient is presented with the Stanford Pain Scale chart and asked to rate their overall pain and discomfort during the treatment. This is documented in the treatment session record.
- A waist circumference measurement of the abdominal region is made using the constant-tension tape measure. This measurement is recorded in the Post-Treatment Measurement record.
- The patient is provided with a study diary form, and instructed to document in the diary any unusual sensations, events, or bodily responses and to bring the form with them for the next visit.
- At the final treatment session only, the patient is provided with the Q-Post Survey to fill out.
- The patient is then scheduled for the next study session, and then excused.

FOLLOW-UP (approximately 15 minutes):

- The patient is scheduled for and arrives at the study site at the appropriate time points 1, 4, or 12 weeks after the final treatment.
- The date of the visit and the time of arrival will be documented Post-Assessment Measurement and Anticipated Effects forms.
- For the 1-week and 4-week visits, the patient fills out the WaistQ-Post Survey. For the 12-week follow-up, the WaistQ-12 Survey is filled out.
- The patient will have their waist circumference measured and documented by an investigator assigned to the post-treatment measurement and assessment activity, as described in the Measurement Tools and Methods section of this protocol. The patient will be asked for the study diary, and the investigator shall review the diary and discuss with the patient the items that are recorded about any abnormal body functions or observations that may be relevant to the treatment. If any are indicated, these will be documented in the patient record. The

investigator will also review observations and adverse events documented in previous sessions and discuss these with the patient. Additional record shall be made by the investigator as to whether past events and observations have been resolved or are still unresolved.

- For the 4-week or 12-week follow-up visits, the patient is provided with a study diary form, and instructed to document in the diary any unusual sensations, events, or bodily responses and to bring the form with them for the next visit. The patient is then scheduled for the 4-week or 12-week follow-up visits as appropriate.
- At the 12-week follow-up, additional review is made of the patient's Medical History and Medication forms and any new content for these forms is noted therein and discussed with the patient. If the 12-week follow-up visit is concluded and Adverse Event forms indicate any unresolved adverse results for the patient, additional follow-up is scheduled as appropriate until all adverse results are concluded.

DATA ANALYSIS AND FINAL REPORTS (approximately 2-3 weeks)

- Once all study participants have concluded the 12-week follow-up, unless participants are unable to be contacted or unwilling to participate in the follow-up, and all adverse interactions have a documented conclusion, analysis of the study data shall be conducted according to the Data Analysis section below.
- Data documented in the physical study records are populated into electronic spreadsheets.
- Data integrity is validated, and then data analysis is performed.
- Once data analysis is complete, final study reports shall be prepared, presenting the results of the study.

Monitoring

The study shall be monitored in the following manner:

- Training and Initial Inspection: The sponsor shall visit the study site and shall conduct an inspection of the site to determine that the site is adequate and appropriate for the activities described in this protocol. In particular, attention shall be given to ensuring that separation of treatment and the two assessment activities are accommodated to ensure patient and staff blinding is maintained, and that patient safety is ensured. Training shall be conducted for the investigators and staff regarding the study protocol, methods of measurement, and operation of the device.
- Informed Consent: The sponsor shall obtain copies of the signed informed consent forms prior to delivery of study devices.
- Surprise Inspection: At least twice during the course of study treatments, an independent third-party shall conduct a surprise inspection of the study site while treatments are being conducted, to review that the protocol is being followed, confirm that protection of human subjects is being employed, and ensure that treatment records are properly filled and maintained. Since the total duration of the device treatment period is anticipated to be only 4-6 weeks, the mandated 2 surprise visits are considered appropriate. If records or actions of investigation staff are found to be inconsistent, additional site inspections and possible additional training shall be employed.
- Collection of devices and records: Once all device treatments are completed, the sponsor shall retrieve the study devices and obtain copies of all treatment records. The sponsor shall review the patient records, and make especial note of adverse interactions contained in the records.
- Follow-up Records: At the conclusion of the follow-up visits, the sponsor shall collect the patient records from the follow-up visit. The sponsor shall compare and confirm that all adverse

interactions that have been documented have been resolved at this point. If any adverse interactions are still unresolved, the sponsor shall work with the investigator to continue follow-up with the affected patients to achieve resolution.

- Audit of the Records: An independent third-party shall perform a sampling review of the study records during the surprise visits, and shall conduct a full audit of all study records at the conclusion of the study. This audit shall include quality control verification of data uploaded to electronic format. The audit shall confirm that all indicated adverse effects, whether anticipated or not, have been followed through to conclusion and such conclusions are documented in the Case Record Forms.

Efficacy & Safety variables, including collection of adverse event data and anticipated adverse reactions

EFFICACY

Input variables related to the determination of efficacy include the patient's age, gender, ethnicity, height, and weight (to determine the relative extent of obesity). Study output variables collected during this trial included the change in patient waist circumference as a primary indicator. Capture and collection of patient images may be useful for informational purposes, but are not a requirement of this study and shall not be used for any data analysis or study conclusions.

SAFETY

Input variables related to safety are reflected in the exclusion criteria. Primary among these is the effect the ultrasound treatment has on existing illnesses or conditions. There is concern that the delivered energy could adversely affect these conditions, making the patient's overall status worse. Other input variables for safety include the effect on localized tissue directly exposed to the ultrasound, as well as possible systemic effects that may occur.

Output data collected for safety include the patient's assessment of pain or discomfort experienced during or following the treatment, and questioning regarding any observations about systemic health that the patient may make during the overall trial. Any such comments or concerns from the patient are documented in the patient treatment records. Additionally, observations by the clinician about localized tissue response and patient general behavior were collected if any occurred and made part of the patient record.

ADVERSE INTERACTIONS

Anticipated adverse responses include:

- Localized inflammation, edema, and elevated temperature of the treatment area: Based on the proposed mechanism of action, the application of ultrasound and the disruption of cells could result in a localized increase in tissue temperature, accompanied by increased blood circulation through the insulted tissue.
- Localized pain or discomfort: The action of ultrasound of this frequency and intensity on nerve cells is not well understood. The same mechanism of action could trigger nervous cell responses interpreted by the brain as a pain response. The disruption and cavitation of adjacent adipose cells could also trigger a pain response.
- Possible system disruption: The applied ultrasound energy is intended to provide localized energy at the typical depth of tissue where sub-dermal adipose cells are found. The ultrasound energy could continue to propagate further into the body, reaching gastro-intestinal organs beneath the treated area. In generalized terms, there could be disruptions to digestive processes resulting from exposure to the ultrasound energy.

- Possible elevation of blood glucose levels: The disruption and lysis of adipose cells may release an increased amount of glycogen into the blood stream. Patients who present with diabetes or pre-diabetes or who are susceptible to variations in blood glucose levels may experience side-effects consistent with elevated glucose levels wherein they are unable to metabolize and/or process the glucose products adequately.
- Possible pigmentation changes of the skin: The action of ultrasound on the skin may alter the amount or chemical nature of pigments in the skin, causing discolorations relative to the surrounding skin.
- Possible aggravation of surgically compromised or wounded tissue: The action of ultrasound on tissue that has been disrupted, such as through surgical procedures, previous wounds, or previously or presently inflamed tissue, could create mechanical stresses on the wound boundaries an possible overcome connective tissues across the wound boundaries, resulting in re-opened or inflamed sites.
- Possible increased compromise of immuno-suppressed patients: Patients who are or have recently experienced systemic procedures or conditions that weaken or suppress the immune system may be further weakened as a result of this treatment, as the process is expected to utilize immune responses to clear away the treated sub-cutaneous tissues.

If any adverse events occur, such will be immediately reported to the principal investigator who will assess the events and make determinations about whether the study should be halted or modified, or whether the participant should be excluded from further participation in the study. The study sponsor shall also be notified of the adverse event and of the principal investigator's assessment and decision regarding any action towards the participant or the study.

DATA ANALYSIS

Data quality assurance

Patient and study data will be documented on physical, printed patient treatment session sheets. The completed sheets will be provided to the sponsor, who has the responsibility of uploading the data into electronic files for statistical analysis and processing. Calculations made from the data will be conducted in the electronic files, and the accuracy of calculations provided by the electronic spreadsheets shall be relied on. The clinicians and those who participate in the data collection will not have access to the electronic files, calculations, or analyses. Electronic data management tools are also employed to perform the statistical analysis of the key data.

Accuracy of the measurements made by the tape measure are verified by the sponsor. This is confirming the calibration status of the device via the manufacturer's certificate of calibration, as well as verification by measuring rigid metal cylinders with the device and comparing the measurements with NIST traceable calibrated calipers to measure the sample diameter from which the circumference can be calculated.

Where patients discontinue participation in the study, or key data is not documented properly on the treatment sheets, data from such patients shall not be incorporated in the statistical analysis of the data.

Statistical methods planned, including handling data exclusion, analysis of sub-groups if any

Any patient that did not conclude all three treatments shall be excluded from the analysis. If there are fewer than 37 patients remaining after the study concludes and exclusions are applied, than no analysis shall take place and additional study subject enrolled until a minimum of 40 completed participants exist. In the event that a patient concludes at least one treatment session, but afterwards discontinues

participation in the study, the presence of the patient in the study shall be acknowledged for data analysis purposes. Missing data for such acknowledged participants shall be fulfilled by inserting the least favorable result obtained realized from among all test subjects who did complete the study. Statistical analysis shall then proceed on the fully populated study data.

Primary analysis shall consist of applying the Anova single-factor acceptance test evaluating the change in waist circumference based on the data collected by the tape measure. A confidence of P=0.05 shall be applied. The results of this data shall be directly applied to determining if the primary objective of the study has been met.

A correlation analysis shall be applied to all variable data recorded, as well as key calculations from the data, to identify any potential two-way connections or relationships between sets of data. Any correlations scoring a magnitude of 0.85 or greater shall be discussed and if appropriate, Anova single-factor analysis applied to determine if there is statistical significance to the relationship and any statements that might be supported by the data. Of primary interest is any impact the starting BMI might have on the amount of change, and any impact that gender may impart to the outcome.

Sample size

A total sample size of a minimum of 40 patients was established for this study. This number is based on

$$n = \frac{2 \cdot (Z_\alpha + Z_\beta)^2 \cdot \sigma^2}{d^2}$$

where:

- Z_α = 1.96, based on a confidence of P=0.05 (false rejecting of a true null hypothesis),
- Z_β = 0.84, based on a power of 80% (failure to reject a false null hypothesis),
- d = 1.0 inch reduction in waist circumference as a meaningful/successful change in measurement,
- σ = 1.50 inches, based on the evidence presented by a published study[5] under similar treatment parameters and conditions reporting a typical response of 0.75 inches through the umbilical region and assuming some of the population may not respond at all.

The resulting calculation gives a population size of 37. Accounting for possible attrition and adding a generous margin of safety, a recruitment size of 50 individuals is arrived at. Review of the data analysis shall be conducted by an independent entity to confirm the handling and calculation of data, and the conclusions drawn from the data regarding statistical significance.

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