INVESTIGATOR-INITIATED CLINICAL TRIAL

Food Allergen Eliminations and Combined Protocols for Obesity Reduction: a Preliminary Comparison Study.

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1 SYNOPSIS

TITLE: **Food Allergen Elimination and a Brief, Therapeutic Exercise for Obesity Reduction**

OBJECTIVES:
Primary: The purpose of this study was to determine if removing food allergens alone or combined with a multi-discipline weight loss protocols are effective in obesity reduction.

NAME OF COMPANY: Galveston Clinical Research Foundation

STUDY SITES: Galveston, Texas, USA

METHODOLOGY: Randomized, Controlled, Cross-over Trial

NUMBER of PATIENTS: 18

Inclusion:

1) Healthy adults, ages 20 – 75
2) Body Mass Index ≥ 30

Exclusion:

1) Cardiovascular Heart Disease
2) Hypothyroid Secretion (Can reapply after Treatment and normal TSH, T3, T4 levels)
3) Hypogonadal syndrome (Can reapply after Treatment and normal testosterone levels.)
4) CVA, TBI or any neural injury or disease
5) Diabetes Mellitus (Type I or II)
6) Other weight loss protocols, diets, or medication (30 day wash out)

Randomized Categorization

- N/A

STATISTICAL METHODS:

A Repeated Measures AVOVA will measure changes in weight, body composition, and BMI.

SAFETY:

Serious Adverse Events associated with study procedures (ALCAT Blood draw, dietary changes and 2-minute exercise sessions) will be reported to the IRB within 24 hours and to the FDA as required by IRB.

2. TABLE OF CONTENTS

2.1 List of Abbreviations

AE	Adverse Event
BMI	Body Mass Index
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
HR	Heart Rate
Ig	Immunoglobulin(s)
IRB	Institutional Review Board
PI	Principal Investigator
SAE	Serious Adverse Event
SOPs	Standard Operating Procedures
US	Unites States of America
VO2	Maximal Volume of Oxygen (Consumed)
WHO	World Health Organization

3.1 Introduction

The current national incidence rate of obesity in America is 118 million adults (36.5%), based on Body Mass Index (BMI) > 30 [1,2]. The cost of the Obesity Disease is over $150 Billion dollars in the USA last year, and the CDC states that "The medical costs for people who have obesity were $1,429 higher" (than non-obese adults). The demographics for American obesity can be seen in Figure 1, and the greatest percentage by ethnicity is among non-hispanic black Americans and obesity is greatest in the 40-59 age group.[1] The WHO estimated that the United States is the fattest country on the planet.

Hidden food allergies are estimated to affect up to 75% of the US population [3-5]. While food allergy testing and elimination have been studied for reduction of migraine headaches and intestinal disorders, the research has not been extensive in determining the efficacy of food allergy elimination in obesity reduction [3-11]. Many weight loss protocols have been examined [6-41] but studies identifying and reversing the causes of obesity are few. Without identification and reduction of the cause, protocols only treat the symptoms.

However, one definitive, food allergen elimination study for over-weight reduction was conducted by Lewis et al [6]. They tested 120 overweight subjects (mean age 45.5, BMI = 29) for IgG food allergens and foods that had a positive reaction were removed for experimental subjects diet for 90 days. These subjects saw significant changes in weight and waist/hip circumferences, and the mean changes are as follows: Weight (– 5kg, $P<0.01$) and Waist circumference (-5.4cm, $P<0.01$). However that study did not use leukocyte testing to also measure reactions from IgE an IgA, nor did it test other protocols for weight reduction, and the subjects were not all obese [6].

"PAN-5" is an effective combination of separately proven protocols and the acronym includes the following: **Portion** sized meals (<2 cups, 5/d) [12-14], **Aerobic**-surge Exercise for 2-minyes, 5/d) [15-39], and **Natural** foods which must be eaten 5 times/day [40-44]. A portion-sized study with adult subjects was completed by Kesman et al who examined 65 obese adults (Mean age 55.8 and BMI > 30 to 40) and their intervention included a Portion-size plate (2 cups) with separations for food groups. After six months there was a significant change between experimental vs control groups ($P=0.41$) and the mean changes were -2.4% Δ body weight for Portion Plate vs +2.2% Δ body weight for the control group [12].

A landmark study examining the frequency of eating and portion size was done by Iwao et al. on Olympic boxing athlete's weight loss in a two week study. Twelve boxers were separated into two categories, one group ate 6 meals/day (6M) and one group ate 2 meals/day (2M). The exact same daily food quantities were divided into 6 meals or two meals, and all boxers had similar exercise and training regime [14].

Both groups lost weight and while there was not significant difference in the weight change, the 6M group had a 90% greater change in body fat than the 2M group. This study showed that eating smaller portions sizes, more frequently, yielded greater fat reduction in the 6M group [14].

High intensity exercises with brief durations have been a recent addition to the protocols used in weight loss. A study by Fisher et al, titled "High Intensity Interval - vs Moderate Intensity - Training for Improving Cardiometabolic Health in Overweight or Obese Males: A Randomized Controlled Trial" showed resounding evidence supporting this protocol. High Intensity Interval Training (HIIT) vs a common moderate intensity training (MIT) for weight loss were tested in twenty-eight healthy, overweight or obese males, (age 20, BMI 25 - 35) and this study lasted 6 weeks [15].

The HIIT exercise intervention included 20-minute, interval training on a cycle ergometer at 15% of max anaerobic power (four minutes), followed by 85% of max anaerobic power (30 seconds). The interval cycle was ridden four times per exercise session, three times per week. In comparison the MIT group trained at 55% of peak VO_2 for 45-60 minutes of continual cycling, five days/week. The total time training over the 6-week study duration was as follows: HIIT = 360 minutes vs. MIT = 1,350 minutes. Even with a 3.75 times lesser duration in training the, there was only significant difference in one of eight variables.

The mean change in peak VO^2 was greater for MIT (MIT 11% vs HIIT 2.3%, P=0.0185), however there was no significant difference (all > 0.05) in all of the other outcome measures: Weight mean change (Δ) was -0.9kg; BMI (Δ was -0.29); Total Cholesterol (Δ was -10.8mg/dL); Triglycerides (Δ was -26.4mg/dL); HDL (Δ was -1.65mg/dL); LDL (Δ was -6.08mg/dL); Insulin Sensitivity (Δ was -0.71). HIIT was almost equal to all changes vs MIT, even though HIIT only required 27% of the time dedicated to exercise vs MIT. Brief high intensity exercise should increase compliance.

A new, brief Aerobic-surge protocol includes exercising at ≥ 75% of a deconditioned person's estimated maximum heart rate for only two minutes, (5/day) and this formula is (220-age) x .75 = target Aerobic-surge heart rate. This brief 2-minute bout was performed five times/day and was tested in a randomized, controlled, multi-centre trial by Willis et al [16]. Fifty four obese subjects (mean age 39, BMI > 30) completed participation at three sites in this 60-day trial. The Aerobic-surge subjects (N=23) were taught how to elevate their HR to the target heart rate (≥ 75% of max HR) and measure this from their carotid pulse. The exercises used included stationary bicycling, stair climbing, and other similar exercises to elevate their HR to the target rate. In comparison the Control subjects (N=23) were simply told to "exercise more" which is a common instruction given in primary care.

The dependent variables in that study were changes in weight (pounds) and 'Body Summation' of 10 girth measurements (neck, shoulders, waist, his, etc.). A 2x2 ANOVA was used to calculate differences. There was a significant difference between groups (P<0.0001), and the mean changes were as follows: Exp = -18.0lbs and -18.7" vs. Control +1.3lbs and +1.7."[16]

Eating natural, unprocessed foods was discussed in a portion size study [13] and Polsen et al examined differences from changing one's diet to natural foods in the New Nordic Diet [40]. They compared the 'Average' diet (control) to the New Nordic Diet (NND) which removed the processed foods and substituted with natural foods (high in fruits, vegetables, fish, and whole grains). In that study 181 obese subjects (mean age 42) were randomly selected to either the NND group or the Average diet for 26-weeks.

The dependent variable was change in weight and there was a significant difference for the NND vs Average group. The NND subjects showed a mean change of -4.7 kg vs the Average group lost only a mean -1.5kg (P < 0.001). There were also significant differences seen in Fat change (1.87% difference, P < 0.001), Waist circumference (-2.94cm difference, P < 0.001), and in Systolic blood pressure (-5.13 mm Hg difference, P < 0.001) [40].

These studies show the overlapping benefits from reducing Portion sizes (with greater meal frequency) and Aerobic-surge or brief, high intensity exercise, plus the befits of eating Natural foods. The **purpose** of this study was to determine if removing food allergens alone or combined with a multi-discipline weight loss protocols are effective in obesity reduction.

This proposed study will have two volunteer, unrandomized groups: Food Allergen elimination (alone) vs. Combined Treatment (Food Allergen elimination + PAN-5). After 90-days the changes will be tested and copared.

Prevalence of Obesity

The current national incidence rate of obesity in America (36.5% or 118 million adults) and they are obese based on Body Mass Index (BMI) > 30 [1,2].

Diagnosis of Obesity

Diagnosis of obesity is done with the Body Mass Index scale and readings over 30.

The formula for BMI is: Body Weight (kg) ÷ Height^2 (m) = BMI

Current Standard of Care for Obesity

The current standard of care for obesity includes medication such as phentermine, dietary food reductions and/or products, calorie reduction, and eventually gastric surgery bypass or sleeve insertions. However none of these treatments address or reduce the cause of this disease.

3.1 Summary of Potential Risks

This is a randomized, cross-over study that will not include and invasive treatments methods or pharmaceutical products. The risks in this study are as follows:

- Drawing blood for diagnostic testing by venipuncture is an invasive procedure that may occasionally be associated with adverse events such as pain, bleeding, syncope, or ecchymosis.
- The Aerobic-surge, 2-minute exercise bouts will have potential risks for injuries such as falls, scrapes, bruises, pulled connective tissue injuries, etc. However this risk is no greater than the risk in daily ambulation and activity.

3.2 Summary of Potential Benefits

The potential benefits include the benefits seen in a Pilot study of food allergy elimination plus addition of this Aerobic-surge exercise as seen in a pilot study [46]:

- Reduction in Weight and BMI
- Reduction in Body Fat percentage
- Reduction in Blood Pressure
- Reduction in Total Cholesterol
- Reduction of Triglycerides
- Reduction of LDL
- Reduced health care expenses
- Improved HDL/LDL ratio
- Improved repertory rate
- Increased energy
- Improved self-image

4 STUDY OBJECTIVES

The **purpose** of this study was to determine if removing food allergens alone or combined with a multi-discipline weight loss protocols are effective in obesity reduction.

5 INVESTIGATIONAL PLAN

5.1Endpoints

5.1.1 Efficacy Endpoints

This study is a preliminary, case/comtrol study comparing food allergen elimination and food allergen elimination with PAN-5 for metabolic acceleration. The endpoint will be the 90-day completion of this trial

5.1.2 Safety Endpoints

Only serious adverse events (SAEs) associated with study procedures will be recorded.

5.1.3 Other Endpoints

N/A

5.2 Study Design

This is a pilot study comparing these two categroies for weight loss.

5.2.1 Completion of a Subject's Participation and the Overall Study Completion

Each subject's will be followed through completion of the 90-day trial.

5.2.1.1 Completion of a Patient's Participation in the Study

Participation in the study will be completed after 3 months. Each patient will have daily communication (text) with the clinical staff in the first 30 days, and weekly communication through the first 3 months.

5.2.1.2 Premature Patient Discontinuation from the Study

Patients are free to withdraw consent and/or discontinue participation in the study at any time, without prejudice to further treatment. A patient's participation in the study may also be discontinued at any time at the discretion of the Principle Investigator or Primary Care Physician.

5.2.1.3 Overall Study Completion

The study will be considered to be complete after 90-days.

5.3 Discussion of Study Design

This is an Investigator-Initiated, preliminary case/control study. A

6. SUBJECT Population and Selection

6.1 Inclusion Criteria

A subject must meet all of the following criteria to be eligible for this study.

1. The subject is > 20 years of age and is willing and able to provide signed informed consent.
2. The subject has a BMI > 30.
3. The subject is between the ages of 20 to 75 years of age.

6.2 Exclusion Criteria

A patient who meets any of the following criteria will be excluded from this study.

1) Cardiovascular Heart Disease and this includes but is not limited to history of myocardial infarction, atrial fibrillation, etc.
2) History of cerebral vascular event (stroke), traumatic brain injury, Parkinson's disease or any other neural injury or disease.
3) Diabetes Mellitus (Type I or II)
4) Pregnancy
5) Participation in any other weight loss protocols, diets, or medication.

7 TREATMENTS

The treatments will include the following:

- Elimination of foods showing any leukocyte reaction (tests all Ig reations).
- Restructure of dietary schedule of eating to < 2 cups of food, five times each day.
- Participation in the 2-minute Aerobic-surge exercises (with HR ≥ 75% max HR) performed at home or work, five times per day.
- Consuming Natural foods five times a day, (included in the total 2 cup volume), 5/day.

7.1 Prior and Concomitant Medications and Therapeutic Procedures

Concomitant medications and therapies the patient has taken in the week prior to enrollment will be recorded during the first study visit. Patients will be excluded from enrollment in the study if they have taken or are currently taking medication for weight loss such as Phentermine, medication for insulin control, or medication for HR (propranolol).

7.2 Method of Assigning Patients to Treatment

All subjects will receive food allergen testing and they can volunteer to also be treated with the PAN-5 protocol.

7.3 Blinding and Randomization

- No blinding or randomization will occur.

7.4 Treatment Compliance

Compliance will be tracked through daily communication with staff (daily text messages or paper recordings of daily compliance to be turned in weekly) and this will be done through the 90-day duration of this trial.

8. STUDY ASSESSMENTS

8.1 Study Schedule of Events

A screening phone call will evaluate the following:

- Eligibility (BMI, age, etc.)
- Medication eliminations (i.e. insulin, propranolol, etc.)

The initial meeting at the clinic will include the following:

- Informed consent and description of the study
- Confirmation of study eligibility
- Review of full medical history

Meeting for Enrolment will include the following:

- Consultation with PI
- Collection of blood through venipuncture for Food Allergen testing
- Demonstration of Aerobic-surge protocol and testing that subject understands
- Physical Examination
- Enrolment
- Initial Body Composition Testing.

8.2 Demographic and Screening Assessments

Demographic data will be collected at the first study visit, and a physical examination will be conducted by a licensed physician.

A targeted medical history will be collected for all patients enrolled in the study. It includes any history of or current manifestation of signs and symptoms commonly associated with endocrine pathologies.

8.3 Efficacy Assessments

Not applicable.

8.4 Safety Assessments

Any SAEs associated with study procedures will be recorded and reported.

9 ADVERSE EVENT REPORTING

9.1 Adverse Event

For the purpose of this protocol, the following definitions and reporting requirements will apply:

An adverse event (AE) is any untoward medical occurrence associated with the performance of a study procedure in a clinical investigation patient.

AEs may include:

- Symptoms described by the patient
- Clinically significant changes in the patient's physical exam or other signs observed by the Investigator or medical staff

Definition of a Procedure-related AE:

A procedure-related AE is an AE occurring during a clinical study that is considered by the Investigator or the Medical Monitor (or designee) to be related to a research procedure (i.e., related to the fact that a patient is undergoing a procedure in the study). For example, a procedure-related AE may be an event related to a medical procedure required by the protocol (a single blood draw or an injury from an Aerobic-surge performed in the normal course of this study).

Only serious procedure-related adverse events will be recorded.

9.2 Serious Adverse Event

A serious adverse event (SAE) is an AE that results in any of the following:

- Death: The patient died as the result of the event.
- Is life-threatening: An AE that places the patient, in the view of the Investigator or clinician, at immediate risk of death from the AE as it occurred, i.e., does not include an AE that had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing hospitalization
- Persistent or significant disability/incapacity: An AE that results in a substantial disruption of a person's ability to conduct normal life functions.
- Congenital anomaly/birth defect: A congenital anomaly/birth defect that occurs in the offspring of a patient exposed to the study procedure.

- Other Medically Important Serious Medical events: An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the patient or patient may require medical or surgical intervention to prevent one of the outcomes listed above.

9.3 Evaluation of Adverse Events/Serious Adverse Events

SAEs will be assessed by the PI. Only SAEs determined by the Investigator to be possibly related or related to study procedures will be recorded. Any study-procedure-related or possibly-related SAEs ongoing after the Screening/Baseline visit will be followed until resolution.

9.3.1 Causality Assessment

For each SAE the Investigator must determine whether, based on available evidence, there is a reasonable possibility that the study procedure caused the event. Causal relationship will be classified according to the following criteria:

- Not Related: There is no suspicion of a causal relationship between the procedure and the AE.
- Unlikely Related: There is no evidence for a causal relationship between the procedure and the AE; however, such a relationship cannot be ruled out.
- Possibly Related: There is some evidence supporting the possibility of a causal relationship between the procedure and the AE.
- Related: There is strong evidence that there is a causal relationship between the procedure and the AE.

A causality assessment must be provided for each SAE recorded even if there is only limited information at the time.

Upon receipt of follow-up information, the Investigator may change his/her assessment of causality and amend the SAE report accordingly.

9.3.2 Severity

Severity indicates the intensity of the event and should not be confused with seriousness (i.e., Section 9.2), which is an event outcome applied for the purpose of event classification and regulatory reporting.

Severity Grading

The Investigator will assess the severity of all study-procedure-related SAEs as Mild, Moderate, or Severe, based on the following definitions.

Definitions:

- Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant.
- Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

9.3.3 Outcome

Outcome describes the status of the SAE.

The Investigator will provide information regarding the patient outcome of each SAE that is judged to be possibly related or related to a study procedure.

Definitions for possible results of an AE outcome:

- Recovered/Resolved: the event has improved or recuperated
- Recovering/Resolving: the event is improving
- Not Recovered/Not Resolved: the event has not improved or recuperated
- Recovered/Resolved with sequelae: the patient recuperated but retained pathological conditions resulting from the prior disease or injury
- Fatal: termination of life as a result of an adverse event; there should be only one AE marked with this outcome
- Unknown: not known, not observed, not recorded or refused

9.3.4 Action Taken Regarding the Investigational Product

Not applicable.

9.4 Reporting Serious Study-Procedure-Related Adverse Events

9.4.1 Initial Reporting of Serious Study-Procedure-Related Adverse Events

Study-procedure-related or possibly related SAEs should be recorded using appropriate medical terminology. When recording, it is preferable to provide a diagnosis. In the absence of a diagnosis, each sign and symptom should be captured as a unique SAE. Sufficient information should be sought to assist the Investigator both in determining the diagnosis and in making a causality assessment.

The necessity and time requirements for reporting of study-procedure-related or possibly related SAEs to the Sponsor or its designee and/or regulatory agencies are as follows:

- All SAEs related or possibly related to study procedures must be reported to the IRB or designee within 24 hours of the Investigator's first knowledge of the event.
- A completed Clinical Study SAE Report Form containing a detailed written description of the event along with available supporting documents (e.g., discharge summary, autopsy report, diagnostic test results, etc.) should be provided by fax or e-mail to the following address:

Safety Reporting Christine Smikal, RN, BSN, CPN
Fax: 409-750-7239 E-mail: christinesmikal@yahoo.com

- Additional information that is not available at the time the initial SAE Report Form was completed, must be promptly reviewed and provided to the IRB within 48 hours of receipt. Full supporting documentation should be solicited by the investigative site even if the SAE occurred at another institution. Such documentation may include copies of relevant patient/hospital records, discharge summaries, laboratory/test results or autopsy reports.
- If, at any time after the patient has completed participation in the study (as defined in Section 0), the Investigator or study staff becomes aware of an SAE that they suspect is related to the study procedures (see Section 9.3.1), then the event and any known details must be reported promptly to the Sponsor or its designee, following the reporting instructions in Section 9.4.3.

9.4.2 Follow-Up of Serious Adverse Events

All SAEs related or possibly related to study procedures will be followed until resolution, the condition stabilizes, or the Investigator and Sponsor agree that follow up is no longer necessary.

Rules for SAE follow up apply to all patients, to the extent allowed by the patient's consent. The Investigator will ensure that follow up includes further investigations consistent with appropriate medical management to understand the nature and/or causality of the SAE. The Sponsor/designee, or regulatory authorities may also request additional information regarding an SAE at any time.

All follow-up information must be promptly reviewed by the Investigator and provided to the Sponsor within the specified timelines. Additional procedure-related SAEs may be identified during the review of follow-up information and should be processed in accordance with requirements defined throughout Section 10.

9.4.3 Reporting to Regulatory Authorities, Investigators and IRB

The Sponsor will ensure that processes are in place for provision of study-procedure-related or possibly related SAE reports to Investigators and institutional review boards (IRBs) or institutional ethics committees as required, within the specified timelines.

The NIH or its designee will submit study procedure-related or possibly related SAE reports to the Investigator as required. In the US, Investigators will report study procedure-related or possibly related SAEs to their IRB in accordance with applicable standard operating procedures and/or local reporting requirements.

Investigators must forward copies of the IRB notification to the Sponsor or its designee, when applicable.

9.5 Pregnancy Reporting

Pregnancy will be reported to the PI immediately upon receipt of this test by the subject.

10 DATA COLLECTION, QUALITY ASSURANCE, AND MANAGEMENT

10.1 Recording of Data

Copies of pertinent records in connection with the study, including all source documents, will be made available to the NIH, IRB, or its designee on request with due precaution towards protecting the privacy of the patient.

The PI may elect to have data entered by the clinical site directly into an electronic system using electronic case report forms, eCRFs.

Data will be entered by the site into the eCRFs in the electronic data capture (EDC) system that is 21 Part 11 compliant. Unless explicitly directed, blank data fields are not acceptable. Any erroneous entries made in the eCRFs must be corrected. Changes made to the data after initial entry into the eCRF will be captured via an electronic audit trail, and include the reason for change. Incomplete entries or entries needing additional explanation will be highlighted or queried to the Investigator for clarification.

Adverse events may be coded with the latest version of the medical dictionary for regulatory activities (MedDRA) available at study initiation. Similarly, prior and concomitant medications and concomitant therapies may be coded using the latest version of World Health Organization (WHO) drug available at study initiation. If used, the versions employed at study start will be maintained throughout the project.

10.2 Data Quality Assurance

Study monitors will perform source document verification according to the clinical monitoring plan to ensure there are no inconsistencies between the eCRFs and source documents. Discrepancies will be resolved in accordance with the principles of Good Clinical Practice.

10.3 Data Management

Data management will be coordinated by Galveston Clinical Research or its designated representative in accordance with the study data management plan.

11 STATISTICAL METHODS AND PLANNED ANALYSES

11.1 General Considerations

The dependent variables will include change in Body Composition (weight, Fat%), and BMI.

Independent variables will include" age, gender, ethnicity, and duration of obesity.

11.2 Determination of Sample Size

This is a pilot study and will recruit 20 potential subjects.

11.3 Analysis Sets

All study patients for whom data are available will be included in the analysis dataset. All study patients who complete the 30-day assessments will be included in the safety dataset.

11.4 Demographics and Baseline Characteristics

Demographic and baseline characteristics (age/age categories, sex, race, ethnicity, and baseline characteristics) will be analyzed using the safety analysis set. Summary statistics (n, mean, median, standard deviation, minimum, and maximum) will be reported. Information collected from the targeted medical history will be summarized in the same fashion as the other baseline characteristics.

11.5 Subject Accountability

Subject compliance and accountability will be monitored with daily text transmission for the two month and then weekly communications through completion of the first six months in this study.

11.6 Study Treatment Usage and Compliance

Subject compliance and accountability will be monitored with daily text transmission for two months and then weekly communications through completion of the first six months in this study.

11.7 Body Composition Testing

Testing will be done in compliance the testing protocol supplied by the Electrical Impedence test guidelines.

11.8 Safety Analyses

Any SAEs related or possibly related to study procedures will be listed.

11.8.1 Physical Examination and Vital Signs

Physical Examination will be performed as a screening method to exclude cardiovascular pathologies such as arrhythmia or history of myocardial infarction before initiation of the subject's participation in this study.

11.8.2 Clinical Laboratory Tests

Laboratory testing for food allergies will be conducted by the Cell Science System laboratory using the ALCAT method for all subjects.

11.8.3 Adverse Events

Only SAEs related or possibly related to invasive study procedures will be recorded.

11.8.4 Other Statistical Issues

Deviations from the statistical plan, along with the reasons for the deviations, will be described in protocol amendments, the complete statistical plan, the clinical study report, or any combination of these, as appropriate.

11.8.5 Significance Levels

Differences beyond 0.05 will be considered significant.

11.8.6 Missing or Invalid Data

N/A

11.9 Interim Analysis

N/A

12 SPECIAL REQUIREMENTS AND PROCEDURES

This protocol was designed and will be conducted, recorded, and reported in compliance with the International Conference on Harmonization/Good Clinical Practice guideline. These requirements are stated in the International Conference on Harmonization Guideline Topic E6 entitled "Guideline for Good Clinical Practice."

12.1 Institutional and Ethics Review

This protocol, informed consent form, participant information sheet, and any proposed advertising material will be submitted to an appropriate ethics committee, applicable regulatory authorities, and host institution(s) for written approval (where applicable). These documents will also be submitted to and approved by the above parties for all substantial amendments to the original approved documents (where applicable). Documentation of any applicable approval(s) and the approved consent form must be received by the Sponsor or its designee prior to enrollment of patients.

12.2 Data Monitoring Committee

The data monitoring committee will include one study investigator paired with one scientist who is not an investigator in this trial.

12.3 Changes to the Conduct of the Study or Protocol

Any changes in the study protocol, such as changes in the study design, objectives or endpoints, inclusion and exclusion criteria, and/or procedures (except to eliminate an immediate hazard) will be implemented only after the mutual agreement of the Investigator, IRB, and NIH or its designee. All protocol changes must be documented in protocol amendment(s). Protocol amendment(s) must be signed by the Investigator and approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) prior to implementation. Any changes in study conduct that result from a pending amendment will be considered protocol deviations until IRB or IEC approval is granted. Documentation of IRB or IEC approval must be returned to the Sponsor or its designee.

12.4 Investigator's Responsibilities

- The investigator will comply to guidelines provided in the following documents: Good Clinical Practice (GCP) Guidelines (ICH-E6) and the Title 45 Code of Federal Regulations (CFR) Part 46 in addition to central IRB guidelines.

12.4.1 Patient Informed Consent

Investigators must adhere to GCP, which includes ethical principles that have their origin in the Declaration of Helsinki, when developing the patient informed consent form and when obtaining consent from the patient. Written informed consent is required prior to enrollment in the study. It is the responsibility of the Investigator to document the consent process within the source documents and obtain consent using an IRB or IEC approved consent form.

The subject (20 years of age), is willing to provide written, informed consent. Confidenality will be maintained during the study. Data and information collected during this study, including information on subject's race and ethnicity, are required by government regulatory authorities and may be published but will not include any personal identity. The study will use unique study identifiers to maintain confidentiality. Any study-related records that identify patients will be kept confidential, and to the extent permitted by applicable laws and/or regulations will not be made publicly available. Any results that are published from this study will not include a patient's personal identity.

12.4.2 Case Report Forms

Copies of pertinent records in connection with the study, including all source documents, will be made available to the Sponsor or its designee on request with due precaution towards protecting the privacy of the patient.

Data will be entered by the site into the eCRFs in the EDC system. Unless explicitly directed, blank data fields are not acceptable. Any erroneous entries made in the eCRFs must be corrected. Changes made to the data after initial entry into the eCRF will be captured via an electronic audit trail, and include the reason for change. Incomplete entries or entries needing additional explanation will be highlighted or queried to the Investigator for clarification.

12.4.3 Record Retention

The Investigator is responsible for oversight and maintenance of the study records and patient source documents. These records must be readily available for audit or inspection.

The Investigator must retain study records for at least 2 years after completion of the clinical study. However, these documents should be retained for a longer period, if required by other applicable requirements (e.g., applicable local regulatory requirement) or by an agreement with the NIH, IRB, or its designee. The Investigator will contact the NIH, IRB, or its designee prior to any record destruction.

Patient records or other source data must be kept for the maximum period of time mandated by the hospital, institution, or private practice, but not less than 15 years.

If off-site archiving is used, all records must be retrieved and made available for review at the time of an audit or regulatory authority inspection.

12.4.4 Monitoring

A Monitor will remotely interface with and may visit the Investigator periodically for the purpose of monitoring the progress of this study in accordance with the protocol, GCP, and local regulations. A monitor, auditor, IRB and or/other regulatory authority, such as the United States Food and Drug Administration (FDA), would have access to study-related medical documents for the purposes of the study. Non-compliance with the protocol, GCP, and local regulations will be documented and corrective actions implemented, as necessary. It is the responsibility of the Investigator to be present or available for consultation during remote or on-site monitoring visits. In order to complete remote or on-site monitoring visits, all data pertaining to a patient's participation in this clinical investigation must be made available to the monitor.

At any time prior to, during, or after completion of the clinical study, an audit may be performed by the NIH or its designee or a representative of a national regulatory agency may choose to inspect a study site; this includes FDA. The FDA may inspect all records related to the study. Investigators must notify the NIH or its designee upon notification of inspection by a representative of a national regulatory agency. A Sponsor or designee representative will be available to assist in the preparation for study site inspections. All pertinent study data must be made available for verification, audit, or inspection purposes.

12.4.5 Study or Site Termination

If the NIH or its designee, the Investigator, or regulatory authorities discover any conditions during the study that indicate that the study or study site should be terminated, this action may be taken after appropriate consultation between the Sponsor or its designee and the Investigator. The Sponsor or its designee has the right to terminate the participation of either an individual site or the study at any time, for any reason which may include the following:

- The incidence and severity of AEs in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- Investigator(s) do(es) not adhere to the protocol or applicable regulatory guidelines in conducting this study.

- Submission of knowingly false information from the study site to the Sponsor or its designee, or regulatory authorities.

In the event that the study is terminated early, the Sponsor or its designee will provide specific guidance to investigational sites regarding the end-of-study procedures.

12.4.6 Investigational Product Control

Not applicable.

12.4.7 Disclosure of Data

All details related to the disclosure and publication of study data will be addressed in the Investigator's study contract.

11.4.9 Clinical Study Report

Reports will be made and submitted annually.

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14 APPENDIX

Figure 1. Obesity in the USA