Pennington Biomedical IRB FWA 00006218
Approved March 27, 2020

March 18, 2020

PROTOCOL

Tissue selective estrogen complex to prevent metabolic dysfunction in women

(RISE)

Principal Investigator: **Eric Ravussin, Ph.D.**
Medical Investigator: Frank Greenway, M.D.

Version Number: Eleven
Version Date: March 18, 2020

Table of Contents

BACKGROUND

With the dramatic increase in life expectancy, many women will spend a large part of their lives in a post-menopausal state. Apart from causing degeneration of the cardiovascular, skeletal and central nervous systems, estrogen deficiency also increases risk for metabolic syndrome, type 2 diabetes (T2D) and non-alcoholic fatty liver diseases (NAFLD). Overall, the role of estrogen deficiency to the pathophysiology of chronic metabolic diseases in women is emerging as a novel therapeutic challenge that parallels the increased risk associated with conventional estrogen replacement protocols. It is against this backdrop that novel therapeutic strategies targeting estrogen receptor in non-reproductive tissues may offer therapeutic benefits that reduce metabolic dysfunction associated with both ovarian failure and estrogen deficiency.

Selective estrogen receptor modulators (SERMs) are compounds that exert tissue-selective estrogen receptor (ER) agonist or antagonist activity. For example, *bazedoxifene (BZA)* is a novel SERM that exhibits estrogen agonist activity in bone but estrogen antagonist activity in breast and uterus. *Tissue-selective estrogen complexes (TSECs) are drugs in which a SERM and an estrogen are combined to produce mixed estrogen agonist and antagonist activity.*[7] The goal of a TSEC regimen containing BZA with conjugated equine estrogens (CE) is to provide the benefits of estrogen such as reducing hot flashes and vulvar–vaginal atrophy, preventing menopausal osteoporosis and promoting favourable effects on cardiovascular risk while simultaneously protecting the endometrium and breast from estrogen stimulation without the need for a progestin. The *TSEC therapy combining CE and BZA was approved in September 2013 by the Food and Drug Administration [Duavee™, Pfizer, Inc]* for treatment of postmenopausal symptoms and prevention of osteoporosis.

Dr. Mauvais-Jarvis' laboratory was a pioneer in demonstrating that estrogen protects pancreatic islet β-cell insulin secretion and synthesis, nutrient homeostasis, and survival via three estrogen receptors, ERα, ERβ and the G-protein coupled ER. They showed that ER actions were relevant to human islet function and survival, and had major clinical impacts in the diabetes field by enabling the design of a novel estrogenic peptide that targets β-cells without unfavorable gynecological effects in females. Most ***recent preliminary data, derived from a mouse model of post-menopausal metabolic syndrome, demonstrate that TSEC prevent estrogen deficiency-induced metabolic dysfunction – including obesity, T2D and NAFLD- as efficiently as CE alone*** and importantly, without causing endometrial hyperplasia. Because SERMs like Tamoxifen or Raloxifene have never shown such a beneficial metabolic effect in preclinical models and in humans, these data underscore the clinical significance of this line of investigation and they also provide a firm rationale for the next series of experiments that are proposed in this application.

We propose to assess the therapeutic potential of TSEC in preventing metabolic dysfunction in postmenopausal women and to explore the hypothesis that the metabolic effect of TSEC relies on the activation of the production and sensitivity of the hepatic hormone *fibroblast growth factor 21 (FGF21). These findings will have a direct and immediate scientific impact that* fits with the LACaTS Center theme. The knowledge that will be generated from this work will fill key gaps in our understanding of the mechanism of estrogen action on metabolic diseases that will be used for therapeutic purposes. Thus, there is direct, clinically relevant translational impact to the proposed work. As part of this LACaTS grant, Dr. Mauvais-Jarvis will continue to investigate the potential molecular mechanisms of TSEC on the metabolic syndrome in mice only.

There are several innovative features to our proposed study. ***First,*** a novel feature of our approach is that *translational studies of TSEC action in preventing metabolic dysfunction in postmenopausal women have never been performed*. A ***second,*** innovation is to propose a novel paradigm in which the metabolic actions of TSEC are *mediated via a stimulation of FGF21 production and action*.

STUDY DESIGN

Study Overview

The goal of this pilot clinical study is to perform a **randomized crossover study** to test the beneficial effect of 8 weeks of treatment with *TSEC or BZA/CE/(a clinical image of Duavee™) vs. placebo* on insulin sensitivity and energy metabolism in eight post-menopausal women. There will be a two-month washout period in-between the 2 treatments (TSEC vs. Placebo). The recruitment will be performed at the Pennington Biomedical Research Center with the help in recruiting from the Baton Rouge General Obstetrics and Gynecology Physicians (see below). The drugs will be provided by Pfizer, Inc. without charge.

Objective: Assess the therapeutic potential of TSEC in preventing metabolic dysfunction in postmenopausal women.

Primary Endpoint: Insulin sensitivity by a 2-step hyperinsulinemic euglycemic clamp

Secondary Endpoints: a) Resting metabolic rate by a metabolic cart (Deltatrac) and;
b) Body composition by DXA and MRS

Exploratory Endpoints: Molecular work (at Tulane) in animal model only.

Hypothesis

TSEC treatment will improve both whole body insulin sensitivity (high insulin dose) and liver insulin sensitivity (suppression of HGP) in postmenopausal women.

Power Calculation

Although a formal power analysis was not feasible in this pilot study, in light of the high precision that is achieved using the clamp, we can expect similar improvements to be detectable with up to 10 or less completers for whole body and hepatic insulin sensitivity respectively in this randomized crossover trial.

Timeline

After passing the screening process, the expected duration of an individual subject's participation in the study is about 26 weeks.
We anticipate that it will take up to 6 months to complete enrollment for the study.

STUDY POPULATION

Target Population

Post-menopausal women (<5y post last period) aged 50-60 years old

Number of Participants

We expect up to 10 post-menopausal women to complete this study.

Inclusion/Exclusion Criteria

Inclusion Criteria

- Post-menopausal women (<5y post last period)
- Age between 50-60y
- Symptomatic (hot flashes, vaginal dryness) or asymptomatic
- BMI 30-40 kg/m^2
- Normal mammogram past 12 months
- Physician clearance (Ob/Gyn, Baton Rouge General Physician Primary Care Network, or PBRC)
- Be willing to accept own blood during IV procedure

Exclusion Criteria.

- Amenorrhea other causes (excess androgen)
- Diabetes mellitus
- Medications: (diabetes, antipsychotics, oral steroids, weight loss drugs†)
- History of major depressive episodes requiring treatment
- Tricyclic antidepressants (TCAs)
- Current smokers, or having smoked within last 3 months
- ≤ 3 month washout of birth control pill, estrogen, and/or progestin
- Hysterectomy (total and partial)
- Contraindications to estrogen treatment ††
- Unable or unwilling to do an MRS
- Additional exclusion for optional BIA procedure only:
  - Medications: Diuretics, glitazones
  - Congestive heart failure
  - Chronic kidney disease
  - Pacemaker or other metal implants

† *other chronic medications are acceptable as long as stable for 2 months, and may need MI approval before screening.*

†† *for unusual vaginal bleeding, blood clots, hepatic disease, bleeding disorder, past/present history of breast or uterine cancer, pregnant, breastfeeding*

Recruitment

Baton Rouge General Obstetrics and Gynecology Physicians will be the primary site for recruiting patients for the study. Gynecology and the Baton Rouge General Physician Primary Care Network will also be asked to provide additional recruits through a series of educational

presentations by Dr. Evelyn K. Hayes, MD, FACOG, NCMP. At these clinics, patients are seen for routine wellness exams in addition to problem visits. Women in the target population for recruitment frequently present with symptoms of estrogen deficiency. Over the past 12 months, the Baton Rouge General Obstetrics and Gynecology practice has seen over 800 women aged 50-60 with a BMI between 30 and 40 and with menopause according to the definition adopted in this study. Further delineation of such group can identify those women who are naïve to estrogen and symptomatic with hot flashes and/or vaginal dryness. After an explanation of the study by Dr. Hayes or her staff, interested participants who meet initial screening criteria (date of final menstrual period, last mammogram, confirmation of medication use, age, BMI,) will be cleared by the primary-care Obstetrician-Gynecologist and a referral form will be sent by REDCap to PBRC. All this information will be transferred to a study coordinator at PBRC in accordance with HIPAA and eligible patients will be contacted to schedule a screening visit at PBRC.

Additionally, PBRC will be a secondary site for recruitment. Participants will be recruited continuously by our recruitment core via media advertising, listserv, health promotion events, databases, and referral sources. Similarly, once potential participants are identified via PBRC recruitment methods and recorded in PBRC tracking databases, PBRC staff will schedule a screening visit at PBRC. We anticipate no problem in rapidly recruiting the 8 women needed to complete the study.

STUDY DESCRIPTION

Location

After referral of potential participants from Dr. Hayes (and her staff) or participant recruitment via PBRC, the screening visit will be conducted in the Outpatient Unit at PBRC.
The Insulin sensitivity testing (clamp), energy expenditure testing, blood drawing will be performed in the Inpatient Unit at PBRC.

Consent Process

The informed consent process will be conducted in the Outpatient Unit at PBRC during the screening visit and will be conducted primarily by the study coordinator, but also on occasion by the study PI or by a trained staff member of the Outpatient Clinic. Written informed consent will be obtained before any procedures are performed. Potential subjects will be given ample time to read the Informed Consent and to ask questions during the screening visit.

Screening Process

Prospective volunteers will be both referred from Dr. Evelyn Hayes or via PBRC recruitment efforts (see above) and invited to the Center for a complete screening visit conducted in the morning after an overnight fast. Height, metabolic weight, waist and hip circumference as well as blood pressure will be measured. This will be followed by a complete medical history including reproductive function and medications, a physical exam, and an ECG. If eligible, a blood sample (CBC, chemistry panel with electrolytes, total testosterone, estradiol and FSH)

and a urine sample (urine analysis for general health) will be collected. Potential participants will then be interviewed by trained staff that is overseen by a licensed Clinical Psychologist to identify potential barriers to adherence and study completion. We will work with potential participants to determine if the study is consistent with their lifestyle/time demands and if adhering to and completing the study is feasible. The final enrollment will then be determined on the basis of the laboratory results (blood and urine).

Randomization

Participants will be randomized to the order in which they complete the two parts of the study (Active drug (TSEC) and placebo).

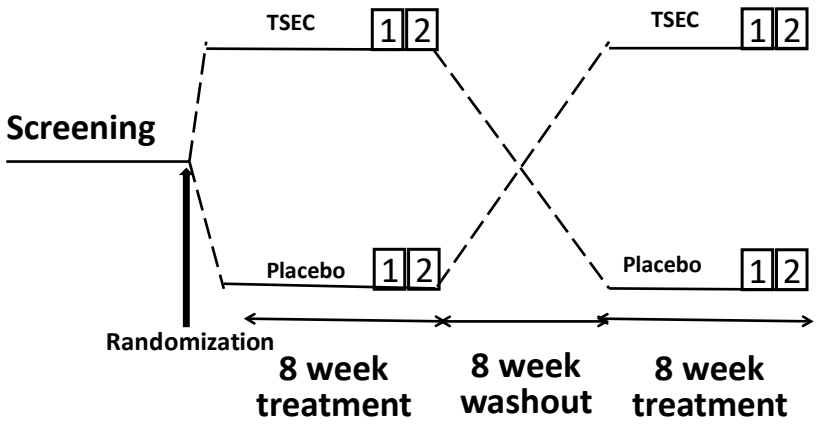
Study Visits

The study visits are described in this section, while the study procedures that will be performed during these visits are detailed in the latter section "Study Procedures".

This study involves is a randomized crossover study with two 8-week treatment period separated by an 8-week washout period. Each participant will participate in this randomized scheme with two evaluation periods (see Figure 1)

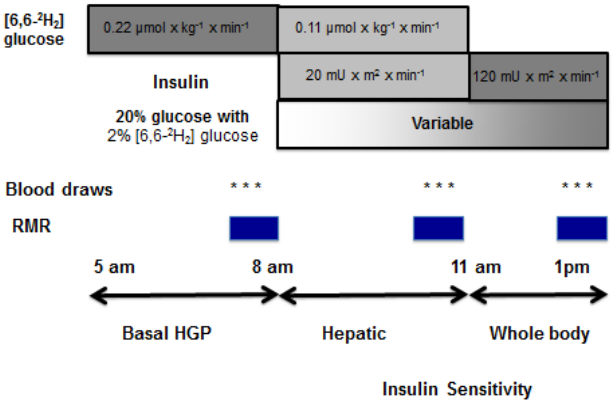
a) End of 8 weeks of treatment with PLACEBO or TSEC
b) End of 8 weeks of treatment with TSEC or PLACEBO

Figure 1. **TSEC Experimental Protocol**



After screening, enrollment and randomization (Week 0) , participants will come for a brief (<1 hour) clinic visit after 1, 3 and 5 weeks (± 3 days) into the first treatment phase (TSEC or Placebo) to get their pills, record weight, waist and hip, and vital signs and collect adverse events. During the eight week (Day 53 & 54 ± 2 days), participants will be admitted to our inpatient unit at ~4pm for measures of total body fat (DXA) and ectopic fat (intra-hepatic and intra-myocellular fat) by ^{1}H Magnetic Resonance Spectroscopy (^{1}H –MRS) as well as visceral fat (VAT by MRI). These measures will be followed by a standard dinner (see Study procedures below) at ~7pm. The following morning at ~4.45am, an IV infusion of D2-glucose will be started to measure splanchnic glucose output (SGO) over 3 hours followed by a 150 min low dose insulin clamp followed by a 2-hour high dose insulin clamp (Figure 2). Also, at this time metabolic weight, blood pressure, and pulse will be collected. At around 6am, during the baseline of the clamp procedure, an adipose and skeletal muscle biopsy will be collected. Additionally, an optional Bioelectrical Impedance Analysis procedure will be offered and, if consent is given, will be completed following voiding and metabolic weight. Participants will be discharged ~2 hours after the end of the clamp procedure.

Figure 2. CLAMP PROCEDURE



After an 8 week washout period, the exact same 8-week treatment and clinic visit protocol will be repeated, this time with the opposite treatment (Placebo or TSEC).

Study Procedures

Body Composition and Ectopic Fat

DXA scans will be performed using a GE Lunar iDXA whole-body scanner. The scan takes about 10 minutes and the radiation dose is less than 1 mrem, equal to about 12-h of background radiation. The scans are analyzed with the QDR for Windows V11.1 software. We will also use the DXA data to estimate muscle mass.

Visceral (VAT) and subcutaneous (SAT) adipose tissue (MRI): Determination of body fat, visceral fat (intraperitoneal and retroperitoneal fat), and subcutaneous fat will be obtained using a 3T GE Signa magnet with measurements from a series of 8 T1-weighted MRI slices positioned axially across the abdomen. Measuring VAT and SAT from only 8 non-contiguous slices has been shown in our hands to be as accurate as measuring it from a much larger number of contiguous slices

Intrahepatic and intramyocellular lipid by ^{1}H –MRS: The magnetic resonance scans will be performed at the Imaging Facility of the Center (part of the NORC, NIDDK funded Center Grant; PI Ravussin). The subject will be asked to lie supine on the patient table of the 3.0 T magnet for approximately 20 min. A ^{1}H body coil will be used to measure intrahepatic fat stores. A single PRESS box (30 x 30 x 30 mm) will be collected in an area of the liver that is free from heavy vascularization. Similar measures will be acquired from 3 voxels in the soleus muscle. Data are analyzed using the jMRUi software package.

2-Step Hyperinsulinemic (20 & 120 mU·m^{-2}·min^{-1}) Clamp at 90 mg/dl Glucose with RMR

The glucose clamp will be performed at the end of the two 8-week treatment periods as shown in Figure 2. At approximately 7pm the night before, participants will eat a standard meal (30% of energy requirements calculated on the basis of their (Harris-Benedict predicted RMR x 1.4; 50% CHO, 30% fat, and 20% protein). Between 4am and 7am, a catheter will be inserted into a hand vein heated at 56°C to obtain arterialized blood samples and into an antecubital vein of the contralateral arm for infusion of labeled glucose. Approximately 30 minutes after inserting the IV lines, a skeletal muscle biopsy and an abdominal adipose tissue biopsy will be collected. After baseline blood samples are obtained, [6,6-$^2H_2$]glucose (22 μmol/kg prime and 0.22 μmol·kg^{-1}·min^{-1} constant infusion basal) will be started between 4am and 7am. Three hours later, the 2-step euglycemic (20 and 120 mU m^{-2} min^{-1}) hyperinsulinemic clamp is initiated and continued for 5 hours. Euglycemia (90 mg/dl) is achieved by a variable infusion of 20% dextrose enriched to approximately 2.0% with [6,6-$^2H_2$]-glucose to minimize changes in glucose isotopic enrichment. The plasma insulin concentrations achieved with these insulin infusion rates provide optimal ranges for evaluating insulin's effect on hepatic glucose production (HGP) (20 mU m^{-2} min^{-1}) and glucose uptake by skeletal muscle (120 mU m^{-2} min^{-1}). The infusion of [$^2H_2$]-glucose will be decreased by 50% (0.11 μmol·kg^{-1}·min^{-1}) of basal during stage 1 and completely during stage 2 (full suppression of HGP). Three blood samples will be taken during the last 30 min of each step and at each step including baseline, a blood sample will be drawn to be archived (20 ml at baseline and 10 ml at steps 1 and 2).

Calculations. At low dose insulin infusion, plasma insulin levels are expected to increase to ~50 μU/ml, and HGP is expected to be ~50% suppressed in these participants. During this steady state (Rd), peripheral glucose uptake equals the glucose infusion rate (GINF) plus the measured residual HGP (Rd = St-state GINF + residual HGP). At 120 mU m^{-2} min^{-1} of insulin, HGP will probably be totally suppressed, and Rd = Steady State GINF. At each step, insulin sensitivity (SI) will be calculated using the formula: SI = Rd / (St-state insulin level - basal insulin level).

Resting metabolic rate (RMR) will be measured 3 times during the clamp procedure. At each step, O_2 consumption and CO_2 production will be measured by indirect calorimetry for 40 min (last 30 min will be used for calculations) using a metabolic cart (DeltaTrac, SensorMedics, Yorba Linda, CA). Patients will void before the test, and urine will be collected to determine urinary nitrogen and substrate oxidation.

Fat and muscle biopsy

Fat biopsy will be collected during the baseline of the clamp for cell sizing and numbering using osmium fixation, and to extract DNA and RNA for future work. The biopsy will be obtained as follows: After cleansing the skin on the right side of the abdomen with povidone-iodine solution, and placing a sterile drape, topical anesthesia will be administered with a mixture containing 50%/50% lidocaine 2% and bupivacaine 0.5%. A 0.75-cm incision will be made in the skin and a Bergstrom needle inserted to collect, under aspiration, approximately 500-750 mg of adipose tissue. The sample will be washed in sterile PBS and 75% of it snap frozen in liquid nitrogen, the remaining placed in osmium tetra oxide. Upon completion of the biopsy, the incision will be closed with a sterile bandage, and antibiotic ointment / sterile dressing applied.

Muscle biopsy. Similarly, after the adipose tissue biopsy, a Vastus Lateralis muscle biopsy will be performed using the technique of Bergstrom in order to measure the impact of treatment on insulin signaling pathway gene expression, measure in vitro the impact on glucose uptake, and to look at skeletal muscle morphology after TSEC vs. placebo treatment. After cleansing the skin with povidone-iodine solution, the skin, adipose tissue and skeletal muscle

fascia of the right leg will be anesthetized using a 50%/50% mixture of bupivicaine 0.5% and lidocaine 2%. The skin will be incised (0.75cm) with a #11 scalpel. The fascia fibers are separated with the blunt edge of the scalpel and the Bergstrom needle inserted into the vastus lateralis. After suction is applied, approximately 100 mg of tissue is cut and removed. Several passes will be used to obtain at least 250mg of muscle. Pressure is applied and the skin is closed with sterile tape. After cleaning the sample, the muscle will be snap frozen in liquid nitrogen and stored for subsequent analysis.

Bioelectrical Impedance Device (BIA) (optional procedure)
Participants should remain resting in the supine position for 10 minutes prior to testing, after voiding, in the fasting state. Once a participant's Subject ID, date of birth, age, sex, height, and weight are entered into the device, the procedure can be conducted. The BIA device automatically tests and analyzes with imbedded software. BIA will be performed according to the recommendations (ESPEN Guidelines, Kyle et al. Clin Nutr 2004). Two disposable BioTekna electrodes will be applied to the dorsal surface of the right hand: the first (injector electrode) on the distal end of the third metacarpal bone between the 2nd and 3rd finger (metacarpophalangeal joint), and the second (sensor electrode) between the distal prominences of the radius and ulna of the wrist (radio-ulnar joint). Another couple of electrodes will be placed on the dorsal surface of the right foot: the first electrode on the distal end of the third metatarsal bone between the 2nd and 3rd toe (metatarsophalangeal joint), and the second electrode) between the medial and lateral malleolus of the ankle (tibiotarsal joint).
The distance between the electrodes is 5 cm. Two pairs of cables will be connected to the right hand (it is irrelevant which of the two pairs of cables). Similarly, the other pair of cables will be connected to the right foot. The red alligator clip shall be connected to the injector electrode (metatarsophalangeal joint and metacarpophalangeal joint), while the black clip to the sensor electrode (tibiotarsal joint and radio-ulnar joint). Note: the two pairs of cables should not touch or cross each other, or touch the floor. Failure to observe these precautions may distort the test results.

DATA MANAGEMENT

Data and Biospecimen Storage

Electronic data will be stored securely on computers at PBRC. Paper forms will be kept securely in locked storage at PBRC. Blood samples will be stored in the Clinical Chemistry Core at PBRC. Specimens will be labeled with a unique de-identification code if transported outside of Pennington Biomedical.

Statistical Analysis

Using SAS® Version 9.4 and/or other statistical software, the data will be analyzed using mixed model or other appropriate statistical approaches and using post-hoc comparison adjustments for multi-testing. Descriptive analysis will also be performed. Statistical significance is set at $\alpha=0.05$.

Confidentiality and Privacy

Records that identify study participants will be kept confidential as required by law, and every effort will be made to maintain the confidentiality of participants' study records. Except when required by law, study participants will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in records disclosed outside of Pennington Biomedical Research Center.

All biological specimens will be stored and analyzed at Pennington by the Clinical Chemistry Core, and will be labeled with a unique code number ("A number"). Some samples may be analyzed by outside labs or outside personnel. All samples analyzed externally from Pennington Biomedical will be sent with no identifying information and in compliance with Pennington Biomedical policies and procedures.

To protect subjects' privacy interests and to make subjects feel at ease, all participants will be assured of their anonymity and confidentially. In addition, the amount of personal information collected by the study will be minimized and limited to the study's endpoints; all data will be used only for research purposes.

Access to participants' data and biological specimens will be limited only to the study's investigators, including investigators external to Pennington Biomedical, to clinical support staff for this study, and to the overseers of clinical facilities at Pennington. This will be ensured through stringent security measures (assured by the Research Computing Core), including the use of subject ID numbers, storage of medical records in locked areas, and electronic security systems required by HIPAA.

BENEFITS, RISKS, AND SAFETY

Potential Benefits

Given the short duration of the study, we do not promise any benefits to participants from participating in this study. Possible benefits to participants include learning about their current health status.

Risks

This study involves the following risks to subjects:

- **Risks associated with TSEC (Bazedoxifene/Conjugated Estrogens (BZA/CE):** The most common side effects reported in at least 1 out of 100 postmenopausal women receiving BZA/CE include abdominal (stomach) pain, vaginal discharge, vaginal yeast infection, and leg cramps. Other adverse events reported in less than 1 out of 100 subjects were gall bladder disease and increase in liver function tests. More serious but rare side effects associated with the use of BZA/CE included venous thromboembolic events (blood clot in a vein of the legs or lungs), reported in less than 1 out of 100 subjects. If you experience sudden pain in legs or difficulty breathing, notify the Medical Investigator immediately.

  Precautions
  You may be allergic to conjugated estrogens or bazedoxifene. If you experience allergy symptoms such as rash, hives, or itching, notify your study doctor immediately.
  All estrogens and SERMS should be stopped prior to and during prolonged

immobilization (such as bed rest, surgery or long-distance travel). It is recommended that study drug be discontinued four to six weeks prior to prolonged immobilization.

You may not use hormone products during the study; including any oral, transdermal, vaginal or other products containing estrogens, progestins, androgens, testosterone, DHEA or SERMs.

- **Blood Draws:** There is the possibility of discomfort, pain, and bruising at the arm vein or site where the needle is inserted. There is also a small risk of bleeding and a very small risk of infection at the site of the blood draw. Aseptic (sterile) technique and trained personnel minimize these risks.

- **DXA:** The DXA scan involves extremely low levels radiation exposure. This technique has been demonstrated to be safe and approved for children. There are no known risks of the body scan.

- **MRI & MRS:** There are no known serious risks associated with MR studies provided that prescreening is performed to exclude individuals with internal ferromagnetic foreign bodies. MR prescreening will be performed according to PBRC policy and procedure and as part of the inclusion exclusion criteria. PBRC policy includes informed consent prescreening, a pre-screen by the study coordinator, a screening questionnaire reviewed by the MR technologist with the participant, and a hand wand search for ferromagnetic bodies where necessary by procedure. Claustrophobia is a potential non-serious adverse event. The proposed RF energy deposition is within limits set forth by the FDA [calculated on the MR instrument as SAR].

- **Two-Step Clamp Procedure:** The use of sterile procedures and good technique will minimize the risks. The intended site of catheterization will be cleaned thoroughly with an antiseptic and, after insertion, the area will be dressed so that it is not in contact with the outer environment. There has been no incidence of catheter infection in any of the hundreds of experiments we have already performed using this technique. A risk during the measurement of insulin sensitivity is hypoglycemia (low blood sugar) which will be minimized by: 1) constant supervision by a qualified physician at all times during the procedure; 2) constant monitoring of blood glucose during the procedure (every 5 min) and after (every 15 min for 2 hr); and 3) immediate availability of corrective measures (IV 50% dextrose). There is no risk to the measurement of RMR by indirect calorimetry during the different steps of the clamp.

- **Fat biopsy:** There is a small risk of infection, bleeding, and scarring of the skin at the site of the biopsy. Our inpatient medical team has performed more than 500 subcutaneous adipose biopsies.

- **Muscle biopsy:** Muscle biopsy risks include: pain from the injecting local anesthesia and cramping during the biopsy, bleeding, scarring of the skin at the site of the biopsy, cutaneous anesthesia from cutting a subcutaneous sensory nerve (1 in 70), the latter is almost always temporary but occasionally can become permanent. Our inpatient team has performed more than 1,000 muscle biopsies with no serious complications.

- **Bioelectrical Impedance Analysis (BIA):** There is no risk associated with the BIA measurement provided that prescreening is performed to exclude individuals with medical implants such as pacemakers or metal joint replacements. Measurements will not be performed on any subject who is pregnant, and all females should inform the technologist if there is any possibility that they are pregnant.

In addition to the risks listed above, participants may experience a previously unknown risk or side effect.

Monitoring Subject Safety

Participants will be asked about any adverse events following each stay in the respiratory chamber. Any instances adverse events and safety concerns will be documented and reported to the PBRC IRB per standard procedures.

Once every 4 months, the PI will review the progress and safety of the study with the MI, and write a summary of the results of those discussions as applicable.

Compensation

Participants will be compensated $1000 if they complete the entire study. $350 of the compensation will be paid at the end of their first phase treatment and the remaining $650 at the end of the study. There is no compensation available for research-related injury.

Withdrawal of Subjects

There is no risk associated with withdrawal from TSEC. Participants may be withdrawn from the study if either the PI or MI feels that their continued participation would jeopardize either the subject's health or the results of the study.