THE PHYSIOLOGIC EFFECTS OF INTRANASAL OXYTOCIN ON SARCOPENIC OBESITY

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Clinical Sites:

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List of Abbreviations

OGTT – Oral Glucose Tolerance Test
SPPB – Short Physical Performance Battery
SIADH – Syndrome of Inappropriate Antidiuretic Hormone Secretion
TUG – Timed Up and Go
Mini-Cog – Mini-Cog test - Screening for Cognitive Impairment in Older Adults
MoCA – Montreal Cognitive Assessment
GDS – Geriatric Depression Scale
CES-D – Centers for Epidemiologic Studies Depression Scale

Study Summary

Title	The Physiologic Effects of Intranasal Oxytocin on Sarcopenic Obesity
Protocol Number	OXY-SAR-001
Phase	II
Methodology	Eligible subjects self-administer 24 IU intranasal oxytocin q.i.d. for 8 weeks. The study will examine whether the intervention will promote weight loss and preserve muscle mass, thereby preserving and/or improving physical function in older subjects with sarcopenic obesity.
Study Duration	12 weeks ± 1 week
Study Center(s)	UTHSCSA, UTHealth (Houston), UTMB (Galveston)
Objective	1) To assess the effect of intranasal oxytocin administration on body weight, adiposity, muscle mass, and glucose tolerance in older adults. 2) To assess the effect of intranasal oxytocin on physical function and physiologic parameters of insulin signaling, inflammation, and muscle regeneration in older adults.
Number of Subjects	36 completers (3 sites, up to 15 at each site, allowing for attrition)
Inclusion Criteria	Age ≥60 years BMI 30.0-43.0 kg/m^2 Sedentary (≤2 strenuous exercise/week) Gait speed < 1 meter/second
Exclusion Criteria	1) Diabetes with Hemoglobin A1c (HbA1c) ≥ 6.5 and/or taking medications known to effect glucose homeostasis 2) Significant heart disease (history of MI within the last year or New York Heart Classification grade III-IV) 3) Poorly controlled hypertension (SBP > 170 or DBP >95 mm/Hg) 4) Anemia (Hematocrit <34%) 5) Renal Disease (glomerular filtration rate [GFR] < 30 mL/min, abnormal serum sodium levels, ≥ 5 white blood cells or ≥ 5 red blood cells on urinalysis) , or physical exam findings indicative of fluid imbalance; individuals with underlying disorder of sodium/water balance, such as SIADH, diabetes insipidus, or psychogenic polydipsia) 6) Liver Disease (AST/ALT/AlkPhos > 2 x upper limit of normal) 7) Use of systemic steroid, androgens, or anti-coagulants 8) Active/unstable conditions: inflammatory, thyroid, autoimmune, gastrointestinal (GI), hematologic, or neoplastic disorders (Exclude subjects with clinical lab values outside the normal range (other than as specified above)) 9) Individuals with underlying seizure disorder or underlying neurologic disorder that increases seizure risk 10) Cognitive impairment (MiniCog <3), unstable mental illness or GDS > 7, substance abuse, or history of eating disorder
Study Product, Dose, Route, Regimen	Intranasal Oxytocin 24 IU self-administered q.i.d. (3 puffs each nostril 4x/day) for 8 weeks
Statistical Methodology	Generalized linear mixed effects model will be used to evaluate the effect of oxytocin on the change of each continuous measure. The effect of oxytocin will be assessed by whether the time by oxytocin interaction is significantly different from 0 with a 2-sided p-value<0.05

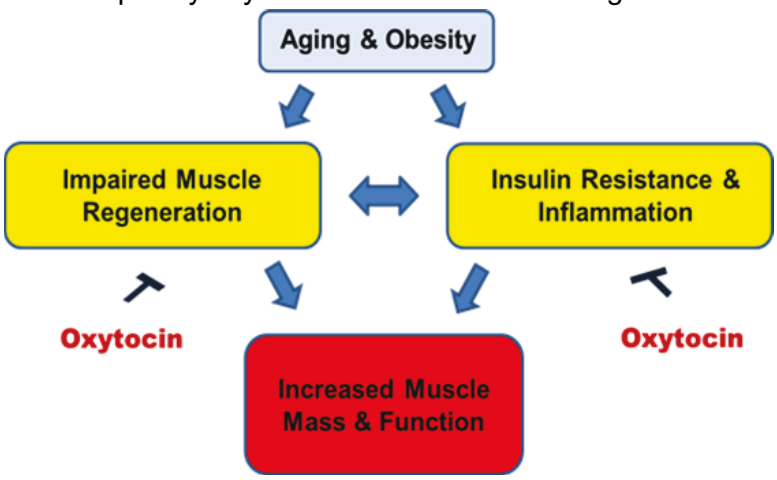
1. Introduction

This document is a protocol for a human research study. This study is to be conducted according to Good Clinical Practice guidelines as adopted by FDA, applicable government regulations, and Institutional research policies and procedures.

1.2. Background

Recent small studies in humans have shown that intranasal oxytocin reduces appetite, caloric intake, body mass index (BMI), and waist circumference (WC), while simultaneously improving physiologic parameters such as fat and carbohydrate utilization, respiratory quotient, and total cholesterol[1-3]. In a study of 25 young-middle aged (18-45 years old) men and across a spectrum of weights (13 normal-weight and 12 overweight/obese), a one-time intranasal dose of 24 international unit (IU) oxytocin resulted in reduced total caloric intake and reduced fat intake at a subsequent meal[1]. This one-time dose of oxytocin also resulted in reduced cholecystokinin (an anorexigenic hormone), fasting insulin and homeostatic model assessment of insulin resistance (HOMA-IR). Another longer term, 8-week study of 24 IU intranasal oxytocin four times daily in 24 young-middle aged adults (60% women, mean age 35 years) resulted in reduced body weight, BMI, WC, and total cholesterol [2].

Oxytocin is a nine amino acid polypeptide produced by the supraoptic and paraventricular hypothalamic nuclei and released into the circulation via the posterior pituitary gland. Oxytocin induces uterine contractions during delivery and promotes lactation in women; however, oxytocin continues to be produced and released into the circulation in men and non-pregnant women and its plasma concentration decreases with age[4]. The physiologic relevance of oxytocin in men and non-pregnant women is not clear, as oxytocin administration does not induce uterine contractions or lactation in these instances. In the brain, oxytocin is thought to play a role in aspects of social cognition and fostering prosocial behaviors. Consequently oxytocin administration is being evaluated for the treatment of a variety of mental disorders, including autism, depression, and anxiety[5]. Oxytocin regulates feeding and metabolism at multiple sites and through multiple mechanisms involving both central and peripheral mechanisms[6]. It has recently been demonstrated that oxytocin also suppresses appetite in both rodents[7] and humans [1,3]. These effects are likely mediated by oxytocin receptors that are expressed on gastric vagal nerve endings and throughout the gastrointestinal tract[3]. Intranasal oxytocin is safe and well tolerated[8]



1.3. Significance

With aging, the capacity of human tissues to maintain homeostasis and regenerate gradually declines and eventually fails, leading to degenerative age-related diseases and geriatric syndromes [9]. Reduction in muscle mass in humans begins in the third decade of life, resulting in a progressive decrease in strength[10]. Muscle aging is characterized by a deficiency in muscle regeneration after injury and by muscle atrophy leading to altered muscle function. The limiting step in muscle regeneration appears to be reduced activation of the muscle stem cells with age[11]. To regenerate muscle, these stem cells break quiescence and proliferate to form new myofibers. Stem cells from aged muscle have the potential to repair damaged muscle, but are reversibly inhibited from doing so by the effects of aging. Interestingly, aged muscle stem cells can be rescued for tissue repair by a number of experimental methods including oxytocin exposure.

1.4. Preliminary Data

Recent work in mice showed that short-term systemic oxytocin delivery restores muscle regeneration in old mice by improving aged muscle stem cell function [4]. This work also demonstrated that oxytocin acts directly on muscle stem cells (in vitro and in vivo) and that the pro-myogenic effects of oxytocin are mediated by MAPK/ERK signaling. The effect of oxytocin on skeletal muscle in humans has not yet been studied to our knowledge.

1.5. Investigational Agent

Intranasal oxytocin was marketed in the U.S. beginning in 1957 and used in the 1960's in the postpartum period for milk let-down. It was taken off the market in 1995 in the U.S., but it is still available in Europe. There are several clinical trials now listed on clinicaltrials.gov studying the use of intranasal oxytocin for autism, dementia, and other psychological and developmental conditions.

1.6. Dose Rationale and Risk/Benefits

With assistance from the College of Pharmacy of University of Texas at Austin, we performed a literature review of the safety and side effect profile of intranasal oxytocin. To summarize, the studies reviewed that studied lactation did not have any significant adverse events or complications related to the use of intranasal oxytocin. The side effects noted with intranasal oxytocin were rare instances of headache, nausea, and allergic dermatitis (between 1/1000 and 1/10,000 occurrences, and occasional abnormal uterine contractions (between 1/100 and 1/1000 occurrences). In our study we expect fewer uterine contraction occurrences since none of the women enrolled will be postpartum and all will be post-menopausal. In studies that examined the use of intranasal oxytocin for conditions other than lactation, a recent article on this subject was also recently published [22]. This article reviewed 38 trials and 3 case reports of both men and women. Mild adverse events were reported by 279 out of 1529 subjects and consisted of light headedness, vertigo, drowsiness, sleepiness, dry mouth, nasal irritation, runny nose, abdominal pain, anxiety, euphoria, increased energy, relaxation, calm sensation, and headache.

In this study we propose to use intranasal oxytocin to examine its physiologic and metabolic effects. We propose to use the same dose as recently reported by Zhang et al. [2]. In this recently conducted study, 24 subjects were randomized to intranasal oxytocin (24 IU QID) or placebo for a period of 8 weeks. They demonstrated that there was no cardiovascular, hepatic or renal dysfunction related to the use of intranasal oxytocin. In fact, they noted improved hepatic function in the oxytocin group. Based on the review of the literature and recent published studies indicating the safe use of intranasal oxytocin, we feel that intranasal oxytocin can be safely administered in a research study to examine its physiologic effects such as we propose.

2. Study Objective

a) To assess the effect of intranasal oxytocin administration on body weight, adiposity, muscle mass, and glucose tolerance in older adults.

b) To assess the effect of intranasal oxytocin on physical function and physiologic parameters of insulin signaling, inflammation, and muscle regeneration in older adults.

3. Study Design

3.1. Subjects

Population: 36 obese (BMI 30.0-43.0 kg/m2) sarcopenic (see below) subjects aged ≥60 years will be randomized to either intra-nasal oxytocin or placebo (saline) for 8 weeks. Oxytocin (Syntocinon; Novartis-Switzerland via

Victoria Apotheke Zürich) will be dosed at 24 IU four times a day (before each meal and at bedtime)[2].

Additional inclusion criteria (as shown in Table 1), are stable body weight, sedentary lifestyle (within the last 6 months), and sarcopenic obesity.

Sarcopenic obesity is defined using a modified consensus criteria of gait speed <1 meter/second [12]. Exclusion criteria are shown in Table 1. Muscle mass will be measured as an outcome variable, however, it will not be used as a basis for inclusion.

able 1. Inclusion and exclusion criteria
Inclusion Criteria
Age ≥60 years
BMI 30.0-43.0 kg/m2 with stable body weight (±2%) in past 3 months
Sedentary (≤2 strenuous exercise/week)
Gait speed < 1 meter/second
Exclusion Criteria
Diabetes with HbA1c ≥ 6.5 and/or taking medications known to effect glucose homeostasis, Significant heart disease (history of MI within the last year or New York Heart Classification grade III-IV) Poorly controlled hypertension (SBP >170 or DBP >95 mmHg), Anemia, Renal Disease (as specified in Section 4 below), or Liver Disease
Use of systemic steroids/androgens or anticoagulants
Active/unstable inflammatory, thyroid, autoimmune, gastrointestinal (GI), hematologic or neoplastic disorders (Exclude subjects with clinical lab values outside the normal range (other than as specified above))
Cognitive impairment (Mini-Cog <3), unstable mental illness or GDS > 7, substance abuse, or history of eating disorder

3.2. General Design

Randomized, placebo controlled Phase II pilot study to be conducted at 3 clinical sites: UTHealth San Antonio, UTHealth in Houston, and UTMB in Galveston.

3.3. Study Endpoints

Primary outcomes: Change in weight, adiposity, muscle mass, and physical function (gait speed, strength, SPPB). This is a pilot study which will examine change in these outcomes prior to and after treatment.

Secondary outcomes: Change in glucose tolerance as measured by OGTT, measures of systemic inflammation (TNF-α, C-reactive protein, IL-6, adiponectin), muscle inflammation (IL-1β, IL-6, MCP-1, TNF-α mRNA), and muscle stem cell number and proliferative capacity. This is a pilot study which will examine change in these outcomes prior to and after treatment.

Table 2. Outcome variables	
Primary Outcome	**Secondary Outcome**
Adiposity (BMI, DEXA)	Glucose tolerance (OGTT)
Muscle mass (DEXA, MRI, muscle fiber CSA)	Systemic and muscle inflammation
Physical function (gait speed, muscle strength, balance)	Muscle stem cell number and proliferative capacity

3.4. Potential Risks to Subject Safety

Drug Administration.

Rare: (observed in <1% of subjects)

- Nausea
- Allergic dermatitis
- Occasional abnormal uterine contractions (unlikely in post-menopausal subjects)
- Hyponatremia and or fluid imbalance

Mild: (observed in 10-20% of subjects)

- Light-headedness
- Vertigo
- Drowsiness
- Sleepiness
- Dry mouth
- Nasal irritation
- Runny nose
- Abdominal pain
- Anxiety
- Euphoria
- Increased energy
- Relaxation
- Calm sensation
- Headache

Blood withdrawal.

All studies involve the withdrawal of blood. Any subject who has donated blood in the previous two months will not be studied. The subjects will be instructed not to donate blood for two months after the study. Any subject with a hematocrit of < 34% will not be studied. Blood will be collected for safety and for research purposes at screening and study visits. Upon subject-reported history of any condition (known or suspected) that would be exclusionary, investigator may choose to order appropriate additional clinical labs to ensure eligibility.

IV lines.

Catheters will be placed in an antecubital vein or a hand vein for the OGTT. Local hematomas occur in about 1% of catheterizations. Infection is possible (<1%), but we have not experienced this complication.

Muscle biopsy.

At the time of biopsy, subjects may feel pain, discomfort, or pressure (variably described by different subjects) for about 5-10 seconds. Pain or discomfort ceases as soon as the cannula is withdrawn. Local hematomas occur in <2% of subjects. One patient experienced a moderately painful hematoma that resolved within 2 weeks (0.1%). About 1 in 50 subjects report non-clinically evident numbness or altered sensation at the biopsy site, which is transient.

There is the possibility that a future biopsy may not be done at the discretion of the PI in case the subject did not tolerate well a prior biopsy.

Radiation exposure.

Subjects will be exposed to a small amount of radioactivity during DEXA.

4. Subject Selection and Withdrawal

4.1. Inclusion Criteria

- Both genders.
- Age ≥ 60 years.
- All ethnic groups.
- BMI 30.0-43.0 kg/m2 with stable body weight (±2%) in past 3 months.
- Sedentary (≤2 sessions of strenuous exercise/week)

- Gait speed < 1 meter/second
- Labs: HCT ≥34%, GFR ≥ 30 mL/min, liver enzymes (AST < 2 x upper limit of normal, ALT < 2 x upper limit of normal, alkaline phosphatase < 2 X upper limit of normal), normal electrolytes, urinalysis with < 5 red blood cells (RBCs) and < 5 white blood cells (WBCs) , and normal PT and PTT. If additional labs are drawn to ensure eligibility, inclusion requires lab results fall within the normal range per local lab. Note: Some sites may draw additional clinical labs per local standard operating procedure, which are not collected as research data for this study.

4.2. Exclusion Criteria

- Diabetes with HbA1c ≥ 6.5 and/or taking medications known to effect glucose homeostasis
- Significant heart disease (history of MI within the last year or New York Heart Classification grade III-IV)
- Poorly controlled hypertension (SBP >170 or DBP >95 mmHg)
- Anemia (HCT <34%)
- Renal Disease (GFR < 30 mL/min), abnormal serum sodium levels, urinalysis with ≥ 5 RBCs or ≥ 5 WBCs, or physical exam findings indicative of fluid imbalance; individuals with underlying disorder of sodium/water balance, such as SIADH, diabetes insipidus, or psychogenic polydipsia
- Liver Disease or abnormal liver enzymes (AST > 2 x upper limit of normal, ALT > 2 x upper limit of normal, alkaline phosphatase > 2 X upper limit of normal)
- Use of systemic steroids or androgens
- Individuals with underlying seizure disorder or underlying neurologic disorder that increases seizure risk
- Current treatment with anticoagulants (i.e. warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsies.
- Active/unstable conditions: inflammatory, thyroid, autoimmune, gastrointestinal (GI), hematologic, or neoplastic disorders. Exclude subjects with clinical lab values outside the normal range (other than as specified above).
- Cognitive impairment (Mini-Cog <3)
- Unstable mental illness (GDS > 7)
- Substance abuse
- History of eating disorder
- Subject is considered unsuitable for the study in the opinion of the investigator for any other reason

4.3. Subject Recruitment and Screening

Subjects will be recruited through the institutional volunteer registries and advertisement (newspaper, flyers, etc.). All study visits will be performed in the Clinical Research Centers (CRCs) of UTHealth San Antonio, UHS, UTMB and UTHealth-Houston. At some sites, subjects will call-in to an advertised number and will be pre-screened using an institutionally-approved process. Callers then provide permission for a research team member to follow up with phone screening using an IRB-approved form (sample contained in Attachment D section) and staff will schedule the screening visit if the potential subject agrees If potentially eligible following the telephone screening process, an in-person screening visit will be conducted.

Screening evaluation will follow informed consent at Visit 1 and will include: medical history, physical examination (including anthropometric measurements), Mini-Cog, and screening blood tests (complete blood count, chemistry, lipid profile, hemoglobin A1c, coagulation tests, urinalysis).

4.4. Early Withdrawal of Subjects

4.4.1. When and How to Withdraw Subjects

Subjects have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to his or her future medical care by the physician or at the institution.

Withdrawal of full consent for a study means that the subject does not wish to receive further investigational treatment and does not wish to or is unable to continue further study participation including any follow-up in person, by phone, through third parties including relatives or friends, via discussion with other treating physicians, and by use of medical records; subject data up to withdrawal of full consent will be included in the analysis of the study. Any subject may withdraw full consent to participate in the study at any time during the study. The Investigator or sub-investigator will discuss with the subject appropriate procedures for withdrawal from the study.

Final Study Visit for Withdrawn Subjects

At the time of receiving notification of intended withdrawal, researchers will ask subject to complete a final visit. The informed consent document advises subjects to discuss withdrawal decision with the principal investigator. This visit includes a repeat history and physical examination, weight, functional testing, Bioimpedance (UTMB site only), DEXA, questionnaires, and laboratory tests (safety labs, inflammatory markers). Subjects may choose to complete all or part of the final study visit.

4.4.2. Data Collection and Follow-up for Withdrawn Subjects

If subjects are withdrawn prematurely from the study, appropriately designated research staff will make efforts to collect at least survival data throughout the protocol defined follow-up period for that subject.

Investigator will consult with Study Statistician with regard to any incomplete data set as compared to the full data set that fully supports the analysis. If a subject withdraws consent to participate in the study, attempts will be made to obtain permission to record survival data up to the protocol-described end of subject follow-up period.

Investigator and designated research staff make it a high priority to obtain survival data on all subjects lost to follow up. Lost to follow up will be defined as a subject missing 2 or more consecutive visits, not answering or responding to 3 follow up phone calls to subject or emergency contacts, or returned receipt of 1 certified letter.

5. Study Drug

5.1. Description

See Attachment D. Victoria Apotheke Zurich: Syntocinon, Drug information

5.2. Treatment Regimen

Subjects are instructed to administer 3 sprays per nostril (24 IU) 4 times a day for 8 weeks (56 days).

Method for Assigning Subjects to Treatment Groups

The randomization key will be designed by a biostatistician or sponsor's designee not directly engaged in the research and key will be provided to the research pharmacy for coding study drug. After the key is created, the research pharmacy will keep the randomization key in case unblinding is necessary at any point during the

study.

Subject ID numbers will be generated in REDCap based on the data management design. The Study Coordinator at each site stores the Enrollment key, which is kept in a locked cabinet or on a secure server with limited access.

Study staff maintains data records, and specimens, in a de-identified manner using only the unique Subject ID/Randomization number. Only the secure source documents may contain subject identifiers (name, DOB, SSN, etc.)

5.3. Preparation and Administration of Study Drug

Intranasal Administration Protocol.

To prime a new bottle:

- Hold the bottle upright and away from you, then pump it several times until you see a faint spray.
- Do not prime the pump again before each daily use.

To use the nose spray:

- Before using the spray, blow your nose gently to clear the nostrils.
- Keeping your head in an upright position, carefully place the nozzle into one nostril, aiming straight back toward the base of the skull. Do not aim the nozzle straight up toward the top of the head.
- Press the pump toward the bottle and spray as directed.
- Do not inhale while spraying. Breathe or sniff gently through the nose after spraying and do not blow nose again for at least 30 minutes.
- To keep the nosepiece clean, wipe it with a clean tissue and replace the dust cap after use.

Subject Compliance Monitoring

A drug diary will be provided to all subjects at the time of drug dispensing at Visit 3 (or Visit 4 if optional MRI is completed). Subjects will be instructed to log their use of the study drug daily and bring the drug diary to each visit.

5.4. Prior and Concomitant Therapy

Exclusionary medications:

Drugs that affect glucose homeostasis, systemic corticosteroids, androgens.

Treatment with anticoagulants (warfarin). Aspirin (up to 325 mg) and clopidogrel will be permitted if these can be held for seven days prior to the biopsies.

5.5. Packaging

Nature and Contents of the Container

Victoria Apotheke Zurich will supply blinded product and placebo, package for refrigerated shipping, and manage customs and importation documents.

5.6. Receipt, Storage and Handling of Study Drug

Local site Research Pharmacy will be responsible for receipt, inventory, storage, and destruction of Study Drug.

Receipt of Drug Supplies

Any damaged or unusable study drug in a given shipment will be documented by the receiving Pharmacy. The Research Pharmacist will notify the IND Sponsor of any damaged study drug.

Special instructions upon receipt

Open package, review and compare to shipping documents, log inventory counts and refrigerate.

Storage

Nasal spray: Store in a refrigerator (2–8°C or 35.6°F–46.4F).
Shelf life: After opening the bottle, store the nasal spray at room temperature (15–25°C) and use within 1 month.

Dispensing of Study Drug

Designated staff from the site Research Pharmacy maintains the Drug Accountability Logs to track how, when and to whom the investigational drug was prescribed, dispensed and assigned to subjects. Study clinical staff will keep drug administration records regarding drug that is damaged or wasted.

Routine study drug administration and reconciliation will be performed based on clinical site policy and standard operating procedures.

Return or Destruction of Study Drug

The procedures for final reconciliation of the site's drug supply at the end of the study will be in accordance with local site Pharmacy standard operating procedures. There are no special precautions cited for disposal of Intranasal Oxytocin.

6. Study Procedures

Study visits. All studies will be performed in the Clinical Research Center at the designated site(s).

6.1. Visit 1 (Consent, Screening for Eligibility, and Baseline Testing – Day 0)

Subjects present in fasting state to the site CRC at appointed time for informed consent processing, medical history and physical exam to assess inclusion and exclusion criteria, including measurements of weight, gait speed, waist circumference, hematology, coagulation studies, chemistry with liver function, lipid panel, urinalysis, and serum and urine osmolality. A 2-hour OGTT will be performed to assess glucose tolerance. . Screening mental status assessments (Mini-Cog and depression scale) will be performed to ensure valid consenting process. If subjects meet initial eligibility criteria based on walking speed and history & physical examination. Individuals meeting eligibility will proceed to a 2-hour OGTT. During the 2-hour OGTT, baseline testing will be performed, including labs, food recall and appetite assessments and psychometrics. After the 2-hour OGTT, the participant will be given a meal or substantial snack (per local clinical research unit) and continue with the functional testing. A screening window of 30 days is allowed so that all laboratory and test results can be returned and reviewed.

Estimated length of this visit is 6 hours.

- Vital signs will be assessed for blood pressure, heart rate, respiratory rate and temperature
- Walking speed will be assessed at usual speed over a 15-foot course
- Waist circumference will be measured in inches and converted to centimeters.
- Weight in pounds will be measured or calculated in kilograms and used in BMI calculation.
- History & physical examination, targeted to the inclusion & exclusion criteria will be performed to determine study eligibility.

- Laboratory tests: CBC, CMP with liver function, Hemoglobin A1c, coagulation test (PT/INR, PTT), urinalysis, serum osmolality, urine osmolality, lipid panel. Order additional labs, if necessary, based on pertinent medical history.
- Psychometrics: Mini-Cog scoring < 3 will not be studied; Geriatric Depression Scale (GDS) scoring > 7 will not be studied and warrants follow up assessment with mental health professional
- Electrocardiogram (ECG): ECG with definitive evidence of prior myocardial infarction will not be studied
- 2-hour OGTT: Baseline (fasting) samples for determination of glucose and insulin concentrations will be drawn at -30, and -15 minutes (these basal values are averaged). At time zero, each subject will ingest 75 g of glucose. Glucose and insulin are determined every 30 minutes for 2 hours following glucose ingestion. The Matsuda index of insulin sensitivity will be calculated from the OGTT results, as described [13].
- Food recall and appetite assessments: A 24-hour food recall, 3-day food diary, and appetite will be assessed using standardized questionnaires[14, 15].
- Psychometrics: The Montreal Cognitive Assessment (MOCA) and The Centers for Epidemiologic Studies Depression Scale (CES-D) will be administered for baseline measurement.
- Functional Testing: Timed Up and Go (TUG) and Short Performance Physical Performance Battery (SPPB, modified) will be measured using standardized methods [23, 24]. Grip strength will be assessed using handheld digital dynamometer.

6.2. Visit 2: Optional MRI (within 7 days after V1)

MRI (optional): Image of the abdomen and dominant lower extremity will be done if funding is available to examine the effect of oxytocin on abdominal visceral fat and thigh muscle volume as described [25]. If participant has a pacemaker or any other reason that would preclude obtaining an MRI, this procedure can be omitted at the discretion of the PI.

6.3. Visit 3 – Physiological measures and Randomization (within 7-14 days after V2)

Subjects present to the site CRC in fasting state to assess body composition, bioimpedance, calorimetry, muscle biopsy, and systemic inflammation. An intravenous (IV) line is placed for access during muscle biopsy.

- Vitals signs, weight and BMI
- DEXA: Whole body dual-energy X-ray absorptiometry is performed to measure lean and fat body mass
- Bioimpedance: Measures weight, body fat percentage, and body mass index (BMI). The BF-350 is an FDA-cleared body composition monitor is an affordable solution for mainstream healthcare and fitness providers. Bioimpedance will be performed at UTMB Galveston site only.
- Calorimetry: Indirect calorimetry is performed in fasting state at rest for 30 minutes. Calorimetry will not be performed at UT Houston due to unavailability of required equipment. Since this is not a major outcome of the study, this does not significantly affect the scientific integrity of the study.
- Muscle Biopsy: Vastus lateralis muscle needle biopsy will be performed to assess muscle fiber type, satellite cells, and inflammatory markers.
- Laboratory and blood draw: Systemic inflammatory markers (see Analyses)

- Adverse event review questionnaire will be completed.

Analyses.
Muscle:
A biopsy of the vastus lateralis muscle will be performed as described using aseptic technique [17]. The following measurements will be performed:

- *Muscle fiber cross sectional area (CSA) [18]*
- *Satellite (stem) cell number and proliferative capacity [19, 20]*
- *Inflammatory genes / proteins. Measurements of NFκB and MAPK signaling, two key mediators of the inflammation state, will be conducted as described [21]. We will also assess cellular inflammation by measuring the levels of the mRNA transcripts for IL-1β, IL-6, MCP-1, and TNFα mRNA in skeletal muscle before and after oxytocin treatment [21].*

Blood/plasma:

- *Systemic inflammation - measuring plasma concentration of TNF-α, C-reactive protein, IL-6, and adiponectin, using commercially available ELISA kits.*

Randomization. Subject will be randomized to study drug or placebo and dispensing occurs at the end of V3.

6.4. Visit 4. Safety Labs (+1 week since start of study drug, within ±3-day window) and drug tolerability

Subjects present in fasting state to the site CRC to assess:

- Vital signs, weight, BMI, waist circumference
- Safety labs: Chemistry (including serum sodium), liver function, urine osmolality and serum osmolality
- Food recall: repeat 24-hour food recall, 3-day food diary and appetite assessments (same as Visit 1)
- Drug tolerability: subjects will be assessed for drug tolerability, including any side effects or adverse effects. Special attention will be made for signs and symptoms suggestive of hyponatremia including lethargy, muscle spasms, seizures, confusion/delirium, nausea and vomiting

6.5. Visit 5 Safety Labs (+4 weeks of drug administration, within ±7-day window) and drug tolerability

Subjects present in fasting state to the site CRC to assess:

- Vital signs, weight, BMI, waist circumference
- Drug compliance and tolerability, AE review
- Psychometrics: repeat MOCA and CES-D
- Food recall: same as Visit 1
- Safety labs: chemistry with liver function, urine osmolality and serum osmolality

6.6. Visit 6; Post treatment measurements (6-7.5 weeks on treatment)

Subjects present in fasting state to the site CRC to assess:

- Vital signs, weight, BMI, waist circumference
- Laboratory: CBC, chemistry with liver function, lipid panel, urine and serum osmolality, hemoglobin A1c, coagulation tests (PT/INR, PTT)
- Functional testing: Gait speed, grip strength, modified SPPB, TUG
- Food recall: same as Visit 1

- 2-hour OGTT
- Drug compliance and tolerability, AE review

6.7. Visit 7: Optional MRI (if done at V2) – (7-7.5 weeks on treatment)

MRI (optional): Image of the abdomen and dominant lower extremity will be done if completed at Visit 2. If participant has a pacemaker or any other reason that would preclude obtaining an MRI, this procedure can be omitted at the discretion of the PI.

6.8. Visit 8: Post treatment measurements (7.5-8 weeks of treatment)

Subjects present in fasting state to the site CRC to assess:

- Vitals signs, weight, BMI, waist circumference
- MOCA, CES-D
- DEXA body composition, Bioimpedance (UTMB Galveston only) and Calorimetry
- Muscle biopsy (satellite cell, fiber type, fat infiltration, inflammation, gene expression)
- Blood draw: Systemic inflammation
- Drug tolerability, compliance, and AE review

See Summary of Visits Table on next page.

Summary of Visits Table

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	Screen Day 0 (fasting)	**MRI + 7d**	**V2 +7-14d (fasting)**	**1 week on treatment (fasting)**	**4 weeks on treatment (fasting)**	**6-7.5 weeks on treatment (fasting)**	**MRI 7-7.5 weeks on treatment**	**7.5-8 weeks on treatment (fasting)**
Medical history and physical examination, ECG	X							
Vital signs, Weight, BMI	X		X	X	X	X		X
Waist circumference	X			X	X	X		X
Mini-Cog	X							
Montreal Cognitive Assessment (MOCA)	X				X			X
Geriatric depression scale (GDS)	X							
Centers for Epidemiologic Studies Depression Scale (CES-D)	X				X			X
Gait speed, 15-foot course	X					X		
Electrocardiogram	X							
**CBC, HgbA1c, and Coagulation testing (PT/INR, PTT)	X					X		
**Chemistry with liver function, serum osmolality (other safety labs if clinically indicated by history)	X			X	X	X		
**Lipid Panel	X					X		
Urinalysis	X							
Urine osmolality	X			X	X	X		
OGTT, 2-hour	X					X		
Body composition (DEXA)			X					X
Bioimpedance (UTMB only)			X					X
Calorimetry			X					X
Randomization*			X					
Body composition (MRI) - *optional*		X					X	
Functional tests: mSPPB, TUG, grip strength	X					X		
24-hour food recall, 3-day food diary, appetite	X			X	X	X		
Muscle biopsy			X					X
Systemic inflammation			X					X
Drug tolerability, compliance,AE review			AE review only	X	X	X		X

*** After Visit 4, participants may receive periodic phone calls by study staff between study visits to assess any symptoms and encourage adherence to study drug.**

**** Any marginally abnormal lab findings may be repeated if clinically indicated to ensure initial or continued eligibility.**

7. Statistical Plan

7.1. Sample Size Determination

Our power and sample size estimation is based on findings from an 8-week study of intranasal oxytocin in 24 adults, which found a greater reduction in body weight and BMI with oxytocin. Based on their findings, our proposed study (12 completers each site for 36 completers total, 18 completers in each arm) is expected to have 96.4% power to detect the oxytocin effect if the standard deviation (SD) associated with weight loss is 6.0 kg in the oxytocin group and 1.5 kg in the control group. For a more conservative estimate assuming a SD of 6.0 in both the oxytocin and control groups, our study would have 78.2% power. For both these power analyses, we assume type 1 error of P=0.05.

7.2. Statistical Methods

Analytical Approach.

Generalized linear mixed effects model will be used to evaluate the effect of oxytocin on the change of each continuous measure. The effect of oxytocin will be assessed by whether the time by oxytocin interaction is significantly different from 0 with a 2-sided p-value<0.05. Normal transformed score of an outcome will be used in the analyses when appropriate.

8. Safety and Adverse Events

8.1. Definitions

Adverse Event (AE)

In general, AE is used very broadly and encompasses physical and psychological harms and includes:

> Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A serious adverse event is any AE that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such

events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated Problems Involving Risk to Subjects or Others (UPIRSO)
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 7 days following the last administration of study treatment.

Pre-existing Condition
A preexisting condition is one that is present at the start of the study. A pre-existing condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for and adverse event. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition.
- Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2. Recording and Reporting of Adverse Events and Protocol Deviations

At each contact with the subject, the investigator or study staff will seek information about adverse events by specific questioning and, if appropriate, by examination. The principal investigator will review the safety and progress of this study on a continuing basis. Safety data will be reviewed at each study visit. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form.

- AEs will be tracked using the HSC IRB AE tracking form or data management tool (See Section 9.3) to be reviewed by Site Investigator weekly and IND Sponsor monthly.

- All AEs occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

- All adverse events will be summarized annually and submitted to the IRB.

- Deviations from the approved protocol (PDs) will be recorded in the site Protocol Deviation Log. When a deviation occurs site investigators or research staff will notify the site IRB and the IND sponsor as appropriate per local policy and procedure.

- The IND Sponsor records every PD in a study-wide log and reviews within 48 hours of notification to determine if prompt reporting to IRB of record is required.

8.3. Recording and Reporting of Serious Adverse Events and Unanticipated Problems

SAEs and UPIRSOs will be reported per governing IRB policy and procedure.

- UPIRSOs that are a result of study participation are reported to the IRB and the Medical Safety Officer within 24 hours. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All adverse events will be graded as mild, moderate, or severe.
- SAEs will also be submitted to the IRB and the safety officer within 24 hours.
- SAEs that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported per Institutional policy and according to FDA requirements.

Any action resulting in a temporary or permanent suspension of this study (e.g. local site IRB actions) will be reported to the IND Sponsor.

Investigator reporting: notifying the IND sponsor

Any study-related UPIRSO, and any type of SAE, must be reported to IND Sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to IND sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site.

Report SAE/UPIRSOs by email or facsimile to:

Sara E. Espinoza, MD
Associate Professor, Department of Medicine

University of Texas Health Science Center at San Antonio
Barshop Institute for Longevity and Aging Studies
7703 Floyd Curl Drive
Mail Code 7886
San Antonio, Texas 78229-3900
(210) 617-5197 (phone) – (210) 562-6130 (fax)
Email: espinozas2@uthscsa.edu

Within the following 48 hours, the investigator must provide further information on the SAE or the UPIRSO in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic information that will assist the understanding of the event.

Reporting Process: Notifying the UTHSCSA IRB

Adverse events not requiring immediate reporting may be tracked at each site on UTHSCSA Adverse Event Tracking Form (Track both UPIRSO and Events and Problems Not Requiring Prompt Reporting) and will be collected in REDCap. For SAE and UPIRSO, use UTHSCSA Prompt Reporting Form.

Sponsor reporting: Notifying the FDA

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening
    -or-
  - previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Sponsor Reporting Process

Serious adverse events may be submitted on FDA Form 3500A or in a narrative format. The contact information for submitting IND safety reports is noted on the next page.

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Metabolism and Endocrinology Products
5901-B Ammendale Road
Beltsville, MD 20705-1266
Phone: (301) 796-2290
Fax: (301) 796-9712

8.4. Medical Monitoring

The IND Sponsor will review the safety and progress of this study on a monthly basis or when needed if SAE

or SAE-UPIRSO occurs. The Medical Safety Officer isDean L. Kellogg, Jr.,, M.D., Ph.D., Professor, Division of Geriatrics, Gerontology and Palliative Medicine in the Department of Medicine.

Contact information: (see next page)
Dean L. Kellogg, Jr., , M.D., Ph.D., *Professor, Department of Medicine*
Division of Geriatrics, Gerontology and Palliative Medicine
University of Texas Health Science Center at San Antonio
7703 Floyd Curl Drive
Mail Code 7875
San Antonio, Texas 78229-3900
(210) 617-5197 (phone) – (210) 562-6130 (fax) Email: kellogg@uthscsa.edu

8.5. Unblinding Procedures

See Section 8.3 – For SAE or SAE-UPIRSO, the site investigator must report within 24 hours of the event to IND Sponsor who confers with Medical Safety Officer (Section 8.4) to assess the need for initiation of stopping rules or unblinding procedures. Responsibilities of each individual are bulleted below.

Medical Safety Officer

- Assess SAE or SAE-UPIRSO to determine if unblinding is required and, if yes, notify pharmacist to request subject's assignment
- Compare event data to intervention/assignment to determine if SAE is related to study intervention
- Assess study and subject safety to determine if stopping rules should be invoked
- Create the Safety Officer record in a blinded report format and communicate to Sponsor and with overall impression and recommended plan of action
- Responsible for determining the need to "unblind" the site investigator

Research Pharmacist

- Upon request, if necessary, unblind subject assignment and communicate to Medical Safety Monitor
- Suspend dispensing of study drug until SAE reporting is complete

Sponsor

- Receive and review Medical Safety Officer report with Pharmacist and Study Statistician
- Determine and implement stopping rules by communicating to site investigators, if required
- Ensure that IRB, FDA, and funding sponsors are notified as appropriate to local or agency policy
- Provide a copy of Medical Safety Officer report/record to appropriate personnel to file in eRegulatory Binder

Clinical Study Coordinator

- Ensure appropriate medical care and follow up is scheduled and implemented until SAE resolved

8.6. Stopping Rules

In the unlikely event that a study-related death or SAE occurs, the decision to stop the trial, either temporarily or permanently, will be the responsibility of the Medical Safety Officer in collaboration with the IND Sponsor.

Clinical excursions that a subject may experience that may warrant stopping study participation or drug administration either temporarily or permanently include but are not limited to:

- Clinical criteria:
  - Fever with a temperature > 101°F or other clinical indication of active illness
  - Blood pressure >170/95 or <90/50
  - Allergic reaction to drug
  - Serious nosebleeds or other localized side effects of drug administration
  - Hyponatremia (serum sodium < 135 mmol/L) and/or low serum osmolality (serum osmolality < 275 mOsm/kg).
    - Obtain serum sodium levels and osmolality at any time hyponatremia is suspected based on symptoms lethargy, muscle spasms, seizures, confusion/delirium, nausea and vomiting or physical examination
    - Any patient withdrawn from the study or has low sodium levels at the final visit will be evaluated until full normalization of the electrolyte abnormality occurs, and clinical and laboratory evaluations will be made available to medical safety monitor for review.
  - Other Serious Adverse Event adjudicated by medical safety monitor and invoking stopping rules

9. Data Handling and Record Keeping

9.1. Confidentiality

Information learned about all subjects will be kept confidential. All data and protected health information (PHI) in paper form will be kept confidential by assigned anonymous identifier and kept secured (password protected and/or double locked). Subjects will not be identified in any way in any publication.

9.2. Source Documents

Source data are contained in source documents found in paper subject files at the research site and in site electronic medical records. If source data are handwritten, print all entries legibly in black ink. Erasures and white-out material are prohibited. If any entry error has been made on paper, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.3. Data Management

Data System: REDCap

All data will be input using a web front-end interface. All users are individually assigned authorization for access to specific components of the database application. Information that is input is checked for logical and range consistency and mandatory data fields must be entered in order to input a record. Reports are available as Excel exports to each site staff with access to reports.

Case Report Forms

CRFs will be developed in REDCap and may be printed in PDF for handwritten use. Radiology and lab reports may be printed or copied for use as source documents, while questionnaires and validated instruments may be copied from the Manual of Operations (MOP) for use in the paper research record. Data entry personnel will be appropriately trained before study is initiated.

9.4. Records Retention

The IND Sponsor is responsible for maintaining the Trial Master File by way of access to and storage in the eRegulatory Binder at Clinical Trials Express.

The local site Principal Investigator is responsible for maintaining study essential documents for at least 3 years after study participation ends for the last subject at the last site, or according to the IND Sponsor's institutional

retention period, whichever is greater.

These documents should be retained for a longer period if required by a funding agency, the FDA or other institutional retention policy. In such an instance, it is the responsibility of the IND Sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10. Study Monitoring, Auditing, and Inspecting

10.1. Study Monitoring Plan

The Sponsor will ensure that the designated study monitor, compliance specialist or other quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the study monitoring visits.

Monitoring elements will be reviewed at least once during the study period as described below:

- Consent process
- Accuracy of the collection and processing of laboratory specimens
- Forms and source documents in the subjects' research records will be current and maintained in a timely manner.
- Physical and mental condition of the subject and any type of AE will be assessed and monitored prior to beginning the study by the investigator(s) and staff, and during the study period.

Areas of focus for study monitoring by the designated study monitor:

- Regulatory documentation and compliance with local policy will be monitored once during the study period at each site.
- The safe handling and accountability of drugs, which includes the use of temperature logs and disposal of waste, will be maintained upon receipt of study drug, upon dispensing, and at the close out of the study period.
- Compliance with the protocol and conduct of the study, including the consenting process, along with source data compared to collected data (SDV), will be monitored once at each site during the study period.
  - *30% of subject records and 100% of primary outcome data for each subject at each site will be monitored.*
  - *The first and the last subject enrolled will be monitored for consent practices and 100% of eligibility data collected.*
- Adverse event logs will be monitored and research records analyzed for AEs that were overlooked or otherwise not documented properly in the study files.
- The safety assurance and condition of any electrical, radiological, magnetic, gaseous, and hazardous equipment, tools or apparatus that will be used by the investigators and subjects at each site will be properly maintained and certified by the site's biomedical safety staff on at least an annual basis. The local site PI will attest to this assurance prior to enrolling the first subject.

10.2. Auditing and Inspecting

The site Investigator will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The site investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as a site investigator in this study implies acceptance of potential inspection by government

regulatory authorities and applicable University compliance and quality assurance offices.

11. Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312) applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the governing Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator(s) before the study is initiated.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachments, Section 15, Attachment B., Sample Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally authorized representative, and the IRB-approved research professional obtaining the consent.

12. Study Finances

12.1. Funding Source

In San Antonio, this study is financed through a grant from the US National Institutes of Health/National Center for Advancing Translational Sciences (NIH/NCATS). Each site is responsible for its own funding of the study, including study drug supply.

12.2. Conflict of Interest

None reported (see Forms FDA 3455 on file in CTX eRegulatory Binder)

12.3. Subject Stipends or Payments

This study will reimburse subjects for time and transportation. A schedule of payments is shown below. The total potential reimbursement to a subject is $630 for the study for all visits including both optional MRIs ($480 without MRI). Payments may be prorated to include the last visit completed if study participation is terminated early. Manual payments for additional or partial visits, if necessary, will be handled on an ad-hoc basis with prior approval from the funding sponsor.See Payment Schedule in the table below.

Visit 1	$ 75
Visit 2 (optional)	$ 75
Visit 3	$ 150
Visit 4	$ 15
Visit 5	$ 30
Visit 6	$ 60
Visit 7 (optional)	$ 75
Visit 8	$ 150
Manual Payment for an Interim Visit	Up to $ 30

13. References

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14. Attachments

ATTACHMENT A. PLACEHOLDER FOR SAMPLE CONSENT FORM

ATTACHMENT B. EVENT SCHEDULE

	Visit 1	Visit 2	Visit 3	Visit 4*	Visit 5	Visit 6	Visit 7	Visit 8
	Screen Day 0	MRI +7 days	V2 +7-14 days	1 week on Rx	4 weeks on Rx	6-7.5 weeks on Rx	7-7.5 weeks on Rx	7.5-8 weeks on treatment
Medical history and physical examination, ECG	X							
Vital signs, Weight, BMI,	X		X	X	X	X		X
Waist circumference	X			X	X	X		X
Mini-mental status exam (Mini-Cog)	X							
Montreal Cognitive Assessment (MoCA)	X				X			X
Geriatric depression scale (GDS)	X							
Centers for Epidemiologic Studies Depression Scale	X				X			X
Gait speed, 15-foot course	X					X		
**Hematology (CBC, HgbA1c) and Coagulation testing (PT/INR, PTT)	X					X		
**Chemistry with liver function and serum osmolality	X			X	X	X		
**Lipid Panel	X					X		
**Urinalysis	X							
**Urine osmolality	X			X	X	X		
Electrocardiogram	X							
OGTT, 2 hour	X					X		
Body composition (DEXA)			X					X
Bioimpedance (UTMB only)			X					X
Calorimetry			X					X
Randomization			X					
Body composition (MRI)		X	X				X	
Functional tests: mSPPB, TUG, grip strength	X					X		
24-hour food recall, 3-day food diary, appetite	X			X	X	X		
Muscle biopsy: satellite cell, fiber type, fat infiltration, inflammation, gene expression			X					X
Systemic inflammation			X					X
Drug tolerability/ compliance, adverse event monitoring			AE review only	X	X	X		X

*** After Visit 4, participants may receive periodic phone calls by study staff between study visits to assess any symptoms and encourage adherence to study drug.**

**** Any marginally abnormal lab findings may be repeated if clinically indicated to ensure initial or continued eligibility.**

ATTACHMENT C. PLACEHOLDER FOR VICTORIA APOTHEKE ZURICH

ATTACHMENT D. PLACEHOLDER FOR QUESTIONNAIRES, INSTRUMENTS, & FORMS

ATTACHMENT E. SUMMARY OF PROTOCOL CHANGES

Date of Change	Version #	Section Modified	Before Change	After Change
11-21-2016	1.1	Title Page	IND Number: Pending	IND Number: 132460-0001
		All pages		General spacing, formatting, pagination and header/footer changes
		3.4 Potential Risks	Rare: Nausea, dermatitis, uterine contractions	(*FDA requested change*) – Rare: added hyponatremia and or fluid imbalance
		4.2 Exclusion Criteria	Renal disease (Creatinine >1.4)	(*FDA requested change*) Renal disease: abnormal serum sodium levels, abnormal urinalysis, or physical exam findings indicative of fluid imbalance; individuals with underlying disorder of sodium/water balance, such as SIADH, diabetes insipidus, or psychogenic polydipsia; Individuals with underlying seizure disorder or underlying neurologic disorder that increases seizure risk
		6.1 Consent and Screening Visit 1, page 9	Screening labs did not include urinalysis, urine or serum osmolality	(*FDA requested change*) Added urinalysis, urine and serum osmolality to screening labs
		6.3 Visit 3, page 10	No safety labs mentioned at this visit	(*FDA requested change*) Visit 3a, 3b, 3c created and safety review/labs added at 3c. Visit 3a: Blood draw for systemic inflammatory markers Visit 3b: Optional MRI Visit 3c. Safety review/labs: Chemistry, including serum sodium and serum osmolality and drug tolerability assessment
		6.4 Visit 4, page 11	Safety labs: CBC and CMP	(*FDA requested change*) Added urinalysis and serum osmolality at 4 weeks of drug therapy; removed CBC
		6.5 Visit 5,+4 weeks from V4	Labs: CBC, CMP, cholesterol panel, HgbA1c, coagulation tests, and systemic inflammatory markers	Assigned safety labs to 6.5.1 Visit 5a – Laboratory: CBC, CMP, urinalysis, serum osmolality, lipid panel, HgbA1c, coagulation tests (PT, INR, PTT)
		Section 6, Study Visits	No embedded Visit and Procedures Schedule	Added Table 3, page 13.
		8.6, Stopping Rules	No mention of hyponatremia or fluid imbalance	(*FDA requested change*) Added to clinical criteria the clinical values for stopping due to hyponatremia or changes in fluid balance

12-15-16	1.2	Throughout document		Formatting and space revisions
		Title Page	Title of Study: Physiological…	Changed title of study to “Physiologic”
			Clinical Sites and Coordinating Center	Changed UTHSCSA to new brand name UTHealth San Antonio Added Clinical Trials Express - Coordinating Center
			No Attachment E.	Added Attachment E. Summary of Protocol Changes
		Table of Contents	Page numbering not hyperlinked	Added hyperlinks to page numbers
		3.2 General Study Design	Phase I/II	Changed to “Phase II pilot”
		3.4 Potential Risks to Subject Safety	Blood Withdrawal, page 4 includes details about blood being drawn.	Removed detailed lab tests and inserted more general statement that blood will be drawn for safety and for research purposes at screening and or study visits.
			IV Lines, page 4: …antecubital vein and a hand vein…	Deleted “and” and inserted “or”
		4.3 Subject Recruitment and Screening	…performed in CRCs…Subjects will be screened for eligibility using an initial phone screen.	Changed to read: At some sites, subjects will call-in to an advertised number and will be pre-screened…callers provide permission for research team to follow up with phone screening using an IRB-approved form…
		6.1 Study Procedures	6.1 Visit 1, Consent and Screening Day 0	6.1 Visit 1 (Consent and Screening for Eligibility – Day 0) Added estimated length of this visit is 5 hours at the end of paragraph 1.
		6.2 Baseline	6.2 Baseline	Changed to read Baseline Testing Moved Grip Strength bullet to fall within Functional Testing along with other assessments.
		6.3.	6.3 Visit 3a.	Changed subheading to 6.3 Visit 3a-c: Physiological measures, Randomization and optional MRI
		6.5	6.5 Visit 5	Changed subheading to 6.5 Visit 5a-c:
			Table 3. Study Visits and Procedures missing a few items	Table 3. Added history and physical, added MMSE, separated blood and urine labs into separate rows and visits accordingly
		10.1, Study Monitoring Plan	Areas of focus for study monitoring…	Added to 2nd bullet on drug accountability to insert “upon dispensing”…
		12, Study Finances	12.3, Subject Stipends or Payments, …total potential reimbursement of $770	…changed total to $660 with both MRIs, and $510 without MRIs. Subject payment table changed per visit accordingly.
		14, Attachments	Attachments A, B, C, D	Changed title of Attachment D

12-15-16	1.2.1			*Internal changes only:* Incorporated into and saved as v1.2 above
12-19-16	1.2.2	6.1-6.5 Study Procedures		Changed Visit 1 MMSE to Mini-Cog, extended Gait measure from 10 feet to 15-foot course. Added MoCA to Visit 2 Added food and appetite measures at Visit 3c and Visit 4 and deleted them at Visit 5a. Modified Visit table accordingly.
12-20-16	1.2.3	ATTACHMENT E. Summary of Protocol Changes Table		Updated all changes to date by version in the Summary table.
		8.4 Medical Monitor	8.4, Page 16	Added “Contact Information: (see next page)” before listing his address, etc. on page 17
		ATTACHMENT B. Placeholder for Event Schedule	ATTACHMENT B., Event Schedule	Inserted Event Schedule from Page 12, Table 3, Study Visits and Procedures; corrected event schedule in 2 places to be consistent with ICF
03-17-17	1.2.4	Table of Contents	Update Visit Numbers	Renumbered Visit 3a-c, 4, 5a-c to visits 3-9
		3.1	Definition of Sarcopenic Obesity	Updated definition of Sarcopenic Obesity to reflect modified consensus. Muscle mass will be measured as an outcome.
		6.3-6.5.3	Update Visit Numbers	Renumbered Visit 3a-c, 4, 5a-c to visits 3-9
		Table 3	Study Visits and Procedures	Renumbered Visit 3a-c, 4, 5a-c to visits 3-9
		Table 12.3	Subject Stipend	Renumbered Visit 3a-c, 4, 5a-c to visits 3-9
		ATTACHMENT B	ATTACHMENT B	Renumbered Visit 3a-c, 4, 5a-c to visits 3-9
05-16-17	2.0	4.2 Exclusion Criteria 4.3 6.1c	GDS > 5	GDS > 7
		4.3	Screening tests	Remove ECG
		6.1	Visits 1 and 2	Combined to Visit 1, remove ECG, deleted unnecessary language
		6.1-6.8	Update Visit Numbers	Renumbered Visit 1-8
		Table 3	Study Visits and	Renumbered Visit 1-8, removed ECG,
		Table 12.3	Subject Stipend	Renumbered Visit 1-8
		ATTACHMENT B	ATTACHMENT B	Same as Table 3,
		Summary of Protocol Changes Table.		Updated to V2.0 all changes to protocol shown above

06-20-17	2.1	6.1	Visit 1	Added ECG back in
		6.2 and 6.3	Visits 2 and 3	Swapped visits 2 and 3 to have optional MRI occur before randomization. Provided PI discretion to forego optional MRI if necessary
		6.3	Visit 3	Baseline visit added to establish IV access for biopsy and UT Houston no calorimetry due to no equipment.
		6.7 and 6.8	Visits 7 and 8	Swapped visits 7 and 8 to have optional MRI occur before completion of study drug and final visit
		Visit Table	Visit Table	Swapped visit procedures to be consistent with narrative protocol
		Summary of Protocol Changes Table		Updated to V2.1 all changes to protocol shown for 06-20-17 version
10-03-17	2.2	Title Page, headers and footers	Version number and IND series #	Updating version numbering in header and footer to 2.2; IND# 0003 = v2.0 protocol, 0004 = v2.1 protocol, 0005 = v2.2 this version to be consistent with recent IND reporting to FDA
		Study Summary page 1	Inclusion/Exclusion	Removed ADA criteria from diabetes exclusions, using A1c only; changed Serum creatinine measure to GFR for renal exclusion, changed urinalysis criteria for WBC and RBC; Significant heart disease (history of MI within the last year or New York Heart Classification grade III-IV)
		Page 2 and 4		Spacing or format changes
		6.1	Visit 1	Inserted screening window of 30 days
		6.2 and 6.7	Visits 2 and 7	Inserted abdomen to MRI as well as dominant lower extremity
		6.3 and 6.8, and Visit table page 15	Visits 3 and 8	Changed bioimpedance to be offered only at UTMB Galveston
		Reference List	Citation 25	Corrected the reference to MRI of abdomen and lower extremity, adding citation number 25
10-08-17	2.2.1	Title page and Summary of Changes table	Administrative changes	Added 03-24-17 as original approval; removed “(placeholder)’ from CTX address; updated versioning in headers and footers

10-17-17	2.2.2	Summary of Visits Table	Repeat labs language	Added spaces to create table on single page of its own. Added language to allow repeating marginally abnormal labs if clinically indicated to ensure initial or continued eligibility Changed periodic phone calls to occur after Visit 4 Removed Optional from MRI and Baseline from V3
		Title page and footers	Versioning	Updated versioning throughout document to 2.2.2.
11-17-17	2.3	Title page and footers	Versioning	Updated versioning throughout document to 2.3; IND series -0006 added to title page
		Visit Section 6 and Summary Visit Table	Procedures	Added vital signs to Visits 1,3,4,5,6 and 8 to narrative section 6
		Visit Section 6.4, 6.5, 6.6	Labs	Remove lipid panel from Visits 4 and 5 narrative to be consistent with Summary Visit Table. Add urine osmolality to Visit 4 narrative to be consistent with other visits assessing osmolality and Summary Visit Table Change Visit 4, 5 and 6 referencing Visit 2 related to Food Recall to Visit 1.
		Visit Section 6.8	Procedures	Add Vital signs, weight, BMI, MOCA and CES-D to narrative to be consistent with Summary Visit Table Add drug compliance/tolerability, and AE review to Visit 8 narrative to be consistent with Summary Visit Table Add waist circumference to Visit 8 narrative and to the Summary Table of Visits for consistency
		Section 12	12.1	Rephrasing each site responsible for its own funding, “including study drug supply.”
		General		Revised pagination issues throughout the document
04-03-18	2.4	Visits 1,3,4,5,6,8 narrative	Fasting state	Revised all visits to be consistent whenever there are labs drawn that subject presents to CRC in fasting state
		Visit Schedule	(fasting)	Change table to reflect fasting at Visits 1,3,4,5,6,8
		Eligibility criteria	30-40 kg/m2	Change BMI range to 30.0-43.0

08-03-18	2.5	Section 8.3-8.4	Medical Monitoring	Change Medical Safety Monitor to replace A. Fisher with Dean Kellogg, MD, PhD Add oversight by Pepper Center DSMB to review study progress and overall safety