Study Protocol Title:
BAT as a therapeutic for the metabolic and cardiac dysfunction with senescence (BATSR)

Study Sponsor:
AdventHealth

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List of Abbreviations:

ACSM: American College of Sports Medicine's
BAT: Brown Adipose Tissue
BMI: Body Mass Index (kg/m^2)
BP: Blood Pressure
CBC: Complete Blood Count
CMP: Complete Metabolic Panel
DEXA: Dual-energy X-ray absorptiometry
DO: Doctor of Osteopathic Medicine
DVT: Deep Vein Thrombosis
ECG: Electrocardiogram
EMR: Electronic Medical Record
eGFR: estimated Glomerular Filtration Rate
FDA: Food and Drug Administration
HbA1c: Hemoglobin A1c
HDL: High-Density Lipoprotein
HIPAA: Health Insurance Portability and Accountability Act
HIV: Huma Immunodeficiency Virus
IRB: Institutional Review Board
LDL: Low-Density Lipoprotein
MD: Medical Doctor
MI: Medical Investigator
MMSE: Mini Mental State Examination
MRI: Magnetic Resonance Imaging
NP: Nurse Practitioner
OS: Old Sedentary
PA: Physician Assistant
PASE: Physical Activity Scale for the Elderly
PC: Personal Computer
PI: Principal Investigator
RER: Respiratory Exchange Ratio
RM: Repetition Maximum
SOP: Standard Operating Procedure
SPPB: Short Physical Performance Battery
TRI: Translational Research Institute
TSH: Thyroid Stimulation Hormone
UF: University of Florida
VLDL: Very Low-Density Lipoprotein
VO_2: Volume of Oxygen Consumed
WI: Working Instruction
YA: Young Active

Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with applicable Federal regulations and institutional research policies and procedures.

The US population is rapidly aging. It is projected by 2030, more than 20% of the population will be over 65 years of age (An Aging Nation: The Older Population in the United States. *Current Population Reports (US Census Bureau)*, 1-28 (2014)). Aging per se is the strongest risk factor for a multitude of diseases including both metabolic and cardiovascular disease. Increased age is also associated with a reduction on physical activity levels and exercise intolerance, resulting in decreased cardiorespiratory fitness (VO2max). Many studies have shown that VO2max strongly correlates to all-cause mortality (21). The effects of aging on the body include a loss of functional independence, a decrease in the quality of life, and an increased risk of mortality. The increase in the aged population has elevated the significance of elucidating potential therapeutic treatments.

Increasing age is directly correlated to greater prevalence of both metabolic and cardiovascular disease (6). Aging results in impaired glucose metabolism and increased insulin resistance; a third of the population over 60 years of age has diabetes (elderly-onset diabetes), and an additional third of this population presents with prediabetes (18). There is also a higher prevalence of cardiovascular mortality in older adults with impaired glucose metabolism compared to those with normal glucose tolerance. An important tissue that combats metabolic disease and is cardioprotective is brown adipose tissue (BAT) (3, 8, 31, 36, 39). BAT is a thermogenic tissue that contains large amounts of mitochondria to dissipate chemical energy as heat. When activated, either by cold-stimulation or increasing the amount of BAT by transplantation, BAT increases energy expenditure, decreases plasma glucose and lipids, and improves systemic metabolism. Previous work have demonstrated that increasing the amount of BAT by transplantation improves metabolic health and has an endocrine effect on the heart (14, 36, 38). Additionally, studies have identified that cold-activation or exercise releases from BAT a “batokine”, the lipid 12,13-diHOME, which has a robust effect on metabolism (25, 35)

BAT has great potential as a therapeutic target to combat both metabolic and cardiovascular disease, but BAT mass decreases with age (8, 12, 30-32, 39). The objective of this study is to define the effect of aging on BAT mass in a cohort of older sedentary adults and older athlete adults. A secondary objective is to define how changes in BAT mass with aging relate to changes in BAT derived lipokines (12,13 diHOME). Finally, we would like to further validate the method for determining BAT mass using MRI.

This work embodies a multidisciplinary and multi-Investigator approach and is the product of an ongoing successful collaboration between experts in magnetic resonance imaging, BAT, and aging at AdventHealth Translational Research Institute (TRI), Orlando, FL.

The proposed study is a prospective, two group, controlled exercise training study consisting of three distinct but connected phases:
I) Screening Phase and Pre-Intervention testing,
II) Exercise Training Intervention Phase, and
III) Post-Intervention testing.

Eligible participants will be assigned to one of two groups:

1. Older adults who are regularly engaged in endurance exercise (OA-Older Athlete) and

2. Older adults who are sedentary (OS-Older Sedentary).

The expected duration for a participant in this study is approximately 120 days.

A series of physiologic and clinical/health outcomes will be assessed before and after the exercise intervention period:

1. Morning resting fasted variables: blood pressure; blood glucose and lipids
2. Physical Examination, Resting ECG, Urine collection
3. Cardiorespiratory fitness (VO2peak) determined via a graded cycle ergometry test.
4. Physical function: The Short physical performance battery (SPPB) will be used to measure lower extremity performance as a secondary outcome.
5. Body composition by DEXA scan - bone mineral density, total and partitioned lean mass, estimated visceral fat.
6. Muscle strength and power: Quadriceps muscle performance testing (maximal strength and power) will be performed using a Biodex dynamometer.
7. Hyperinsulinemic euglycemic glucose clamp to assess whole body insulin sensitivity.
8. Echocardiograph measurements to assess cardiac function.
9. MR Imaging for BAT mass.

Participants in the OS group will enter a progressive 8-week supervised aerobic and resistance training program at the exercise training facility at the AdventHealth TRI. Blood will be collected before and after the intervention to assess circulating Batokines.

Background Information and Scientific Rationale

Metabolic and cardiovascular dysfunction with age. It is well established that metabolic and cardiovascular diseases are closely related with the aging process. Obesity, visceral adiposity, and insulin resistance are observed much more frequently in the elderly, resulting in a greater incidence of type 2 diabetes. These metabolic disorders contribute to the cardiovascular disease in the elderly. There is also a progressive decline in diastolic and systolic function, a blunted contractile reserve and hypertrophy, which manifests as Heart Failure with Preserved Ejection Fraction (HFpEF). The collective effect is that ~75% of people over 60 have a cardiovascular disease, with mortality upwards of ~40%(46).

Physical inactivity and aging. Together, the collective effect of aging on metabolism, the cardiovascular system, and other organ systems concomitant with a reduction on physical activity levels leads to exercise intolerance. This inactivity decreases VO2max, a leading risk factor in cardiovascular disease and all-cause mortality (21). This decrease in physical activity can result in a loss of functional independence and a decrease in quality of life.

Brown adipose tissue (BAT) and aging. BAT is a thermogenic tissue that produces energy as heat. It is characterized by a multilocular appearance, increased mitochondria, and increased expression of uncoupling protein-1 (UCP1)(30). Modulation of BAT metabolism influences whole body energy balance, and increased BAT activity counteracts obesity and diabetes (2, 7, 11, 13, 14, 24, 30, 36, 39). BAT is influenced by cold exposure through increased sympathetic activity (20, 28), various secreted factors (20, 25, 28), or exercise (4, 15-17, 22, 23, 27, 29, 34, 37, 41-45, 47). BAT mass and activity are decreased in humans and mice with aging (7, 12, 31-33). The decrease in BAT with age is due to blunted sympathetic activity (32, 33), altered endocrine control (32), and the loss of brown adipocyte regenerative capacity due to dysfunctional stem cells (32,

33). This decreased BAT activity is manifested in a diminished response to cold temperatures (i.e., reduced heat generation via non-shivering thermogenesis) (26, 31). There is also an inverse relationship of BAT to body mass index and visceral fat mass (7, 31). These data suggest that the loss of BAT contributes to the metabolic dysfunction with aging.

BAT and metabolism. Previous studies have demonstrated that increasing the amount of BAT in rodents using a transplantation model improved whole-body and metabolic health and increase insulin-stimulated glucose uptake (14, 36). BAT transplantation reduced body weight and adiposity and improved whole-body glucose metabolism. BAT transplantation increases glucose metabolism specifically in the heart, white adipose tissue (WAT), and the endogenous BAT (36).

BAT and cardiovascular function. Recent studies have investigated the endocrine role of BAT to influence cardiovascular function. UCP1-/- knockout mice had greater cell death, greater adverse remodeling, and worse survival in catecholamine-induced cardiomyopathy (38). These detrimental effects in UCP1-/- knockout mice were prevented with transplantation of BAT from wild-type mice, suggesting that thermogenically active BAT has a cardioprotective effect. Additionally, BAT transplantation increases glucose uptake to the heart (36). Taken together, these studies indicated that BAT can influence heart function.

BAT as an endocrine organ. BAT functions as a secretory organ, releasing various secreted factors that act on other tissues and organs (2, 14, 36, 40). Several BAT-derived molecules, called "batokines" have been identified to function in an autocrine, paracrine, and endocrine manner (40). Previous studies have demonstrated that increasing the amount of BAT through a transplantation model improved whole-body metabolic health and increased insulin-stimulated glucose uptake in the heart and WAT (14, 36). Recent work has identified a batokine, the lipid 12,13-diHOME, to be increased in circulation in response to various stimuli in both rodents and humans and increases fatty acid uptake in BAT and skeletal muscle (25, 39). In line with a reduction in BAT, 12,13-diHOME is decreased in older adults in comparison to young counterparts (35). These studies suggest the inability of BAT to secrete batokines with aging may impair whole-body metabolism and cardiovascular function in older adults.

Study Objectives

Primary Objective

To define the role of BAT and 12,13-diHOME in the age-induced impairments in metabolism and cardiac function, and determine if increasing BAT mass or its endocrine function is a viable therapeutic strategy to improve metabolic and cardiovascular function in the elderly.

Study Design

Research Design

The proposed clinical study is a single site prospective, two group, controlled exercise training study of older athletes (OA) (n=15 completers) and older sedentary (OA) (n=15 completers) consisting of three distinct but connected phases:

Phase I: Pre-Intervention, Screening and Baseline testing
Phase II: Intervention, Exercise Training

Phase III: Post-Intervention, Follow-Up testing
A series of physiologic and clinical/health outcomes will be assessed before and after the exercise intervention period:

1. Morning resting fasted variables: blood pressure; blood glucose and lipids
2. Physical Examination, Resting ECG, Urine collection
3. Cardiorespiratory fitness (VO2peak) determined via a graded cycle ergometry test.
4. Physical function: The Short physical performance battery (SPPB) will be used to measure lower extremity performance as a secondary outcome.
5. Body composition by DEXA scan - bone mineral density, total and partitioned lean mass, estimated visceral fat.
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7. Hyperinsulinemic euglycemic glucose clamp to assess whole body insulin sensitivity.
8. Echocardiograph measurements to assess cardiac function.
9. MR Imaging for BAT Mass

Participants in the OS group will enter a progressive 8-week supervised aerobic and resistance training program at the exercise training facility at the TRI. Blood will be collected before and after the intervention to assess circulating Batokines. A complete Schedule of Activities (SOA) can be found in the Appendix.

The expected duration for a participant in this study is approximately 120 days.

Males and females in the age ranges 65-90 years will be recruited from AdventHealth's electronic medical record (EMR), senior centers, television, radio and social media advertisements, mass mailing lists from the Orlando, FL catchment area, and by contacting potential participants on the AdventHealth TRI's participant database.

Study Site(s)/Location(s) and Number of Participants

AdventHealth Orlando site locations: Translational Research Institute, 301 E. Princeton Street. Orlando, Florida 32804.

Estimated number of participants at AdventHealth Orlando site (including screenings): 36
Estimated number of participants at external sites: 0
Total number of all sites: 1

Estimated number of participants at all sites combined (including screenings): 36

Multi-Site Research Logistics/Communication Plan

N/A

Research Conducted in a Foreign Country

N/A

Community-Based Participatory Research

N/A

Participants Selection

Vulnerable Populations (if applicable)

AdventHealth Orlando Employees: Recruitment efforts will follow AdventHealth Orlando recruitment SOPs for research. AdventHealth Orlando Employees will not be individually targeted nor excluded from study participation based on employment. AdventHealth Orlando employees who engage the AdventHealth TRI asking to participate in the study will be processed per standard consent procedures for participants. In addition, during the consent process, the study staff will review standard consent language stating that an employee's participation or lack of participation in the study will not affect their employment status or relationship with AdventHealth Orlando.

Inclusion Criteria

1. Males and Females between the ages of 65 to 90 years of age.
2. BMI ≤ 35kg/m^2, inclusive at time of screening.
3. Stable weight (No gain/loss of ≥ 10 lbs within 6 months prior to screening).
4. Non-smokers as defined by not smoking any tobacco or using nicotine-containing products and not using vape pens or vaporizers within 3 months prior to screening.
5. Participant must have renal function with an estimated glomerular filtration rate (eGFR) > 45 ml/min/1.73m^2 determined at screening.
6. Triglyceride level is < 350 mg/dl and LDL cholesterol is ≤ 150 mg/dl at screening.
7. States willingness to follow protocol as described, including the prescribed exercise level and completing any forms needed throughout the study.
8. Voluntarily signed and dated an informed consent form, approved by an Institutional Review Board/Independent Ethics Committee, and provided Health Insurance Portability and Accountability Act authorization (HIPAA) or other privacy authorization prior to any participation in study.

Group Specific Inclusion Criteria:

9. Older Athletes (**OA**) Only: Endurance trained athletes, defined as exercising (running, cycling, swimming) >3days/wk for >6 months without layoff. This will be verified by self-report and triaxial accelerometry.
10. Older Sedentary (**OS**) Only: Defined as <1day/wk of structured exercise and determined by self-report and triaxial accelerometry.

Exclusion Criteria

1. History of type 1 or type 2 diabetes per self-report at screening visit 1.
2. Actively pursuing weight loss and/or lifestyle changes at time of screening.

3. Untreated or poorly controlled hypertension (SBP > 150, DBP > 95).

4. Mini Mental State Exam (MMSE) <21.

5. Participant has had a significant cardiovascular event (e.g. myocardial infarction, stroke) ≤ 6 months prior to screening visit; or stated history of congestive heart failure; or participant has evidence of cardiovascular disease assessed during the ECG at screening. In the event of a positive stress test, participants are referred to their primary care physician. If the electrocardiogram (ECG) is determined to be a false positive, participant may be allowed to participate in study after confirmatory records obtained.

6. Current infection (requiring prescription antimicrobial or antiviral medication, or hospitalization), or corticosteroid treatment (with the exception of inhaled or topical steroids) in the last 3 months prior to screening visit.

7. Participant is currently taking anti-inflammatory medication or has had anti-inflammatory medication within 1 week prior to screening (including over the counter formulations; e.g. Aleve, Motrin, ibuprofen, naproxen, low dose aspirin).

8. Surgery requiring >2 days of hospitalization in the last 3 weeks prior to screening visit.

9. Participant has an active malignancy (with the exception of basal cell) or autoimmune disease.

10. Participant has a chronic, contagious, infectious disease, such as active tuberculosis, Hepatitis B or C, or HIV, per self-report.

11. Participant is an amputee and/or has presence of partial or full artificial limb.

12. Participant currently has uncontrolled severe diarrhea, nausea or vomiting.

13. Participant has uncontrolled severe (including stage III or above) gastrointestinal absorption-related disorders, within 3 months of screening, such as: obstruction of the gastrointestinal tract, inflammatory bowel disease, short bowel syndrome, gastroesophageal reflux disease, gastroparesis, peptic ulcer disease, celiac disease, intestinal dysmotility, diverticulitis, ischemic colitis and bariatric surgery.

14. Cannot abstain from alcohol for the duration of the testing periods.

15. Subjects who fulfill any of the contraindications for MRI; examples include metal implants, devices, paramagnetic objects contained within the body and excessive or metal-containing tattoos.

16. Unable to participate in MR or DEXA assessments due to physical limitations of equipment tolerances (e.g., MRI bore size and DEXA 450-pound weight limit), claustrophobia, or based on Investigator's judgment at screening.

17. Participant cannot refrain from taking medications/dietary supplements/herbals or substances that could modulate glucose metabolism, or are considered anabolic, or reduce weight (fat mass) in the opinion of the PI or Physician, starting two weeks prior to enrollment and over the entire course of the study. These include progestational agents,

steroids, growth hormone, dronabinol, marijuana, calcium-betahydroxy-betamethylbutyrate (CaHMB), free amino acid supplements and dietary supplements to aid weight loss.

18. Participant has hypothyroidism (TSH ≥10 mIU/L) or hyperthyroidism (TSH < 0.4 mIU/L).

19. Presence of any condition that, in the opinion of the Investigator, compromises participant safety or data integrity or the participant's ability to complete the study.

20. Because all women participating in this project will be post-menopausal, there will be no need for a pregnancy test prior to DEXA procedures. Females currently on hormone replacement therapy (HRT) can participate in the study if they have been on a stable dose of HRT for at least 6 months and will continue to be on HRT during the study.

21. Potential participants taking stable doses of medications for the last 30 days prior to screening for Blood pressure, cholesterol, GERD may be permitted to participate.

22. Participant becomes Covid-19 positive at any point during the study.

Resources Available

We attest that all AdventHealth Translational Research Institute faculty and staff will be trained, and this training will be documented as described in AdventHealth Translational Research Institute Work Instruction 031.100.015 Documentation of Protocol Training.

We will implement regular, ongoing discussions between the PI and coordinator as per the AdventHealth Translational Research Institute SOP 030.000.002 Oversight of Research Studies at the Translational Research Institute. The coordinator will review source and communicate with all applicable study team members involved in the study on a regular basis regarding reportable new information, implementing amendments, study progress, and quality assurance/control.

The AdventHealth Translational Research Institute facilities are state of the art and we have within our building all required resources and staff to execute the study. We have a medical oversight team, Medical Oversight Committee, as well as a Quality Committee to appropriately monitor and address adverse events.

Study Procedures

Participants Recruitment and Screening

Males and females in the age ranges 65-90 years will be recruited from AdventHealth EMR, senior centers, television, radio, social media advertisements, text messaging, emailing, newsletters, and mass mailing lists from the Orlando, FL catchment area, and by contacting potential participants on the AdventHealth TRI's participant database.

Potential participants will be medically screened to determine good health and without any contraindication to exercise. Approximately equal numbers of men and women will be recruited. Neither race nor ethnicity will be exclusions.

We will recruit volunteers through public advertisement by placing advertisements in community newspapers and radio, as well as on social media. Individuals who respond to

the ads will be contacted by telephone for preliminary screening and will be given an opportunity to ask initial questions and be provided additional description of the study. Potential volunteers who seem to meet criteria will be scheduled for an outpatient examination at AdventHealth TRI. Informed, written consent will be obtained at the screening visit.

We anticipate ~400 phone screens to successfully enroll and complete 18 participants per group (anticipating 15 completers per group).

Consent Process

We attest that all study staff delegated the authority to obtain informed consent will follow "Investigator Guidance: Informed Consent (HRP-802)", as well as "Investigator Guidance: Documentation of Informed Consent (HRP-803)".

Non-English Speaking Participants

We will not be enrolling Non-English speaking participants due to the complexity of the visits, number of visits and length of the study. The participant population will be English speaking.

Participants who are not yet adults (infants, children, teenagers)

We are not enrolling participants who are not yet adults.

Cognitively Impaired Adults

We will not enroll cognitively impaired adults.

Adults Unable to Consent

We will not enroll adults who are unable to provide consent.

Documentation of Informed Consent Process

Documentation of the informed consent process is required to establish that the participant was accurately and adequately informed and that no study-related procedures were initiated prior to obtaining informed consent. A research team member will note in the source documentation the consent process, date consent was obtained, and that consent was obtained prior to initiating any research procedures.

Waiver of Written Documentation of Consent or Waiver of Consent

N/A

Randomization

N/A

Study Visits

All study visits will be conducted at the AdventHealth Translational Research Institute.

PHASE I – SCREENING AND PRE-INTERVENTION TESTING

Both older athlete and older sedentary groups will undergo the screening and pre-intervention testing phase. See "Appendix: Schedule of Activities" for a table description of the procedures that will be performed during PHASE I.

Screening: SV1

Approximate visit time: ~ 3 1/2 hours

Volunteers will arrive, after an 8 hour overnight fast, to the AdventHealth TRI. Following voluntary written informed consent, all research participants will undergo a screening process for eligibility. This process may be repeated if necessary, under the discretion of the Medical PI. The following listed below procedures will be performed:

Demographics, Medication & Medical History, Physical Examination, Vital Signs, and Anthropometrics will be obtained. A full physical examination will be performed by a study physician, physician assistant, or nurse practitioner and will include general assessment of the following: head, ears, nose, eyes, throat, heart, lungs, abdomen, extremities, skin, neurological functioning, and any other impressions or problems noted by the provider at the time of exam. This will also include height, weight (metabolic), BMI, blood pressures, heart rate, respirations, temperature, medication, supplement history and a basic assessment of cognitive functioning via the Mini Mental State Examination (MMSE).

Electrocardiogram (ECG). A standard 12-lead ECG will be performed. Electrodes will be placed on the chest to provide a tracing of the electrical activity of the heart.

Physical activity questionnaires will be administered at screening. The Exercise History and Physical Activity Scale for the Elderly (PASE) are instruments that measures the level of habitual physical activity for individuals. The CHAMPS Physical activity health questionnaires will also be administered at screening. The MMSE will be administered at this time too.

Fasting blood draw to measure: a lipid panel; Complete Metabolic Panel (CMP); Complete blood count with differential (CBC w/ diff); TSH and HbA1c. Total blood volume for screen labs 14mls. Safety labs may be repeated per Investigator discretion, if needed. Blood draws may also take place at visit 2 or visit 3.

Urine collection for urinalysis.

Waist Circumference Measurements which are a surrogate measure of abdominal fat will be conducted during screening. The Gulick II tape measure will be used for accuracy in obtaining duplicate waist measurements. All circumferences will be measured in centimeters directly on the skin.

Hand-Grip Strength Test. All participants will perform a hand grip test that will be conducted three times with 1 minute of rest between each repetition using the dominant hand of each participant. The greatest grip strength and the average of the three tests will be recorded.

Lower extremity performance battery. The Short Physical Performance Battery (SPPB) is a brief performance-based test that will be used to measure lower extremity performance. The SPPB consists of three tasks: five repeated timed chair stands, timed standing balance (with feet in parallel, semi-tandem, and tandem positions), and a 4-meter walk to determine usual gait speed.

Step Test: Each participant will be asked to complete a timed step test.

Covid Testing: Participant must have a negative covid test 1 week prior to the next visit. Covid testing may take place on a separate visit when SV1 is not within 1 week of V1.

Screening tests may be repeated if the Investigator deems necessary.

Visit 1
Approximate visit time: ~ 3 hours

Review of Adverse Events, Concomitant Medications
Vital Signs
Body Weight

3-Day Food Diary: Participants will be given a food diary and asked to record what foods and drinks they eat over a 3-day period. Participants will return the completed diary on visit 2.

Fasting blood draw to collect EDTA plasma. Circulating lipids related to BAT activity and mass will be measured in plasma samples. Total blood volume will be 10 mls. Safety labs may be repeated per Investigator discretion, if needed.

MR Imaging for BAT Mass. Brown adipose tissue will be differentiated from white adipose tissue using fat fraction and T2* relaxation time maps generated from a commercially available modified 6-point Dixon (mDixon) water-fat separation method. MRI scans will be performed on a Philips 3T using axial, coronal, and sagittal slice orientations to provide anatomical reference scans in the neck and body area. Quantitative mDixon scans will be used for calculating proton density fat fraction (PDFF) maps. The participant may be removed from the magnet and repositioned during the exam in order to determine the same day variability of this method.

Dual Energy X-Ray Absorptiometry (DEXA). DEXA Scans will be performed to measure body fat and estimate muscle mass using a GE Lunar iDXA whole-body scanner. The participant will remove all metal accessories and may be asked to change into a hospital gown. The participant will lie on the DEXA table while the scanner arm emits low energy X-rays as it passes along the body. The scan takes up to 15 minutes and the radiation dose is less than 1 mrem, less than half the average daily radiation dose in America.

Indirect Calorimetry: Indirect calorimetry will be used during the maximal oxygen consumption test (VO2max) to measure the metabolic rate, respiratory quotient (RQ) and substrate utilization using a MAX II Metabolic cart (AEI Technologies, Pittsburgh, PA). The analyzer will be calibrated before each subject with standardized gases containing 5%

CO2, 21% O2 and balance N2. Calculations of O2 consumption (VO2) and CO2 production (VCO2) will be made by comparing a single measure of the O2 and CO2 concentrations of the testing room to continuous measurements of O2 and CO2 concentrations in expired air diluted in a constant air flow (20-50 L/min) generated by a pump. From the above, energy expenditure standardized for temperature, pressure, and moisture will be calculated at one-minute intervals.

Maximal Oxygen Consumption (VO_{2max}). Aerobic fitness will be determined by measuring maximal O_2 consumption (VO_{2max}) during a stationary bicycle exercise test. Provider monitoring will follow The American College of Sports Medicine's (ACSM) Guidelines for Exercise Testing and Prescription and AdventHealth TRI BATS-Pilot SSP. Heart rate, blood pressure and ECG will be recorded throughout this test. During the test, participants will breathe through a low resistance mouthpiece and wear a nose clip. Expired gases will be measured by indirect calorimetry. Following a standardized warm up, participants will begin exercising at a moderate intensity with the workload (resistance on a cycle ergometer) increased every minute until the participant can no longer continue. VO_2 max tests will take as little time as possible in order to ensure that test termination is due to the participant reaching VO_2 max rather than terminating the test due to muscle exhaustion. A leveling-off (plateau) or decline in oxygen uptake should be demonstrated in order to be reasonably sure that a participant has achieved the maximum capacity for aerobic metabolism. However, there are several additional criteria that will be used to determine if maximal aerobic capacity is achieved. These criteria are: the respiratory exchange ratio (RER) increased to 1.10 or higher and the participant's heart rate increased to within 10 beats of the age-predicted maximum (208 – [0.7 x age]). This test will be supervised by a qualified Medical Provider as stated in AdventHealth TRI SOP 030.100.015 Procedural Staffing.

In the event of a positive stress test, participants are referred to their primary care physician. If the electrocardiogram (ECG) is determined to be a false negative, participant may be allowed to continue participating in the study.

Triaxial Activity Monitor: Two tri-axial accelerometer-based activity monitors will be placed during Visit 2. One will be placed on the wrist and the other on the triceps of the same arm. The monitors integrate motion sensor data from the tri-axial accelerometer to estimate the energy cost of free-living activity. The participant will wear the monitors for 5-7 days, except while showering or bathing. The participant will have the monitors removed and the data uploaded at Visit 2 or Visit 3.

Visit 2

Approximate visit time: ~ 3 hour

Review of Adverse Events, Concomitant Medications

Vital Signs

Body Weight (metabolic)

Collection of Activity Monitors

Cardiac Function- Echocardiogram: Echocardiography measurements (systolic and diastolic function) will be performed using a Philips Epic or comparable level cardiac imaging system. Subjects will be properly hydrated 30 minutes prior to testing. 2D imaging, spectral and Tissue Doppler, and Strain with Heart Model Imaging will be

performed before any exercise testing. Left ventricular mass, left ventricular ejection fraction, left atrial volume and ejection fraction will be measured using Heart Model and estimation of right atrial pressure will be done using the most recent standards of The American Society of Echocardiography. The Doppler measurements and calculations will include aortic valve and left ventricular outflow tract velocity time integrals, E/A ratio, E/e' ratio, D/E slope, left ventricular myocardial performance index, isovolumic relaxation time, and tricuspid regurgitation peak velocity. In the event of scheduling challenges, the Echo can be on the business day before or after this scheduled visit.

Muscle Power Testing. Muscle power testing will be performed using a Biodex pneumatic-driven dynamometer which is equipped with load cells and potentiometers (for helping measure changes in joint angle). The dynamometer is connected to a PC with software to measure muscle power and velocity. After the participant is seated at rest for ~5 minutes, a blood pressure measurement is taken. If the value is below 160/100 mm Hg, the participant continues with the procedures below. If the value is above 160 and/or100 mm Hg, the participant's blood pressure is re-checked after five minutes and one final time after 10 min rest or until it is within an acceptable range. If after three attempts the participant's blood pressure is greater than 160 and/or100 mm Hg, the participant's test is re-scheduled for another day. If the BP is in acceptable range, the participant will warm up with one minute of free pedaling on a cycle ergometer (seat height is adjusted by setting the seat height at standing hip height and adjusting while participant is on the bike for 10^{o} of flexion at the knee when leg is fully extended). Tester then explains the power test and also describes the responses to the pain/discomfort (P/D) scale (range = 0 to 6) and that the participant may decide to discontinue testing at any time. After the ~1-minute bike warm-up the participant will be seated on the Biodex machine with the lateral condyle of the knee lined up with the axis of rotation of the machine arm and the seat belts should then be fastened snugly. Both legs will be tested. The participant will be instructed to keep arms crossed across the chest and to remember to maintain normal breathing patterns. participant will perform three power tests on each leg at each resistance of 60, 120 and 180 degrees per second with a ~2-minute rest between each adjustment in resistance and a ~5-minute rest between the right and left leg trials. The order of the resistance will be selected randomly. The participant will give a P/D rating after each trial. After both legs have been tested BP should be taken again immediately at conclusion of test and after ~5 minutes of rest. If BP reading is normal the visit will continue. If it is above 160/100 mm Hg the participant will rest for ~2 minutes and BP will be taken again. If BP has not normalized to below 160/100 mm Hg, the visit will be discontinued, and the participant will remain in the facility for at least ten more minutes while monitoring blood pressure. When BP finally normalizes, the participant will be sent home with the recommendation to contact their physician. The PI and the Medical PI will also be notified.

Termination criteria for maximal power testing:
- A participant asks to discontinue testing.
- Equipment failure

Relative termination criteria:
- Participant reports >3 on the P/D scale (with explanation that the pain persists after the cessation of movement or if the participant reports >3 in any area besides the knee, upper or lower leg.

Muscle strength (1-RM) assessments. The initial weights for knee extension should be set based on the participant's predicted 1 repetition maximum (1 RM). Based on previous

research, males and females < 65 years of age have a predicted 1 RM of 90% and 60% of their body weight, respectively, for the knee extension. For each additional 10 years of age the 1 RM should decrease 10% (ex. 91 year old male would have a predicted 1 RM of 60% of his body weight). This number should be calculated and recorded on the data collection sheet. One can then calculate the participant's first 5 lifts at 0, 20, 35, 50, and 70 percent of predicted 1 RM. This weight scale is followed, and weight is continually increased until termination of the test. Both legs will be tested, and the first leg will be selected randomly. The participant will be instructed to keep arms crossed across the chest and to remember to maintain normal breathing patterns. Appropriate rest intervals will be provided during testing and the participant will give an RPE rating and P/D if needed after each trial. Prior to 1-RM testing, if participant's blood pressure is less than 160/100 mm Hg, then the participant will warm-up on a bike for ~1-minute with no resistance. If BP was elevated the participant should rest for 5 more minutes. If BP is still elevated greater than 160/100 mm Hg, the participant will be sent home with the recommendation to contact their physician, and this participant will be followed up for potential re-testing. After both legs have been tested a BP will be taken after ~5 minutes.

Termination criteria for maximal strength testing:
- A participant asks to discontinue testing.
- An individual moves a weight through a full range of motion with relative comfort but cannot move through the full ROM with a two-unit increase.
- Participant cannot move through full ROM with proper form (participant lifts lower or upper back away from padded seat, participant kicks pad using acceleration force to lift weight, hands not crossed across chest, uneven or jerky ROM).

Relative termination criteria:
- Participant reports >3 on P/D scale with explanation that the pain persists after the cessation of movement or if the participant reports >3 in any area besides the knee, upper or lower leg.
- Participant reports >18 on RPE scale.

Covid Testing: Participant must have a negative covid test prior to the next visit. Covid testing may take place on a separate visit when V2 is not within 1 week of V3.

Visit 3 Day -7
Approximate visit time: 10 minutes + Evening admission (~6:00pm) overnight.

Review of Adverse Events, Concomitant Medications
Body Weight

Fasting Finger Stick to collect glucose for clamp preparation. Participants will be asked to fast overnight prior to blood draw.

Standardized Meal: Participants will visit the metabolic kitchen at AdventHealth TRI on the evening before the glucose clamp and consume a standardized meal (10 kcal/kg) consisting of 50% carbohydrate, 15% protein, and 35% fat.

Visit 3 Day -6
Afternoon discharge (~2:00pm)

Vital Signs
Body Weight

Resting Metabolic Rate/Respiratory Quotient (RMR/RQ): Indirect calorimetry will used at two times during the hyperinsulinemic-euglycemic clamp to measure the resting metabolic rate/respiratory quotient (RMR/RQ) and substrate utilization using a MAX II Metabolic cart (AEI Technologies, Pittsburgh, PA). The analyzer will be calibrated before each subject with standardized gases containing 5% CO2, 21% O2 and balance N2. Subjects will be instructed to lie in a Semi-Fowler's position and remain motionless and awake during these periods. A transparent plastic hood connected to the device will be placed over the head of the subject. Calculations of O2 consumption (VO2) and CO2 production (VCO2) will be made by comparing a single measure of the O2 and CO2 concentrations of the testing room to continuous measurements of O2 and CO2 concentrations in expired air diluted in a constant air flow (20-50 L/min) generated by a pump. From the above, energy expenditure standardized for temperature, pressure, and moisture will be calculated at one-minute intervals.

Hyperinsulinemic-Euglycemic Clamp: The glucose clamp is the gold standard for measurement of insulin sensitivity and will be performed as previously described by our group (1, 5, 9, 10). After an overnight 8-hour fast, an intravenous catheter will be placed in the vein for infusion of insulin and glucose. A second catheter will be placed in the vein of the contra-lateral arm for blood withdrawal. After baseline blood is collected, a primed (210 mg/m^2), continuous (2 mg/min/m^2) infusion of [6,6-2H2] glucose will be initiated for assessment of endogenous glucose production. A 2-step euglycemic clamp will be started with a 2-hour infusion of insulin (Humulin-R) at 15 mU/m^2/min, followed by 2 hours at 40 mU /m^2/min. Plasma glucose will be measured at 5-10 min intervals via NOVA StatStrip Glucose Meter. Plasma glucose will be allowed to either decrease to ~90 mg/dl or will be increased to ~90 mg/dl in each participant depending on the fasting blood glucose levels. Euglycemia (~90 mg/dL) will be maintained with a variable 20% dextrose infusion enriched with [6,6-2H2] glucose. Rates of glucose disposal (M) and endogenous glucose production (EGP) will be calculated by non–steady-state equations based on plasma [6,6-2H2] glucose enrichment determined by gas chromatography mass spectrometry. A baseline blood sample will be collected prior to -30 mins. Then three blood samples will be collected for determination of plasma insulin, free fatty acid (FFAs), C-Peptide, and glucose concentrations prior to the insulin infusion, and at the beginning of each steady state and every 10 minutes during the two steady state portions of the clamp which will last approximately 4 hours from the time at which the insulin infusion is initiated (-30min, -20min, -10min, 0min, +30min, +60min, +100min, +110min, +120min, +220min, +230min, and +240min). Participants will complete a 24-hour urine collection on days where hyperinsulinemic-euglycemic clamps are run. Total blood volume during this procedure will be approximately 192 mls.

Calculations: Hepatic glucose production is expected to be completely suppressed at this level of insulin, even in diabetic subjects. In this case, peripheral glucose uptake (R_d) should equal the glucose infusion rate (GINF) during steady state (the last 30 minutes of each step of the clamp) after correction for urinary glucose loss.

That is: - R_d = Steady State GINF - urinary glucose loss

Insulin sensitivity (S_I) will be calculated using the formula: $S_I = R_d$ / (steady state insulin level - basal insulin level) where steady state insulin equals the average insulin

concentration during the last 40 minutes of the clamp and basal insulin equals the average insulin level in the 30 minutes before starting the insulin infusion.

Fasting and insulin-suppressed FFA [IS-FFA] will be measured with an ultrasensitive FFA assay from Wako. Glucose disposal rate [GDR] will be expressed as mg/kg FFM [DEXA]/minute.

Metabolic flexibility of substrate oxidation

Before and during the steady state of the clamp, we will measure substrate oxidation using a MAX I Metabolic Cart (AEI Technologies, Pittsburgh, PA) to measure 'metabolic flexibility' as described by Kelley and Mandarino (19).

PHASE II – EXERCISE TRAINING INTERVENTION

Older sedentary participants will undergo 8 weeks of exercise training. Exercise training visits may extend up to 3 weeks through Phase III in rare circumstances to help accommodate staffing scheduling availability. The exercise program will be supervised by a certified exercise physiologist and will take place at the AdventHealth TRI exercise training facility. After the first week of exercise training at TRI, exercise training may take place remotely in rare circumstances, for example, if the participant has a short trip planned or the participant becomes ill and must skip a session during their intervention phase. Remote exercise will be brisk walking outside for a duration and intensity that is intended to be similar to the exercise performed at TRI. For remote exercise, the participant will be given and taught how to appropriately use a portable heart rate monitor to record their exercise sessions and a tracking log to document their session. A certified exercise physiologist will call a remotely exercising participant each day a remote exercise session is scheduled to provide instruction and to assess exercise compliance. Upon return to TRI, the data will be downloaded and added to the participant's exercise session files for analysis.

Exercise compliance will be monitored for exercise training 4 days per week (minimum of 32 total sessions). Aerobic training will be 30 minutes of brisk walking, jogging, cycle ergometry. Walking will be the primary mode of aerobic exercise given its widespread popularity and ease of administration across a broad segment of the older adult population. Walking will be at a moderate intensity and determined based on Heart Rate (50-70% of Maximal HR from most recent VO2max) and rating of perceived exertion using Borg's scale (ranges from 6 to 20). Other forms of endurance activity (e.g., stationary cycling) will be utilized on a limited basis when regular walking is contraindicated either medically or behaviorally. This is the exercise regimen that has demonstrated substantial increases in muscle size and strength in elderly people. See "Appendix: Schedule of Activities" for a table description of the procedures that will be performed during PHASE II.

PHASE III – POST-INTERVENTION TESTING

Following the exercise training intervention, participants in the OS group will participate in post-intervention testing. The testing procedures are a subset of those performed during the pre-intervention testing period (PHASE I). A detailed description of the procedures can be found above. The testing procedures will be taken over a 2-week period following the exercise intervention. See "Appendix: Schedule of Activities" for a table description of the procedures that will be performed during PHASE III.

Study Duration

Estimated start date for enrollment is Sept 30, 2019. Active recruitment and testing will continue until the end of the funding period or until 15 participants per group have been recruited. The study is funded by the National Institute of Aging until 01/31/2024.

Materials of Human Origin: Collection, Preparation, Handling and Shipping

Materials of human origin will be collected in the manner described in the specific study visits section of this protocol.

Biospecimen samples will be stored in ultralow temperature freezers and liquid nitrogen dewars or other storage units located at the AdventHealth TRI Laboratory Room 2404. The AdventHealth TRI facility is secured via key card and equipped with a back-up generator system. Laboratory personnel in the facility have 24/7 key-controlled access to the laboratory. Chain of custody of biospecimen samples is maintained through requisition forms and in the StarLIMS database. Specimen tubes are coded, and specimen requests and distribution are documented.

The plasma/serum samples will be stored indefinitely, or until a sample is fully used. All biological specimens will be stored without identifiers or linkage codes.

After study aims have been achieved and study related endpoints have been measured and analyzed, any remaining biospecimens will be stored at the AdventHealth TRI Laboratory Room 2404 and will then be considered as "archived biospecimens." Archived biospecimens will be used for any additional hypothesis-related experimentation or testing for the **purposes of this study**, **consistent with the original aims**, which cannot be predicted at the time the protocol is developed due to the evolving nature of scientific exploration.

Additionally, archived biospecimen samples may be stored indefinitely for future research. Archived biospecimens could be used for **separate research** by **both** AdventHealth Orlando scientists and scientists outside of AdventHealth TRI. This would be allowed for **research of any type** (without limitation to disease, process, or research methods) if it has scientific merit as determined by the Principal Investigator, with an additional review by the respective Program Director. For research outside of AdventHealth Orlando, a Material Transfer Agreement will be obtained, which will govern the transfer and chain of custody of the biospecimens outside of AdventHealth Orlando.

Study Outcome Measures (Endpoints)

The major outcomes of the study are collection of imaging of BAT by magnetic resonance imaging and circulating lipokines (12,13-diHOME) from 15 participants in each of the older athlete and sedentary groups. In addition, collection of all phenotype data are considered secondary outcome measures, including: Aerobic fitness (VO_2max test, DEXA, Muscle power and strength, Physical function (SPPB), and accelerometry data).

Data Management and Quality Plan

Data De-identification

Participants will be enrolled using Cerner's Patient Protocol Manager; the application assigns each participant a unique participant identifier, or "PID". This PID is a code

consisting of a combination of numerals and letters, which serves as the identifier for this participant for this research study and links them back to their hospital medical record and their protected health information (PHI). Access to the "link" between the PIDs, the PHI, and to the clinical data are only granted to the clinical research team as assigned on the Delegation of Authority Log. All the clinical research data is recorded in a de-identified fashion onto our paper source documents, which is then transcribed into our electronic case report forms, (CRF). The CRF is used for storage (a database) and facilitates analysis. Clinical data generated by research devices also uses the PID, and once the data has been transformed into interpretable results it is stored into the clinical research database. Both storage locations are secured and only assessable to the assigned clinical research team. The "link" will not be used to re-identify participants except in the event of a serious adverse event (SAE). The "link" will be stored in the Patient Protocol Manager and the clinical research database, where only the AdventHealth TRI research team has access. These secure databases are stored/accessed on the AdventHealth Orlando password-protected computer network. No one outside of AdventHealth Orlando investigators or researchers will have access to the databases.

Data Confidentiality, Storage, and Retention

The identity and personal health information will be kept confidential to the extent permitted by the applicable laws and/or regulations and will not be made publicly available. If results of this study are published or presented, the identities will not be revealed. Confidentiality will be maintained during and after the study. This information is also included in the informed consent, which is discussed with the participant prior to enrollment.

Study documentation and paperwork will be stored in our locked medical records room. The data records will also be stored in as electronic records. This data will be safeguarded so that only those on the research team have access to any of the clinical data (both source documentation and data warehouse storage). The electronic data is maintained by Adventist Information Technology (AIT) security controls.

The duration of study data retention will be determined by the AdventHealth TRI Records Management Policy. Electronic de-identified data will be kept indefinitely in our data warehouse.

Data Quality

Data quality control will occur according to our SOPs on Data Entry, Quality Control Procedures and Query Management. All data will be entered into an electronic data capture (EDC) system and checked against the paper source for accuracy by a second party (Data Entry SOP) and errors resolved through the Query Management SOP. Ten percent of the data points will be routinely checked at the beginning, middle, and close of a study for quality control (Quality Control SOP). Finally, all critical endpoints (as determined by the PI or Sub-I) will be assessed using quality control analyses. The data will be loaded into the clinical research database. Data in the warehouse will also be routinely monitored over time.

The device data will directly be imported into spreadsheets or entered and confirmed into the EDC. Data from the VO2max, activity monitors, and DEXA data are exported directly into spreadsheets for further calculations. Data for each of the endpoint analyses will be imported into an SAS database linked with a participant ID.

Data Sharing (outside of AdventHealth Orlando)
This is a collaboration partnership between AdventHealth TRI, and its affiliates. Certain data elements will need to be shared along with the biospecimen samples (primarily plasma samples). Should archived biospecimens be needed for research outside of AdventHealth Orlando and certain data elements that are connected to the archived biospecimen samples be needed to conduct the research, then Data Use Agreement(s) will be obtained. The Data Use Agreement(s) will identify the purpose for data sharing, the specific data elements to be shared, and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by a study coordinator.

Sample Size Determination

The primary purpose of this protocol is to understand the role of physical activity on BAT mass and circulating batokines, and how these variables relate to cardiac and physical function in older adults. A formal sample size calculation was not performed. Rather, we referred to published studies with similar experimental designs (determination of BAT mass by PET scan) as a guide for sample size determination. From the published literature we determine that an n=15 per group (active and sedentary) will provide sufficient statistical power to detect differences in BAT mass.

Statistical Analysis Plan

The analysis will involve multiple steps and draw on statistical methods ranging from simple descriptive statistics to the modeling of repeated measures outcome data accounting for potential missing observations. The major outcomes of the study, the BAT mass, circulating batokines, muscle mass, strength, power and quality, and VO_2max will be compared using an unpaired student t-test to explore baseline differences between older athletes and older sedentary groups. Additionally, repeated measures ANOVA will be performed on the dependent variables as a function of the exercise intervention in the older sedentary group. Multiple linear regression will be used to determine how BAT mass and circulating batokines explain variation in physical characteristics of muscle (strength, quality) and cardiovascular health (VO_2max). Sex will be explored as a biological variable.

Potential Risks and Benefits

Potential Benefits

Participants will likely receive no direct benefit from taking part in this research study.

Potential Risks

RMR/RQ. There is no physical risk associated with RMR/RQ. Other risks include a feeling of claustrophobia experienced by some participants while under the transparent "hood".

Protection Against Risk:

- A member of the study staff will remain with the subject at all times to ensure his/her comfort.

Blood draws and Intravenous lines (lab samples and infusions, e.g.). There is a risk of pain, vasovagal syncope, hematomas, and/or infection at the blood draw site (low risk of serious AEs). The total volume of blood drawn is over the course of the study is approximately 372 mls.

DEXA scan. There is a very small risk of cancer with excessive exposure to any radiation. There are also risks for unborn children associated with radiation exposure. The radiation dose from the scan is less than a chest x-ray, or less than half the average amount a person would receive in a day in America.

Maximal Oxygen Consumption (VO_{2max}). There is a risk of changes to blood pressure, irregular, fast or slow heart rhythm, fainting, and in rare instances (<0.01%) heart attack, stroke, or death with exercise testing, at similar rates to exercising during daily life.

Activity/Heart Rate Monitoring. There are no risks associated with the wearing of activity monitors. However, the Bodymedia armband has sensor pads that touch the skin that may be irritating for some participants. Participants with nickel allergies will not be required to wear the Bodymedia activity monitor.

Biodex/Strength testing/Exercise/Maximal Oxygen Consumption (VO_{2max}). There is a risk of changes to blood pressure, irregular, fast or slow heart rhythm, fainting, and in rare instances (<0.01%) heart attack, stroke, or death with exercise testing, at similar rates to exercising during daily life.

Magnetic Resonance Imaging and Spectroscopy. There are no known biological risks associated with magnetic resonance imaging and spectroscopy. Some short-term discomfort may be experienced. The short-term risks associated with MRI are minimal, but include heating, loud noises and claustrophobia. There are some people who should not undergo MRI; the contraindication is largely based on the presence of metal objects within a person (i.e. pacemaker, aneurysm clip, metal fragments, etc.).

Cardiac Function - Echocardiogram A transthoracic echocardiogram carries no risk. There's a chance for slight discomfort when the electrodes are removed from the skin. This may feel similar to pulling off a Band-Aid.

Hyperinsulinemic-euglycemic clamp. There is a risk of hypoglycemia (low blood sugar) during the clamp.

Mitigation of Risks

Blood draws and Intravenous lines (lab samples and **infusions, e.g.)**. All venipuncture will be conducted by qualified staff using aseptic techniques.

DEXA scan. A urine pregnancy test will be done prior to scans of all women of childbearing potential (all women except those with prior hysterectomy, tubal ligation, or absence of menses for ≥2 years).

Activity Monitoring. Participants with nickel allergies will not be required to wear the Bodymedia activity monitor.

Biodex/Strength testing/Exercise/Maximal Oxygen Consumption (VO_{2max}). Prior to conducting any exercise testing, a history and physical including ECG will be conducted and will be used to determine whether a participant is clear to participate in exercise testing. An MD/DO/NP/PA will be available during exercise testing for participants who are at risk according to the American College of Sports Medicine (ACSM) Guidelines. ECG, blood pressure and heart rate may be monitored during the test.

Magnetic Resonance Imaging and Spectroscopy. There will be a strict safety screening protocol, to ensure any people with contraindications are excluded from volunteering. There will be no diagnostic analysis associated with any of the MR sequences used in this protocol. However, some of the MR images we obtain as part of this protocol may show incidental medical findings. In the case where a medical abnormality is apparent on an image, the image will first be reviewed by an investigator on this protocol. If the abnormality is confirmed, then the participant will be instructed to seek medical attention from their health care provider.

Hyperinsulinemic-Euglycemic Clamp. In-house supervision by a qualified physician or mid-level provider at all times during the procedure. Constant monitoring of blood glucose during the procedure (every 5-10 minutes). Immediate availability of corrective measures (IV 50% dextrose and/or glucagon).

Provisions to Protect the Privacy Interest of Participants

Participants will be assigned unique identifiers for study-related records. All precautions will be taken to make sure that only authorized individuals will access participant research records. The collection of sensitive information about participants will be limited to minimum necessary to achieve the aims of the research, so that no unneeded sensitive information will be collected.

Early Withdrawal of Participants

Investigator Withdrawal of Participants

The participation in this study may be stopped at any time by the study PI without the participant's consent because:
-The study Medical investigator thinks it necessary for participant's health or safety;
-Participant has not followed study instructions;
-The AdventHealth TRI has stopped the study; or
-Administrative reasons require the participant's withdrawal.

Participant Request for Withdrawal from Study

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits.

Data Collection and Follow-up for Withdrawn Participants

Participants who request withdrawal or who are withdrawn by the PI from the study will have their data maintained in the research database. This data and biospecimens from the

withdrawn participant may be included in subsequent analysis. Participant withdrawal of biospecimens is not an option.

Adverse Event Reporting

Adverse Events

An adverse event (AE) is defined as both an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. Each participant is evaluated for adverse events at every study visit. Any event that is reported to the study staff and which meets the criteria of an adverse event will be documented as such and graded as to its attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol) and severity (mild, moderate, or severe).

Recording of Adverse Events

At each contact with the participant, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

Notification of Adverse Events

Adverse events will be reported according to AdventHealth Orlando IRB guidelines.

Safety Monitoring Plan

Safety Monitoring

Research and safety data will be reviewed by the PI. This review will take place at regular meetings with the clinical coordinator and medical investigator where the safety labs for each new participant will be reviewed. Other items discussed will include: progress or adverse events occurring in the following: participant confidentiality, participant recruitment, and consent process. All will monitor response to tolerance of and effectiveness of the exercise program.

Progress reports, including patient recruitment, retention/attrition, and AEs from the reviews will be compiled into an annual report and will include a list and summarization of adverse events. In addition, the annual report will address (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely.

Data and Safety Monitoring Board (DSMB) or Equivalent

N/A

Ethical Considerations

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. No vulnerable populations will be studied in this protocol.

Sharing of Results with Participants

Participants will be offered the opportunity to meet with the PI to review the results of their outcomes. Copies of their testing results will be made available to the participants. In addition, the PI will provide an overview of the study's outcome to the participant if he or she requests the information.

Funding Source

This study will be supported by a sub award from a prime award from the National Institutes of Health, National Institute of Aging (R01 AG060542), P.I., Kristin Stanford, Ph.D. Sub Award PI and Co-Investigator, Paul Coen, Ph.D. The IRBNet # for this grant is 1456947.

Participant Stipends or Payments

Participants will receive a total of $1425.00 (or up to $1641 with exercise extension visits) if in the OS group, and $500.00 if in the OA group, upon the completion of all study visits. Ride shared services, for example Uber Health or Lyft, may be made available to the participants by the study team with PI approval. Travel services will be arranged only by the study team with approval. If participant is unable to complete all study visits, the stipends will be prorated to the following:

Athlete Group **Total Payment: $500**	Phase 1	SV1	$0
		V1	$75
		V2	$75
		V3	$350

Sedentary Group **Total Payment: $1425** *(up to $1641 with Extension Visits)*	Phase 1	SV1	$0
		V1	$75
		V2	$75
		V3	$150
	Phase II	V4, 5, 6, 7	$75
		V8, 9, 10, 11	$75
		V12, 13, 14, 15	$75
		V16, 17, 18, 19	$75
		V20, 21, 22, 23	$75
		V24, 25, 26, 27	$75
		V28, 29, 30, 31	$75
		V32, 33, 34, 35	$75
		Exercise Extension	*$18/visit*
		V36	$75

	Phase III	V37	$150
		V38	$150
		V39	$150

A Mastercard® will be the method of payment and will be requested upon completion of the study or on their last day of participation, should they find they cannot complete the study.

Publication Plan

We attest that the AdventHealth TRI faculty and staff will adhere to POLICY-TRI-ADM-005 (Access to Clinical Trial Data for Publication Purposes). The goal will be to publish novel and interesting findings from this research. Assignment of authorship and the contributions of each author will be determined by the International Committee of Medical Journal Editors (ICMJE) policy to guide authorship.

Appendix: Schedule of Activities

	Phase I: Pre-Intervention					Phase II: Intervention									Phase III: Post-Intervention				
	Screening	Baseline				Exercise Training (~8 wks x 4 sessions/wk)								Exercise Extension	Follow-up				
Visit	SV1	V1	V2	V3		V 4-7	V 8-11	V 12-15	V 16-19	V 20-23	V 24-27	V 28-31	V 32-35	(prn)	V36	V37	V38	V39	
Day	D-54	D-26	D-19	D-7	D-6	D0* - D3	D7 - D10	D14 - D17	D21 - D24	D28 - D31	D35 - D38	D42 - D45	D49 - D52		D56	D60	D64	D70	D71
Windows**	within 28 days of V1+2d	±2	±2	±4 CRU overnight		Wk 1 Mon-Sun	Wk 2 Mon-Sun	Wk 3 Mon-Sun	Wk 4 Mon-Sun	Wk 5 Mon-Sun	Wk 6 Mon-Sun	Wk 7 Mon-Sun	Wk 8 Mon-Sun	*Wk 9-11 Mon-Sun	+3	±2	±3	±2 CRU overnight	
OA Group	X	X	X	X	X/EOS														
OS Group	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X/EOS
Informed Consent	X																		
Inclusion/Exclusion Criteria	X																		
Demographics	X																		
Past Medical History	X																		
Concomitant Medication/Supplements	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X														X				
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X			X														X	
Weight (metabolic)	X	X	X	X	X										X	X	X	X	X
BMI	X														X				
Waist circumference	X														X				
Vital Signs (BP, Pulse, Respiration, Temp)	X	X	X		X										X	X	X		X
ECG (12-lead)	X														X				
Study Specific Questionnaires: MMSE, PASE, CHAMPS, Exercise History	X																		
Blood Collection: Lipid Panel, CMP, CBC with differential, TSH, HbA1c	X														X				
Blood Collection: EDTA Plasma (for		X														X			
Covid testing	X*		X*												X*		X*		
Urine Collection	X																		
Handgrip strength	X														X				
SPPB	X														X				
Step Test	X														X				
Dispense 3 Day Food Diary		X														X			
Collect 3 Day Food Diary			X														X		
MRI		X														X			
DEXA		X														X			
VO2max test/stress test		X														X			
Placement of Accelerometers		X													X				
Collect Accelerometers			X*	or X													X*	or X	
Echocardiogram			X														X		
1-RM Muscle Strength			X														X		
Biodex			X														X		
Blood Collection: POC Glucose (clamp prep)				X														X	
Dinner Meal Consumption				X														X	
24-hr Urine Collection					X														X
Hyperinsulinemic-Euglycemic Clamp					X														X
RMR/RQ					X														X

**Scheduling Windows are noted here as a general guide. Visits can be scheduled slightly outside of noted windows in unique/rare circumstances with PI approval without sacrificing participant safety or scientific data.

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