Study Protocol Title:
Adipogenesis, triglyceride turnover and cellular composition of adipose tissue in response to endurance training (ATLAS).

Study Sponsor:
AdventHealth

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List of Abbreviations:

ACSM – American College of Sports Medicine
AH – AdventHealth
BMI – Body Mass Index
CRF – Case Report Forms
DO – Doctor of Osteopathic Medicine
ECG – Electrocardiogram
EDC – Electronic Data Capture
FDA - Food and Drug Administration
GCP - Good Clinical Practices
ICH - International Conference on Harmonization
IR – Insulin Resistance
IRB – Institutional Review Board
MD – Medical Doctor
MRI – Magnetic Resonance Imaging
PBRC – Pennington Biomedical Research Center
PCOS – Polycystic Ovarian Syndrome
PHI – Protected Health Information
PI – Principal Investigator
PID – Participant Identifier
RER – Respiratory Exchange Ratio
SAE – Serious Adverse Event
SOP – Standard Operating Procedure
TG – Triglyceride
TRI - Translational Research Institute
$VO_{2\,max}$ – Maximal Volume of Oxygen Consumed
WAT – White Adipose Tissue
snRNAseq – single nuclei RNA sequencing

Introduction

This document is a research protocol for a human research study to investigate the effects of exercise training on adipogenesis, triglyceride turnover and cellular composition of adipose tissue, and insulin resistance in subjects with obesity. The described study will be conducted in compliance with the research protocol, Good Clinical Practices (GCP) International Conference on Harmonization (ICH) Guidelines (E6) for GCPs standards as adopted by the Food and Drug Administration (FDA) and associated Federal regulations, and all applicable institutional research requirements.

Background Information and Scientific Rationale

Excess adiposity – obesity – causes insulin resistance (IR) in white adipose tissue (WAT) and other tissues. The role of poor WAT quality in IR, however, is less clear. Low adipocyte triglyceride (TG) turnover, high formation rates of new adipocytes, high senescent cell burden, tissue and cellular inflammation and high circulating free fatty acids (FFAs) all characterize poor WAT quality [1, 2].

Exercise generally improves whole-body IR and decreases fasting FFAs – even without reductions in overall fat mass – thus highlighting the significance of exercise effects on WAT quality. Evidence of the direct impact of exercise – independent of weight loss – on WAT-specific IR is limited and inconclusive. The underlying mechanisms that drive exercise-induced changes in WAT biology, such as new adipocyte formation and TG turnover rates, cell composition, senescence, and inflammation, are poorly understood in humans.

Using a suite of innovative methods, ours will be the first study to comprehensively assess the direct effects of exercise on features of WAT quality both in vivo (formation of new adipocytes and adipocyte TG turnover) and ex vivo (cellular landscape of WAT) and WAT-specific IR (suppression of lipolysis via subcutaneous microdialysis) in humans with obesity. Using a practical approach of isotopic labeling that incorporates deuterium (2H), administered as oral 2H2O, into the DNA and TG moieties of adipocytes, we have compelling published and preliminary evidence that exercise reduces in vivo formation of new adipocytes in both mice and humans, suggesting an important exercise-induced mechanism of WAT remodeling [3-5]. We reason that increased adipocyte TG turnover rates improve adipocyte function, WAT-specific IR and overall WAT quality and thus abrogate the need for increased formation of new adipocytes.

Adipocytes comprise 90% of the tissue volume, but only ~50% of WAT cellular content, with the remainder comprised of immune cells, stem cells, and pre-adipocytes [6, 7]. Using our established method [8], we will employ innovative single nuclei transcriptomics (snRNAseq) to discover the effects of exercise on senescence and inflammation within adipocytes and non-adipocytes of WAT to further understand how exercise impacts WAT quality and WAT-specific IR via in vivo microdialysis during insulin suppression.

Study Objectives

The overall aim of this study is to investigate the effects of exercise training on adipogenesis, TG turnover and cellular composition of adipose tissue, and insulin resistance in subjects with obesity.

Primary Objective/Aim/Goal/Hypothesis

The primary aim of this study is to investigate the effects of 12-weeks of endurance exercise training on *in vivo* formation of new adipocytes, TG turnover rates, and cellular composition of the WAT.

Approach: A subcutaneous WAT biopsy from the abdomen will be collected before and after 12 weeks of deuterium (2H) labelled water administration and endurance exercise training in men and pre-menopausal women with obesity. Collected WAT samples will be utilized for *in vivo* measurements of adipocyte formation and TG turnover. The remaining WAT samples will be frozen for future analyses that include snRNAseq and histology to assess cellular composition, senescence, and inflammation of the WAT

Secondary Objective/Aim/Goal/Hypothesis

The secondary aim of this study is to assess the effects of 12-weeks of endurance exercise training on WAT-specific IR and fasting FFA levels and molecular adaptations in skeletal muscle that may contribute to the alterations in WAT.

Approach: Blood samples and dialysates from abdominal subcutaneous WAT will be collected during hyperinsulinemic clamp to assess whole-body IR and WAT-specific suppression of lipolytic rates under physiological insulin in men and pre-menopausal women with obesity. A skeletal muscle biopsy from the vastus lateralis will be collected before and after 12 weeks of deuterium (2H) labelled water administration and endurance exercise training to assess transcriptomic and proteomic adaptation that may associate with WAT adaptations.

Study Design

Research Design

This is a double-site (AH TRI and PBRC), longitudinal (12 weeks) randomized clinical trial with two groups; a control group (CTRL) and an exercise training group (EX)

Research Intervention Description

Subjects will be submitted to 12 weeks of supervised aerobic exercise training intervention and oral deuterium-labelled water administration OR oral deuterium-labelled water administration only.

This study consists of a Screening Period, Baseline Period, Intervention Period, and Follow-up Period, detailed below.

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

AdventHealth Orlando Sites: Translational Research Institute (TRI)

Estimated number of subjects at AdventHealth Orlando site: 35 men and women (accounting for a 20% dropout; we expect 29 to complete the study)

Name of external site(s) outside of AdventHealth Orlando: Pennington Biomedical Research Center

Estimated number of subjects at external sites: 35 men and women (accounting for a 20% dropout; we expect 29 to complete the study)

Total number of all sites: 2
Estimated number of subjects at all sites combined: 70

Multi-Site Research Logistics/Communication Plan

AdventHealth IRB will be the IRB of record for both sites, and AdventHealth will be the coordinating center. The two participating sites will meet via teleconference or phone call on a regular basis to discuss any issues, recruitment strategies, enrollment status, safety concerns, and any changes to the study protocol or supporting documents. Dr. Sparks and Dr. White have known each other for over a decade and have complementary skill sets in adipose tissue metabolism, as well as working closely together on the Program Planning Committee for The Obesity Society (TOS) for the past several years. Both investigators have done in-person site visits in the last 2 years in order to align their common laboratory practices related to this project, in addition to meeting virtually on a bi-weekly basis to plan this project. This project represents a continuation of the strong collaborations between AH TRI and PBRC. AH TRI and PBRC are both clinical sites in MoTrPAC (U01AR071133) and thus aligned and cross-validated for all clinical assessments, procedures, WAT processing and the exercise intervention. In broader terms, AH TRI and PBRC have long outstanding track records in leading multi-site lifestyle modification interventions in studies funded by the NIDDK (MoTrPAC, DPP, LookAHEAD), NIA (CALERIE 1 and 2, LIFE), and USDA (PROOF). While the proposed study will capitalize on our sites' participations in MoTrPAC and align our overlapping clinical procedures and intervention accordingly, we cannot execute this as an ancillary to MoTrPAC due to the prospective nature of the $^{2}H_2O$ labeling, hyper-insulinemic clamp and large tissue requirements for the downstream assays. We have chosen to conduct this clinical exercise trial at two sites to maximize efficiency through use of common core capabilities and complementary bench-based methods, to ensure successful completion of the enrollment goals, as well as enhance our ability to include African Americans (mainly from the PBRC population) and Hispanics (mainly from the AH TRI population).

Research Conducted in a Foreign Country

N/A

Community-Based Participatory Research

N/A

Subject Selection

Vulnerable Populations (if applicable)

Cognitively Impaired Adults: N/A

Children: N/A

Pregnant Women: N/A

Neonates of non-viable or uncertain viability: N/A

Prisoners: N/A

Employees: Recruitment efforts will follow AdventHealth recruitment Standard Operating Procedures (SOPs) at AH or PBRC recruitment SOPs for research at PBRC. AdventHealth and PBRC employees will not be individually targeted nor excluded from study participation based on employment. AdventHealth and PBRC employees who engage the AdventHealth Translational Research Institute or PBRC 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 or PBRC.

Students: Students will not be individually targeted nor excluded from study participation based on employment.

Inclusion Criteria

1. Men and pre-menopausal women;
2. 18-45 years of age;
3. BMI 30.0-40.0 kg/m^2;
4. For women, if not using pharmaceutical (hormonal) contraception (i.e. birth control pills, vaginal ring, injections, or implant), must agree to use either a double barrier method as a form of birth control to prevent pregnancy (i.e. male condom with spermicide, with or without cervical cap or diaphragm); use implants or intrauterine contraceptive devices; have a tubal ligation (surgically sterile); practice abstinence; or be in an established relationship with a vasectomized or same sex partner during the entire duration of the study;
5. Able to speak and understand written and spoken English;
6. Sedentary defined as self-reporting no more than 1 day per week, lasting no more than 60 minutes, of regular (structured) endurance or resistance exercise in the past year.
7. Must be willing to be randomized to either study group;
8. Must be willing to adhere to all study procedures.
9. Participant has 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.

Exclusion Criteria

1. Self-reported unstable body weight in the last 3 months (> ±5%);
2. Significant changes in the quality of the diet or the level of physical activity within the last 3 months;

3. Weight >396 lb (180 kg);
4. Diagnosis of Type 1 or Type 2 diabetes mellitus;
5. Fasting blood glucose ≥126 mg/dL;
6. HbA1c ≥ 6.5%
7. Blood pressure >140/90 mmHg ;
8. Self-reported history or presence of the following cardiovascular conditions: congestive heart failure, coronary artery disease, significant valvular disease, congenital heart disease, serious arrhythmia, stroke, symptomatic peripheral artery disease, stable angina, myocardial infarction or coronary revascularization within 6 months; clinically significant abnormalities on ECG, presence of cardiac pacemaker, or implanted cardiac defibrillator; inability to complete the VO_2max test;
9. Anemia (hemoglobin <12 g/dl in men or 11 g/dl in women; Hct <34%);
10. Kidney disease (creatinine >1.6 mg/dl or estimated glomerular filtration rate <60 mL/min/1.73m^2);
11. Abnormal liver enzymes (>2 times the upper limit of normal) that are clinically significant;
12. Serious digestive disorders including severe gastroesophageal reflux disease (GERD), irritable bowel syndrome (IBS), Crohn's disease, chronic constipation and/or diarrhea, etc.;
13. Self-reported chronic, active, or latent infection requiring chronic antibiotic or anti-viral treatment; Human Immunodeficiency Virus (HIV); active hepatitis B or C undergoing antiviral therapy;
14. Current or history of eating disorders;
15. Abnormal bleeding or coagulopathy (self-report) or history of a bleeding disorder or clotting abnormality;
16. Clinical diagnosis of Chronic Obstructive Pulmonary Disease (COPD);
17. Dyslipidemia (fasting triglycerides >500 mg/dL; low-density lipoprotein cholesterol (LDL-C) >190 mg/dL);
18. Whole blood donation in the last 3 months or platelet/plasma donation in the last week or plans for donation during entire protocol period;
19. Receiving active treatment (including monoclonal antibodies) for autoimmune disorders within the last 6 months;
20. Alcohol consumption >7 drinks per week for women or 14 drinks per week for men or history of binge drinking (≥5 drinks for males or 4 drinks for females in a 2-hour period more than once per month);
21. Current smokers (smoking or use of any tobacco or e-cigarette/e-nicotine/THC/cannabis products in the last 3 months); stopped smoking <10 years at time of screening for those with a >20 pack-year smoking history or stopped smoking <5 years at time of screening for those with a <20 pack-year smoking history;
22. Night shift work in the last 6 months or planning night shift work during the study period;
23. Known allergy to lidocaine or other local anesthetic;

24. Hyperthyroidism including those with normal TSH (0.35-4.5) on pharmacological treatment; individuals with hypothyroidism may be referred to their primary care provider for evaluation and retested; any medication change for hypothyroidism must be stable for ≥3 months prior to retesting;
25. Previous bariatric or other surgery for obesity;
26. History of cancer (other than non-melanoma skin cancer) within the last 5 years (not in remission); anti-hormonal therapy (e.g., for breast or prostate cancer) within the last 6 months;
27. Diagnosed psychotic or psychiatric conditions prohibiting adherence to study protocol; hospitalization for any psychotic or psychiatric condition within one year;
28. Currently pregnant (pregnancy test performed on day of DEXA scan in women of child-bearing potential); post-partum during the last 12 months; lactating during the last 12 months; planning to become pregnant during the participation period;
29. Polycystic Ovarian Syndrome (PCOS) (self-report);
30. Metabolic bone disease (self-report) including history of non-traumatic fracture from a standing height or less and/or current pharmacologic treatment for low bone mass or osteoporosis, other than calcium, vitamin D, or estrogen;
31. Hospitalization for COVID-19 infection in the past 12 months; individuals who tested positive for COVID-19 but were not hospitalized must be symptom-free at least 14 days;
32. Partial and/or full hysterectomy (self-report);
33. Not willing to archive biospecimens for future use;
34. Not willing to undergo the microdialysis procedure;
35. Participants who fulfill any of the contraindications for MRI including metal implants, devices, paramagnetic objects contained within the body and excessive or metal-containing tattoos;
36. Unable to participate in MR assessments due to claustrophobia or physical limitations of equipment tolerances (e.g., MRI bore size) based on investigator's judgment at screening;
37. Positive toxicology result from screening;
38. The following medication use is exclusionary:

- Dose change for any chronic-use drug in the last 3 months;
- Cardiovascular: beta blockers and centrally acting anti-hypertensive drugs, anticoagulants, antiarrhythmics, and antiplatelet drugs (other than aspirin ≤100 mg/day);
- Psychiatric: chronic use of medium- or long-acting sedatives and hypnotics, including all benzodiazepines (short-acting non-benzodiazepine sedative-hypnotics are allowed), mood stabilizers, antiepileptic drugs, stimulants, Attention-

Deficit/Hyperactivity Disorder (ADHD) drugs, anti-psychotic drugs;

- Pulmonary/Inflammation: chronic oral steroids (inhaled steroids are allowed), burst/taper oral steroids more than once in the last 12 months, B2-agonists (allowed if on stable dose at least 3 months);
- Genitourinary: 5-alpha reductase inhibitors, daily phosphodiesterase type 5 inhibitor;
- Hormonal: androgenic anabolic steroids, anti-estrogens, anti-androgens, estrogens and/or progestins used for reasons other than birth control, growth hormone, insulin like growth factor-I, growth hormone releasing hormone, any drugs used to treat diabetes mellitus or lower blood glucose, metformin for any indication, any drugs used specifically to induce weight loss, any drugs used specifically to induce muscle growth/hypertrophy or augment exercise-induced muscle hypertrophy;
- Pain/Inflammation: narcotics and narcotic receptor agonists, regular use of non-steroidal anti-inflammatory drugs (NSAIDs) or acetaminophen, muscle relaxants ≥2 days per week;
- Other: chronic systemic antimicrobials (antibiotic, antiviral, antifungal, antiparasite, etc.) for any reason, high-potency topical steroids if ≥10% surface area using rule of 9s, continuous/chronic use of antibiotics or other anti-infectives for treatment or prevention, monoclonal antibodies, anti-rejection medications/immune suppressants;

39. 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.

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". All PBRC investigators and staff involved in the project will be appropriately trained to conduct each element of the research in accordance with standard operating and quality control procedures, with knowledge of racial, ethnic, and cultural appropriateness. In addition, the study team has extensive expertise in topics aligned with the project, including nutrition, obesity, physical activity, physiology, adipose tissue, energy metabolism, and statistics. All methods and procedures in the project have been meticulously authenticated and are well established in the laboratories of AH TRI and PBRC, and

the analyses will be conducted in compliance with established Good Laboratory Practice methodological protocols.

We will implement regular, ongoing discussions between the principal investigator (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. PBRC will remain in communication with TRI through the duration of the study to ensure that all required approvals have been obtained, that each site has the most current version of the protocol, consent document, and HIPAA authorization that includes any recent modifications, and that all data will be safeguarded as required by local information security policies

The AdventHealth Translational Research Institute facilities are state of the art, and we have within our building all the 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. The multidisciplinary team at PBRC is well suited to conduct the study in alignment with the experimental design and to provide appropriate safety, oversight, and assessment of adverse events with all procedures (e.g. Medical Investigator and nurse practitioners).

Other members of the study team include Dr. Robbie A. Beyl (biostatistician at PBRC) and Dr. Marc Hellerstein (consultant). Dr. Beyl's primary responsibilities for this research will be clinical intervention design, creation of the randomization scheme, and conducting the statistical analyses. Dr. Hellerstein developed the method of 2H-labeling for quantification of adipocyte formation and TG turnover rates *in vivo* in mice and humans and has a long history of collaborating with Dr. White. Dr. Hellerstein will provide support for the data analyses and interpretations.

Study Procedures

Subject Recruitment and Screening

Recruitment methods utilized may include, but will not be limited to, the following: recruitment from within the AdventHealth TRI or Pennington Biomedical Research Center patient population, electronic medical records and database searches (including third party recruitment vendors); advertising in multiple media such as print ads, flyers, brochures, posters; radio ads; television spots; and internet/social media advertising. All advertising materials will be submitted to the AdventHealth Institutional Review Board (IRB) for review prior to using or publishing them. Recruitment efforts will follow AdventHealth Orlando recruitment SOPs for research.

Consent Process

We attest that all study staff delegated the authority to obtain informed consent will follow CW AHC 216, "Informed Consent Process and Written Documentation of Informed Consent". Upon consent, a research team member will provide the participant a copy of the signed consent and the original consent form placed in the participants chart.

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

- N/A

Cognitively Impaired Adults

- N/A

Adults Unable to Consent

- N/A

Documentation of Informed Consent Process

Documentation of the informed consent process is required to establish that the subject 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

Waiver of Written documentation of Consent (consent will be obtained but signatures will not be required)

N/A

Waiver or Alteration of the Consent Process (consent will not be obtained, required information will not be disclosed, or the research involves deception)

N/A

Waiver or Alteration of HIPAA Authorization

Partial waiver of HIPAA authorization will be requested.

Non-English Speaking Subjects

We intend to enroll only English-speaking participants due to the complexity of the study procedures and the lack of translational capacity that could be adequately provided throughout the duration of the study.

Randomization

Men and women will be randomized to the CTRL and EX groups on a 1:1 basis following successful completion of the VO_2max test and imaging tests. Participants will be stratified by sex to ensure balance between each of the groups. Stratifying allows for different sample sizes in each sex, but ensures that within each sex, there are an equal number of treatments and controls.

Study Visits

Study visits will be conducted at the AdventHealth Translational Research Institute or Pennington Biomedical Research Center.

<u>Screening Visit (SV)</u> (Outpatient; Approximate visit time: 2 hours)

Participants will arrive for this outpatient visit after a 10 hour overnight fast. After obtaining informed consent, data collection for assessing eligibility will commence. The screening visit will include:

[1]Urine for pregnancy test (if applicable), urinalysis, and toxicology screen (Amphetamine, Barbiturate, Benzodiazepine, Cocaine, Methadone, Opiate, THC, Cannabis, Tricyclics, and Oxycodone)

[2]Demographics and complete medical history (including, but not limited to, alcohol use, concomitant medications, health conditions, allergies and exercise habits)

[3]Vital signs (respiratory rate, temperature, heart rate, blood pressure)

[4]Standard physical examination performed by a study physician, physician assistant, or nurse practitioner

[5]Anthropometrics including height, weight, waist circumference, and BMI

[6]Screening blood draw to measure CBC with differential and platelets, comprehensive metabolic panel (CMP), lipid profile, blood glucose, HbA1C, ALT, AST, alkaline phosphatase, insulin level, and TSH

[7]Electrocardiogram (ECG)

[8]Consent Process Checklist assessing the potential participants' understanding of and willingness to perform all study activities.

Upon review of all clinical and laboratory-based screening endpoints, eligible participants will then be scheduled for baseline assessments. Laboratories can be repeated and participants may be rescreened for the study at the PI or provider's discretion.

Baseline Period – Visit 1 (V1)

Day -14 (±2): Outpatient. Approximate visit time at TRI: ~ 3-4 hours

[1]**Anthropometrics** including height, weight, waist circumference, and BMI.

[2]**Vital signs** including respiratory rate, temperature, heart rate, and blood pressure

[3]**MR assessment of visceral and subcutaneous fat (VAT/SAT) and thigh fat (~1 hour)*:** Volumetric measurement of fat, muscle and bone in the participant's abdomen and thigh will be completed using an Achieva 3T MRI/Multinuclear MRS (Philips, Andover MA), a Siemens 3T Prisma (Siemens Healthcare, Erlangen, Germany) or a GE Discovery 750w. Scans will be performed under standardized conditions with subjects in a supine position using the quadrature body coil. Each scan will take approximately 45 minutes to perform. To minimize the potential for confounding factors to affect the measurements, MR scans will be conducted following a fast (except water) of at least 4 hours. Low resolution scans will be taken to determine appropriate positioning for high resolution images. Then, T1 weighted imaging sequences will be performed covering the entire abdomen, ensuring complete coverage from the diaphragm through the pubic symphysis. Participant will be repositioned supine feet first, to scan similar images over the thighs.

Fat Quantification: Resultant images will be analyzed using Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester MN) to segment depots of fat (eg. subcutaneous vs. visceral) as well as bone and muscle volume.

Incidental Findings: 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.

[4]**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. A urine pregnancy test will be completed on all women of childbearing potential

(all women except those with prior hysterectomy, tubal ligation, or absence of menses for ≥2 years) prior to the DEXA scan for safety.

[5]**Maximal Oxygen Consumption**: Aerobic fitness will be determined by measuring maximal volume of oxygen consumed (VO_{2max}) during a stationary bicycle exercise test. Provider monitoring will follow The American College of Sports Medicine (ACSM) Guidelines for Exercise Testing and Prescription. During the test, participants will breathe through a low resistance mouthpiece and wear a nose clip. Expired gases will be measured by indirect calorimetry and heart rate/rhythm monitored by 12-lead EKG. Participants will perform an exercise test on a stationary bicycle ergometer (Lode Excaliber, Gronigen, The Netherlands) at the local site. The test will begin with a warm-up of light pedaling. After the 2-minute warm up at a constant workload, the workload will increase in a ramped manner that is individualized to the participant (either 10watts/minute or 15watts/min) based on participant characteristics. VO_{2max} tests will take as little time as possible in order to ensure that test termination is due to the subject reaching $VO_{2\ max}$ rather than terminating the test due to muscle exhaustion. 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 1.05 or higher and the subject'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 "TRI SOP 030.100.015 Procedural Staffing.**"**. All study investigators and staff involved in the project are appropriately trained to conduct each element of the research in accordance with standard operating and quality control procedures.

[6]**Randomization**: Once a participant passes screening, completes the VO_2max test, and is considered eligible to continue in the study, they will be randomized in a 1:1 ratio into either the control (CTRL) group or the exercise group (EX). Randomization will be conducted by a statistician.

Baseline Period – Visit 2 (V2)

Day-8 (± 2): In patient. Participants will report to the local site the evening of day -8 for an overnight stay. A single urine collection will be done for measurements of urea, nitrogen, and creatinine. Participants will then receive a standardized dinner meal (10 kcal/kg; 50% carbohydrate, 15% protein, 35% fat), and be strongly encouraged to consume all food provided. Any unconsumed food will be recorded. Participants will remain fasting from completion of evening dinner meal at the local site until WAT biopsy and hyperinsulinemic-euglycemic clamp protocol are completed the following day.

[1]**Surveys (Optional):**

With the exception of the Diet History Questionnaire (DHQ) III, all surveys will be administered to participants via the password protected computer-based software REDCap.

- **Diet History Questionnaire (DHQ) III:** Approximate duration: 10 min; the DHQ-III, owned by the NIH National Cancer Institute will be completed through the NIH-developed secured platform specific for this instrument. Participants will complete the DHQ-III under their assigned PID during the clinic visit and no personal identifiers will be obtained.
- **Retrospective Visual Analog Scale (VAS):** Approximate duration: 5 min; an 8-item retrospective VAS assessing appetite, including hunger, fullness after meals, thoughts of food, cravings, and urges to eat.
- **Three Factor Eating Questionnaire (TFEQ):** Approximate duration: 5 min**;** an 18-item questionnaire assessing cognitive and behavioral components of eating and comprises 3 different scales corresponding to cognitive restraint, emotional eating, and uncontrolled eating.
- **Food-Craving Inventory (FCI):** Approximate duration: 5 min**;** a 33-item self-report measure of general and specific food cravings relating to overeating and binge eating.
- **MacArthur Socioeconomic Status Index:** Approximate duration: 6 min; an 11-item questionnaire regarding participants' perception of their socioeconomic status relative to their community and country, their living situation, level and degree of education obtained, job status, income, and debt.
- **Perceived Stress Scale (PSS):** Approximate duration: 3 min; a 10-item questionnaire measuring the degree of perceived stress regarding certain life events during the past month.
- **Generalized Anxiety Disorder 7-item (GAD-7) scale:** Approximate duration: 3 min; a 7-item brief measure for symptoms of anxiety, based on the generalized anxiety disorder diagnostic criteria described in the Diagnostic and Statistical Manual of Mental Disorders (DSM).
- **Center for Epidemiologic Studies Depression Scale (CES-D):** Approximate duration: 3 min; a 20-item measure for symptoms associated with depression, such as restless sleep, poor appetite, and feeling lonely. If a participant's score is ≥16, the REDCap survey platform will trigger an alert to the study coordinator. A study provider will meet with the participant and direct them to resources/referrals as needed based on provider assessment and discretion.

[2]Hair Collection (Optional): A hair sample will be cut with fine scissors from the posterior vertex position of the scalp and as close to the scalp as possible. Approximately 40 hair follicles will be cut (slightly larger than the diameter of a pencil) to reach the needed weight of 50 mg. The sample will be stored for future analyses.

Day-7: Early morning.

[3]WAT Biopsies: The abdominal skin 6-10 cm lateral to the umbilicus will be cleansed with chlorhexidine. A sterile drape will be placed, and the skin and adipose tissue will be anesthetized using 1%- Lidocaine HCl and a 0.09% Tumescent Lidocaine solution. A 3-4 mm Mercedes Liposuction needle will be inserted for aspiration of a minimum of 1.5g up to 10g of adipose tissue. Once the sample is aspirated, the needle will be removed. In the instance where the use of a needle under aspiration yields inadequate amounts of adipose

tissue, additional adipose tissue may be collected using forceps and a scalpel to excise visible superficially located subcutaneous adipose tissue. The incision will be closed with steri-strips (suture(s) if allergy to steri-strips), and sterile dressing will be applied. An MD, DO, NP, or PA will conduct this procedure. The adipose tissue will be processed as follows:

1. Fresh procedures and storage
    a. Adipose tissue cell isolation and flash frozen for isotope labeled DNA extraction.
2. Histology (Formalin fixed)
    a. Fat cell size
    b. Other
3. Frozen
    a. snRNAseq
    b. TG turnover quantification

[4]**Muscle Tissue Biopsies (Optional):** A biopsy of the Vastus Lateralis muscle will be performed using the Bergstrom technique. Subjects will be placed in a supine position and the skin will be cleansed with chlorhexidine solution. After a sterile drape is placed over the skin, local anesthesia (1%-Lidocaine HCl) will be administered,. The skin will be incised (approximately 0.75 cm) with a #11 scalpel, and the Bergstrom needle will be inserted into the Vastus Lateralis. Up to 250mg of muscle tissue will be obtained under suction. After the biopsy, pressure will be applied to stop bleeding and the skin will be closed with Steri strips (suture(s) if allergy to steri strips). A sterile dressing will be applied. An MD, DO, NP, or PA will conduct this procedure. Collected tissue will be snap-frozen for future analyses.

[5]**Hyperinsulinemic-euglycemic Clamp:** Hyperinsulinemic-euglycemic clamp: The glucose clamp is the gold standard for measurement of insulin sensitivity and will be performed similarly to the procedure initially described by Defronzo in 1979. The overall concept of the euglycemic clamp is that insulin is infused to stimulate glucose uptake and inhibit endogenous glucose production, and the rate of 20% glucose infusion necessary to maintain euglycemia can be used to assess whole body insulin sensitivity.

After an overnight 10-hour fast, an intravenous catheter will be placed for infusion of insulin and glucose. A second catheter will be placed in a vein of the contra-lateral arm for blood withdrawal. After baseline blood is collected, a 2-step euglycemic clamp will be started with a 2-hour infusion of insulin (Humulin-R) at 10 mU/m^2/min, followed by 2 hours at 40 mU /m^2/min. Whole blood will be used to assess plasma glucose at 5-10 min intervals via NOVA StatStrip Glucose Meter or YSI. 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 by measuring blood glucose at 5-10 min intervals via a NOVA glucometer or YSI and using a variable 20% dextrose infusion.

Throughout the clamp blood samples are collected at -30min, -20min, -10min, 0min, +45min, +90min, +100min, +110min, +120min, +180min, +220min, +230min, and +240min of insulin infusion. Total blood volume during this procedure will be approximately 260 mls.

Calculations:

Insulin sensitivity (M/I) will be calculated using the formula: M/I = R_d / (steady state insulin level - basal insulin level) where steady state insulin equals the average insulin concentration during the last 30-40 minutes of each steady state phase of the clamp.

Fasting and insulin-suppressed FFA [IS-FFA] will be measured as a measure of adipose tissue insulin sensitivity with an ultrasensitive FFA assay from Wako.

[6]**Subcutaneous Microdialysis**: To assess antilipolytic sensitivity of WAT to insulin and production of hormones, proteins, and other factors (including, but not limited to amyloid precursor protein (APP) TNF-a, leptin, and adiponectin), up to four microdialysis catheters will be inserted into the periumbilical subcutaneous adipose tissue. Topical anesthesia (EMLA cream) or ethyl chloride will be applied to each insertion site.

The catheters come as a sterilized kit (CMA Microdialysis) with a sterilized cutting needle (20-gauge) and introducer needle (54mm long X 1.4 mm diameter). The insertion site will be cleaned with chlorhexidine and a small incision made using the provided cutting needle. The catheter will be inserted using the introducer needle, which is then removed, and the catheter secured under a clear adhesive dressing. Flow to the microdialysis catheters will be initiated and dialysate collected in approximately 30-minute samples; the catheters will be calibrated using zero-net flow methods. Perfusate will consist of sterile 10 mM ethanol in ringers injection to account for changes in adipose tissue blood flow. The microdialysis collection will proceed during the glucose clamp. Approximately 66 mL of blood and less than 8 mL of interstitial microdialysate will be obtained.

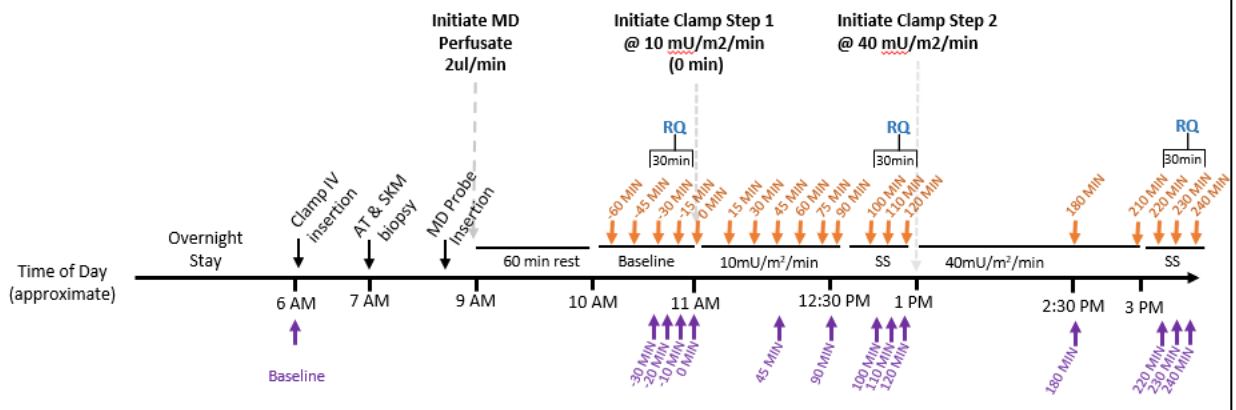
The rates of carbohydrate and lipid oxidation will be calculated as described by Elia and Livesey. The basal rate will be calculated by averaging the data for the time= -20 to time=0 minutes (fasting RQ), and insulin-stimulated glucose and lipid oxidation rates will be calculated by averaging the data for the time=40 to time=60 minutes (post glucose administration RQ).

[7]**Blood Draw**: After the IV catheters are inserted and prior to the clamp procedures beginning, a baseline blood draw will be collected from the venous catheter. The following will be measured: CBC with differential and platelets, CMP, lipid profile, blood glucose, ALT, AST, alkaline phosphatase, insulin level, TSH, and HbA1C.

[8]**Respiratory Quotient (RQ):** Indirect calorimetry will measure the respiratory quotient (RQ) and substrate utilization using either a ParvoMedics Metabolic Cart at TRI (Salt Lake City, UT) or Omnical Cart at PBRC (Maastricht Instruments BV). The ParvoMedics Metabolic Carts are calibrated daily before each subject with standardized gases containing

1% CO_2, 16% O_2 and balance N_2. The Omnical Carts are also calibrated daily before each subject with standardized gases containing 0.8% CO_2, 18% O_2 and balance N_2 and then self-calibrate continuously through the test approximately every 15 minutes. After inserting the clamp catheters, completing the WAT and muscle biopsies, and inserting the microdialysis catheters, and allowing flushing of the microdialysis catheters for 5 minutes, participants will rest for 60 minutes laying down in a Semi-Fowler's position. After 30 minutes of dialysate collection, a transparent plastic hood connected to the device will be placed over the head of the subject. Calculations of O_2 consumption (VO_2) and CO_2 production (VCO_2) will be made by comparing measures of the O_2 and CO_2 concentrations of the testing room (measured every 5 minutes at TRI and every 2 minutes at PBRC) to continuous measurements of O_2 and CO_2 concentrations in expired air diluted in a constant air flow (15-30 L/min at TRI and 85-90 L/min at PBRC) generated by a pump. From the above, energy expenditure standardized for temperature, pressure, and moisture will be calculated at one-minute intervals. After ~30 minutes, the remaining clamp procedures will resume. This will be repeated during steady state 1 (+90-120min) and steady state 2 (+210-240mins).

Figure. Visit 2 and Visit 4 Biopsies, Clamp, Microdialysis, and Respiratory Quotient Measurements Timeline



Note: Each blood draw will be 20ml total (10 ml red top tubes; 10 ml purple top tubes)

[9]**Standardized Meal and Accelerometer**: After all procedures are complete, participants will be provided a standardized meal along with post procedure instructions. Participants will also be provided with a triaxial activity monitor (Actigraph GT3X) which will be worn for 7-10 days. The monitor integrates motion sensor data from the tri-axial accelerometer to estimate the energy cost of free-living activity. The participant will wear the monitor at

all times, except while showering or bathing. After the period of wear time, the participant will return the monitor and the data will be uploaded.

[10]7-Day Food Record (optional): Participants will be asked to record their food and beverage intake for 7 days and return the form to the research site at their next in-person visit.

Phone Follow-up After Baseline Visit 2

The participant will be contacted by phone within 24-72 hours after baseline visit 2 discharge to ensure no adverse events have occurred on both biopsy and arterial line sites.

Intervention Period (Weeks 1-12)

Biopsy and catheter insertion site check: A biopsy site and catheter site check will be done when the participants pick up their first supply of $^{2}H_2O$.

H_2O Administration and Return (12-week labelling period) and Urine Collections: $^{2}H_2O$ will be prepared by the Mass Spectrometry Core. Participants will receive 50 mL aliquots of 70% $^{2}H_2O$ or 35 mL aliquots of 99.9% $^{2}H_2O$ in individual amber prescription containers. Participants will drink three 35 mL or 50 mL doses per day for the first week, and two doses per day from the second through the twelfth week. Participants will receive the $^{2}H_2O$ aliquots containers bi-weekly, a log sheet to record consumption, and at-home urine collection cups with instructions. Participants will be instructed to keep urine samples taken at home in the refrigerator until their next in-person visit (every other week). Urine samples will be used to verify compliance weekly (at AH TRI or PBRC). Compliance with $^{2}H_2O$ intake for both groups will be monitored through the weekly urine collections and return of empty containers for counting. ^{2}H enrichments in urine, reflecting ^{2}H body water enrichments, will be measured by isotope ratio mass spectrometry. Enrichments are expected to be between 1-2.5%. If a participant's urine sample is <1%, the study team will encourage compliance with the participant. If enrichment is <1% for a period of 3 weeks, the PIs will discuss participant withdrawal. Participants will be instructed to maintain their normal daily and weekly routines.

Participants in the EX group will engage in 12-week endurance exercise training.

Endurance Exercise Training: Outpatient. Approximate visit time: ~ 1.5 hour
Participants in the EX group will engage in endurance exercise training three times a week for approximately 12 weeks. The exercise program will be supervised by a certified exercise physiologist and will take place at the TRI exercise training facility or at PBRC. Each session will last approximately 75 minutes with a 50-60 minute stimulus phase and the remaining time being used to warm up and cool down. Participants will use a cycle ergometer and/or treadmill for the duration of the intervention period. During all sessions, heart rate will be monitored using Zephyr heart rate monitors to ensure that participants maintain the target exercise intensity during training. Intensity will be set as a percentage

of heart rate reserve (calculated from pre-training VO_{2max}) and will increase during the intervention: 60% ± 5 bpm for weeks 1-4; 70% ± 5 bpm for weeks 5-8; 75% ± 5 bpm for weeks 9-10; and 80% ± 5 bpm for weeks 11-12. Self-reported perceptual data will be recorded periodically during exercise sessions and will be used to track the subjective experience of participants and in interpreting adherence data.

Body Weight Management and Health Education: All participants will be expected to maintain weight within ±2kg throughout the study. Should a participant gain or lose weight >2kg, the study team will be instructed on how to reduce or increase caloric intake to maintain body weight (Appendix). Participants in the EX group will be weighed onsite weekly and the study team will advise on weight maintenance, if needed. Participants in the CTRL group will be weighed onsite biweekly when they return and pick up $^{2}H_{2}O$. During biweekly on-site weigh ins, CTRL participants will be instructed on weight maintenance, if needed. Additionally, the study team will review the participant's physical activity habits and dietary habits, adverse events, and medication/medical history changes.

DEXA (Weeks 4-6): In addition to the pre- and post-intervention MRI and DEXA scans, during Weeks 4-6, one DEXA scan will be performed as described above to confirm maintenance of fat mass.

Review of Adverse Events: Study staff will assess for any adverse events at each exercise visit.

Accelerometers: Following the last bout of exercise training, participants will be provided with a triaxial activity monitor (Actigraph GT3X) which will be worn for 7-10 days. The monitor integrates motion sensor data from the tri-axial accelerometer to estimate the energy cost of free-living activity. The participant will wear the monitor at all times, except while showering or bathing. After the period of wear time, the participant will return the monitor and the data will be uploaded.

7-Day Food Record: Participants will be asked to record their food intake for 7 days during Week 12 and return the form at Visit 3.

Follow-up Period – Visit 3

Day 85 (±1): Outpatient. Approximate visit time: ~3-4 hours. Scheduled 48-72 hours after the last exercise bout.

The following assessments will be performed as described in **Baseline Period – Visit 1**:

[1]**Anthropometrics** including height, weight, waist circumference, and BMI.

[2]**Vital signs** (respiratory rate, temperature, heart rate, blood pressure)

[3]**MR assessment of visceral and subcutaneous fat (VAT/SAT) and thigh fat (~1 hour)**

[4]Dual Energy X-Ray Absorptiometry (DEXA)

[5]Maximal Oxygen Consumption

Follow up Period – Visit 4

The following assessments will be performed as described in **Baseline Period – Visit 2**:

Day 87 (± 2): In patient. Participants will report to the local site the evening of day 87 for an overnight stay. Urine collection will also be done for measurements of urea, nitrogen, and creatinine. Participants will then receive a standardized dinner meal (10 kcal/kg; 50% carbohydrate, 15% protein, 35% fat), and be strongly encouraged to consume all food provided. Any unconsumed food will be recorded. Participants will remain fasting from completion of evening dinner meal at the local site until WAT biopsy and hyperinsulinemic-euglycemic clamp protocol are completed the following day.

[1]Surveys (Optional)
- **Diet History Questionnaire (DHQ) III**
- **Retrospective Visual Analog Scales (VAS)**
- **Three Factor Eating Questionnaire (TFEQ)**
- **Food-Craving Inventory (FCI)**
- **Perceived Stress Scale (PSS)**
- **MacArthur Socioeconomic Status Index**
- **Generalized Anxiety Disorder 7-item (GAD-7) scale**
- **Center for Epidemiological Studies-Depression Scale (CES-D)**

[2]Hair Collection (Optional)

Day 88 (± 2): Early morning.

[3]WAT Biopsies

[4]Muscle Tissue Biopsies (Optional)

[5]Hyperinsulinemic-euglycemic Clamp

[6]Subcutaneous Microdialysis

[7]Blood Draw

[8]Respiratory Quotient (RQ)

Phone Follow-Up After Visit 4

The participant will be contacted by phone within 24-72 hours after Visit 4 discharge to ensure no adverse events have occurred on both biopsy and arterial line sites.

A table identifying the procedures in relation to the study timeline can be found at the end of this document.

Study Duration

Projected start date: August 2024

Estimated duration to enroll all study subjects: 4 years

Total length of time participants will remain in the study: approximately 14 weeks

Estimated date for investigators to complete the study (including analysis): April 2029 (5 years).

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 drawers, or other storage units located at each facility. The 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 PBRC facility is secured via key card and each building is equipped with a back-up generator system. Laboratory personnel within each core of the facility have 24/7 key-controlled access to the respective core laboratory with freezer storage spaces. The chain of custody of the biospecimen is maintained through requisition forms that are specific to each core and on the PBRC OneDrive. All biospecimen tubes are coded and labeled appropriately, and biospecimen requests and distribution are documented.

The TRI facility will be the main repository for biospecimen samples. PBRC will ship all tissue and blood samples (with exception of WAT samples needed for triglyceride turnover and adipocyte formation analysis and screening/safety visits) to AH TRI for testing and storage. The AH TRI will ship WAT samples needed for triglyceride turnover and adipocyte formation analysis. Material Transfer Agreements will be in place prior to transferring any samples between study sites. The MTA will govern the transfer and chain of custody of biospecimens outside of AdventHealth.

The adipose tissue specimens and 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 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 and PBRC scientists and scientists outside of AdventHealth and PBRC. 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 PI, with an additional review by the respective Program Director. For research outside of AdventHealth or PBRC, a Material Transfer Agreement will be obtained, which will govern the transfer and chain of custody of the biospecimens outside of AdventHealth or PBRC.

Study Outcome Measures (Endpoints)

Primary outcomes:

1. In vivo adipogenesis in WAT. New adipocyte formation will be assessed two times (pre and post intervention) on the frozen isolated $^{2}H_2O$ labelled adipose tissue cells.
2. In vivo TG turnover. WAT TG turnover rates will be assessed two times (pre and post intervention) on abdominal subcutaneous WAT on the extracted lipid from the frozen $^{2}H_2O$ labelled adipose tissue.
3. Cellular composition of WAT. Cell composition and gene expressions in WAT will be assessed two times (pre and post exercise intervention) by snRNAseq on the frozen adipose tissue.
4. Adipocyte morphology, senescence, and inflammation. Adipocyte size, markers of cellular senescence and inflammation will be assessed two times (pre and post exercise intervention) by immunohistochemistry on the formalin-fixed paraffin-embedded adipose tissue.

Secondary outcomes:

1. Whole body and WAT-specific insulin resistance: Whole-body insulin resistance and WAT-specific antilipolytic sensitivity to insulin will be evaluated pre and post exercise intervention *in vivo* using blood and dialysate samples collected during glucose clamp and subcutaneous microdialysis.

Data Management and Quality Plan

Data De-identification

Participants will be enrolled and assigned a participant identification number (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 and survey data is recorded in a de-identified fashion onto our paper source documents and/or electronic case report forms (CRF). Both sites will enter data into REDCap, a HIPAA-compliant, web-based forms and survey platform approved for use by AdventHealth. REDCap will be used for storage and to facilitate analysis. All data not entered into REDCap (e.g., data collected from individual exercise sessions) will be stored on a shared OneDrive folder only accessible to the assigned study-specific research team at PBRC and AH TRI.

Clinical data generated by research devices (e.g., accelerometer) also uses the PID, and once the data has been transformed into interpretable results it is stored into the clinical research database (either REDCap or OneDrive). Both storage locations are password-protected, secured and only assessable to the assigned clinical research team from AH TRI and PBRC. The "link" will not be used to re-identify participants except in the event of a serious adverse event (SAE) requiring "unblinding" to treat the participant. The "link" will be stored in the Patient Protocol Manager and in the OneDrive folder, where only the research team has access.

Data Confidentiality, Storage, and Retention

The identity and personal health information of study participants 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 participant prior to enrollment.

Study documentation and paperwork will be stored in our locked medical records room at AH TRI and PBRC. The data records will also be stored as electronic records in REDCap and OneDrive. 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.

AdventHealth Translational Research Institute retention policy is maintained in the Records Management Policy. Electronic de-identified data will be kept indefinitely in our data warehouse.

Per the institutional policy, investigator records must be kept for a minimum of 7 years after completion of discontinuation of the study, or for longer if required by applicable local regulations, or what is applicable per the Sponsor.

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 (REDCap) 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.

Data Sharing

Some of the endpoint testing will be conducted at outside laboratories/institutions. To perform these analyses/testing/etc. and to interpret results, certain data elements will need to be shared along with the biospecimen samples. Data Use Agreements will be obtained, which will identify 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 aim is powered to detect differences in the change in *in vivo* formation of new adipocytes (i.e., fraction of newly formed adipocytes; "f") of subcutaneous WAT between the CTRL and EX groups. We hypothesize that the EX-group will have a decrease in the fraction of newly formed adipocytes in response to the EX intervention as compared to the CTRL group. Analysis of women from our previous VIVA study (NCT03530111) indicates that the mean value of "f" in the physically active (exercise) group is 11.4%, while the sedentary group has an "f" value of 17.8%. Because VIVA only has cross-sectional data (assessment at one time-point), we must also use data from the Apple & Pear study (NCT01748994) 9 to estimate the change in fraction of newly formed adipocytes, as the proposed study includes an exercise intervention. By utilizing data from the CTRL-group in the Apple & Pear study 9 (baseline assessment) and increasing the estimate to ensure that the study is well-powered, we can estimate the CTRL and EX groups from the proposed study to have an overall mean "f" value of 17% (at baseline) with a standard deviation of 6.0%. A total sample size of 58 (assuming a ~17% overall lost-to-follow-up rate) will provide at least 80% power to detect significant differences in "f" between the CTRL and EX groups, with a 5% significance level.

Single nuclei RNAseq Power Calculation. To date the most comprehensive analysis of human WAT has been conducted by Emont and colleagues who performed snRNAseq in whole frozen WAT and VAT from humans with obesity and established 17 subpopulations of cells. However, these data were generated using the 10X Genomics platform. We have published preliminary snRNAseq data in WAT from 22 individuals with overweight and obesity. We expect to capture nuclei from the WAT that may reveal up to 12 different cell populations (Fig. 4). If we assume the rarest cell type is 2% and require at least a 100 of the of rarest cell type to accurately quantify and describe it, we require a total of 6,792 nuclei to achieve a 99% probability of meeting these requirements. Our current technique permits 1400 cells per participant to be analyzed which equates to 28,000 cells for 20 participants at each timepoint in EX-group and 14,000 nuclei for 10 participants at each timepoint in the CTRL-group. This would allow us to accurately define subpopulations within WAT and generate sufficient data to compare the impact of endurance exercise on adipocyte populations and non-adipocyte populations.

Statistical Analysis Plan

Primary and Secondary Objective Analysis

Shapiro-Wilk test will be initially performed to assess the normality of data and homogeneity of variances. Pre- and post-intervention results will then be tested by paired t-test or Wilcoxon signed-rank test, as appropriate. Results will be expressed as mean ± standard deviation and significance will be established, *a priori*, at $p \leq 0.05$.

Potential Risks and Benefits

Potential Benefits

The potential benefits to an individual participant in this study are not known. Exercise training can trigger many beneficial adaptations, but individual responses and adaptations vary. All participants will acquire knowledge about their physiological status (e.g., cardiorespiratory fitness, body composition, etc.) that may be of personal benefit.
The risks presented to participants are minimal, and information gained from this project will be useful to identify mechanisms responsible for exercise effects on adipose tissue quality and insulin resistance in subjects with obesity.

Potential Risks

Described below are all the known risks of being in this study.

Dual Energy X-ray Absorptiometry (DEXA): 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.

Magnetic Resonance Imaging: There are no known biological risks associated with magnetic resonance imaging. 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.).

Maximal Oxygen Consumption (VO_{2max}) test and exercise training: 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.

Hair Collection: There is minimal risk associated with obtaining hair samples. A rare risk includes minor scalp laceration during sample collection and related infection.

Adipose Tissue Biopsy: There is a risk of pain from the local anesthesia, and a risk of bruising, bleeding and infection at the site of the biopsy. In addition, there is a risk of skin irritation due to the steri strips and dressing.

Muscle Tissue Biopsy: There is a risk of pain from the local anesthesia, and a risk of bruising, bleeding (hematoma) and infection at the site of the biopsy. In addition, there is a risk of skin irritation due to the steri strips and dressing. Local sensory loss may occur by cutting a subcutaneous sensory nerve (<1 in 100), which is almost always temporary but occasionally can become permanent. On rare occasion (<1 in 1000) bleeding, requiring hospitalization, may occur. The procedures are well tolerated when performed properly.

Intravenous lines/blood draws (lab samples, e.g.): there is a risk of pain, vasovagal syncope, hematomas, and/or infection at IV insertion/blood draw site (low risk of SAE).

Subcutaneous Microdialysis: There is a risk of pain, hematomas, and/or infection at insertion site (low risk of serious AEs).

Deuterium-labeled water (^2H_2O) administration: ^2H_2O-labeled water is water that is labeled with the stable, non-radioactive isotope deuterium. Deuterium-labeled water poses minimal health risk associated with drinking it. Participants may rarely experience transient dizziness or nausea following the initial intake.

Hyperinsulinemic-euglycemic clamp: There is the risk of bruising at the site of needle insertion in the veins. The risk of receiving an insulin infusion is that it could produce hypoglycemia (rare). Hypoglycemia may cause one to feel nervous, shaky, sweaty, hungry, and dizzy and have a rapid heart rate. If severe, hypoglycemia can cause coma, seizure or even death (all rare).

Resting Metabolic Rate/Respiratory Quotient (RMR/RQ): There is no physical risk associated with RMR/RQ. Other risks include a feeling of claustrophobia experienced by some subjects while under the transparent "hood".

Surveys: There are no risks involved beyond what would reasonably be encountered in everyday life. It is possible that participants may experience the following during completion of surveys:

1. Loss of privacy as a result of a breach of confidentiality
2. Undue stress related to content on the survey instruments

Mitigation of Risks

Dual Energy X-ray Absorptiometry (DEXA): 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).

Magnetic Resonance Imaging: There will be a strict safety screening protocol, to ensure any people with contraindications are excluded from volunteering.

Incidental Findings: 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.

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

Hair Collection: Hair collection will be performed by trained and qualified study staff.

Adipose Tissue Biopsy: Adipose biopsies will be conducted by qualified staff following institutional policies and procedures including sterile techniques and sterile dressing to the site. Prior to the biopsies, participants will be asked for allergies including lidocaine and the use of anticoagulants. Subjects that have an allergy to lidocaine or have used anticoagulants in clinically significant amounts will be excluded. Sutures will be used to close the site, for those with a known allergy to steri strips.

Muscle Tissue Biopsy: Muscle biopsies will be conducted by qualified staff following institutional policies and procedures including sterile techniques and sterile dressing to the site. Prior to the biopsies, participants will be asked for allergies including lidocaine and

the use of anticoagulants. Subjects allergic to lidocaine or that used anticoagulants in clinically significant amounts will be excluded. Sutures will be used to close the site, for those with a known allergy to steri strips.

Intravenous lines/blood draws (lab samples, e.g.): All venipuncture will be conducted by qualified staff using aseptic techniques.

Subcutaneous microdialysis: Insertion will be conducted by qualified staff using aseptic technique to reduce these risks.

Deuterium-labeled water (^2H_2O) administration: Separated doses (35 mL) for ^2H_2O consumption will minimize symptoms.

Hyperinsulinemic-euglycemic clamp:
It is very unlikely that severe hypoglycemia will occur during the insulin infusion because blood glucose will be monitored every 5 to 10 minutes at the bedside and glucose infusions of 20% dextrose will be adjusted to maintain euglycemia (plasma glucose ~ 90 mg/dl). The participant is also kept awake at all times during the insulin infusion so that they can report or be observed for any changes. If the participant's blood glucose is nearing hypoglycemic levels, the plasma glucose will be checked even more frequently, and the 20% dextrose infusion rate increased. If hypoglycemia occurs, the insulin infusion will be discontinued, and intravenous glucose continued at higher rates along with the intake of oral carbohydrates. The volunteers will then be monitored for at least 2 hours during the IV glucose infusion and oral supplementation, then for at least 1 hour after discontinuing the IV glucose. Additionally, there will be in-house supervision by a qualified physician or mid-level provider at all times during the procedure and immediate availability of corrective measures (IV 50% dextrose and/or glucagon).

Resting Metabolic Rate/Respiratory Quotient (RMR/RQ): A member of the study staff will remain with the subject at all times to ensure his/her comfort.

Surveys: Surveys will be offered via password protected computer-based software REDCap. IP addresses will not be collected. Survey results will be aggregated, no individual responses will be shared. Participants may opt out of survey questions they develop discomfort with.

Provisions to Protect the Privacy Interest of Subjects

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 the minimum necessary to achieve the aims of the research, so that no unneeded sensitive information will be collected.

Early Withdrawal of Subjects

Investigator Withdrawal of Subjects

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 subject's health or safety;
- Participant has not followed study instructions;
- The AdventHealth Translational Research Institute has stopped the study;
- Administrative reasons require the participant's withdrawal;
- Participant is unable to complete a procedure;
- We are unable to place a venous line or place a catheter for the subcutaneous microdialysis.

Subject 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. If a participant leaves the study before the final regularly scheduled visit, she/he may be asked by the study doctor to make a final visit for some 'end-of–study' procedures. This is to make sure that there are no safety concerns

Data Collection and Follow-up for Withdrawn Subjects

Participants who request withdrawal or who are withdrawn by the PI from the study will have their data maintained in the research database up to the point of withdrawal. The available data will be included in subsequent analysis because keeping these participants in the analysis is essential for study validity.

Adverse Events - Recording and Reporting

Each participant will be 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). Any severe and/or unanticipated adverse event will be immediately reported to the IRB according to AdventHealth IRB guidelines.

At each contact with the subject, the study team will seek information on adverse events by specific questioning and, as appropriate, by the Provider through examination. Information on all adverse events will be recorded immediately in the source document, 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. All adverse and unexpected events will be reported according to AdventHealth IRB guidelines.

Safety Monitoring Plan

Safety Monitoring

Adverse events will be documented and reported by the study coordinator, Medical Investigator or other TRI/PBRC staff. Research and safety data will be reviewed by the PI. This review will take place at regular meetings with the research coordinator and Medical Investigator. Other items discussed will include progress or adverse events occurring in the following: participant confidentiality, participant recruitment, and consent process. Both the TRI and PBRC have a standing committee at each institution that meets monthly to review all adverse events in the clinical trials and will additionally be charged with review of the study.

A Data and Safety Monitoring Plan (DSMP) Committee will also be established. A group of researchers at AH TRI and PBRC will oversee the DSMP. It will include:

1. MPIs: Lauren Sparks, Ph.D. and Ursula White, Ph.D.
2. MIs: Frank Greenway, M.D. and non-MD Provider (PA or ARNP level)
3. Biostatisticians: Robbie Beyl, Ph.D. and Fanchao Yi, M.S.
4. Study Coordinators (TBD)
5. Independent Safety Monitor (TBD) not directly involved in the study

The DSMP Committee meetings may be conducted in-person, via conference call, or webinar. The main focus will be on participant accrual, protocol compliance, review of lab safety data, and review of all AEs/SAEs. No outcome data/results will be discussed until after completion of the data collection. The DSMP committee will meet once at least annually and an *ad hoc* meeting will be held with the Mis in case of a serious adverse event or death. Minutes will be recorded and forwarded to all members of the committee. The Independent Monitor will review and identify any deficiencies in the DSMP and provide the results of the reviews and advise guidance and recommendations concerning the study. Following each meeting, the DSMP committee will provide written documentation regarding findings for the study as a whole and any relevant recommendations related to continuing, changing, or terminating the study. A copy will be provided to the IRB.

Data and Safety Monitoring Board (DSMB) or Equivalent

N/A

Ethical Considerations

Participation in this study is voluntary. Subjects 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 Subjects: Participants will be offered the opportunity to meet with the Principal Investigator or designated medical staff to review the results of their lab assessments or other standard clinical data. Copies of their testing results will be made available to the participants upon request. In addition, the Principal Investigator or designated study staff 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 research program grant (RPG) from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK133529, P.I. Lauren M. Sparks, PhD). The IRBNet # for this grant is 1949440.

Subject Stipends or Payments

Participants at TRI may receive up to $1,000 for completion of all procedures. If participant is unable to complete all study visits, the stipends will be prorated to the following:

Visit	Prorated amount
Screening visit	$25
Visit 1 & 2	$200
Muscle Biopsy (Optional)	$50
12-week intervention	$450
Visit 3 & 4	$225
Muscle Biopsy (Optional)	$50
Total for all completed visits	**$1,000**

Participants at PBRC may receive up to $1,000 for completion of all procedures. If participant is unable to complete all study visits, the stipends will be prorated to the following:

Visit	Prorated amount
Visit 1 & 2	$200
12-week intervention (Weeks 1, 3, 5, 7, 9, 11)	$50 biweekly
Visit 3 & 4	$500
Total for all completed visits	**$1,000**

Payment for each visit is contingent on completing the visits and procedures listed above. A prepaid card will be the method of payment and will be requested upon completion of each study visit/phase or on their last day of participation, should they find they cannot complete the study.

Participants' payment will be requested within 3 business days from completion of each study visit/phase.

For participants who are not AdventHealth Employees

If participants receive more than $600 in payments in a calendar year from AdventHealth, this income will be reported to their IRS. The participant may be required to pay tax on this income.

For participants who are AdventHealth or AdventHealth Medical Group Employees

All payments will be reported as added income to the participant's base salary and will be taxed on a future paycheck. If the participant is an employee of AdventHealth, their decision to participate will not affect their employment or relationship with AdventHealth.

Publication Plan

We attest that the TRI faculty and staff will adhere to POLICY-TRI-ADM-005 (Access to Clinical Trial Data for Publication Purposes). 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.

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Appendix:

The following table identifies the procedures in relation to the study timeline for CTRL group:

	Screen	Baseline			^2H_2O Intervention Period												Follow-up		
					Week														
Visits	**SV1**	**V1**	**V2**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**V3**	**V4§**	
Days		-14	-8	-7	0-6	7-13	14-20	21-27	28-34	35-41	42-48	49-55	56-62	63-69	70-76	77-83	85	87	88
Windows	Within 28 days of V1	±2	±2		±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	
Informed Consent	X																		
Eligibility Assessment	X																		
Randomization		X																	
Urine Collection (urea, nitrogen and creatinine)			X															X	
Urine Collection (pregnancy, if applicable)	X	X							X								X		
Urine Collection (toxicology)	X																		
Physical Exam + Medical History	X																		
Vital Signs	X																		
Anthropometrics	X	X															X		
Screening Blood Draw	X																		
Electrocardiogram	X																		
Surveys			X															X	
MRI		X															X		
DEXA		X							X*								X		
VO2max Test		X															X		
Overnight Stay with Standardized Meal			X															X	
7-Day Food Record Start				X												X			
7-Day Food Record Return					X												X		
Hair Collection			X															X	
WAT Biopsy				X															X
Muscle Biopsy				X															X
Respiratory Quotient (Hood)				X															X
Pre-Clamp Blood Draw				X															X

Hyperinsulinemic-euglycemic Clamp + Microdialysis				X															X
Accelerometer Provided			X													X			
Biopsy and Catheter Insertion Site Check					X														X
On-site Body Weight Measurement							X		X		X		X		X				
Weight Maintenance and Health Education							X		X		X		X		X				
$^{2}H_2O$ Pick-up					X		X		X		X		X		X				
$^{2}H_2O$ Container Return							X		X		X		X		X	X			
$^{2}H_2O$ Urine Collection					X	X	X	X	X	X	X	X	X	X	X	X			
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*DEXA can take place between Weeks 4-6 to increase scheduling flexibility

[§]Visit 4 procedures must be done within 36-48 hours after the VO2max test

On-site Body Weight Measurement, Weight Maintenance and Health Education, and $^{2}H_2O$ activities must occur on the first day of exercise for that week.

The following table identifies the procedures in relation to the study timeline for EX group:

	Screen	**Baseline**			**$^{2}H_2O$ and Exercise Intervention Period**												**Follow-up**		
					Week														
Visits	**SV1**	**V1**	**V2**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**V3[§]**	**V4**	
Days		-14	-8	-7	0-6	7-13	14-20	21-27	28-34	35-41	42-48	49-55	56-62	63-69	70-76	77-83	85	87	
Windows	Within 28 days of V1	±2	±2		±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	
Informed Consent	X																		
Eligibility Assessment	X																		
Randomization		X																	
Urine Collection (urea, nitrogen and creatinine)			X															X	
Urine Collection (pregnancy, if applicable)	X	X							X								X		
Urine Collection (toxicology)	X																		
Physical Exam + Medical History	X																		
Vital Signs	X																		
Anthropometrics	X	X															X		
Screening Blood Draw	X																		
Electrocardiogram	X																		
Surveys			X															X	
MRI		X															X		
DEXA		X							X*								X		
VO2max Test		X															X		
Overnight Stay with Standardized Meal			X															X	
7-Day Food Record Start				X												X			
7-Day Food Record Return					X												X		
Hair Collection			X															X	
WAT Biopsy				X															X
Muscle Biopsy				X															X
Respiratory Quotient (Hood)				X															X
Pre-Clamp Blood Draw				X															X
Hyperinsulinemic-euglycemic Clamp + Microdialysis				X															X
Accelerometer Provided			X													X			
Biopsy and Catheter Insertion Site Check					X														X

Supervised Exercise Training					X	X	X	X	X	X	X	X	X	X	X	X			
On-site Body Weight Measurement					X	X	X	X	X	X	X	X	X	X	X	X			
Weight Maintenance and Health Education					X	X	X	X	X	X	X	X	X	X	X	X			
$^{2}H_2O$ Pick-up					X		X		X		X		X		X				
$^{2}H_2O$ Container Return							X		X		X		X		X	X			
$^{2}H_2O$ Urine Collection					X	X	X	X	X	X	X	X	X	X	X	X			
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*DEXA can take place between Weeks 4-6 to increase scheduling flexibility

[§]Visit 3 must be done 48-72 hours after the last exercise session. Visit 4 must be done within 36-48 hours after the VO2max test.

On-site Body Weight Measurement, Weight Maintenance and Health Education, and $^{2}H_2O$ activities must occur on the first day of exercise for that week.

Weight Maintenance and Health Education will occur as needed following body weight measurement.