Study Protocol Title:
Exercise training as an intervention to improve muscle function and recovery following bed rest in older adults with type 2 diabetes (RECOVER)

Study Sponsor:
AdventHealth Orlando

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List of Abbreviations:

AE - Adverse Event
AF - Activity Factor
AH – AdventHealth
AH TRI – AdventHealth Translational Research Institute
AMP - Adenosine Monophosphate-Activated Protein
ATP - Adenosine Triphosphate
BMI - Body Mass Index
BP - Blood Pressure
BR - Bed Rest
BUN - Blood Urea Nitrogen
CaHMB - Calcium-Beta-Hydroxy-Betamethylbutyrate
CON – Ambulatory (Non-Exercised) Control Group
CRF - Case Report Form
CRU - Clinical Research Unit
DBP - Diastolic Blood Pressure
DEXA - Dual-Energy X-ray Absorptiometry
DNA - Deoxyribonucleic Acid
DO - Doctor of Osteopathic Medicine
DPP - Dipeptidyl Peptidase
DSMB - Data Safety Monitoring Board
DVT - Deep Vein Thrombosis
ECG - Electrocardiogram
EGP - Endogenous Glucose Production
EX – Exercise Intervention Group
FDA - Food and Drug Administration
FFA - Free Fatty Acid
eGFR - Estimated Glomerular Filtration Rate
GDR - Glucose Disposal Rate
GLP - Glucose-Like Peptide
HbA1c - Hemoglobin A1c
HDL - High-Density Lipoprotein
HFD - High Fat Diet
HIPAA - Health Insurance Portability and Accountability Act authorization
HIT - Heparin Induced Thrombocytopenia
HIV - Human Immunodeficiency Virus
HR - Heart Rate
HRT - Hormone Replacement Therapy
HsCRP – high sensitivity C-Reactive Protein
ICF - Informed Consent Form
IMCL - Intramyocellular Lipid

IR - Insulin Resistance
IV - Intravenous
LDL - Low-Density Lipoprotein
LMWH - Low Molecular Weight Heparin
MD - Medical Doctor
MRI - Magnetic Resonance Imaging
MRS - Magnetic Resonance Spectroscopy
NP - Nurse Practitioner
PA - Physician Assistant
PBR - Pre-Bed Rest
PCP - Primary Care Physician
PE - Pulmonary Embolism
PI - Principal Investigator
PID - Participant Identification Number
QD - Per Day
REE - Resting Energy Expenditure
RER - Respiratory Exchange Ratio
RM - Repetition Maximum
RMR - Resting Metabolic Rate
ROM - Range of Motion
ROS - Reactive Oxygen Species
RQ - Respiratory Quotient
SAE - Serious Adverse Event
SAS - Statistical Analysis System
SBP - Systolic Blood Pressure
SDH - Sorbitol Dehydrogenase
S_I - Insulin Sensitivity
SOP - Standard Operating Procedures
SPPB - Short Performance Physical Battery
T2D - Type 2 Diabetic
TRI - Translational Research Institute
TSH - Thyroid Stimulating Hormone
VO_2max - Maximal Oxygen Uptake
VTE - Venous Thromboembolism
400MWT - 400-Meter Walk Test

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.

Older adults with type 2 diabetes (T2D) experience an accelerated rate of sarcopenia, which is the deterioration in muscle mass, strength, and physical performance. Periods of disuse caused by illness or hospitalization causes rapid loss of muscle mass and strength, which negatively impact physical function upon re-ambulation. In fact, without adequate rehabilitation, physical function does not fully recover in older adults following disuse, reducing the quality of life and loss of independence

There is a lack of effective therapeutic options to aid muscle recovery following disuse, in part, because the myocellular mechanisms underlying the atrophy and recovery programs in human muscle are not well characterized. Our long-term goal is to identify specific molecular targets for the development of evidence-based clinical interventions to counteract the negative health outcomes associated with muscle atrophy induced by illness, injury, or hospitalization. Our overall objectives are to employ highly innovative methods in muscle biopsy specimens in order to decipher the temporal sequence by which mitochondrial dysfunction and lipotoxicity in older adults with T2D impact atrophy and recovery of muscle mass, strength and physical function following bed rest. Studies in the clinically relevant context of aging, T2D and exercise will provide a physiological framework in which the temporal and mechanistic relationships between mitochondria, intramyocellular lipid and protein synthesis/breakdown can be interrogated during atrophy and recovery.

Our primary approach is to conduct a prospective, parallel two arm, randomized controlled study consisting of five discrete but connected phases:

I) Screening, II) Pre-Bed Rest, III) Bed Rest, IV) Ambulatory/Exercise Recovery and V) Resistance Exercise (OPTIONAL)

Eligible participants will be Older Adults with T2D and will be randomly assigned to one of two groups: Exercise recovery (EX) or Ambulatory recovery (CON).

The expected duration for a subject in this study could be up to 16 weeks.

A series of physiologic and clinical/health outcomes will be assessed before, during and after the bed rest intervention period and recovery period:

1) Morning resting fasted variables: blood pressure; blood glucose and lipids. 2) Physical Examination, Resting EKG, Urine collection (for urinalysis, BUN, creatinine). 3) Cardiorespiratory fitness (VO_2peak) determined via a graded cycle ergometry test. 4) Body composition by; DXA scan - bone mineral density, total and partitioned lean mass, estimated visceral fat; and MRI – left and right leg mid-thigh muscle, bone and fat volume and area. 5) Muscle strength and power: Quadriceps muscle performance testing (maximal strength and power)

will be performed using a Biodex dynamometer. 6)Physical function: The Short physical performance battery (SPPB) will be used to measure lower extremity performance as a secondary outcome. 7) MRS: Mitochondrial energetic capacity (ATP_{max}) will be determined by ^{31}P MRS with a brief (24 sec) bout of isometric exercise protocol. 8) Hyperinsulinemic euglycemic clamp to determine insulin sensitivity.

Participants will enter a 10-day strictly controlled bed rest intervention at the Translational Research Institute (TRI) clinical research unit and a progressive 4-week aerobic and resistance training recovery program at the exercise training facility at the TRI, if not randomized to the ambulatory recovery group. Both groups can also receive additional optional resistance exercise training following 4 weeks of the ambulatory or exercise recovery phase. Blood and skeletal muscle biopsies will be collected immediately followings days 10 of the bed rest period and after 1, 2, 3 and 4 weeks of the recovery period. Adipose tissue biopsies will also be collected on bed rest day one, immediately following 10 days of bed rest, and after 4 weeks of recovery if the participant elects to do this. The adipose biopsy is available to be chosen by the participant but not required.

Background Information and Scientific Rationale

Premise: Skeletal muscle atrophy is a clinically significant problem that occurs during disuse or immobilization due to hospitalization, illness and injury, and leads to a loss of muscle strength and physical function (28, 42, 66). This is a particular public health problem for older adults who comprise the majority of hospital patients in the US (26, 38) and who may lose more muscle mass during bed rest (64, 88).

Older adults do not adequately recover following bed rest without adequate rehabilitation (57, 112, 113), which likely contributes to their reduced functional status and ambulation upon discharge (20, 22), a loss of independence, nursing home placement (39) and an increased risk for falls and fractures (80). Older adults are also more likely to develop type 2 diabetes (T2D). Indeed, ~33% of all hospital patients in the US have T2D. Evidence from the longitudinal Health ABC study showed that T2D is associated with more rapid loss of muscle mass and strength in older adults (89). Furthermore, older adults with T2D are at increased risk of injurious falls (78) and physical disability (25). Despite the potentially serious clinical implications however, T2D has not been investigated as an important factor in muscle atrophy and recovery response with immobilization.

Mechanisms underlying disuse atrophy and poor muscle recovery in humans are not well characterized. Most immobilization studies in humans have been limited to measuring changes in muscle only before and after disuse atrophy and recovery (20, 21, 64, 65, 113, 114). This has contributed to discordant views in the field regarding the causal roles that deranged protein synthesis or degradation play in atrophy responses (43, 94, 96). Supported by evidence from preclinical rodent models (8), clinical investigations of early time course changes during atrophy and recovery responses could reveal temporal mechanistic relationships, leading to identification of muscle targets in humans. For example, only 24-48 hrs. of inactivity can reduce muscle protein synthesis (115), and reduce phosphorylation of AKT, a key upstream regulator of mTOR/p70S6K (111, 118).

The role of mitochondrial energetics in disuse atrophy in humans is unknown. The metabolic correlates to muscle atrophy include skeletal muscle insulin resistance (21, 23, 75), decreased fatty acid oxidation and a shift to glucose oxidation (9), intramyocellular lipid accumulation (23, 110) and impaired protein synthesis (36, 42, 87). While mitochondria have been implicated in the etiology of these metabolic dysfunctions in the context of aging and diabetes (44, 92), the role of mitochondria in human muscle disuse atrophy and loss of function is poorly understood.

Mitochondrial reactive oxygen species as an upstream mediator of impaired protein synthesis. Release of pro-apoptotic factors (1, 73) morphological alterations (fission, swelling), energy stress via reduced ATP (100), and increased mitochondrial reactive oxygen species (ROS) emission (1, 60, 79, 82) have all been reported during muscle atrophy in pre-clinical studies. It is not clear whether these alterations in muscle mitochondria are simply a consequence of myofiber atrophy or actively contribute to muscle atrophy. Pre-clinical evidence indicates that mitochondrial ROS can depress protein synthesis by decreasing phosphorylation of 4E-BP1 and impairing mTOR assembly (93, 104, 120) and also induce muscle insulin resistance in part by impairing postprandial AKT phosphorylation, a major driver of the mTOR pathway and protein synthesis (5).

Mitochondria activate protein degradation systems. Mitochondria-targeted antioxidant treatment in rodents support a crucial role for mitochondrial ROS in mediating muscle atrophy (79, 95). Increased ROS production can also exacerbate muscle atrophy (59). Mitochondrial ROS stimulate proteolytic degradation pathways (autophagy & proteasome system) (7, 24, 49, 55, 70, 74, 106) and energetic stress (reduced ATP production), which can activate the AMP kinase (AMPK)-FoxO3 pathways leading to increased expression of ubiquitin-proteasome system and lysosome-autophagy system (48, 100). Taken together, multiple lines of compelling pre-clinical evidence, including data from our group presented later, implicate a central role for mitochondrial energetics in muscle atrophy (Fig. 1). *A handful of small, underpowered human studies provide circumstantial evidence that mitochondrial dysfunction occurs during limb immobilization (11, 46, 47, 111) but* ***none*** *have adequately interrogated a potential role for muscle mitochondria during disuse atrophy and recovery.*

The mechanisms by which intramyocellular lipids contribute to human muscle atrophy is unknown. Evidence from both pre-clinical models and humans suggest that impaired mitochondrial fatty acid oxidation (41) and intramyocellular lipid (IMCL) may play a role in muscle atrophy (23). Specific lipotoxic species including ceramide are associated with poor anabolic response to exercise in older adults (99). High fat diet (HFD) fed rodents and muscle cell culture models consistently link ceramide with exacerbated muscle atrophy or impaired anabolic signaling (58, 72, 98, 101, 108). Excess intramyocellular lipids are also linked with poor muscle quality, and with slower myofiber contraction, force and power development in obese older adults (13, 45). *The role of IMCL in general and ceramides specifically in human muscle atrophy has not been examined.*

T2D may be a critical factor linking mitochondrial energetics, imcl and disuse atrophy. Older adults are becoming more obese along with having an increased incidence of T2D. Findings from our group and others indicate that skeletal muscle insulin resistance in T2D is mediated by lipid induced mitochondrial stress (83). T2D, Obesity and IR have been shown to contribute to blunted control of sub-fraction specific-protein synthesis by insulin, amino acids and other anabolic stimuli (12, 50-53, 84, 86). Although mechanistic studies are limited, studies reporting reduced muscle

protein synthesis by experimental elevation of circulating fatty acids (85, 108) support a putative connection between IR and an exacerbated atrophy response. These impairments could also impact functional recovery following periods of inactivity. *Moreover, we will show preliminary data that* ***older obese subjects have an accelerated loss of muscle mass*** *during bed rest.*

Mitochondrial dysfunction has been hypothesized to be involved with skeletal muscle insulin resistance in older adults with T2D, possibly by affecting IMCL accumulation (71, 90, 91). Our group has shown that obesity and T2D is characterized by impaired fatty acid oxidation (17), ceramide accumulation (2, 14, 17), and mitochondrial dysfunction (97, 116). These are all factors that likely contribute to lower protein synthesis in obese individuals during disuse, resulting in greater muscle atrophy and poor recovery. Indeed, recent evidence suggests that the link between mitochondria and IR extends to mitochondrial cardiolipin remodeling (68), oxidative stress and cellular redox state (5, 67). The contribution of dysfunctional mitochondrial energetics and lipotoxicity to muscle atrophy in humans has not been elucidated. Studies in older adults with T2D will provide a pathophysiological context and parallax view of mitochondria's relationship with muscle atrophy and recovery that will allow us to delineate novel mechanisms.

The role of adipose tissue and crosstalk with skeletal muscle. Recent evidence suggests that adipose tissue is not just a "storage depot" for fat but is a highly active endocrine organ, which plays a pivotal role in whole body energetics (63). In particular, the adipose tissue of older adults and T2D becomes infiltrated with macrophage and becomes "inflamed" releasing adipokines (TNF) that impact metabolic health of other tissues in the body. Adipose tissue inflammation and the release of factors (adipokines) mediate insulin resistance in muscle, which in turn prevents protein synthesis and contributes to loss of muscle mass in sarcopenia (61). While these mechanisms have been relatively well described in animal models, evidence in humans is lacking. An exploratory aim of this protocol will be to examine adipose tissue metabolism/signaling and its role in disuse and exercise recovery.

Effective therapeutic strategies to prevent muscle atrophy and aid in recovery are lacking. Pharmacological therapies to date have focused on increasing muscle mass and include testosterone, selective androgen receptor modulators (SARMS), growth hormone, and myostatin antibodies that are currently at early phase clinical trials (81). Dietary supplements enriched with leucine and essential amino acids can promote protein synthesis (119). There is a critical need, however, to test mechanism-based approaches to prevent or attenuate atrophy and aid recovery following periods of inactivity for older adults.

Exercise as a strategy to aid recovery. Exercise interventions can clearly improve mitochondrial energetics, alleviate IR and stimulate protein synthesis resulting in improved muscle mass and strength (10). However, older adults with T2D are reported to have an attenuated improvement in health outcomes following exercise (109). *In context of recovery from bed rest, the efficacy of exercise to facilitate recovery of muscle mass, strength and function in older adults with T2D has not been determined.* We have shown that aerobic exercise improves insulin sensitivity, mitochondrial function and lowers sphingolipid in muscle from older adults and in obesity (33), and others have demonstrated reductions in oxidative stress (102). Here we propose to test the efficacy of exercise to facilitate recovery following bed rest, and to use exercise as a physiological tool to examine the relationships between improved mitochondrial energetics, lipid partitioning,

protein synthesis and IR during recovery. This could also emphasize mitochondria as an adjunct target to anabolic therapies.

Study Objectives

The overall objective of this project is to decipher the temporal sequence by which exercise training alters mitochondrial dysfunction and muscle protein synthesis in older adults with T2D and how an exercise intervention can aid in recovery of muscle mass, strength and physical function following bed rest. Studies in the clinically relevant context of aging, T2D, and exercise will provide a physiological framework in which the temporal and mechanistic relationships between mitochondria, intramyocellular lipid and protein synthesis/breakdown can be interrogated during atrophy and recovery.

Study Design

Research Design

The proposed clinical study is a prospective, parallel two arm, randomized controlled study consisting of five discrete but connected phases:

I) Screening, II) Pre-Bed Rest, III) Bed Rest, IV) Ambulatory/Exercise Recovery and V) Optional Resistance Exercise

Eligible participants will be Older Adults with T2D and will be randomly assigned to one of two groups: Exercise recovery (EX) or Ambulatory recovery (CON).

The expected duration for a subject in this study could be up to 16 weeks.

A series of physiologic and clinical/health outcomes will be assessed before, during and after the bed rest intervention period and recovery period.

Males and females with pre-diabetes or type 2 diabetes in the age range of 60-80 years will be recruited from AdventHealth's electronic medical record (EMR), the TRI recruiting database, senior centers, television, radio and social media advertisements, mass mailing lists from the Orlando, FL catchment area, and by contacting potential participants on the TRI's participant database.

Study Intervention Description

Bed Rest Intervention. The participant will remain in bed rest for 10 days. The metabolically balanced study diet will be individually formulated for each participant based on their energy needs and food preferences. Each subject's protein intake will equal to 0.8g/kg body weight /d. The remaining macronutrients will be distributed approximately to the subject's typical diet from the 3-day food diary. Required energy intake (kcal/d) will be based on RMR results with an activity factor (AF) of 1.1. Diets will be prepared in the

Metabolic Kitchen at the TRI. Participants will maintain strict bedrest and remain flat as much as possible during bed rest; however, participants can have the head elevated to 30 degrees (by study staff) for meals. Participants will be free to change position while lying in bed by rolling side to side. Participants may elevate legs when lying flat in bed for comfort. Participants may also sit up during use of incentive spirometer. Participants may be transferred on to a gurney/stretcher, or wheelchair for transportation. Participants will be allowed to use a bedside commode for urination and will be taken to a toilet in a wheelchair for bowel movements. Participants will be allowed to shower as long as subjects use a shower chair or wash up at the sink while sitting in the wheelchair, and/or they can take a sponge bath while in bed. Participants may have their hair washed while in bed. Participants will not be allowed to take a traditional bath. Participants will be allowed to shower normally after becoming ambulatory. This is an intervention that has been shown by our group to produce robust reductions in lean mass in older adults (IRBNet #514957-98) (107).

Exercise Recovery Program. The exercise recovery program will be supervised by a certified exercise physiologist and will take place at the TRI exercise training facility. Exercise compliance will be monitored carefully. We are examining the muscle-specific effects of the recovery exercise program on the muscle groups of interest. We will use an exercise recovery program that has demonstrated substantial increases in quadriceps muscle size and strength in elderly people (37, 40, 105). During the first 4 weeks of recovery, participants will perform a combination of aerobic and resistance exercise training with a certified exercise physiologist 3 days per week at the TRI exercise training facility. Exercise compliance and adherence will be monitored carefully. Aerobic training will be 30 minutes of brisk walking, jogging, and 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 ratings of perceived exertion. Three sets of 8–12 repetitions of each resistance exercise will be performed to target the major lower body muscle groups, including the vastus lateralis of the quadriceps.

Ambulatory Recovery. Participants in the ambulatory recovery group will not receive any exercise intervention or advice on exercise. Rather these participants will return to their regular daily routine that they engaged in prior to the bed rest intervention.

(Optional) Resistance Exercise Program. The resistance exercise program will be supervised by a certified exercise physiologist and will take place at the TRI exercise training facility. Exercise compliance will be monitored carefully. Strength training will consist of upper and lower body exercises. Participants will participate in strength training 3 days per week (12 total sessions). Each resistance exercise will be performed with a resistance that will allow 8–10 repetitions (approximately 70-80% of 1 RM). Three sets of 8–12 repetitions of each exercise will be performed to target the major upper and lower body muscle groups after appropriate rests. Each repetition will employ a slow speed (approximately 5 seconds) to maximize muscle hypertrophy and minimize risk of a muscle pull or injury. The subject

will walk at their usual pace before and after strength training to allow for a warm up and cool down. Strength training (with an appropriate warm up and cool down) will take approximately 1 hour. Speed of contraction for the concentric component will be approximately 2 seconds for full extension and the eccentric component will be 4 to 6 seconds in duration. The one repetition maximum will be determined weekly to ensure that each participant is exercising at the appropriate duration. This is the exercise regimen that has demonstrated substantial increases in muscle size and strength in elderly people.
If the participant is unable to come in for a scheduled exercise appointment, alternate arrangements for the visit may be made within the visit window with Principal Investigator approval.

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

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

Name of external site(s) outside of AdventHealth: N/A
Estimated number of subjects at external sites:0

Total number of all sites: 1
Estimated number of subjects at all sites combined: 75

Multi-Site Research Logistics/Communication Plan
N/A
Research Conducted in a Foreign Country
N/A
Community-Based Participatory Research
N/A

Subject Selection

Vulnerable Populations (if applicable)

AdventHealth Employees: Recruitment efforts will follow AdventHealth recruitment SOPs for research. AdventHealth Employees will not be individually targeted nor excluded from study participation based on employment. AdventHealth employees who engage the 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.

Inclusion Criteria

1. Participant must be male or female, ages 60 through 80 years of age.
2. Participant has pre-diabetes or has been diagnosed with type 2 diabetes and taking 0-3 oral hypoglycemic agents, which include basal insulins, incretin mimetics, DPP-4 inhibitors, Sulfonylureas and/or Metformin therapy, and has an Hgb A1C < 9.0%.
    a. Participants on Insulin, , SGLT2 inhibitors, and Thiazolidinediones will be excluded.
    b. Pre-Diabetics are defined as having an HgbA1c of greater than or equal to 5.7% and less 6.5%, or a fasting glucose of greater than or equal to 100 mg/dl and less than 126 mg/dl or has a glucose of greater than or equal to 140 mg/dl and less than 200mg/dl at the 2 hour blood draw during OGTT
3. Participant must have renal function with an estimated glomerular filtration rate (eGFR) > 45 ml/min/1.73m^2 determined at screening.
4. Participant's triglyceride level is < 350 mg/dl and LDL cholesterol is ≤ 150 mg/dl at screening.
5. Participant states willingness to follow protocol as described, the prescribed activity level and completing any forms needed throughout the study.
6. 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. Participant has type 1 Diabetes.
2. BMI > 40.0 kg/m^2
3. Participant is actively pursuing weight loss and/or lifestyle changes.
4. Participant has a history of pressure ulcers.
5. Participant has a stated history of Deep Vein Thrombosis (DVT), pulmonary embolism, or a known hypercoagulable condition, or other clotting or bleeding disorders.
6. History of gastrointestinal or intracranial hemorrhage.
7. History of stroke or cerebrovascular accident within the last 6 months.
8. Recent history of major trauma (within 3 months).

9. Thrombocytopenia (<100,000/microL) or hyperkalemia (K > 5.2) on screening laboratory assay. May repeat lab value per PI discretion.
10. Untreated or poorly controlled hypertension (SBP > 150, DBP > 95), or hypotension (SBP <100 DBP <60)
11. Participant has hypothyroidism (TSH ≤(0.5mIU/L) or hyperthyroidism TSH ≥ 10mIU/L.
12. Participant has 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.
13. 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 asprin).
14. Participant has had surgery requiring > 2 days of hospitalization in the last 1 month prior to screening visit.
15. Participant has an active malignancy or autoimmune disease.
16. Participant has current significantly impaired liver function in the opinion of the study Medical Investigator (mild asymptomatic fatty liver is acceptable), or hepatic enzyme tests are ≥ 2.5 times normal limit.
17. Participant has a chronic, contagious, infectious disease, such as active tuberculosis, Hepatitis B or C, or HIV, per self-report.
18. Participant is an amputee and/or has presence of partial or full artificial limb.
19. 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.
20. Participant currently has uncontrolled severe diarrhea, nausea or vomiting.
21. Participant has an obstruction of the gastrointestinal tract, inflammatory bowel disease, short bowel syndrome or other forms of gastrointestinal disease such as stage III or above gastroesophageal reflux disease, gastroparesis, peptic ulcer disease, celiac disease, intestinal dysmotility, diverticulitis, ischemic colitis and bariatric surgery.
22. Participant cannot abstain from alcohol for the pre-bed rest and bed rest portion of the study. For the recovery portion of the study the participant must agree to consume no more than ~14gm of alcohol per day (equivalent to ~ 1 glass of wine (4-5 oz 12% ABV) or ~bottle of beer (12 oz 5% ABV) /day).

23. Participant cannot refrain from taking medications/dietary supplements/herbals or substances that could modulate glucose metabolism (other than oral hypoglycemic medications), or are considered anabolic, or reduce weight (fat mass), or that may interact with low-molecular weight heparin or induce hypo- or hyper-coagulable state, in the opinion of the PI or medical investigator, starting one week prior to Pre-bed rest phase and over the entire course of the study. These include progestational agents (except prescribed birth control), steroids, growth hormone, dronabinol, marijuana, calcium-beta-hydroxy-betamethylbutyrate (CaHMB), free amino acid supplements and dietary supplements to aid weight loss.
24. Participant has a mini-Mental State Examination score < 21.
25. 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.
26. 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.
27. Participant has a sensitivity or allergy to lidocaine.
28. Participant has a sensitivity or allergy to heparin, enoxaparin, or other low molecular weight heparin.
29. History of allergy to pork products.
30. History of heparin-induced thrombocytopenia.
31. Hemoglobin < 10.0 g/dL for females; < 11.0 g/dL for males; or participant has clinically significant signs/symptoms of anemia in the opinion of the PI or medical provider.
32. Concomitant medications with known contraindication or interaction with low-molecular weight heparin (including anti-platelet agents, anti-coagulant agents, non-steroidal anti-inflammatory drugs)
33. 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.
34. Participant experiences symptoms of claudication. Symptoms include cramping pain in the legs and/or difficulty walking.

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.

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.

Resources Available

We attest that all TRI faculty and staff will be trained, and this training will be documented. We will adhere to AdventHealth ORA SOP 06 (Research Personnel Selection, Qualification, Responsibilities, and Training) and Working Instruction (WI) 031.100.015 Documentation of Protocol Training.

Protocol development meeting, with follow-up meetings, will occur regularly to ensure that all persons assisting in the research are adequately informed. Periodic meetings will be scheduled to maintain and update any new information/issues.

Study Procedures

Subject Recruitment and Screening

Males and females with pre-diabetes and type 2 diabetes in the age range of 60-80 years will be recruited from AdventHealth 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 TRI's participant database.

Potential participants will be medically screened to determine good health, weight stable (no gain/loss of ≥ 10 lbs in 6 months prior to screening), and without any contraindication to exercise. Approximately equal numbers of men and women will be recruited. Neither race nor ethnicity will be exclusions. Re-screening of participants may be repeated per Medical Investigator discretion, if needed.

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 TRI. Informed, written consent will be obtained at the screening visit.

We anticipate ~400 phone screens to successfully complete 18 participants per group.

Consent Process

We attest that all study staff delegated the authority to obtain informed consent will follow The institutional "SOP: 401.116 Informed Consent Process and Written Documentation of Informed Consent "and Investigator Guidance: Remote Informed Consent Process in Non-Exempt Research (HRP-831)" and Investigator Guidance: Remote Informed Consent Process in Non-Exempt Research (HRP-831)".

Potential subjects will be given time to read the consent document and will be interviewed by a clinical coordinator. The coordinator will give a detailed overview of the study and then ask if the potential subject has questions and if they might be interested. The candidate is then encouraged to ask questions. After all questions have been addressed and interest remains, the candidate then signs the consent if he/she chooses to do so. After the subject has signed the consent or in the scenario of the remote Inform Consent process once the original signed copy has been received, inclusion and exclusion criteria will be reviewed. The new subject is given a photocopy of the signed consent form for his/her records and the PI/Sub-I will review all participant Inclusion Exclusion Worksheets. Once deemed eligible, the PI/Sub-I will sign and date the Inclusion Exclusion Worksheet. Participants will be free to withdraw at any time for any reason without consequence.

All study staff delegated the authority to obtain informed consent will follow
SOP: 401.116 Informed Consent Process and Written Documentation of Informed Consent.

Non-English Speaking Subjects

The participant population that we recruit will be primarily English speaking. In the unlikely event that a non-English speaking participant meets criteria for enrollment, the AdventHealth IRB Investigator Guidance: Short-Form Consent Process in Research (HRP-804), will be followed.

Subjects 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 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. In the scenario of Remote Informed Consent Process documentation of all actions will be maintained as described in the

Investigator Guidance: Remote Inform Consent Process in Non-Except Research (HRP-831).

Waiver of Written Documentation of Consent or Waiver of Consent

N/A

Randomization

A randomization schedule containing the subject treatment group assignment (EX or CON) will be generated by the study statistician. A permuted blocks approach will be used with subjects stratified by gender. We will use blocks of random sizes of 4 to reach the total sample size of 20 to start (18 completers) in each group.

Study Visits

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

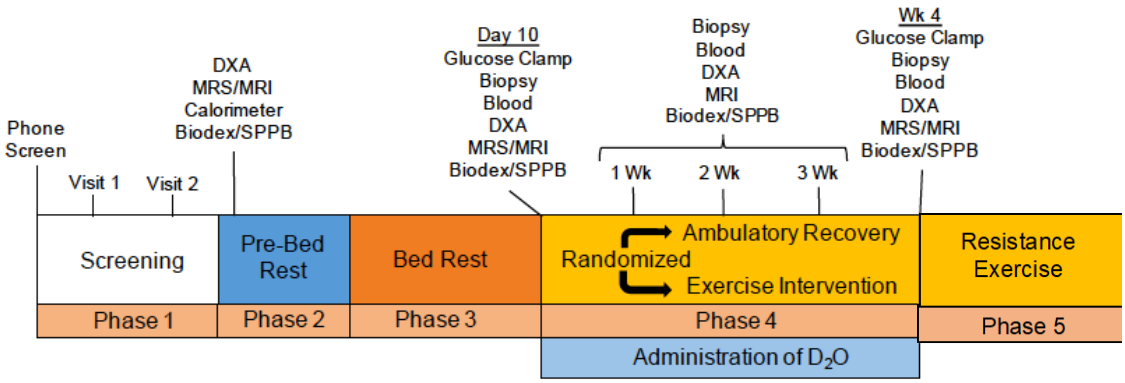

Figure 1: Schematic depiction of the study protocol.

Phase	Description	Duration	Subject at home	Subject Ambulatory	Visit Window
I	Screening	< 35 days	Yes	Yes	35 days within PBR1
II	Pre-Bed Rest	5 days	Yes	Yes	-
III	Bed Rest	10 days	No	No	-
IV	Ambulatory/Exercise Recovery	4 weeks	Yes	Yes	± 1 days
V	Optional Resistance Exercise	4 weeks	Yes	Yes	± 2 days

Phase I – Screening.

Screening Visit 1

Approximate visit time: ~ 4 hours

The purpose of the staged screening process is to identify and verify eligible participants over a series of contacts. Interested participants are first screened by phone. The phone interview is designed to exclude individuals who are clearly ineligible or unlikely to benefit from participation in the study. Potentially eligible volunteers will arrive at TRI in the morning in a fasted state (at lease or ~8-hour overnight fast). Following provision of voluntary written informed consent, the following tests will be conducted. The study menu will be reviewed with each subject at screening and reasonable substitutions will be made for diet intake compliance.

	Screening Visit (days)	
Assessment	1	2
Review and collect signed ICF	X	
Review Eligibility (may occur remotely)	X	
Health history(may occur remotely) and full physical examination[1]	X	
Medication/supplement history (may occur remotely)	X	
Age	X	
Metabolic Body Weight[16]	X	X
Height	X	
Vital Signs[11]	X	
BMI	X	
Waist circumference[4]	X	
Questionnaires (CHAMPS, Mini-Mental, etc.)[12]	X	
SPPB[7]	X	
Step Test[15]	X	
400m walk test (usual pace)[13]	X	
Handgrip strength[10]	X	
Fasted blood draw[2]	X	
OGTT (Pre-Diabetics only)	X	
Resting 12 lead ECG[5]	X	
Urine Collection[3]	X	X
Dispense 3-day food diary	X	
Collect 3-day food diary		X

Placement of Accelerometers		X
Resting Metabolic Rate/RQ[9]		X
VO2max test/stress test[8]		X

[1]A Health History will be obtained. A standard full physical examination will be performed by a study physician, physician assistant, or nurse practitioner. Age, weight, height and BMI and hand grip strength will also be determined.

Medical Health History and Physical will be conducted by a study physician or medical provider (nurse practitioner or physician assistant) for enrollment into the study.

[2]A fasting blood draw for determination of lipid profiles (cholesterol, HDL, LDL, VLDL, triglycerides); Complete Metabolic Panel (CMP) which includes electrolytes, glucose, kidney and liver function (ALT, AST, Alk phos); Complete blood count with platelet and differential (CBC); kidney function (BUN and creatinine); HbA1c, Thyroxine (T4), Thyroid stimulating hormone (TSH). Total blood volume for screen labs is approximately 18mls. Safety labs may be repeated per Medical Investigator discretion, if needed.

[3]Urine Collection for urinalysis will be collected during SV1. Urine collection for urea nitrogen and creatinine will be completed during SV2.

[4]Waist Circumference Measurements which are a surrogate measure of abdominal fat will be conducted. 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.

[5]Resting 12 lead ECG: Electrodes will be placed on the chest to provide a tracing of the electrical activity of the heart.

Oral Glucose Tolerance Test (OGTT): Glucose tolerance will be assessed only in potential pre-diabetics with a 75 g oral glucose tolerance test (OGTT). Subjects will be studied after an overnight fast. Samples may be collected from an indwelling IV line or by venipuncture. (Consider the necessity of multiple time points/analytes for the given experiment) Two baseline samples will be drawn at approximately times= -10 and -1 minutes. The subjects will then ingest a 75 g glucose beverage at time=0 minutes (first sip of beverage) and will be asked to consume the entire beverage in <5 minutes. Blood samples will be collected at approximately times= 30, 60, and 120 minutes. Each blood sample will be analyzed for plasma or serum glucose, insulin, free fatty acid, C-peptide, and incretins (GLP-1 and GIP). Total blood volume collected during this procedure is approximately 48 mL.

a. Participants will be considered pre-diabetic if their HgbA1c is greater than or equal to 5.7% and less 6.5%, or a fasting glucose of greater than or equal to 100

mg/dl and less than 126 mg/dl or has a glucose of greater than or equal to 140 mg/dl and less than 200mg/dl at the 2 hour blood draw during OGTT

[7]Short Physical Performance Battery (SPPB)
Briefly, this test measures standing balance (feet side by side, semi-tandem, and tandem), habitual gait speed (4-meter walk test), and chair stand time (how rapidly an individual can stand from a seated position). Additionally, each participant will be asked to complete a timed step test.

- Standing Balance should be performed on a flat non-slip surface and without assistance, such as a cane or walker.
- The Four Hundred Meter Walk Test (400MWT) is a practical, simple test that requires an obstacle free hallway. This test measures the walking speed in meters per second over 4m. For the 4MWT, clearly mark the 4 m course using high visibility, or similar tape.
- For the Chair Stand test the subject is seated, with their arms crossed. The subject must be able to stand upright and sit back down from this seated position.

[13]A 400-meter walk test at usual pace will also be conducted. The 400-meter walk test is a measure of cardiorespiratory fitness, lower extremity muscle function and general mobility. During this self-paced walking test, patients are instructed to walk 400 meters at their usual pace or without any expectation of time. The ability to walk 400 meters in less than 15 minutes has been suggested as an indicator of sufficient capacity for community ambulation. The goal of this outcome measure is to determine the amount of time required to walk 400 meters. The patient may rest whenever necessary but should understand the aim is to walk the entire 400-meter distance as soon as possible. The time needed to complete the test will be recorded on source documentation. If the person is unable to walk the full 400 meters because of needing to stop or not completing the distance within 20 minutes, the distance (in meters) and time walked during the test will be recorded.

The 400mwt should be performed using a 20 m course. The 400-meter walk test will be performed and timed using a stopwatch. Subjects will be instructed to walk as quickly as possible for 10 laps around markers placed 20 meters apart. Standard encouragement will be given by study personnel after the completion of each lap.

[10]Subjects will perform a hand grip test that will be conducted three times with 1 minute of rest between each repetition using the dominant arm. The greatest grip strength and the average of the three tests will be recorded.

[11]Vital signs consist of body temperature, pulse rate (or heart rate), blood pressure, and respiratory rate.

[12]A number of health questionnaires will be administered at screening, including: CHAMPS Physical activity, Life Space, Lifestyle, Functional Status, Pittsburgh Fatigability Scale.

[15]The Step Test is conducted by recording the time it takes for a participant to complete a series of repeated steps. The participant will be asked to complete a timed step test where they will be repeatedly stepping up and down from an 8-inch high step.

[16] Body Weight is Metabolic Weight. Metabolic weight is completed during fasting and with an empty bladder while wearing a gown and underwear. An average weight between two measurements will be used. The metabolic weight will be the difference between of the average subject weight and weighted gown.

Screening Visit 2

Approximate visit time: ~ 2 1/2 hours

[9]Resting Metabolic Rate/Respiratory Quotient (RMR/RQ): Indirect calorimetry will measure the resting metabolic rate/respiratory quotient (RMR/RQ) and substrate utilization after an 8-hour overnight fast using a MAX II Metabolic cart (AEI Technologies, Pittsburgh, PA). The analyzer will be calibrated before each subject with standardized gases containing 5% CO_2, 21% O_2 and balance N_2. 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 O_2 consumption (VO_2) and CO_2 production (VCO_2) will be made by comparing a single measure of the O_2 and CO_2 concentrations of the testing room to continuous measurements of O_2 and CO_2 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.

[8]Graded exercise testing: Volunteers must pass the medical screen before they will be allowed to undergo the exercise test. If the resting ECG prior to the exercise test shows clinically significant abnormalities, the test will not be conducted. If abnormalities develop during the test, the test will be stopped. On both occasions, the participant will be referred to their Primary Care Physician for further evaluation. Before and after exercise activities are performed, the participant's that are type 2 diabetics will have a blood glucose checked using their home glucose monitor or glucose monitor at TRI. Blood glucose will be measured prior to and immediately following each exercise session for safety. If blood glucose is > 300mg/dl, study team will notify TRI provider immediately for review of glucose values, as well as medications. If the glucose values are consistently > 400mg/dl, participant will be instructed to follow up with their primary care physician. If blood glucose is < 100mg/dl, a snack will be given, and the participant will be allowed to exercise. Self-monitored blood glucose measurements should be checked anytime a patient has symptoms of hypoglycemia or hyperglycemia.

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. Heart rate, blood pressure and ECG, if applicable, will be recorded throughout this test. During the test, subjects will breathe through a low resistance mouthpiece and wear a nose clip. Expired gases will be measured by indirect calorimetry and heart rate monitored by a portable heart rate monitor and/or ECG.

Following a standardized warm up, subjects will begin exercising at a moderate intensity with the workload (resistance on a cycle ergometer or speed and grade on a treadmill) increased every minute until the subject 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 subject 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 subject 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 subject's heart rate increased to within 10 beats of the age-predicted maximum (208 – [0.7 x age]).

The test will also be stopped, and subjects excluded from further participation if there is >2mm ST-segment depression on ECG or evidence of cardiovascular instability, such as hypotension, dangerous arrhythmias or angina.

Prior to beginning the exercise test, the Borg Rating of Perceived Exertion (RPE) Scale will be presented to participants. The RPE Scale is a rating of how hard a person feels their body is working and is an accepted supplementary tool for prescribing exercise programs in both healthy and special populations. Throughout the test, participants will be asked to point to the numerical rating of how they feel and will be encouraged to remember a rating of how "somewhat hard" (13) feels. The rating of "somewhat hard" should coincide with a heart rate of approximately 50-70% of their maximal exertion level. During maximal testing participants should report a rating of 19-20 ("maximal exertion"). A cardiologist will review the ECG, if the medical Provider deems necessary. If a study physician or cardiologist identifies any artifacts observed from the exercise test that may render the participant ineligible for participation in an exercise intervention, that subject will be referred to his/her Primary Care Physician (PCP) or cardiologist for follow-up care and further examination of test results. If participants are referred for further examination, a written approval to perform unsupervised exercise must be obtained by PCP or cardiologist in order for subject to continue participation in the research study. If written approval from the PCP or cardiologist is not able to be obtained, participant will be excluded from further participation in the investigation. The test will take approximately 30-40 minutes.

Accelerometers to assess free-living physical activity over approximately 5 days will be provided, along with instructions on how to use the devices. They will be placed on the arm and wrist. The device will be returned by the participant after at least 5 days of recording and prior to Pre-Bed Rest Day 1.

Phase II – Pre-Bed Rest (PBR).
This phase of the study should be initiated no more than 35 days after the first screening visit. PBR 1 will be considered the study baseline. The Table below summarizes the schedule of assessment during Pre-Bed Rest.

The five days prior to bed rest is considered the pre bed rest period and will be abbreviated as PBR 1 to PBR 5. Subjects will be required to report to the study site for meals and assessments during these 5 days. To prepare subjects for the bed rest diet (stabilize diet), subjects will be provided with a breakfast, lunch and dinner, for consumption at the study site or as packaged meals for each of the 5 days. Subjects will consume breakfast at the study site and will have the option to consume lunch and dinner at the study site, or they will be provided with a packed lunch and dinner for later consumption.

The metabolically balanced study diet will be formulated for each subject to have each subject's protein intake equal to 0.8g/kg body weight/d, and kcal intake to be based on their measured total daily energy expenditure from the armband accelerometer. The remaining macronutrients will be distributed approximately to the subject's typical diet from the 3-day food diary.

Body Weight during the Bedrest phase only, will be taken using the bed scale during fasting and with an empty bladder while wearing a gown or light clothing and undergarment(s).

	Pre-Bed Rest (days)				
Assessment	1	2	3	4	5
Medication changes	X	X	X	X	X
Metabolic Body Weight[16]	X	X	X	X	X
Evaluate DVT Risk					X
MR Assessments[1] (ATPmax//IMCL/IMAT/)		X*			
Biodex/Strength assessment[2]			X*		
DEXA[3]			X*		
Eucaloric Diet Stabilization - Adequate protein	X	X	X	X	X
Admit patient for bed rest intervention					X

*The imaging and exercise procedures may be performed as baseline measurements on any Pre-Bed Rest day

[1]MR Assessments to occur preferentially on PBR2, however may occur on any PBR day to accommodate scheduling. Assessments defined below:

Maximal ATP Production (ATPmax): ATP_{max} will be measured in order to determine in vivo mitochondrial capacity. Participants will complete the MR Screening for ATP_{max}, and

the information will be verified with the participant by a member of the TRI Imaging Core to determine if data acquisition can be performed safely. The magnetic resonance scans to measure mitochondrial energetics will be performed on a 3T Philips Acheiva at the TRI. The participant will be informed that the imaging scans will produce loud "knocking" noises, but they will be provided both ear plugs and earphones to minimize this noise.

OVERVIEW: The Phosphocreatine (PCr) recovery time constant (τ) and the PCr level in oxygenated muscle at rest $(PCr)_{rest}$ will be used to calculate maximum mitochondrial capacity (ATP_{max}). Participants will be asked to lie supine on the patient table of the MRI for approximately up to one hour. After set of survey images is acquired to locate the muscle group of interest, a fully relaxed ^{31}P spectra will be collected. The ATPmax experiment will be performed by obtaining a set of sequential ^{31}P spectra; one spectrum will be acquired every 6-7.5 seconds for the duration of the ATPmax experiment. For the first two minutes, the participant will remain still in order to obtain baseline [PCr], [ATP] and [Pi] measurements. After the baseline is established, the participant will be asked to perform contractions of the quadriceps (by kicking against straps around the lower leg) for up to 45 seconds. After kicking is stopped, the participant will remain still for the remainder of the experiment in order to allow the PCr peak to return to baseline. The ATPmax experiment may be repeated. The scan time for the ATPmax experiment will take approximately 45 minutes.

Intramyocellular Lipid *In Vivo* ^{1}H Magnetic Resonance Spectroscopy (IMCL): The participant will be asked to lie supine on the patient table of the magnet (Philips Acheiva 3T) for approximately 45 min. The participant's leg will be positioned inside a radio frequent (RF) ^{1}H knee coil with the knee in extension and the ankle in a neutral position. Standard clinical MR Imaging, including 3-place localization and T2 weighted images, will be completed to obtain anatomical images for voxel / slice localization. The PRESS (point resolved spectroscopy) sequence with water suppression will be used to obtain spectroscopic measurements of the intramyocellular lipid (IMCL). A PRESS single voxel sequence will be used to obtain spectra from separate voxels (up to 15 x 15 x 15 mm^3) within largest volume of the calf muscles (echo time (TE) = 35 msec and a resonance time (TR) = 1500 seconds). Voxels will be positioned to avoid fascia, vascular structures and gross marbling as determined using the anatomical images.

MR assessment of Intramuscular and Subcutaneous Fat (IMAT): Volumetric measurement of fat, muscle and bone in the participant's mid-thigh leg will be completed using an Achieva 3T MRI/Multinuclear MRS (Philips, Andover MA). Scans will be performed under standardized conditions with subjects in a supine position. The torso XL coil will be placed around their legs (encompassing both knees and thighs). If the participant and the coil cannot fit into the bore of the magnet, the coil will be removed and the exam will be completed using the quadrature body coil. Each scan will take approximately 30 minutes to perform. Low resolution scans will be taken to determine the approximate midpoint of the participant's femur. Then T1 weighted imaging sequences will be performed centered at the midpoint.

[2]Strength assessment. The initial weights for knee extension should be set based on the subject'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 subject's first 4 lifts at 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. If, prior to exercise, the blood pressure is less than 160/100 mm Hg, then the subject will warm-up on a bike for ~ 1 minute with no resistance. If BP is elevated the subject should rest for 3 more minutes. If BP is still elevated greater than 160/100 mm Hg, the subject will be sent home with the recommendation to contact their physician, and this subject will be followed up for potential re-testing.

Termination criteria for maximal strength testing:
- A subject 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.
- Subject cannot move through full ROM with proper form (subject lifts lower or upper back away from padded seat, subject kicks pad using acceleration force to lift weight, hands not crossed across chest, uneven or jerky ROM).

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

[2]Biodex 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. If the BP is in acceptable range prior to exercise, the subject 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 subject is on the bike for 10^{o} of flexion at the knee when leg is fully extended). Tester explains the power test and also describes the responses to the pain/discomfort (P/D) scale (range = 0 to 6) and that the subject may decide to discontinue testing at any time. The technician will then explain the test: "This is a power test and we want to see how quickly you can move an amount of weight through a distance." After the 1-minute bike warm-up the subject 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 belt should then be fastened snugly. Both legs will be tested, and the first leg will be selected randomly. Familiarization of the Biodex will be performed at the first day of testing. Familiarization at future visits is not required. The subject will be instructed to keep arms crossed across the chest and to remember to maintain normal breathing patterns. Next the subject performs a power test with 60, 120

and 180 degrees on each leg and gives a rating of P/D. Subject will perform three power tests on each leg and a 2 min rest between each increase in resistance. The subject will give a P/D rating after each trial. Before each test the tester will remind the subject to keep their back against the seat. If necessary other constant reminders will include – "Breathe normally," "Look straight ahead," or "Try to move your leg up to here (tester indicates end of movement)." The tester will also provide standard encouragement such as – "Move your leg as quickly as you can," "Give me your best effort". After both legs have been tested BP will be taken at conclusion of test and after 5-10 minutes of rest. If BP reading is normal, testing will continue. If it is above 160/100 mm Hg or has decreased by more than 15 mm Hg (top or bottom number) from the resting values, the subject will rest for 2 minutes and BP will be taken again. If BP has not normalized to below 160/100 mm Hg, further testing will be discontinued, and the subject will remain in the facility for at least ten more minutes while monitoring blood pressure. When BP finally normalizes, the subject will be sent home with the recommendation to contact their physician. The PI, Dr. Coen, will also be notified.

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

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

Before and after exercise activities are performed, the participant's blood glucose will be checked (type 2 diabetics only) using their home glucose monitor or glucose monitor at TRI. Blood glucose will be measured prior to and immediately following each exercise session for safety. If blood glucose is > 300mg/dl, study team will notify TRI provider immediately for review of glucose values, as well as medications. If the glucose values are consistently > 400mg/dl, participant will be instructed to follow up with their primary care physician. If blood glucose is < 100mg/dl, a snack will be given, and the participant will be allowed to exercise. Self-monitored blood glucose measurements should be checked anytime a patient has symptoms of hypoglycemia or hyperglycemia.

[3]Dual Energy X-Ray Absorptiometry (DEXA). DEXA Scans will be performed to measure body fat and estimate muscle mass using a GE Lunar iDEXA 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. One 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. DEXA scan will occur preferentially on PBR3, however may occur on any PBR day to accommodate scheduling.

Phase III –Bed Rest.
This phase of the study may be initiated either the evening of PBR 5 or during BR 1. The subject should remain on bed rest for approximately 10 days. A metabolically balanced study diet will be formulated for each subject to have each subject's protein intake equal to 0.8g/kg body weight/d. The remaining macronutrients will be distributed approximately to the subject's typical diet from the 3-day food diary. Required energy intake (kcal/d) will be based on RMR results with an activity factor (AF) of 1.1.

It is recommended that subjects maintain strict bed rest and remain flat as much as possible during bed rest, however subjects can be propped up (by study staff) with pillows (for eating, etc.). Subjects are free to change position while lying in bed by rolling side to side. Subject may be transferred onto a gurney/stretcher, or wheelchair for transportation. Subjects will be allowed to use a bedside commode for urination and will be taken to a toilet in a wheelchair for bowel movements. Subjects will be allowed to shower as long as they are sitting and wash up at the sink while sitting in the wheelchair, and/or they can take a sponge bath while in bed Subjects may have their hair washed while in bed. Subjects will not be allowed to take a traditional bath. Subjects will be allowed to shower normally after becoming ambulatory. Body Weight during the Bedrest phase only, will be taken using the bed scale during fasting and with an empty bladder while wearing a gown or light clothing and undergarment(s).

The following procedures, evaluations and/or assessments will be obtained daily during bed rest: 1) Medication/supplementation changes, 2) Vital signs consisting of body temperature, pulse rate (or heart rate), blood pressure, and respiratory rate will be monitored routinely (3 times daily). 3) Subjects will be interviewed to determine if any AEs/SAEs occurred in the last 24 hours. Medication to help mitigate reflux problems, constipation, and other issues associated with being supine will be available and administered per site standard of care or study physician discretion. All medications administered will be recorded.

Following 10 days of bed rest, the post bed rest recovery will consist of assisting the participant with ambulation and ensuring the participant is capable of showering, walking and dressing without assistance. The participant may stay an extra night at the study site for further recovery if it is deemed medically necessary by the study physician. A follow up phone call will be made that evening to ensure the subject is ambulatory and to inquire about any adverse events. The participant will return to the TRI on day 11 (+ 1 day) following discharge for additional post-bedrest assessments as listed below.

Before and after exercise activities are performed, the participant's blood glucose will be checked (type 2 diabetics only) using their home glucose monitor or glucose monitor at TRI. Blood glucose will be measured prior to and immediately following each exercise session for safety. If blood glucose is > 300mg/dl, study team will notify TRI provider immediately for review of glucose values, as well as medications. If the glucose values

are consistently > 400mg/dl, participant will be instructed to follow up with their primary care physician. If blood glucose is < 100mg/dl, a snack will be given, and the participant will be allowed to exercise. Self-monitored blood glucose measurements should be checked anytime a patient has symptoms of hypoglycemia or hyperglycemia.

Capillary blood glucose will be measured four times a day for type 2 diabetics, before each meal and at bedtime. If two consecutive results of > 240mg/dl are recorded, the study physician will review and change the patient's current treatment regimen (DPP-4 inhibitors, sulfonylureas and/or Metformin) to prevent further hyperglycemia. If two consecutive results of >270mg/dl are recorded, the study physician may initiate insulin therapy. Insulin therapy will continue until the participant reaches a target glucose range of 140–180 mg/dL (7.8–10.0 mmol/L). The participant's treatment regimen and diet will be reviewed and changed as necessary to prevent hypoglycemia (<70 mg/dL), although this is not expected with medications like, DPP-4 inhibitors and/or Metformin.

The following table summarizes the schedule of assessment during Bed Rest.

Assessment	Bed Rest (Days)										
	1	2	3	4	5	6	7	8	9	10	11 (post BR)
Placement of Accelerometers	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Medication/supplement history	X	X	X	X	X	X	X	X	X	X	
Eucaloric Diet - Adequate protein	X	X	X	X	X	X	X	X	X	X	
Vital signs	X	X	X	X	X	X	X	X	X	X	X
Passive Exercise[4]	X	X	X	X	X	X	X	X	X	X	
Incentive spirometer[5]	X	X	X	X	X	X	X	X	X	X	
Evaluate DVT risk	X	X	X	X	X	X	X	X	X	X	
Body Weight	X		X		X		X		X	X	
Metabolic Body Weight[16]											X
Glucose Clamp - 4Hr[6]										X	
RMR[10]										X	
24-Hour Urine										X	
Blood draw (hemoglobin and hematocrit)[7]							X			X	
Deuterated H2O administration[8]										X	X
Fasting Muscle Biopsy/Blood draw[1]										X	
Fasting Adipose Biopsy[2] (optional)	X									X	
Anticoagulant Therapy		X	X	X	X	X	X	X	X		

DEXA[3]										X	
MR Assessments (ATPmax/IMCL/IMAT[3])										X	
Orthostatic Hypotension Assessment[11]										X	
VO_2max test											X
400m walk test											X
Biodex/Strength testing										X	
SPPB										X	
Step Test										X	
Questionnaires[9]											X

[1]**Muscle Tissue Biopsy:** For this study fasted is defined as consuming no food (except for water and allowed medications/dietary supplements) for at least 8 hours the before blood draw/muscle biopsy. Fasting status should be verified verbally with the subject. A venipuncture blood draw will also be taken prior to each muscle biopsy (Approximately 44ml on Day 10)). Blood will be processed, aliquoted and stored at -80C.

Our group has performed more than 1,500 muscle biopsies during the past 20 years (600 during the past 4 years since the move to the TRI), including same day serial biopsy studies and studies in men and women over 90 years old (14, 17-19, 34, 35). We will conduct 1 biopsy immediately post bed rest (day 10) and 4 biopsies of the vastus lateralis during the ambulatory or exercise recovery interventions. The approximate locations of the biopsies will be balanced as indicated to avoid interference from previous muscle biopsies (See Figure). 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 will be administered using lidocaine. The skin will be incised (approximately 0.5 cm) with a #11 scalpel, and the Bergstrom needle will be inserted into the Vastus Lateralis. Approximately 150 mg 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. This procedure is well-tolerated in older adults. Subjects will not perform physical exercise 48 hours prior to the muscle biopsy to help prevent acute effects of exercise on muscle lipid and the other primary outcomes. Muscle specimens will be trimmed of any visible adipose and connective tissue using a dissecting microscope and processed. The following assays will be performed on the muscle biopsy specimens:

1. Mitochondrial respiration will be measured in permeabilized muscle fibers from vastus lateralis biopsies using the Oxygraph 2K system (Oroboros, Innsbruck, Austria). This approach permits a comprehensive evaluation of mitochondrial respiratory function upon titration with ETC complex-specific

inhibitors and substrates (56). We assess complex I+II supported respiration and palmitate ß-oxidation supported respiration in separate assay protocols. Submaximal and maximal, coupled and uncoupled respiration will be evaluated along with coupling control ratios. This technique is well established in our laboratory (16, 19, 35) and we have conducted respirometry assays in over 500 muscle biopsy specimens in recent years.

2. Mitochondrial H_2O_2 emission will be measured as an index of redox state in permeabilized muscle fiber bundles by real time monitoring of Amplex Red oxidation using a spectrofluorometer, equipped with a thermojacketed cuvette chamber (Horiba Jobin Yvon, Edison, NJ). Dr. Coen has implemented the permeabilized muscle fiber method to quantify H_2O_2 emission rates as an index of cellular redox state (5) in over 400 biopsies to date with a high degree of within-sample reproducibility (CV<10%).

3. Calcium retention capacity of mitochondria will be determined by spectrofluorometry. Change in free Ca^{2+} in the cuvette during mCa^{2+} uptake will be calculated using the known Kd for Calcium Green 5N and the equations established by Tsien (117) for calculating free ion concentrations using ion-sensitive fluorophores. Dr. Coen has implemented the permeabilized muscle fiber method to quantify H_2O_2 emission rates as an index of cellular redox state and calcium retention capacity (5, 6) in over 400 biopsies to date with a high degree of within-sample reproducibility (CV<10%). Assays will be conducted by trained technicians in a blinded manner.

4. Muscle protein synthesis and breakdown rate will be determined. Total and individual protein synthesis will be assessed in muscle biopsy specimens using deuterium oxide (D_2O) according to their previously published procedures in humans (4, 103). 50mg of muscle biopsy specimen will be fractionated into myofibrillar, cytosolic and mitochondrial protein fractions according to their previously published procedures (30-32, 76). After protein hydrolysis derivatized amino acids will be analyzed on an Agilent 7890A GC coupled to an Agilent 5975C MS (30-32, 76). To assist in the determination of protein turnover during non-steady state conditions, the group recently established a mathematical model to determine rates of synthesis and breakdown using changes in enrichment over time (77).

5. Multidimensional 3D shotgun lipidomics will be conducted. Briefly, lipids will be extracted from N2-frozen biopsy sample (20mg) by a modified Bligh and Dyer method (54). Internal standards will be used to quantify individual molecular species of lipid classes (54).. Mass spectrometry-based lipidomics will be performed on a TSQ Vantage QpQ mass spectrometer equipped with an Triversa Nanomate automated nanospray device and operated with Xcalibur software (69). Identification and quantification of individual molecular species

will be performed using an automated software program. This powerful approach will enable us to analyze over 30 lipid classes and hundreds to thousands of individual lipid species collectively representing more than 95% of the total content of the lipidome in human muscle. Total content and individual species of sphingolipid will be quantified. Cardiolipin will be quantified as an index of mitochondrial content as will oxidatively modified phospholipids malondialdeyde and 4-hydroxy-2,3-trans-nonenal.

6. Immunoblot for activation of protein synthesis/degradation pathways. Key mediators of protein synthesis (AKT/P-AKT, P-mTORC2, P-4EBP, P-eIF4B P-S6K, P-S6), autophagy/proteasome mediated breakdown (P-FOXO3a, MuRF-1, Atrogin-1, LC3B1/2, Beclin-1, p62), and mitochondria content (OXPHOS) among others will be quantified by immunoblot with validated antibodies and +/- controls. This is a standard assay that is routinely performed in our group.

7. Histochemical analysis for fiber type and muscle lipid will be determined by Oil red O staining intensity and cross-sectional area will be determined in type I and type II myocytes performed on serial sections using a modified version of methods previously used in our laboratory (14, 17). Other histological staining may also be performed, including but not limited to SDH and Glycogen.

[2]**Adipose Tissue Biopsy:** This is an optional procedure that the participant will consent to if they would like to have it done. After vital signs are obtained, subjects will be placed in a supine position. The abdominal skin 6-10 cm lateral to the umbilicus will be cleansed with chlorhexidine. A sterile drape is placed, and the skin and adipose tissue will be anesthetized using Tumescent lidocaine. Depending on adipose deposits available and the clinician's judgment, either a 3-4 mm Mercedes Liposuction needle, 13g Yale needle, or a 4-6 mm Bergstrom needle will be inserted for aspiration of approximately 1000 mg 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.

[3]**Imaging Assessments** (DEXA, IMAT, ATPmax and IMCL) previously defined in PreBed Rest Phase.

[4]We will mitigate risk of VTE with daily passive exercise and through prophylactic anticoagulation with low-molecular weight heparin. Daily passive exercise will be given throughout bed rest. Low-molecular weight heparin will be given throughout bed rest, except on days when patient will undergo biopsy.

[5]Participants will be instructed to use an incentive spirometer 5-10 times per day (every 1-2 hours) during bedrest to help clear their lungs.

[6]**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 (3, 15, 27, 29). 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 U13C 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 10-30 mU/m^2/min, followed by 2 hours at 80-100 mU /m^2/min. Plasma glucose will be measured at ~5 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 U13C glucose. Rates of glucose disposal (M) and endogenous glucose production (EGP) will be calculated by non–steady-state equations based on plasma U13C 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. Bedrest ends after the completion of the hyperinsulinemic-euglycemic clamp on Day 10.

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 (62).

[7]A blood collection will be done to test the participant's hemoglobin and hematocrit levels on Day 7 and Day 10. If the hemoglobin level is < 10.0 g/dL for females or < 11.0 g/dL for males on either day, the medical provider may repeat the tests and/or withdraw the participant from the study.

[8]Administration of D_2O. 25ml of 99% D2O will be consumed 2x/day for the first week following bed rest and 25 ml once a day for the next 3 weeks, with the last dose being given the day before week 4, visit 16.This regimen will provide a stable, continuous precursor pool enrichment of body water.

[9]A number of health questionnaires will be administered following bed rest, including: Life Space, Lifestyle, Functional Status, Pittsburgh Fatigability Scale.

[10]Resting Metabolic Rate/Respiratory Quotient (RMR/RQ) will be determined that morning in the fasting state.

[11]An orthostatic hypotension assessment will be completed once participants become ambulatory. This will be completed by measuring blood pressure while in lying down, sitting, and standing positions. The assessment will be completed to ensure participant safety prior to being discharged from TRI.

Phase IV-Ambulatory/Exercise Recovery Program

Exercise Recovery. The **exercise recovery program** will be supervised by a certified exercise physiologist and will take place at the TRI exercise training facility. Exercise compliance will be monitored carefully. We are examining the muscle specific effects of the recovery exercise program on the muscle groups of interest. We will use an exercise recovery program that has demonstrated substantial increases in quadriceps muscle size and strength in elderly people (37, 40, 105). During the 4 weeks of recovery, participants will perform a combination of aerobic and resistance exercise training with a certified exercise physiologist 3 days per week at the TRI exercise training facility. Exercise compliance and adherence will be monitored carefully. Aerobic training will be 30 minutes of brisk walking, jogging, cycle ergometry and will be at 60-80% of post bed rest VO2max peak HR. Participants will wear a Heart Rate Monitor during Aerobic exercise sessions. 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. Three sets of 8–12 repetitions of each resistance exercise will be performed to target the major lower body muscle groups, including the vastus lateralis of the quadriceps.

Ambulatory Recovery. Participants in the ambulatory recovery group will not receive any exercise intervention or advice on exercise. Rather these participants will return to their regular daily routine that they engaged in prior to the bed rest intervention.

Participants in both Exercise and Ambulatory Recovery will be admitted to TRI the evening before Week 4 / Visit 16 for an overnight stay. The participants will be served dinner and type 2 diabetics will be asked to bring their own home glucometer to test glucose.

The following table summarizes the schedule of assessment during Ambulatory/Exercise Recovery Phase. Descriptions of assessments can be found above (Phase III).

Week	**Week 1**				**Week 2**				**Week 3**				**Week 4**			
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Day of Week	F	M	T	Th	F	M	T	Th	F	M	T	Th	F	M	T	F
Window Days	+1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±1	±3
Fasting Muscle Biopsy/Blood Draw[1]				**X***^				**X***^				**X***^				**X***^
Fasting Adipose Biopsy[2] **(optional)**																**X***^
24-Hour Urine																**X***^
Glucose Clamp - 4Hr[6] with RMR																**X***^
Vital Signs	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^
Medication/Supplement Intake & Changes	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^
Adverse Events	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^	**X***	**X***	**X***	**X***^
Metabolic Body Weight[16]				**X***^				**X***^				**X***^		**X***^		**X***^
BIODEX/Strength Testing												**X***^				
Handgrip Strength												**X***^				
SPPB												**X***^				
Step Test												**X***^				
Questionnaires[2]														**X***^		
DEXA				**X***^				**X***^				**X***^				**X***^
MR Assessments (ATPmax/IMCL/IMAT)				**X***^				**X***^				**X***^				**X***^

Accelerometer Handout															X*	X^
Accelerometer Collection						X*		X^								
VO_{2max} Test														X*^		
Cardio Exercise Program	X*	X*	X*		X*	X*	X*		X*	X*	X*		X*	X*	X*	
Strength Exercise Program	X*	X*	X*		X*	X*	X*		X*	X*	X*		X*	X*	X*	
Deuterated H2O adminstration[8]	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	X*^	
400m Walk Test														X*^		

* = only on this visit for exercise cohort

^ = only on this visit for ambulatory cohort

Phase V – Optional Resistance Training.

Following Phase IV of the study, to expedite complete recovery, subjects may attend three resistance exercise training sessions per week over a four-week period, for a total of 12 visits. Resistance exercise training will include weight training of all major muscle groups. Subjects will perform three sets of eight repetitions at 70-80% of their 1 RM for each exercise, which will include upper body and lower body muscle groups. During all exercise sessions, subjects must be supervised by qualified personnel with the appropriate training to identify if the subject is under any type of distress. Study assessments will also be performed during the 4-week resistance training according to the table below:

	Resistance Training (Optional)											
	Week 1			Week 2			Week 3			Week 4		
Assessment	1	2	3	4	5	6	7	8	9	10	11	12
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Medication/supplement intake and changes	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Metabolic Body Weight[16]			X			X			X			X
Resistance Training[1]	X	X	X	X	X	X	X	X	X	X	X	X
BIODEX/Strength Testing	X											X
Handgrip Strength						X						X
SPPB						X						X
Step Test						X						X
Questionnaires[2]						X						X
Accelerometer Collection		X										

Before and after any exercise activities are performed, the participant's blood glucose will be checked (type 2 diabetics only) using their home glucose monitor or glucose monitor at TRI. Blood glucose will be measured prior to and immediately following each exercise session for safety. If blood glucose is > 300mg/dl, study team will notify TRI provider immediately for review of glucose values, as well as medications. If the glucose values are consistently > 400mg/dl, participant will be instructed to follow up with their primary care physician. If blood glucose is < 100mg/dl, a snack will be given, and the participant will be allowed to exercise. Self-monitored blood glucose measurements should be checked anytime a patient has symptoms of hypoglycemia or hyperglycemia.

[1]Resistance training will include weight training of all major muscle groups 3 times per week for 4 weeks.

[2]A number of health questionnaires will be administered during the ambulatory recovery phase, including: Life Space, Lifestyle, Functional Status, Pittsburgh Fatigability Scale. CHAMPS will be administered.

Study Duration

Estimated start date for enrollment start is August 1st, 2019. Enrollment is projected to take ~3 years. Patients will be studied over a ~16-week period. The study is funded by the National Institute on Aging until 05/31/2023.

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 TRI Laboratory Room 2404. 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 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 Orlando scientists and scientists outside of AdventHealth Orlando. 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 primary outcomes, including leg lean mass, protein synthesis rate, and mitochondrial respiration, will be measured at 5 time points (immediately following bed rest, and on weeks 1, 2, 3, and 4 of Recovery Phase).

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 source documents, which is then transcribed into the 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) requiring "unblinding" to treat the participant. The "link" will be stored in the Patient Protocol Manager, where only the 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. Some records will be stored as electronic records. This data will be safeguarded so that only those on the research team have access to the clinical data. The electronic data is maintained by AdventistHealth Information Technology (AIT) security controls.

The duration of study data retention will be determined by governing FDA regulations and/or sponsor contract mandate (if applicable). TRI retention policy is maintained in the Records Management Policy. Electronic de-identified data will be kept indefinitely in our database.

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 exported into the study database.

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 TRI, the Sponsor, and its affiliates. Certain data elements will need to be shared along with the biospecimen samples (primarily satellite cell cultures). 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

Fifteen (15) participants are expected to complete the study after conservatively estimating a 25% drop-out rate. This will be sufficient to provide 80% power to detect a significant mean difference in leg lean mass between groups during recovery, based on previously published data (REF), and

from out bed rest and recovery study in older adults (recovery in leg lean mass over 4 weeks (n=20; mean ± SD) = +1.2 ± 0.5 kg). Sex will be explored as a biological variable, however, based on data from our previous bed rest and recovery study we do not expect a difference in rate of recovery between men and women (n=10 men +1.1 kg, n=10 women +1.0 kg lean mass recovery). The sample size will also provide 80% power to detect a significant mean difference of 0.38 AU in muscle mitochondrial OXPHOS protein expression between groups during recovery. The power calculations are based on two-sample t-tests for mean difference with unequal variance and a P-value of 0.05.

Statistical Analysis Plan

The ***primary*** outcomes, including leg lean mass, protein synthesis rate, and mitochondrial respiration, will be measured at 5 time points following 10 days of bed rest. 2x5 repeated-measures ANOVA will be performed on the dependent variables as a function of intervention (2 levels: EX and CON groups) and time (3 levels: 5x biopsy timepoints). We will also fit mixed models (fixed and random effects) to interrogate the longitudinal data. This will allow us to better account for the longitudinal nature of the data and to explore the influence of other potential predictors of changes in muscle mass. In particular, the relationship between change in IR, protein synthesis and mitochondrial function will be explored. Since many of these outcome variables are from biopsies, e.g., various mitochondria parameters will be correlated with one another, covariance will be explored, and we will also use the relative change measures of these as a bivariate outcome. Sex, race and ethnicity as biological variables will also be explored. Sex will be explored as a biological variable, however, based on data from our previous bed rest and recovery study we do not expect a difference in rate of recovery between men and women (n=10 men +1.1kg, n=10 women +1.0 kg leg lean mass recovery).

Potential Risks and Benefits

Potential Benefits

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

Potential Risks

Blood draws and/or IV (lab samples, e.g.). There is a risk of pain, vasovagal syncope, hematomas, and/or infection at the blood draw site or IV insertion site (low risk of serious AEs).

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 one scan is less than a chest x-ray. There are six scans in this study; the total radiation is less than 4 mrem, or less than the same average amount a person would receive over 3 days in America.

Muscle 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.

Optional 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.

Oral Glucose Tolerance Test. There is a risk of pain, hematomas, and/or infection due to venipuncture (low risk of serious AEs). Nausea from glucose ingestion may occur.

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.

Activity/Heart Rate Monitoring. There are no risks associated with the wearing of monitors, however the armband/chest band that holds the monitor in place may be irritating to the skin for some subjects. Participants with nickel allergies may have irritation at the site of the monitor.

Bed Rest Intervention. The 10-day bed rest intervention will result in a mild deconditioning that may increase fatigability and lower muscle strength (10-20% decrease) and mass (-3% approximately) and increased body fat mass of participants. Individuals with prolonged immobility are at risk for venous thromboembolism (VTE). There is also a risk of pressure ulcers – extrinsic pressure greater than capillary perfusion pressure (30mm/Hg) for a prolonged period of time results in ischemia to the affected tissue. Both hyperglycemia and hypoglycemia are risks for hospitalized patients with T2D and are associated with adverse health outcomes. However, these risks are mitigated by close clinical monitoring during the bed rest intervention (VTE prevention and glycemic control plans) and completion of the exercise recovery program. The participants will be informed that they may abort the study at any time. There are no alternative procedures/methods available with less risk or cost that provide comparable information to that obtained by the procedures described in this proposal.

Enoxaparin. There is a risk of bleeding, nausea, diarrhea, fever, swelling of the hands or feet, and injection site reactions including swelling, pain, bruising, and redness. Concomitant medications with known contraindication or interaction with low-molecular weight heparin (including anti-platelet agents, non-steroidal anti-inflammatory drugs) will be exclusionary.

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".

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.).

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

Mitigation of Risks

Blood draws and/or IV (lab samples, e.g.). All venipuncture will be conducted by qualified staff using aseptic techniques. Total blood volume throughout the entire study will be approximately 550 mls.

Muscle 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 to 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.

Optional 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 to 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.

Oral Glucose Tolerance Test. All venipuncture will be conducted by qualified staff using aseptic technique.

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 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 may be monitored during the test.

Activity/Heart Rate Monitoring. Participants with nickel allergies will not be required to wear the monitor.

Bed Rest Intervention. During bed rest, the participants will be closely monitored by CRU nursing staff and medical oversight will be provided by Dr. Richard Pratley, a physician/scientist with over 30 years of experience in conducting clinical investigations in diabetes and obesity.

Venous Thromboembolism. (VTE) risk mitigation. Individuals with prolonged immobility are at risk for VTE. We have used the American College of Chest Physicians (ACCP) Evidence Based Clinical Practice Guidelines in understanding the risk profile of enrolled participants for the development of venous thromboembolism as well as the risk of major bleeding with thromboprophylaxis.

According to the ACCP, the Padua Risk Prediction score provides the best available model for assessing risk – based on the Padua Risk Prediction model, [which assigns 3 points to reduced mobility, 1 point to Age ≥ 70, and 1 point to Obesity (BMI ≥ 30)] the majority of participants in this study will have a score ≥4, which qualifies as "high risk of VTE". We will mitigate risk of VTE in our participants by employing a comprehensive prophylactic plan that includes anticoagulation with low-molecular weight heparin and daily passive exercise throughout bed rest.

Non-treated baseline risk of bleeding was defined as 0.4% based on the control arm of trials of thromboprophylaxis in medical patients. Subsequent analysis of any anticoagulant vs. none to prevent VTE and low-molecular weight heparin (LMWH) vs. low-dose unfractionated heparin (LDUH) demonstrate a significant reduction in fatal pulmonary embolisms (PEs) and a reduction in symptomatic deep venous thrombosis (DVT). The effect of anticoagulation on major bleeding was not statistically significant (and relatively low, from baseline risk of 4 per 1,000 to 1 more per 1,000). There was no statistically significant effect of anticoagulation on all-cause mortality.

In addition to the risk of bleeding described above, heparin-based anticoagulation is associated with a rare entity called heparin induced thrombocytopenia (HIT). As per the ACCP Evidence Based Guidelines, medical patients who are only receiving LMWH will not have routine platelet count monitoring and the associated risk of HIT is <0.1%.

Mechanical methods of thromboprophylaxis were evaluated and associated with an unfavorable rate of skin issues and less risk reduction for symptomatic DVT as compared to anticoagulation in high risk patients. Intermittent pneumatic compression devices had not been studied in hospitalized medical patients; data reviewed through a meta-analysis failed to demonstrate a beneficial effect on mortality or PE but reduced the risk of DVT primarily in surgical patients. Thus, compression devices will not be utilized.

Therefore, we will mitigate risk of VTE in our high-risk participant population through prophylactic anticoagulation with low-molecular weight heparin given its safety profile and advantageous dosing frequency (qd vs. BID/TID for LDUH). Participants will receive enoxaparin 40mg subcutaneous injection, daily while immobile, with dose held only on the day of biopsies to minimize risk of bleeding or oozing intra- and post-procedurally. We will employ the daily passive exercise, throughout bed rest.

Every effort to prevent a study-related injury will be taken. If a DVT is suspected, the participant will be assessed by a provider, withdrawn from the study, and instructed to seek emergency treatment immediately. Sources: Prevention of VTE in Nonsurgical Patients - https://journal.chestnet.org/article/S0012-3692(12)60124-X/fulltext

HIT - https://journal.chestnet.org/article/S0012-3692(15)31496-3/pdf

Pneumonia Prevention
Participants will also use an incentive spirometer 5-10 times (every 1-2 hours) daily to prevent pneumonia.

Glycemic management protocol. Hyperglycemia and hypoglycemia are risks for hospitalized patients with T2D and are associated with adverse health outcomes. To mitigate this risk patients with HA1C < 8.0% will be recruited. In addition, a glycemic management protocol will be administered for type 2 diabetics by the study physician. Capillary blood glucose will be measured four times a day, before each meal and at bedtime. If two consecutive results of > 240mg/dl are recorded, the study physician will review and change the patient's current treatment regimen (DPP-4 inhibitors, sulfonylureas and/or Metformin) to prevent further hyperglycemia. If two consecutive results of >270mg/dl are recorded, the study physician may initiate insulin therapy. Insulin therapy will continue until the participant reaches a target glucose range of 140–180 mg/dL (7.8–10.0 mmol/L). The participant's treatment regimen and diet will be reviewed and changed as necessary to prevent hypoglycemia (<70 mg/dL), although this is not expected with medications like, DPP-4 inhibitors and/or Metformin.

In addition, the following procedures, evaluations and/or assessments will be obtained daily during bed rest: 1) Medication/supplementation changes, 2) Vital signs consisting of body temperature, pulse rate (or heart rate), blood pressure, and respiratory rate will be monitored routinely. 3) Subjects will be interviewed to determine if any AEs/SAEs occurred in the last 24 hours. 4) Subjects will be offered medication to help mitigate reflux problems, constipation, and other issues associated with being supine. Following 10 days of bed rest, the post bed rest recovery will consist of assisting the participant with ambulation and ensuring the participant is capable of showering, walking and dressing without assistance. The participant may stay an extra night at the study site for further recovery if it is deemed medically necessary by the study physician. A follow up phone call will be made to ensure the subject is ambulatory, remind the subject about the exercise

schedule, and to inquire about any adverse events. Participants receive supervised exercise recovery program.

Skin Care. Turning and adjustment in bed will be the primary preventative measure. Skin assessments will be performed twice per day during the bedrest phase of the study. If there is a loss of skin integrity a skin management plan will be employed as directed by the medical providers.

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

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 minutes). Immediate availability of corrective measures (IV 50% dextrose and/or glucagon).

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 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 TRI has stopped the study; or
-Administrative reasons require the participant's withdrawal.

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.

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. 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 subject, 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 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 subject will be reviewed. Other items discussed will include: progress or adverse events occurring in the following: subject confidentiality, subject 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. The annual report will be forwarded to the Advent Health IRB and the TRI.

Data and Safety Monitoring Board (DSMB) or Equivalent

A Data and Safety Monitoring Board (DSMB) will be assembled by the NIH Program and study investigators. The DSMB will meet at a regular cadence that will be decided by the members of the DSMB. The DSMB will provide oversight and monitoring to ensure the safety of participants and the validity and integrity of the data and will be empowered to provide recommendations related to all aspects of protocol integrity as well as factors affecting participant safety. We propose that the DSMB consist of members external to the institution, without conflicts of interest and with the following qualifications:

1. An expert in the science of exercise and effects of physical activity.
2. A physician who can lead the effort in monitoring the overall event rate of various adverse events (AE) and serious adverse events (SAE), as well as balance between intervention groups.
3. An external statistician who can monitor data collection, critique analysis plans, request additional data analysis, and provide interpretation of results to the DSMB members.

For the first DSMB meeting, the board will review the DSMB plan, study protocol, quality control measures, and analysis plans. Subsequent DSMB teleconferences will focus on participant recruitment, intervention session attendance rates, retention rates, adverse event (AE) and serious adverse event (SAE) summaries and classifications, data quality control, and response to previous DSMB recommendations. Additional information will be provided to the DSMB with break down by randomized group assignment and these will be reviewed in the closed session with the study statisticians. Separate systems will be put in place to handle the report of AEs and SAEs to the Chair of the DSMB and the AdventHealth IRB at the study site in a timely fashion after adjudication.

Data and Safety Monitoring Plan

As this project has relatively few subjects, the study progress and safety will be reviewed weekly by the PI, one of the study physicians, and/or research coordinator including progress or adverse events occurring in the following: subject confidentiality, subject recruitment, and consent process. All will monitor response to tolerance of and

effectiveness of the intervention program. Overall, data integrity and participant safety will be monitored and maximized by the study investigator, clinician, and research coordinator involved who are experienced at conducting bed rest and exercise trials and specifically in the methods of collection of biospecimens to study the molecular changes in response to an inactivity and exercise challenge.

Monitoring Plan

The DSMB and Study Investigators will be responsible for the following:

1. Review the research protocol and plans for data and safety monitoring, including all proposed revisions.
2. Review methodology used to help maintain the confidentiality of the study data and the results of monitoring by reviewing procedures put in place by investigators to ensure confidentiality.
3. Monitor study design, procedures and events that will maximize the safety of the study participants and minimize the risks.
4. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the study site(s), and other factors that may affect study outcome.
5. Consider factors external to the study when relevant information becomes available, such as scientific developments that may have an impact on the safety of the participants or the ethics of the study.
6. Review serious adverse event documentation and safety reports and make recommendations regarding protection of the safety of the study participants.
7. Evaluate and report on perceived problems with study conduct, enrollment, and/or data collection, including:
8. Progress reports, including patient recruitment, retention/attrition, and AEs from the weekly reviews.

In addition, the DSMB reports 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.

In addition to the above, among the most comprehensive and effective method of monitoring risks in studies with relatively few subjects is a weekly and biweekly individual case that will be reviewed by the PI, one of the study physicians, and/or research coordinator including progress or adverse events occurring in the following: subject confidentiality, subject recruitment, and consent process. All will monitor response to tolerance of and effectiveness of the exercise program. Files will be kept in a locked file cabinet in the laboratory of the PI to assure individual privacy and confidentiality of subject records. The research coordinators will monitor the following items: the timeframe of recruiting subjects, quality of data being entered, any external factors relevant for the safety

of participants throughout the entire study and is also instructed to make the PI aware of all events, expected or unexpected.

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

Copies of the participant's testing results may be made available to the participants. In addition, the Principal Investigator will provide an overview of the study's outcome to the participant if he or she requests the information.

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Subject Stipends or Payments

We have budgeted an honorarium of $3,550 for participating. In the event the participant does not complete all study visits or procedures, payments will be prorated.

Participants will be given a Mastercard® as a means to receive payments for this study. The terms and conditions for this card will be provided for the participant for review. Payment will be requested within 3 business days from the completion of the study visits.

Payments will be requested after completion of Bed Rest and Resistance Week 4 for a total of 2 study payments. If the participant does not consent to Resistance Exercise, the second payment will be requested after completion of Recovery Week 4. If participant is unable to complete all study visits, the stipends will be prorated to the following:

Visit	Prorated Amount	
SV2	$160.00	
PBR Day 1-5 ($50/day)	$250.00	
BR Day 1-9 ($110/day)	$1100.00	Payment 1
BR Day 10	$160.00	$1770.00
Post Bed Rest Day 11	$100.00	
Recovery Week 1	$250.00	
Recovery Week 2	$250.00	Payment #2
Recovery Week 3	$250.00	Up to
Recovery Week 4	$250.00	$1780.00

Optional Resistance Week 1 ($65/session)	$195.00	
Optional Resistance Week 2 ($65/session)	$195.00	
Optional Resistance Week 3 ($65/session)	$195.00	
Optional Resistance Week 4 ($65/session)	$195.00	
Total for all completed visits	**Up to $3,550.00**	**Up to $3,550.00**

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.

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