Protocol Title: Resistance Exercise and Low-Intensity Physical Activity Breaks in Sedentary Time to Improve Skeletal Muscle and Cardiometabolic Health in Older Adults–REALPA Breaks in Sedentary Time Pilot Study

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PROJECT SUMMARY / ABSTRACT

Although awareness of the detrimental impact that sedentary behavior has on skeletal muscle and cardiometabolic health has increased over the last 20 years, more than 60% of older adults remain sedentary for greater than 8 hours per day. Moreover, 80% to 90% of adults 60 years of age or older do not meet the current public health guidelines for aerobic exercise (AE) or resistance exercise (RE) based physical activity (PA). Collectively, these adverse health behaviors contribute to the development of multiple chronic medical conditions commonly afflicting older adults, including type 2 diabetes, cardiovascular disease, sarco/dynapenia, frailty, and premature mortality. Emerging evidence suggests that breaking up sedentary time with light intensity PA (LPA) improves muscle and cardiometabolic health. Recent data also suggest that RE combined with moderate intensity AE effectively improves muscle and cardiometabolic health in older adults. However, the impact that RE combined with LPA breaks in sedentary time has on muscle and cardiometabolic health in older adults remains unknown. The *overall objective* of this pilot study is to determine the effect of 16 weeks of RE alone or RE combined with LPA breaks in sedentary time on muscle and cardiometabolic health. The *central hypothesis* is that the addition of LPA breaks in sedentary time will enhance RE-induced improvements in muscle and cardiometabolic health in sedentary older adults. Our overall *approach* to test our central hypothesis is to recruit and study 36 physically inactive community dwelling older adults (65-80 years) who are randomized to 16 weeks of either (i) RE (2 x/wk), (ii) RE (2 x/wk) and LPA breaks in sedentary time (5 d/wk, 6x10 min breaks per day at 2 METS, ~500 kcal/wk above resting metabolism), or (iii) RE (2 x/wk) and moderate intensity AE (3 d/wk, 50 min/session at 4 METS, ~500 kcal/wk above resting metabolism). The effect that these PA interventions have on muscle health will be measured by changes in muscle strength, mass, and quality (strength/mass) using isokinetic dynamometry and dual-x-ray absorptiometry (DXA) (primary outcomes). In addition, improvements in muscle oxidative capacity will be measured using high-resolution respirometry and oxidative damage to muscle proteins by immunoblotting (secondary outcomes). The effect

that these PA interventions have on cardiometabolic health will be measured by changes in body composition by DXA, fasting blood glucose and lipids by a clinical chemistry panel and glucose tolerance by a mixed meal tolerance test (primary outcomes). Additionally, improvements in immunometabolic health will be measured by changes in low-grade systemic inflammation (e.g., TNFα) using immunoassays and immune cell oxidative capacity by high-resolution respirometry (secondary outcomes). The proposed studies will provide preliminary evidence that LPA breaks in sedentary time enhance RE- induced improvements in muscle and cardiometabolic health in older adults, will further evaluate efficacy in a follow-up R01 randomized clinical trial.

OBJECTIVES

The *overall objective* of this pilot study is to determine the effect of 16 weeks of resistance exercise (RE) alone or RE combined with low intensity physical activity (LPA) breaks in sedentary time (ST) on muscle and cardiometabolic health in older adults.

HYPOTHESIS AND SPECIFIC AIMS

We *hypothesize* that the addition of LPA breaks in ST will enhance RE-induced improvements in muscle and cardiometabolic health in sedentary older adults.

Specific Aim 1. To evaluate the effect of resistance exercise (RE) alone or with regular low intensity physical activity (LPA) breaks in sedentary time (ST) on skeletal muscle health in older adults. Our *working hypothesis* is that regular LPA breaks in ST will enhance RE-induced improvements in muscle health in older adults. *Outcomes*: We will measure muscle strength, mass, and quality (strength/mass) using isokinetic dynamometry and dual-x-ray absorptiometry (primary outcomes). We will measure muscle OXPHOS using high-resolution respirometry and oxidative damage to muscle proteins by immunoblotting (secondary outcomes).

Specific Aim 2. To evaluate the effect of resistance exercise (RE) alone or with regular low intensity physical activity (LPA) breaks in sedentary time (ST) on cardiometabolic health in older adults. Our *working hypothesis* is that regular LPA breaks in ST will enhance RE-induced improvements in cardiometabolic health in older adults. *Outcomes*: We will measure body composition by dual energy x-ray absorptiometry, fasting blood glucose and lipids by a clinical chemistry panel and glucose tolerance by a mixed meal tolerance test (primary outcomes). Additionally, we will assess immunometabolic health by measuring low-grade systemic inflammation (e.g., TNFα) using enzyme-linked immunosorbent assays and isolated immune cell OXPHOS by high-resolution respirometry (secondary outcomes).

BACKGROUND AND SIGNIFICANCE

Impact of Physical Inactivity and Excessive Sedentary Time (ST) on the US's and Louisiana's Public Health. Physical inactivity (i.e., <150 min/week of moderate or <75 min/week of vigorous intensity physical activity (PA)) and excessive ST (i.e., sitting/lying ≥ 10 h/d) are major public health concerns affecting approximately 20% of US adults.[1] In 2015, Louisiana had ~1 million physically inactive adults, of which greater than 200,000 were 65 years of age or older.[1] We estimate that the excess health care expenditures attributed to physical inactivity in older Louisianans alone approaches $305 million per year.[2] Physical inactivity and excessive ST contribute to several of the chronic conditions commonly afflicting older adults, including insulin resistance, type 2 diabetes, cardiovascular disease, sarcopenia/dynapenia, mobility disability, frailty, and premature mortality.[3-9] These factors likely contributed to Louisiana ranking last in overall senior health in 2015.[1] Physical inactivity and excessive ST continues to rise among those 65 years age or older creating an important health care disparity that urgently needs to be addressed.[1]

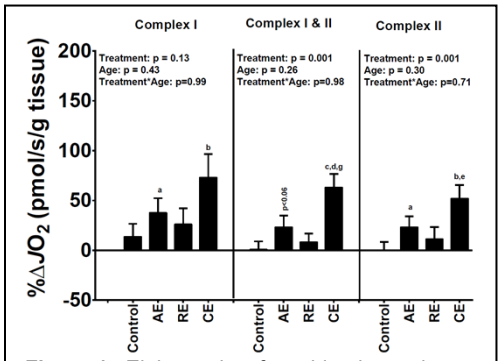

Figure 1. Eight weeks of combined exercise (CE) consisting of whole-body resistance (RE) plus moderate-intensity aerobic exercise (AE) increased muscle oxidative capacity (state-3 respiration, JO_2) as or more effectively than either treatment alone in young (18-30 y) and older (65+ y) adults, independent of age.

Gap in Knowledge Addressed by Specific Aims. Although awareness of the detrimental impact that sedentary behavior has on skeletal muscle and cardiometabolic health has increased over the last 20 years, greater than 60% of adults 60 years of age or older remain

sedentary for greater than 8 h/d.[10] Moreover, 80% to 90% of older adults do not meet the current public health guidelines for aerobic exercise (AE) or resistance exercise (RE) based PA.[11] Collectively, these adverse health behaviors contribute to the development of poor muscle and cardiometabolic health and contribute to the development of type 2 diabetes, cardiovascular disease, sarco/dynapenia, mobility disability, and frailty in older adults.[3-8] Prior reports suggest that achieving the current public health guidelines for AE based PA may not fully protect against the increased risk of developing these chronic conditions associated with excessive ST.[12, 13] The current public health guidelines also do not adequately address the potential health benefits of breaking up ST with light intensity PA (LPA), especially when combined with RE. Recent data suggest that LPA breaks in ST improves muscle and cardiometabolic health.[14] We recently demonstrated that combined exercise (CE) consisting of RE plus moderate intensity AE is as or more effective at improving muscle strength, mass, quality, and OXPHOS capacity than either treatment alone in young and older adults despite lower training volumes **(Figure 1)**.[15] ***However, the impact that RE combined with LPA breaks in ST has on muscle and cardiometabolic health in older adults remains unknown.*** Thus, there is a *critical, unmet need,* to address this gap in knowledge, which may facilitate the identification of novel strategies to prevent, delay, or reverse declines in muscle and cardiometabolic health among older adults. To address this critical, unmet need, we propose a ***pilot study*** of our novel exercise intervention that combines RE and LPA (REALPA) breaks in ST to improve muscle and cardiometabolic health in older adults.

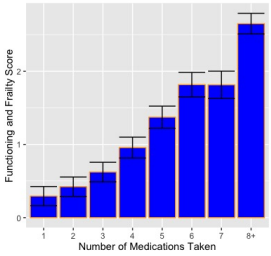

Figure 2. Impact of polypharmacy on physical functioning in NHANES 1999-2016. Higher score represents lower physical function and higher frailty.

Critical Barriers Addressed by Specific Aims. Successful implementation of an exercise regimen in older adults requires careful consideration of barriers to initiating and maintaining an exercise regimen. In addition to the common barriers for initiating and maintaining an exercise regimen (e.g., time, social support, etc.), older adults face additional barriers including low peak VO_2, reduced physical function, impaired mobility, exertional dyspnea, and fear of adverse events. The proposed Specific Aims directly examine a patient-centered approach designed to reduce the impact of these barriers. Finally, REALPA breaks in ST may be a more practical long-term intervention to improve muscle and cardiometabolic health in older adults, especially among those with additional barriers associated with chronic medical conditions.

NIA Priority Addressed by Specific Aims. The NIA is currently interested in identifying non-pharmacological approaches to improve muscle and cardiometabolic health in older adults, because polypharmacy (i.e., ≥ 5 medications) associated with treating age-related medical conditions is significant health concern. Our preliminary data suggest that polypharmacy is associated with impaired physical function and frailty in older adults **(Figure 2)**. We propose to examine a non-pharmacological approach to improve muscle and cardiometabolic health in older adults.

RESEARCH DESIGN (APPROACH)

We will use state-of-the-art methodologies to test our central hypothesis that the addition of regular LPA breaks in ST will enhance RE-induced improvements in skeletal muscle and cardiometabolic health in sedentary older adults. To test our central hypothesis, we will conduct a ***pilot study*** that addresses the following parallel Specific Aims:

Specific Aim 1. To evaluate the effect of resistance exercise (RE) alone or with regular low intensity physical activity (LPA) breaks in sedentary time (ST) on skeletal muscle health in older adults.

Specific Aim 2. To evaluate the effect of resistance exercise (RE) alone or with regular low intensity physical activity (LPA) breaks in sedentary time (ST) on cardiometabolic health in older adults.

Overall Approach to Address Specific Aims 1 and 2. We will capitalize on Louisiana State University's (LSU) and Pennington Biomedical Research Center's (PBRC) robust infrastructure for recruiting and retaining volunteers into exercise- and nutrition-related studies. Up to 45 physically inactive community dwelling older adults (65-80 years) will complete a screening visit, 2 pre-training visits, and 3 post-training visits as depicted in **Figure 3 and Table 1.** Here we will briefly outline the study population, recruitment, screening and study

visits, while providing a more detailed description of pertinent methodologies in the Detailed Methods and Analysis Section.

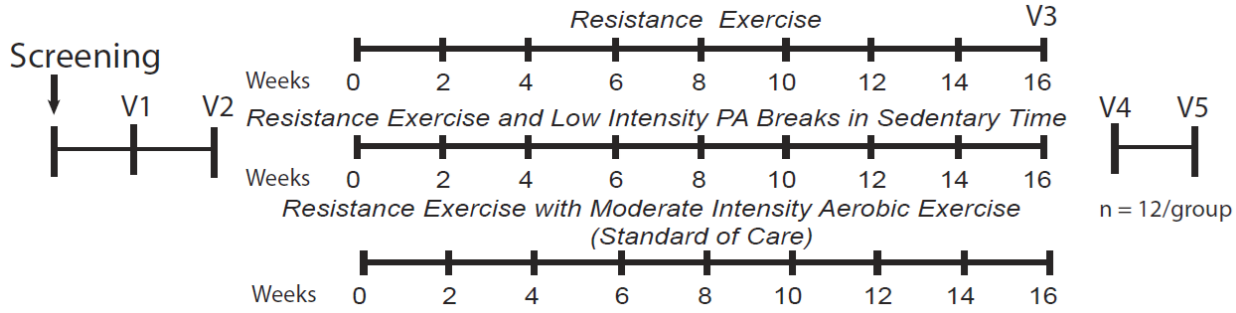

Figure 3. Overall subject flow.

	Screening		Pre-Training		Exercise	V3		Post-Training	
	Part I	Part II	V1	V2	16 wks	Part I	Part II	V4	V5
Informed Consent	P								
Inclusion/Exclusion Criteria	P								
Height and Weight	P	L	L	P	L	P	L	L	P
Vital Signs (blood pressure and heart rate)	P		L	P	L	P		L	P
Fasting Blood Draw	P		L					L	P
- Complete Blood Count, Blood Chemistry and Lipids	P								P
- Glucose, Hormones, & Pro-Inflammatory Cytokines			L					L	
- High Resolution Respirometry – T-Cells			L					L	
Screening Questionnaires	P								
- PARQ+	P								
- Saint Louis University Mental State (SLUMS) Exam	P								
Resting Electrocardiogram	P								
Medical History and Physical Exam	P								
Short Physical Performance Battery (SPPB)	P					P			
Exercise Test – VO_2 Peak	P					P			
Dual X-Ray Absorptiometry (DXA)			L					L	
Physical Activity and Function Questionnaires		L						L	
- International Physical Activity Questionnaire-Elderly		L						L	
- Sedentary Behavior Questionnaire		L						L	
- PROMIS Physical Function Questionnaire		L						L	
- Pittsburgh Fatigability Questionnaire		L						L	
Body Composition Assessments			L	P**				L	P**
- Circumferences				P**					P**
- Optical Imaging				P**					P**
- Bioelectrical Impedance			L	P**				L	P**
Strength and Endurance Testing		L					L		
- Isokinetic		L					L		
- 1-Repitition Maximum		L					L		
- Grip Strength		L					L		
Mixed Meal Tolerance Test and Post Meal Blood Draws			L					L	
- Glucose, Hormones, & Pro-Inflammatory Cytokines			L					L	
Magnetic Resonance Imaging (MRI) – Leg Muscle Mass				P					P
Phosphorous Magnetic Resonance Spectroscopy – ^{31}P-MRS				P					P
Muscle Biopsy (*Vastus Lateralis*)				P					P
Physical Activity Monitoring			X		X				
Baseline Dietary Assessment			X						
Weekly Exercise Sessions (According to Assignment)					L				
OPTIONAL: Carbon-13 Magnetic Resonance Spectroscopy—^{13}C-MRS				P					P

Table 1. Schedule of Events. P = performed at PBRC, L = performed at LSU and X = performed on subjects own. *Visit 3, Part II can be done prior to a regularly scheduled exercise session. ** These measurements will be performed in Dr. Heymsfield Laboratory.

STUDY POPULATION

Study Population. We will recruit, randomize, and study up to 45 physically inactive, community-dwelling, older adults (65-80 years) with a BMI between 18.5-34.9 kg/m^2 from the greater Baton Rouge community to complete 36 subjects (12/group) accounting for a 20% attrition rate. The Baton Rouge community has between 30-40,000 adults 65 years of age or older. (See Provisions to Monitor the Data to Ensure the Safety of Subjects)

Inclusion and Exclusion Criteria

Enrolled subjects will meet the following inclusion and exclusion criteria:

Inclusion Criteria.

1. Capable and willing to give written informed consent, and understand inclusion and exclusion criteria
2. 65-80 years of age inclusive
3. Body Mass Index (BMI) between 18.5-34.9 kg/m^2, inclusive
4. Physically inactive as determined by self-report
5. Stable medical therapy for allowable medications for 30 days defined as:
    a. No addition or removal of a medication
    b. No change in dosage of a medication
6. Have no life-threatening conditions or diseases
7. Willing to allow researchers to use data, biospecimens (blood and muscle tissue), and images (e.g., magnetic resonance imaging) for research purposes after study participation is completed
8. At least 2 weeks post-completion of the COVID19 vaccine regimen.
    a. Acceptable proof of vaccine includes a completed vaccine card and/or letter from a healthcare provider indicating the date that the COVID19 vaccine was completed.

Exclusion Criteria.

1. Nursing home resident
2. Physically Active:
    a. > 100 min/wk of moderate OR > 50 min/wk vigorous intensity PA
3. Saint Louis University Mental State (SLUMS) score < 21
4. Evidence or self-report history of deep vein thrombosis, pulmonary embolism, cardiovascular, peripheral vascular, cerebral vascular, pulmonary, or renal disease
5. Evidence or self-report history of type 1 or 2 diabetes mellitus
6. Evidence or self-report history of a bleeding disorder
7. Evidence or self-report history of recurrent vasovagal episodes
8. Evidence or self-report history of Schizophrenia or bipolar disease
9. Evidence or self-report history of mobility disability requiring a walker, wheel chair, or inability to walk across a small room.
10. Evidence or self-report history of orthopedic limitations that would preclude them from participation in a dynamic exercise program
11. Evidence or self-report history of severe arthritis (either osteoarthritis or rheumatoid arthritis) that would preclude them from participation in a dynamic exercise program
12. Evidence or self-report history of untreated thyroid dysfunction.
13. Weight loss of > 10% in the last 3 months prior to screening
14. History of weight loss surgery.
15. Use of medications known to influence study outcomes, such as:
    a. Insulin
    b. Oral antidiabetic medications (e.g., metformin)
    c. Corticosteroids within the past 14 days (topical or inhaled are allowed)
    d. Beta-blockers
    c. Anticoagulants
16. Allergy to lidocaine
17. Active smoking

18. Current consumption of > 14 alcoholic drinks per week based on self-report
19. Regular participation in resistance or aerobic exercise training within 3 months of initial screening
20. Absolute Contraindication to Exercise as Defined by the American College of Sports Medicine,[16] including:
    a. Resting diastolic blood pressure > 100 mm Hg
    b. Resting systolic blood pressure > 180 mm Hg
    c. Resting heart rate > 100 beats per min
21. Having a body weight greater than 440 pounds
22. Having medical implants such as a pacemaker or metal joint replacements
23. Having tattoos or permanent makeup completed <30 days prior to the visit
24. Recent (past 3 months) cancer diagnosis, undergoing immunotherapy, taking immune suppressants
25. Presence of allergies or infections requiring antibiotics within the past 14 days
26. Recent (past 3 months) major surgery on the abdomen, pelvis, or lower extremities
27. Any other condition that in the judgement of the Principal Investigator and/or the Medical Director of this protocol may interfere with study participation and adherence to the protocol.
28. Evidence or self-report history of severe depression in the last 5 years.

RECRUITMENT METHODS

Number of Subjects. We expect to screen about 150 potential volunteers, to recruit and study up to 45 subjects to complete at least 36 subjects (12/group) accounting for a 20% attrition rate (See Sample Size, Power Analysis and Randomization section for further details on sample size).

Source of Subjects. The greater Baton Rouge community has between 30-40,000 adults 65 years of age or older. We will identify potentially eligible study subjects via advertising. Specifically, with the assistance of the PBRC Recruiting Core, we will recruit potential subjects from advertisements placed on the bulletin boards, electronic bulletin boards, social media, and in local and regional newspapers (e.g., the Advocate, the LSU Reveille,) and magazines. We will advertise on regional radio stations as needed. We will post flyers in senior housing establishments as well as attend senior expos. All recruitment materials will be approved by the IRB prior to their use.

SCREENING AND STUDY VISITS

Screening Visits.

Potentially eligible subjects will complete a screening visit, which we will split into two parts. We will try and schedule Part I and II on the same day, but these visits may be conducted on separate days.

Screening Visit, Part I (about 3 hours) will be conducted at PBRC and will include IRB approved written informed consent, review of inclusion/exclusion criteria, medical history, physical exam, vital signs, resting electrocardiogram, height, weight, fasting blood draw (~ 10 ml, 2 teaspoons), and Physical Activity Readiness Questionnaire for Everyone (PARQ+), the Short-Physical Performance Battery (SPPB),[17] and Saint Louis University Mental Examination.[18] We will peak oxygen consumption (VO_2 peak) using a cardiopulmonary exercise test[16] in the Exercise Testing Core. *Prior to the completion of Part I of the screening visit, eligible subjects will be provided a map and directions on how to get to the LSU Exercise Physiology Laboratory and the School of Kinesiology Exercise Training Facility. There are reserved parking spots available through the LSU School of Kinesiology that will be reserved for Part II of the screening visit as well as study visits conducted in the LSU Exercise Physiology Laboratory or the School of Kinesiology Exercise Training Facility. We will also provide the subjects the contact information (office and cell phone number) of the study personnel assigned to meet them at each study visit and training session.*

Screening Visit, Part II (about 2 hours) will be conducted in LSU Exercise Physiology Laboratory and will include height, weight, the completion of the International Physical Activity Questionnaire-Elderly,[19] Sedentary Behavior Questionnaire,[20] and PROMIS Physical Function Questionnaire[21, 22], and the Pittsburgh Fatigability Questionnaire. We will also measure height and weight. We will measure isokinetic strength and endurance of the knee extensors (Biodex System 3) and the 1-repetition maximums (1-RMs)

for the knee extension, leg press, and chest press. Note: the 1-RM measurements may be performed prior to your regularly scheduled exercise session during the first and last week of the intervention. We will also measure grip strength using a hand dynamometer.

Baseline Physical Activity Monitoring. At the end of part II of the screening visit, all subjects who are deemed eligible to continue onto the pre-training visits described below will be provided two devices to measure their physical activity leading up the their pre-intervention testing. A wrist-worn physical activity monitor (Actigraph, GT9X) will be issued to the subjects to wear 24-h/d for 7-days (±3 days) prior to their pre-training visits to quantify objectively their PA and ST. Subjects will also be asked to wear a second physical activity monitor (Actigraph, GT9X) on their thigh 24-h/d for 7-days (±3 days). Subjects will be asked to return their physical activity monitors during the Pre-training Visit 1 (V1). In the event that a subject is deemed ineligible after part II of the screening visit, we would request that the subject return his/her physical activity monitors at their earliest convenience. We will screen raw data from these monitors for periods of valid wear-time with a required minimum wear-time of 10 h per valid wear-day and 4 valid wear-days per week.[23, 24] We will use standard algorithms to characterize the patterns of PA and ST.[25]

Baseline Dietary Record. Subjects will be asked to record their dietary intake for the 24 hrs prior to their pretraining visit 1. They also will be asked to replicate their dietary intake before posttraining visit 4. Moreover, they will be asked to refrain from consuming caffeine or participating in vigorous exercise 48 hours prior to these visits.

Pre-training Visits 1 (V1). (About 5 hours). V1 will be scheduled at least 7 days after screening visit Part II.

After an overnight fast (> 10 hours), subjects will report to the LSU's Exercise Physiology Laboratory to complete V1. During V1, we will measure height, weight and vital signs. We will measure body composition using Dual Energy X-Ray Absorptiometry (DXA) (Hologic, Horizon A)[15] and bioelectrical impedance analysis (BIA). During V1, we will acquire a fasting blood sample (~50 ml or 10 teaspoons) for the measurement of glucose, insulin, lipids, pro-inflammatory cytokines, and isolation of T-cells. We will then perform a standardized Mixed Meal Tolerance Test (MMTT) with serial blood draws (~100 ml total or 6.8 tablespoons) to measure glucose and insulin area under the curves (AUCs), insulin sensitivity (SI), and beta cell responsivity (Φ).[26] We will draw about 150 ml or 10-12 tablespoons of blood for this this test in total. We will use a portion of the fasted blood samples collected to characterize each subject's immune compartment using four-color flow cytometry,[27-29] while also assessing negatively-sorted T-cell OXPHOS using HRR.

Pre-training Visits 2 (V2). (About 3.5-4 hours). V2 will be scheduled at least 24 hours after V1, but within 2 weeks of V1.

After an overnight fast (> 10 hours), study subjects will report to PBRC to complete V2. During V1, we will measure height, weight and vital signs. We will measure body composition using standard circumferences, optical imaging, and BIA, which will be conducted in Dr. Steven Heymsfield's Laboratory. During V2, subjects will undergo a magnetic resonance imaging (MRI) study to measure leg muscle mass and phosphorus magnetic resonance spectroscopy (^{31}P-MRS), to measure *in vivo* mitochondrial oxidative capacity.[30] During V2, subjects who agree to the optional Carbon-13 magnetic resonance spectroscopy procedure will do that procedure. During V2, a percutaneous muscle biopsy from the *vastus lateralis* (~500 mg) will be acquired under local anesthesia to assess muscle OXPHOS using HRR,[15, 31] mitochondrial enzyme activities, and oxidative damage to muscle proteins by immunoblotting. Note: the muscle biopsy will be acquired from the opposite leg, as the MRS study was performed.

Exercise Interventions. After completing V2, we will randomize the subjects to one of three 16-week interventions: (i) RE, (ii) REALPA breaks in ST, or (iii) RE+AE **(Figure 3)**. The RE+AE group serves as the standard of care. The RE group will complete supervised RE (2 x/wk). The REALPA breaks in ST group will complete a supervised RE (2 x/wk) and regular unsupervised LPA breaks in ST (5 d/wk, 6x10 min breaks/d at 2 METS (~30-40% VO_2 peak), ~500 kcal/wk above resting metabolism). The RE+AE group will complete supervised RE (2 x/wk) and calorically matched moderate intensity AE (3 d/wk, 50 min/session at 4 METS (~60-75% VO_2 peak), ~500 kcal/week above resting metabolism). The RE component for all 3 groups will consist of 3 sets of 10-12 repetitions to failure for a total of 8 exercises targeting the large muscle groups **(Table 2)**. The AE component for the RE+AE group will consist of treadmill walking at ~4 METs for 50 min

(Table 2). The RE and AE sessions will take place in LSU School of Kinesiology's Exercise Training Facility under direct supervision (Drs. Irving, Johannsen, and Spielmann with the assistance of trained graduate students). All supervised exercise sessions will include a 5 minute warm-up and cool-down on either a bike or treadmill. We will provide subjects flexibility on the days per week that they can complete their exercise sessions. Note: The start of the exercise intervention will be at least 5 days post V2 to allow for adequate recovery from the muscle biopsy, but no more than 6 weeks after V2. Up to 2 weeks may be added to a subjects exercise intervention, to allow for potential make-up of missed exercise sessions and to provide flexibility for scheduling study visits.

The LPA breaks in ST will consist of walking at ~2 METS for 6x10 min bouts, which will be completed throughout the day, with ~1 bout per hour. Alternative 2 MET mobility-based activities may also be provided using the Compendium of Physical Activities to promote adherence. We may use text/email messaging to send reminders, the "Stand Up! The Work Break Timer" (Apple/Android App) to promote adherence to the LPA breaks in ST protocol. We may also use the CentrePoint Cloud-Based Data Capture and Management Platform to send reminders to study participants. We will initially send these reminders on the days in which the subjects do not report for their RE sessions, the frequency of these reminders may be increased or decreased based on adherence. To reduce the risk of injury and muscle soreness, we will progressively increase the intensity and volume of the exercise prescriptions to the targeted training intensities over the first month of the intervention. Prior to each training session, we will assess resting blood pressures and heart rates. We will not allow subjects to exercise if they have a resting diastolic blood pressure > 100 mm Hg, resting systolic blood pressure > 180 mm Hg, or resting heart rate > 100 beats per min. We will also assess heart rates using heart rate monitors (e.g., Polar or Zephyr Bioharness) and ratings of perceived exertion (RPE, Borg 6-20) throughout the supervised exercise sessions. We will also measure the subjects' weight once per week during one of their scheduled exercise visits.

	Mon	Tue	Wed	Thu	Fri
A. Resistance Exercise (RE)		• Leg Press • Leg Extension • Leg Curls • Chest Press • Lat Pulldowns • Shoulder Press • Triceps Extension • Biceps Curls 3 Sets 10-12 Reps to Failure		• Leg Press • Leg Extension • Leg Curls • Chest Press • Lat Pulldowns • Shoulder Press • Triceps Extension • Biceps Curls 3 Sets 10-12 Reps to Failure	
B. LPA Breaks in Sedentary Time*	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS	6x10 min bouts at 2 METS
C. Aerobic Exercise	1 x50 min bouts at 4 METS		1 x50 min bouts at 4 METS		1 x50 min bouts at 4 METS

Table 2. The RE group will complete supervised RE (2x/wk) (A). The REALPA breaks in ST group will complete supervised RE (2x/wk) and regular unsupervised LPA breaks in ST (5x/wk) (A+B). The RE+AE group will complete supervised RE (2x/wk) and calorically matched moderate intensity AE (3x/wk) (A+C).

Physical Activity Monitoring During Intervention. To quantify total physical activity throughout the intervention, we will ask that subjects wear a physical activity monitor (Actigraph, GT9X) on their wrist 24 h/d for the duration of the study and a second physical activity monitor (Actigraph, GT9X) on their thigh during weeks 1, 4, 8, 12, and 16 for further quantification. We will screen raw data from these monitors for periods of valid wear-time with a required minimum wear-time of 10 h per valid wear-day and 4 valid wear-days per week.[23, 24] We will use standard algorithms to characterize the patterns of PA and ST.[25]

Adherence Monitoring During Intervention. We will monitor each subject's attendance to the prescribed exercise training sessions and exercise training logs to assess adherence to the exercise intervention. We will also use the physical activity monitoring data to help assess adherence to the exercise intervention. De-identified physical activity monitoring data will be uploaded to the CentrePoint Cloud-Based Data Capture and Management Platform to facilitate data collection and monitoring.

Visit 3 (V3) (End of Exercise Intervention). During last week of the intervention (i.e., week 16), subjects will be asked to complete V3, with two parts. Part I will occur PBRC and Part II at the LSU Exercise Physiology Laboratory on the main campus of LSU.

V3, Part I (about 2 hours).

After an overnight fast (> 10 hours), subjects will report to PBRC to complete V3, Part I. During this visit, we will measure height, weight and vital signs. We will then perform the SPPB and Exercise Test (VO_2 Peak).

V3, Part II (about 1 hour).

Subjects will report to LSU Exercise Physiology Laboratory to complete V3, Part II (non-fasted). The visit procedures for this visit can be done at a regularly scheduled exercise session and would be completed prior to the start of that day's exercise. During this visit, we will conduct the Biodex, 1-RM, and Handgrip Strength and Endurance Testing.

Posttraining Visit 4 (V4) (About 5 hours).

After an overnight fast (> 10 hours), study subjects will report to LSU Exercise Physiology Laboratory to complete V4. V4 will be scheduled 48 hours after the subject's last training session. During this visit, we will measure height, weight and vital signs. During this visit, we will ask subjects to complete the International Physical Activity Questionnaire-Elderly,[19] Sedentary Behavior Questionnaire,[20] and PROMIS Physical Function Questionnaire[21, 22], and the Pittsburgh Fatigability Questionnaire. We will also measure the body composition by DXA and BIA. We will also perform the MMTT as described for V1. We will also the draw fasting and MMTT associated bloods as described for V2 (total blood volume of about 150 ml or 10-12 tablespoons total). The analyses performed on these blood samples collected in V4 will be as described for V2.

Posttraining V5 (About 3.5-4 hours)

After an overnight fast (> 10 hours), study subjects will report to PBRC to complete V5. V5 will be scheduled about 72 hours after the subject's last training session. During this visit, we will measure height, weight and vital signs. We will measure body composition using standard circumferences, optical imaging, and BIA, which will be conducted in Dr. Steven Heymsfield's Laboratory. During this visit, we will collect a fasting blood sample (~ 10 ml). We will also perform the MRI for leg muscle mass, Phosphorous Magnetic Resonance Spectroscopy – ^{31}P-MRS, and Muscle Biopsy as described for V3. During V5, subjects who agree to the optional Carbon-13 magnetic resonance spectroscopy procedure will do that procedure.

STUDY ENDPOINTS

Primary Endpoints.

1. Comparison of within and between group changes in muscle strength, mass, and quality (strength/mass) using isokinetic dynamometry and DXA (primary outcomes Aim 1) between treatment groups.
2. Comparison of within and between group changes in body composition by DXA, fasting blood glucose and lipids by a clinical chemistry panel and glucose tolerance by a mixed meal tolerance test (primary outcomes Aim 2)

Safety Endpoints.

1. Safety will be monitored by adverse event reporting (See Data Safety Monitoring Plan).

DETAILED METHODS AND ANALYSIS

Exercise Test – VO_2 Peak (about 1 hour). Prior to the first exercise test, subjects will complete a physical exam, medical history, resting EKG which will be reviewed by Dr. Greenway (Medical Investigator) and/or other qualified practitioner. Exercise test will only proceed if subject qualifies. We will use an incremental treadmill protocol with indirect calorimetry (Parvo-Medics TrueOne) to measure VO_2 peak at PBRC in the Exercise Testing Core.[16] Subjects will be asked to initially walk at 1.5 miles per hour at 0% grade for 5 minutes. Following this initial warm-up, subjects will begin walking at self-selected brisk walking speed at 0% grade. Thereafter the speed will remain constant and the grade will increase by 2% every 2 minutes until

volitional exhaustion. Once this is reached, a 5 minute cooldown starting at 1.5 mph and 0% grade will be initiated. During the cool-down, the speed will be gradually lowered by 0.5 mph each minute until 0 mph is reached. We will assess heart rate continuously using a 12 lead EKG, while blood pressure and ratings of perceived exertion (RPE, Borg 6-20) will be assessed every 2 minutes. According to PBRC SOP 1303 for exercise testing, a physician will be available in the building and may be present at the time of testing should any complications arise. During the test, subjects will be told that they can lightly grip the hand rails at any time if it makes them feel more secure while walking. Stopping rules for this test are consistent with other exercise testing procedures performed in the Exercise Testing Core as described in SOP 1303. The exercise EKG will be read by the study physician for any signs of abnormalities and will be documented and reported to the PI and the study medical investigator. The medical investigator will contact the subject to discuss any abnormal findings, and with the subjects permission may contact the subject's physician to facilitate appropriate follow-up.

Dual Energy X-Ray Absorptiometry (DXA) (about 10 minutes). This scan measures the amount of bone, muscle, and fat in the body. The scan will be performed using a whole-body scanner (Hologic, Horizon A) in the LSU Exercise Physiology Laboratory. Each subject will be required to wear light weight clothes without zippers (e.g., t-shirt and shorts) or other DXA approved attire for each scan. They will be asked to remove all metal-containing objects from his/her body, and to lie down on the table. The subject will be carefully positioned on the table, and his/her legs will be placed together using Velcro-like straps. A scanner emitting low energy X-rays and a detector will pass along the subject's body. The subject will be asked to remain completely still while the scan is in progress. The scan takes less than four minutes. An additional lumbar spine scan will also be performed, which also takes less than four minutes. These scans are for research purposes only and not for diagnostic treatment.

Strength Testing (30-60 minutes). Strength testing will take place in the LSU Exercise Physiology Laboratory and the LSU School of Kinesiology's Exercise Training Facility. Before strength test begins, the subjects will walk on the treadmill for 5 minutes to warm-up.

Biodex Strength Testing (About 20 minutes). We will measure strength (peak torque, N◦m) of the knee extensor muscles using isometrically (0◦/s) isokinetic dynamometry (Biodex System 3) at 60◦/s[32]. Prior to each test, the subject will be provided with a short period of practice so that they can acclimate to the testing protocol. During each test, the subject will be asked to work as hard as they can for each repetition.

Biodex Endurance Testing (About 10 minutes). We will measure knee extensor endurance (or fatigue) in response to 120 maximal voluntary contractions at 240 ◦/s (one every 2 seconds for 4 minutes)[33]. All contractions will be performed over a 70 degree range of motion (100-170 degrees, 180 degrees = full knee extension). During each test, the subject will be asked to work as hard as they can for each repetition.

One-Repetition Maximum Testing (About 15 minutes). Skeletal muscle strength will be assessed using a 1-RM measurements obtained for the leg extension, leg press, and chest press. Subjects will be provided a dynamic warm-up consisting of weight they can lift at least 6 times. Subjects will then complete about 4-6 trials to reach the maximum weight they can lift one time. Subjects will start the first trial at relatively low weight, followed by 2 to 3 min rest. Each subsequent attempt, the weight the subjects lift will increase until your 1-RM is achieved. **Note:** these measurements may be performed prior to a regularly scheduled exercise session during each subject's first and last week of the intervention. In some subjects, we may use a submaximal test (e.g., 3 RM) to estimate their 1RM.

Handgrip Strength Testing (about 5 minutes). Handgrip strength will be measured with a hand dynamometer. This test involves squeezing on the hand grip dynamometer as hard as you can for several seconds. This assessment will be repeated 2-3 times per hand.

Body Composition Assessments at LSU (about 10 minutes). The following body composition assessments will take place in the LSU Exercise Physiology Laboratory during V1 and V4).

Bioelectrical Impedance Analysis (BIA) - LSU (about 5 minutes) The BIA test will measure the amount of total body water, fat free mass, and fat mass in the body. The subject will be asked to remove all footwear and socks/stockings. Once changed and barefoot, the subject will be asked to stand on a scale

(similar to a large gym scale) and to hold on to hand electrodes on each side of the scale. The subject will be asked to step off of the scale once the measurement is complete (less than one minute).

Body Composition Assessments at PBCR (about 30 minutes). The following body composition assessments will take place in Dr. Steven Heymsfield's Laboratory during V2 and V5.

Circumferences (about 10 minutes). Circumferences of the waist, hip, upper arms and thighs will be measured using the National Health and Nutrition Examination Survey (NHANES) protocol. Measurements will be made by a trained study personnel using a calibrated tape measure. We have a circumference training and validation program in place from earlier studies.

Optical Imaging (about 5 minutes). Body shape will be imaged and measured (circumferences and body volumes) will be using 2D and 3D imaging techniques. We will scan each subject 2-3 times using the Fit3D scanner. The Fit3D is a rotating platform that takes under 45 seconds to complete one scan.

Bioelectrical Impedance Analysis (BIA) - PBRC (about 5 minutes). The subject will also be asked to lay quietly for about 15 minutes. During this time, electrodes from a second BIA system will be positioned on the ankles and fingers according to manufacturer's protocol. Another measurement will be taken with the second system.

Magnetic Resonance Imaging (MRI) Leg Muscle Mass (about 15 minutes). This scan will take an image of the muscles in each subject's thigh from the hips to the knees. These scans will be performed in the PBRC Imaging Core. The subject will change into a hospital gown and remove all objects containing metal from his/her body. The subject will lie on his/her back on the scanner table in a comfortable position with his/her arms resting on his/her stomach. The subject will then be moved into the magnet, and the scan will proceed. The scan will take approximately 15 minutes. During the scan, the subject will hear loud tapping noises. The subject will be given head phones for protection from the scanner noise and can listen to music during the scan if desired. The subject will also be given a call button should he/she need the MRI tech during the exam. This scan is for research purposes only and not for diagnostic treatment.

Phosphorous Magnetic Resonance Spectroscopy – ^{31}P-MRS (about 45 minutes). We will use phosphorus magnetic resonance spectroscopy (^{31}P-MRS), to measure *in vivo* mitochondrial oxidative capacity.[30] These measurements will be performed in the PBRC Imaging Core. This test involves measurement of skeletal muscle phosphocreatine during resting conditions and after performing leg contractions in the MRI scanner. The aim of this test is to calculate the rate of phosphocreatine recovery after dynamic leg extension inside of a MRI, which is used as an *in vivo* marker of oxidative capacity in the skeletal muscle. Prior to testing, study subjects must be rested for a minimum of 3 hours after their last exercise regimen. This scan will take approximately 45 minutes in total to complete. Subjects will be positioned supine on the 3.0 T magnet with only the lower extremity inside the bore. The ^{31}P RF coil will be positioned over the right *vastus lateralis* (or left if the person has a medical reason not to use the right leg). The ankles and femur will be secured to each other with Velcro or nylon sports straps to create some resistance for the dynamic kicking exercise required for the test. A scout scan is performed first in order to visually locate the *vastus lateralis* muscle. Next, ^{31}P spectra is acquired every 60 seconds for 2 minutes to achieve baseline [PCr], [ATP] and [Pi] while the subject rests in the scanner. The subject will then be prepared for the dynamic leg extension exercise. The dynamic 1D-^{31}P MRS scan will be set up as follows: Time to repetition 1.5 s, acquire average spectra at a rate of 6 seconds per spectrum for a total of 75 spectra (estimated prescription time, 7 minutes 30 seconds). This pulse sequence will run for 36 seconds (i.e. 6 spectra) before instructing the subject to perform the leg extension exercise. When instructed, the subject will be asked to kick the right leg (or the leg surrounded by the coil) hard and fast for approximately 28 seconds against the Velcro or nylon strap. The ^{31}PCr peak will be monitored in real-time. We will determine if the reduction in PCr is 35 to 45 percent of baseline ^{31}PCr and will instruct the subject to stop at this moment. Alternatively, if this 35-40% reduction is not met, we may start the dynamic scan until the first four spectra are acquired then pause the scan and calculate 40% of the baseline PCr peak. This value can then be used for the target signal in the real-time display mode and we will start the scan with the exercise and instruct the subject to stop at the moment the target signal is observed. In the event that we overshoot the target signal, we will allow the subject to rest and relax for 10 minutes then repeat the leg extension exercise. This scan is for research purposes only and not for diagnostic treatment.

PBRC Fasted Blood Draw. During the Screening Visit, Part I, and post-training V5, blood will be drawn to measure complete blood counts (CBC), clinical chemistry (Chem 15) and blood lipids. These measurements will be conducted in the PBRC Clinical Chemistry Core.

LSU Mixed Meal Tolerance Test (MMTT), LSU Blood Draws, and Storage (about 4 hours). After an overnight fast (≥10 h), an intra-venous (IV) line will be placed in the arm vein for blood drawing purposes by trained and medically certified personnel (e.g., a certified phlebotomist, licensed medical personnel, Dr. Neil Johannsen, PhD, or Dr. Guillaume Spielmann). The IV will remain there throughout the testing. A fasting blood sample (~50 ml or 10 teaspoons) will be drawn and then the subject will drink about 2 cups of Boost (82 g of carbohydrates, 8 g of fat, and 20 g protein).[34] Blood samples will be drawn at 0, 10, 20, 30, 60, 90, 120, 150, and 180 min post-ingestion to measure plasma glucose, insulin, and c-peptide. We will draw about 150 ml or 10-12 tablespoons of blood for this test in total, including the fasting blood draw. During the IV procedure, a small amount of the subjects own blood (less than 1 teaspoon) may be immediately returned into his/her vein through the IV after each specimen is collected. During the MMTT the subjects will be in a semi-recumbent position on an examination table. In the event of a vasovagal response, we will place the subject in a supine position with their legs elevated. The subjects will be provided water to increase fluid intake. We will also monitor his/her blood pressure closely. The PI and medical investigator will notified. We will also assess their standing pulse rate and blood pressure and ensure they feel okay again before releasing them. After initial processing, fasting and post-ingestion plasma glucose will be measured immediately using the glucose oxidase method (Anolox GL5) and a portion of the fasting and post-ingestion plasma samples will be stored at -80°C for batch measurements of hormones (e.g., insulin and c-peptide). We will use the trapezoidal rule to determine the glucose and insulin AUCs, and the Oral Minimal Model to calculate the SI and Φ.[26, 35] A portion of the fasting whole blood sample will be used to isolated PBMCs and T-Cells. Moreover, after initial processing, fasting serum samples will be stored at -80°C for batch measurements of pro-inflammatory cytokines. Left over samples will be stored at -80°C for future research purposes. We may also assess heart rates using heart rate monitors (e.g., Polar or Zephyr Bioharness) during the MMTT.

> **Resting Metabolic Rate (RMR) and Fuel Oxidation Before and After MMTT.** Indirect calorimetry will be performed prior to and during each MMTT. Resting energy expenditure and substrate utilization will be assessed using the ParvoMedics, TruOne 2400 metabolic cart (Sandy, UT) calibrated with standard gas mixtures. A transparent plastic hood connected to the metabolic cart will be placed over the participant's head and calculations of energy expenditure and carbohydrate and fat oxidation rates will be made from expiratory gases diluted to produce a constant fraction of expired carbon dioxide (~1.0%). RMR measurements will be assessed before the MMTT and at 1 and 2 hours after ingestion of the mixed meal, each measurement will last about 30 minutes.

Muscle Biopsy Procedure (about 30 minutes). A *vastus lateralis* muscle biopsy will be performed using the technique of Bergstrom as described in SOP 906 from the PBRC Inpatient Unit by a trained medical professional. After cleansing the skin with povidone-iodine solution, the skin, adipose tissue and skeletal muscle fascia are anesthetized using < 5mL of a 50%/50% mixture of bupivicaine and lidocaine (final concentrations 1.0% and 0.125%). The skin is incised (0.75cm) with a #11 scalpel. The fascia fibers are separated with the blunt edge of the scalpel and the Bergstrom needle (4mm) inserted into the *vastus lateralis*. After suction is applied, approximately 500 mg of tissue is cut and removed. Four to 6 passes may be required to obtain a goal of 500 mg of muscle. Pressure is applied and the skin is closed with sterile tape.

Muscle Biopsy Processing and Storage. We will immediately place a portion of the muscle biopsy sample (~50-100 mg) in ice-cold (0-4 °C) relaxation and biopsy preservation buffer (BIOPS: 10 mM Ca^{++}-EGTA, 0.1 µM free Ca^{++}, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM MgCl2, 5.77 mM ATP, 15 mM phosphocreatine)[31]. This portion of the biopsy will be immediately transferred on ice (0-4 °C) to the LSU Exercise Physiology Laboratory for measurement of skeletal muscle OXPHOS using HRR (see Transport of Muscle Tissue and Blood). The BIOPS buffer has been shown to preserve the integrity of skeletal muscle OXPHOS measured using HRR for a minimum of 4 hours.[36] The remaining muscle will be flash-frozen in liquid nitrogen, transported to the LSU Exercise Physiology Laboratory on dry ice, and stored at -80°C (see Transport of Muscle Tissue and Blood).

HRR-OXPHOS Protocol. Using HRR (Oroboros Oxygraph-O2k) and a standard substrate inhibitor titration protocol, we will measure muscle OXPHOS (State 3 respiration, HRR-OXPHOS) in saponin permeabilized muscle fibers.[31] Specifically, we will assess muscle OXPHOS using substrates specific for respiratory chain complex I (10mM glutamate, 2mM malate) and complex I and II (10mM glutamate, 2mM Malate, 10mM succinate) in the presence of 2.5mM ADP. We will normalize the oxygen flux (JO_2) per gram muscle wet weight (pmol/s/g tissue). We will use a similar SUIT protocol in digitonin permeabilized immune cells. We may also assess OXPHOS using alternative SUIT protocols and/or in isolated mitochondria.

Citrate Synthase, Complex I, and Complex II Activities. We will measure citrate synthase activity spectrophotometrically in tissue/cell homogenates using a commercially available citrate synthase activity kit (Sigma Aldrich) and Complex I and Complex II activities using commercially available ELISA kits (Abcam).

Immunoblotting. We will quantify protein abundance of the electron transport chain using standard immunoblotting techniques with the MitoScience Total OXPHOS Antibody Cocktail (Mitosciences) as previously described.[15] We will also measure oxidation of muscle proteins using the Oxyblot kit (Millipore).

PBMCs and T-cell Isolation. Total blood lymphocyte numbers will be quantified by flow cytometry (BD Accuri C6, BD Biosciences, Ann Arbor, MI, USA). Fresh PBMCs will be isolated by gradient centrifugation as described previously.[27] Pan T-cell isolation kits (Miltenyi Biotech, Germany) will then be used to sort T-cells.

Flow Cytometry. We will assess T-cell phenotypes by four-color flow cytometry on a BD Accuri C6 flow cytometer. We will label isolated pan T-cells (1.0×10^6) with pre-diluted monoclonal antibodies (mAbs) and incubated at room temperature in the dark for 30 min. The mAb combinations will consist of anti-killer cell lectin-like receptor G1 (KLRG1) Alexa488 or CD45RA FITC, CD28 PE or CCR7 PE, CD4 PerCP or CD8 PerCP and CD3 APC to allow comprehensive characterization of the level of T-cell differentiation.[37]

Glucose, Hormones, Lipids, and Pro-inflammatory Cytokine Assays. We will measure plasma glucose using the glucose oxidase method (Anolox GL5) and insulin and c-peptide using commercially available ELISA kits (Linco Research, St. Charles, MO). We will measure total, high-density lipoprotein, low-density lipoprotein cholesterol and triglycerides by a clinical chemistry panel. We will measure serum IL-6, TNF-α, IFN-γ and C-reactive protein using commercially available enzyme-linked immunosorbent assay kits.

Transport of Muscle Tissue and Blood. The fresh and flash-frozen muscle biopsy samples will be transported for analysis and storage at the LSU Exercise Physiology Laboratory (co-directed by Drs. Irving, Spielmann, and Johannsen) by approved study personnel. The fresh muscle will be transported on ice (0-4 °C), while the flash-frozen muscle biopsy samples will be transported using dry-ice to ensure the integrity of the muscle samples. When the flash-frozen muscle samples arrive at LSU Exercise Physiology Laboratory they will be placed in a -80°C freezer for future analyses. In the event that the flash-frozen samples cannot be transported from PBRC to LSU immediately, they will be temporarily stored in the Clinical Chemistry Laboratory at -80°C as agreed upon with Stephen Lee or in Dr. Jackie Stephen's Laboratory at -80C. All samples will be transported in a leak-proof primary receptacle within leak-proof secondary packaging inside a rigid outer package. The outer package will be clearly labeled with biohazard labels and when appropriate with dry-ice labels. Moreover, a spill kit will also be available throughout the transport process. Currently, there is no *a priori* plan to transport blood samples from PBRC to LSU or from LSU to PBRC. However, in the event that we do, we will transport the cryopreserved blood samples (e.g., plasma or serum) on dry ice as described above for the flash-frozen muscle tissue. Once the samples are in the LSU Exercise Physiology Laboratory they will be handled according to procedures detailed in our LSU IBRDSC protocol (registration16036).

Biological Safety Training. All study personnel acquiring, handling, transporting, and analyzing the blood and tissue samples collected as part of this study will have up to date training on blood borne pathogens, basic biosafety, basic laboratory safety, hazard communication, hazardous waste training through LSUs Environmental Health and Safety Office. PBRC personnel who are involved in acquiring and handling the blood and muscle tissue (e.g., Nurse Practitioner/Physician for muscle biopsies) are not required to undergo the LSU training, however, they are required to take comparable training through PBRC.

OPTIONAL: Carbon-13 Magnetic Resonance Spectroscopy – ^{13}C-MRS (about 95 minutes). The participant will be asked to undergo an infusion via IV. The solution will be made of a non-radioactive labelled

molecule called 13C-acetate. An IV line will be placed in a vein in of the subject's arm. A port will be placed in a vein of the subject's other arm so that a 2 mL (0.4 teaspoons) sample of blood may be drawn before and every 10 minutes during the procedure for a total of 18-20 mL (3.5-4 teaspoons) of blood. The IV line will be used to infuse the solution at a predetermined rate (e.g. 15 mg/kg/min as bolus followed by 6 mg/kg/min for the remainder of the scan). The solution will begin to enter the vein and may continue to flow until the scan is over. We will then use Carbon-13 Magnetic Resonance Spectroscopy (^{13}C-MRS), to measure *in vivo* skeletal muscle biochemical dynamics. These measurements will be performed in the PBRC Imaging Core. This test involves measurement of ^{13}C-labeled TCA cycle metabolites and acetylcarnitine-acetyl CoA shuttling in the muscle. The aim of this test is to calculate the rates of TCA cycle metabolic flux and levels of acetylcarnitine and acetyl. Prior to testing, study subjects must have fasted for at least 10 hours. This scan will take approximately 95 minutes in total to complete. Subjects will be positioned supine on the 3.0 T magnet with only the lower extremity inside the bore. The ^{13}C RF coil will be positioned over the right *vastus lateralis* (or left if the person has a medical reason not to use the right leg). The ankles and femur will be secured to each other with Velcro or nylon sports straps to create some resistance for the dynamic kicking exercise required for the test. A scout scan is performed first in order to visually locate the *vastus lateralis* muscle. Next, ^{13}C spectra are acquired for 20 minutes while the subject rests in the scanner. The subject will then be infused with the ^{13}C-acetate followed by ^{13}C spectra acquisition for 75 minutes. After 60 minutes of infusion, the participant will be instructed to kick against the strap or a force sensor for 12 second periods followed by 12 second rest periods in repetition for the remainder of the scan (about 15 minutes). It is possible that the participant may experience muscle fatigue earlier than the 15 minutes. This scan is for research purposes only and not for diagnostic treatment.

STUDY TIMELINES

The anticipated duration that each subject will actively participate in this study is about 5-6 months, which includes initial screening, baseline assessments (V1, V2), 16 week intervention period, and post testing assessments (V3, V4, V5).

Our goal is to enroll all subjects within the first 18 months of this study. We plan to complete preliminary analyses at the completion of this 2 year pilot study (Summer/Fall 2020). We anticipate leveraging the preliminary data from this pilot study to submit a follow-up R01 at the completion of this study.

DATA AND SPECIMEN MANAGEMENT

Data collection will take place at PBRC and at LSU.

At PBRC, we will have PBRC assign each study subject a unique identification (ID) number, which we will use on all data collection forms, questionnaires, biological specimens, and electronic records. Only the PI and approved study personnel will have access to the master list that directly links the individual study subjects to their respected ID. This list will maintained electronically at PBRC.

Likewise at LSU, to preserve confidentiality and data integrity all data collection forms obtained will be secured in locked filing cabinets, or on password protected computers. Access to the primary data collection forms will be under the control of the PI and approved study personnel. Consistent with good clinical practice we will keep hard copy data records for a minimum of 3 years at which time they may be destroyed or kept indefinitely.

Biological samples (e.g., blood and muscle) will be immediately assessed as described in study protocol or stored frozen (-80°C) in the LSU Exercise Physiology Laboratory until analyses can be completed. In the event that biological samples are moved off-site for further analysis, the samples will be de-identified. Specifically, we will be label the samples their subject ID numbers, sample collection date, and time. Staff at these sites will not have access to the master list at any time. Biological samples may be stored indefinitely for the purposes of future research.

Data Management and Primary Statistical Analyses. We will collect, manage, and securely back-up study data daily using Research Electronic Data Capture (REDCap) hosted by LSU.[38] Data sharing will use PBRC's file transfer system or other encrypted HIPAA compliant data transfer service for data transfers between Dr.

Irving (LSU) and Dr. Greenway (PBRC). We will screen all data for distributional properties (mean, median, range) and departures from normality prior to substantive analyses. To test the main effects of time, treatment, and their interaction on the primary outcomes, we will use mixed-effects two-way Analysis of Variance (ANOVA) models (α=0.05). The models will include parameters to estimate the main effects of time (pre- and post-training) and treatment (RE, REALPA breaks in ST, RE+AE) on the dependent variables. We will use *Fisher's Least Significant Differences test* to examine within and between group mean differences (α=0.05). We will test all models for the main effect of sex. If the main effect of sex is significant, we will perform additional exploratory analyses stratified by sex. Other covariates that may be included in the models include race, change in body weight, and baseline SPPB scores. We will also compare adherence rates across the study groups.

Sample Size, Power Analysis and Randomization. We will use the data collected in this ***pilot study*** to estimate effect sizes for the primary and secondary outcomes, which we will use to inform our power analyses for a follow-up R01 application designed to test the efficacy of our novel exercise intervention on skeletal muscle and cardiometabolic health in sedentary older adults. We will use a fixed sample size of 12 subjects per group, because increasing the sample size above 12 (i.e., 12 to 15) has diminishing benefit in estimations effect sizes and 95% confidence intervals often referred to as the "rule of 12".[39, 40] Thus, we will recruit up to 15 subjects per group to account for a 20% attrition rate. The NIH's Clinical and Translational Science Awards Biostatistics, Epidemiology, and Research Design (BERD) units recommend 12 subjects per group for estimating effect sizes and 95% confidence intervals.[39] Following baseline testing, we will randomize the study subjects to one of the three interventions using a randomized, permuted, block design stratified by sex and race.

PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

This Human Subjects Research meets the definition of a Clinical Trial. This study does not involve major risk to subjects. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research.

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics, and Design

Study Population. We will recruit, randomize, and study up to 45 physically inactive, community-dwelling, older adults (65-80 years) with a BMI between 18.5-34.9 kg/m^2 from the greater Baton Rouge community to complete 36 subjects (12/group) accounting for a 20% attrition rate. We outline the inclusion and exclusion criteria below. We will study subjects on the medications they currently utilize for the treatment of their age-related comorbidities except for those known to interact with the study outcomes.

Inclusion Criteria.

1. Are capable and willing to give written informed consent, and understand exclusion criteria
2. 65-80 years of age inclusive
3. Body Mass Index (BMI) between 18.5-34.9 kg/m^2, inclusive
4. Physically inactive determined by self-report
5. Stable medical therapy for allowable medications for 30 days defined as:
    a. No addition or removal of a medication
    b. No change in dosage of a medication
6. Having no life-threatening conditions or diseases
7. Willing to allow researchers to use data, biospecimens (blood and muscle tissue), and images (e.g., magnetic resonance imaging) for research purposes after study participation is completed

Exclusion Criteria.

1. Nursing home resident
2. Physically Active:
    a. > 100 min/wk of moderate OR > 50 min/wk vigorous intensity PA
3. Saint Louis University Mental State (SLUMS) score < 21
4. Evidence or self-report history of deep vein thrombosis, pulmonary embolism, cardiovascular, peripheral vascular, cerebral vascular, pulmonary, or renal disease
5. Evidence or self-report history of type 1 or 2 diabetes mellitus
6. Evidence or self-report history of a bleeding disorder
7. Evidence or self-report history of recurrent vasovagal episodes
8. Evidence or self-report history of severe depression, Schizophrenia, bipolar disease
9. Evidence or self-report history of mobility disability requiring a walker, wheel chair, or inability to walk across a small room.
10. Evidence or self-report history of orthopedic limitations that would preclude them from participation in a dynamic exercise program
11. Evidence or self-report history of severe arthritis (either osteoarthritis or rheumatoid arthritis) that would preclude them from participation in a dynamic exercise program
12. Evidence or self-report of history untreated thyroid dysfunction.
13. Weight loss of > 10% in the last 3 months prior to screening
14. History of weight loss surgery.
15. Use of medications known to influence study outcomes, such as:
    a. Insulin
    b. Oral antidiabetic medications (e.g., metformin)
    c. Corticosteroids
    d. Beta-blockers

c. Anticoagulants
16. Allergy to lidocaine
17. Active smoking
18. Current consumption of > 14 alcoholic drinks per week based on self-report
29. Regular participation in resistance or aerobic exercise training within 3 months of initial screening
20. Absolute Contraindication to Exercise as Defined by the American College of Sports Medicine,[16] including:
    a. Resting diastolic blood pressure > 100 mm Hg
    b. Resting systolic blood pressure > 180 mm Hg
    c. Resting heart rate > 100 beats per min
21. Having a body weight greater than 440 pounds
22. Having medical implants such as a pacemaker or metal joint replacements
23. Having tattoos or permanent makeup completed <30 days prior to the visit
24. Recent (past 3 months) cancer diagnosis, undergoing immunotherapy, taking immune suppressants, and presence of allergies or infections requiring antibiotics
25. Recent (past 3 months) major surgery on the abdomen, pelvis, or lower extremities
26. Any other condition that in the judgement of the Principal Investigator and/or the Medical Director of this protocol may interfere with study participation and adherence to the protocol

Source of Subjects. The greater Baton Rouge community has between 30-40,000 adults 65 years of age or older. We will identify potentially eligible study subjects via advertising. Specifically, we will recruit potential subjects from advertisements placed on the bulletin boards, electronic bulletin boards, social media, and in local and regional newspapers (e.g., the Advocate, the LSU Reveille,) and magazines. We will advertise on regional radio stations as needed. We will post flyers in senior housing establishments as well as attend senior expos.

Retention Strategies. Retention will be a challenge in the proposed studies, as it is in all clinical trials. Our proposed study provides a variety of benefits in an effort to maximize the number of subjects who complete all follow-up visits. These benefits include the interventions and medical monitoring. Essential aspects of maximizing participation and promoting retention are: 1) carefully screening and assessing barriers to adherence and retention prior to randomization; 2) carefully monitoring adherence problems (which often predict retention problems), trying to identify these problems early before subjects refuse further study contact; and 3) applying specific strategies to address these problems. Some strategies include providing social and emotional support, flexibility in scheduling exercise training session and flexibility in scheduling study visits.

Randomization. After completing both pre-training visit 1 and 2, study subjects will be randomized to one of three 12 week intervention groups i) RE alone, ii) REALPA breaks in sedentary time, or iii) RE+AE. We will prepare randomization scheme based on a permuted block design stratified by sex and race.

Exercise Interventions. The RE group will complete 16 weeks of supervised RE training (2 x/wk). The REALPA breaks in ST group will complete 16 weeks of supervised RE training (2 x/wk) and regular unsupervised LPA breaks in sedentary time (5 d/wk, 6x10 min breaks per day at 2 METS (~30-40% VO_2 peak), ~500 kcal/wk above resting metabolism). The RE+AE group will complete 16 weeks of RE (2 x/wk) and calorically matched moderate intensity AE (3 d/wk, 50 min/session at 4 METS (~60-75% VO_2 peak), ~500 kcal/week above resting metabolism). The RE component for all three groups will consist of 3 sets of 10-12 repetitions to failure for a total of 8 exercises targeting the large muscle groups (See Research Strategy for Details). The RE and AE sessions will take place in LSU School of Kinesiology's Exercise Training Facility under direct supervision (Drs. Irving, Johannsen, and Spielmann with the assistance of trained undergraduate and graduate students). Over the first month of the intervention, we will be progressively increase the intensity and volume of the exercise prescriptions to the targeted training intensities and volumes to reduce the risk of injury and muscle soreness. Prior to each RE and/or AE training session, we will assess resting blood pressures, heart rates, and clinical symptoms. We will not allow subjects to exercise if they have an absolute contraindication to exercise (see Potential Risks Section

Below). We will also assess heart rates using heart rate monitors (e.g., Polar or Zephyr Bioharness) and ratings of perceived exertion (RPE, Borg 6-20) throughout the supervised exercise sessions.

b. Sources of Materials.

Throughout the course of the proposed studies, we will acquire blood and muscle samples exclusively for research purposes. We will acquire data on physical activity, body composition, muscle strength, cardiorespiratory fitness (VO_2 peak), and physical function exclusively for research. We will also acquire data on mitochondrial oxidative capacity from muscle and peripheral blood mononuclear cells (e.g., T-cells) exclusively for research. We will provide all study subjects with a unique study ID, which we will be use to label all study samples and data. Only the PI and approved study personnel will be able to link the study ID to individually identifiable private information about the study subjects. No use will be made of pre-existing specimens. We will collect, manage, and securely back-up study data daily using Research Electronic Data Capture (REDCap) hosted by LSU/PBRC. Some data may also be stored in excel and/or statistical analysis data sets (e.g., SAS). We will store all research and/or medical records in locked areas, with only authorized study personnel having access to the confidential research and/or medical records. Samples and/or data sent outside of study team will also be de-identified. Presentation of data will be in aggregate form, with all identifying subject level characteristics removed prior to publication or presentation.

c. Potential Risks.

Overall, the study procedures do not involve significant risks to either the study subjects, research investigators, or study personnel. The following are potential risks associated with this study.

1. **Height and Weight.** There is no risk to subjects for measuring their height or weight.
2. **Vital Signs (blood pressure and heart rate).** Subjects may experience temporary discomfort during blood pressure recordings due to the pressure of the cuff on their arm. There are no risks associated with measuring heart rate.
3. **Fasting for about 10 hours.** There is a possibility that fasting for 10 hours may make subjects feel nauseous. Light snacks such as granola bars and juice will be available for subjects to eat once the fasting procedures are completed.
4. **Electrocardiogram (EKG or ECG).** There are minimal risks associated with this test. There is a small possibility there may be some redness or irritation while cleaning the skin prior to applying the electrodes or if the subject happens to be allergic to the adhesive on the electrodes.
5. **Short Physical Performance Battery (SPPB).** The risks associated with the SPPB are risks that may accompany low-to-moderate intensity exercise. These possibly include muscular fatigue, soreness, breathlessness, rapid heart rate, elevated blood pressure, dizziness, lightheadedness, or falls. All precautions will be taken to avoid any injury or harm. All tests will be monitored by trained personnel and emergency procedures and equipment are in place.
6. **Questionnaires.** There are no anticipated risks from completing self-report questionnaires (e.g., International Physical Activity Questionnaire – Elderly, Sedentary Behavior Questionnaire, PROMIS Physical Function Questionnaire, and Pittsburgh Fatigability Questionnaire). If signs of minor stress or fatigue are apparent, subjects will be given time to take a break from completing the questionnaires. It is estimated that the questionnaires will take 30 minutes to complete. Responses to the questions will be coded to protect confidentiality, and subjects may choose to not answer questions.
7. **Exercise Test – VO_2 Peak.** We will perform all VO_2 Peak tests in the PBRC Exercise Testing Core. There is minimal risk of injury or a cardiovascular event during VO_2 Peak. We minimize the risk of an event during VO_2 Peak by performing pretest a review of the medical history, physical examination by a physician, use of a highly trained staff, and well-defined emergency procedures. Subjects may experience temporary discomfort during blood pressure recordings due to the pressure of the blood pressure cuff on the arm. We will perform all tests in the presence of an exercise physiologist with extensive experience in conducting maximal exercise tests. All laboratory staff are trained in basic CPR and/or ACLS. In the event of a life threatening emergency, the subject would be treated with ACLS (advanced cardiac life support) by a staff physician, research nurses and subsequently be

transported to the nearest acute care medical-surgical facility via Emergency Medical Services which is a parish wide paramedic response unit. The closest facility is approximately 0.25 miles away.

8. **Isokinetic, 1-Repititon Maximum, and Grip Strength Testing.** We will perform isokinetic, 1-repitition maximum, and grip strength testing in the LSU Exercise Physiology Laboratory. There is minimal risk of injury to isokinetic strength testing. Occasionally, subjects experience mild muscle soreness following isokinetic, 1-repitition maximum, and grip strength testing.
9. **Physical Activity Monitoring.** There is no risk associated with measuring activity with accelerometers. The accelerometers fit comfortably on the subject's wrist and thigh. The accelerometers can easily be removed should they become uncomfortable or in rare cases when they cause skin irritations.
10. **Venipuncture, Intravenous Catheters and Blood Draws.** Blood draws will be completed during this study that may be associated with pain, light-headedness, infection, or bleeding or bruising at the site of venipuncture; however, the trained staff will use proper aseptic technique while taking blood samples in order to reduce the risk of these unwanted effects. Subjects may feel hungry or weak during the times they are required to fast. The total blood withdrawn during the screening visit, pre-training visit 1 and pre-training visit 2 will be between 150-200 mL (10-13.5 tablespoons). Moreover, the total blood withdrawn during the post-training visit 3 and 4, 16 weeks later, will be between 150-200 mL (10-13.5 tablespoons). Note: participants electing to participate in the optional C13 Magnetic Resonance Spectroscopy Study will have an additional 20 mL of blood during the during visit 1 and visit 5, bringing the total pretraining blood draw volumes between 170-220 mL (11.5-15.0 tablespoons) during the pre- and posttrianing periods. Subjects should refrain from donating blood or being in another research study eight weeks prior to the study and until eight weeks after the completion of the study. These blood draw volumes of fall well within the range recommended by the American Red Cross (500 mL or 1 pint per 8 weeks).
11. **Muscle Biopsies.** A *vastus lateralis* muscle biopsy will be performed using the technique of Bergstrom as described in SOP 906 from the PBRC Inpatient Unit by a trained Nurse Practitioner or physician. For *vastus lateralis* muscle biopsies, the risks are mild to severe pain, soreness, bruising and a small scar are common risks. There is a small risk of a hematoma (collection of blood in the tissue) or infection at the biopsy site. There is also a slight risk that a superficial nerve may be cut; the nerve may heal, or it may result in a permanent loss of sensation in the skin at the biopsy site. Sterile technique will be used to minimize infection risks and the biopsy site will be monitored closely. To reduce the risk of hematomas and bleeding, we ask that subjects stop taking anticoagulants, non-steroidal anti-inflammatory (NDSAIDs), and aspirin for a week before and after the biopsy procedure. We will exclude individuals who cannot stop their anticoagulants. Previous data suggest that the rates of adverse events following a percutaneous muscle biopsy in older adults (60-76 years) are similar to those of younger adults.[41] Moreover, Dr. Eric Ravussin previously conducted a study at PBRC that included biopsies in elderly adults (70-84 years) with no serious adverse events.[42] The PI has led and been involved in multiple studies that included muscle biopsies in both young (18-30) and older adults (65-85 years).[15, 43-45]
12. **Archive of Biological Sample (blood and muscle biopsy tissue).** The primary risk to subjects who donate bio-samples to be banked for future research (molecular in this case) is the risk of loss of confidentiality and/or privacy. Most banks need to maintain a link between the identities of donors and coded specimens to be able to collect valuable clinical follow-up information about the donor. We will, however, create and maintain a firewall between the source and the researcher so that the protected health identifiers are never given to the researcher(s). Storage and disposal of tissue will be conducted in a manner conforming to the appropriate care and handling of biological specimens as outlined through the Institutional Biohazard Committee Guidelines.
13. **Dual Energy X-Ray Absorptiometry (DXA).** We will perform the DXA scans within the LSU Exercise Physiology Laboratory. We will use the DXA scans to measure body composition (fat and lean mass content). DXA involves minimal x-ray exposure, about the same amount from 12 hours background

radiation from the sun. Although exposure to radiation can harm an unborn child, all women in the proposed studies will be post-menopausal.

14. **Circumferences.** There are no known risks associated with the circumference measurements.
15. **Optical Imaging.** The 3D optical scans are not a standard procedure for patients; however, it does not involve using the imaging cameras in an unusual way. The optical scans require subjects to stand straight for approximately 1 minute. The scanner involves standing on a small rotating platform. The faces of each subject will be blurred at the time of acquisition such that their identity will not be possible from the saved images.
16. **Bioelectrical Impedance Analysis (BIA).** There is no risk associated with the BIA measurement. However, subjects with medical implants such as a pacemaker or metal joint replacements cannot be measured on the machine. Measurements will not be performed on any subject who is pregnant, and all females should inform the technologist if there is any possibility that they are pregnant.
17. **Magnetic Resonance Imaging and Spectroscopy (MRI and MRS Risks).** Measurements will not be performed on any subject who is pregnant, and all females should inform the technologist if there is any possibility that they are pregnant. There is no risk associated with the BIA measurement. However, subjects with medical implants such as a pacemaker or metal joint replacements cannot be measured on the machine.

    There are no known biological risks associated with magnetic resonance scanning. It has been used routinely for over 20 years. It produces side effects in very few situations. Those situations include:

    Metal: Because the magnetic resonance machine uses a magnetic field, it can move any metallic objects that are inside the body. This disruption of metal inside the body is extremely dangerous to you and may even be life threatening. If you think you may have a cardiac stent, metallic implant, metallic piercings, shrapnel, or any other metallic material in your body, it is of utmost importance that you alert the study coordinator or MR technician. If you have metallic materials in your body that cannot be removed, we will exclude you from this study for your safety.

    Electronics: Magnetic resonance imaging involves the use of radio frequency energy that can disrupt the functioning of electronic devices. If you think you might possess a pacemaker or any other electronic medical device inside your body, it is of utmost importance that you inform the study coordinator or MR technician. If you have any such electronic devices we will exclude you from this study for your safety.

    Tattoos and cosmetics: Some tattoos and cosmetics contain metallic materials that can heat up during scanning, especially if they are located on the part of the body being scanned. If the metallic material heats up enough, you may feel an uncomfortable burning sensation, and a skin burn may develop. If you have any tattoos or cosmetics that might contain metallic materials, please alert the study coordinator or MR technician. If you feel a burning sensation on your skin, alert the study coordinator or MR technician. In some cases, the amount of metallic material in the area being scanned is so excessive that the scan must be stopped. In other cases, a cold compress placed over the metallic material will be used to prevent the burning sensation.

    Confinement: During the MR scan, you will be lying down on a table inside of a metal tube. The metal tube is a confined place. This might produce a feeling of claustrophobia, which can be distressing. If you have experienced claustrophobia in the past, you might become too distressed to complete the scan. If you become distressed during the scan due to confinement in the scanner tube, please alert the MR technician and the scan will be halted.

    Noise: The MRI machine creates a loud, rhythmic noise that sounds like grinding or churning. This can be distressing to those who are sensitive to loud noises. You will be provided with earplugs to reduce the noise. But if you find the machine noises distressing, alert the MR technician and the scan can be halted.

Peripheral nerve stimulation: During the MR scan, the magnetic field around your body goes through rapid changes. These changes are all within safety limits set by the Food and Drug Administration. But, some people experience twitching in the nerves of their arms or legs as a result of these magnetic field changes. This twitching is generally not painful, and it stops at the end of the MR scan. But the feeling of inadvertent muscle twitching may make you feel disoriented or uncomfortable. If you experience this and wish to stop the scan as a result, please tell the MR technician.

Physical frailty: The MR technologist performing the scan has received extensive training in how to position all subjects, including elderly ones, in the MRI machine safely and comfortably. However, some older people have a more difficult time walking or moving their bodies due to arthritis and other conditions. There is a slight chance that these individuals could feel discomfort or fall during transitions into or out of the MRI scanner. The technologist will insure that the walkway to the scanner is safe for you to walk on, will place cushioning on the scanner table for your comfort, and will carefully guide your movements around the scanner to minimize this risk.

Venous thromboembolism: In some elderly or obese individuals, lying down perfectly still for multiple hours can slightly increase the risk that blood clots develop in the blood vessels. These blood clots can be hazardous to your health. The technologist will make every effort to keep your time in the MRI machine as short as possible to reduce this risk. Also, you will have breaks during your time in the MRI machine, and during these breaks the technologist will ask you to move your arms and legs and reposition your body to get comfortable. Moving around in this way reduces your risk of blood clots.

18. **Exercise Interventions**. The risks associated with the three exercise training interventions are those commonly associated with moderate to vigorous physical activity in older adults. The proposed exercise interventions are unlikely to cause major problems. We have conducted numerous exercise training studies and have never had a serious adverse event. There is the possibility of adverse events ranging from minor musculoskeletal problems to, in very rare cases, cardiovascular events. Occasionally study subjects experience minor orthopedic problems, but most are self-correcting with rest and standard first aid. These orthopedic injuries will be minimized by gradually progressing subjects to their prescribed dose at the start of the study and, if necessary, alternating exercise sessions between cycle ergometers and treadmills. Exercise supervisors are trained in first aid and basic CPR. All RE and AE sessions will be supervised by trained personnel in the LSU's School of Kinesiology's Exercise Training Facility. The LSU School of Kinesiology's Exercise Training Facility is well equipped with emergency response equipment including automatic defibrillator. Although some study subjects will be at moderately elevated risk for CVD, they will receive a thorough health screen including a physical examination by a study physician and a maximal exercise test. According to the available data on adverse events resulting from the types of exercise proposed here, risk should be low in this study. Fatal events during exercise are extremely rare.[16] All resistance exercise (RE) and aerobic exercise (AE) training sessions will be supervised by a trained exercise specialist. The risk associated with the low intensity physical activity breaks in sedentary time are unlikely to cause any major problems. Prior to each exercise session, resting blood pressure, heart rate, and clinical symptoms will be assessed. Subjects will not be allowed to commence their exercise session if they have an absolute contraindication exercise,[16] which includes
    a. Unstable Angina
    b. Resting systolic blood pressure > 200 mm Hg or diastolic blood pressure > 110 mm Hg
    c. Uncontrolled sinus tachycardia (resting HR >120 beats/min)
19. **MMTT.** There is a possibility of pain, bruising, or infection at the site of the needle insertion for the IV line. Trained personnel minimize this risk. The drink may make cause nausea, vomiting, abdominal bloating, or a headache.
20. **RMR.** There is no known risk in having a RMR (resting metabolic rate). If you are claustrophobic, it may be uncomfortable to have the plastic hood over your upper body.

21. **Confidentiality.** All volunteers are assured of their confidentiality both verbally and in the informed consent form. All research and/or medical records are securely stored in locked areas. Access to these areas is limited to the approved research support staff, director of the clinical facilities, and the PIs. Volunteers' medical records are filed according to ID numbers generated by PBRC. All forms on the chart, with the exception of consent form and laboratory results, display only the ID number. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name OR other identifying information.
22. **OPTIONAL: C13 Magnetic Resonance Spectroscopy.** There is a possibility of pain, bruising, and/or infection at the sites of the needle insertion for the IV line, and the port placement for the blood draws. Trained personnel minimize this risk. The risks associated with MRI scanning are the same as listed above. The risks associated with receiving the IV with 13C-acetate solution include the following:
    a. 13C is a natural type of carbon which is present in all living things. 13C is commonly used in research studies. There are no known risks connected to receiving 13C.

2. Adequacy of Protection against Risks

a. Recruitment and Informed Consent

Recruitment. Study subjects are volunteers, who we will recruit through advertisements placed on bulletin boards, social media, and in local and regional newspapers, and magazines. We will also post flyers in senior housing establishments as well as attend senior expos. We may also advertise on regional radio stations. Following a brief telephone interview that will be conducted by the Pennington Biomedical Research Center's Recruiting Department, prospective volunteers will report to the Pennington Center for a Screening Visit to confirm eligibility.

Informed Consent. The PBRC's Institutional Review Board will approve the study protocol and consent form prior to study initiation. Potentially eligible study subjects will meet with one of the study investigators or approved study personnel, who will explain the scientific rationale of the study, the procedures, potential risks involved and rights of the study subject. Eligible subjects will provide informed written consent prior to their participation in the study. We will provide a copy of the consent to the study subject and will keep the original consent in the subject's study records at securely stored at PBRC.

Human Subjects Training. The key personnel identified in this proposal have completed the required education on the protection of human research subjects. The LSU uses the National Institute of Health – Protection of Human Subjects online training program to certify that all study personnel meet the minimum requirements for Human Subjects Training. In addition, the key personnel on this proposal have also completed the formal training program entitled the "Collaborative Institutional Training Initiative (CITI)". CITI is a web based educational course designed to provide formal training in human subjects' research for all personnel involved in human subject research. Key study personnel at the non-LSU sites will be required to complete their institutions' IRB training program.

b. Protections Against Risk

Protections against Risk Associated Assessments and Study Interventions. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research. The study team has extensive experience in all procedures and aspects of the study. (See Data Safety Monitoring Plan below).

Confidentiality and HIPAA Requirements. We will strictly maintain confidentiality of all medical records and research data collected as part of this study using established procedures. We will keep the original data in the subject's record that is securely stored within PBRC for data collected at PBRC and LSU for data

collected at LSU, and we will enter the data into a RedCap database under the direction of the study team's biostatistical consultant. We will routinely transfer the original data collection forms (e.g., questionnaires) from LSU to PBRC to ensure that each subject's records are up to date. The PI, Co-I's, medical consultant, and biostatistical consultant will review all data. The privacy of the study subjects is very important to PBRC, LSU, our study team, and we will protect the privacy of the study subjects as much as possible. In compliance with HIPAA requirements, we will not send out the names, addresses, phone numbers, social security numbers, or any other identifying information on our study subjects. If study data and / or study samples are sent outside of the LSU for further analysis, these data and / or samples will be de-identified. Similarly, any publications generated from the data collected from the proposed studies will exclude identifiers.

3. Data Safety Monitoring Plan (DSMP)

Overview of DSMP. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators to assure that no volunteer suffers any adverse effects from participating in the research. The study team has extensive experience in all procedures and aspects of the study. The study team will review any subject safety issues as they occur and monthly research meetings. We will use an investigators committee to review the safety data in aggregate once a month. Our investigators committee will include the PI, Co-I's, and medical director (Dr. Frank Greenway). The PBRC's Institutional Review Board will also continuously monitor the proposed study for subject safety throughout the study period.

Safety Monitoring. In this study, an **adverse event or experience** is defined as any health-related unfavorable or unintended medical occurrence that happens throughout study participation. Examples of adverse events include but are not limited to the following:

- A clinically significant laboratory or clinical test result.
- An event that results in missing a study visit.
- An event that requires a visit to a physician.
- An event that occurs as a result of a study procedure.
- Unanticipated or untoward medical events that are not plausibly pregnancy related and may be study related.

LSU and PBRC is committed to ensuring and maintaining the safety of its subjects. We will use the AAHRPP provided definitions of Adverse Events and Serious Adverse Events. We will report adverse events according to the institutional reporting policy provided by AAHRPP.

A **serious adverse event** (SAE) is defined as an unanticipated medical occurrence that is deemed associated with study participation by the study Medical Investigator that results in one of the following:

- Death
- Life-threatening event
    - Life threatening events in the mother or fetus are defined as those that in the view of the research staff and PI put the individual patient at imminent substantial risk of dying, or if continued participation in the study might have resulted in death.
- Hospitalization
- Disability or permanent damage
- Medical Intervention to prevent permanent impairment or damage

All AEs from date of consent will be reported. Adverse events will be documented during the scheduled visits. For each sign, symptom or adverse event, the following information will be recorded:

- A brief descriptor of the adverse event
- Start and stop dates
- Intensity (mild / moderate / severe)
- Whether the AE was "serious" or not (as defined below)

- Causal association with the intervention assigned (none / doubtful / possibly / probably / very likely)
- Outcome (resolved / resolved with sequelae / improving / still present and unchanged / death)
- Action taken with respect to the intervention (none / intervention temporarily discontinued / medical therapy required / intervention permanently discontinued / other).

Adverse event data will be collected from the date of consent until the final visit. Adverse event data will be analyzed quarterly, but serious or life-threatening adverse events may require immediate reporting and follow-up. We anticipate most adverse events will be mild and the subject will be able to resume activities within a day or two of reporting the event. Adverse Event reporting will follow the requirements of the IRB. Only adverse events that qualify as unanticipated problems will be reported to the IRB. Unanticipated problems involving risks to subjects or others include incidents only if the incident is unexpected, related or possibly related to participation in the research, and indicated that subjects or others are at a greater risk of harm than was previously known or recognized.

Withdrawal of Subjects. Subjects could be withdrawn from the study if the Dr. Irving (PI) or Dr. Greenway (Medial Investigator) feel that doing so would be in the subject's best interest medically. Subjects could be withdrawn for lack of compliance to the protocol. The IRB and/or sponsor could stop the trial early. Any Subject can withdrawal upon request.

Vulnerable Populations. No vulnerable populations are involved in this study.

Sharing of Results with Subjects. The results of the tests done in the study will be analyzed and published in a medical journal. The results will be summarized and shared with the study subjects. After the study, individual results will be made available to the study subjects and, at their request, with their primary physician.

Incidental Findings. We will follow the recommendations of a Presidential panel in handling incidental findings on MRI scans [46]. First, we indicate to the subject whether or not there is a well-established set of incidental findings for the scan they are undertaking, and if so what those incidental findings are. For scans without a well-established set of incidental findings, we will provide a list of incidental findings that we feel may be possible. We will then describe the difference between clinically actionable incidental findings and non-clinically-actionable incidental findings. We will then ask the subject to decide whether they want to be informed of non-clinically-actionable incidental findings. Subjects will be told that informing them of clinically-actionable incidental findings is required for participation in the study. In the event that study personnel identify MRI scan abnormalities, they will consult with a radiologist who will determine the clinical relevance of the abnormalities. Subject identity will not be shared with the radiologist in this event. If the radiologist determines that an MRI scan abnormality is relevant to personal health, the radiologist will then determine whether the finding is clinically actionable. In the event that the finding is clinically actionable, or if the subject consented to be informed of non-clinically actionable incidental findings, study personnel will provide the information to the study Medical Investigator so that he can discuss the relevance of the finding with the subject. In the event of an incidental finding that is to be released to the subject, the imaging findings flow from the study staff, to a radiologist, to the Medical Investigator who explains the findings with the subject.

4. Potential Benefits of the Proposed Research to the Study Subjects and Others.

The proposed studies will advance our understanding of the potential beneficial effects that LPA breaks in sedentary time have on RE induced improvements in muscle and cardiometabolic health in older adults. At study completion, we expect to have demonstrated that LPA breaks in ST enhance RE induced improvements in muscle and cardiometabolic health in older adults. Combined RE and LPA (REALPA) breaks in ST may be a more practical long-term exercise regimen to improve muscle and cardiometabolic health in older adults than more traditional exercise regimens that combine RE with moderate intensity AE, especially among those with chronic medical conditions. We anticipate that subjects participating in all three exercise regimens (RE only, REALPA, RE+AE) will receive some improvement in their muscle and cardiometabolic health. Moreover, using

state-of-the-art methodologies we will also comprehensively assess the impact of all three exercise regimens on muscle and cardiometabolic health, including muscle and immune cell mitochondrial oxidative capacity and whole-body glucose homeostasis during a MMTT.

In our opinion, the risks are justified by the expected knowledge gained from this study.

5. Importance of the Knowledge Gained.

Physical inactivity and excessive sedentary time are major public health concerns affecting millions of US older adults. Physical inactivity and excessive sedentary time contribute to several of the chronic conditions commonly afflicting older adults, including insulin resistance, type 2 diabetes, cardiovascular disease, sarcopenia/dynapenia, mobility disability, and frailty. Moreover, few older adults meet the current public health guidelines for aerobic exercise (AE) or resistance exercise (RE) based physical activity. The current public health guidelines also do not adequately address the potential health benefits of breaking up sedentary time with light intensity PA (LPA), especially when combined with RE. We recently demonstrated that combined exercise (CE) consisting of whole-body RE plus moderate-intensity AE is as or more effective at improving muscle strength, mass, quality, and OXPHOS capacity than either treatment alone in young and older adults despite lower training volumes.[15] However, the impact that RE combined with LPA breaks in sedentary time has on muscle and cardiometabolic health in older adults remains unknown. Moreover, the NIA is also currently interested in identifying non-pharmacological approaches to improve muscle and cardiometabolic health in older adults, because polypharmacy associated with treating age-related medical conditions is significant health concern. Thus, to address this gap in knowledge, we propose a pilot study of our novel, non-pharmacological, intervention that combines REALPA breaks in sedentary time to improve muscle and cardiometabolic health in older adults. REALPA breaks in sedentary time may be a more practical long-term exercise regimen to improve muscle and cardiometabolic health in older adults than more traditional exercise regimens that combine RE with moderate intensity AE, especially among those with chronic medical conditions. We will leverage the results from this study to develop a *highly competitive NIH R01* to test the efficacy of our novel exercise intervention to improve muscle and cardiometabolic health in older adults. Preservation of muscle and cardiometabolic health in older adults will have a *positive impact* on the quality of life and health span of the US's aging population, which addresses a priority of the National Institute of Aging.

SETTING

The study will be done at LSU by Drs. Brian A. Irving, PhD, (PI), Guillaume Spielmann, PhD, (CoI), and Neil Johannsen, PhD, (CoI) in the School of Kinesiology and by Dr. Frank Greenway, MD (CoI and Medical Investigator) at Pennington Biomedical Research Center who will provide medical oversight. Dr. Steven Heymsfield, MD, Owen Carmichael, PhD, and Nick Broskey, PhD are at Pennington Biomedical Research Center and will also serve as collaborating investigators on this protocol.

RESOURCES AVAILABLE

The LSU School of Kinesiology and Pennington Biomedical Research Center has all the equipment necessary to conduct this study and has expertise in the methods described in the protocol. The greater Baton Rouge community has between 30-40,000 adults 65 years of age or older. We will identify potentially eligible study subjects via advertising. Specifically, we will recruit potential subjects from advertisements placed on the bulletin boards, electronic bulletin boards, social media, and in local and regional newspapers (e.g., the Advocate, the LSU Reveille,) and magazines. We will advertise on regional radio stations as needed. We will post flyers in senior housing establishments as well as attend senior expos. All recruitment materials will be approved by the IRB prior to their use.

PRIOR APPROVALS

This study will be sponsored by National Institute of Aging.

COMPENSATION

We will compensate subjects $500 for completion of the study. If subjects do not complete the entire study, subjects will be compensated $100 for completion of the following visits: pretraining V1, pretraining V2,

posttraining V3, posttraining V4, posttraining V5. Subjects will not be compensated for screening visits or the supervised exercise visits. Subjects who complete the optional C13 Magnetic Resonance Spectroscopy procedure will be compensated an additional $50. The check will be requested from the LSU Payroll Department and will be directed to the LSU School of Kinesiology when the subject completes the study or at the appropriate milestone if the subject is compensated during the course of the study. It usually takes about 3-4 weeks for it to arrive at Louisiana State University.

PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF SUBJECTS

All discussions will be held with study subjects in examination rooms to protect their privacy. The study will be published, but the subjects will not be identified. The study will be explained verbally as well as on the informed consent document. Each subject's signed informed consent document will be kept in subject's record securely stored within PBRC for possible inspection by regulatory authorities or by the Sponsor. The subject will receive a copy of the written informed consent document once he/she has signed. Any written records collected at PBRC and/or PBRC will be kept in the secured as previously described.

COMPENSATION FOR RESEARCH-RELATED INJURY

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center or LSU. In the event of injury or medical illness resulting from the research procedures in which a subject participates in, the subject will be referred to a treatment facility. Medical treatment may be provided at the subject's expense or at the expense of his/her health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should a subject require ongoing medical treatments, they must be provided by community physicians and hospitals.

ECONOMIC BURDEN TO SUBJECTS

If subjects are assigned to an intervention group that requires text messaging/email/reminders the subject will be responsible for his/her own personal data and messaging rates.

CONSENT PROCESS

Written informed consent will be obtained from the subjects taking part in this study. The consent process will be initiated prior to screening by Drs. Irving, Spielmann, Johannsen, Greenway or IRB approved personnel but the process will continue throughout the study and subjects will be encouraged to ask any questions they may have. If subjects wish to take the consent home for further reflection or to discuss with their family or counselors, this will be permitted.

DRUGS OR DEVICES

No drugs or devices are being tested in this study.

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