**Official Title:** Interactive roles of cardiorespiratory fitness and adiposity on recovery of impaired glucose and vascular control after physical inactivity

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## 1. Significance

### 1.1 Purpose Statement

Our primary purpose is to examine the interactive role of health status (cardiorespiratory fitness and adiposity) on the ability to restore impaired glucose control following short-term reduced physical activity (RPA). We also seek to examine vascular function as a mechanism of recovery of impaired glucose control following a return to normal physical activity.

### 1.2 Scientific Premise (Significance)

1.2.1 Reduced Physical Activity and Obesity Epidemics: Physical activity (PA) comprehensively improves overall health in a manner that cannot be duplicated pharmaceutically (1, 2), whereas physical inactivity weakens the function of multiple organ systems and accelerates chronic disease risk, functional decline, and premature mortality (1). Unfortunately, 25% of adults in the United States (CDC) are physically inactive, and most adults spend 9+ hours a day being sedentary (3). RPA is now considered the 4$^{th}$ leading cause of death (4). The rapid 28% increase in obesity prevalence since 2008 is also alarming (5). Currently, over 40% of the US population is considered obese and at high risk for many preventable pathologies (5). A primary driver of this impaired health with obesity is increased adipose tissue, specifically visceral adipose tissue (VAT), altered cardiometabolic function, and impaired glucose control (5). Together, RPA and obesity contribute to an annual healthcare burden of over $1 trillion and the deaths of millions (5). Although the adverse effects of RPA and obesity are becoming more recognized, there are no signs of the resolution of these epidemics. Impaired cardiometabolic health (which contributes to elevated chronic disease risk) is a consequence of both obesity and RPA. Although obesity is well studied, the mechanisms behind reversing RPA-induced health issues, particularly metabolism and vascular control (discussed later), are poorly understood.

1.2.2 Reduced Physical Activity and Metabolism: RPA (~<1.5 times resting metabolic rate) initiates several physiological changes, including loss of muscle mass and strength, reduced central and peripheral cardiovascular function, and metabolic dysfunction (4). These effects occur at different rates, but metabolic dysfunction and some cardiovascular changes may occur rapidly (i.e., within hours and days) (6). The more immediate cardiovascular changes are decreased plasma and stroke volumes, reduced blood flow to the tissues attributed to increased arterial stiffness, and decreased vascular endothelial function (1). RPA ultimately causes a reduction of energy demands from the skeletal muscle due to the lack of use (1). Reduced use results in fewer changes in circulation to the muscle and reduced substrate utilization characterized by less blood glucose and lipid uptake and oxidation. This results in less turnover of substrates and less insulin sensitivity (1). The mechanisms of diet-induced cardiometabolic dysfunction have been thoroughly studied, but the causes of RPAinduced insulin resistance (IR) are not as well understood (6). While several primary mechanisms responsible for diet-induced IR have been identified, these mechanisms may not play a significant role in the initial cardiometabolic dysfunction induced by RPA, especially concerning blood glucose control. This implies that the primary mechanism responsible for the initial RPA-induced metabolic dysfunction is yet to be elucidated. Unlike nutrient-induced IR, RPA-induced changes in blood glucose control occur much more rapidly (6, 7). Yet these RPA-induced changes are reversible with a return to normal physical activity or exercise in most healthy people (6, 7). If the detrimental effects of RPA were not rapidly reversible with the resumption of physical activity, the prevalence of cardiometabolic disease would be astronomical. Fortunately, most evidence demonstrates that metabolism is restored with physical activity in healthy individuals, but the exact reasons are unclear. Evidence does indicate that RPA accelerates the development of metabolic syndrome (8) and that inconsistent patterns of physical activity are associated with a greater risk for metabolic syndrome (9, 10). Indeed, specific apparent periods, such as winter seasons (11, 12), holidays (13, 14), and illness/injury (7), are associated with increased sedentary behavior (11, 15). During these periods of RPA, individuals rapidly develop impaired blood glucose control (described above), followed by adverse changes in body composition (e.g., increased fat mass and potentially reduced lean mass) (16) and eventually weight gain. This has been demonstrated with holiday weight gain (17).

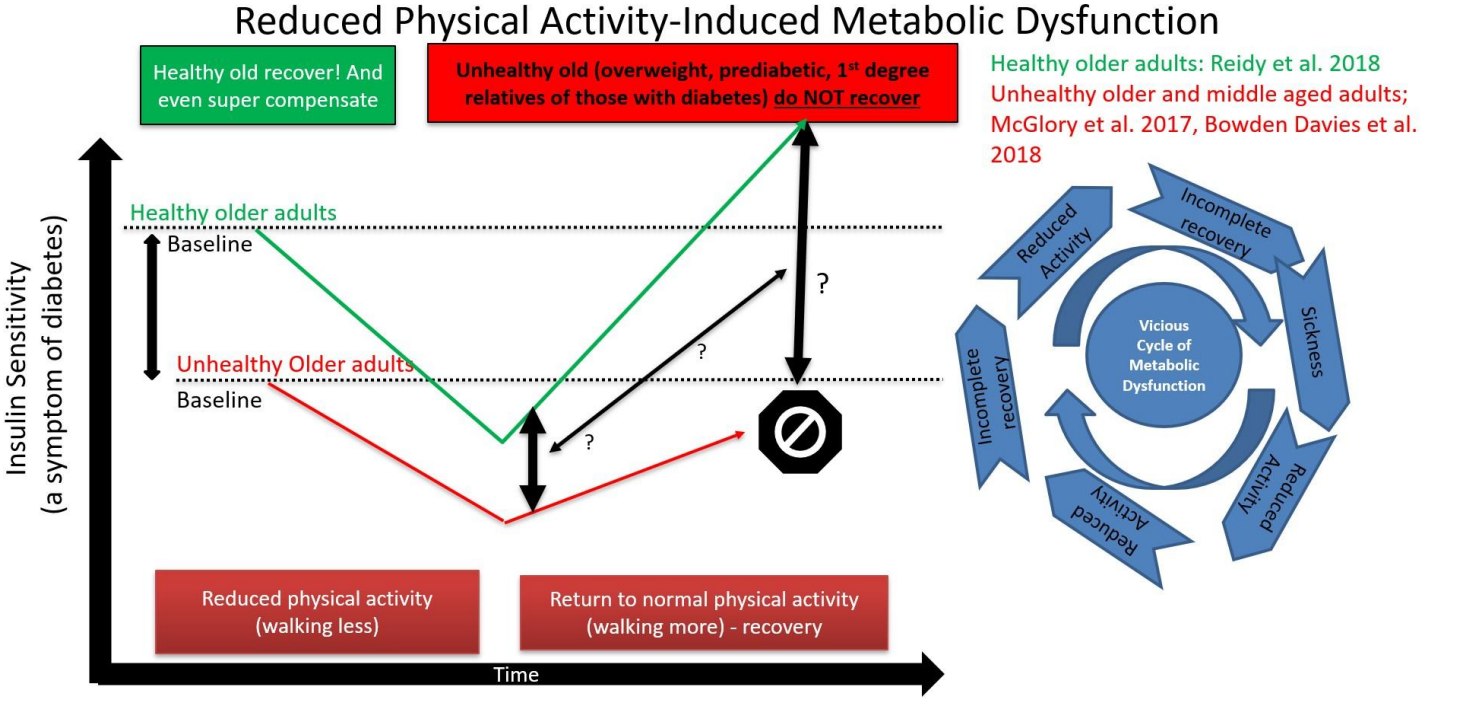
1.2.3 Effect of CRF and Adiposity on Health

Cardiorespiratory fitness (CRF) is well recognized as the most significant predictor of premature mortality and CVD and a strong predictor of metabolic disease (18). Rather than BMI-assessed obesity, adiposity is emerging as a better indicator of poor health (19) and risk of CVD (20), metabolic disease (21, 22), and mortality (23, 24). CRF and adiposity may be interactive. Low CRF is a predictor of developing abdominal obesity (25). This state of low CRF and high adiposity could further worsen an individual's health and has been classified as metabolically unhealthy obesity (MUO). High CRF - Low adiposity (HCLA) individuals are likely at the lowest level of health-related risk. High CRF - High adiposity (HCHA) individuals have been classified as metabolically healthy obese (MHO). Although they have more risk than HCLA individuals, they maintain some protection against cardiometabolic disorders compared to Low CRF - High Adiposity (LCHA) individuals and maybe even Low CRF - Low Adiposity (LCLA) individuals for some health conditions (26). The HCHA condition may be relatively healthier but is considered an unstable transition state towards LCHA and a greater health risk (26– 29). The health stability of the HCHA condition seems to be linked to the conditional low presence of central adiposity and active engagement in MVPA (26). How CRF and adiposity interact to impact the metabolic response and recovery following RPA remains unknown but could be vital to our understanding of cardiometabolic disease progression.

1.2.4 Effect of CRF and Adiposity Health Status on Recovery of Metabolism Following Physical Inactivity:

The time course of development of these symptoms of impaired glucose control in response to RPA is unknown, especially in the context of divergent cardiometabolic states and health conditions like CFR or adiposity, on their own or in combination. What also remains unknown is the time course over which these changes return to the pre-inactivity condition, if they do at all. Using the hyperinsulinaemic-euglycaemic clamp to measure IR, KroghMadsen et al. have shown a 17% reduction in insulin sensitivity after two weeks of 85% step reduction in young adult males (30). We demonstrated similar findings in a small cohort of healthy older adults experiencing 67% step reduction (31). Interestingly, this older but healthy cohort displayed a super-compensation of their insulin sensitivity during a two-week return to normal physical activity levels (recovery). Specifically, following the twoweek return to normal activity, their insulin sensitivity was greater than before starting the step reduction (31). The reasons for this super-compensation are unknown.

**Figure 1.** Schematics demonstrate that the negative reduction and recovery of insulin sensitivity due to reduced physical activity are potentially different by the initial level of health.



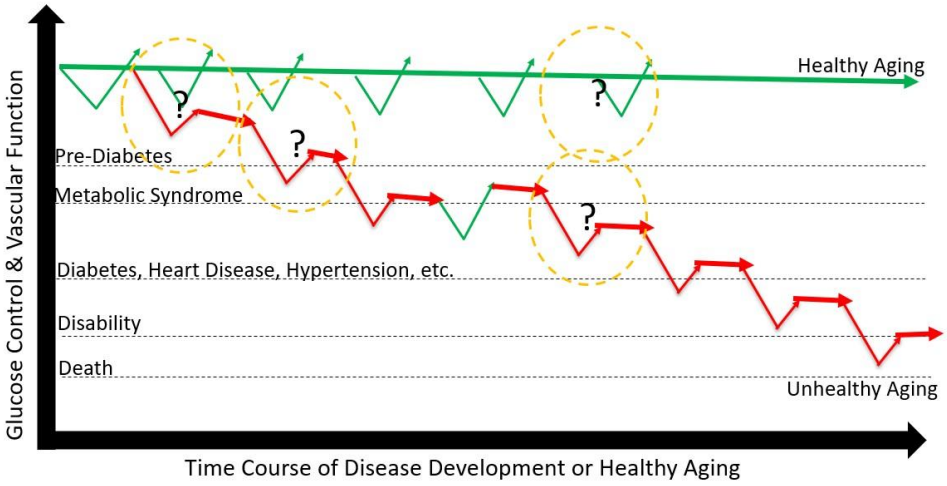
In contrast, relatively unhealthy individuals, such as older adults with pre-diabetes (32) and first-degree relatives of those with diabetes (33), do not fully recover their insulin sensitivity after 2-weeks of recovery (return to normal patterns of ambulation) following a period of step reduction. Interestingly, Henrickson and Tischler mentioned unpublished findings that super-compensation of muscle blood glucose uptake during reloading was attenuated in diabetic animals compared to controls (34). Thus, the evidence suggests that those individuals with compromised cardiometabolic health have impaired recovery of blood glucose control following inactivity (**Figure 1**). Yet, the recovery period in the above human studies showed this disparity only two weeks after returning to normal physical activity. We have no information on glucose control during other time periods following a return to normal physical activity in those with divergent health statuses.

1.2.5 Potential Acceleration of Cardiometabolic Disease by Incomplete Recovery from Physical Inactivity?

Metabolic disease may be hastened by the inability to recover from periods of transient insulin resistance like physical inactivity. Individuals with compromised health have impaired recovery of blood glucose control following inactivity (32–34) (Figure 1). This implies an inability to reverse RPA-accelerated cardiometabolic dysfunction and, thus, a more rapid progression toward the development of cardiometabolic disease in these atrisk populations **(Figure 2**). Investigations to elucidate the time course of the development of cardiometabolic dysfunction following inactivity and the recovery of cardiometabolic function after resumption of normal activity may provide inroads towards delaying or even preventing the progression of cardiometabolic diseases, especially in more susceptible populations.

**Figure 2.** Schematic of the hypothetical impact of periods of reduced physical inactivity during the life course on metabolic and vascular health between healthy and unhealthy (those with glucose intolerance). The healthy person is able to recover from bouts of reduced physical inactivity and maintain their metabolic and vascular health. However, the person with failing health does not fully recover from periods of reduced physical activity, which accelerates their progression toward cardiometabolic diseases.

1.2.6 Link Between Vascular and Metabolic Function: Although the link between vascular and metabolic function is well established, it is not well understood in the context of RPA. The signaling pathways regulating blood glucose uptake and endothelial nitric oxide (NO) production are very similar and potentially interactive (35). Insulin is a stimulator of endothelial NO synthase to promote perfusion and dysregulated insulin signaling, which leads to increased production of the vasoconstrictor endothelin1(ET-1) (35, 36). Increased blood flow to contracting muscle elevates arterial shear stress, stimulating NO production to enhance blood flow further. NO-dependent blood flow contributes 20-40% of insulin-stimulated blood glucose uptake. Thus, in an endothelium-dependent manner, insulin directs the delivery of blood glucose via the increase of capillary surface area for glucose transport (37). An imbalance between ET-1 and NO production decreases blood flow, worsening insulin resistance (38). The relationship between vascular function and insulin sensitivity is complementary in that enhanced insulin signaling and sensitivity improve vascular function to reduce insulin resistance. We propose this link is an initial regulator of RPA-induced cardiometabolic dysfunction and its restoration upon the resumption of normal activity.




Although vascular function has been shown to be impaired during RPA, the exact mechanisms behind these changes are unconfirmed. Reductions in shear stress-induced NO production may explain the observed vascular

and metabolic dysfunction with RPA. Studies in healthy adults show that transient RPA impairs vascular function. Specifically, 1.5 to 3 hours of sitting (a model of transient RPA) blunts the largely NO-dependent reactive hyperemic response (39, 40) induced by passive leg movement (PLM) (41–43) demonstrating the detrimental effects of RPA on the lower limb vasculature. The reactive hyperemia-induced shear stress resulting from PLM has not been examined in the context of more prolonged exposure to RPA or the recovery from RPA. Further, 3 hours of sitting negatively affects central arterial (i.e., aortic) stiffness as shown by an increase in carotid-femoral pulse wave velocity (cf-PWV) (44, 45), demonstrating that RPA has broader effects on vascular function beyond the lower limb vasculature. However, the generalizability of these findings remains limited to healthy individuals transiently (i.e., up to 3 hours), reducing their PA. To our knowledge, no studies have investigated the effects of decreases in daily PA (and resumption of normal PA) on PLM-induced reactive hyperemia and central arterial stiffness in individuals differing in adiposity and CRF. Studies are needed to determine whether the adverse vascular effects of RPA recover upon resumption of normal PA in at-risk (i.e., high adiposity, low fitness) populations. These data would provide support for the development of interventions to decrease RPA and improve health outcomes.

1.2.7 Supporting Data: Our team of undergraduate and graduate research students generated pilot data from a few participants. For this study, we placed a physical activity monitor and continuous glucose monitor (CGM) on three participants during a one-week run-in of normal physical activity (baseline) followed by a week of RPA and then a week of return to normal physical activity (recovery). During the RPA week, we provided an electric scooter to the participants to enable them to reduce their physical activity by ~2000 steps per day from their normal activity of ~9000 steps per day. Using the CRF and adiposity cutoffs described above, we classified these participants as HCLA, LCLA, and LCHA. Using CGM, the participant's mean 24-hour glucose increased in the RPA week compared to the run-in week (baseline) (**Figure 3**). Interestingly, mean 24-hour glucose remained elevated in the recovery week and was highest in the LCHA individual, suggesting a worsening rather than recovery of glucose control.

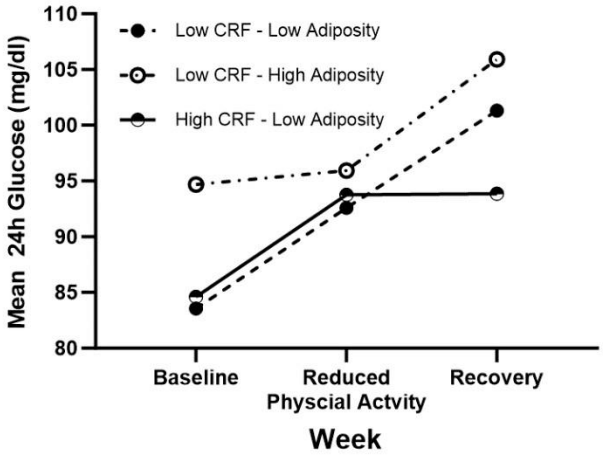



**Figure 3.** Daily 24-h mean glucose for a week before, during and after a week of physical inactivity.

## 2. Innovation

The core innovations of our proposed studies are based on our novel explanation underlying the recovery of glucose control and vascular function following RPA. We designed a study adopting important methodological innovations that will explore the effects of alterations in physical activity on cardiometabolic health outcomes in at-risk groups. Each of these innovations is detailed below:

2.1 Examination of the **interactive effect** of important health indicators **(CRF and adiposity)** on the **recovery of glucose control and vascular function following RPA**: CRF and adiposity have been shown to impact

numerous health indicators (18–24). Yet the impact of CRF and/or adiposity has never been examined as factors regulating the role of recovery from RPA-induced impairment of glucose control and vascular function. Our novel study design allows us to isolate the separate and combined effects of CRF and adiposity on recovery of RPAinduced impairment of glucose control and vascular function.

2.2 Examination of the **passive leg movement (PLM) as a novel and physiologically relevant approach to assessing vascular function** following RPA and during recovery of RPA: Passive leg movement (PLM) is a technique that induces reactive hyperemia via mechanical deformation and stretch of the skeletal muscle to assess resistance vessel function. Studies show that PLM-induced reactive hyperemia is blunted following 1.53 h of sitting, a model of transient RPA (46). We propose the NO-dependent PLM technique is a physiologically relevant approach to examine the link between glucose control and vascular function. Furthermore, the PLM technique has not been extended to longer RPA interventions or those with divergent health conditions.

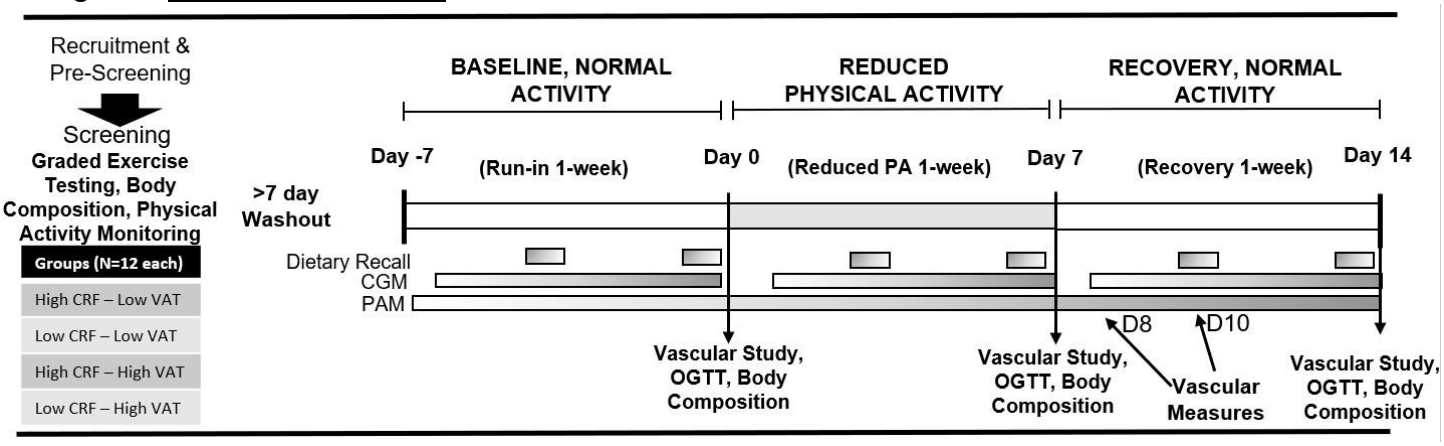
2.3 Use of Continuous Glucose Monitoring to Determine the **Time-Course of Recovery of RPA-induced Glucose Control in a Field Setting**: The studies that infer that differential health status causes a divergent recovery of glucose control following RPA (31–33) all used a timepoint at two weeks into return to normal activity. The time course of recovery by health condition is a gap in the literature. The use of continuous glucose monitoring will allow for 24-hour monitoring of glucose control for three consecutive weeks. This will enable us to determine how rapidly RPA impairs glucose control and how quickly glucose control is recovered daily (**Figure 4**). This rarely utilized ecological approach combined with laboratory OGTT assessment will provide a comprehensive evaluation of glucose control.

## 3. Approach

3.1 Specific Aim 1: **To determine the interactive effect of CRF and adiposity on recovery of impaired glucose control after RPA.** We hypothesize that 7-d of RPA (>2000 fewer steps + 10% more sitting daily) will impair GC and that recovery of GC will be delayed in those with lower CRF and/or higher adiposity (high CRF/low adiposity > high CRF/high adiposity > low CRF/low adiposity > low CRF/high adiposity). To test this, we will examine GC with continuous glucose monitoring and oral glucose tolerance tests during 7-d of normal PA, 7-d of RPA, and 7-d of resumption of normal PA with N=12 (6 male and six female) adults in each of the four groups.

3.2 Specific Aim 2: **To determine the interactive effect of CRF and adiposity on vascular function improvements during physical activity recovery as a mechanism of recovered glucose control.** We hypothesize that 7-d of RPA will impair vascular function and that individuals with lower CRF and/or higher adiposity will have prolonged RPA-induced impairment of vascular function during the 7-d recovery period. To test this, we will assess passive leg movement (PLM)-induced hyperemia and arterial stiffness during 7-d of normal PA, 7-d of RPA, and 7-d of resumption of normal PA. We expect these studies to demonstrate that differences in the recovery of impaired vascular function after RPA mediate the beneficial cardiometabolic effects of resumption of normal physical activity.

**Figure 4** Clinical Study Design



3.3 Methodology: We propose the design shown below in **Figure 4** with undergraduate student researchers mentored by Drs. Reidy, Ballard, and Timmerman, with support from graduate research assistants from the Department of Kinesiology, Nutrition, and Health (KNH) at Miami University. Study Design: With assistance from a clinical study recruiting firm (TrialFacts), we will recruit, screen, and enroll eligible participants (18-40y) across the following four groups: high CRF/low adiposity; high CRF/high adiposity; low CRF/low adiposity; low CRF/high adiposity. We will prescreen interested participants to narrow the selection group to find eligible participants. Potential participants who passed the prescreening will be given an activity monitor to wear for five days and scheduled for a screening of VAT with body composition testing and CRF via a treadmill-graded exercise test (47, 48). Final participants will be selected based on the American Medical Association cutoff for low and high CRF is <9 and >14 METS (18) and the cutoff for low and high VAT ( <130 $cm^2$ or ≥130 $cm^2$ for men and <100 $cm^2$ or ≥100 $cm^2$ for women) (49). We anticipate the possibility of sex-based differences in older adults (31). So, in this population of young adults, we will have each group split N=6 male and N=6 female with collected information on menstrual status in females. Each participant will undergo a three-week study. For the first week (day -7 to day 0), participants will be instructed to maintain their normal level of physical activity. For the second week (day 0 to day 7), the RPA week, participants' schedules will be examined, and strategies designed to reduce steps (by at least 2000 steps) and physical activity (by taking elevators and short-cuts, etc.) by increasing sitting time >10%. To assist in reducing walking and increased sitting, participants will be given seated electric scooters to use whenever they normally walk. The scooters will be returned on the third week (day 7 to day 14), and participants will be instructed to resume their normal physical activity. During the entire three weeks, participants will wear a continuous glucose monitor (CGM) and a physical activity monitor (ActivPal). Dietary records are recorded by participants on a weekday and a weekend day for three weeks with the National Institute of Health NIH Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool. At the end of each week (i.e., on days 0, 7, and 14), participants will arrive at the laboratory to assess body composition (BIA), popliteal and femoral artery shear stress, and blood will be collected before and during an oral glucose tolerance test. On days 8 and 10, popliteal artery shear stress will be assessed, and fasting blood will be gathered.

3.4. Outcomes and Analyses:

*RPA and activity monitoring:* As part of the screening assessment, physical activity, transportation levels, and patterns that could be adjustable and reduced with electric scooter use will be determined. To modify physical activity sufficiently, participants must have a baseline physical activity of >7000 steps and walk outside a mile or more daily. Suppose the team determines that it is feasible to modify the participant's physical activity with electric scooter use and strategic adjustment to their activity patterns. In that case, they will be included in the study. The participants will be counseled on strategies (using elevators, altering routes of travel, etc.) with and without the scooter to reduce their steps during the RPA week. Also, during the RPA week, a team member will contact the participants daily to assist and strategize to ensure the reduction goals for steps (>2000 steps per day reduction) and physical activity (increased sitting time by 10%) are met. Each participant will be given a wearable activity monitor to assist with this goal and provide visual feedback on steps and sitting time. After enrollment, each subject will wear an accelerometer (ActivPal, Company, Model) for three weeks (Day -7 to Day 14). The accelerometer will be scheduled to start at midnight on Day -6. The device will be checked weekly to verify it is recording. On the morning of the final day, the accelerometer will be removed. Data processed through

PALanalysis (company. Version 8.11.8.75) to calculate the number of steps, sitting time, stepping time, primary lying time, secondary lying time, total sedentary time, and activity score (week 1 = Run-In, week 2 = Scooter, week 3 = Recovery). Steps, seated transport time, and sitting time will be calculated per hour per day each week. To calculate the number of steps per hour and transport time per hour per day of each week, the data will be exported in PALanalysis v8.11.8.75 with 1-hour Epochs. Days with less than 1000 min of wear time will be excluded. To track the electric scooter use during the RPA week that the scooter is provided, participants will be instructed to GPS log their scooter use on their phone with a commercial phone application in private mode.

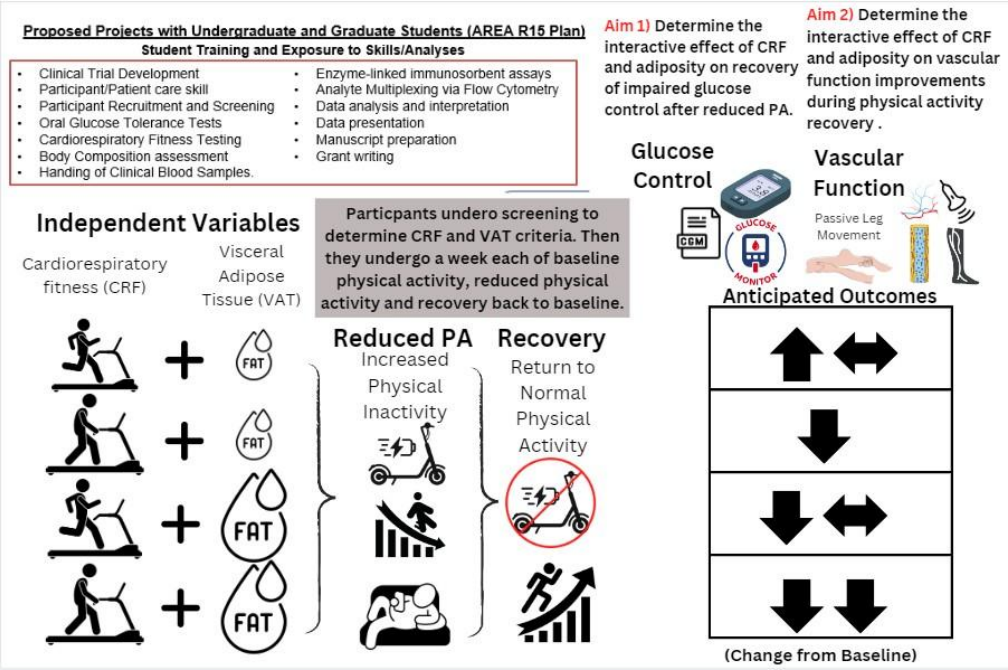



**Figure 5.** Overall Project Summary Highlighting Training Opportunities/Outcomes, Specific Aims and Hypotheses.

*Oral glucose tolerance test (OGTT):* Participants will be asked to consume their habitual diet the day before their first OGTT (Day 0) and keep a detailed food log. Participants will be instructed to consume the same foods the day before the remaining two OGTT trials (Days 7 and 14). Food logs will be assessed for energy and nutrient intake using the ASA24. On the morning of each OGTT, participants will arrive in the lab following an 8–10 h fast. An indwelling intravenous catheter will be placed into a forearm vein. Baseline (0 min) blood samples will be collected into heparinized and serum separator evacuated tubes following 15 min of seated rest. Participants will consume a volume of glucose tolerance beverage (TRUTOL, Fisher Diagnostics) containing 1 gram of glucose·kg−1 of lean mass to control for expected differences in body and fat mass between individuals. Participants will remain rested in the reclined position for the duration of the OGTT. Blood samples will be collected at 30, 60, 90, and 120 min post drink. Heparinized blood samples will be utilized to assess blood glucose in real-time during the OGTTs using the Contour Next blood glucose monitoring system (Ascensia Diabetes Care, Parsippany, NJ). Following collection, serum separator tubes will be allowed to clot (30 min, four °C) and then centrifuged (1450 x g, 15 min, four °C). Samples will stored at −80°C until analysis for PAI-1, Insulin, Leptin, and cortisol concentration with the LEGENDplex™ Human Diabesity Panel (Biolegend, San Diego, CA) on an Attune Flow Cytometer (Invitrogen, Carlsbad, CA). We have previously conducted OGTTs (50, 51).

*Body composition*: Body Composition will be determined using bioelectrical impedance (INbody 720, Company, City) and dual x-ray absorptiometry (Norland Elite, Swissray, Mahwah, NJ) on days 0,7, and 14. The scans will be standardized by having participants wear the same clothes, following the dietary instructions (mentioned above), and voiding the bowel and bladder right before the test. Participants will be asked to consume a glass of water at 10 pm and fast overnight to control for the influence of fluid and food intake on these measurements.

*Continuous glucose monitoring:* Participants will wear CGM (Freestyle Libre Pro, Company, City) sensors for the duration of the study, with the first sensor being placed on day -7 and a second sensor replacing it on day 0. After the skin is cleaned with an alcohol wipe, sensors will be put on the back of an arm. Care will be taken to

select the arm/side that the participant does not sleep on. To prevent falsely altered sensor glucose values, participants will be screened and instructed to avoid using salicylic acid products, take supplements with more than 500mg of ascorbic acid and acetaminophen, and refrain from lying on the sensor. The sensors collect data every 15 minutes, beginning the hour after the sensor is placed. Data on the first day of wear time will be excluded due to the potential of lower, less accurate results (52, 53). Data will be exported from the sensors using Freestyle Libre Pro software. Before beginning data analysis, a quality check in the following will be conducted: 1) any CGM readings <50 mg/dL during sleep time as defined by ActivPal laying down; 2) 3 or more consecutive readings of <50 mg/dL flanked by CGM readings of >80 mg/dL within 30 minutes before or after the string; 3) 3 or more consecutive readings in which readings of >200 mg/dL are interspersed with readings of <70 mg/dL. If the data meets these criteria, those points will be excluded or averaged using surrounding data points. Data will be excluded if two or more sequential values meet the previous criteria and averaged if only one value meets the criteria. Data will be compared with blood glucose levels collected during the fasting blood sample for calibration between differences in interstitial glucose levels (as collected by the CGM) and blood glucose levels. Quality-checked data will then be imported into the IGlu Application in R Studio and analyzed using the following parameters: Night hours set from 10 PM to 7 AM; Time above (high) set to 140 mg/dL; Time above (low) set to 120 mg/dL; Time in range set to 70-120 mg/dL; Time below (high) set to 70 mg/dL; Time below (low) set to 54 mg/dL; Data reported using this software includes weekly and daily means, standard deviations (SD), coefficient of variations (CV), medians, time below range < 54 mg/dL (TBR <54), time below range < 70 mg/dL (TBR <70), time in range 70-120 mg/dL (TIR 70-120), time above range >120 mg/dL (TAR >120), time above range >140 mg/dL (TAR >140), mean amplitude of glycemic excursions (MAGE), and area under the curve (AUC).

*Femoral artery blood flow (shear stress):* Passive leg movement (PLM)-induced reactive hyperemia will be measured to assess peripheral vascular function. Briefly, the PLM technique involves passive movement of the leg to elicit a reactive hyperemic response measured by high-frequency ultrasonographic imaging. Following published technical guidelines (54, 55), diameter and blood velocity in the common femoral artery will be measured following 15 minutes of seated rest using a 5 to 12MHz multi-frequency linear array transducer connected to a high-resolution ultrasound (T3300; Terason, Burlington, MA). Arterial diameter and velocity will be recorded continuously for 60 seconds at baseline and 60 seconds during PLM. The PLM technique involves a research team member passively moving the participant's leg through a 90° range of motion at a rate of 60 movements per minute. The participant's skin will be marked, and a photo will be taken to ensure the transducer is consistently placed throughout the study visits. Offline analyses will be performed using automated edgedetection software with end-diastolic gating (Medical Imaging Applications, Iowa City, IA). Femoral artery blood flow and shear rate (estimate of shear stress) will be calculated from arterial diameter and blood velocity measurements. PLM-induced reactive hyperemia across intervention days will be measured by Dr. Ballard, who has significant experience in vascular function testing.

*Central arterial stiffness and blood pressure:* Carotid-femoral pulse wave velocity (cf-PWV) and pulse wave analysis (PWA) will be measured using the SphygmoCor XCEL system (AtCor Medical, Naperville, IL) following 10 min of rest to assess central arterial stiffness and blood pressure, respectively. PWA involves using a brachial cuff to measure peripheral blood pressure and waveforms. Central waveforms are deducted using a transfer function built into the Sphygmocor system. These waveforms are evaluated to obtain aortic systolic and diastolic pressure, mean arterial pressure, and aortic pulse pressure. Augmentation pressure is calculated, and augmentation index (AIx) is reported as a percentage of the central pulse pressure. Arterial PWV and PWA across intervention days will be measured by Dr. Ballard, who has significant experience in vascular function testing.

*Cardiorespiratory fitness testing*: On the screening day, participants will perform a Balke-graded exercise treadmill test to assess cardiorespiratory fitness (maximal oxygen consumption, VO2 max) (47, 48). This test will be conducted on a treadmill and lasts between about 10 and 20 minutes. Heart rate (Polar T31 HR monitor (Kempele, Finland)), oxygen consumption (via indirect calorimetry, TrueOne® 2400, ParvoMedics, Sandy, UT), and rating of perceived exertion (RPE) will be monitored every minute during the test.

*Blood collection and analysis:* A blood sample will be collected during fasted conditions on Days 0, 7, and 14. Participants will be instructed to refrain from eating or drinking anything besides water after 10 pm the night before their visit. A 40ul whole blood sample will be analyzed using an Alere Cholestech LDX (Alere San Diego, Inc., San Diego, CA) and an Alere Cholestech TC HDL GLU Cassettes to analyze total cholesterol (TC), highdensity lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and blood glucose (GLU).

3.5.1 Statistical Analyses: Our primary goal (Aim 1) is to characterize how glucose control changes in participants differing by level of CRF and adiposity (group) during the resumption of normal physical activity (recovery) following a week of RPA. Our next goal (Aim 2) is to determine the impact of vascular shear stress as a mechanism of improved glucose control during physical activity recovery by the level of CRF and adiposity (group).

Our primary outcomes are CGM derived 24hr glucose Mean, Median, SD, CV, MAGE, Time above range (TAR) >120, >140, Time below range (TBR) <54, <70, Time in range (TIR) 70-120, and vascular shear stress. Secondary outcomes are step count, sitting time, standing time, dietary intake of energy and macronutrients, lean body mass, fat mass, visceral adipose tissue, central arterial stiffness, and blood pressure. For all outcomes, assumptions of residual normality, variance homogeneity, and sphericity will be checked and verified.

For Aim 1, the effects of glucose control (mean, median, variability measures, and time in and out of range) will be tested with a three-way ANOVA with between-subjects factors of CRF (high/low), adiposity (high/low), and a within-subjects repeated measure of time. Dunnet comparisons of (Baseline (day 0) vs. RPA (day 7) and Baseline (day 0) vs. Recovery (day 14) but conditioned on the group (CRF/adiposity combination) will be conducted following appropriate high-order interaction tests to confirm that the time effect changes with the group. If there are no missing values, repeated measures of GLM-based ANOVA will be used. In the case of missing values, a mixed-effects model will be utilized. We hypothesize that 7-d of RPA will impair glucose control and that recovery of glucose control will be attenuated and delayed in those with lower CRF and/or higher adiposity (high CRF/low adiposity > high CRF/high adiposity > low CRF/low adiposity > low CRF/high adiposity). Specifically, we chose a priori to compare baseline week (day 0) vs RPA week (day 7) by group to assess the effect of RPA. Then, we will compare the baseline week (Day 0) vs recovery week (day 14) by group to assess group-specific differences in recovery of glucose control. Lastly, as a secondary analysis to test differences by group in CGM variables between days during the recovery week (days 8-14), a Three-Way ANOVA will be used with between-subjects factors of Groups (CRF by Adiposity) and the within-in-subjects repeated measure of time as days. Appropriate post-hoc multiple comparison procedures will be employed to assess significant main or interaction effects.

For Aim 2, we will examine changes in vascular blood flow (shear stress) in response to passive leg movement (PLM) as a mechanism of improved glucose control during physical activity recovery by CRF and adiposity (group) using a similar statistical model. We will also include time points for days 8 and 10 to assess the time course of recovery of vascular function following RPA. Thus, we are measuring vascular function at Day 0 (baseline week), Day 7 (RPA week), Day 8 (recovery day 1), Day 10 (recovery day 3) and Day 14 (recovery day 7). We hypothesize that individuals with lower CRF and/or higher adiposity will have greater and prolonged RPA-induced impairment of vascular function during the 7-d recovery period. To test this, we chose a priori to use appropriately planned contrasts of interaction effects to compare Baseline week (day 0) vs. RPA week (Day 7) to assess the impact of RPA, and then we will compare the day 0 to day 8, 10, and 14 (Recovery week) to evaluate group-specific differences in the magnitude and time course of recovery of vascular control. All figures and statistics will be conducted in Graph Pad Prism (Version 10.) Alpha will be set at $p<0.05$.

3.5.2 Sample Size and Power Calculations: In our preliminary data, we had eight low CRF participants who underwent a week of baseline testing and a week of RPA, followed by a week of average recovery. Their 24hour glucose levels did not return to baseline glucose levels (change of 7.1±7.8 mg/dl from baseline). All the values below were estimates based on your hypothesis. We will use the recovery back to the baseline of mean 24-hour glucose for sample size calculations, as the main goal of this proposal is to investigate the effect of recovery on glucose control. Based on this preliminary data, we anticipate low CRF individuals will have not recovered their

mean 24-hour glucose back to baseline (7.1±7.8 mg/dl high compared to baseline after a week of return to normal activity). We theorize that increased adiposity, in addition to Low CRF, will worsen the response more consistently (10±6 mg/dl change). Previous data suggest healthy adults recover or supercompensate glucose control in the weeks following inactivity (31, 33, 56). Thus, we anticipate no change or a slight improvement from baseline (-2±3 mg/dl) in High CRF, Low Adiposity, and a slight change (5±8 mg/dl) with more variability in High CRF, High Adiposity participants. Using an alpha of 0.05 and 80% power, the anticipated difference between Low CRF High Adiposity and High CRF Low Adiposity could be captured with N=6 per group. The anticipated difference between High CRF High Adiposity and High CRF Low Adiposity could not be captured with n=20 per group. The anticipated difference between Low CRF Low Adiposity and High CRF Low Adiposity could be captured with N=12 per group. Thus, we will use N=12 per group as this could detect potential differences in half of our comparisons and is feasible to complete in the three-year time frame.

3.6 Strategies to Ensure Robust and Unbiased Results: We have carefully considered strategies to ensure robust and unbiased results in designing the proposed study.

1. Pilot experimentation indicates the project is feasible
2. Effective use of seed grant funding through our division at Miami University to successfully pilot all outcomes and demonstrate their robustness when conducted by trained undergraduate student researchers (1.2.7 & 3.3.1)
3. Appropriate sample size calculations (3.5.2)
4. Use a manual of operating procedures (MOOP) that outlines the standard operating procedures for all study aspects.
5. Complete and document the training of study personnel by Drs. Reidy, Ballard, and Timmerman are on MOOP procedures.
6. The research team uses logbooks (reviewed weekly by Dr. Reidy) for all procedures.
7. Having multiple persons trained together on each protocol to ensure someone is always available.
8. Regular equipment calibration (e.g., indirect calorimetry, flow cytometer, etc.).

3.7 Consideration of Relevant Biological Variables: The impact of CRF and adiposity is well understood regarding disease risk (see section 1.2.4), but the effect of these relevant biological variables on recovery of glucose control and vascular function following RPA is not known. Thus, we aim to address that in this proposal. Additionally, we anticipate the possibility of sex-based differences as seen in older adults (31). So, in this population of young adults, we will have each group split N=6 male and N=6 female with collected information on menstrual status in females to adjust for if needed. This will provide preliminary data to conduct sample size calculations for the aims of future grants on sex differences.

3.8 Team We propose maximizing student research opportunities by conducting efficient and comprehensive real-world and laboratory experiments designed to analyze physical activity behavior and physiological and functional biomarkers. These studies will be performed on the Miami University campus with undergraduate research assistants mentored by Drs—Reidy, Ballard, and Timmerman and supervised by graduate research assistants at Miami University.

Dr. Paul Reidy (PI, Miami University) is an expert in human RPA models and insulin sensitivity measures (31, 50, 57, 58).

Dr. Kevin Ballard (Co-PI, Miami University) is an expert in human models of RPA, assessment of glucose control and vascular function (51, 59, 60), and phlebotomy.

Dr. Kyle Timmerman (co-PI, Miami University) is an expert in human models of RPA assessment of glucose control and phlebotomy (51, 59, 61).

Michael Hughes (consultant, Miami University Statistical Consulting Center) will serve as a statistical consultant.

Undergraduate and graduate student researchers: We request funding for two graduate and eight undergraduate research assistants. Given the coordination for this study and the variability of undergraduate schedules, we anticipate needing two graduate students (Exercise and Health Sciences concentration) and

about eight undergraduate students to assist with this research project. The plan for our goal of student engagement is highlighted in section 3.9 below.

3.9 Plan to Engage Students in Meritorious Research in Alignment with AREA Grant Goals: As stated in its mission, **Miami University "values teaching and intense engagement of faculty with students through its teacher-scholar model, by inviting students into the excitement of research and discovery."** This sentiment is further reinforced in the College of Education, Health, and Society: "Students are encouraged to explore ways in which they may learn through direct experience via internships, study abroad, undergraduate research, and other experiential learning options." To ensure the quality of the research and research engagement that encourages careers in biomedical science, students will undergo the Research Readiness Pathway and Research Engagement Pathway, as described below.

Students engaging in this proposed project would progress through a Research Readiness Pathway, including:

1. *Basic IRB Training:* Before participating in the proposed project, all those participating in the research must complete the *Collaborative Institutional Training Initiative (CITI) Training* for education on the ethical conduct of research and policies involved in human research.
2. *Miami University's Responsible Conduct of Research (RCR) Training*: Miami is committed to the development and continued enhancement of sound and ethical research among faculty, staff, and students. This program focuses on research misconduct, data acquisition and management, mentor/trainee responsibilities, human research, conflict of interest commitment, publication practices, and responsible authorship.
3. *Laboratory Safety Training*: All students participating in this research project will go through Miami University's Laboratory Safety Program Training. This program focuses on preventing and managing physical, chemical, and biological hazards within the lab.

Undergraduate student researchers will be involved in every level of the proposed project. Drs. Reidy, Ballard, and Timmerman currently supervise about 20 students in undergraduate research yearly. Thus, conducting this project with current and future students in their research labs is feasible. Ideally, undergraduate student researchers are recruited in their first and second years so that they have 2-3 years dedicated to the proposal. These undergraduate students will benefit from direct interaction with Drs. According to the model described below by Reidy, Ballard, and Timmerman, graduate students provide additional tutoring. Once the Research Readiness Pathway is complete, students will enter the Research Engagement Pathway including:

1. *Onboarding/Supervision to Ensure Robust Data Collection*: Students will first observe the standard protocols, then they will conduct the research under supervision, and lastly, they will demonstrate their ability to conduct the procedures independently. Student supervision will occur 1) weekly during laboratory meetings and 2) during more specific orientation sessions that will be conducted for specific procedures. Students will specifically receive hands-on training for the following: clinical coordination, exercise testing, continuous glucose monitoring, ultrasound imaging (limited to 1-2 dedicated students to reduce variability), oral glucose tolerance testing, physical activity monitoring, diet recall, and analysis, data analyses, and manuscript preparation.

2. *Learning Path to Independence*: Students seeking to expand their experiences further will be able to conduct honors theses and independent studies and be given the opportunity to develop ideas for internal undergraduate research awards and summer undergraduate research sub-projects. These student leaders will have bi-weekly individual meetings with the PIs and take the lead on critical aspects and methodologies of the proposal. With this proposed model, there are several possible sub-projects for students to take the lead on and generate posters for presentations and local, regional, and national conferences.

3. *Presentation*: Drs. Reidy, Ballard, Timmerman, and their students regularly submit abstracts to the regional and annual American College of Sports Medicine conferences. Students working on this project will gain

experience collecting, analyzing, and presenting data at these national conferences. Additionally, Miami University sponsors research forums for graduate and undergraduate students.

4. *Preparation of Peer-Reviewed Manuscripts*: Drs. Reidy, Ballard, and Timmerman have collectively authored and co-authored over 150 peer-reviewed manuscripts and will mentor their students in the publication of data generated from this proposed project. We will target the following peer-reviewed journals: *Journal of Applied Physiology; Skeletal Muscle; Physiology; Medicine and Science in Sports and Exercise; Journal of Clinical Endocrinology and Metabolism.* Developing a research manuscript for a peer-reviewed journal will be invaluable for students, particularly those desiring to pursue careers in academia and research.

The timing of the procedures outlined in the proposal is critical to overlapping participants. This means that conflicts can arise with variability in student course schedules. Thus, the consistent availability of graduate students throughout the year and having 3-4 undergraduate students trained on each procedure will be essential to completing these aims. The combined training of several students on procedures and the consistent presence of graduate and undergraduate students make this proposal feasible. The preliminary data presented above in this proposal was generated by students under Drs. Reidy, Ballard, and Timmerman's mentorship models signify the viability of this plan and the prospect of continued success.

This proposal will provide the high-quality undergraduate students of Miami University exposure and hands-on involvement in clinical research design and methodologies typically unavailable to most undergraduate-focused research institutions. This will launch those students into careers in the biomedical sciences and health professions.

3.10 Expected Outcomes and Interpretation: For **specific aim one,** we expect that 7-d of RPA will impair glucose control and that recovery of glucose control will be delayed in those with lower CRF and/or higher adiposity. These findings could help identify impaired recovery from RPA as a pivotal transition period accelerating disease development in individuals based on the level of CRF and adiposity. Thus, identifying those at most significant risk of impaired recovery of cardiometabolic control would stress the importance of avoiding RPA and, if unavoidable, seeking timely therapies to restore the cardiometabolic losses to blunt the acceleration of disease development. For **specific aim two,** we expect individuals with lower CRF and/or higher adiposity to have prolonged RPA-induced vascular function impairment during the 7-d recovery period. These findings would further stress the importance of avoiding RPA and making physical activity or other vascular therapies a top priority in the clinical setting.

3.11 Potential Problems and Solutions: A potential issue for this study is finding the diverse range of CFR and adiposity for our independent variable. To mitigate this concern, we have budgeted for the assistance of the recruitment firm Trialfacts. Dr. Timmerman has had success with Trialfacts with his previously funded NIH AREA R15 award. Another concern with these free-living physical activity reduction studies is compliance with the reduced activity during the RPA week. We have addressed this concern by the clever inclusion of an electric scooter as a means to enable transportation while lowering activity. As the use of these devices is becoming increasingly popular, this also provides a more generalizable model of RPA. The risk of injury with standup scooters is high. However, we mitigate that risk by providing a seated electric scooter and conducting a scooter safety clinic before giving the participant the scooter. Also, as part of the inclusion process, the participant's ability to safely navigate an obstacle course will be determined.

3.12 Future Directions: This AREA (R15) application would seek to elucidate how CRF and/or adiposity status impact recovery of impaired glucose control and vascular function from RPA. Although we include both sexes, we do not have enough information to generate sample size calculations aiming toward sex differences. Successful completion of the proposed project would allow for the development of future proposals that enable us to generate preliminary data for studies to examine the interactive effect of health status by sex, 1) age, 2) or 3) to explore interventions to recover impairments in glucose control

and vascular function following RPA. 1) A future direction would examine how sex may interact with health status (CRF and adiposity) on recovery of glucose control and vascular function following RPA.
2) A future direction would examine how age may interact with health status (CRF and adiposity) on recovery of glucose control and vascular function following RPA.
3) A future study would serve to explore interventions to facilitate recovery of impairments in glucose control and vascular function following RPA.

Conducting the research outlined in this proposal will **significantly enrich the research environment in our department**. It will result in an ongoing, externally supported line of research that will have a substantial impact on 1) peer-reviewed research related to metabolic and vascular dysfunction, RPA/activity, and health and 2) engage undergraduate students in hands-on research through experiments, data analysis, and data dissemination via publications and presentations. This would be an effort encompassed within a training plan to prepare them for research-focused graduate work in biomedical sciences.

3.13 Timeline: Some highly motivated graduate and undergraduate students who generated the preliminary data are ready and trained. They and the PIs would be able to bring in and train new students seamlessly to start the project soon. Upon notification of funding, we would begin recruiting on July 1, 2024, with enrollment open until November. Enrollment will be paused during the winter months each year (November-February) due to cold weather. During the cold weather pauses, we will conduct an annual review, inventory, preliminary data analysis, and abstract and manuscript preparation. Open recruitment and testing will occur from March to November each year. We anticipate completing ~15 participants per year based on conservative due diligence estimates in consultation with the Trialfact recruitment firm. **Students will submit and present preliminary data** at university, regional, and national conferences during each of the three years. They are following year 2 and 3, Drs. Reidy, Timmerman, and Ballard will begin submitting manuscripts associated with the study and preparing an NIH submission extending the results of the data generated from this project.

| Time-line | Year 1, 2 and 3 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Goal** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** | **Jan** | **Feb** | **Mar** | **Apr** | **May** | **Jun** |
| Recruitment | ■ | ■ | ■ | ■ | | | ■ | ■ | | | | |
| Enrollment | | | ■ | ■ | ■ | | | | ■ | ■ | ■ | ■ |
| Pause: Review & Report | | | | | | ■ | ■ | ■ | | | | |
| Abstracts/Manuscripts | | | | | | | | | | | ■ | ■ |

This proposal would facilitate additional impactful undergraduate and graduate research experiences and skills and further enhance Drs—Reidy, Ballard, and Timmerman's independent research programs. We are well prepared to conduct this proposal. The undergraduate student population, particularly those seeking research opportunities, is exceptional. The environment for facilitating student research is excellent.

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