Title of Study: Prescribed Exercise to Reduce Recidivism After Weight Loss Pilot (PREVAIL-P) study*:*

Clinical Trials ID: NCT03685123

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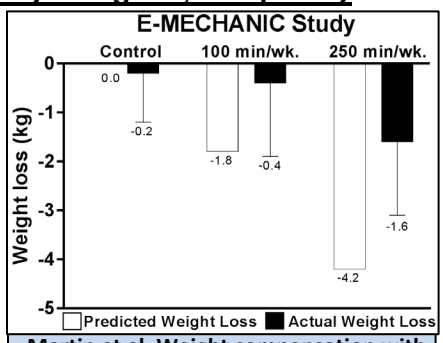
Specific Aims: Achieving clinically significant weight loss (CWL) of 5-10% has been shown to improve important cardiometabolic risk factors in overweight and obese adults (e.g. insulin sensitivity, dyslipidemia, systemic inflammation, blood pressure). However, only 20% of individuals achieve weight loss maintenance and even mild weight regain (2-6%) is associated with regression of risk factors toward pre-weight loss levels. Obtaining high amounts of physical activity (PA) are critical for successful weight loss maintenance. However, the current recommendation for the amount of PA needed to maintain CWL (200-300 min per wk. at moderate intensity) was derived primarily from retrospective studies where PA level was determined by questionnaire and other important design features were not included (e.g. standardization/supervision of exercise levels, randomization after clinically significant weight loss). The American College of Sports Medicine position stand on Physical Activity and Weight Maintenance states that "**there are no correctly designed, adequately powered, energy balance studies to provide evidence for the amount of physical activity to prevent weight regain after weight loss**." This is important given that increased compensation for weight loss (losing less weight than predicted) has been observed with high levels of aerobic exercise training (Figure). Similarly, changes in cardiometabolic risk factors (e.g. insulin sensitivity, lipids, arterial stiffness) have **rarely** been reported across PA levels during weight maintenance, which may be more influential on cardiovascular disease risk status compared to only evaluating changes in weight. Thus, there is a need for tightly controlled prospective interventions to test the extent to which the weight maintenance physical activity recommendations improve weight maintenance and cardiometablic risk factors compared to other exercise levels.



Martin et al. Weight compensation with different exercise levels (preliminary

The Prescribed Exercise to Reduce Recidivism After Weight Loss Pilot (PREVAIL-P) study will evaluate the effect of aerobic exercise training amount on weight maintenance following CWL. In concert with goals of the R56 mechanism, this one-year project will provide data to support a future R01 application where overweight and obese (BMI: 25-40 kg/m^2) men and women (18-65 years old) complete an OPTIFAST diet (7%-10% weight loss). Participants that obtain CWL will be subsequently randomized to aerobic exercise training consistent with the minimum physical activity guidelines (~150 min of moderate intensity exercise) or weight maintenance guidelines (200-300 min per wk. at moderate intensity) for 9 additional months. Therefore, we have proposed 2 studies to address the feasibility of this intervention and demonstrate differences in weight and cardiometabolic health parameters to justify the funding of a future R01 proposal. In both studies, exercise training will be supervised by study staff to confirm exercise-related caloric expenditure. Non-exercise physical activity will be assessed objectively through accelerometry during the entire intervention.

Specific Aim 1: To *demonstrate the efficacy of the weight loss program in producing CWL and retention/adherence of the exercise intervention.* Overweight and obese adults (N=39) will participate in an OPTIFAST weight loss program and supervised aerobic exercise training (~550 MET min. per week.) for 10 weeks. Participants who obtain CWL will be subsequently randomized to 18 weeks of aerobic training consistent with the minimum physical activity recommendations (~550 MET min per week.) or weight maintenance guidelines (~970 MET min per week). We will evaluate the percentage of participants that obtain at least 7% weight loss following OPTIFAST treatment, retention rates in the weight loss program, adherence to exercise levels, and changes in weight and cardiometabolic risk factors in response to the intervention.

Specific Aim 2: To *test the hypothesis that exercise levels consistent with weight maintenance recommendations leads to greater weight maintenance after CWL compared to the minimum physical activity recommendation levels.* Overweight and obese adults (N=30) enrolled in VIDANT health's OPTIFAST program and have achieved at least 7% weight loss will be randomized to 36 weeks of aerobic exercise training consistent with the minimum public health guidelines for physical activity (~550 MET min. per week.) or weight maintenance levels (~970 MET min per week). We will evaluate the effect of the intervention on weight (primary) as well as main secondary measures (e.g. body fat, visceral fat, lipids, lipoprotein particles size/class, insulin sensitivity, blood pressure, arterial stiffness, systemic inflammation, fitness, and quality of life). The aforementioned cardiometabolic risk factors were selected because they can be improved specifically by weight loss and thus may respond differently to weight maintenance or regain.

The PREVAIL-P study will provide data on the effect of exercise level on weight maintenance and cardiometabolic risk factors after CWL. This study has high public health importance and will yield meaningful results to justify additional funding for this clinical trial as an R01 application.

1. Background and Significance: Clinically significant weight loss (CWL) is defined as a weight loss of at least 5%-10% of initial body weight and has been shown to elicit improvements in important cardiometabolic risk factors (e.g. lipids, blood pressure, insulin resistance) [1]. However, the vast majority of individuals (80%) [2] are not able to maintain CWL, and the recidivism back to the initial weight is not only associated with increased cardiovascular disease (CVD), but also regression in CVD risk factors [3, 4]. Potential rationales for the high prevalence of weight regain after the initial weight loss include increases in appetite hormones (e.g. ghrelin), decreases in anorexigenic hormones (e.g. Leptin, GLP-1), reductions in compliance with self-monitoring/weighing habits, and decreases in resting metabolic rate with weight loss [3]. Importantly, it has been well established that obtaining a high amount of physical activity is a critical factor for the maintenance of CWL [1]; however the actual level of physical activity needed to optimally promote weight maintenance following CWL has not been adequately quantified or tested in a prospective intervention. In the American College of Sports Medicine (ACSM) position stand (*Appropriate Physical Activity Strategies for Weight Loss and Prevention of Weight Regain for Adults*) [1] a committee of experts attempted to determine the amount of physical activity that was necessary to promote weight maintenance after CWL. The committee determined that approximately 200-300 minutes per week of physical activity at a moderate intensity was necessary to promote weight maintenance. These levels are based on data from studies from the National Weight Loss Registry and retrospective analyses from prospective studies where the amount of physical activity was quantified primarily through questionnaires [5-9]. The ACSM position stand emphasized that although this was the best estimation that could be determined based on the limited available literature, **"major flaws"** existed in the design of these studies". Further, the position stand expressed the need for more research as it states, "***there are no correctly designed, adequately powered, energy balance studies to provide evidence for the amount of physical activity to prevent weight regain after weight loss***."

The **primary limitations** of the physical activity amount and weight maintenance literature are listed below:

- Many studies were **retrospective**, meaning that physical activity levels were self-selected [5-7]
- Physical activity levels were quantified through subjective physical activity questionnaires [5-7, 10-12], which have reduced accuracy and may be over-reported compared to objective measures [13-15]
- Lack of randomization to different physical activity levels **after the attainment of CWL**
- Physical activity and weight maintenance was evaluated across a wide range of PA levels. Studies evaluating physical activity level and weight maintenance often use tertile analyses which collapse many physical activity levels together (e.g. <150 (low), 150-250 (moderate), >250 min. per week (high). Thus, if level of exercise is being compared to weight maintenance and one of the categories is <150 minutes per week. This means that an individual with 2 min. per week and 145 min. per week are both categorized as having low physical activity, which are very different exercise levels.
- Little data are available on how physical activity levels after CWL affect clinically relevant traditional and non-traditional **cardiometabolic** risk factors

To further highlight the need for a prospective intervention on physical activity amount and weight maintenance, previous exercise studies **without a weight loss component** have shown that there is greater amount of weight loss compensation (losing less weight than predicted) with very high levels of aerobic training. Church et al. [16] observed greater amounts of weight compensation in obese postmenopausal women after 6 months of aerobic exercise at around ~140 min. per week compared to ~193 min. per week (Figure 1A). Similarly, preliminary data from the E-MECHANIC study (Co-PI: Corby Martin [consultant on this grant]) show a greater amount of weight compensation after 6 months of aerobic training in overweight and obese adults exercising at ~250 min. per week) compared to ~100 min. per week) (Figure 1B). Weight compensation occurred in 90% of exercisers in the 250 min. per week group. Thus, it possible that weight compensation may be an issue for those exercising at weight maintenance guidelines after CWL, but at this time there are no prospective interventions, which have the necessary design components to fully study this issue.

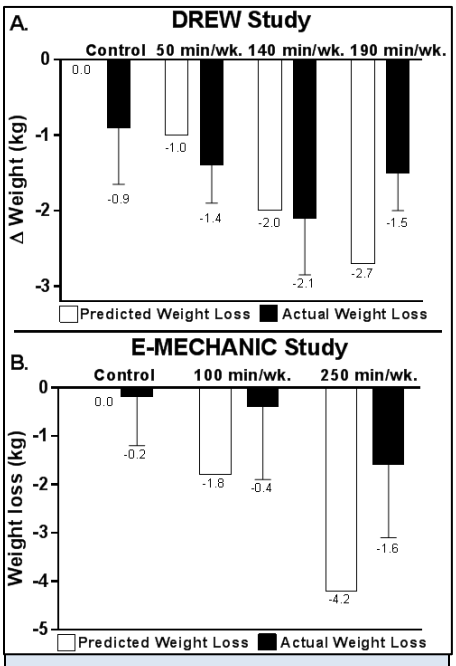


Figure 1. Weight compensation and exercise level in DREW (Panel A) and E-MECHANIC (Panel B) studies

While weight maintenance is an important endpoint, it may be **equally or more important** to determine the impact of different exercise levels after CWL on major independent cardiovascular and metabolic risk factors. Weight loss alone has notable effects on lipids (LDL-C: ~↓5 mg/dL, triglycerides: ~↓15 mg/dL, HDL-C: ~↑2-3 mg/dL) [17-19], but also

other clinically relevant cardiometabolic risk factors such as insulin sensitivity [20, 21], arterial stiffness [22, 23], c-reactive protein [24, 25] and resting blood pressure [18, 19]. Independently, aerobic exercise training has a much more limited effect on lipids [26], but has been shown to have independent effects on insulin sensitivity [27, 28], arterial stiffness [29-31], lipoprotein particle size [32] and absolute fitness [33, 34]. It also important to highlight that cardiorespiratory fitness and change in cardiorespiratory fitness [35]) are independent risk factors for cardiovascular disease [35-37]. Higher levels of aerobic training improves fitness in a dose response manner [33]. Thus, along with prevention of weight regain, exercising at weight maintenance guidelines (200-300 minutes of moderate physical activity per week) has the potential to maintain CWL more effectively than lower levels of exercise, but also offer more cardiometabolic health benefits. This is particularly important given that even mild weight regain (2-6%) [4] has been associated with regression in major cardiovascular risk factors such as total cholesterol [38, 39], LDL cholesterol [39, 40], triglycerides [38, 41, 42], blood pressure [41-43], glucose [38, 39, 44] and insulin levels [40, 43]. Along with cardiovascular complications, quality of life is lower in obese compared to normal weight individuals [45-47]. QOL improves with aerobic training in a dose response manner [48] and also improves with weight loss [49-51] and is another important variable to understand the health benefits of the weight maintenance physical activity recommendations.

Thus, there is value in understanding the impact of aerobic training to counter the negative cardiovascular and behavioral impacts of weight regain after weight loss. The clinical and public health implications of this research will provide physicians, clinicians and health professionals greater information on how to help their patients maintain their weight loss and what the direct impact of these exercise levels are on major traditional and non-traditional cardiometabolic variables.

2. Innovation: The Prescribed Exercise to Reduce Recidivism After Weight Loss Pilot (PREVAIL-P) study will provide data to support a large-scale trial on the impact of aerobic exercise training level on weight maintenance and cardiometabolic risk factors. To address the design limitations of previous studies on physical activity level and weight maintenance, the PREVAIL-P study will: 1) Directly supervise exercise training to confirm exercise-related energy expenditure (indirect calorimetry will be used to enhance the accuracy to exercise caloric expenditure). 2) Non-exercise physical activity will be objectively measured during the entire intervention through accelerometry. 3) Randomizing participants to study groups after clinically significant weight loss. 4) Obtain data on cardiovascular risk factors, which have rarely been reported in the weight maintenance literature (as much of the focus is solely on weight). This R56 application and subsequent R01 application will help clarify how aerobic exercise level impacts weight maintenance, but also clinically relevant traditional (brachial blood pressure, lipids, insulin sensitivity, body fat) and non-traditional risk cardiometabolic risk factors (central blood pressure, arterial stiffness, lipoprotein particle size/class and fitness), which have both high clinical and public health relevance. These studies have the potential to impact future obesity and physical activity guidelines for weight maintenance. An innovative aspect of our design for this initial one-year period is that we will: 1) demonstrate efficacy and the utility of our experimental design and 2) provide a participant pool for future studies on physical activity and weight maintenance.

3. Approach:

Rationale for the studies proposed: Since the R56 mechanism has one year of research funding, a full study on weight loss (12 weeks) and subsequent exercise/physical activity intervention typical of weight maintenance studies (9 to 12 months) is not possible. However, we believe that we can obtain the necessary data for an R01 on this topic by: 1) Demonstrating that the study team can successfully deliver the weight loss component of the trial with high achievement of CWL in overweight/obese participants and subsequent delivery of the exercise training intervention (albeit shorter than a full weight maintenance trial). 2) Obtaining a sample of participants that have achieved 7%-10% weight loss at the beginning of the award from participants completing a weight loss program and randomize them to study groups and subsequently provide data to support our experimental approach.

Study 1: To *demonstrate the ability of the study team to deliver the weight loss program producing clinically significant weight loss and retention/adherence of the exercise training intervention:*

Brief Description: We will recruit 39 sedentary overweight with elevated metabolic risk (e.g. pre-diabetes, hypertension, dyslipidemia, elevated waist circumference) and obese (Class I and II) adults (age: 18-65 yrs., BMI: 25-39.9 kg/m^2) from the Pitt county, NC area. We selected these criteria based on the 2013 AHA/ACC/TOS Guidelines for the Management of Overweight and Obesity in Adults [19]. We will exclude individuals with diagnosed T2D or significant cardiovascular diseases, and/or those taking medications or have conditions that confound weight loss or regain (e.g. hypo/hyperthyroidism [both medicated or untreated], bariatric surgery, etc.). Participants will undergo an OPTIFAST weight loss program and supervised aerobic

exercise training (~550 MET min.) for 10 weeks. Participants who obtain clinically significant weight loss (≥7%) will be subsequently randomized to 18 weeks of aerobic training consistent with the minimum physical activity recommendations (PA-REC) (~550 MET min.) or weight maintenance recommendations (WM-REC, ~970 MET min.). We will evaluate the percentage of participants that obtain at least 7% weight loss following OPTIFAST treatment, retention rates in the weight loss program and the study as whole, and adherence to exercise levels of the participants. We will also evaluate cardiovascular risk factor changes due to the intervention. A flow diagram for **Study 1** is shown in **Figure 2.**

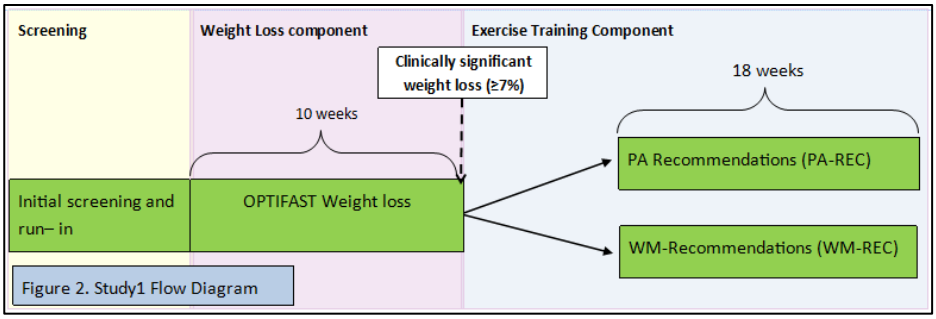


Figure 2. Study1 Flow Diagram

Rationale for inclusion/exclusion: For the inclusion criteria, we made several key decisions in regard to BMI levels, postmenopausal women, and those with advanced cardiovascular diseases/type 2 diabetes. We decided to include individuals who were overweight (with an indication of increased risk). This was based on the 2013 AHA/ACC/TOS Guidelines for the Management of Overweight and Obesity in Adults [19], which provided recommendations for those who needed to lose weight. Since the secondary aims of the study focus on changes in CVD risk factors, it is necessary to recruit a population that has some derangement in these factors at baseline. We decided to exclude participants with morbid obesity (BMI ≥ 40 kg/m^2) to maintain some consistency between absolute (kg) and relative weight loss (%) across participants and these individuals may have joint/gait issues that may affect long term exercise [52]. In regard to those with established CVD or other conditions, the PREVAIL study represents a **preventive strategy** to reduce future CVD events and/or metabolic disease. Therefore, we feel that the examination of the project objectives in higher risk populations (e.g. diabetes, secondary prevention CVD, etc.) would be better addressed in a separate study with outcomes measures specific to those conditions. We decided to include postmenopausal women in the present application since the current weight maintenance guidelines for physical activity do not exclude them or have different exercise recommendations based on menopausal status [1]. Additionally, previous physical activity studies evaluating weight maintenance have included postmenopausal women [5, 53-55].

Table 1. Major Inclusion Criteria for Study Participation	
Age/sex	Men and women 18-65 years of age, Postmenopausal females permitted
BMI/Physical activity	25-39.9 kg/m^2 at enrollment, current sedentary status
Informed Consent	The capability and willingness to provide written informed consent
Exclusion Criteria	
Significant CVD or disorders	Including but not limited to serious arrhythmias, cardiomyopathy, congestive heart failure, stroke or transient ischemic attacks, peripheral vascular disease, acute, chronic or recurrent thrombophlebitis or myocardial infarction/stroke, postmenopausal women
Diabetes	Previous diagnosis or taking medication for type 1 or 2 diabetes, fasting glucose >125 mg/dL
Blood pressure	Systolic blood pressure >180 and diastolic blood pressure >100 [56].
Other medical conditions	Including but not limited to chronic or recurrent respiratory, gastrointestinal, neuromuscular, neurological, HIV or psychiatric conditions. Hospitalization over the last 5 years or currently treatment for mental illness. Conditions which are life-threatening or can be aggravated by exercise training
Other exclusions	Plans to be away from the Pitt county area more than 2 weeks for the next 3 months. Pregnant or plans to become pregnant. Currently engaging a diet or a weight loss program. Do not own smartphone for MyFitnessPal and Centrepoint Apps. Non-compliance during screening visits.

Recruitment: Participants will be recruited in the Pitt County, NC area through newspaper advertisement, targeted Facebook advertisement (based on age and location), emails to East Carolina University (ECU) employees, web-screening from a study website, targeted direct mailers, and flyers in local physician offices. Potential participants will be phone screened for major aspects of the study inclusion/exclusion criteria (summarized in Table 1). Based on a completed exercise training study in obese adults in our laboratory, we had a phone screen to randomization ratio of about 1:5. Therefore, we estimate that it will require 195 phone screen contacts during the award to obtain a sample of 39 participants. This should be feasible, as with limited resources, we have screened ~250 individuals per year, which is 110% of the contacts needed for the present study. Individuals who remain eligible following phone screen will participate in a screening visit (SV1). The PREVAIL-P study coordinator will consent the participant (e.g. provide information about the study, structure of the study, study-related procedures, risks/benefits, etc.). Individuals interested in the study following the

consent process will fill out a calendar where they will indicate when they can attend the OPTIFAST weight loss class and the required exercise sessions. Participants will also identify potential back-up times for exercise training. In addition, the coordinator will interview the participant to assess for major barriers for study completion (e.g. distance from home to the facility, available time for participation, weekly time commitments, acceptability for participation in both weight loss and exercise components of the study). We plan to recruit 13 overweight adults, 13 adults with Class I obesity and 13 adults with Class II obesity. We have based these numbers on the 1 year period of this initial grant application and the budget provided.

An ActiGraph accelerometer (Pensacola, FL) will be used to measure steps, moderate to vigorous physical activity, and sleep over the course of 7 consecutive days. Participants will also wear an ActivPAL accelerometer (PAL technologies, Glasgow, UK) to measure the amount of sitting time. Participants will be instructed to wear both devices 24 hours per day and to perform their normal activities. In addition, participants will log their food using My Fitness Pal App (described below) for 7 days. Those that demonstrate non-compliance (<5 days of physical activity monitor wear or fail to monitor food) will be excluded from further participation. After 7 days, the participant will return both physical activity monitors and a nurse will draw blood to evaluate for hepatic, renal, hematological, endocrine, and metabolic function for inclusion/exclusion purposes. Premenopausal women will take a urine-based pregnancy test to confirm that they are not pregnant. After this, the participant will be scheduled for all outcome measures *(Discussed in Section 5: Assessments).*

OPTIFAST Weight Loss Program (Initial Weight Loss): OPTIFAST is a comprehensive medically supervised weight loss program that combines lifestyle education and medical monitoring with portion-controlled, nutritionally-balanced meal replacement products (shakes, bars and soups). The major goal of the OPTIFAST program is to provide CWL in PREVAIL participants equal to or exceeding 7% of weight loss. While involved in the OPTIFAST program, participants will also perform supervised exercise training at ECU.

Participants will receive a nutrition assessment by a registered licensed dietician/nutritionist. The active weight loss phase of the program consists of 10 weeks with the first 8 weeks consuming full meal replacement. Each OPTIFAST product provides 160 – 170 calories, 14 gm protein (whey, casein, and/or soy), 3 gm total fat, 0 trans-fat, ~20 gm carbohydrate, 220 mg sodium, 470 mg potassium, < 1 gm lactose, and 10 – 30% of the RDI for vitamins and minerals. Participant nutrient goals are based on BMI. For the purposes of this study (BMI range: 25-39.9 kg/m^2), participants will consume approximately 5 OPTIFAST products per (800-820 calories per day, protein 70g). Measured resting metabolic rate and the exercise level of the supervised exercise training (described below) will also be considered when developing caloric goals. At week 8, participants have the option of eliminating 2 products per day and introducing 350 calories of food from a Healthy Food Exchange list. During this time, caloric intake usually reaches 1300–1500 calories per day. By week 10, participants have been transitioned, at an individual pace, to all self-prepared food except for 1 or 2 products daily if desired. VIDANT Wellness Clinic has over 10 years of experience delivering this intervention and the dieticians in the program are certified by Nestle. The cost of the weight loss program and the OPTIFAST products will be provided by the grants' resources to help recruit a generalizable sample and include participants from socioeconomic groups who would otherwise could not afford the program.

Classes will be delivered in a rolling fashion (e.g. participants can enter the classes at any week and go through a 10-week progression), which will allow the assessment schedule to be feasible when participants complete the weight loss component. The class typically has about 20-25 participants (and will only contain research participants) and will occur once per week. At each class, participants will weigh in, fill out a questionnaire from Nestle (regarding the number of products consumed, fluid intake, and any physical changes) and receive a didactic topic relevant to weight loss. About 15 minutes before the end of the class is left open for participants to ask individual questions with the dieticians. Participants must reach at least 7% weight loss at the end of the program to progress into the exercise component of the study. Those who do not meet this threshold will be excluded from further study participation. Data from VIDANT Wellness Center indicate that the OPTIFAST program has achieved CWL (≥7.0%) in 71.4% of patients who enroll in the program and 81.3% of those who complete the program. In the present study, we hope to increase the number of participants meeting CWL by providing supervised exercise training during the initial weight loss phase of the study [19, 57, 58] and only enrolling participants that do not have major barriers to treatment.

My Fitness Pal: During the screening and the weight loss component, participants will enter data through a mobile App called MyFitnessPal (MFP). The App allows subjects to track the nutritional values of their diets, by either scanning in the barcode or searching an extensive database for their chosen food and/or drink. It incorporates elements of social cognitive theory, including self-monitoring, goal setting, and feedback. Total daily intake can be viewed in the form of caloric, nutrient or macronutrient values as graphs and pie charts, with warnings when preset calorie or nutrient limits are being approached. Caloric goals will be entered into

MFP by research staff and all participants will receive training on how to utilize the app. Research staff will also check to assure participants are entering data into MFP at exercise training sessions. In addition, the research staff will enter and track compliance to entering MFP data (days entered/days required), caloric intake, and macronutrient composition (% carbohydrates, fat and protein). MFP data will be used to assist participants in tracking dietary intake. Energy intake study outcomes will be based on food frequency questionnaire.

Exercise training during weight loss component: Participants will come to ECU 2-3 times per week for supervised aerobic training during the weight loss component. Exercise facilitates a greater magnitude of initial weight loss when combined with caloric restriction [19] and should therefore increase the likelihood of obtaining CWL after the weight loss component. The initial exercise level will be 300 MET minutes per week and increased by 50 MET minutes per week until the participant reaches the full amount of exercise during the initial weight loss component of 550 MET minutes per week (general public health guidelines [59]) (Table 2). Similar exercise volumes have been used during the initial weight loss phase [5, 60]. See "Exercise training procedure" for further details on exercise sessions.

	Exercise amount
Week 1	300 MET min.
Week 2	350 MET min.
Week 3	400 MET min
Week 4	450 MET min.
Week 5	500 MET min.
Week 6-10	550 MET min.

Table 2. Exercise progression in the weight loss component

Rationale for the selection of CWL of ≥7%: The range for CWL is generally considered to be 5-10% [1] and 7% was criteria for weight loss in the Diabetes Prevention Program [61]. Therefore, we believe that our selection of 7% is a good balance between a magnitude of weight loss that confers a clinical benefit, but not so small that after minimal weight regain a participant would no longer be classified as having CWL (e.g. a participant with an initial weight loss of 5% and regain to 4.7%).

Tracking of weight loss during initial weight loss phase: During the weight loss component, percent weight loss, magnitude of weight loss, OPTIFAST product data, estimated intake from MFP data, and weight loss class/exercise attendance rates will be entered into a RedCap database. These data will be monitored weekly at study meetings using a SAS script that will query the main RedCap database and automatically generate the percent weight loss and other fidelity data of all active participants.

Randomization after attainment of CWL: Participants who achieve 7% weight loss will be randomized to exercise levels consistent with the minimum physical activity recommendations **(PA-REC)** or weight maintenance recommendations **(WM-REC)** for 18 additional weeks. The randomization list will be generated by the study biostatistician. This list will be maintained with a ECU staff member not associated with the study and with no participants contact. PREVAIL-P staff and the PI will not have access to the randomization list.

Rationale for the selected exercise amounts during exercise component: The rationale for the PA-REC group is this is the recommended amount of exercise by AHA and ACSM to promote improved cardiovascular health [59, 62]. Based on previous data, this should support improvements in cardiometabolic risk factors, but be less optimal for weight maintenance. For the WM-REC group, we decided to target an exercise volume (MET min.) consistent with 250 minutes per week, which is consistent the WM guidelines (200-300 minutes per week) [1]. We believe this is feasible in a supervised format of 4 to 5 times per week. To inform this decision, we reviewed data from two aerobic training studies performed in our laboratory. Based on expected speed/grade of treadmill exercise at week 12 (similar to week 12 of the present proposal), we estimated that the average participant would exercise at a speed of 2.6 mph and 3.0% grade. Based on the proposed exercise level of the WM-REC group, this participant would exercise about 60 minutes 4 times per week or 50 minutes 5 times per week. We estimated that our least fit participants would exercise at 2.4 mph and 2.0%, which would amount to 56 minutes 5 times per week (see derivation below).

Since the weight maintenance recommendations (200-300 min/wk. at moderate intensity) is based solely [1] on **moderate** intensity physical activity, we had to translate this for a program composed of aerobic exercise training, which can be performed at moderate and vigorous intensities. Therefore, we decided to prescribe the exercise levels in the proposed study using MET min. The derivation of the exercise requirements in each study group are summarized in Table 3. Based on our laboratory data in obese adults, we estimated that the average $VO_{2\,max}$ of participants in PREVAIL would be 23.7 ml·kg·min. We further estimated that 50% of VO_2 max (deemed moderate intensity) [63, 64] would correspond to a VO_2 during exercise of 11.5 ml·kg·min or 3.3 MET min. Based on this, it would require 494 MET min. per week at moderate exercise intensity to correspond with the current minimum physical activity recommendations of 150 min/week of moderate intensity exercise. Similarly, we estimated that moderate exercise at 825 MET min. per week would correspond to approximately 250 min. per week of moderate intensity exercise for the WM-REC group. Next, we needed to factor in the expected adherence rates for each group. Based on our laboratory's data in obese adults, we expect at least 92% adherence to MET min. goals in the PA-REC group. Due to the

additional exercise requirements of the WM-REC group, we expect at least 85% adherence to MET min. goals. Therefore, we set the exercise level of the PA-REC group at 550 MET min. per week and the WM-REC group at 970 MET min. per week. (Table 3).

Study Group	Physical Activity Guideline	Equivalent MET min.	Expected adherence	Exercise levels	Expected time commitment
PA-REC	150 min/wk. mod. intensity	494	92%	550 MET min.	36 min., 3 sess. per wk.
WM-REC	200-300 min/wk. mod. intensity	825	85%	970 MET min.	40 min., 5 sess. per wk.

Table 3. Summary of exercise amounts in weight maintenance phase of PREVAIL for a participant with a VO_2 max of 23 ml·kg·min

Exercise training: Participants will be required to attend supervised exercise training sessions during the weight loss component (10 weeks) and following OPTIFAST treatment (additional 18 weeks) at ECU. We have two different exercise training centers located at the FITT building (located centrally in Greenville) and at the (East Carolina Heart Institute, East Greenville area). Both centers are primarily used for research participants and we regularly train adults with obesity and CVD risk factors. Study staff will supervise training sessions at both sites. The weekly MET min. requirements will be divided into 3-5 sessions per week depending on group requirements/participant preferences. After the weight loss component, all participants in Study 1 should be accustomed to exercise at 550 MET min. (since there was supervised exercise training during the weight loss component). Therefore, participants in the PA-REC group will exercise immediately at the required levels. The WM-REC group, will start exercise at 600 MET min. and increase 75 MET min. per week until they reach the required exercise levels of 970 MET min. Every week, staff will weigh participants using a calibrated scale. Following a 5-minute warm-up, participants will exercise at the heart rate range associated with 50%-75% of VO_2 max (determined from the baseline exercise test). Heart rate will be monitored and collected continuously (every second) during exercise sessions with Zephyr Bioharness 3 HR monitors (Medtronic Annapolis, MD). Following each session, heart rate data will be downloaded and mean heart rate will be averaged for database entry. Rating of perceived exertion will be collected every 5 minutes of the exercise session. Training data (e.g. mean heart rate, total energy expenditure, rating of perceived exertion, MET min. exercised and total exercise time) will be entered into the study database after each session.

To enhance the accuracy of the calculation of exercise-related energy expenditure, we will perform monthly submaximal exercise tests to directly measure the energy expenditure rate during steady state exercise at the same intensity as the training sessions. A correction factor will be obtained (actual energy expenditure/predicted energy expenditure) to account for individual variability in prediction equations for energy expenditure. Indirect calorimetry has been used in previous NIH trials evaluating the effects of exercise training on weight to improve the accuracy of energy expenditure from aerobic exercise training [65-67]. The PI is currently using this methodology in an ongoing NIH funded trial (HI-PACE study, PI: Swift).

In the event that a participant cannot make an exercise session (e.g. travel, time commitments, unexpected life events etc.), we will provide them a Fitbit charge accelerometer to exercise on their own to objectively document their exercise. Participants will be instructed to take a walk and use a Fitbit Charge 3 GPS function (associated with their smart phone) to log their mean heart rate, time walked and distance. Study staff will use this information to estimate the caloric value of this walk using the ACSM walking equation [68]. Although this is not as accurate as supervised exercise in our facility, we felt this is a better alternative to no exercise training. We felt this back-up mechanism of exercise was important to maintain as high an energy expenditure difference between the PA-REC and the WM-REC groups as possible and offer flexibility to participants, which is important for both the primary outcomes of the study and overall study retention.

Exercise training data quality: Exercise adherence will be quantified in the amount of MET min. exercised divided by the amount required. Exercise compliance will be defined as the amount of sessions attended divided by the amount of sessions required. We will actively monitor exercise adherence/compliance and other indicators of intervention fidelity (e.g. target heart rate compliance, wear rate accelerometers, participant morale, progression rate of speed/grade) on a weekly basis in study meetings. Our database will be designed to compute intervention fidelity measures in real time using a SAS script that queries the RedCap database and produces customized intervention fidelity reports. Proactive monitoring of intervention fidelity measures has helped us to obtain excellent training adherence in the past (>90%) by allowing our research team to quickly respond to participants demonstrating major risk factors for low adherence or protocol non-compliance.

Nutrition management after weight loss component: By the end of the weight loss component, the participants will be transitioned to all self-prepared food. Participants will be placed on a weight maintenance diet based on the resting metabolic rate (assessed by indirect calorimetry) obtained after the weight loss component (with the physical activity level of the study group taken into account). In both study groups, participants will be provided a caloric range to support maintenance and encouraged to follow the 2010-2015

Dietary Guidelines for Americans [69], which includes consuming less than 10 percent of calories per day from added sugars, 10 percent of calories per day from saturated fats, and less than 2,300 mg per day of sodium. Dr. Matarese (Ph.D., R.D.) will use resting metabolic rate (RMR) data to prescribe caloric ranges.

Behavioral management of participants in exercise groups: Several strategies will be utilized to promote high exercise adherence. Extensive pre-screening will be performed inclusive of run-in visits (evaluation of available time to exercise, exercise calendars, assessment of time commitments) to screen-out participants with very high drop-out risk. We will provide monthly progress reports to maintain intrinsic motivation for exercise (e.g. attendance rate, changes in speeds/grade, miles walked, etc.). Other strategies for reducing attrition/ poor adherence will be modeled after the retention recommendations provided by the NIH [70].

We will monitor adherence weekly in study meetings. Participants with adherence levels <75% or that display major warning signs for low adherence (e.g. frequent rescheduling, missed sessions, low morale) will meet with the research coordinator to problem-solve the causes of poor adherence and to create a plan to improve adherence. If adherence levels remain less <75% or issues related to the low adherence remain unresolved, the participant will be referred for motivational enhancement therapy (i.e. motivational interviewing) to increase intrinsic motivation for exercise which will be performed at the same time of the training sessions to not increase participant burden. Motivational enhancement therapy [71] will be provided by a doctoral student in clinical health psychology trained in the administration of motivational Interviewing. Numerous reviews and meta-analyses support that motivational interviewing is effective at promoting and maintaining health behavior change, including exercise [72]. Should adherence remain low or deteriorate further, booster motivational interviewing sessions will be employed. If the previous methods are not effective or drop-out risk is determined to be especially high, the participant will be referred to the lead behaviorist of the trial (Robert Carels, Ph.D.).

Tracking of changes in diet and non-exercise physical activity: Non-exercise physical activity will be tracked using a GT9X Link accelerometer (ActiGraph, Pensacola, FL). Devices will be blinded using the ActiGraph software, so participants will not be able to see their data. During exercise training sessions, **accelerometers will be removed from participants** to avoid mixing exercise and non-exercise physical activity data. Recharging of the device (14-day battery life) will be performed during the exercise session by study staff while participants are exercising. Study staff will use ActiGraph's database program (Centrepoint, Pensacola, FL) to monitor and download non-exercise physical activity data (e.g. steps, moderate to vigorous physical activity, sleep, wear/non-wear rates). The Centrepoint program will update in our database using an app in the participants' mobile device or can be uploaded manually at our facility. Our research team has used accelerometers to monitor non-exercise physical activity in a similar manner with excellent device wear compliance in a completed AHA funded study (~96%) and an on-going NIH study (~97%). Block food frequency questionnaires [73] will be used to measure energy intake at each study time point.

Table 4. Schedule of assessments Study 1	**Baseline**	**Weight loss component** (1-10 weeks)	**Attainment of CWL** (Week 10)	**Mid-Exercise Component** (Week 18)	**Follow-up** (Week 26)
Weight, Anthropometrics	X		X	X	X
DEXA, RMR	X		X		X
Blood pressure	X		X		X
Lipids, lipoprotein sub-fraction, CRP, fitness	X		X		X
OGTT, arterial stiffness, central blood pressure	X		X		X
ActivPAL (sitting behavior and total EE)	X		X	X	X
ActiGraph accelerometers (Non-exercise PA)	X	X	X	X	X
Energy intake (FFQ)	X		X	X	X
SF-36	X		X		X

4. Study 2: *The major goal of **Study 2*** is to provide preliminary data to *demonstrate that the weight maintenance physical activity recommendations will have a greater impact on weight and cardiometabolic risk factors compared to the minimum physical activity recommendations.* Given that the R56 mechanism is a 1-year grant, it is not possible to test the full weight loss intervention proposed in the R01 (12 weeks of OPTIFAST treatment and subsequent randomization to exercise for 36 weeks [48 total weeks]), especially when considering anticipated study start-up and recruitment time. Therefore, our plan is to recruit 30 overweight or obese individuals who are currently in, near completion or who have very recently completed the VIDANT OPTIFAST program (<2 weeks with maintenance of at least 7% weight loss) and exercise participants for 36 weeks. VIDANT Health documents each participant's BMI and weight at the beginning of the program. Therefore, we can determine the percent weight loss retrospectively and whether the participant was

overweight or obese at the start of the program. Our consent forms will be written to inform participants that if enrolled, we will contact VIDANT Health about their baseline weight/BMI. The recruitment criteria for the Study 2 study is very similar to Study 1 and is summarized in Table 5. Major inclusion criteria include previously overweight or obese (BMI: 25-40 kg/m^2) at enrollment in the VIDANT OPTIFAST program and documented 7% weight loss at the conclusion of the program. Major exclusions include diagnosis of T2D, cardiovascular disease, previous history of cardiovascular events, thyroid conditions, or contraindications to exercise training.

Table 5. Major Inclusion Criteria for Study Participation in Study 2	
Age/sex	Men and women 18-65 years of age, Postmenopausal females permitted
BMI/Physical activity	25-39.9 kg/m^2 at OPTIFAST enrollment and documented 7% weight loss
Informed Consent	The capability and willingness to provide written informed consent
Exclusion Criteria	
Significant CVD or disorders	Including but not limited to serious arrhythmias, cardiomyopathy, congestive heart failure, stroke or transient ischemic attacks, peripheral vascular disease, acute, chronic or recurrent thrombophlebitis or myocardial infarction/stroke, postmenopausal women
Diabetes	Previous diagnosis or taking medication for type 1 or 2 diabetes, fasting glucose >125 mg/dL
Blood pressure	Systolic blood pressure >180 and diastolic blood pressure >100 [56].
Other medical conditions	Including but not limited to chronic or recurrent respiratory, gastrointestinal, neuromuscular, neurological, HIV or psychiatric conditions. Hospitalization over the last 5 years or currently treatment for mental illness. Conditions which are life-threatening or can be aggravated by exercise training
Other exclusions	Plans to be away from the Pitt county area more than 2 weeks for the next 3 months. Pregnant or plans to become pregnant. Do not own smartphone for MyFitnessPal and Centrepoint Apps. Non-compliance during screening visits.

Recruitment: Upon receiving IRB approval, PREVAIL-P staff will do brief presentations at VIDANT health's OPTIFAST program to classes to present information to potential participants. Interested participants will contact our research staff and will set-up a screening visit to be consented for Study 2 by the research coordinator. Potential participants will be notified that if they complete the OPTIFAST program with CWL (≥7%). A nurse will perform a blood draw to evaluate for hepatic, renal, hematological, endocrine, and metabolic function for inclusion/exclusion purposes. Outcome measures for Study 2 will not be assessed until the participant completes the OPTIFAST program with CWL. Participants will also be offered the use of our ECU exercise facility to enhance the likelihood of CWL with a goal of 550 MET min. per week or greater and offered use of Fitbits to help them increase physical activity levels. Within 2 weeks of completion of the OPTIFAST program and obtainment of CWL, all baseline outcome measures (Table 4) will be assessed and participants will be randomized to the PA-REC or the WM-REC groups (Figure 3).

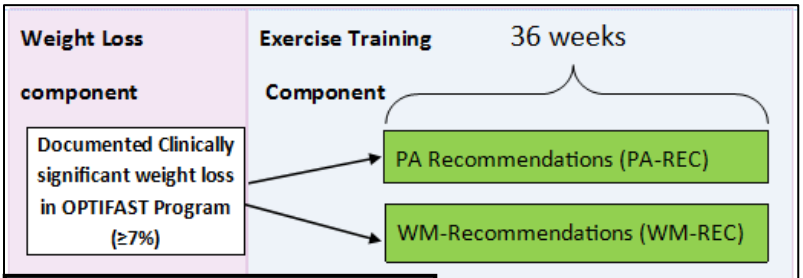


Figure 3. Study 2 Flow diagram

Exercise training intervention: Participants in Study 2 will start exercise training at 300 MET min. per week and increase by 75 MET min. until they reach the maximum MET min. goal of their group assignment. Participant in the PA-REC group will reach the required exercise volume (550 MET min.) by week 5 and the WM-REC group will reach the required exercise volume of 970 MET min. by week 9. Both groups will continue at this exercise volume till the end of the intervention. The energy gap (difference in total MET min. between both exercise groups) is 12,340 MET min. In other words, it would take the PA-REC group 22 additional weeks of exercise

Table 5. Schedule of assessments in the Study 2	**Baseline (After CWL)**	**Mid-intervention (Week 18)**	**Follow-up (Week 36)**
Weight	X	X	X
DEXA, Anthropometrics	X	X	X
Blood pressure	X		X
Lipids, lipoprotein sub-fraction, CRP, fitness	X		X
OGTT, arterial stiffness, central blood pressure	X		X
RMR, ActivPAL (sitting behavior and total EE)	X	X	X
ActiGraph accelerometers (Non-exercise PA)	X	X	X
Energy intake (FFQ)	X	X	X
SF-36	X		X

training at 550 MET min. per week to equate the exercise volume of the WM-REC group. All exercise training sessions methodology will be the same as described for Study 1. In brief, participants will perform exercise training 3-5 times per week dependent on group assignment. The aerobic exercise intensity will be the heart rate associated with 50%-75% VO_2 max and heart rate will be evaluated continuously during the exercise sessions. RPE will be taken every 5 minutes. Participants will wear accelerometers during the entire intervention to assess non-exercise physical activity (devices will be removed during exercise sessions). Exercise compliance and adherence will be monitored in all research participants.

Behavioral management/nutritional management: Behavioral and nutritional management of participants will be the same procedures as described in Study 1.

5. Assessments: Methodology for primary and secondary measurements are shown below. Train schedules for both the Study 1 and Study 2 are shown in Table 4 and Table 5 (shown above), respectively.

Primary Outcome- Change in Weight: The primary outcome measure of the PREVAIL study is change in weight (as a continuous variable) after obtaining clinically significant weight loss to follow-up of the intervention. Weight will be evaluated using a calibrated scale to the nearest tenth of kg with the participant in a fasted state (12 hours), wearing only a hospital gown and after the participant has voided.

Secondary measures: Secondary measures will be collected at all 3 assessment points with the exception of accelerometry/doubly labeled water (obtained prior to each assessment period). We specifically chose cardiometabolic risk factors which have been shown in the literature to **improve with weight loss, exercise training (independent of weight loss), or a combination of both.** The rationale for this is that it is possible that even in the presence of weight regain after CWL, aerobic training may attenuate the deleterious effect on cardiometabolic risk factors. Thus, we will evaluate the effect of the proposed intervention on traditional (lipid concentrations, brachial systolic/diastolic blood pressure), non-traditional (fitness, lipoprotein particles size and subclass, c-reactive protein, pulse wave velocity, augmentation index, central blood pressure) CVD risk factors and a type 2 diabetes risk factor (insulin sensitivity). In addition, we will obtain total energy expenditure, sedentary behavior, non-exercise physical activity, energy intake and macro/micro nutrient information to inform energy balance apart from exercise training. We will also obtain quality of life (QOL) via the SF-36 to have a behavioral measure of the impact of the intervention. The PI has published on weight loss and insulin sensitivity [74, 75], fat loss [75] and c-reactive protein [76] and lipoprotein particle size [75]. Assessments will be performed approximately 48 hours following the last exercise session.

Categorical weight maintenance: We will compare the percentage of individuals in each group that are able to maintain at least 3% of the initial weight loss [1]

Oral glucose tolerance test: An intravenous (IV) line will be placed in an antecubital vein for blood draw purposes and will remain there throughout the testing. A resting fasting blood sample will be drawn after which, participants will drink a sugar solution consisting of 75 grams of glucose. Additional blood samples will be drawn 0, 30, 60, 90, and 120 minutes after drinking the glucose beverage. Samples will be archived at -80°C until sample analysis. Insulin $_{AUC}$ and glucose $_{AUC}$ will be determined following sample analysis. Insulin sensitivity will be estimated using the Matsuda index [77]. At baseline, OGTT measurements will occur prior to exercise testing to avoid confounding these measurements with acute exercise. At follow-up, OGTT will occur 48 hours following the last exercise session, and precede exercise testing.

Maximal Exercise Test: A modified Balke treadmill (Trackmaster 425, Carefusion, Newton Kansas) protocol will be used to determine cardiorespiratory fitness, and the appropriate heart rate range for aerobic exercise training. Participants will walk at an initial speed of 2.0 mph with 0% grade for the first 2 minutes after which the treadmill speed will increase to 3.0 mph for the next 2 minutes. Treadmill grade will be increased by 2.5% every 2 minutes until volitional exhaustion. Respiratory gases (VO_2, CO_2) and ventilation will be measured continuously using a True Max 2400 Metabolic Measurement Cart (Parvomedics, Salt Lake City, Utah). A physician will be present to supervise exercise testing at all study assessment points. The electrocardiogram during the baseline exercise test will be cleared by a physician prior to randomization to study groups.

Pulse wave velocity and Aortic blood pressure parameters: Participants will refrain from large meals and caffeine for at least 2 hours and alcohol, vigorous exercise and vasoactive medication for at least 12 hours. Testing will occur in a quiet temperature controlled room [78]. Carotid to femoral pulse wave velocity (PWV) and aortic blood pressure parameters will be measured using a SphygmoCor XCEL (Itasca, IL). PWV will be obtained in the supine position after a 15-minute rest. PWV parameters will be obtained in duplicate in concert with current guidelines [78]. Aortic blood pressure parameters (brachial blood pressure, aortic blood pressure, augmentation index) will be obtained in the seated position after a 5-minute rest.

Dietary data: FFQ questionnaire data will be assessed using the NutritionQuest (Berkley Inc.) data-on-demand system, which will facilitate immediate scoring and estimation of caloric and macronutrient intake.

Other secondary outcomes: Body composition will be measured using dual energy x-ray absorptiometry (DEXA). Waist circumference will be evaluated with a gulick tape measure at the natural waist. Quality of life will be measured by the short form health survey (SF-36) [79]. Blood glucose, insulin, lipids, c-reactive protein, and lipoprotein particle size/class will be measured through standard analytical techniques. Resting metabolic rate (RMR) will be assessed via indirect calorimetry using a ventilated hood in the fasted state and in a supine position. RMR data will be collected for 30 minutes after 15 minutes of rest. An ActivPAL accelerometer will be worn for 7 days to assess time spent sitting/standing and total energy expenditure.

6. Data management: Study data will be stored in a Red Cap © database [80]. The database will be designed to develop recruitment reports (e.g. total contacts, reasons for exclusion during screening, screen to randomization ratio for recruiting projections), weight loss program data (% weight loss, class attendance, etc.) and exercise training data (e.g. exercise adherence, heart rate compliance) and outcome data. Recruiting, weight loss program and exercise training data reports will be reviewed to monitor the intervention fidelity.

7. <u>Statistical Design</u>: Primary analysis will first check for baseline differences between the groups to help determine possible covariates that need to be taken into account. Intent to treat analysis will be conducted first followed by those that had high adherence to the exercise group (>75%). The analysis for the primary aim (weight change) will be a mixed effect linear model with change in weight as the outcome. In brief summary, this model will use the baseline, weight loss midpoint, and the follow-up weights all awhile accounting for the baseline covariates as well as the weekly weight changes. Results will be reported as least square means with t-test used to test for differences both between and within groups. Changes in cardiovascular risk factors will be evaluated in a similar manner as weight, with mixed effect linear models. Particular focus will be given to both the baseline changes and the changes after the clinically significant weight loss. Both set of covariates will be used with variable selection methodology to determine if there is a set of covariates that can predict the outcome of the weight maintenance phase. To evaluate categorical differences between groups in regards weight maintenance, we will use a mixed effect logistic model to model the probability of achieving weight maintenance, with results presented as adjusted odds ratios.

8. <u>Preliminary data and experience of the study team</u>: Collectively, the study team has significant experience implementing weight loss and exercise training interventions. VIDANT Wellness Center has documented success in achieving 7% weight loss (71% of enrolled participants, 81% of participant completing the program). Dr. Matarese will oversee the delivery of the nutritional intervention. She has experience in nutritional interventions as she was the Co-PI of a recently completed multi-center study sponsored by Abbott (N= 652). In addition, she uses OPTIFAST treatment within her clinical practice and both Drs. Matarese and Pories have a completed multicenter randomized trial evaluating OPTIFAST weight loss treatment [81]. Dr. Robert Carels is the psychology departments' director of clinical training. He has used motivational interviewing for weight control in obese populations [82] and has performed both weight loss and maintenance interventions [82-89]. His role on the present study will be to provide behavioral support to participants demonstrating risk of drop-out or low adherence. Dr. Walter Pories will be the medical investigator and has served this role on studies using an OPTIFAST diet and bariatric surgery. Dr. Corby Martin (expertise in energy balance) will serve as a consultant on the grant.

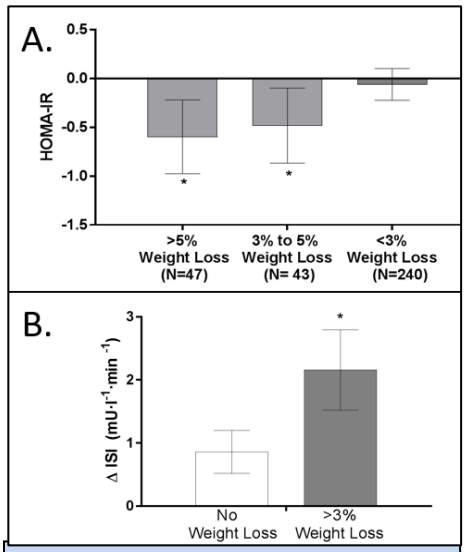


Figure 4. Changes in insulin sensitivity with weight loss A) Swift et. al. *Obesity* 2016. B) Swift et. al. *Plos One* 2018

The PI and Dr. Houmard have a history implementing supervised exercise studies (Table 5). The PI has 44 publications in exercise training and CVD/T2DM risk factors. He is a former NIDDK T-32 postdoctoral fellow in Preventive Medicine (Pennington Biomedical, Mentor: Timothy Church, M.D.), which focused specifically on <u>large scale exercise training trials</u>. In terms of independent extramural funding, Dr. Swift has obtained NIH and AHA funding as the PI of exercise trials (AHA scientist development award, NIH R03). In addition, the Dr. Swift is a Co-I on the NIH common fund MoTrPAC study. The PI has published 10 articles evaluating the effect exercise training level on CVD risk factors [75, 90-97]. In specific reference to weight loss and exercise, the PI has published 2 review articles on the effect of physical activity on weight loss and weight maintenance [95, 98]. The PI has published 2 papers showing that weight loss amplifies health benefits even in participants performing aerobic training. Figure 4A shows the change in HOMA-IR in overweight/obese women participating in 6 months of aerobic training. Those that achieved greater than 5% weight loss (and even 3% weight loss) had greater reductions in

HOMA-IR compared to those that did not lose weight (<3%). Figure 4B shows that insulin sensitivity (measured via an intravenous glucose tolerance test) in overweight/obese adults participating in 8 months of aerobic training. While insulin sensitivity was improved in participants that did not lose weight (<3% weight loss), a greater improvement in insulin sensitivity was observed in participants who achieved at least 3% weight loss. In the same paper, the PI showed that modest weight loss is associated with improvements in triglycerides, non-HDL cholesterol, LDL particle size, total LDL particles and HDL particle size compared to those that do not lose weight [75]. These results informed incorporating these variables in this proposal.

Dr. Houmard has been the ECU site PI on multi-center NIH funded exercise trials (e.g. STRRIDE I, STRRIDE AT/RT) including being an exercise training site PI on the MoTrPAC study (see Table 6). The PI will assume complete responsibility for leading this trial. However, Dr. Houmard will provide senior mentorship for the PI given that he is an early stage investigator. Dr. Houmard is the PI's faculty mentor. Mentorship of the PI is included within Dr. Houmard's job responsibilities of his faculty appointment at ECU. Thus, no additional effort is needed from this grant for mentorship activities.

Investigator	Study	N	Population	Role	Adherence
Swift, D	HI-PACE (NIDDK)	36†	Obese African Americans	PI	98.6%
Swift, D.	I-CAN (AHA)	45	Obese adults	PI	92.1%
Swift, D.	Walkmore (AHA)	120	Postmenopausal women	NIH T-32 Postdoc	NSE,NPI
Swift, D.	Fit for Life (NIH)	57	Elderly adults >70 yrs.	NIH T-32 Postdoc	NPI
Swift, D.	E-Mechanic (NIH)	198	Adults 18-65 yrs.	NIH T-32 Postdoc	NPI
Houmard, J.	POWER (NIH)	48*	Adults after gastric bypass	ECU site PI	NSE
Houmard, J.	STRRIDE I (NIH)	67*	Overweight adults	ECU site PI	90.6%
Houmard, J	STRRIDE II (NIH)	75*	Overweight adults	ECU site PI	86.9%

Table 6. Previous experience in NIH and AHA funded exercise and physical activity trials *We have reported the n of participants that have been recruited through the ECU site of the study; †ongoing study, NSE: not supervise exercise, NPI: Swift not the PI

9. <u>Study Timeline</u>: The timeline of PREVAIL-P is shown in Table 7. Study start-up will occur during month 1 and 2 of the grant. The start-up will include the creation of a manual of procedures (MOP), development of standard operating procedures (SOPs), training of all research staff on procedures/visits, mock run-throughs of clinical visits and exercise training methodology. In addition, we will develop a custom-made RedCap database to include all study outcome measurements, tracking of recruitment and creation database reports for monitoring variables suggestive of the overall intervention fidelity. We plan to begin recruitment of both Study 1 and Study 2 in month 1-4 of the grant award. We plan to complete the intervention from month 3-11 of the grant award. Lastly, we plan to complete data collection by the end of the 11th month of the study after which the research team will then verify study data, perform data cleaning/statistical analyses and write/submit the primary outcomes paper.

Table 7. PREVAIL-P Timeline	Study Month											
	1	2	3	4	5	6	7	8	9	10	11	12
MOP/SOP development, training	■	■										
Recruitment of Study 1		■	■	■								
Recruitment of Study 2		■	■									
Intervention			■	■	■	■	■	■	■	■	■	
Follow-up testing							■	■	■	■	■	
Data management/cleaning			■	■	■	■	■	■	■	■	■	■
Data analysis												■
Paper writing and submission												■

10. <u>Rigor and Reproducibility</u>: Most blood analyses will utilize LabCorp Inc., which has a CLIA certification for accuracy in methods of analysis. Gas calibration will occur prior to submaximal or maximal indirect calorimetry. Current guidelines will be followed for arterial stiffness measures procedures and the machine will be calibrated annually. Weight will be measured on a calibrated scale in the fasted state. Glucose and insulin from OGTT samples will be measured in duplicate. During study start-up, all procedures and methods will be documented in the MOP and investigators will approve the MOP before the initiation of data collection.

11. <u>Potential Pitfalls</u>: 1) <u>Low adherence and sufficient separation between exercise groups:</u> Obtaining high adherence and adequate differences in the exercise levels are critical to the success of the study. The investigative team has demonstrated the ability to implement exercise training with excellent adherence to the 6-month time point (>90%) based on our current AHA/NIH funded exercise trials. We have established several safeguards to reduce the enrollment of participants with high drop-out risk by evaluating for indicators of low adherence in our screening procedures (semi-structured barriers interview) and we have an intervention plan lead by behavioral personnel to intervene on participants exhibiting warning signs for low compliance (overseen by Robert Carels, Ph.D.). The research team monitors adherence in all participants weekly to

quickly intervene on participants who are not meeting exercise goals. NIH R01s using similar procedures have obtained high exercise adherence (>90%) and retention (>85%) [33, 99, 100]. 2) The PI is an early stage investigator: The PI has a strong history of publication in exercise amount and CVD risk factors, and has obtained significant research funding as the PI for a junior investigator including a career development grant from the AHA and a R03 from NIDDK (both exercise training interventions). While the PI will assume complete and total responsibility for leading the trial, Joseph Houmard (co-investigator) has substantial experience in NIH sponsored exercise trials, and will provide senior mentorship to the PI for any other study-related issues (along with the other co-investigators). Drs. Pories and Matarese are Co-PIs of a published 52-week OPTIFAST diet study [81]. Dr. Martin will visit ECU during study start-up and the intervention period to assist with MOP development and early protocol issues (see letters of support). 3) Failure to recruit: We are confident that we can recruit the proposed study as described. To assure that we are able to meet our recruitment goals, we have budgeted ($7,000 dollars) for recruitment material and for a 20 hour a week staff member who will perform recruiting calls after hours (5PM) and during weekends during the recruitment phase of the study.

12. Alternative approaches: We considered using the exercise volume corresponding to 300 minutes per week for the exercise prescription in the WM-REC group (which is the maximum value recommended by the weight maintenance guidelines), but this may be burdensome to participants with very low fitness, who would have to come more than 5 times per week to exercise or have very long exercise sessions (>60 mins. per session). Therefore, we felt that the selection of targeting the exercise volume associated with 250 minutes per week struck a good balance between an amount of exercise that would promote weight maintenance, but was also feasible in a supervised setting. In addition, we felt that this was the best choice to support high retention and adherence to the exercise levels in the WM-REC group.

13. Limitations: An important limitation is the PREVAIL-P study will occur under laboratory conditions in which staff will ensure high compliance to the amount of exercise. Thus, the study is not designed to determine methods of motivating participants to achieve these exercise levels in a real-life setting, which represents an important research question. However**,** given the lack of data of prospective trials in this area and that these **recommendations have been disseminated**, we feel that it is necessary to first determine the effects of different exercise levels on weight maintenance and cardiometabolic risk factors as this may better inform how to properly design future effectiveness studies. Another limitation is that there are many nutritional and physical activity interventions to induce CWL. Thus, the proposed study cannot address whether an interaction effect exists between the weight loss regimen utilized and magnitude of weight maintenance. Due to 1-year of funding in the present mechanism, we cannot evaluate the impact of the intervention on long-term weight loss outcomes. However, we plan to study the cohort of participants within PREVAIL-P and the subsequent R01 (future grants applications) to begin to address long term weight loss.

14. Conclusion: The PREVAIL-P study will provide valuable data to inform the design of an R01 on the impact of exercise level on weight loss and cardiometabolic risk factors after CWL. We are confident that both projects will demonstrate that are approach is robust and will justify further funding of this clinical trial. Study 1 will demonstrate the ability of the study team to implement the weight loss intervention and demonstrate adherence to exercise training after CWL. Study 2 will allow the study team to adequately power primary and secondary outcomes for weight maintenance trial for the R01 application. A fully powered R01 on the impact of exercise training level on weight, body composition and cardiometabolic risk factors directly addresses limitations in this research area identified by a recent ACSM position stand [1] and an NIH sponsored working group [101] on weight maintenance by randomizing individuals to groups following a clinically significant weight loss, prospectively measuring exercise training amount (supervised exercise), non-exercise physical activity (objective physical activity monitors) throughout the entire intervention. The results of the PREVAIL study will have high clinical implications given the high rates of regain following weight loss treatment and further inform future physical activity guidelines on weight loss maintenance. If funded, subsequent renewals of the R01 application could aim at following these cohorts to examine long-term weight loss, incorporating apps to increase physical activity levels in weight maintenance or determine the effect of other exercise programs after CWL (e.g. intensity, modality, etc.). We believe the results of this R56 proposal and subsequent R01 proposal have the potential to be published in a top tier medical or obesity journal and will be of great interest to physicians, clinicians and public health professionals treating overweight and obese adults.

References

1. Donnelly, J.E., et al., *Appropriate Physical Activity Intervention Strategies for Weight Loss and Prevention of Weight Regain for Adults.* Medicine and Science in Sports and Exercise, 2009. **41**(2): p. 459-471
2. Wing, R.R. and S. Phelan, *Long-term weight loss maintenance.* The American Journal of Clinical Nutrition, 2005. **82**(1): p. 222S-225S.
3. Soleymani, T., S. Daniel, and W.T. Garvey, *Weight maintenance: challenges, tools and strategies for primary care physicians.* Obesity Reviews, 2016. **17**(1): p. 81-93.
4. Kroeger, C.M., K.K. Hoddy, and K.A. Varady, *Impact of Weight Regain on Metabolic Disease Risk: A Review of Human Trials.* Journal of Obesity, 2014. **2014**: p. 8.
5. Jakicic, J.M., et al., *Effect of exercise duration and intensity on weight loss in overweight, sedentary women: A randomized trial.* JAMA, 2003. **290**(10): p. 1323-1330.
6. Jakicic, J.M., et al., *Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: A randomized trial.* JAMA, 1999. **282**(16): p. 1554-1560.
7. Andersen, R.E., et al., *Effects of Lifestyle Activity vs Structured Aerobic Exercise in Obese Women.* JAMA: The Journal of the American Medical Association, 1999. **281**(4): p. 335-340.
8. Klem, M.L., et al., *A descriptive study of individuals successful at long-term maintenance of substantial weight loss.* The American Journal of Clinical Nutrition, 1997. **66**(2): p. 239-46.
9. Schoeller, D.A., K. Shay, and R.F. Kushner, *How much physical activity is needed to minimize weight gain in previously obese women?* Am J Clin Nutr, 1997. **66**(3): p. 551-6.
10. Borg, P., et al., *Effects of walking or resistance training on weight loss maintenance in obese, middle-aged men: a randomized trial.* Int J Obes Relat Metab Disord, 2002. **26**(5): p. 676-83.
11. Ewbank, P.P., L.L. Darga, and C.P. Lucas, *Physical activity as a predictor of weight maintenance in previously obese subjects.* Obes Res, 1995. **3**(3): p. 257-63.
12. Fogelholm, M., et al., *Effects of walking training on weight maintenance after a very-low-energy diet in premenopausal obese women: A randomized controlled trial.* Archives of Internal Medicine, 2000. **160**(14): p. 2177-2184.
13. Hagstromer, M., et al., *Comparison of a subjective and an objective measure of physical activity in a population sample.* J Phys Act Health, 2010. **7**(4): p. 541-50.
14. Leenders, N., W.M. Sherman, and H.N. Nagaraja, *Comparisons of four methods of estimating physical activity in adult women.* Medicine and science in sports and exercise, 2000. **32**(7): p. 1320-1326.
15. Sallis, J.F. and B.E. Saelens, *Assessment of physical activity by self-report: status, limitations, and future directions.* Research quarterly for exercise and sport, 2000. **71**(sup2): p. 1-14.
16. Church, T.S., et al., *Changes in Weight, Waist Circumference and Compensatory Responses with Different Doses of Exercise among Sedentary, Overweight Postmenopausal Women.* PLoS ONE, 2009. **4**(2).
17. Dattilo, A.M. and P.M. Kris-Etherton, *Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis.* The American Journal of Clinical Nutrition, 1992. **56**(2): p. 320-8.
18. Weiss, E.P., et al., *Effects of matched weight loss from calorie restriction, exercise, or both on cardiovascular disease risk factors: a randomized intervention trial.* The American Journal of Clinical Nutrition, 2016. **104**(3): p. 576-586.
19. Jensen, M.D., et al., *2013 AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults.* A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society, 2013.
20. Gower, B.A., et al., *Effects of weight loss on changes in insulin sensitivity and lipid concentrations in premenopausal African American and white women.* The American Journal of Clinical Nutrition, 2002. **76**(5): p. 923-927.
21. Mason, C., et al., *Dietary weight loss and exercise effects on insulin resistance in postmenopausal women.* Am J Prev Med, 2011. **41**(4): p. 366-75.
22. Petersen, K.S., et al., *Effect of Weight Loss on Pulse Wave Velocity.* Systematic Review and Meta-Analysis, 2015. **35**(1): p. 243-252.

23. Rider, O.J., et al., *The Effect of Obesity and Weight Loss on Aortic Pulse Wave Velocity as Assessed by Magnetic Resonance Imaging.* Obesity, 2010. **18**(12): p. 2311-2316.
24. Tchernof, A., et al., *Weight Loss Reduces C-Reactive Protein Levels in Obese Postmenopausal Women.* Circulation, 2002. **105**(5): p. 564-569.
25. Selvin, E., N.P. Paynter, and T.P. Erlinger, *The effect of weight loss on C-reactive protein: a systematic review.* Arch Intern Med, 2007. **167**(1): p. 31-9.
26. Leon, A.S. and O.A. Sanchez, *Response of blood lipids to exercise training alone or combined with dietary intervention.* Med Sci Sports Exerc, 2001. **33**(6 Suppl): p. S502-15; discussion S528-9.
27. DiPietro, L., et al., *Exercise and improved insulin sensitivity in older women: evidence of the enduring benefits of higher intensity training.* Journal of Applied Physiology, 2006. **100**(1): p. 142-149.
28. Houmard, J.A., et al., *Effect of the volume and intensity of exercise training on insulin sensitivity.* Journal of Applied Physiology, 2004. **96**(1): p. 101-106.
29. Collier, S.R., et al., *Effect of 4 weeks of aerobic or resistance exercise training on arterial stiffness, blood flow and blood pressure in pre- and stage-1 hypertensives.* J Hum Hypertens, 2008. **22**(10): p. 678-686.
30. Madden, K.M., et al., *Short-Term Aerobic Exercise Reduces Arterial Stiffness in Older Adults With Type 2 Diabetes, Hypertension, and Hypercholesterolemia.* Diabetes Care, 2009. **32**(8): p. 1531-1535.
31. Yokoyama, H., et al., *Short-term aerobic exercise improves arterial stiffness in type 2 diabetes.* Diabetes Research and Clinical Practice, 2004. **65**(2): p. 85-93.
32. Kraus, W.E., et al., *Effects of the Amount and Intensity of Exercise on Plasma Lipoproteins.* New England Journal of Medicine, 2002. **347**(19): p. 1483-1492.
33. Church, T.S., et al., *Effects of Different Doses of Physical Activity on Cardiorespiratory Fitness Among Sedentary, Overweight or Obese Postmenopausal Women With Elevated Blood Pressure.* Journal of the American Medical Association, 2007. **297**(19): p. 2081-2091.
34. Duscha, B.D., et al., *Effects of Exercise Training Amount and Intensity on Peak Oxygen Consumption in Middle-Age Men and Women at Risk for Cardiovascular Disease.* Chest, 2005. **128**(4): p. 2788-93.
35. Blair, S.N., et al., *Changes in Physical Fitness and All-Cause Mortality: A Prospective Study of Healthy and Unhealthy Men.* JAMA, 1995. **273**(14): p. 1093-1098.
36. Blair, S.N., et al., *Physical Fitness and All-Cause Mortality.* Journal of the American Medical Association, 1989. **262**(17): p. 2395-2401.
37. Myers, J., et al., *Exercise Capacity and Mortality among Men Referred for Exercise Testing.* New England Journal of Medicine, 2002. **346**(11): p. 793-801.
38. Wang, P., et al., *Circulating ACE is a predictor of weight loss maintenance not only in overweight and obese women, but also in men.* Int J Obes, 2012. **36**(12): p. 1545-1551.
39. Linna, M.S., et al., *Successful weight maintenance preserves lower levels of oxidized LDL achieved by weight reduction in obese men.* Int J Obes (Lond), 2007. **31**(2): p. 245-53.
40. Thomas, T.R., et al., *Exercise and the metabolic syndrome with weight regain.* Journal of Applied Physiology, 2010. **109**(1): p. 3-10.
41. Delbridge, E.A., et al., *One-year weight maintenance after significant weight loss in healthy overweight and obese subjects: does diet composition matter?* The American Journal of Clinical Nutrition, 2009. **90**(5): p. 1203-1214.
42. Matsuo, T., et al., *An increase in high-density lipoprotein cholesterol after weight loss intervention is associated with long-term maintenance of reduced visceral abdominal fat.* Int J Obes (Lond), 2010. **34**(12): p. 1742-51.
43. Márquez-Quiñones, A., et al., *Adipose tissue transcriptome reflects variations between subjects with continued weight loss and subjects regaining weight 6 mo after caloric restriction independent of energy intake.* The American Journal of Clinical Nutrition, 2010. **92**(4): p. 975-984.
44. Crujeiras, A.B., et al., *Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients.* Metabolism, 2014. **63**(4): p. 520-31.
45. Hassan, M.K., et al., *Obesity and health-related quality of life: a cross-sectional analysis of the US population.* International Journal Of Obesity, 2003. **27**: p. 1227.
46. Jia, H. and E.I. Lubetkin, *The impact of obesity on health-related quality-of-life in the general adult US population.* Journal of Public Health, 2005. **27**(2): p. 156-164.

47. Zia, U.H., et al., *Meta-analysis of the association between body mass index and health-related quality of life among adults, assessed by the SF-36.* Obesity, 2013. **21**(3): p. E322-E327.
48. Martin, C.K., et al., *Exercise dose and quality of life: A randomized controlled trial.* Archives of Internal Medicine, 2009. **169**(3): p. 269-278.
49. Fontaine, K.R., et al., *Impact of weight loss on health-related quality of life.* Qual Life Res, 1999. **8**(3): p. 275-7.
50. Fontaine, K.R., et al., *Weight loss and health-related quality of life: results at 1-year follow-up.* Eat Behav, 2004. **5**(1): p. 85-8.
51. Warkentin, L.M., et al., *The effect of weight loss on health-related quality of life: systematic review and meta-analysis of randomized trials.* Obes Rev, 2014. **15**(3): p. 169-82.
52. Ling, C., et al., *Gait and Function in Class III Obesity.* Journal of Obesity, 2012. **2012**: p. 8.
53. Jakicic, J.M., et al., *Effect of exercise on 24-month weight loss maintenance in overweight women.* Arch Intern Med, 2008. **168**(14): p. 1550-9; discussion 1559-60.
54. Voils, C.I., et al., *Maintenance of weight loss after initiation of nutrition training: A randomized trial.* Annals of Internal Medicine, 2017. **166**(7): p. 463-471.
55. Svetkey, L.P., et al., *Comparison of strategies for sustaining weight loss: The weight loss maintenance randomized controlled trial.* JAMA, 2008. **299**(10): p. 1139-1148.
56. National Institutes of Health, *The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.* 2004.
57. Foster-Schubert, K.E., et al., *Effect of Diet and Exercise, Alone or Combined, on Weight and Body Composition in Overweight-to-Obese Postmenopausal Women.* Obesity, 2012. **20**(8): p. 1628-1638.
58. Johns, D.J., et al., *Diet or Exercise Interventions vs Combined Behavioral Weight Management Programs: A Systematic Review and Meta-Analysis of Direct Comparisons.* Journal of the Academy of Nutrition and Dietetics, 2014. **114**(10): p. 1557-1568.
59. U.S. Department of Health and Human Services, *2008 Physical Activity Guidelines for Americans.* 2008: p. 1-3.
60. Jakicic, J.M., et al., *The effect of physical activity on 18-month weight change in overweight adults.* Obesity (Silver Spring), 2011. **19**(1): p. 100-9.
61. The Diabetes Prevention Program Research, G., *The Diabetes Prevention Program (DPP): Description of lifestyle intervention.* Diabetes care, 2002. **25**(12): p. 2165-2171.
62. Haskell, W.L., et al., *Physical Activity and Public Health: Updated Recommendation for Adults from the American College of Sports Medicine and the American Heart Association.* Medicine and Science in Sports and Exercise, 2007. **39**(8): p. 1423-1434.
63. Morss, G.M., et al., *Dose-Response to Exercise in Women Aged 45-75 yr (DREW): Design and Rationale.* Medicine & Science in Sports & Exercise, 2004. **36**(2): p. 336-344.
64. Kraus, W.E., et al., *Studies of a targeted risk reduction intervention through defined exercise (STRRIDE).* Med Sci Sports Exerc, 2001. **33**(10): p. 1774-84.
65. Myers, C.A., et al., *Examination of mechanisms (E-MECHANIC) of exercise-induced weight compensation: study protocol for a randomized controlled trial.* Trials, 2014. **15**: p. 212.
66. Donnelly, J.E., et al., *Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: The midwest exercise trial.* Archives of Internal Medicine, 2003. **163**(11): p. 1343-1350.
67. Donnelly, J.E., et al., *Aerobic exercise alone results in clinically significant weight loss for men and women: Midwest exercise trial 2.* Obesity, 2013. **21**(3): p. E219-E228.
68. American College of Sports Medicine, *ACSM's Guidelines for Exercise Testing and Prescription*. 8th ed, ed. M. Whaley. 2010, Baltimore: Lippincott Williams& Williams. 139-140.
69. Agriculture, U.S.D.o., *Dietary Guidelines for Americans (8th edition).* 2015.
70. Coday, M., et al., *Strategies for retaining study participants in behavioral intervention trials: retention experiences of the NIH Behavior Change Consortium.* Ann Behav Med, 2005. **29 Suppl**: p. 55-65.
71. Miller, W.R. and S. Rollnick, *Motivational interviewing: Helping people change*. 2012: Guilford press.
72. Martins, R.K. and D.W. McNeil, *Review of Motivational Interviewing in promoting health behaviors.* Clin Psychol Rev, 2009. **29**(4): p. 283-93.
73. Block, G., et al., *Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period.* Journal of the American Dietetic Association, 1992. **92**(6): p. 686-93.

74. Swift, D.L., et al., *Effects of clinically significant weight loss with exercise training on insulin resistance and cardiometabolic adaptations.* Obesity, 2016. **24**(4): p. 812-819.
75. Swift, D.L., et al., *Effects of aerobic training with and without weight loss on insulin sensitivity and lipids.* PLOS ONE, 2018. **13**(5): p. e0196637.
76. Swift, D.L., et al., *Effect of Exercise Training Modality on C-Reactive Protein in Type 2 Diabetes.* Medicine and Science in Sports and Exercise, 2012. **44**(6): p. 1028-1034
77. Matsuda, M. and R.A. DeFronzo, *Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp.* Diabetes Care, 1999. **22**(9): p. 1462-70.
78. Townsend, R.R., et al., *Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association.* Hypertension, 2015.
79. Ware, J.E., Jr. and C.D. Sherbourne, *The MOS 36-Item Short-Form Health Survey (SF-36): I. Conceptual Framework and Item Selection.* Medical Care, 1992. **30**(6): p. 473-483.
80. Harris, P.A., et al., *Research Electronic Data Capture (REDCap) - A metadata-driven methodology and workflow process for providing translational research informatics support.* Journal of biomedical informatics, 2009. **42**(2): p. 377-381.
81. Ard, J.D., et al., *Effectiveness of a total meal replacement program (OPTIFAST® program) on weight loss: results from the OPTIWIN study.* Obesity (Silver Spring), In Press.
82. Carels, R.A., et al., *Using motivational interviewing as a supplement to obesity treatment: a stepped-care approach.* Health Psychol, 2007. **26**(3): p. 369-74.
83. Carels, R.A., et al., *A randomized trial comparing two approaches to weight loss: differences in weight loss maintenance.* J Health Psychol, 2014. **19**(2): p. 296-311.
84. Carels, R.A., et al., *Applying a stepped-care approach to the treatment of obesity.* J Psychosom Res, 2005. **59**(6): p. 375-83.
85. Carels, R.A., et al., *Reducing cardiovascular risk factors in postmenopausal women through a lifestyle change intervention.* J Womens Health (Larchmt), 2004. **13**(4): p. 412-26.
86. Carels, R.A., et al., *Step-down approach to behavioural weight loss treatment: a pilot of a randomised clinical trial.* Psychol Health, 2013. **28**(10): p. 1121-34.
87. Carels, R.A., et al., *Taking control of your personal eating and exercise environment: a weight maintenance program.* Eat Behav, 2008. **9**(2): p. 228-37.
88. Carels, R.A., et al., *Successful weight loss with self-help: a stepped-care approach.* J Behav Med, 2009. **32**(6): p. 503-9.
89. Carels, R.A., et al., *Transforming your life: an environmental modification approach to weight loss.* J Health Psychol, 2011. **16**(3): p. 430-8.
90. Swift, D.L., et al., *The effect of different doses of aerobic exercise training on endothelial function in postmenopausal women with elevated blood pressure: results from the DREW study.* British Journal of Sports Medicine, 2012. **46**(10): p. 753-758.
91. Swift, D.L., et al., *Effect of Different Doses of Aerobic Exercise Training on Total Bilirubin Levels.* Medicine and Science in Sports and Exercise, 2012. **44**(4): p. 569-574.
92. Swift, D.L., et al., *Racial Differences in the Response of Cardiorespiratory Fitness to Aerobic Exercise Training in Caucasian and African American Postmenopausal Women.* Journal of Applied Physiology, 2013. **114**(10): p. 1375-82.
93. Johannsen, N.M., et al., *Effect of Different Doses of Aerobic Exercise on Total White Blood Cell (WBC) and WBC Subfraction Number in Postmenopausal Women: Results from DREW.* PLoS ONE, 2012. **7**(2): p. e31319.
94. Swift, D.L., et al., *Exercise Training and Habitual Physical Activity: A Randomized Controlled Trial.* American Journal of Preventive Medicine, 2012. **43**(6): p. 629-635.
95. Swift, D.L., et al., *The Role of Exercise and Physical Activity in Weight Loss and Maintenance.* Progress in Cardiovascular Diseases, 2014. **56**(4): p. 441-447.
96. Earnest, C.P., et al., *Dose Effect of Cardiorespiratory Exercise on Metabolic Syndrome in Postmenopausal Women.* The American Journal of Cardiology, 2013. **111**(12): p. 1805-11.
97. Swift, D.L., et al., *The effect of different doses of aerobic exercise training on exercise blood pressure in overweight and obese postmenopausal women.* Menopause, 2012. **19**(5): p. 503-509
98. Swift, D.L., et al., *The Effects of Exercise and Physical Activity on Weight Loss and Maintenance.* Progress in Cardiovascular Diseases, 2018.

99. Church, T.S., et al., *Exercise without Weight Loss Does Not Reduce C-Reactive Protein: The INFLAME Study.* Medicine and Science in Sports and Exercise, 2010. **42**(4): p. 708-716.
100. Church, T.S., et al., *Effects of Aerobic and Resistance Training on Hemoglobin A1c Levels in Patients With Type 2 Diabetes.* JAMA: The Journal of the American Medical Association, 2010. **304**(20): p. 2253-2262.
101. MacLean, P.S., et al., *NIH working group report: Innovative research to improve maintenance of weight loss.* Obesity (Silver Spring), 2015. **23**(1): p. 7-15.

Statistical Analysis Plan

Title: Prevail R-56

Principal Investigators: Swift

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1 INTRODUCTION

1.1 Preface

Achieving clinically significant weight loss (CWL) (5-10%) has been shown to improve important cardiometabolic risk factors in obese adults (e.g. insulin sensitivity, dyslipidemia, systemic inflammation, blood pressure). However, only 20% of individuals achieve weight loss maintenance and even mild weight regain (2-6%) is associated with regression of risk factors toward pre-weight loss levels. Obtaining high amounts of physical activity (PA) are critical for successful weight loss maintenance. The Prescribed Exercise to Reduce Recidivism After Weight Loss pilot (PREVAIL-P) study will evaluate the effect of aerobic exercise training amount on weight maintenance following CWL in obese (BMI: 25-40 kg/m^2) men and women ages 18-65 years old. In study 1, volunteers will complete a 10-week OPTIFAST weight loss program. Individuals who achieve a weight loss of at least 7% from baseline weight will be subsequently randomized to levels of aerobic training consistent with half of physical activity recommendations (PA-REC) or weight maintenance recommendations (WM-REC) group for 16 additional weeks. Exercise sessions will be directly supervised by study staff to confirm exercise-related caloric expenditure. Non-exercise physical activity will be assessed objectively through accelerometry during the entire intervention in all randomization groups. For study 2, participants who are currently in or have nearly completed the VIDANT OPTIFAST program and achieve at least 7% weight loss will be randomized to either the PA-REC group or the WM-REC group for the next 36 weeks.

The primary outcome of PREVAIL-P for both study 1 and study 2 is the change in weight from the completion of the OPTIFAST program (achievement of clinically significant weight loss) to follow-up. Secondary measures include the proportion of participants achieving weight maintenance, changes in clinically relevant cardiometabolic risk factors (insulin sensitivity, arterial stiffness, fitness, fat mass, lipids, lipoprotein particle size and c-reactive protein), and energy intake between study groups.

1.2 Purpose of the analyses

The analysis will test if participants whether a physical program after clinically significant weight loss consistent with weight maintenance recommendations (WM-REC group) will have better health outcomes compared to participants in the physical activity recommendations (PA-REC) group.

1.3 Study aims and endpoints

The overarching goal is to test the effectiveness recommendations effect weight maintenance and cardiovascular risk factors.

1.3.1 Aim 1

To *demonstrate the efficacy of the weight loss program in producing CWL and retention/adherence of the exercise intervention.* Overweight and obese adults (N=39) will participate in an OPTIFAST weight loss program and supervised aerobic exercise training (~550 MET min. per week.) for 10 weeks. Participants who obtain CWL will be subsequently randomized to 16 weeks of aerobic training consistent with the minimum physical activity recommendations (~550 MET min per week.) or weight maintenance guidelines (~970 MET min per week). We will evaluate the percentage of participants that obtain at least 7% weight loss following OPTIFAST treatment, retention rates in the weight loss program, adherence to exercise levels, and changes in weight and cardiometabolic risk factors in response to the intervention.

1.3.2 Aim 2

To *test the hypothesis that exercise levels consistent with weight maintenance recommendations leads to greater weight maintenance after CWL compared to the minimum physical activity recommendation levels.* Overweight and obese adults (N=30) enrolled in VIDANT health's OPTIFAST program and have achieved at least 7% weight loss will be randomized to 36 weeks of aerobic exercise training consistent with the minimum public health guidelines for physical activity (~550 MET min. per week.) or weight maintenance levels (~970 MET min per week). We will evaluate the effect of the intervention on weight (primary) as well as main secondary measures (e.g. body fat, visceral fat, lipids, lipoprotein particles size/class, insulin sensitivity, blood pressure, arterial stiffness, systemic inflammation, fitness, and quality of life). The aforementioned cardiometabolic risk factors were selected because they can be improved specifically by weight loss and thus may respond differently to weight maintenance or regain.

1.4 Exploratory aims

We have planned the proposed research program such that our Primary Aims, described above, are the foundation of a rigorously designed study with mechanistic hypotheses. Such a study design requires focus and derivation of hypotheses that are strongly supported by preliminary data and adequate study power. Through this process we have identified many promising hypotheses that did not meet our rigorous criteria for being a Primary Outcome. These outcomes are of great significance but have less supporting evidence and we have classified them as secondary and exploratory outcomes. These additional outcomes are presented below and are referenced in the methods and herein. They are not, however, developed to the same extent as the Primary Aims.

1.4.1 Initial weekly weight loss

To examine the baseline factors of the participant achieving the clinically significant weight loss of 7% in the first 12 weeks of the study (Study 1 only)

1.4.2 Mediator and moderator analyses

To determine if there are any baseline or after initial weight loss have a mediating/moderating the effect on weight maintenance and cardiovascular risk factors. Association between weight loss, weight maintenance, and energy expenditure

To determine if initial weight loss and weight maintenance in the second phase is related to energy expenditure.

2 STUDY METHODS

The PREVAIL-P study will be divided into Study 1 and Study 2. Study 1 will use a parallel arm design with two intervention arms. Each patient will be followed for 10 weeks during the initial weight loss and an additional 16 weeks of weight maintenance. Measurements on the primary outcomes will be conducted at weeks 0, 10, and 26.

In Study 2, participants already in or very recently completed the OPTIFAST program in VIDANT and achieve a 7% weight loss will be randomized exercise groups (36 weeks afterwards). Study 2 will use a parallel arm design with two intervention arms. Primary outcomes in study 2 will be performed at 0,18, and 36 weeks.

2.1 Sample

The inclusion criteria for a participant include the following:

- The ages of 18-65,
- BMI between 25.0-39.9 kg/m^2,
- Current sedentary status (<2 days per week of exercise)
- Physically capable of exercise

2.2 Randomization

After initial weight loss (in both Study 1 and Study 2), participants who achieve 7% weight loss in the OPTIFAST program will be randomized to exercise levels consistent with physical activity recommendations (PA-REC) or weight maintenance recommendations (WM-REC) . The randomization for the proposed study will be generated by the study biostatistician, who will also be responsible for randomizing participants to study groups. No other research staff (including the PI) will have access to the study randomization. The randomization will be stratified by BMI Class (overweight, Class I and Class II) and gender to assure equal distribution to study groups.

2.3 Sample size and study power

The goal of the PREVAIL-P study is to provide feasibility of the weight loss intervention/subsequent exercise (Study 1) and power primary and secondary variables for a definitive trial (Study 2). Thus, this R56

will be used to inform the design of a subsequent. The originally sample size calculation called for 225 subjects to have 80% power. Based on Cocks, we will need at least 9% of the original sample to obtain proper estimates. This initial percent is doubled to 18% to account for the increased complexity as compared to the instance described by Cocks.

2.3.1 Aim 1

To *demonstrate the efficacy of the weight loss program in producing CWL and retention/adherence of the exercise intervention.* Overweight and obese adults (N=39) will participate in an OPTIFAST weight loss program and supervised aerobic exercise training (~550 MET min. per week.) for 10 weeks. Participants who obtain CWL will be subsequently randomized to 16 weeks of aerobic training consistent with the minimum physical activity recommendations (~550 MET min per week.) or weight maintenance guidelines (~970 MET min per week). We will evaluate the percentage of participants that obtain at least 7% weight loss following OPTIFAST treatment, retention rates in the weight loss program, adherence to exercise levels, and changes in weight and cardiometabolic risk factors in response to the intervention.

The goal of this aim is to demonstrate feasibility of the research team to successfully deliver the weight loss program and subsequent exercise training. We estimate that we can obtain at least 76.0% of the participants to achieve clinically significant weight loss. Thus, we expect at least 30 of the 39 to reach the exercise component of the trial. We felt that at least 15 participants per group would be a good sample to demonstrate the adherence of the subsequent exercise training program. We expect to complete 90% of the participants who have obtained significant weight loss.

2.3.2 We will evaluate several metrics of feasibility from Study 1 including the percent of participants meeting the weight loss goal of 7%, attendance of the weight loss classes, attendance at exercise classes, adherence to exercise goals during (MET minutes exercised vs. MET minutes required).

2.3.3 Aim 2

To *test the hypothesis that exercise levels consistent with weight maintenance recommendations leads to greater weight maintenance after CWL compared to the minimum physical activity recommendation levels.* Overweight and obese adults (N=30) enrolled in VIDANT health's OPTIFAST program and have achieved at least 7% weight loss will be randomized to 36 weeks of aerobic exercise training consistent with the minimum public health guidelines for physical activity (~550 MET min. per week.) or weight maintenance levels (~970 MET min per week). We will evaluate the effect of the intervention on weight (primary) as well as main secondary measures (e.g. body fat, visceral fat, lipids, lipoprotein particles size/class, insulin sensitivity, blood pressure, arterial stiffness, systemic inflammation, fitness, and quality of life).

2.3.4 The major goal of Study 2 is to power main and secondary measures for a definitive trial on physical activity level after clinically significant weight loss. Our goal is to complete at least 10 participants per group (90% retention) for power analyses for a definitive trial.

3 STATISTICAL PRINCIPALS

Analyses will use SAS Version 9.4 (SAS Institute, Cary NC). The primary methodology will be linear mixed effect models. Repeated effects will account for the patient's correlations over time. Further details about the covariance structures is given below. Results will be given in the form of both the raw beta coefficient when the predictor is continuous and as least square means when the predictor is categorical. Overall F-tests will be done for sets of beta coefficients that correspond to the same grouping variable (i.e. gender or race interactions). When the overall F-test is significant, pairwise T-test will be investigated, with alpha adjusted for the number of comparisons. Whenever possible visualization of these results will be given by scatter plots with the overall beta coefficients used as slope estimators overlaid on the plots.

3.1 Distributional assumptions

Preliminary statistical testing will be performed to investigate homoscedasticity and normality of model residuals. The Shapiro–Wilk test will be used to test for normality of residuals for outcomes modeled with a linear mixed model. For non-normal data, a log or square root transformation will be applied and retested. If these transformed data again indicate non-normality, non-parametric tests will be used. The Mann-Whitney U test will be the primary non-parametric test. This test uses the sum of the ranks to determine if the samples are from the same distribution. Due to the large number of possible ties in the data, the Mann-Whitney U test may not be the best test. In such situations, the Kolmogorov–Smirnov test will be used. This test is based on the largest difference in the empirical distribution function of the two groups. As such, it is not as sensitive to ties in the data.

3.2 Outcome measures

3.2.1 Primary

The primary outcome measure of the PREVAIL-P study is change in weight after obtaining clinically significant weight loss to follow-up of the intervention. Weight will be evaluated using a calibrated scale to the nearest tenth of kg with the participant in a fasted state (12 hours), wearing only a hospital gown and after the participant has voided.

3.2.2 Secondary

For Study 1, secondary measures will be collected at all 3 assessment points with the exception of accelerometetry (obtained prior to each assessment period). We specifically chose cardiometabolic risk factors which have been shown in the literature to improve with weight loss, exercise training (independent of weight loss), or a combination of both. The rationale for this is that it is possible that even in the presence of weight recidivism, that adherent exercise training may attenuate the deleterious effect of weight regain on cardiometabolic risk factors. Thus, we will evaluate the effect of the proposed intervention on traditional (lipid concentrations, brachial systolic/diastolic blood pressure), non-traditional (fitness, lipoprotein particles size, c-reactive protein, pulse wave velocity, augmentation index, central blood pressure) CVD risk factors as well as a type 2 diabetes risk factor (insulin sensitivity), total energy expenditure, energy intake and quality of life. The PI has published on significant weight loss and insulin sensitivity [6], weight/fat loss and c-reactive protein [7] and lipoprotein particle size. Quality of life (QOL) will be measured via the SF-36 questionnaire. QOL improves with aerobic training in a dose response manner [8] and also improves with weight loss [9-11], and thus may vary between groups in the present proposal.

For Study 2, secondary measures will be assessed at baseline and follow-up (with the exception of weight and body composition which will also be assessed at mid-intervention). Covariance structures
The variance-covariance structure of the repeated effects will be dependent on the variability among all participants. A preliminary test will be conducted to assess significance of the homogeneity of variances [10-12] for between treatment arm differences. The within-patient variability will be modeled with an unstructured covariance matrix. Correlation patterns will be assessed with Akaike's Information Criterion (AIC) to make final selections.

3.3 Covariates

Covariates in the generalized linear mixed model will include, but not limited to the follow:

- time point
- time point*treatment interaction
- age
- gender
- initial weight
- race/ethnicity
- socioeconomic status

Other potential covariates may include using the baseline measures of several of the primary and secondary outcomes.

Variable selection in select linear models will be determined though model fit statistics such as AIC.

3.4 Mediation and moderator analyses

Mediations will investigate how the covariates affect the relationship between weight gain and the intervention [13]. Mediation analyses will be applied using both structural mean models and principal stratification [14]. Preacher and Hayes bootstrapping methods, as well as the Sobel test, will be used to test potential mediations of the outcomes. The advantage of using the Preacher and Hayes bootstrapping methods over just the Sobel test include greater power and distributional free assumptions. Mediation will be done using the CALIS procedure in SAS along with the MIXED procedure and IML for the bootstrapping methods.

We will use the Baron and Kenny method and bootstrapping methods for moderator analysis. A statistically significant interaction will define age as a moderator. This interaction will only be removed from the model when not statistically significant.

While any of the covariates could be potential mediator or moderators, we only investigate a subset of these variables. Weight change will be the primary variables of interest.

3.5 Subgroups

Heterogeneity of variance effects for genders for each of the treatments will be analyzed first as potential subgroups. While these sample sizes are not powered to detect differences between each subgroup, these results will be invaluable by providing directionality for future hypotheses.

3.6 Missing data

Data may be missing because participants drop out or fail to complete an instrument or assessment. Three patterns of missing data will be considered: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR). Data are MCAR if events that lead to all missing values are independent of both the observed and missing values. Data are MAR if the events depend on the observed values but are independent of the missing values. If the missing data mechanism depends on specific reasons related to the missing data themselves, then the values are MNAR.

Sensitivity analyses will be performed to investigate whether different analyses under different assumptions provide robust inferences. Four analyses will be used: 1) a mixed effects linear model for repeated measures that makes use of all data irrespective of missing data; 2) completers analysis with participants who have complete data on the primary outcomes; 3) mixed effects linear time trend model with random coefficients; and 4) multiple imputation analysis using the MCMC methods. The robustness of the inferential findings to the analytic methods will be assessed by comparing differences in analytical findings across the four analyses.

3.7 General reporting conventions

Descriptive and summary statistics will be generated for outcomes and covariates of interest. For all outcomes, all continuous variables will be summarized using n (non-missing sample size), mean, standard deviation (SD), standard errors (SE), correlations (R), minimum (Min), maximum (Max), and coefficient of variation (CV). The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures, unless otherwise specified.

All tables, figures and data listings will be presented in portrait orientation, unless landscape orientation suggests that the information is easier to view. All titles will be centered on a page. For tables, sample sizes for each treatment group will be presented as totals in the column header (N=xx), where appropriate. Sample sizes shown with summary statistics are the number (n) of patients with non-missing values. Summaries for categorical variables will include only categories that patients had a response in. Percentages corresponding to null categories (cells) will be suppressed.

3.8 Statistical summary conventions

The mean and SD will be reported to one decimal place greater than the original data. Minimum and maximum will use the same number of decimal places as the original data. All percentages will be rounded and reported to a single decimal place (xx.x%). If percentages are reported as integers, percentages greater than 0% but <1% will be reported as <1%, whereas percentages greater than 99% but <100% will be reported as >99%. A percentage of 100% will be reported as 100%. No value of 0% will be reported. Any computation

of percent that results in 0% is to be reported as a blank. Summaries that include p-values will report the p-value to three decimal places with a leading zero (0.001). P-values <0.001 will be reported as "<0.001".

3.9 Interim analyses

No interim analyses are planned.

4 ANALYSIS

All primary outcomes are analyzed as using change scores. The linear mixed effect models will use the outcome as the response for the primary outcomes. Full details about each set of models is given in Section 3.

4.1 Analysis Populations

The primary statistical analyses will rely on intent-to-treat (ITT) methodology, with all patients' data included who have a measurement at baseline and follow-up. A per protocol analysis will also be conducted as a secondary analysis.

4.2 Demographic and Baseline Variables

These data will include (but are not limited to) height, weight, BMI, body composition, and blood pressure lipids, fitness, sitting behavior, total energy expenditure, accelerometers results, food frequency questionnaire, and SF-36. F-tests followed up by pairwise T-tests and chi-squared test will be used to determine if any differences occur between groups. Baseline variables for all psychological, behavioral, and physical measures will also be investigated to ensure a random and balance sample.

4.3 Analyses of the primary aims

4.3.1 Aim 1

4.3.2 To *demonstrate the efficacy of the weight loss program in producing CWL and retention/adherence of the exercise intervention.* Aim 2

Although the primary purpose of the present study is to evaluate feasibility/efficacy of the weight loss intervention/exercise (Study 1) and powering weight maintenance variables (Study 2). We will still evaluate the impact of the intervention outcome variables. For both Study 1 and Study 2, weight will be the primary outcomes measure and the other cardiometabolc variables will be considered secondary outcomes. In addition, the percent of participants maintain weight (categorical analysis) will also be considered a secondary endpoint.

Primary outcome: To determine the magnitude of weight maintenance (as a continuous variable) in response to different levels of exercise training after CWL

Initial results will be based on a linear mixed effect model with the change scores as the outcome. The model will use random effects to account for the correlation in a participant over time. Results from this model will be reported as least square means, with p-values based on t-tests. Two different sets of models will be used. The first set of models will contain only treatment, while the second is will also contain the covariates based on the model selection process. Pairwise post-hoc comparisons of the category variables will be corrected for multiple comparisons will be made when necessary.

Secondary outcomes:

To determine the percentage of individuals (as a categorical variable) who are able to maintain weight loss maintenance in response to different levels of exercise training after CWL

Logistic mixed effect model with the probability of achieving weight maintenance, with results presented as adjusted odds ratios. The model will use random effects to account for the weight differences at baseline. Results from this model will be reported as odd ratios, with p-values based on Chi-squared statistics. Two different sets of models will be used. The first set of models will contain only treatment, while the second is

will also contain the covariates based on the model selection process. Pairwise post-hoc comparisons of the category variables will be corrected for multiple comparisons will be made when necessary.To determine the change in clinically relevant cardiovascular risk factors in response to different levels of exercise training after CWL

Models similar to the ones used in Aim 1 will be used here as well. Outcomes for this aim include both traditional (lipid concentrations, brachial systolic/diastolic blood pressure), non-traditional (fitness, lipoprotein particles size, c-reactive protein, pulse wave velocity, augmentation index, central blood pressure) CVD risk factors as well as a type 2 diabetes risk factor (insulin sensitivity), total energy expenditure, energy intake and quality of life.

4.4 Analyses of the Exploratory Aims

4.4.1 Initial weekly weight loss

Mixed effect models will be used to model the weekly weight changes in the first 10 weeks. Baseline covariates will be used to determine which set of covariates best predicts the weight at week 10.

4.4.2 Mediator and moderator analyses

Baseline covariates will be tested as both mediating/moderating effects on weight maintenance and cardiovascular risk factors. See section 3.4 for full details of these tests.

4.4.3 Outcome transformation

Almost all primary outcomes will be based on change from baseline. However, this model makes assumptions about the data that may not be valid when the final data is reviewed. The primary assumption of concern is that change from baseline is not dependent on the starting value since all arms should have approximately equal due to randomization. This assumption will be investigated using a two-sample t-test on the baseline values. Whenever this assumption is violated, two other models will be investigated. The first alternative model will use the baseline time in addition to the follow-up times. This model allows for testing of change in group means over time as opposed to the primary model that tests individual change over time. The second alternative model will use only the follow-up times and with initial time as a covariate. This model assumes that baseline group means are equal and the follow-up values are adjusted for this overall baseline value. Comparisons and contrasts will be made between all three model for similar tests when possible.

5 REFERENCES

1. Andersen, R.E., et al., *Effects of Lifestyle Activity vs Structured Aerobic Exercise in Obese Women.* JAMA: The Journal of the American Medical Association, 1999. **281**(4): p. 335-340.
2. Jakicic, J.M., et al., *Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: A randomized trial.* JAMA, 1999. **282**(16): p. 1554-1560.
3. Church, T.S., et al., *Effects of Different Doses of Physical Activity on Cardiorespiratory Fitness Among Sedentary, Overweight or Obese Postmenopausal Women With Elevated Blood Pressure.* Journal of the American Medical Association, 2007. **297**(19): p. 2081-2091.
4. Kraus, W.E., et al., *Effects of the Amount and Intensity of Exercise on Plasma Lipoproteins.* New England Journal of Medicine, 2002. **347**(19): p. 1483-1492.
5. Church, T.S., et al., *Effects of Aerobic and Resistance Training on Hemoglobin A1c Levels in Patients With Type 2 Diabetes.* JAMA: The Journal of the American Medical Association, 2010. **304**(20): p. 2253-2262.
6. Swift, D.L., et al., *Effects of clinically significant weight loss with exercise training on insulin resistance and cardiometabolic adaptations.* Obesity, 2016. **24**(4): p. 812-819.
7. Swift, D.L., et al., *Effect of Exercise Training Modality on C-Reactive Protein in Type 2 Diabetes.* Medicine and Science in Sports and Exercise, 2012. **44**(6): p. 1028-1034
8. Martin, C.K., et al., *Exercise dose and quality of life: A randomized controlled trial.* Archives of Internal Medicine, 2009. **169**(3): p. 269-278.
9. Fontaine, K.R., et al., *Impact of weight loss on health-related quality of life.* Qual Life Res, 1999. **8**(3): p. 275-7.
10. Fontaine, K.R., et al., *Weight loss and health-related quality of life: results at 1-year follow-up.* Eat Behav, 2004. **5**(1): p. 85-8.
11. Warkentin, L.M., et al., *The effect of weight loss on health-related quality of life: systematic review and meta-analysis of randomized trials.* Obes Rev, 2014. **15**(3): p. 169-82.
12. Conover, W.J., M.E. Johnson, and M.M. Johnson, *A Comparative-Study of Tests for Homogeneity of Variances, with Applications to the Outer Continental-Shelf Bidding Data.* Technometrics, 1981. **23**(4): p. 351-361.
13. Hayes, A.F., *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. 2017.
13. Hayes, A.F., *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. 2017.
14. Hayes, A.F., *Beyond Baron and Kenny: Statistical Mediation Analysis in the New Millennium.* Communication Monographs, 2009. **76**(4): p. 408-420.
15. Cocks, K., & Torgerson, D. J. (2013). S*ample size calculations for pilot randomized trials: a confidence interval approach*. Journal of clinical epidemiology, 66(2), 197-201.