Study Title: A Novel Mobile Health Exercise Intervention in Aging: Brain Perfusion and Cognition

NCT03058146

Date protocol last approved by the IRB: 06/15/2022

This protocol contains only the relevant portions of the IRB approved study protocol.

OBJECTIVE

Physical activity interventions with older adults can improve brain health; however, most interventions have been performed in gym-like settings that reach a small sector of the senior population. Since not everyone can access a gym, it is important to study whether brisk walking in real-world environments can also help brain health. This study will use mobile health devices to help older adults independently walk for brain health, thus representing a critical step towards the dissemination of physical activity intervention programs aimed at preserving cognitive function in healthy aging.

SPECIFIC AIMS

Aim 1: To perform a randomized controlled trial of walking (N=60) to investigate if the use of mobile health (mHealth) technology in real-world environments will help increase cardiorespiratory fitness, time spent in moderate to vigorous physical activity (MVPA), cerebral blood flow (CBF) and resting state brain connectivity, and cognitive function, and decrease sedentary time compared to a healthy aging education control condition in cognitively normal older adults aged 65-80 over a 3 month period.

> **Hypothesis 1:** Compared to the healthy aging education control condition (N=30), older adults assigned to the mHealth walking condition (N=30) will have greater increase in cardiorespiratory fitness, MVPA, regional CBF (hippocampal, frontal, anterior and posterior cingulate cortex, and inferior parietal lobule), cognitive function, and strengthened resting state connectivity, as well as greater decreases in sedentary time, relative to baseline.

Aim 2: To investigate if changes in regional CBF (frontal and hippocampal) mediate the relationship between changes in MVPA/sedentary time and changes in cognitive function (executive functions and memory) for those assigned to the mHealth walking condition.

Hypothesis 2: Changes in CBF will partially mediate the relationship between changes in MVPA/sedentary time and changes in cognitive functions within the mHealth walking condition.

Exploratory Aim: To explore whether the effects of the mHealth walking intervention on the outcomes of interest differ as a function of genetic risk for Alzheimer's disease (apolipoprotein E [APOE] ε4 carriers versus noncarriers). We believe that those at risk for AD (APOE ε4 carriers) will display greater increases in CBF compared to noncarriers as a function of the walking mHealth intervention.

METHODS

MEASURES

Fitness Testing (30 minutes): Fitness will be measured before (baseline) and after the intervention (3 months) by trained personnel at UCSD EPARC. We will measure fitness using a Submaximal-Graded Exercise Test (GXT). The GXT will be conducted on a treadmill at UCSD EPARC. Heart rate will be plotted against the workload and its corresponding estimated VO_2 (American College of Sports Medicine, 2006), and maximal VO_2 will be estimated plotting the slope of the line to estimated maximal heart rate (220-age). Although the research team recognizes that direct measurement of VO_{2max} is considered the criterion measure of cardiorespiratory fitness, a sub maximal exercise test with estimations of oxygen consumption is appropriate for this study for several reasons. The proposed intervention is designed to promote regular, moderate-intensity physical activity, which may have minimal effect on maximal aerobic power, but significant effects on submaximal endurance capacity. Thus, we would expect to see an increase in total treadmill time and a decrease in heart rate at a given submaximal workload. By eliminating the need to wear a mask to directly gather metabolic data, the degree of subject burden, and the investigators' effort to avoid stressful procedures relevant to the exercise experience, are minimized. Both of these are important considerations for this older, sedentary population.

Four outcome measures will be determined from this test: 1) Total time to 85% of estimated maximal heart rate (with the exception of participants taking beta blockers—see below); 2) heart rate corresponding to a moderate (i.e. 3+ METS) workload; 3) heart rate recovery (HRR) measured at 1, 2, and 3 minutes of recovery; and 4) rate-pressure product (RPP), which is the product of heart rate and systolic blood pressure [SBP x HR] / 100 measured toward the end of each of the first three stages of exercise. RPP is an indirect assessment of cardiac work. A decrease in RPP for a given amount of aerobic work signifies an improvement in cardiac function.

The heart rates measured during the final ten seconds of minutes seven (7) and/or nine (9) will be utilized to determine heart rate zones for exercise prescription during the intervention period. These measurement times occur during stage 3 (3.0 mph) at which participants are

estimated to be working at a level equivalent to 3.3 METS; a threshold slightly higher than the minimum level to be considered "moderate" activity. Minutes three and five of that stage (minutes 7 and 9 of the total protocol) have been chosen because of the likelihood that the participant will have reached "steady state" after 3+ minutes at that workload. Specifically, steady state is the point at which the heart's work (i.e. RPP) is equal to the workload, and is unlikely to change with increased exercise duration. For exercise prescription purposes, the two heart rate values gathered during stage 3 of the protocol (i.e. minutes 7 and 9 of the total protocol) will be averaged if the difference is three beats per minute (bpm) or less; and the greater of the two heart rates will be used if the difference is greater than three bpm.

Quality assurance for this procedure is approached as follows: Conditions known to affect heart rate will be controlled to the best of our ability. Environmental conditions (room temperature; relative humidity) will be monitored and kept stable for repeated measures. Participants will be asked to refrain from caffeine or other stimulants on the day of their test. The treadmill will be calibrated monthly. Participants will not be asked to withhold beta blocker medications. Individuals who are taking beta-blockers will be asked to exercise to a workload corresponding to 80% of their age predicted maximal heart rate. This precaution should account for the approximately 5% reduction in heart rate observed with beta blockade and will allow the research team to provide accurate/scaled exercise prescription. Furthermore, participants using beta blockers will also be coached on the Rating of Perceived Exertion (RPE) scale and will be counseled to exercise at a level corresponding to 5-7 (moderate exercise). The mHealth device to be used in the intervention, will also be used during fitness testing to compare heart rate accuracy.

Physical activity (PA) assessment: Prior to participation, participants will be phone screened to evaluate inclusion criteria. Part of this initial evaluation includes a 1-week accelerometer monitoring period to ascertain sedentary status (< 10 minutes per day of moderate levels of physical activity as measured by accelerometry) and establish baseline levels of MVPA. Participants will be mailed research grade accelerometers with instructions (and will be coached via phone), which they will wear for 1 week while performing their everyday activities. Accelerometers will be brought back to the research group during visit 1 for analyses of the sedentary time data. Participants will also undergo cognitive and fitness testing during visit 1. During the RCT, the intervention and control conditions, participants will undergo 3, 1-week long PA measurement periods using accelerometers (baseline, mid-study, and post study). Triaxial accelerometers provide information regarding the frequency and intensity of PA and sedentary time and it is a small device that is worn comfortably on the hip, which has been validated for use with older adults. Accelerometers will allow us to determine if potential participants qualify as sedentary (< 10 minutes per day in moderate levels of PA) as well as to compare sedentary and physically active time between the intervention and control conditions at baseline, 6 weeks (mid), and 3 months (post). During each measurement period, participants will be asked to wear

the accelerometer on their hip, for a minimum of 12 hours a day, for 1 week, while they perform their everyday activities. Accelerometers will not be worn during sleep or when there is a probability of getting the device wet (during showering, swimming, etc). Outcomes from this assessment include: daily average sedentary time and light, moderate, and vigorous physically active time.

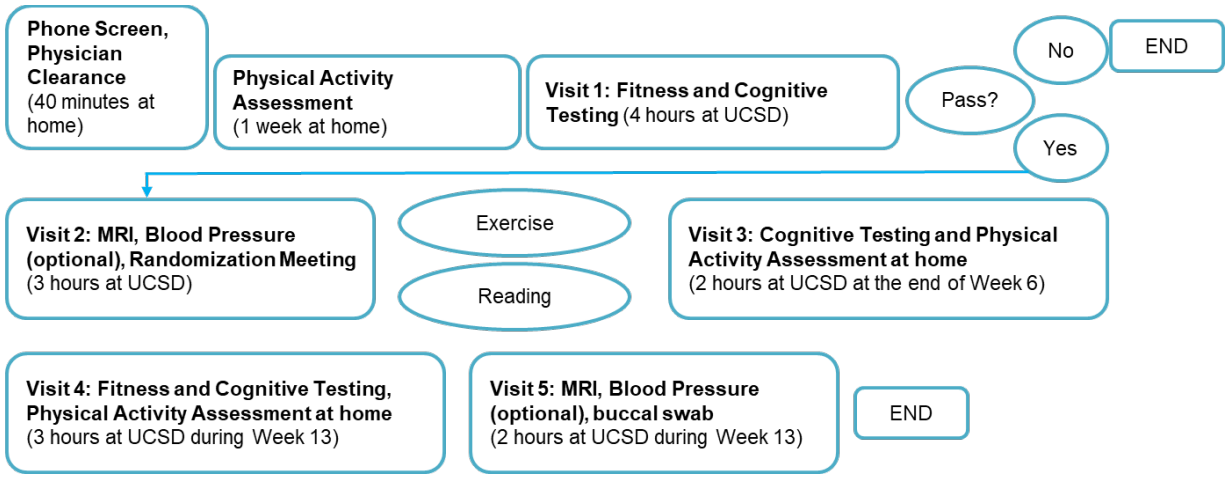
Cognitive Testing (~3 hours): All participants will undergo cognitive testing at baseline, mid, and post intervention assessments. To exclude individuals with dementia or mild cognitive impairment, those who do not meet established cutoffs on the Mattis Dementia Rating Scale and also show evidence of MCI (based on Jak et al. 2009 comprehensive criteria) on cognitive testing at baseline will be disqualified from further participation. The NIH Toolbox Cognition Battery will be implemented to measure 5 cognitive domains as the main cognitive outcomes: Memory (Picture Sequence), Executive Functions (Flanker Inhibitory Control & Dimensional Change Card Sort), Language (Picture Vocabulary & Oral Reading Recognition), Processing Speed (Pattern Comparison), Working Memory (List Sorting), and Attention (Flanker Inhibitory Control). Since Memory and Executive Functions are most sensitive to PA-related changes, we will supplement Executive Functions testing with the Delis Kaplan-Executive Function System: Trail Making, Color-Word Interference, and Verbal Fluency Tests and Memory with the California Verbal Learning Test-II, the Wechsler Memory Scale (WMS-IV) Logical Memory Test, and a face recognition test developed by our research group. Individual test scores will be used to evaluate cognitive status for inclusion/exclusion criteria, and to create the composite scores for each of the 5 cognitive domains identified above by averaging their z-scores. Alternate forms of each test will be administered when available to avoid practice effects. Mental health symptoms will be assessed with the NIH Toolbox PROMISE measures available on REDCap.

Buccal Swab for Genotyping (5 minutes): For participants whose genotype is unknown, Dr. Zlatar or a research assistant will collect necessary buccal swabs (saliva samples) using the Oragene DNA sample collection kit for PCF analysis of Apolipoprotein (APOE) genotype. PCR analysis for APOE genotyping. Buccal swabbing will be done by the project research technician from participants recruited from sources outside of the UCSD Alzheimer's disease research center (ADRC). All samples will be stored in a -80° freezer located in a locked laboratory at the Stein Institute for Research on Aging. All samples will be labeled using the unique study identifier assigned to each patient, visit date, and visit number. They will be logged using this information and no other identifiers. The results of any genetic analysis will be kept confidential in a secured, locked location and used for research purposes only. No identifiable information is part of the information. Genotyping information will not be shared with research participants.

Brain Imaging Assessment (60 minutes): Arterial Spin Labeling (ASL) MRI will be used to measure whole-brain resting CBF before and after the intervention. Analyses will focus on the following regions of interest (ROIs), which are sensitive to changes in PA: bilateral hippocampi (hippocampal ROI), inferior/middle frontal gyri and frontal pole (frontal ROI), anterior and posterior cingulate cortex (ACC/PCC ROI), and the angular and supramarginal gyri (inferior parietal lobule ROI). Right and left ROIs will be averaged to reduce the number of comparisons, with exception of the hippocampus. To acquire resting CBF, the MPPCASL sequence developed at the UCSD Keck Center (GE MR 750: 3 Tesla scanner) for fMRI will be utilized. It is optimal for white and gray matter perfusion measurement since it reduces some of the previous limitations and has improved tagging efficiency (which translates to higher temporal signal to noise ratio and well-controlled temporal bolus leading to robust CBF quantification) tagging duration=2 sec, TI=3.6 sec, TR=4.2 sec, TE=minimum, reps=64, FOV=22x22 cm, 20 5 mm axial slices with a single shot spiral acquisition, collecting 8 cycles where each cycle consists of 8 images acquired with unique phase offsets, acquisition time =4:46 minutes. The structural MRI sequence includes a high resolution T1-weighted Fast Spoiled Gradient Recall (3D FSPGR) scan: 172 1 mm contiguous sagittal slices, FOV=25 cm, TR=8 ms, TE=3.1 ms, flip angle=12, T1=600, 256 x 192 matrix, Bandwidth=31.25 kHZ, frequency direction= S-I, NEX=1, scan time=8 min 13 sec, as well as a T2-weighted FLAIR sequence for assessment of white matter hyperintensities (to be used as a covariate) collected in the axial plane: TR=8650 msec, TE=136 msec, TI=2250 msec, FOV=24 cm, 27 x 5mm slices, Bandwidth=31.25, 1.00 NEX, Acquisition time=4 min.

DESIGN & PROCEDURES

Randomized Controlled Trial (RCT): All potential participants will be pre-screened over the phone to determine if eligibility criteria are met (obtain physician's clearance and assess MRI scanning eligibility and medical history which could exclude them from participation). They will then provide verbal feedback to undergo the physical activity assessment (1-week accelerometer measurement) to determine sedentary status and provide baseline physical activity levels. Participants will bring the accelerometers back to the team during session 1 where they will be consented and enrolled in the study. Following cognitive and fitness testing (session 1), eligible participants will be randomly assigned to one of two conditions ("mHealth walking" or "healthy aging education" control conditions). During session 1, if participants were unable to complete the fitness or cognitive protocols, did not meet the cognitive criteria, or if there was indication of high risk to perform fitness testing (based on cardiovascular risk assessment), participation will be discontinued. Since this intervention is individualized and takes place in their real-world environment, group sessions or synchronized start/end points are not needed (there is rolling recruitment and enrollment).



A) MHEALTH WALKING CONDITION: Older adults randomized to this condition (N=30) will perform 3 months of prescribed brisk walking in their real-world environments, using mHealth during each exercise session, to objectively measure and help track activity, with the goal of increasing cardiorespiratory fitness and achieving/maintaining a minimum of 150 minutes of moderate to vigorous physical activity (MVPA) per week over time. The mHealth walking intervention condition will consist of the following active components: 1) an individualized and gradually increasing exercise prescription based on individual fitness testing; 2) study monitoring/compliance phone calls to provide motivation, track goal achievement, and assess/manage any barriers to adherence; 3) and the use of mHealth to objectively track adherence and measure PA behaviors as well as aid with maintaining MVPA by providing real-time feedback about PA performance (heart rate target feedback). Each active component, all of which together comprise a novel and multifaceted PA intervention, are described in detail below:

1. Exercise prescription: Based on their estimated maximum HR during fitness testing, each participant assigned to the mHealth walking condition will be prescribed a PA regimen targeting between 60-75% of their -estimated HR, which is equivalent to MVPA intensity (~3 METS). Since our participants will be sedentary at onset, the exercise prescription will gradually increase PA levels and HR targets by setting realistic goals in order to promote adherence and minimize risk until participants achieve at least 150 minutes of MVPA per week at 60-75% target HR. As such, prescribed walking times and target intensity will increase over time. Goals will be revised during monitoring/compliance calls (described below) according to participants' performance and whether or not they achieved their prescribed goals for each stage. Goal revision will follow protocols successfully implemented by Dr. Kerr (Co-Investigator) and her group in previous community-based exercise interventions. Given the use of immediate targeted feedback from the mHealth device, it is expected that participants will be able to achieve and maintain

prescribed levels of MVPA, since they will be able to immediately correct their walking pace when provided with a tactile vibration from the device. During each exercise session, the mHealth device will record participants' heart rate which will be automatically saved to the smartphone application. Study staff will ask participants to log the data gathered by the app and share it with the study staff to provide targeted feedback during monitoring/compliance calls as well as to provide motivation and positive reinforcement when goals have been achieved.

2. Monitoring/Compliance calls: Participants will receive monitoring/compliance calls from the research team which will vary in frequency throughout the length of the study. These calls are designed to measure participants' progress, assess for barriers to exercise and problem-solve with participants, revise goals if needed, set new goals, provide positive feedback and motivation when goals have been reached, provide motivation when goals have not been reached, to implement incentives when goals are not being reached, and to review objective data gathered from the mHealth device directly with participants. These calls will also serve as an opportunity for participants to report any adverse events to the research team. Compliance calls, which are an active and important component of this novel intervention, will take place weekly during the first month and bi-weekly during months 2 and 3 with the goal of reducing contact with participants to a minimum to encourage sustained adherence and self-monitoring after contact with staff ends. Each phone call will last between 10 and 20 minutes. Participants in both groups will receive monitoring phone calls (to equate for attention from study staff). If participants cannot be reached by phone, follow-up emails will be sent to them. Similarly, weekly email surveys will be sent to obtain self-reported exercise data from the intervention participants and to ask questions about the readings for the control participants.

3. Use of mHealth: Another active component of this novel PA intervention is the use of the Mio Fuse system to help track and objectively measure heart rate (HR) during the intervention. This device provides immediate tactile feedback to the user regarding their preferred heart rate zone to encourage the participant to remain in that specific zone as long as possible. A Polar HR band will also be used to track HR during fitness testing, which will be used for analyses of amount of time spent within the target heart rate zone. Previous intervention studies that have used mHealth devices have focused on providing the participants with personalized plans and exercise reminders, but they have not taken advantage of collecting objective data from these devices. Furthermore, the Mio Fuse will be individually programmed for each participant in accordance to their own prescribed HR target zones (based on estimated maximum heart rate from fitness testing) to alert them if they are deviating from their prescribed heart rate targets in real time. We believe this capability will aid participants in maintaining their prescribed HR target zones by adapting their behavior immediately. The Mio Fuse can be manually re-programmed in order to adjust HR goals according to the gradual nature of the intervention. Even though the use of mHealth is an important active component of the intervention, it has not been conceptualized as

the sole agent of change in this study. Rather, all three components described in this section are expected to provide a propitious environment for increasing cardiorespiratory fitness and MVPA within this multifaceted framework. Information from the Mio Fuse application will be shared by the participants with the research staff on a weekly basis. If the Mio Fuse device poses significant difficulties, we will implement a similar, more user-friendly mHealth device to track heart rate activity.

B) HEALTHY AGING EDUCATION CONTROL CONDITION: The education control condition (N=30) will provide participants with printed materials and homework assignments (quizzes) on issues related to successful aging, such as nutrition, social activity, cognitive and social engagement. This type of control condition was chosen rather than a no-contact condition in order to equate for attention from study staff. As such, participants in both conditions will undergo the same amount of contact time with staff members in person and during compliance phone calls. Topics will not cover PA to avoid changes in PA within the control group. Compliance phone calls for this group will focus on checking on homework assignments and briefly testing knowledge of the reading materials as a means to assess adherence to the control condition. Given that the education control condition will not be given a mHealth device to perform prescribed exercise, we will also conduct an accelerometer PA measurement period at three time points (baseline, mid, and post intervention) with both conditions. This measurement will allow us to assess whether individuals assigned to the control condition have incurred any changes in PA levels, above and beyond changes in cardiorespiratory fitness measured before and after the intervention. Participants in this control condition will also receive monitoring phone calls at the same frequency as those in the mHealth condition to equate for attention from stud staff. Follow-up emails will be sent when no contact is achieved via phone and weekly surveys will be sent to assess knowledge of the reading materials.

RCT Outcomes and Analyses

Brain Image Processing: Neuroimaging data processing is performed with Analysis of Functional NeuroImages (AFNI), FMRIB Software Library (FSL, Oxford, United Kingdom), SPM5, FreeSurfer and locally created MatLab and R scripts.

High Resolution Structural Scan: T1-weighted MRI is used for cortical and subcortical segmentation and parcellation to obtain volumetric data. Skull stripping of the high-resolution T1-weighted data image is performed using AFNI 3dSkullstrip. Scans are manually edited to remove residual non-brain material when necessary. Tissue segmentation is performed using FSL's Automated Segmentation Tool (FAST) algorithm to define cerebrospinal fluid (CSF), gray matter (GM), and white matter (WM) regions.

Whole Brain Resting Cerebral Blood Flow (CBF): For each subject, a mean arterial spin labeling (ASL) image is formed from the average difference of the control and tag images. This mean ASL image is then converted to absolute units of CBF (mL/100 g tissue/min) with use of the CSF image. To correct the CBF measures for partial volume effects, we use the method previously reported by Johnson and colleagues. These calculations assume that CSF has zero CBF and that CBF in GM is 2.5 times greater than that of WM. The following formula is used to compute partial volume corrected CBF signal intensities: CBFcorr = CBFuncorr/(GM + 0.4 * WM). CBFcorr and CBFuncorr are corrected and uncorrected CBF values, respectively. GM and WM are gray matter and white matter partial volume fractions, respectively. Information from the high resolution structural image and the FSL FAST tool are used to determine the tissue content of each perfusion voxel. To distinguish between GM and WM CBF, only voxels that survive masking after we apply the participant's resampled probabilistic GM or WM mask created using FSL FAST are submitted to voxel-wise analysis. CBF images are then spatially normalized to MNI space to obtain regions of interest (ROIs) using the Harvard-Oxford cortical and subcortical brain atlases available in FSL. ROIs to be examined include: bilateral hippocampi, inferior/middle frontal gyri/frontal pole (prefrontal), anterior and posterior cingulate cortex (ACC/PCC), and the angular and supramarginal gyri (inferior parietal lobule) given their sensitivity to exercise-related changes.

Data Analyses: Data will be screened for outliers, missing values, and normality of variable distribution. IBM SPSS, R statistical software, and MATLab, will be used to analyze the data. Preliminary analyses will be used to assess the influence of outliers and non-normally distributed variables for possible exclusion or data transformation. Linear mixed effects models, within an intent to treat framework, will be used to test specific hypotheses. Mixed effects models account for both fixed and random effects and are less susceptible to missing data. For outcomes with 2 time points, mixed effects models are conceptually equivalent to a two-sample t-test on change scores, thus we will use mixed effects models for all outcomes to maintain a unified analytical framework. When multiple analyses are conducted for a given hypothesis, correction for family-wise Type I error rates will be made using Bonferroni adjustments for the number of analyses being performed.

Hypothesis 1: Compared to the healthy aging education control condition (N=30), older adults assigned to the mHealth walking condition (N=30) will have greater increase in fitness levels, MVPA, regional CBF (hippocampal, prefrontal, anterior and posterior cingulate, and inferior parietal lobule), and cognitive function, strengthened resting state connectivity, and greater decreases in sedentary time, relative to baseline, after 3 months.

Analysis 1: For the MVPA/sedentary time and cognitive testing outcomes (for which 3 time points will be collected), we will investigate the longitudinal effect of intervention using linear mixed effects models. We will include fixed effects for condition (mHealth walking or healthy aging education control), time (0, 6 weeks, and 3 months, treated as a continuous variable), and their interaction (condition X time). To account for within subject correlations, we will include a random intercept over time term. Prior to analyses, trajectory plots for each of the outcome variables will be created as a function of time to check for linearity and to determine whether it is necessary to allow for individual rates of change (random slopes). This would allow us to investigate if there are different individual rates of cognitive change between the intervention and control conditions over time. Similarly, for fitness level and regional CBF, (for which 2 time points will be collected), we will investigate the longitudinal effect of intervention using linear mixed effects models by including fixed effects for condition (mHealth walking or healthy aging education control), time (0 and 3 months, treated as a continuous variable), and their interaction (condition X time). Inference will be focused on the condition X time interaction term for all analyses.

Hypothesis 2: Changes in regional CBF in the hippocampus and prefrontal cortex will partially mediate the relationship between changes in MVPA/sedentary time and changes in cognitive functions (memory and executive functions) within the mHealth walking condition.

Analysis 2: We will use the methods described by Preacher and Hayes to perform a mediation regression between the difference of MVPA/sedentary time and cognitive functions at baseline and 3 months, with the difference between CBF at baseline and 3 months acting as the mediator.

Exploratory/Training aim: This aim is not powered to detect significance but rather it is purely formative in nature given the PI's research trajectory and future interests. This training aim will explore if APOE genotype modified the relationship between changes in MVPA and CBF and changes in MVPA and cognition. For this purpose, two multiple regression models will be conducted. 1) Change in MVPA (post-pre from accelerometers) and changes in sedentary time (post-pre from accelerometers), APOE genotype (e4 carrier versus non-carrier), and the interaction term between MVPA/sedentary time change and APOE genotype will be entered as predictors of changes in CBF (post-pre) in those assigned to the mHealth walking intervention group only. 2) For the second regression model, the same predictors will be entered, but with changes in cognition as the outcome variable (post-pre). These analyses will allow us to detect

effect sizes (beta coefficients) to power future studies to further examine the effects of APOE genotype on intervention outcomes.

Power Analysis: Large effect sizes have been observed in the literature for CBF (Cohen's d=1.34) and cognitive function (d=.75) following a 3 month-long PA intervention; however, this was a supervised intervention with a no-contact control group, thus we expect our effect sizes to be in the medium to large range. The proposed sample size of 30 participants per group will have 80% power to detect a medium to large effect size=.65 on CBF with a one-tailed test and α=.05 and 80% power to detect an effect size=.75 on cognitive performance with α=.05.