Study Protocol and Statistical Analysis Plan

Study Title: The Effect of Physical Activity on Cognition Relative to APOE Genotype (PAAD-2)

Date: May 24, 2021

NCT: ID not yet assigned

Taken from the Specific Aims Section: The purpose of this study is to test the following specific aims.

- Aim 1: Test the causal link between PA and cognitive performance in persons with a family history of Alzheimer's disease (FH+)
- Aim 2: Determine if the effect of PA on cognitive performance is moderated by APOE4 status.
- Aim 3: Assess the extent to which the effects of PA on cognition are explained by a moderated mediation model with cerebral structure, white matter integrity, and resting state connectivity as mediators and APOE4 status as a moderator.
- Aim 4: Assess putative biomarkers as mediators and APOE4 status as a moderator of the effects of PA on cognitive performance.

3.c APPROACH

Our methods are based on our successful Phase I proof-of-concept trial (PAAD) in which we demonstrated cognitive benefits in persons with FH+ in association with 8 months of PA [1]. To logically extend that work, we will conduct a 12-month RCT, include MRI and more biomarkers, and incorporate further cognitive measures for comparison with findings from IGNITE. Participants will be randomly assigned to a PA condition (PAC) or a usual care control (UCC). Participants will be tested at 3 time points (pre, mid, and post) relative to the treatment period. We will use latent growth curve modeling to test questions related to trajectories of change.

3c.1 Overview

We will recruit 240 sedentary, middle-aged (40-65 years), cognitively normal adults with a FH+ to participate. Research staff will initially assess eligibility during a telephone interview. Prior to beginning the program, individuals will complete additional screening and sign an informed consent form at the pre-test. Those who remain eligible will be randomly assigned to the PAC or the UCC. Testing will take place at 0, 6, and 12 months. Following program completion, PAC participants will be encouraged to continue with their exercise independently, and UCC participants will be given a short-term YMCA membership or cash equivalent.

3c.2 Participants

3c.2.1 Recruitment. Cognitively normal, community-dwelling men and women 40-65 years of age will be recruited from six counties in North Carolina: rural (Randolph, Rockingham), regional city and suburban (Davidson, Alamance), and urban (Guilford, Forsyth) and other counties within a 2-hour distance of Greensboro, NC. This recruitment strategy will increase the diversity of our sample and contribute to the reproducibility of the results. The 6 counties have a combined population of approximately 469,588 in this age range [2]. We will advertise the study via local television, radio, social media, and newspapers and flyers distributed to support groups (see Letters of Support). Advertisements will request participation by adults (40-65 years) with a FH+ (defined as one first-degree relative diagnosed with AD). Although *APOE4+* make up approximately 24% of the general U.S. population <65 years of age [3], based upon our own [1, 4] and other research [5], recruiting adults with a FH+ will increase the percent of *APOE4+* to ~35%. Thus, by recruiting 240 people with a FH+, we anticipate successfully enrolling about 80 *APOE4+* participants. Given demographics of the recruitment region, we will include approximately 125 women and 115 men. In our past work, we have successfully recruited, screened, and enrolled participants from a similar age group (50-65 years) using these methods. We successfully enrolled 54 participants in Guilford County alone (target population=76,000) in a shorter period of time and with less funds allocated for recruitment. Assuming a similar success rate (0.07%) and given the size of the target population in the 6 counties (N=469,588), we will readily meet our target sample size (0.07%*N=328). We have letters of support from all of the YMCAs indicating their willingness to work with us in the enrollment of participants and the implementation of the program.

3c.2.2 Inclusion criteria. Inclusion criteria will be assessed during the telephone interview. Eligible participants must have a self-reported FH+ and be between 40-65 years of age, able to

communicate in English, and sedentary (30 min of moderate intensity PA fewer than 3x/week for the last 3 months by self-report) as defined by the Guidelines of the American College of Sports Medicine (ACSM**)** [6]. We will not include currently active adults because they are unlikely to benefit further from the intervention, and are likely to exhibit ceiling-level cognitive performance that could bias statistical models. Previously, when recruiting people who are "not regularly physically active", only 6% were excluded because of being too active [1].

3c.2.3 Exclusion criteria. Exclusion criteria will be assessed during the telephone screening, through surveys, and during pre-testing. Decisions regarding inclusion/exclusion will be made by the Project Coordinator and the Cognitive Testing Post-Doc in consultation with the PI and Dr. Tomika Williams (Adult Gerontological Primary Care Nurse Practitioner). See **Figure 2** for percentages excluded based upon these criteria in our previous study [1]. Individuals will be excluded if they:

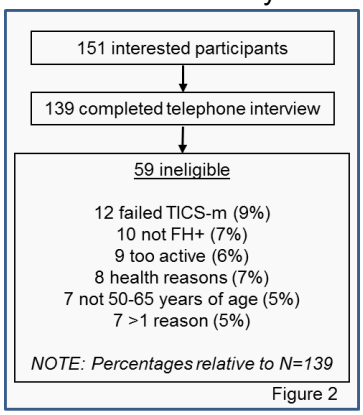

Figure 2

- Meet the criteria for clinical cognitive impairment (MCI, AD, or other forms of dementia) assessed *in two stages*. *First*, during telephone screening, we will use the modified Telephone Interview for Cognitive Status (TICS-m). The TICS-m has acceptable sensitivity and specificity in the detection of dementia [7] and amnestic MCI [8] and does not have the same ceiling constraints as other measures of cognitive impairment [8, 9]. Participants who score <36 will be excluded [8]. *Second*, at the pre-test, participants will complete the Montreal Cognitive Assessment (MoCA). We include people scoring equal or higher than 25 and exclude people scoring equal or lower than 22 without additional consideration. For people scoring 23 or 24, the MoCA-MIS cutoff (< 11) is additionally considered to screen out individuals with potential mild cognitive impairment (MCI). For people meeting both MoCA-TS (≤ 22) and MoCA-MIS (≤ 10) may be close to MCI rather than normal cognition based on Kaur, Edland, and Peavy (2018)'s data [10]. These stratified and double-layered criteria will maximize true positives and minimize false positives for the detection of MCI as distinct from normal cognition. Participants excluded based upon these criteria will be referred to Dr. Williams and advised to discuss their cognitive performance with their physician. Previously, 9% of interested participants (50-65 years) were excluded for this reason (see **Figure 2**). We anticipate fewer will be excluded in this study because of the expanded age range (40-65 years).
- Cannot perform PA because of known cardiovascular, metabolic, or renal disease and are symptomatic (assessed using the 2014 PAR-Q+) or because of orthopedic limitations as per the ACSM Guidelines [11].
- Self-report any history of confounding neurologic (e.g., traumatic brain injury, prior stroke, myelopathy, myopathy, peripheral neuropathy, brain tumors), psychiatric (e.g., active major depression, any history of schizophrenia or bipolar disorder), or active severe or functionally disabling neurologic or medical diseases (e.g., Parkinson's disease, active treatment for cancer), or any other conditions that might limit exercise or pose a danger to the patient – assessed using a Medical Health History (MHH).
- Report the current use of medications to treat symptoms of AD or that adversely affect cognition (MHH).
- Meet the criteria for depression (score >16 and anhedonia or dysphoria nearly every day for the past 2 weeks, plus additional symptoms in 2 or more other DSM symptom groups reported as occurring nearly every day for the past 2 weeks or 5-7 days in the past week) using the short form of the Center for Epidemiological Studies Depression Scale Revised (CESD-R). Report suicidal ideation on the CESD-R.
- Are not able to be compliant with the study protocol (i.e., are unwilling to be assigned to either condition, will not live in the area through the completion of testing, are unwilling to complete all testing, are unable to attend the exercise sessions)

3c.3 Procedures

3c.3.1 Telephone interview. Interested individuals will be asked to contact project staff by telephone. Staff will describe the study procedures. Individuals who remain interested will answer questions relative to the inclusion and exclusion criteria and will complete the TICS-m to ascertain initial eligibility for the study. Eligible participants will be scheduled for pre-testing. Following the telephone interview, participants will be contacted by the Cognitive Testing post-doctoral fellow to complete additional surveys (e.g., the Medical Health History [MHH], the CHAMPS, demographics). This will provide additional contact with participants between telephone screening and pre-test which should reduce dropout, will reduce participant burden at the pre-test by reducing the time requirement, will help establish a rapport with the staff, and will allow participants more time to obtain a physician consent before the pre-test. Upon review of the MHH, participants will be identified as: (1) apparently healthy, (2) having known disease but asymptomatic or with managed symptoms, or (3) symptomatic. Participants in category 3 who have known disease that is poorly managed (i.e., they are symptomatic and unstable) will not be allowed to participate. Other participants in category 3 and participants in category 2 must provide physician consent prior to participation. Those in category 1 will be asked to obtain physician consent for participation.

3c.3.2 Pre-, mid-, and post-test. At the pre-test, participants will read and sign an informed consent approved by the Institutional Review Board, will complete measures regarding exclusion criteria (see 3c.2.3), will complete the MoCA, and will provide a saliva sample for *APOE* genotyping. At the pre-test, mid-test, and post-test, participants will come to the lab for two days of testing. On the first day, participants will perform cognitive tests (see 3c.4.3), have resting heart rate (HR) assessed, and complete a submaximal exercise test (see 3c.4.4). On the second day, participants will provide a blood sample and complete an MRI scan (see 3c.4.5). On this day, testing will begin before 9:00 am to control for diurnal variability and with participants in a fasted state. Participants will provide a blood sample and then be offered a light breakfast. Participants will then complete an MRI scan (see 3c.4.5).

3c.3.3 Experimental conditions. Following the pre-test, participants will be randomly assigned to treatment conditions. Random assignment will be conducted using a computerized randomization procedure implemented by Labban (Co-I); participants will be contacted and informed of their group assignment by the Project Coordinator. Staff conducting testing (post-doctoral fellows, graduate research assistants) will be blinded to group assignment throughout the study. Participants will be instructed not to discuss group assignment during testing sessions. These steps will contribute to the scientific rigor of the proposed study.

3c.3.3a Physical activity condition (PAC). The PAC was used in our previous research in which we found improvements in memory in association with the program. It was originally based on meta-analytic evidence [26] indicating that in RCTs the largest effects of PA on cognition in older adults were in programs that include both aerobic and strength training (g=0.59). Hence, the PAC will include both modes of activity. Subjects will be asked to attend 3x/week for 1 year and exercise will take place in a group setting. Each subject will be encouraged to walk at a moderate intensity (target HR = 40-59% HR reserve) dependent on resting heart rate and age [168]. At every session, exercise specialists will record the amount of time spent in the aerobic and strength training portions of the program, HR (assessed by palpation for 20-seconds) and RPE from each participant during the middle portion of aerobic activity, and RPE during the middle portion of strength training. Participants will record the number of sets and reps and the color of the exercise band or the weight (if/when individuals progress to handheld weights) used for each exercise during strength training.

Every 3 weeks, Karper will observe a session to confirm that prescribed elements are included and non-prescribed elements have not been introduced. At this time, data from exercise logs and exercise specialist records will be reviewed for evidence of progression, consistent attainment of moderate intensity, and with respect to the prescribed duration of the aerobic and strength training components. Karper will also assess resting heart rate (HR) and average HR during exercise using Polar HR monitors. This information will be examined and used to recalculate target HR. Action values are operationalized as moderate intensity exercise (RPE = 12-13, HR = 40%-59% HR reserve) with time in aerobic exercise increasing from 15-min/day in week 1 to 30 min/day by week 4 and with time in strength training at a minimum of 30/min day throughout. In the first weeks of the PAC, more time will

be needed to teach the participants the exercises and to identify appropriate resistance levels. However, by week 8, strength training will be completed in 30 min; this will be maintained throughout the 12-month intervention. For strength training, elastic Therabands and dumbbells will be used. Subjects will begin with Therabands with the least resistance, completing one set of 10 repetitions for each of 10-15 exercises. As they can complete 15 repetitions for any given exercise in proper form, they will be progressed to the next higher resistance band for that exercise. If remediation is necessary, Karper will work with the exercise specialist to identify barriers and facilitators to treatment fidelity and will develop solutions to identified problems.

This PA program is inexpensive, safe, and suitable for community adult programs and home-based exercise recommendations. The program was initially implemented at local YMCAs but has been offered via Zoom since resuming post-COVID-19 pandemic. Fidelity across sites will be ensured by using qualified and experienced Exercise Specialists who will be trained by Karper (Co-I) to implement the program. Fidelity to the program and consistency of implementation across sites will be further ensured by Karper's regular (once / 3 weeks) visits to each site to observe, evaluate, and provide feedback on sessions. Measures of compliance and adherence will be obtained at all exercise sessions; sites will be compared monthly to ensure no substantive differences across sites. If such differences occur, we will determine their causes and make necessary changes.

The exercise specialists will have an appropriate educational background, exercise leadership experience, ACSM Group Exercise Instructor Certification (or equivalent), and CPR Certification. All exercise specialists will be vetted by Karper in consultation with the PI (Etnier), will be provided up to 4 hours of training by Karper to administer the program, and will be observed every 3 weeks. As described, we also have a well-thought out plan to ensure the fidelity of the intervention delivery.

3c.3.3b Usual-care control (UCC). We will use a UCC in which we ask participants to maintain their normal health practices (e.g., diet, annual physicals) for 1 year. Because the inclusion criteria require that participants be sedentary, we anticipate that these individuals will not show consistent increases in PA over the year. Importantly, we considered the use of a comparison condition that controls for aspects of our intervention not specifically tied to the exercise itself (e.g., social interactions, attention, feelings of accomplishment). For the two conditions to be equal in all ways except the physical activity, this comparison condition would have to be engaging enough to ensure adherence for 3 days/week for one year. Thus, we considered non-exercise conditions such as health education, music lessons, and art lessons. However, interest in participating in these activities for an entire year might be very individualized; participants might not be willing to be randomly assigned to either group and might have low adherence if assigned to an "undesirable" condition. Further, these activities might themselves prompt participants to become more physically active and/or lead to cognitive gains, thereby reducing the expected treatment effect [12]. Alternatively, we also considered an exercise condition with less intense physical activities, such as a stretching and toning condition (STC). But given the evidence that an STC results in cognitive benefits indistinguishable from those in an exercise condition [13, 14], we determined an STC might limit our ability to observe treatment effects. Thus, we elected to use a UCC. To reduce effects from experimenter attention, to minimize attrition, and to assess possible cross-contamination, we will contact them once per week. We will provide educational materials (covering health topics, but not PA) to UCC participants biweekly. We will also contact UCC participants once per month to inquire about their health and to entertain questions about the educational materials. Once per month, we will assess their self-reported PA [15]. In this fashion, UCC participants will be contacted by staff every week. To further encourage retention, we will provide UCC participants with a short-term YMCA membership or cash equivalent after the post-test. When participants are offered an intervention after a UCC waiting period, cross-contamination is low (7.1% of studies) and fewer participants drop out from a UCC than an exercise treatment (4.7% fewer) in trials up to 1 year [15].

3c.3.4 Adherence. In our past research (PAAD), we anticipated 15% dropout; actual dropout was 13% before program completion (all for reasons unrelated to the study) [1]. Completers attended 76% of prescribed sessions. We will incorporate the same methods to ensure high levels of adherence: 1) use a group-based program; 2) offer incentives for adherence; and 3) provide compensation for testing

sessions. If participants drop out prior to pretest, we will recruit more participants to achieve our target sample size. Overall, we anticipate that 204 of the 240 participants enrolled will complete the study.

3c.4 Measures

3c.4.1 Genotype. Saliva samples will be collected using Oragene-500 kits. Genomic DNA will be extracted from saliva samples for SNP testing. The SNPs associated with the two amino acid residues (codons 112 and 158) will be used to identify participants as *APOE*+ or *APOE*-. Remaining DNA material will be stored indefinitely for future analyses.

3c.4.2 Biomarkers. We will use standard protocols for collection and storage of blood samples and assays and analyses. Blood samples will be collected from an arm vein at rest [16]. Plasma and serum will be separated by centrifugation. Samples will be stored at -80^0. Glucose will be analyzed using a commercially available assay kit and requires <10 µL of serum. All other assays will be conducted using a multiplex system (Luminex 200S), which uses very small (20-50 µL total) volumes of blood. BDNF will be given priority in any insufficient samples. Samples will be stored indefinitely.

3c.4.3 Cognitive Test Battery. We will assess performance across cognitive domains, specifically including tests assessing cognitive abilities sensitive to the early stages of dementia [17] and to PA interventions [18]. We will include measures from our previous PAAD study [1] as follows: information processing speed will be assessed with the Wechsler Adult Intelligence Scale (WAIS-IV) Digit Symbol Task (raw score); memory (immediate recall, delayed recall, and retroactive and proactive interference) will be measured using the Auditory Verbal Learning Test; delayed visuospatial memory and constructional praxis will be measured using the Rey-Osterrieth Complex Figure Test; attention will be assessed using the Paced Auditory Serial Addition Test (3- and 2-second tests) and forward and backward Digit Span measures from the WAIS-IV (raw scores). Executive function will be measured using the Trail-Making Test A and B and the Stroop Test. The Wide Range Achievement Test (WRAT) will be used to assess premorbid intelligence and may be used as a covariate if necessary.

We will use an additional measure of episodic memory since: 1) memory measures were most sensitive to effects of PA in our previous work [19], and 2) episodic memory is most sensitive to early stages of AD [20]. We will administer a primed cued-fragment completion task that has been used to assess the independent contributions of controlled and automatic memory processes in healthy older adults and individuals with very mild AD [21]. This measure is key for examining potential gains in memory ability conferred by the intervention, because age-related memory deficits often show the largest effects on the controlled aspect of memory performance [e.g., 22]. Thus, this measure would determine whether predicted gains from PA will have a selective influence on this aspect.

To allow comparisons across studies, we will include measures from the NIH toolbox and measures in the IGNITE study. From the toolbox, we will include Dimensional Change Card Sort, List Sort Working Memory, Flanker, and Picture Sequence. To facilitate comparisons with IGNITE, we will use measures of logical memory, spatial relations, spatial working memory, and matrix reasoning from the WAIS-IV, and a measure of paired associate learning from the Virginia Cognitive Aging Project.

All measures have been used extensively, have well-established psychometrics, are not reliant on a particular reading level, and have age-appropriate normative data adjusted for education level. These characteristics ensure that the measures are rigorous and reproducible. Normative data [23], assessment target age groups (NIH Toolbox measures), our preliminary data [24], evidence of change across the lifespan [25], and evidence of sensitivity to *APOE4* status [26-28] suggest that we can expect variability in performance and that there will be room for improvement on these cognitive measures within this age group. For cognitive domains with 3 or more measures, composite scores will be used in the first level of analyses. For all tests, alternate forms will be used when available.

3c.4.4 Submaximal Aerobic Fitness. Aerobic fitness will be estimated using a submaximal exercise test. Participants will complete a submaximal exercise test on a treadmill while using a metabolic cart to collect expired gases. Changes in aerobic fitness will provide an indicant of the physiological responsiveness to the PA intervention.

3c.4.5 MRI. The collection of MRI data will allow for the assessment of cerebral structure and

function. These measures are expected to be sensitive to the PA manipulation [13, 29-31] and to *APOE4* status [20, 32-37]. MRI exams will be conducted at the Gateway MRI Center. Images will be acquired on a Tim Trio Siemens 3T MRI Scanner with a 12 channel receive-only head coil. Images will be acquired parallel to the IGNITE trial and sequences will be written by Jung (consultant). Only participants who meet criteria for MRI will be scanned. Exclusion criteria include: may be pregnant, have previous head or neck surgery, have any metallic or magnetic implants that are contraindicated for MRI, have had surgery in the last 6 weeks, or weigh over 450 lbs.

3c.4.5a Acquisition. A high resolution T1-weighted image will be collected using a 3D volumetric MPRAGE sequence with 0.9 mm isotropic resolution (TR=1900ms, TE=2.93ms, TI=900ms, flip angle=9 degrees, 176 slices) to assess brain volumes. Images will be immediately re-acquired if artifacts due to motion or other sources are detected. Whole-brain connectivity will be assessed using blood oxygenation level-dependent (BOLD) imaging during resting state. Participants are asked to view a cross in the middle of a screen with eyes open. Images will be collected parallel to the anterior commissure-posterior commissure (AC-PC) line using multi-slice gradient-echo planar imaging (EPI) (TR=2000 ms; TE=40ms; field of view=24cm (frequency) x 15cm (phase); matrix size=96 x 86, 40 slices, 3mm thickness, no skip; voxel resolution=3 X 3 X 3 mm). Diffusion tensor imaging (DTI) will be collected to assess white matter microstructure with a dual spin-echo echo-planar imaging (EPI) sequence (30 directions, b=1000 s/mm^2 TE = 90ms effective; NEX = 1, FOV=25.6, matrix size=128x128x40, slice thickness=3mm no gap; TR=5300ms).

3c.4.5b Image Processing. All images will be processed using freely available software (Freesurfer, SPM, FSL) in a customized workflow. T1-weighted anatomical images will be processed using the Freesurfer longitudinal pipeline and recommended manual editing for optimization [38]. fMRI data will be aligned to the T1 data, then warped to template space by applying the transforms computed on the T1 data. Distortion correction will be performed using TOPUP in FMRIB's Software Library (FSL) [39, 40]. To control for physiological noise, all data will be preprocessed to remove white matter and cerebrospinal fluid signal using the CompCorr method implemented through the Conn Toolbox [41, 42]. Fluctuations in signal remaining after controlling for head motion will be identified using ART software [42] and time points with meaningful deviations in signal will be regressed out. DTI data will be processed using FSL software. Tools from FMRIB's Diffusion Toolbox will be used to correct for eddy current distortion and calculate diffusion tensors from which fractional anisotropy (FA) will be computed. FA maps generated from the DTI sequence will be normalized to standard space using SPM12.

3.c.4.5c Analysis. Hippocampal volumes will be estimated using Freesurfer, including quality control and manual editing as recommended. Regions of interest will be extracted from other image formats using WFU Pickatlas [43] for inclusion in SEM models.

3c.4.6 Covariates. Because cognitive performance is expected to be associated with age [e.g., 23, 44, 45] and education [e.g., 23, 45], these variables will be included as covariates in the models. Identifying additional individual difference variables that discriminate levels of responsiveness to the intervention may provide important insights for subsequent research. Data on variables that have been identified as important in past research [46-50] will be collected and explored as potential covariates. These include: sex, blood pressure, smoking, alcohol use, medications and supplements, sleep quality, body mass index, menopausal status, hormone therapy use, diabetes, and cardiovascular risk factors. When assessing FH, detailed information regarding blood-related relatives suspected to have AD or diagnosed with AD will be collected. This will include relationship to participant, age at diagnosis, and method of diagnosis. We will also collect data on parents who have not been diagnosed with AD (age and health status currently or at time of death). Because a FH+ that is maternal and with a younger age of onset [51] results in higher risk, these variables will be included as covariates in statistical analyses. Participants also will complete the International Physical Activity Questionnaire [52] at pre, mid, and post-tests (monthly for UCC).

3c.5 Data Management, Quality Control, Analytical Plan, and Power Analysis

Procedures described here contribute directly to the rigor and reproducibility of the proposed study,

to ensure the quality of data recording and analysis and appropriate determination of sample size.

3c.5.1 Data Management. Data will include hardcopy surveys and data collection sheets and results of cognitive testing, MRI scans, genotype, and blood assays recorded electronically. We will use subject ID #'s and remove names as soon as possible to ensure that participant confidentiality is maintained. All electronic data will be de-identified, and managed using REDCap software. This system allows us to control data access at the individual level and apply other rules and constraints that promote data quality. Pre-test data from the first subjects will be examined to identify data gathering problems to be addressed immediately. The dataset will be cleaned using standard methods to identify impossible and improbable data [53] including frequency distribution checks for outliers and problems in data gathering or entry. Validity checks will be performed as recommended [53]. MRI structural and white matter integrity data will be assessed using Freesurfer, and CONN will be used for RSC analyses. Data will be exported to SPSS and a codebook for variables will be developed. Linear growth curve (LGC) analyses will be conducted using MPLUS [54].

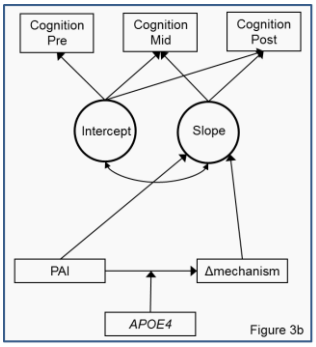

Figure 3b

3c.5.2 Quality Control. Standard Operating Procedures (SOPs) for cognitive testing, the PAC intervention, submaximal exercise testing, and blood draws are in place from our Phase I clinical trial (PAAD) [1]. Modifications to the SOP to reflect the addition of cognitive measures and updates to procedures will be made prior to participant recruitment. MRI SOPs will be developed by Etnier (PI) in close consultation with Hugenschmidt and Jung (Co-Is) and based upon the existing MRI SOPs from IGNITE to maximize compatibility with the IGNITE study. Post-doctoral fellows will be trained to implement the SOPs. Throughout the study, Etnier (PI) will regularly observe testing sessions for quality control relative to SOPs, Karper will regularly observe PAC sessions, and Labban (Co-I) will conduct regular checks of the data to ensure quality control.

3c.5.3 Analytical Plan. The conceptual model driving our research is inherently a model of change: *changes* in PA lead to *changes* in cognitive performance. The current state of the art in statistical models for studying change is LGC analysis [55-57] which models trajectories of observed change as reflecting an underlying ("latent") developmental process. LGC analysis is an ideal statistical tool for testing our conceptual model because it allows us to 1) quantify both mean and individual variation of pretest levels of our outcome variables (e.g., cognitive performance, neurological function/structure, and biomarkers), as well as mean and variance of change in those outcomes over the intervention period; 2) test whether the PA intervention (PAI) and *APOE4* status predict latent trajectories of change in these outcomes; 3) determine whether change in cognitive performance is mediated by putative mechanisms; and 4) test whether *APOE4* status moderates the mediational models.

Figure 3a illustrates our conditional LGC model to test **Specific Aims 1 and 2**. In this model, the intercepts capture participants' initial status; whereas, the slopes capture their change over time. The path arrow from PAI to the slope growth factor is quantified as a regression coefficient which we will use to explore the main effect of PA, *APOE4* status, and sex (not shown) on change in outcomes over the intervention. We do not expect sex to affect the influence of PAI on relevant outcomes, but we will control for sex in our analyses; and, if sex is a significant predictor, we will conduct additional exploratory analyses to guide future research. We do expect that participants assigned to the PAI will exhibit greater improvements in cognitive performance than will controls, but that *APOE4* status will exert minimal direct effects on these behavioral measures. This expectation arises from data gathered in our previous study, which suggests that *APOE4+* who are not yet exhibiting behavioral signs of cognitive decline should not be expected to demonstrate steeper rates of improvement on cognitive measures compared to non-carriers [24]. However, we do expect both PAI assignment and *APOE4* status to impact changes in neuroimaging and biomarker outcomes. Therefore, we will also test whether changes in neuroimaging (**Specific Aim 3**) and biomarkers (**Specific Aim 4**) are mediators of change in cognitive

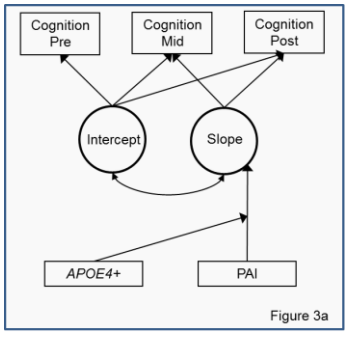

Figure 3a

performance (**Figure 3b**). Because putative mechanisms will be measured at pre-test and post-test only, we will estimate residualized change in these variables using univariate ordinary least squares regression. We will use the resulting change scores to test whether changes in these putative mechanisms mediate changes in cognitive measures. PAI assignment and *APOE4* status will serve as predictors of slopes for cognitive measures, but will also be regressed on mechanism change scores, which will in turn be regressed directly on the slope growth factor. This new parameterization of the model will allow us to formally test the indirect effect of PAI assignment on cognitive outcomes, via their direct effects on the putative mechanisms. Finally, *APOE4* status will be tested as a moderator of the association between PAI and mechanism change scores. The final parameterization of the model will thus be a test of moderated mediation, and is a logical extension of the previous model: *APOE4* status moderates the association between PAI and change in mechanistic outcomes, which are in turn directly predictive of change in cognitive outcomes. We expect participation in the PAI will produce improvements in cerebral structure, cerebral function, and biomarkers, and that these improvements will lead to improved cognitive performance.

The portions of **Specific Aims 3 & 4** that refer to putative mechanisms as outcomes will be tested using hierarchical multivariate regression techniques. Covariates and pre-test scores will be entered into the models at steps 1 & 2, and group assignment and *APOE4* status will be entered at steps 3 & 4. Finally, the PAI x *APOE4* status interaction will be tested at step 5. Entering predictor variables in this fashion will allow us to assess the relative importance (adjusted R^2 and change in R^2) of each to the prediction of post-test neuroimaging and biomarker status.

All dependent variables are continuous, and expected to be normally distributed. Strategies to address violations of model assumptions may include sensitivity analysis, and computation of bootstrapped standard errors and confidence intervals. Missing data will be handled within growth models using maximum likelihood estimation when assumptions that data are missing at random or completely at random are met. If not, alternative strategies to handle missing data will be employed, such as the Diggle-Kenward selection model [58] or pattern-mixture model [59].

3c.5.4 Power Analysis. We estimated sample size requirements using preliminary data from our previous study [24]. Coefficients on which we based our analysis included expected values for slope factors, regression estimates of association between predictors (PAI, *APOE4* status) and slope factors, and reasonable estimates of associations of the PAI-by-*APOE4* status interaction with slope factors. We conducted Monte Carlo simulations with 5000 replications, requiring estimate and variance bias of <10%, and coverage $\geq$95% [60], to estimate power for our target sample size of N=240 with attrition rates of 10-30%. An initial sample of 240, provided the assumed attrition rates, would have an approximate power of 0.97 to detect a mean slope factor of 0.10, power of 0.86-0.96 to detect parameter estimates of 0.075-0.10 for slope regressed on PAI and *APOE4* status, and power of 0.82-0.92 to detect interaction effect estimates of 0.065-0.10 for PAI-by-*APOE4* status regressed on slope.

3c.6 Potential problems, alternative approaches, and limitations

Although we have recruited, enrolled, and retained 54 participants over 1.5 years in our previous trial, we could have challenges enrolling 240 participants (120 PAI, 120 UCC) over 4 years. If so, we will increase recruitment efforts through face-to-face meetings with local support groups and at community centers, places of business, places of worship, and other relevant locations. Additionally, we will recruit from 2 additional adjacent counties (Caswell, Chatham) which would result in 32,594 additional potential participants in the targeted age range. Lastly, we will focus on retaining participants by maintaining regular contact with the UCC and fostering a sense of belongingness and commitment by those in the PAC. The focus on FH+ is purposeful and reflects our interest in understanding the potential of PA for these individuals. However, we recognize that the results may not generalize to persons who are FH-. Additionally, the use of a UCC was carefully considered. However, we acknowledge that with a UCC we cannot differentiate effects of the PAC specific or not specific to exercise. That is, participants in the PAC will be different from those in the UCC in more ways than simply the exercise itself (e.g., they will receive more regular attention, they will have social interactions, they will have a sense of accomplishment). As such, if our hypotheses are supported and suggest that PA benefits cognition by persons with a FH+, future studies will be needed to identify the

specific agents of change in the PAC causally linked to these benefits.

3c.7 Rigor and Reproducibility

Recognizing the NIH's commitment to rigor and reproducibility, our study meets the highest standards [61, 62]. We have estimated sample sizes conservatively and considered expected dropout based on our previous Phase I clinical trial (PAAD). We have strategies in place to handle missing data. We will use random assignment and will ensure that research staff collecting cognitive, neuroimaging, and biomarker data will remain blinded to group assignment throughout the study. We have procedures in place to ensure the fidelity and consistency of implementation of the PA program across sites. We also have procedures to provide quality control at the level of data collection, data analysis, and data reduction. We will assess PA in the UCC group to assess possible cross-contamination. We will use validated, well-established measures of cognition, commercially available assays of biomarkers, and currently accepted methods to collect, reduce, and analyze neuroimaging data. We will consider sex as a biological covariate and pursue its effects from an exploratory perspective if it is a significant predictor. Lastly, when we disseminate our findings, we will heed the reporting standards guidelines relative to issues of rigor and reproducibility [63].

3c.8 Timetable (Figure 4)

Because this study extends our previous work, most SOPs are already in place; thus, we require 4 months to finalize SOPs, obtain approval from the Institutional Review Board, train experimenters, prepare a REDCap data collection system, and finalize data collection materials. Anticipating 15% dropout and with a target of 200 completing the program, we will enroll 240 participants. Initially, we intended to recruit in cohorts and we used this model prior to the COVID-19 pandemic. Using this model, for each cohort, we planned to use 6-8 months (longer period allowed for the later cohorts and to minimize overlap of testing sessions across cohorts) for recruitment and screening. We would then use two months to complete pretesting. After pretesting, participants would be randomly assigned to conditions for the 12-month intervention which would include mid-testing and post-testing at 6-month intervals. We planned to ultimately enroll 4 cohorts (n=60 each). After the COVID-19 pandemic, were were no longer able to implement the exercise program at YMCAs. As such, we changed our recruitment model to strive for 10 participants per month with randomization occurring after approximately 20 people were tested. This allowed for exercise groups to start on a more rolling basis, for the catchment area to be increased due to the virtual exercise, and still allows us to meet our recruitment goals. The modified time table is shown below.

Data reduction and preliminary analyses will be ongoing, but will move towards completion in the final 5 months of Year 5. We anticipate disseminating findings at national and international conferences based upon data collected during the trial as shown in Figure 4. We will publish a paper describing the trial in Year 2, papers on the pre-test data will be prepared in Year 4, and we will prepare manuscripts from the entire data set and plan future studies in Year 5.

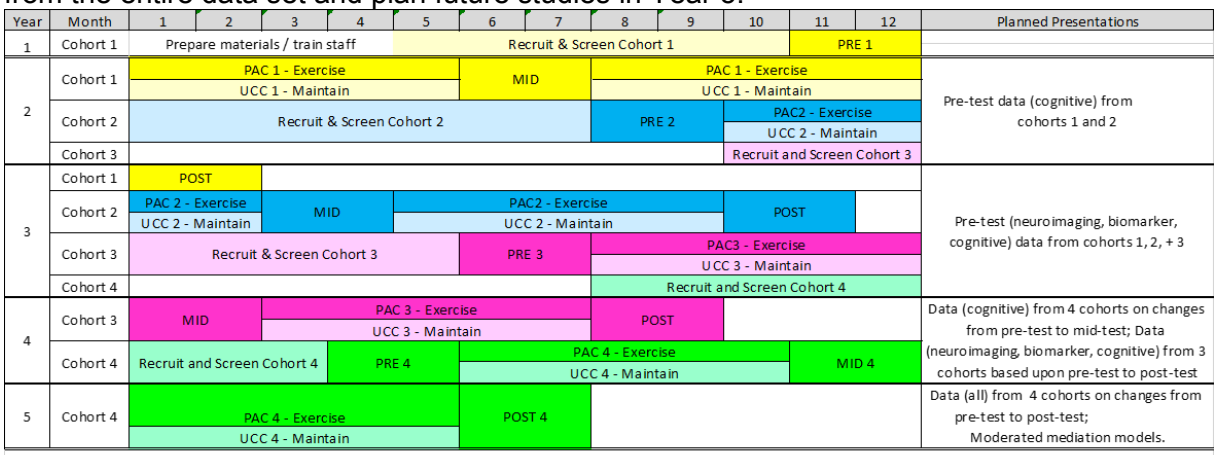

Figure 4. Timetable showing conductance of proposed study over a 5-yr period. PRE=pre-test, MID=mid-test, POST=post-test, PAC = physical activity condition, UCC = usual care control

4.1 Protection of Human Subjects
4.1.1 Risks to Human Subjects
4.1.1.a. Human Subject Involvement, Characteristics, and Design.
Volunteers will be recruited from six counties (Guilford, Forsyth, Randolph, Rockingham, Davidson, Alamance) in the Piedmont region in North Carolina and from other locations within 2 hours drive of Greensboro. The combined population of the six counties is 1,388,480, of whom approximately 469,588 are in the target age range of 40-65 years [77]. Recruiting from these counties, a representative sample in this age range would be predominantly white (67%) and black (23%). Approximately 9% of the population are Hispanic or Latino and approximately 52% are women.

Participants will be recruited using a multi-pronged approach which will include newspaper advertisements, radio announcements, social media, electronic newsletters, and posting of flyers. Local area agencies (please see Letters of Support) and the Trial Match service through the Western Chapter of the Alzheimer's Association will also assist with recruiting. Participants from all ethnic groups will be included in the study. The representativeness of the sample with respect to race will be monitored periodically and, if necessary, additional recruitment efforts will be targeted towards underrepresented racial groups.

The goal of the selection criteria is to include middle-aged English-speaking adults (40-65 years) with a family history of AD (FH+) who are cognitively normal, who are not otherwise clinically impaired, who are healthy enough for exercise, and who are identified as sedentary according to ACSM Guidelines [ACSM, 83]. Sedentary is defined as participating in physical activity at a moderate intensity for 30 min or more fewer than 3x/week over the last 3 months and is assessed by self-report. Participants who will be excluded are those who:

- meet the criteria for clinical cognitive impairment (MCI, AD, or other forms of dementia) assessed in two stages. First, during the telephone screening, we will use the modified Telephone Interview for Cognitive Status (TICS-m). The TICS-m has acceptable sensitivity and specificity in the detection of dementia [110] and amnestic MCI [111] and does not suffer the same ceiling constraints as do other measures of cognitive impairment [111, 112]. Participants who score <36 will be excluded [111]. Second, at baseline screening, participants will complete the Montreal Cognitive Assessment (MoCA). We include people scoring equal or higher than 25 and exclude people scoring equal or lower than 22 without additional consideration. For people scoring 23 or 24, the MoCA-MIS cutoff (< 11) is additionally considered to screen out individuals with potential mild cognitive impairment (MCI). For people meeting both MoCA-TS (≤ 22) and MoCA-MIS (≤ 10) may be close to MCI rather than normal cognition based on Kaur, Edland, and Peavy (2018)'s data. These stratified and double-layered criteria will maximize true positives and minimize false positives for the detection of MCI as distinct from normal cognition.
- are unable to perform PA because have known cardiovascular, metabolic, or renal disease and are symptomatic (assessed using the 2014 PAR-Q+) or because of orthopedic limitations as per the Guidelines of the ACSM [112].
- self-report any history of confounding neurologic (e.g., traumatic brain injury, prior stroke, myelopathy, myopathy, peripheral neuropathy, brain tumors), psychiatric (e.g., active major depression, any history of schizophrenia or bipolar disorder), or active severe or functionally disabling neurologic or medical diseases (e.g., Parkinson's disease, active treatment for cancer), or any other conditions that might limit exercise or pose a danger to the patient – assessed using a Medical Health History **(MHH)**.
- report the current use of medications to treat symptoms of AD or that adversely affect cognition (MHH).
- Meet the criteria for depression (score >16 and anhedonia or dysphoria nearly every day for the past 2 weeks, plus additional symptoms in 2 or more other DSM symptom groups reported as occurring nearly every day for the past 2 weeks or 5-7 days in the past week) using the short form of the Center for Epidemiological Studies Depression Scale Revised (CESD-R). Report

suicidal ideation on the CESD-R.

According to the American College of Sports Medicine, individuals categorized as participating in regular exercise must perform "planned, structured physical activity at least 30 min at moderate intensity on at least 3 d*wk-1 for at least the last 3 mo" [11, p. 33]. We will be recruiting participants *who do not meet* this definition and hence would be categorized as "not" participating in regular exercise. As per the ACSM Guidelines [11], individuals who do not participate in regular exercise must be further categorized as a) having no diagnosis of cardiovascular, metabolic, or renal disease and no signs or symptoms suggestive of these diseases (apparently healthy); b) having known cardiovascular, metabolic, or renal disease, but no signs or symptoms suggestive of these diseases (asymptomatic); or c) signs or symptoms suggestive of cardiovascular, metabolic, or renal disease (symptomatic). This determination will be based upon the medical health history and the participants answers to questions from the 2014 PAR-Q+. As per the ACSM Guidelines:

- apparently healthy individuals will be asked to provide physician consent, but can participate in the exercise program and exercise testing without medical clearance and can progress to vigorous intensity exercise (following ACSM Guidelines)
- asymptomatic individuals will be required to receive medical clearance from their personal physician prior to being allowed to participate in the exercise program or exercise testing. These individuals may perform light to moderate intensity exercise and may be progressed to vigorous intensity exercise as tolerated (following ACSM Guidelines) and as permitted by their physician.
- symptomatic individuals who have managed their disease (stable) will be required to receive medical clearance from their personal physician prior to being allowed to participate in the exercise program or exercise testing. These individuals would likely require a diagnosis related to their specific symptoms. If these individuals were prescribed medication, they would have to be on the medication for 3 months and show signs of stabilization before being permitted to enroll in the study. Once enrolled, these individuals may perform light to moderate intensity exercise and may be progressed to vigorous intensity exercise as tolerated (following ACSM Guidelines) and as permitted by their physician. Symptomatic individuals who do not have good disease management (unstable) will not be allowed to participate.

To maximize adherence, we will take the following steps which have been identified as important: 1) use a group-based program whereby participants complete the program in a cohort; 2) offer incentives for adherence to the PA program; and 3) provide compensation for each testing session.

4.1.1.b. Sources of Materials

The research materials will be obtained from identifiable living human subjects in the form of blood samples, saliva samples, physical measurements, questionnaire data, neuroimages, and cognitive performance data.

4.1.1.c. Potential Risks

All of the testing procedures used have minimal associated risks.

Cognitive testing and questionnaires: There are no known potential risks involved with completion of the cognitive testing or completion of the questionnaires. However, some participants may be identified by the research staff as meeting the criteria for clinical cognitive impairment or for depressive symptoms. These participants will contacted by Dr. Tomika Williams (Gerontological Primary Care Nurse Practitioner) who will encourage them to schedule an appointment with their personal physician.

Blood draws: Risk of infection is minimal with the blood draws. Only slight discomfort should occur when the blood is drawn and the pain should be approximately equivalent to a mosquito bite. Bruising may occur following the blood draw and may result in mild-to-moderate soreness to the touch for several days.

Exercise program and submaximal fitness tests: As described in 4.1.1.a, all participants will be

considered non-exercisers by our inclusion criteria and we will further identify participants as apparently healthy, asymptomatic, or symptomatic based on ACSM guidelines [11]. Those who are apparently healthy can safely participate in moderate-to-vigorous intensity exercise (as will be used in this intervention and in the exercise testing) according to the ACSM guidelines. Those who are asymptomatic, must provide evidence of medical clearance for participation, but then may participate in moderate intensity exercise and progress to vigorous intensity exercise as tolerated and as permitted by their physician. Those who are symptomatic, must provide evidence of medical clearance for participation and must show that their symptoms have stabilized, but then may participate in moderate intensity exercise and progress to vigorous intensity exercise as tolerated and as permitted by their physician. Those who are symptomatic and unstable may not participate. For those who participate in the PA intervention, risks associated with participation are minimal and include: (a) muscular fatigue and/or muscle soreness during and after exercise and (b) risk of falling while exercising. As with any type of exercise, there is a very minor risk of injury as abnormal changes in heart function may occur and, in extremely rare instances, heart attack may occur. However, the risk of this happening is reported as 1 in 10,000 deaths during maximal exercise tests and risks are dramatically lower for the submaximal physical activities and submaximal fitness tests that will be used in this program.

In response to COVID-19, additional safety precautions have been implemented. Anyone who has been diagnosed with COVID-19 will be required to obtain physician consent to complete the submax test. Exercise intervention - (NOTE: For b and c below, if their physician advises them to wait longer than the 2 weeks, that will supersede our guidance) a). Participants who have been diagnosed with COVID-19 and hospitalized will be required to obtain physician consent to resume participation in the exercise program. Once approved, they will begin with very light intensity, will be encouraged to self-monitor physiological responses, and will only be progressed gradually. b). Participants who have been diagnosed with COVID-19 and who experience mild/moderate symptoms that do not require hospitalization will not resume exercise until 2 weeks after cessation of functionally-limiting symptoms (e.g., fever, shortness of breath, fatigue). At that point, they will begin with light intensity, will be encouraged to self-monitor physiological responses, and will only be progressed gradually. c). Participants who have been diagnosed with COVID-19 and who experience no symptoms or very mild symptoms will be allowed to resume exercise at their discretion at a reduced or light intensity, but will be encouraged to self-monitor physiological responses to ensure symptoms do not appear.

Magnetic Resonance Imaging: There are no known risks associated with having a magnetic resonance image as long as requisite safety precautions are taken. The precautions that must be taken are with respect to metal objects (external and internal), potential for burns, potential for hearing loss, muscle twitching and tingling, and risk for pregnant women. We will use a 2-step screening protocol, explicit instructions, and constant availability for communication during the scans as ways to further minimize these risks (see 4.1.2.b). Furthermore, participants will be informed that the MRI images completed at our facility are part of a research study and are not for clinical diagnostic purposes. They will be told that MRI images in this study will not be reviewed by a physician, but they will be given the option to receive a free copy of their images on a CD. Participants will be told in advance that although the scan is not for diagnostic purposes, program staff will notify them if they see a substantial deviation from normal anatomy. If they indicate that they would like to be notified, Dr. Tonya Williams (Gerontological Primary Care Nurse Practitioner) will contact the participant, we will provide him/her with a free copy of their images on a CD, and we will suggest that they contact their physician for follow up. If they indicate that they would not like to be notified, we will not alert them to the deviation. Participants will be regularly reminded that the research team cannot diagnose conditions.

Genotyping: Similar to our previous study, participants will be informed that based upon ethical considerations and in compliance with recommendations [64], they will not be given information about their genotype. Additionally, research staff will explain to participants that their privacy will be protected and the confidentiality of their data will be maintained by insuring that their genetic sample will be identified by ID# only. Genotype information, linked only to an ID#, will only be made available to data analysts and linked only to an ID# and will not be made available to intervention or research staff who interact directly with participants. If participants were to become aware of a heightened genetic risk for Alzheimer's disease, this might result in a negative emotional response. However, we will take the

necessary steps to insure that participants do not gain access to their genotype information (see 4.1.2.b).

4.1.2. Adequacy of Protections against Risks

4.1.2.a. Recruitment and Informed Consent

Participants will be recruited through newspaper advertisements, radio announcements, social media, electronic newsletters, and posting of flyers. Local area agencies (please see Letters of Support) and the Trial Match service through the Western Chapter of the Alzheimer's Association will also be used for recruiting. Interested participants will be asked to contact staff by telephone. Research staff will describe the study procedures. Those who remain interested will complete the TICS-m and answer questions to ascertain initial eligibility (e.g., who in your family has been diagnosed with Alzheimer's disease, what is your age). Because participants will not yet have been consented, none of this information will be recorded in an identifiable fashion (i.e., only descriptive information will be recorded such as xx persons were excluded because of hearing difficulties). Eligible participants will be scheduled for pre-testing. Following the telephone interview, participants will be contacted by the Cognitive Testing post-doctoral fellow to complete additional surveys (e.g., the Medical Health History [MHH], the CHAMPS, demographics, CESD-10, PSQI). At the pre-test, informed consent will be obtained, additional screening will be performed using the Montreal Cognitive Assessment (MoCA), and a saliva sample will be obtained. Participants will be told about all aspects of the study including that their genotype information will not be shared with them and that blood samples, imaging data, and genetic material collected during the study will be stored by ID# and kept indefinitely to allow for future analyses. If a participant does not want his/her blood sample, imaging data, and/or genetic material to be stored for future analyses, he/she will be able to opt out of this aspect of the study while remaining eligible for all other aspects of the study. Participants will be asked to read the consent form and encouraged to ask questions before signing. A copy of the signed consent form will be offered to the participant, and the original signed copy will be kept in the participant's secure file. We will take the following steps to maximize our chances of following subjects after 1-year: 1) include follow-up contact provisions in the consent form, 2) maintain contact with subjects about study progression

4.1.2.b. Protections against Risk

Exercise program and submaximal fitness tests: To ensure participant safety, all physical activity sessions will be administered according to the guidelines established by the American College of Sports Medicine [ACSM, 83]. As described in 4.1.1.a, participants will be identified as apparently healthy, asymptomatic, or symptomatic and we will follow ACSM Guidelines with respect to these qualifiers. We have also implemented safety protocols relative to a person being diagnosed with COVID-19. Although the risks of an adverse event during or in response to exercise are low when following ACSM Guidelines, we will take additional safety precautions. All staff involved in the PA program or in exercise testing will be trained in procedures to handle emergencies or urgent problems and trained in basic cardiopulmonary resuscitation (CPR). As an added safety precaution, an automated external defibrillator (AED) will be available.

Blood draws: Although the risk is minimal, infection is possible when blood samples are taken. The risk of infection will be minimized through the use of sterile techniques by a trained technician. Additionally, because blood and saliva samples are biohazards, all OSHA related guidelines will be followed regarding training of research staff and the disposal of biohazards.

Exclusion criteria: Participants will not be informed of their cognitive performance; however, if a subject's cognitive performance is judged to be of concern (e.g., they do not meet the criteria for participation), the participant will be contacted by Dr. Tomika Williams (Gerontological Primary Care Nurse Practitioner) who will encourage the participant to consult with his/her personal physician. Similarly, if a participant's score on the Center for Epidemiological Studies Depression Scale – Revised (CESD-R) suggests that he or she is experiencing depressive symptoms that might be indicative of clinical depression or suicidal ideation, the participant will be contacted by Dr. Tomika Williams (Gerontological Primary Care Nurse Practitioner) who will encourage the participant to consult a physician.

Magnetic Resonance Imaging (MRI):

MRI is a non-invasive technique that does not have any known risks once necessary safety precautions have been taken. The precautions must be taken with respect to metal objects, potential for burns, potential for hearing loss, muscle twitching and tingling, and risk for pregnant women. The following steps will be taken with regard to each of this risks.

Metal objects: Metal objects within the body or on/in clothing can cause harm to participants, in addition to distorting the quality of the MRI images. Participants will complete the MRI Recruitment Screening Form and the Gateway MRI Screening Form and will go through an extensive screening process to determine if it is safe for them to have an MRI exam. In addition, such things as keys, watches, and credit cards will be kept safely away from the machine. We will ask participants to take off all removable metal (e.g. jewelry, piercings, etc.). People with devices or objects inside the body that are affected by strong magnetic fields (i.e. metallic foreign bodies inside a person's head or eyes, incompatible medical implants, pacemakers, brain stimulators, blood vessel clips, etc.) will not be allowed to participate under any circumstances. Knowingly participating in this study with these types of metallic implants can lead to serious injury or death. Although metal objects sensitive to strong magnetic fields are not allowed in the MRI scanner, there are many metal objects that are not sensitive to strong magnetic fields, such as dental work, pins or screws used during surgery, and even some tattoos contain metal. People with these types of metal objects may safely participate in this study.

Burn risks: In extremely rare cases, metal in the body (e.g., in tattoos) exposed to the powerful radio waves used in MRI may heat up. This heating occurs gradually but if it goes unreported during the MRI exam it could lead to burns. Such burns are easily prevented by reporting any heating sensations to the technologists immediately. For safety, participants will be monitored the entire time you are in the scanner. The study team will be able to communicate with participants during the exam through an intercom. Participants will also be given a ball to squeeze to signal the researcher to stop the exam immediately and for any reason.

Hearing loss: MRI scanners when taking a picture are very loud. Participants will be required to wear Comply Canal tip earplugs during the exam. When the earplugs are used properly, the noise from the MRI scanner is as loud as a garbage disposal or food blender. If the earplugs are not inserted into the ear canal then temporary hearing loss is possible. If at any time the noise from the MRI machine is too loud, participants will be instructed to inform the technologist.

Muscle twitching and tingling: MRI machines turn magnetic fields on and off very quickly to make an image. In rare cases, this may cause a person's muscles to twitch and tingle. The muscle twitching and tingling are temporary and will stop as soon as the scanner stops. In some rare cases, some individuals find the muscle twitching and tingling to be uncomfortable and cannot continue with the MRI exam. If this happens, participants will be instructed to let us know and will be released from the study.

Pregnancy: It is unclear at this time whether strong magnets are a risk to unborn fetuses. Due to the unknown risk and potential harm to an unborn fetus from any MRI scan, pregnant women will be excluded. All women in this study are between the ages of 40-65 and are unlikely to be pregnant, but we will ask this question before entering the scanner.

Data confidentiality and genotyping

All subjects' data will be coded so that the identities of the participants are not contained on the processed data. All data collected in this study will be identified only by subject number, and not by name, and data will only be reported in anonymous or aggregate form. Further, saliva samples are coded based on de-identified information (i.e., subject ID #) so that the WFU lab processes DNA solely on the basis of the ID # and has no access to subject information which is kept by the research staff. Every effort will be made to keep all information confidential. If data are used for publication, no identifying information will be included. The subject name-code number logs will be kept in a locked

cabinet in the Physical Activity and Cognition laboratory at the University of North Carolina Greensboro. All procedures and data handling will be in compliance with HIPAA regulations.

In addition, researchers will request a Certificate of Confidentiality (CoC) from the National Institutes of Health. Information about this CoC will be included in the informed consent. The CoC will allow the researchers to protect participants' privacy. The researchers can use the Certificate to legally refuse to disclose information that may identify participants in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if there is a court subpoena. The researchers will use the Certificate to resist any demands for information that would identify the participant [except as explained below].

The Certificate cannot be used to resist a demand for information from personnel of the United States federal or state government agency sponsoring the project and that will be used for auditing or program evaluation of agency funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA). Participants should understand that a Certificate of Confidentiality does not prevent the participant or a member of your family from voluntarily releasing information. If an insurer, medical care provider, or other person obtains the participant's written consent to receive research information, then the researchers will not use the Certificate to withhold that information.

The Certificate of Confidentiality will not be used to prevent disclosure to state or local authorities of reports of harm to self or others.

To minimize burden, all data collection sessions will be held in a flexible manner to accommodate potential limitations in endurance and abilities. Thus, testing will be discontinued or postponed as needed. To protect against attrition due to participant burden, the investigators will monitor ongoing participation in the study, contact individuals who appear at risk for dropping out, and provide support and encouragement. At all times, they will be encouraged to call with questions or concerns regarding their participation in the study. Participants will be encouraged to seek out medical advice relative to any problems that are detected. Of course, participants may withdraw from the study at any time.

4.1.3. Potential Benefits of the Proposed Research to Human Subjects and Others

The small risks incurred by the participants are outweighed by the potential benefits of this research. Participants randomly assigned to the physical activity condition may benefit directly as a result of participation in the physical activity program which we expect to produce cognitive benefits and other psychological and health benefits. Participants randomly assigned to the usual care control condition will be given a short-term YMCA membership upon completion of post-testing. The general information that will come from this study may provide evidence that the physical activity program can improve cognitive performance in middle-aged adults with a family history of AD. Since the risks associated with participation are minimal, the potential cognitive, psychological, and health benefits to participants outweighs the risks.

4.1.4. Importance of the Knowledge to be Gained

The findings of this study are important because they will advance our knowledge regarding the efficacy of this physical activity intervention for middle-aged adults with a family history of Alzheimer's disease. The findings of this study will contribute to our understanding of the use of this physical activity program as a preventative behavioral intervention that protects the cognitive abilities of middle-aged adults.

4.1.5 Data and Safety Monitoring Plan

We have developed an internal data and safety monitoring plan (DSMP). The DSMP will be implemented through a). communication with and oversight by Safety Officers (Dr. Maryjo Cleveland, Director of Geriatric Consultation Clinic, Wake Forest School of Medicine; Dr. Jeff Williamson, Program Director, J. Paul Sticht Center for Healthy Aging and Alzheimer's Prevention) and the Primary

Investigator (PI, Dr. Jennifer L. Etnier); b). training of research staff; c). monthly conversations with the Safety Officer, the PI, Dr. Tomika Williams (Adult Gerontological Primary Care Nurse Practitioner), relevant co-investigators, and other staff; d). confirmation that no one working on the research funded by this grant has any financial conflict; e). communication with and oversight by the Office of Research Compliance (ORC) at the University of North Carolina at Greensboro (UNCG); and f). communication with the NIA Program Administrator. The primary purpose of the plan is to allow us to monitor safety during the study with secondary charges related to the data collection procedures and the collection of efficacy data. These aspects of the implementation of the DSMP are described in further detail in the Data Safety Monitoring Plan.

Although we are aware of the potential for serious adverse events (SAE) to occur with any type of moderate exercise training, it has been clearly documented that the subjects of the type we will be working with can safely engage in exercise training Even though the risk of a SAE is very low, all personnel who supervise the exercise program or who conduct submaximal fitness tests will be trained in Cardiopulmonary Resuscitation (CPR). Further, all personnel working in the neuroimaging facility will receive appropriate safety training and certification. With an eye to insuring the safe conduct of the study we will review all safety and emergency procedures on a routine basis. As a matter of policy, all study personnel will participate in Responsible Conduct of Research (RCR) education programs.

References

1. Etnier, J.L., et al., *Innovative Research Design Exploring the Effects of Physical Activity and Genetics on Cognitive Performance in Community-Based Older Adults.* J Aging Phys Act, 2015. **23**(4): p. 559-68.
2. United States Census Bureau. *American Community Survey, 2010*. 2010 [cited 2017 April 3]; http://factfinder2.census.gov].
3. Raber, J., Y. Huang, and J.W. Ashford, *ApoE genotype accounts for the vast majority of AD risk and AD pathology.* Neurobiol Aging, 2004. **25**(5): p. 641-50.
4. Etnier, J.L., et al., *Cognitive performance in older women relative to ApoE-ε4 genotype and aerobic fitness.* Med Sci Sports Exerc, 2007. **39**(1): p. 199-207.
5. Caselli, R.J., *Recruiting efficacy for ApoE genotypes*, J.L. Etnier, Editor. 2008: Greensboro, NC.
6. American College of Sports Medicine, *ACSM's Guidelines for Exercise Testing and Prescription*. 8th ed, ed. W.R. Thompson. 2010, Philadelphia, PA: Lippincott Williams & Wilkins.
7. Manly, J.J., et al., *Telephone-based identification of mild cognitive impairment and dementia in a multicultural cohort.* Arch Neurol, 2011. **68**(5): p. 607-14.
8. Cook, S.E., M. Marsiske, and K.J. McCoy, *The use of the Modified Telephone Interview for Cognitive Status (TICS-M) in the detection of amnestic mild cognitive impairment.* J Geriatr Psychiatry Neurol, 2009. **22**(2): p. 103-9.
9. de Jager, C.A., M.M. Budge, and R. Clarke, *Utility of TICS-M for the assessment of cognitive function in older adults.* Int J Geriatr Psychiatry, 2003. **18**(4): p. 318-24.
10. Kaur, A., S.D. Edland, and G.M. Peavy, *The MoCA-Memory Index Score: An Efficient Alternative to Paragraph Recall for the Detection of Amnestic Mild Cognitive Impairment.* Alzheimer Dis Assoc Disord, 2018. **32**(2): p. 120-124.
11. American College of Sports Medicine, *ACSM's Guidelines for Exercise Testing and Prescription*. 10th ed, ed. D. Riebe. 2017, Philadelphia, PA: Lippincott Williams and Wilkins.
12. Sink, K.M., et al., *Effect of a 24-Month Physical Activity Intervention vs Health Education on Cognitive Outcomes in Sedentary Older Adults: The LIFE Randomized Trial.* JAMA, 2015. **314**(8): p. 781-90.
13. Erickson, K.I., et al., *Exercise training increases size of hippocampus and improves memory.* Proc Natl Acad Sci U S A, 2011. **108**(7): p. 3017-22.

14. Voss, M.W., et al., *Plasticity of brain networks in a randomized intervention trial of exercise training in older adults.* Front Aging Neurosci, 2010. **2**.
15. Steins Bisschop, C.N., et al., *Control group design, contamination and drop-out in exercise oncology trials: a systematic review.* PLoS One, 2015. **10**(3): p. e0120996.
16. Knaepen, K., et al., *Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects.* Sports Med, 2010. **40**(9): p. 765-801.
17. Lezak, M.D., D.B. Howieson, and D.W. Loring, *Neuropsychological Assessment*. 4th ed. 2004, New York: Oxford University Press.
18. Colcombe, S. and A.F. Kramer, *Fitness effects on the cognitive function of older adults: A meta-analytic study.* Psychol Sci, 2003. **14**(2): p. 125-30.
19. Etnier, J.L., et al., *The moderating effect of apolipoprotein (ApoE) genotype on cognitive performance in response to chronic physical activity: The Physical Activity and Alzheimer's Disease (PAAD) Study.* , in *North American Society for the Psychology of Sport and Physical Activity*. 2015, J Sport Exerc Psychol: Portland, OR. p. S112.
20. Lind, J., et al., *Reduced hippocampal volume in non-demented carriers of the apolipoprotein E epsilon4: relation to chronological age and recognition memory.* Neurosci Lett, 2006. **396**(1): p. 23-7.
21. Millar, P.R., et al., *Process Dissociation Analyses of Memory Changes in Healthy Aging, Preclinical, and Very Mild Alzheimer Disease: Evidence for Isolated Recollection Deficits.* Neuropsychology, 2017.
22. Jennings, J.M. and L.L. Jacoby, *Automatic versus intentional uses of memory: aging, attention, and control.* Psychol Aging, 1993. **8**(2): p. 283-93.
23. Schmidt, M., *Rey Auditory Verbal Learning Test: A Handbook*. 2004, Los Angeles, CA: Western Psychological Services.
24. Etnier, J.L., et al., *The Physical Activity and Alzheimer's disease (PAAD) study: Cognitive outcomes.* Annals of Behavioral Medicine, In Press.
25. Salthouse, T.A., *Memory aging from 18 to 80.* Alzheimer Disease and Associated Disorders, 2003. **17**(3): p. 162-7.
26. Reynolds, C.A., et al., *Longitudinal memory performance during normal aging: twin association models of APOE and other Alzheimer candidate genes.* Behav Genet, 2006. **36**(2): p. 185-94.
27. Mayeux, R., et al., *Memory performance in healthy elderly without Alzheimer's disease: effects of time and apolipoprotein-E.* Neurobiol Aging, 2001. **22**(4): p. 683-9.
28. Flory, J.D., et al., *Memory performance and the apolipoprotein E polymorphism in a community sample of middle-aged adults.* Am J Med Genet, 2000. **96**(6): p. 707-11.
29. Chapman, S.B., et al., *Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging.* Front Aging Neurosci, 2013. **5**: p. 75.
30. Colcombe, S.J., et al., *Aerobic exercise training increases brain volume in aging humans.* J Gerontol A Biol Sci Med Sci, 2006. **61**(11): p. 1166-70.
31. Colcombe, S.J., et al., *Cardiovascular fitness, cortical plasticity, and aging.* Proceedings of the National Academy of Sciences of the United States of America, 2004. **101**(9): p. 3316-21.
32. Alexander, G.E., et al., *Gray matter network associated with risk for Alzheimer's disease in young to middle-aged adults.* Neurobiol Aging, 2012. **33**(12): p. 2723-32.
33. Burggren, A.C., et al., *Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E e4 carriers.* Neuroimage, 2008. **41**(4): p. 1177-83.
34. Fennema-Notestine, C., et al., *Presence of ApoE epsilon4 allele associated with thinner frontal cortex in middle age.* J Alzheimers Dis, 2011. **26 Suppl 3**: p. 49-60.
35. Tohgi, H., et al., *Reduced size of right hippocampus in 39- to 80-year-old normal subjects carrying the apolipoprotein E epsilon4 allele.* Neurosci Lett, 1997. **236**(1): p. 21-4.
36. Wishart, H.A., et al., *Regional brain atrophy in cognitively intact adults with a single APOE epsilon4 allele.* Neurology, 2006. **67**(7): p. 1221-4.
37. Westlye, L.T., et al., *Effects of APOE on brain white matter microstructure in healthy adults.* Neurology, 2012. **79**(19): p. 1961-9.
38. Reuter, M., et al., *Within-subject template estimation for unbiased longitudinal image analysis.* Neuroimage, 2012. **61**(4): p. 1402-18.
39. Andersson, J.L., S. Skare, and J. Ashburner, *How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging.* Neuroimage, 2003. **20**(2): p. 870-88.
40. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL.* Neuroimage, 2004. **23 Suppl 1**: p. S208-19.
41. Behzadi, Y., et al., *A component based noise correction method (CompCor) for BOLD and perfusion based fMRI.* Neuroimage, 2007. **37**(1): p. 90-101.
42. Whitfield-Gabrieli, S. and A. Nieto-Castanon, *Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks.* Brain Connect, 2012. **2**(3): p. 125-41.

43. Maldjian, J.A., et al., *An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets.* Neuroimage, 2003. **19**(3): p. 1233-9.
44. Boone, K.B., et al., *Rey-osterrieth complex figure performance in healthy, older adults: Relationship to age, education, sex, and IQ.* Clinical Neuropsychologist, 1993. **7**(1): p. 22-28.
45. Fastenau, P.S., N.L. Denburg, and B.J. Hufford, *Adult norms for the Rey-Osterrieth Complex Figure Test and for supplemental recognition and matching trials from the Extended Complex Figure Test.* Clin Neuropsychol, 1999. **13**(1): p. 30-47.
46. Weuve, J., et al., *Physical activity, including walking, and cognitive function in older women.* Jama, 2004. **292**(12): p. 1454-61.
47. Lytle, M.E., et al., *Exercise level and cognitive decline: the MoVIES project.* Alzheimer Dis Assoc Disord, 2004. **18**(2): p. 57-64.
48. Yaffe, K., et al., *A prospective study of physical activity and cognitive decline in elderly women: women who walk.* Arch Intern Med, 2001. **161**(14): p. 1703-8.
49. Abbott, R.D., et al., *Walking and dementia in physically capable elderly men.* Journal of the American Medical Association, 2004. **292**(12): p. 1447-53.
50. Rast, P., et al., *APOEepsilon4 Genotype and Hypertension Modify 8-year Cortical Thinning: Five Occasion Evidence from the Seattle Longitudinal Study.* Cereb Cortex, 2017: p. 1-12.
51. Maye, J.E., et al., *Maternal dementia age at onset in relation to amyloid burden in non-demented elderly offspring.* Neurobiol Aging, 2016. **40**: p. 61-7.
52. Craig, C.L., et al., *International physical activity questionnaire: 12-country reliability and validity.* Med Sci Sports Exerc, 2003. **35**(8): p. 1381-95.
53. Davidson, F., *Principles of statistical data handling*. 1996, New York: Sage.
54. Muthen, L.K. and B.O. Muthen, *Mplus version 6.1*. 2010, Muthen & Muthen: Los Angeles.
55. Curran, P.J. and B.O. Muthen, *The application of latent curve analysis to testing developmental theories in intervention research.* American Journal of Community Psychology, 1999. **27**(4): p. 567-595.
56. Duncan, T.E., S.C. Duncan, and L.A. Strycker, *An introduction to latent variable growth curve modeling: Concepts, issues, and applications (2nd ed.)*. An introduction to latent variable growth curve modeling: Concepts, issues, and applications (2nd ed.). 2006, Mahwah, NJ US: Lawrence Erlbaum Associates Publishers.
57. Duncan, T.E. and S.E. Duncan, *An introduction to latent growth curve modeling.* Behavior Therapy, 2004. **35**(2): p. 333-363.
58. Diggle, P. and M.G. Kenward, *Informative drop-out in longitudinal data analysis.* Applied Statistics, 1994. **43**(1): p. 49-93.
59. Muthen, B., et al., *Growth modeling with nonignorable dropout: alternative analyses of the STAR*D antidepressant trial.* Psychol Methods, 2011. **16**(1): p. 17-33.
60. Muthen, L.K. and B.O. Muthen, *How to use a Monte Carlo study to decide on sample size and determine power.* Structural Equation Modeling, 2002. **9**(4): p. 599-620.
61. Ramirez, F.D., et al., *Methodological Rigor in Preclinical Cardiovascular Studies: Targets to Enhance Reproducibility and Promote Research Translation.* Circ Res, 2017.
62. Landis, S.C., et al., *A call for transparent reporting to optimize the predictive value of preclinical research.* Nature, 2012. **490**(7419): p. 187-91.
63. Vahidy, F., et al., *Reporting Standards for Preclinical Studies of Stroke Therapy.* Stroke, 2016. **47**(10): p. 2435-8.
64. National Heart, L., et al., *Ethical and practical guidelines for reporting genetic research results to study participants: updated guidelines from a National Heart, Lung, and Blood Institute working group.* Circ Cardiovasc Genet, 2010. **3**(6): p. 574-80.