

Study Protocol and Statistical Analysis Plan

Title: Exercise for Brain Health with Increased Genetic Risk for Alzheimer's Disease

NCT Number: NCT03727360

Document Date: September 26, 2025

Participant enrollment (see Figure 1). Our online advertising and recruitment efforts resulted in 5,284 online pre-screening surveys via our laboratory website, which led to 266 individuals who volunteered and completed the in-person informed consent visit (a rate of 5%, which is consistent with the historic and expected rate). Among the 266 older adults who passed the initial pre-screen and were consented, 83 identified as non-white (31.2%), and this included 11 Asian (4.1%), 63 African American (23.6%), 4 Hispanic (1.5%), 5 more than one race (1.9%). Among the 266 consented individuals, 117 (44%) were determined (based on further screening conducted at the consent visit) to not meet the inclusion/exclusion criteria, with the most common reason being identification of cognitive impairment, and 25 voluntarily withdrew from the study, leaving 119 who were randomized to one of two treatment arms, including 26 of the 63 African Americans and (41.3%). A total of 106 of the 266 consented individuals completed the entire 6-month exercise intervention with baseline and follow-up testing. Among the 106 who completed the study there were: 5 Asian (4.7%), 18 African American (17.0%), 80 Caucasian (75.5%), and 3 (2.8%) who preferred not to identify their race/ethnicity.

Confirmation of Intact Cognition. Confirmation of intact cognition was determined by assessing activities of daily living (Lawton and Brody ADL Scale), symptoms of depression (Geriatric Depression Scale)

Neuropsychological Test Battery. Table 1 shows the variables used for screening to determine initial inclusion to warrant an in person visit for written informed consent and further evaluation for intact cognition, the primary memory outcome (Rey AVLT), and secondary fitness outcomes.

Exercise Testing. A submaximal exercise test was conducted on a treadmill using a modified Balke-Ware protocol (2.0 mph, grade increase 1°/min) when expired air was collected and analyzed using open circuit spirometry. The exercise test was conducted/terminated according to the American College of Sports Medicine Guidelines, or it ended when the participant reached 85% of age-predicted maximal heart rate or expressed a desire to stop for any reason.

6-Min Walk. Participants walked along an oval path with instructions to walk as far as possible in 6 minutes. Distance walked was recorded.

MRI Scan. Event-related fMRI was conducted using a gradient-echo echoplanar pulse sequence. High-resolution anatomical images were acquired for registration and localization of functional activity. Physiological monitoring of heart rate and respiratory rate were recorded continuously. A foam pillow was used to limit head motion. We used a 3D-registration algorithm that aligns each volume using six movement parameters.

Famous Name Discrimination Task. The task stimuli consist of 30 names of famous persons and 30 names of individuals who are not well known. Stimuli were originally selected from an original pool of 784 names based on a high rate of correct identification (< 10% error rate). A trial consists of the visual presentation of a single name for 4 seconds (total run time 5 min and 24 sec). Participants make a right index finger key press if the name is famous and a right middle finger key press if the name is not famous. Both accuracy (% correct) and reaction time (ms) are recorded. The 60 name trials are randomly interspersed with 20 4-sec “fixation” trials (to add “jitter”). We exclude occasional incorrect items from the fMRI maps. We developed alternative equivalent forms (based on amount of semantic knowledge, naming accuracy, and ratings of emotional reaction) to allow longitudinal follow-up.

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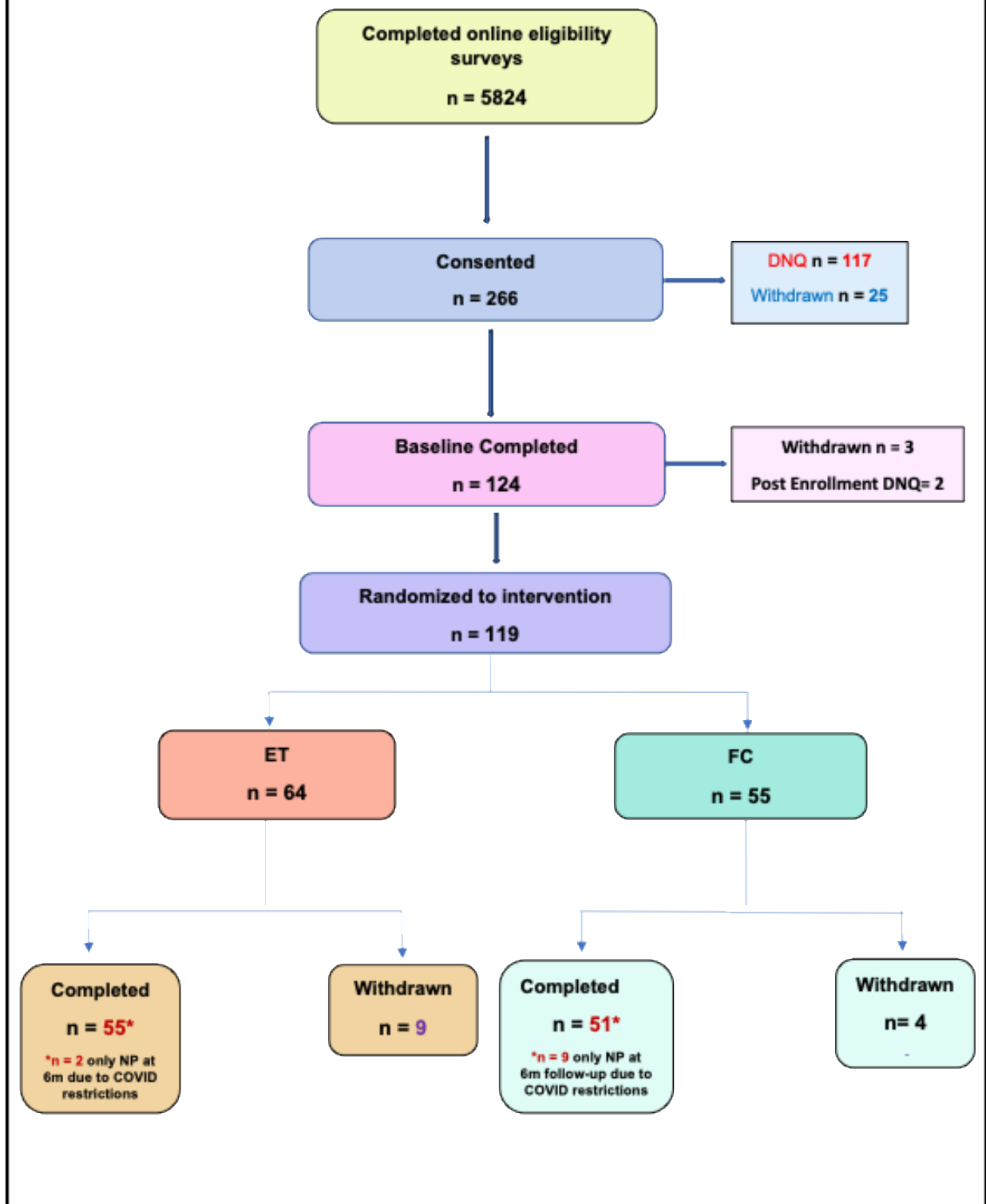


Figure 1. Participant recruitment and retention.

Table 1. Screening and Non-MRI Outcomes Assessments for All Participants					
Outcomes	Screener	30 days Pre-Baseline	Baseline & 6-Months	Baseline Time (min)	6-months Time (min)
Inclusion Criteria					
Screener Questionnaire	x			20	
Geriatric Depression Scale (15 item)	x			5	
Godin Physical Activity History Questionnaire	x			5	
MRI Safety Form	x	x	x	5	5
Physician Approval to Exercise (from PCP)		x			
			EST. TIME:	35	5
Diagnostic Battery					
Lawton and Brody ADL Scale		x		5	
Geriatric Depression Scale		x		10	
MoCA		x		10	
Wechsler Test of Adult Reading		x		2	
			EST. TIME:	27	
Primary Outcomes					
Rey Auditory Verbal Learning Test			x	50	50
			EST. TIME:	50	50
Secondary Outcomes					
Maximal Exercise Stress Test (VO ₂ peak)			x	15	15
6-Minute Walk Test			x	10	10
			EST. TIME:	25	25

Exercise Intervention. In response to the COVID-19 pandemic, in March 2020 we developed an innovative approach to deliver the exercise intervention via Zoom in live sessions offered 4 days per week at two different times (morning and evening), see Table 2. This included an orientation session for each participant to ensure comfort with and ability to use the Zoom platform in their own home, as well as an emergency plan that could be executed remotely. In addition, we developed specific intervention progression steps, and naming convention, that ensures each participant was exercising at the appropriate frequency, duration and intensity. Many undergraduate students served as excellent instructors and provided encouragement to enhance adherence.

Statistical Analysis Plan. We will use the independent samples t-tests to determine if the ET and FC groups differ in the change in RAVLT Trials 1-5 performance before (Baseline) to after (6-Month) the ET vs. FC conditions. Baseline performance will be compared between ET and FC groups using independent samples t-tests. If baseline differences exist, then the baseline values will be used as a covariate in the analysis of change scores. We will also use Independent Samples t-tests to determine if the ET and FC groups differ in the change in famous name discrimination task activation from before (baseline) to after (6-Month) the ET vs. FC conditions. We will use the False Discovery Rate (FDR) to control family-wise error based on the number of functional regions of interest activated by the task. At baseline we expect that the famous name task will activate the hippocampal memory network (overlaps with the default mode network). If baseline differences exist between ET and FC (based on independent samples t-tests), then the baseline activation will be used as a covariate in the analysis. The key finding will be that several brain regions will show decreased activation intensity after the ET intervention. Because activation will be measured during successful memory retrieval both before and after ET and FC, this may suggest improvement in brain network efficiency. The results reported herein should be considered preliminary and taken with caution.

Table 2. Exercise intervention progression from week 1 to week 26.

Week	Days per week	Warm up	stretching	Balance and toning	Marching Time	Obstacle course Time	Exercise Training Group time	Exercise Training Group %HR RPE	Flex. Control Group time	Flex. Control Group %HR RPE
1	2	6 min	4 min	5 min	2 min	6 min	10 min	40-50% 9-11	10 min	20-30% 8-10
2	2	6 min	4 min	5 min	2min	6 min	10 min	40-50% 9-11	10 min	20-30% 8-10
3-4	3	6 min	4 min	5 min	2 min	6 min	10 min	45-50 9-11	10 min	20-30% 8-10
5-6	3	6 min	4 min	5 min	2 min	8 min	20 min	50-55% 10-12	20 min	25-35% 9-11
7-8	4	6 min	4 min	5 min	2 min	8 min	20 min	50-55% 10-12	20 min	25-35% 9-11
9-26	4	6 min	4 min	5 min	2 min	10 min	30 min	60-70% 11-15	30 min	30-40% 9-11