Lifestyle Enriching Activities for
Research in Neuroscience
intervention trial

Lifestyle Enriching Activities for Research in Neuroscience Intervention Trial: LEARNit Study

Statistical Analysis Plan (SAP)

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INTRODUCTION

This document outlines the Statistical Analysis Plan (SAP) for the LEARNit Study. The purpose of this study is to examine the effects of modifiable lifestyle factors including exercise and healthy living education on brain health. The investigators will compare 2 types of interventions, moderate aerobic walking vs. healthy living education reading, over 6 months to evaluate changes in brain function, cognition, and physical function in older adults with cognitive concerns. The trial started recruiting in July 2016 and completed in July 2022. Study was paused between March 19, 2020 and September 2, 2020 due to campus shut-downs during the Covid-19 pandemic.

OVERVIEW

This study evaluates exercise as an intervention for improving brain function assessed by functional magnetic resonance imaging (fMRI), by remediating hippocampal brain dysfunction in patients with early MCI. Based on the current Surgeon General's recommendation for moderate-intensity exercise, participants were randomly assigned to one of two 6-month home-based interventions: 1) aerobic walking for 150 mins/week or 2) healthy living education non-active control. Pre- and post-measurements included fMRI scan (task, resting state), structural MRI scan, cognitive tests of memory and executive function, physical function tests, and blood sample. As an exploratory investigation, participants completed amyloid and tau PET imaging scans at baseline and at 6-month follow-up to examine the contribution of amyloid and tau pathology on brain function and to serve as a positive AD biomarker.

The study addresses following questions:
Primary Objective 1a: To evaluate the primary outcome of change in fMRI BOLD signal during a memory task after a 6-month intervention. (n=59; 1 participant did not complete fMRI scans)
Hypothesis: Change in hippocampal BOLD signal after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Primary Objective 1b: To evaluate the primary outcome of change in Mnemonic Similarity Test (MST) Lure Discrimination Index (LDI) score after a 6-month intervention. (n=60)
Hypothesis: Change in LDI score after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Secondary Objective 2a: To examine the secondary outcomes of change in aerobic fitness and physical function score after a 6-month intervention. (n=60)
Hypothesis: Change in aerobic fitness and physical function score after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Secondary Objective 2b: To examine the secondary outcome of change in plasma BDNF level after a 6-month intervention. (n=60)
Hypothesis: Change in plasma BDNF levels after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Secondary Objective 2c: To examine the secondary outcome of change in amyloid PET level after a 6-month intervention. (n=60)
Hypothesis: Change in amyloid PET level after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Exploratory Objectives 3: To examine the exploratory outcomes of change in brain volume, cognition, tau PET level after a 6-month intervention. (n=60; except MRI in which 59 participants completed pre and post MRI scans)
Hypothesis: Change in hippocampal brain volume, executive function scores, and tau PET levels after the 6-month intervention will be different between the aerobic walking and healthy living education arms.

Exploratory executive function outcomes include digit symbol, Flanker, block design, Trails A and B, and number-letter sequencing. Analyses will be completed on individual tests and an unweighted summed z-score composite of all 6 tests for executive function.

Exploratory brain volumes include left and right hippocampal volumes treated as 2 separate outcomes.

STUDY DESIGN

LEARNit is a single-site, randomized, single-blind trial comparing the effects of moderate aerobic walking (55-80% target HR) versus healthy living education reading in older adults who perform at least 1 standard deviation below normative values for their age (N=66 randomized; N=60 with pre-post data, except 1 participant is missing complete fMRI and MRI scans).

Randomization

We used a randomized, single-blind study design where randomization was achieved using a permuted block schema with block sizes of 2 and 4. Participants were randomized in a 1:1 allocation to either aerobic walking or healthy living education arms and stratified by APOE4 carrier status (yes/no) based on saliva APOE phenotyping.

Power and Sample Size Determination

Given the planned sample size of 30 participants per group, the magnitude of effect observable for this analysis are described below:

This study was powered based on the fMRI primary outcome. Group sample sizes of 30 achieve 80% power for each test to detect an effect size of at least 0.736 and a type I error of 0.05 using a two-sided two-sample equal-variance t-test. There are 2 primary outcomes (cognition, imaging), but because they are very different measures, the alpha level will remain at 0.05 for each primary outcome test. There will be no alpha-level correction for secondary or exploratory outcomes. Preliminary data provide an estimated standard deviation of 0.25 z-score units for fMRI network function, indicating that we will be able to detect differences of 0.184 z-score units in brain function between groups. The effect sizes for fMRI BOLD activity and network function reported in previous physical activity intervention studies in older adults ranged from 1.17-1.24, thus we have high power to detect significant differences in brain function.

Study Aims

Primary Aim

- **Primary 1a.** To test the hypothesis that change in hippocampal BOLD signal after the 6-

month intervention will differ between the aerobic walking and healthy living education arms. Rationale: The Mnemonic Similarity Task (MST) behavioral task was used within an fMRI study of pattern separation, tracing out tuning curves in activity as a function of delta-input (Bakker et al., 2008, Science; Lacy et al., 2011, Learning and Memory). MST is highly sensitive to hippocampal function, placing strong demands on pattern separation processes. MST has been used to identify hippocampal dysfunction associated with healthy aging, dementia, schizophrenia, depression, and other clinical disorders (Stark et al., 2019, Trends in Cognitive Neuroscience).

- **Primary 1b.** To test the hypothesis that change in Lure Discrimination Index (LDI) score after the 6-month intervention will differ between the aerobic walking and healthy living education arms. Rationale: The primary memory outcome of interest in this MST paradigm is the LDI, which tracks the ability to remember details of the stimuli in order to discriminate similar items from repeated/old items (Stark et al., 2019, Trends in Cognitive Neuroscience).

Secondary Aim

- **Secondary 2a.** To test the hypothesis that change in single-stage treadmill test and physical function score after the 6-month intervention will differ between the aerobic walking and healthy living education arms.

- **Secondary 2b.** To test the hypothesis that change in plasma BDNF level after the 6-month intervention will differ between the aerobic walking and healthy living education arms.

- **Secondary 2c.** To test the hypothesis that change in amyloid PET level after the 6-month intervention will differ between the aerobic walking and healthy living education arms.

Exploratory Aim

- **Exploratory 3.** To test the hypotheses that change in hippocampal brain volume, scores on individual tests and composite score of executive function, and tau PET levels after the 6-month intervention will differ between the aerobic walking and healthy living education arms.

Populations of Interest

- **Intent-to-Treat (ITT) Population:** Includes all eligible participants who (1) began the intervention (Intervention Session 1) and (2) completed baseline assessment and 6-month assessment for the primary analysis of fMRI BOLD and MST behavioral. All consented, randomized participants are grouped according to the treatment assigned at randomization, regardless of any protocol violations (n=60 for all outcomes, except n=59 for fMRI primary outcome and hippocampal brain volume exploratory outcome due to missing MRI scans).

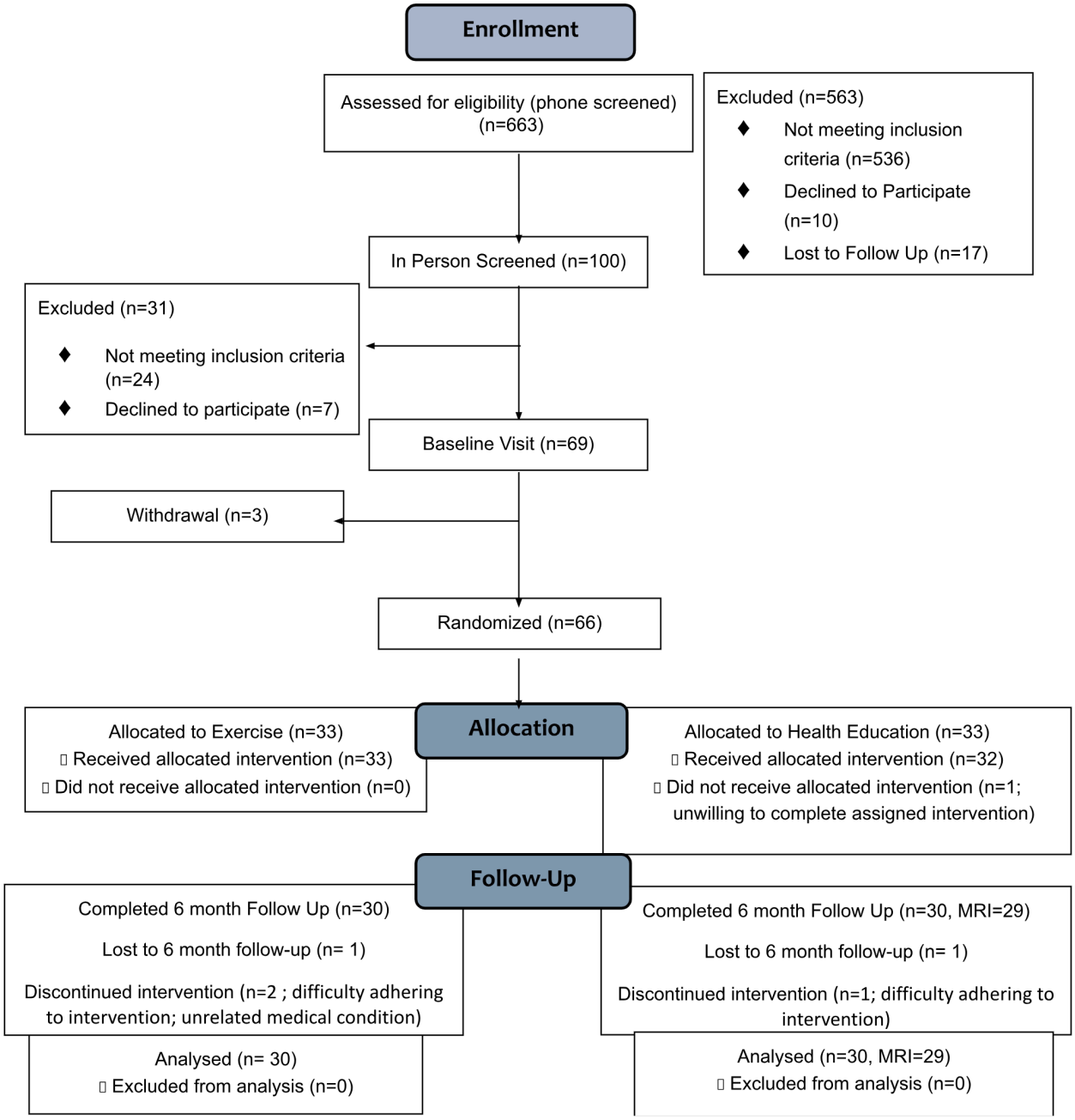
Exploratory sensitivity analyses / subgroup analyses to address whether intervention effects differ by pre-specified sub-groups of the study population

- **Per-Protocol (PP) Population:** Includes all eligible participants in the ITT analysis, plus the following criteria: (1) completed at least 70% of the assigned minutes of aerobic walking or healthy living education reading over the entire intervention period (total duration: 26 weeks X 150 mins/week)
- **Phenotypic differences:** Explore whether there are sex/gender and APOE4 carrier differences by treatment arm.
- **Baseline cognition:** Examine whether those participants with more cognitive impairment at baseline had differential response to the treatment arms.
- **Domain-specific cognitive performance:** Conduct a subgroup analysis based on the participants baseline domain of cognitive impairment.

Study Flow CONSORT Diagram

A total of 60 participants will be analyzed with baseline and follow-up data. One participant is missing their fMRI scan session data due to an MRI-incompatible defibrillator (n=59).

For descriptive purposes only, we include sample sizes of pandemic-related subgroups, 37 participants completed the study before pandemic whereas 23 participants completed the study during the pandemic. 7 of the 23 had extended interventions due to no visits during the 5-month shutdown.



Analyses of Study Outcomes

- Hypotheses regarding the primary cognitive outcome, and secondary and exploratory outcomes will be tested using a linear mixed model for repeated measures (MMRM) including indicator variables for time, intervention, time-by-intervention interaction term, and additional covariates meeting confounding criteria below. A participant-level random intercept will be specified; significance of random slopes will be tested and included in the model if significant. Age and sex/gender will be treated as potential confounders. Potential confounders will be identified from randomization imbalance and based on their effect on the magnitude of treatment. A variable will be treated as confounder if adding it

to the model changes the value of treatment estimate by more than 15%. APOE4 will be included in all models because it was a randomization stratification factor.

- For fMRI hippocampal BOLD analysis, within subject changes in activity will be calculated for each individual subject in those with both baseline and follow-up fMRI data. Subject data will be normalized to group template space which is comprised of timepoint 1 and 2 to reduce measurement error or individual subject bias.

Analyses of Primary Outcomes

- Hippocampal BOLD signal: change in hippocampal BOLD signal during the MST task defined as differences in activity between baseline and 6-month as the outcome measure. Change can be in either direction.

- Lure Discrimination Index (LDI): change in the LDI score, defined as 6-month – baseline, as the outcome measure will be analyzed using the MMRM approach. Higher change scores indicate improvement in LDI performance.

Analyses of Key Secondary Outcomes

Secondary analyses will be conducted using the same MMRM statistical approach described above for the analysis of all secondary outcomes.

- Single-stage treadmill test: change in the VO2 max score from the single-stage treadmill test defined as 6-month – baseline as the outcome measure. Higher change scores indicate improvement.

- Physical function test score: change in the Physical Function test score defined as 6-month – baseline as the outcome measure. Higher change scores indicate improvement.

- Brain-derived neurotrophic factor (BDNF) level: change in BDNF defined as 6-month – baseline level as the outcome measure. Higher change scores are better.

- Amyloid PET SUVR: change in global amyloid composite score including the mean of frontal, temporal, parietal cortex SUVRs defined as 6-months – baseline will be the outcome measure. Lower change scores are better.

Exploratory Analyses

Exploratory analyses will be conducted using the same MMRM approach described above for the analysis of all exploratory outcomes.

- Hippocampal volume: change in hippocampal volume defined as 6-month – baseline will be the outcome measure. Higher change scores are better.

- Tau PET SUVR: change in tau composite score including SUVRs in Braak stages 1-6, separately defined as 6-months – baseline will be the outcome measure. Lower change scores are better.

Special Considerations for described analyses:

- No adjustment to type 1 error level (2-sided α=0.05) will be made for primary, secondary, or exploratory outcomes analyses.

- All primary, secondary and exploratory analyses will be repeated as sensitivity/subgroup analyses for per-protocol subset described below.

- As of the date on this document, the plasma samples are being processed for BDNF. The full or partial data available to our team on or before May 31, 2024 will be used for the planned analysis of BDNF as a secondary outcome.

Sensitivity and Heterogeneity of Effects Analyses

- PET sensitivity analyses – baseline levels of amyloid PET SUVR (continuous and positive/negative status based on established cut-points will be modeled as predictors of intervention responsiveness. Interaction terms of PET measure with treatment-time in the MMRM models will test for heterogeneity of the exercise intervention effect by PET levels of amyloid. For binary subgroups (positive/negative status), estimates of intervention effects will be presented in each subgroup.

- Sex/gender and APOE4 carrier status will be examined in sensitivity analyses to understand whether the treatment effects are differentially affected by sex/gender or APOE4. Interaction terms of sex/gender or APOE4 with treatment-time in the MMRM models will test for heterogeneity of the exercise intervention effect by sex/gender or APOE4.

- Per-protocol subset will also be evaluated for the primary, secondary, and exploratory outcomes described above.

SAFETY

Evaluation of Safety Measures

Event and participant count of the following will be summarized overall and by treatment group for the entire cohort:

- AE: Overall and by MedDRA System Organ Class
- AE: MedDRA Preferred Term
- SAE: Overall and by MedDRA System Organ Class
- SAE: MedDRA Preferred Term
- SAE Definitely Related to Intervention: Overall and by System Organ Class
- Hospitalization
- Deaths

Comparisons of the number of participants with at least one AE, SAE, SAE definitely related to Intervention and Death will be examined between Intervention groups using the Fisher's Exact test.

SOFTWARE

Statistical software R (version 3.5.2) will be used *http:www.r-project.org* and AFNI for image analysis.

SIGNATURES:

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol. I have discussed any questions I have regarding the contents of this document with the principal investigator, biostatistical authors or analysis team. I also understand that any subsequent changes or new approaches to the planned statistical analyses, as described herein, will be described within any publications related to the study.

Judy Pa, PhD – Principal Investigator — Date: 2-6-2024

Wendy Mack, PhD – Biostatistician on record — Date: Feb 6 2024