Protocol & Statistical Analysis Plan

Official Title: Influence of Exercise on Neurocognitive Function in Breast Cancer

NCT02793921

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Title of study:
Exercise Program in Cancer and Cognition (EPICC)

Brief description:
This is a single-site randomized control trial to test whether a moderate-intensity aerobic exercise intervention can prevent cognitive decline in postmenopausal women with breast cancer. Women who meet the eligibility criteria will be randomly assigned to either receive the intervention or usual care. Data will be collected prior to initiating endocrine therapy for breast cancer (TP1) and approximately 7 months later (TP2)and include fitness testing and a battery of neuropsychic tests, blood biomarkers, and other behavioral measures; a subset of women will undergo MR imaging. For women who agree to additional follow-up, data will also be collected approximately12 months post TP1 (TP3).

Specific aims:
We will examine whether a well-controlled and monitored site-based exercise intervention improves cognitive function in postmenopausal women with early stage breast cancer.1. Compared to usual care, examine whether the 6-month exercise intervention improves cognitive function in postmenopausal women with early stage breast cancer. Hypothesis 1. Exercise will improve cognitive function in women receiving AI therapy in a domain specific fashion such that attention, executive and memory functions will be influenced more than other domains.

2. Compared to usual care, examine the direct effects of exercise on neuroimaging metrics of brain health including regional gray matter volume, white matter architecture and functional dynamics of the brain and the pro-inflammatory biomarkers (IL-6, TNF-α), and explore the direct effects of exercise on symptoms (fatigue, sleep problems, depression, anxiety). Hypothesis 2. Exercise will improve neuroimaging metrics of brain health and pro-inflammatory biomarkers.

3. Compared to usual care, explore whether the effects of exercise on cognitive function are mediated by a) neuroimaging metrics of cognitive function, b) IL-6 and TNF-α levels, c) symptoms (fatigue, sleep problems, depression, anxiety), d) and moderated by E2 levels over the first six months of AI therapy.

Inclusion criteria:
1. Postmenopausal women, defined by lack of a menstrual period for an entire year or confirmed by physician 2. 18-80 years of age 3. Able to speak and read English 4. Completed a minimum of 8 years of education 5. Diagnosed with stage 0, I, II, or IIIa breast cancer, confirmed by medical record 6. Within 2 years post-completion of primary treatment 7. Will be at least two weeks post-breast conserving surgery or three weeks post-breast conserving surgery with sentinel lymph node biopsy or four weeks post-mastectomy at the start of participation.

Exclusion criteria:
1. Prior treatment with cancer chemotherapy, central nervous system radiation, of intrathecal therapy 2. Prior cancer involving the central nervous system 3. Clinical evidence of metastatic disease 4. Any of the following breast cancer surgery complications unless approved by the participant's health care provider: persistent seroma requiring aspiration, wound dehiscence, infection, prolonged drain output, lymphedema5. Reconstructive surgery unless approved by the participant's health care provider6. Any significant medical condition that would preclude them from exercising (e.g., uncontrolled diabetes, congestive heart failure, angina, uncontrolled arrhythmia or other symptoms that indicate increased risk for an acute cardiovascular or

Research activities:
Participants: We plan to recruit up to 350 postmenopausal women with early-stage breast cancer: up to 127 will undergo an exercise intervention in this 6-month study described below and up to 127 will be enrolled in an usual care arm. A subsample of women who are randomized will participate in the neuroimaging component of the study(n=150; 75/group).

Locations: Recruitment will take place at St. Clair Budway Surgical Associates and Magee-Women's Hospital of UPMC, Comprehensive Breast Care Center at UPMC Passavant-Cranberry, UPMC South Hills, UPMC McKeesport and Magee Woman care Center in Monroeville. Data collection will take place in participants' homes, at the University of Pittsburgh School of Nursing and Sennott Square or Healthy Lifestyle Institute offices in Oak Hill. The exercise intervention will take place at Sennott Square, Baierl Family YMCA, Sampson Family YMCA, and JCC South Hills Branch or Healthy Lifestyle Institute offices in Oak Hill; alternatively, a participant may exercise in her home or other location of her choosing. MRI Imaging will take place at Carnegie Mellon University SIBR.

Informed Consent: Written informed consent will be obtained from the participant prior to any study-specific activities either in person or remotely.

Assessments: Participants will complete a series of assessments at two timepoints; Time 1(baseline) will occur up to 2 years post-completion of primary treatment and before randomization and initiation of the exercise intervention/usual care. Time 2 will occur approximately within 1 month of completion of the intervention (or 7 months post-Time 1). Data collection at each timepoint will be composed of behavioral assessments, blood samples, neuroimaging and objective measures of physical activity and fitness. We will also objectively evaluate physical activity at 3 time points during participation (approximately every 5 weeks).For participants who agree to additional follow-up via the addendum consent form, Time 3 will take place approximately 6 months post Time 2 and will consist of blood work, fitness testing and behavioral testing as described below. We will also objectively evaluate physical activity at midway (3 months) between Time 2 and Time 3 as well as at Time 3. Participants will also be asked to maintain their edema cap and REDCap diaries during this time. Participants may be asked to complete assessments in two separate sessions at both TP1 and TP2.During one visit (which includes fitness testing), blood samples (approximately 30ccor 6 teaspoons) will be collected for biobanking for future evaluation of the relationships of biomarkers (including genetic biomarkers) with cognitive function, exercise, brain health and symptoms. Blood samples will be transported immediately, under conditions appropriate for preservation of the blood, and stored before the end of the day of testing. Samples will be maintained in a -80°C freezer in the Victoria building under the control of the principal investigators of this research project and will be maintained indefinitely. In the event that we need to send samples to a different facility outside of the University of Pittsburgh for analysis in the future, no identifying information will accompany the sample. Participants will be asked questions prior to the blood draw to determine any factors which may affect the findings. If a recent illness is noted, or certain other factors as noted on the form, are found, the participant may not be eligible for a blood draw at this visit. In this event, we will attempt to collect the blood at another time, either during a subsequent visit or other agreed upon time. Fitness will be objectively measured at Time 1 and Time 2. This test takes 15-30minutes to complete. Subjects will be given exercise testing instructions regarding proper clothing and footwear and will be told to abstain from caffeine or nicotine 2hours prior to the physical activity/fitness assessment. When participants report to the laboratory (Sennott Square) for the fitness assessment they will first be acquainted with the equipment, allowed to ask any questions, and get comfortable with walking on a treadmill. Before the test begins, the participant will get familiar with wearing the mouthpiece and nose clips or facemask required during the test in order to measure cardiovascular fitness. This helps to reduce anxiety associated with exercise and fitness testing. Cardiovascular fitness testing consists of progressively increasing exercise intensity while the subjects exhaled air is collected via a mouthpiece that is connected to a gas exchange chamber via a breathing tube. A comfort fitted head gear supports the mouthpiece. This reduces the effort of the participant to keep the mouthpiece in the correct position throughout the test. Alternatively, the participant may wear a facemask. The gas chamber that is being used is the Parvo Medics True one 2400 metabolic cart, which is one of the most widely accepted systems in scientific research. Next we will provide the participant with adequate warm-up and stretching which will be monitored by the exercise physiologist. After 5-10 minutes of warm-up, the participant will walk on a motor-driven treadmill at a constant speed with increasing increments of the incline. We will conduct a submaximal vo2 fitness test which estimates the maximal oxygen capacity for the individual without reaching an exhaustive level. The sub-maximal graded exercise test is a modified Balke protocol in which the speed remains constant and grade is increased 1% each minute. This protocol has been used extensively in the literature for fitness training for both normal, at-risk, and impaired populations. Verifying maximal vo2 is difficult in at-risk

populations and therefore submaximal vo2 is often the desired method. This method has people walk at a slow - moderate pace with increasing grade increments up to the goal heart rate of 85% of the age-based maximum (220-age) or a rating of perceived exertion equal to or greater than 15 for those individuals whose heart rate response is blunted due to medication. This approach does not require the attendance of a physician because it is within the safe heart rate work zones andic leads do not have to be attached to the participant. The submaximal vo2assessment usually takes about 15 - 30 minutes to complete depending on the starting fitness level of the participant. During the exercise test, heart rate in continuously monitored along with blood pressure readings every two minutes. The blood pressure is obtained by the Sentech Tango 2 automated unit. This blood pressure unit is designed for exercise testing and has been tested as a valid and reliable system to measure blood pressure during exercise. When the subject reaches the endpoint goal of the exercise test, the mouthpiece and head gear will be removed and they will undergo a four minute active cool-down in which they will walk at a slower rate with zero incline grade. After these four minutes the participant will be helped off of the treadmill and undergo a passive cool-down session in which they are seated in a chair. The participant's heart rate and blood pressure will be continuously monitored through the cool-down period and the participant will not be allowed to leave until the vital signs return to normal patterns. Blood pressure will also be monitored both before and after the fitness test to ensure that changes in blood pressure resulting from exercise are all normal. If the participants vital signs remain elevated after a cool down and rest period, the study physician will be called and will provide instructions for further monitoring of the participant. Situations like this are very rare and occur in less than 1/10,000 instances of fitness testing. These assessments will be conducted in Dr. Erickson’s laboratory by an exercise physiologist with >15 years of coaching and exercise testing who is AED and Recertified and has certifications in exercise physiology from the American College of Sports Medicine. Prior to engaging in exercise, participants may be fitted with one or two physical activity monitoring devices (Body Media Sense Wear mini armband or Autographing). The Body Media Sense wear Armband is worn around the upper left arm. Hectograph Link is a wristband worn on the non-dominant arm. Both devices will record physiological data (e.g., heat flux, sweat rate, etc.) over the duration of their exercise test; this information is used in an algorithm to determine intensity and amount of physical activity. The participants will be provided with detailed instructions regarding wearing of the device(s) as well as the option to remove the device(s) if it becomes problematic. Participants may also be asked to wear the physical activity monitoring device(s) for a week before or after the exercise testing session. This way we can get a more comprehensive idea of both activity and fitness levels. In addition, all participants, regardless of group assignment, will be asked to wear the device(s) 3 additional times through their participation in this study(approximately every 5 weeks) and again at the follow-up assessment (TP2) for total of 5 times over the course of participation in the study. Participants who wear the Actigraphy Link will be given a log at the time they receive their device. This log is used to report any time/reason the device is removed as well as information about their sleep. The self-report information contained in the log will be compared to and used in interpreting the device data. Alternatively, in the event that a participant cannot come to Sennott Square for this testing for any reason (e.g., preference, weather, pandemic-related restrictions.), a brief heart-rate monitoring session may take place virtually either by phone or teleconferencing. During this guided session, the participant will be asked to wear study-provided heart rate monitor for approximately 5 minutes and asked to report readings to study staff. Participants will also be asked to report their weight; home scale may provided for participants to use (with instructions to return via pre-paid mailer when complete). Data from the session can provide information to estimate results from a VO2 test. Participants may also be asked to complete questionnaires regarding exercise knowledge and self-efficacy during the physical activity and fitness assessment, time permitting. Alternatively, these may be included in the behavioral assessment. The behavioral assessment includes a battery of standard clinical neuropsychological tests that assess verbal ability, visuospatial ability, attention/processing speed, memory, and executive function, will be administered. These tests have standard administration protocols and will be administered by trained staff. This is expected to take approximately 90 minutes. We will also administer measures of demographics, mood and quality of life. The tasks and measures will include those listed in section 2.8. All of the tests will be identified by the subject ID and not by name. At times these tests can be mentally fatiguing or frustrating. Subjects will be told that the tests are designed to be challenging and that they should just perform to the best of their abilities. They will also be offered breaks as needed between tests to help reduce fatigue and frustration. Data collected will be transported by the research personnel immediately following the assessment if conducted in the participant's home to the office of the PI in the

Department of Nursing for locked storage. Alternatively, neuropsychological tests may be administered virtually via teleconferencing or phone as needed. Other measures may be administered via Redcap or by phone or teleconferencing as needed in the event that the participant cannot be seen in-person. Participants' location data (e.g., zip code or census tract) may be used to gather information from publicly availably datasets to provide information about community/neighborhood characteristics/social determinants of health (such as deprivation, safety, degree of segregation).

Physical Activity Diary: All participants will be asked to provide information related to their physical activity throughout the study. Participants will be offered the option of a paper diary(uploaded in section 2.8) or to complete a weekly electronic mail survey administered via REDCAP.

eDEM Cap: All participants will be provided with the electronic Drug Exposure Monitor (Edema) to measure adherence to hormonal therapy. The Eden consists of a bottle cap fitted with a microprocessor that records the date and time the cap is removed from a medication vial. It also records the duration of time that the cap is off the vial. Eachmonitor has sufficient memory to record and store the dates and times of 2000doses. The Eden will be collected at the time of the follow-up assessment. Neuroimaging** No new MRI participants will be consented after February 2020 ** A subgroup(N=150) will have the following neuroimaging protocol at times 1 and 2. Each session will last approximately 1 hour. Seventy-five women from each group will be randomly sampled to participate in neuroimaging based on age and baseline executive function. On the day of a participant's scan, she will be asked to arrive 30minutes before the start of her scan in order to provide time to review and sign a separate CMU consent form, and to undergo final safety screening by a SIBR MRI technologist. For the sake of brevity, MRI sequence parameters have been omitted but can be found in our prior publications.68,75,78,132,133 Brain Morphology: High-resolution anatomical MPRAGE (1mm3 voxels, 256 slices) images will be used for morphologic and volumetric analyses. The Free Surfer pipeline using its longitudinal option will be employed to compute cortical and subcortical volumes, cortical surface area, and thickness. Intracranial volume (ICV) will be used as a covariate in analysis of brain morphology. Regional values that can be exported into statistical packages (e.g., SAS) will be calculated. Errors and omissions will be corrected according to standard guidelines. Hippocampal subfields: We will use a focal (.4 x .5x 2mm) T2-weighted sequence for hippocampal subfield segmentation146. Since the dentate gyrus and CA1 subfields are more frequently affected byexercise147,148 and the head of the hippocampus may be affected more than thetail,68 this will allow us to segment hippocampal subfields to determine regional specificity of the intervention. White matter imaging will be performed using diffusion-weighted sequence (50 directions; 2.5mm isotropic voxels). Images will be corrected for head motion and eddy current artifacts. For each voxel the first three principle eigenvectors of water diffusion will be calculated using FSL, which will be used to calculate fractional anisotropy (FA), axial and radial diffusivity components. These maps will be transformed into standard space using non-linear normalization. We will also conduct fiber tractography, which identifies tracts between regions and will use computational methods to dissociate crossing white matter fibers withinvoxels.149 Finally, white matter lesions (see next section) will be removed from the calculation of white matter integrity. White matter hyperintensities (WMH): We will use a T2 FLAIR sequence and an automated segmentation method, which uses an algorithm for automated selection of WMH lesions. The segmented WMH are then localized to white matter tracts using the Johns Hopkins White Matter Atlas. Resting state analyses: We will employ standard BOLD sequences used by PI: Erickson and graph theory approaches. Prior to generating brain networks (see150), all images will be motion corrected and registered. To control noise,151 data will be preprocessed to remove white matter, and cerebrospinal fluid signal. Motion scrubbing will be performed.152 Heart rate will be used to correct for physiological confounds. The time series from each node will be compared to every other node using regression analysis, generating a correlation matrix. A threshold is applied to the matrix to retain only the strong connections and is maintained to represent connection strength.153 Once an adjacency matrix is generated, network attributes can be calculated for each node. The data will allow for the identification of changes in network topology that are associated with the intervention. Cerebral Blood Flow(CBF) will be measured with multi-phase pseudo Continuous Arterial Spin Labeling(pals) with background suppression and will be analyzed using BASIL. Task-evoked activation: We selected the n-back working memory paradigm because our preliminary data demonstrates that walking interventions improve performance on this task.154 The task also has high reliability and involves a broad network of brain regions including the PFC, ACC, hippocampus, and basal ganglia.155 Participants determine whether a lowercase letter

matches the letter on the previous trial (1-back) or the letter two trials previously (2-back). We will employ FSL software with standard preprocessing steps including motion correction, temporal filtering, and spatial smoothing and a sample-specific template for registration.75,156,157 We will also use Psychophysiological Interaction (PPI) analyses to examine changes in functional connectivity between regions during task performance.

Randomization: After completing the baseline assessments, subjects may be randomized into one of two groups: (1) Aerobic Exercise Intervention or (2) Usual Care. Subjects will be randomly assigned via a computer using a minimization algorithm with equal allocation to one of two treatment groups: 1) exercise intervention, or 2) usual care. Use of a minimization algorithm will insure treatment balance on two three factors:1) age at study entry (<60, >60 to <70, >70 years) (since age is associated with reduced fitness, cognition, and brain measures), and 2) baseline levels of cognitive function, particularly executive function (based on the composite mean Z-score, <-0.5, > -0.5 to < +0.5, > +0.5), and 3) the time since primary cancer treatment (<= 6months, >6 months to 12 months). Minimization is a form of adaptive treatment allocation in which the probability of assignment to the treatment groups does not remain constant, but is determined by the current balance and/or composition of groups. In this study, the treatment assignment probability will be adjusted in response to the marginal distributions of categories of age at entry and baseline executive function such that the marginal distributions of these categories will be approximately equal between treatment groups. Treatment group assignment will be generated and stored in a secured database. Aerobic

Exercise Intervention Group: An exercise physiologist with a Master's degree in Exercise Science with experience in the proper execution and delivery of the exercise intervention in normal and at risk population while maintaining a safe environment will be the staff member responsible for the training of other staff to deliver the exercise intervention at each site. This individual will also be responsible for developing the exercise protocol and will manage the safety and consistency of the intervention across sites. Exercise staff will have completed a degree in Exercise Science or related field (e.g., kinesiology) and / or have a fitness certification from a reputable organization. All staff will have a minimum of 2 years experience in exercise supervision and prescription. The exercise intervention will be aerobic in nature and performed at a moderate intensity as monitored by heart rate monitors and ratings of perceived exertion(RPE); both will be monitored on-site by a trained member of the research team. Trained study staff will be present at each exercise session at all intervention sites. Occasionally, participants may have heart rate abnormalities which can make it difficult to obtain accurate readings via a heart rate monitor. In these situations, exercise staff will employ additional strategies to monitor exercise intensity as described below:1. Rating of perceived exertion (RPE) – this scale is used for all study participants. Itis a scale that runs 6 (no intensity) to 20 (maximal intensity). It is asked of participants and tracked every 15 minutes during exercise.2. Rating scale of breathlessness – this scale will only be used for the rare case of compromised heart rate response. This scale runs 0.5 (very, very slight breathlessness) to 10 (very, very severe out of breath). If a participant rates 4 and above, the intensity will be decreased.3. Talk Test – this will be used by exercise trainers at every session. For those with heart rate issues, the exercise trainer will proceed through the entire session with ahigh level of attention focused on this procedure. While the trainer is in conversation with the participant, the trainer will be attentive to the level of gasping for breath while talking by the participant. If the participant is struggling to hold or continue a conversation due to the level of work, the intensity will be decreased. Walking on a motorized treadmill will be the encouraged mode of exercise; however participants may use other equipment such as upright bikes, recumbent bikes, ellipticals and indoor walking track, etc. Participants will begin by exercising 10-15 minutes for 3 days/week during the first two weeks of the program and gradually increase the duration for the following 4weeks until they reach 40–50 minutes per session/3 days per week. This level is then maintained for the remainder of the 6 months. This falls within the American College of Sports Medicine (ACSM) guidelines for cancer patients and survivors.106These guidelines (2010) recommend a weekly volume of 150 minutes of moderate intensity or 75 minutes of vigorous intensity exercise, or a combination of thetwo.117 The rate of increase will be tailored based on each subject's baseline cardiorespiratory fitness level and response to exercise. All exercise sessions start and end with a vital sign check and 5-10 minutes of stretching for the purpose of warming up and cooling down. Staff trained in exercise physiology and coaching will supervise all exercise sessions and closely monitor adherence, intensity, and safety. In-

person exercise, as described above, will be the preferred method for intervention delivery. However, participants may also exercise at their home, or other location of their choosing, as needed. When exercising at home, participants will be in contact with a study trainer at least weekly. Participants will receive guidance and coaching related to their exercise sessions - either in real-time or other time as to be accommodating as possible to the research participants. Heart rate during exercise sessions will be monitored via study-provided monitors and will be reported to trainers by the participant. Participants may be provided with online resources/videos for exercise (such as Be Fit Pitt) or may be encouraged to use an exercise resources available to them (such as owned exercise equipment, gym memberships, indoor or outdoor walking, etc.)

Usual Care Group: will not withhold physical activity or limit physical activity in the usual care group but instead will let them engage in activity in the same manner as if they were not part of an active intervention.

Statistical Plan

Randomization: Subjects will be randomly assigned via a computer using a minimization algorithm with equal allocation to one of two treatment groups: 1) exercise intervention, or 2)usual care. Use of a minimization algorithm will insure treatment balance on two factors: 1) age at study entry (<60, >60 to <70, >70 years) (since age is associated with reduced fitness, cognition, and brain measures), and 2) baseline levels of cognitive function, particularly executive function (based on the composite mean Z-score, < -0.5, > -0.5 to < +0.5, > +0.5). Minimization is a form of adaptive treatment allocation in which the probability of assignment to the treatment groups does not remain constant but is determined by the current balance and/or composition of groups. In this study, the treatment assignment probability will be adjusted in response to the marginal distributions of categories of age at entry and baseline executive function such that the marginal distributions of these categories will be approximately equal between treatment groups. Treatment group assignment will be generated and stored in a secured database.

Data analysis: Preliminary Analyses. Exploratory analyses will be performed for data description and screening for anomalies (e.g., outliers, nonnormality). This will be done to: 1)describe univariate and bivariate distributions; 2) identify group imbalances and associations between dependent variables and suspected covariates/ confounders;3) evaluate missing data; and 4) check for violation of statistical assumptions. If assumptions are violated, data transformations or more statistically robust procedures will be considered. Covariates/ confounders will be included in models secondarily and their effects on primary predictors/factors will be evaluated. The randomness of missing data will be investigated using available information on subject characteristics to help discern patterns in missing data, identify possible missing data mechanisms and inform strategies to handle missing data. If the data are ignorable missing, the estimation procedures to be used will produce unbiased estimates while allowing us to retain observations with missing values on the outcome variables. If needed, multiple imputation would be used to impute missing values on covariates. If the data are not ignorable missing, we may use selection or pattern mixture modeling to investigate the sensitivity of results.

Analysis Plan for Aim 1. An "intent-to-treat" (ITT) approach will be used for data analysis. All subjects will be included in the groups to which they were assigned, regardless of adherence to protocol, treatment received, withdrawal or protocol deviation. Although this approach is recommended for efficacy analyses in Retaste sensitivity of the results assuming ITT will be explored using information collected regarding participant’s adherence to the exercise intervention (e.g., protocol, amount of treatment received)) as well as the amount of activity engaged in by the usual care group. The primary endpoint, changes in cognitive function, will be derived from normed(for age, education) composites of neuropsychological test scores (expressed as mean Z-scores).1 (Table 2) The composite scores for the targeted cognitive function domains are empirically derived via exploratory factor analysis. Given the variation in scaling, test scores are converted to Z-scores related to an established cohort of age- and education-matched healthy women, and then averaged into composites, where negative scores indicate poorer cognitive function. Linear mixed-effects modeling with linear contrasts will be used to examine the effect of treatment assignment on cognitive function over time. When fitting models, time will be repeated within-subjects

factor, while treatment assignment will be a between-subjects factor, with an interaction between time and group. Each derived cognitive domain composite will be modeled separately and interaction terms will be included to test our hypothesis that executive, memory, and attention domains will be more affected than other domains. We will consider baseline cognitive function values both as an assessment point and as a possible covariate in analyses. Fixed and/or time-dependent covariates may be included to adjust for group imbalances or variables related to the dependent variables. Standard fit indices will also be used to identify the most appropriate covariance structure. F-tests will test the main and interaction effects included in the model. Individual regression parameters will be computed and reported with standard errors to yield confidence intervals. For each model, residual analysis will be conducted to identify sources of model misspecification, outliers and influential observations. Sensitivity analyses will be performed to discern the impact of influential cases on results. To test specific hypotheses, linear contrasts will be specified and estimated in the repeated measures model to compare changes from follow-up to baseline values between groups. Wald t-statistics will be used to test each contrast. Marginal modeling with generalized estimating equations will also be used as it tends to be more robust to misspecification of the covariance structure and violations in normality assumptions. Results from each modeling approach will be compared via sensitivity analyses.

Analysis Plan for Aim 2. We will use the same analytical approach described for Aim1 to fit models for intermediate outcomes (neuroimaging metrics of brain heath, inflammatory markers, and symptoms) and to test Aim 2 hypotheses regarding the effects of exercise on primary intermediate outcomes (neuroimaging metrics and inflammatory markers of IL-6 and CRP). The only difference will be that initial neuroimaging analyses will be conducted on a voxel-wise basis using FSL, SPM, treasurer, depending on the imaging modality and analysis (e.g., volumetric). In addition to voxel-wise analyses we may apply regions-of-interest (ROI) analyses since our primary hypotheses focus on prefrontal and hippocampal regions. Thesaurus can be generated using anatomical templates from normalized MNI space or from functionally-derived estimates of activation or connectivity. We will use standard cluster-based corrections for multiple comparisons of fMRI results, threshold-free cluster enhancement, and an FDR of $p<.01$ for volumetric and white matter analyses.

Analysis Plan for Aim 3. We will explore neuroimaging metrics of brain health, biomarkers, and symptoms as variables that may mediate the effects of the exercise intervention on cognitive function and E2 as a possible moderator. Initially, we will fit simple mediational models (predictor, single mediator, single outcome) applying the change score method and estimate whether effects of exercise (predictor) on changes in cognitive function outcomes are mediated through changes in the suspected mediators. Goodness-of-fit will be assessed through standard summary indices (e.g., RMSEA, CFI) and residual analyses. Total, indirect and direct effects will be estimated and reported with confidence intervals. Additionally, we will explore mediation applying the half-longitudinal approach proposed by Cole andMaxwell_ENREF_148 as well as an autoregressive mediational approach. Depending on the results from simple mediational modeling, we may also combine mediators into a multiple or serial mediator models to develop a more comprehensive picture of these pathways. As stated previously, voxel-based mediation of neuroimaging results will be conducted using BRAVO by employing the same approach described above. To explore E2 as a moderator of exercise effects on cognitive function, the models developed in Aim 1 will be expanded to also include E2 as main effect as well as interactions of E2 with the other fixed effects. Exploratory analysis. We will explore whether physical activity diary data are related to objective physical activity data. If validated, we will explore the physical activity patterns over 6 months and its relationship to change in outcomes.