Protocol

1. Aerobic Exercise and Cognitive Training in Older Adults

(Short title: Nocera Exercise)

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2. Abstract:

The purpose of this study is to examine the impact of a combined cognitive training and aerobic exercise intervention in sedentary older adults. It is hypothesized that the aerobic exercise will potentiate and increase the generalizability of the cognitive training. Importantly, this study will focus on older adults at-risk for mobility disability. This area is of particular importance considering a large percentage of adults are entering old age and therefore likely to suffer from age-related cognitive decline and mobility disability.

To address our research question 60 adults (age 18-89) will be randomized to one of two 12 week intervention groups: 1) Cognitive Training alone (CT) or 2) Aerobic Exercise + Cognitive Training (AE+CT). The aerobic exercise arm of the study will follow the same format shown to improve a broad range of executive functions in older adults in previous research. The cognitive training arm will consists of a popular commercially-available brain fitness program that has demonstrated specific cognitive improvements and high adherence.

Outcome measures will include changes in the battery of executive function tests which target verbal fluency, response inhibition, and working memory. Additionally, to strengthen our methodology, we will examine tasks that are not expected to change because of their higher reliance on peripheral memory systems or crystallized word knowledge. We will also examine the effects of AE+CT versus CT alone on changes in the hemodynamic response during two fMRI tasks: verbal fluency and verbal working memory. Finally, we will examine the effects of AE+CT versus CT alone on changes in mobility and physical function. To examine the effects of the interventions on mobility we will examine walking speed as our primary outcome.

3. Introduction and Background:

Significantly, declining mental health and mobility disability are primary components of the expected 25 percent growth in health care cost related to the aging crisis in America

[1]. Although, combining aerobic exercise and cognitive training may significantly ease these inevitable features of aging, these findings need to be objectively quantified. Interestingly, in 2002 Fabre and colleagues demonstrated that combining cognitive and exercise training seemed to result in greater effects on cognitive function than either technique alone [2]. However, the Fabre study did not focus on executive functions which are an excellent proxy for cognitive health in older adults [3]. Further, no study to date has examined the significant outcomes of the proposed study in sedentary older adults at risk for mobility disability. Considering that many older adults are sedentary and at risk for functional decline targeted interventions are desperately needed. In the following background sections, we will establish a case for combining aerobic exercise and cognitive training as a potential intervention for executive function impairments in older adults.

Cognitive Impairment and Executive Functions. People over the age of 65 frequently have difficulty thinking abstractly as well as problems with planning, initiating, sequencing, monitoring and stopping complex behaviors. As such, the ability to carry out essential tasks of daily living are compromised. These difficulties are commonly attributed to executive dysfunction demonstrated in aging. In the context of this proposal, executive functions are frontally mediated cognitive functions which require conscious intentional control and include; verbal fluency, inhibition, and working memory. For example, generating words from a category (verbal fluency) requires an individual to initiate a search of words stores while inhibiting competing resources. Further, working memory requires temporarily storing and managing information required to carry out the fluency task.

Cognitive Improvements as a Result of Exercise. Colcombe and colleagues [4] cross-sectionally studied the effects of cardiovascular fitness levels on cortical density in aging humans. They examined the density of gray and white matter in adults ages 55-79 and related those measures to the cardiovascular fitness levels of the participants. Results demonstrated that older adults with greater levels of cardiovascular fitness had significantly less atrophy of gray matter in the frontal cortex as well as significantly less loss of tissues in both the anterior and posterior white matter tracts.

Evidence from animal models has demonstrated several possible mechanisms that may underlie the ameliorative effects of aerobic exercise on cortical structure in aging. For example, in rats exercise has been shown to increase brain neurotrophins such as brain-derived neurotrophic factor, [5] and nerve growth factor. Also, physical activity has been shown to have neuroprotective effects on the brain, leading to neurogenesis in the hippocampus, new cell growth and protection from ischemic damage within the hippocampal formation. Additionally, chronic exercise is associated with growth of blood vessels (angiogenesis) and synaptogenesis in cortex [6]. Collectively, these transformations result in a brain that is more efficient and perhaps more receptive to neuroplastic changes resulting in increased performance and learning.

As indicated, both the animal model and cross sectional humans studies of brain morphology suggested that aerobic activity positively influences cortical structure with

the greatest neuroprotective effects localized to the frontal and prefrontal areas of the human brain [4]. Significantly, several randomized-controlled clinical trials have produced findings consistent with these theorems. For example, research demonstrated that older adults who participated in 6 months of aerobic fitness training significantly increased brain volume in both gray and white matter. Further, no such change was demonstrated for the older adults who participated in stretching or toning (non-aerobic) exercise [7]. These findings are meaningful because frontal areas typically demonstrate the greatest age-related decline.

Equally important, the demonstrated enhancement in frontal integrity highly correlated with the behavioral research demonstrating that aerobic fitness has the greatest impact on frontally-mediated executive functions. For example, the seminal work by Kramer and colleagues demonstrated that 124 older adults randomly assigned to receive aerobic training experienced substantial improvement in performance tasks dependent on executive control processes and the integrity of the prefrontal and frontal cortex [8]. Similarly, neuroimaging studies showed that aerobically trained individuals increased functioning of key aspects of the attentional circuitry during cognitive tasks. More specially, individuals who participated in an aerobic exercise intervention showed greater attention on task-related activity in the prefrontal area (middle frontal gyrus and the superior frontal gyrus) of the cortex when compared to non-exercise controls [9].

Although studies examining the effect of aerobic exercise on cognition have been remarkable, other studies have failed to observe such a relationship. The explanations for these differential findings may be described as followed. First, the manner in which cardiovascular fitness was assessed has differed between studies (e.g. resting heart rate versus the benchmark VO_2 max). Secondly, the cognitive processes examined have differed between studies. For example, cognitive tasks have been evaluated that fall outside the capacity of the frontal cortex previously demonstrated to improve as a result of exercise. And lastly, the age, health, sex and fitness level of the participants have differed between studies. Despite these alternative findings, collectively research demonstrates the relationship between aerobic fitness and cognitive health is encouraging with three key findings. To summarize, first, aerobic exercise can protect the brain from age-related atrophy. Secondly, aerobic interventions increase activity in frontal structures during tasks requiring executive control. Lastly, and equally important, these structural and functional improvements are consistent with behavioral studies demonstrating improvements in executive control processes reliant on the frontal cortex.

Cognitive Training in Older Adults. A growing body of research demonstrates the beneficial effects of cognitive stimulation in later life to promote neural plasticity. Research from both the human and animal models demonstrates that environmental stimulation can enhance and maintain cognitive functioning. Similarly, cognitive remediation has been found to positively impact neural structure while limited education is a predictor of dementia. Importantly, there is a sizable body of research supporting various cognitive training paradigms that have demonstrated large and durable effects on cognitive functioning in older adults. For example, Ball and colleagues demonstrated cognitive interventions targeting memory, reasoning and speed of processing were

effective to the magnitude equivalent to the amount of decline expected in older persons without dementia over a 7-14 year interval.

Unfortunately, despite these promising findings, the generalizability of cognitive training is limited. For example, Owens and colleagues recently examined cognitive training tasks designed to improve reasoning, memory, planning, visuospatial skills and attention in over 11,000 participants. Interestingly, the researchers found improvements in every one of the observed cognitive tasks trained, however, “no evidence was found for transfer effects to untrained tasks, even when the tasks were cognitively closely related” [10].

While cognitive training does appear to impact specific trained cognitive functions **strategies are needed to potentiate and increase generalizability of the outcomes**. Interestingly, a recent article in the October 2010 edition of *Gerontology* [11] suggested the need to combine both exercise and cognitive-focused intervention to “enhance improvements in functional independence over and above the interventions being used separately.” While the cognitive benefits of cognitive training has been demonstrated, it cannot be assumed that aerobic exercise will potentiate the effect. Unfortunately, studies have yet to adequately investigate the effect of cognitive training used in conjunction with aerobic exercise in older adults. **This proposal specifically addresses this gap in the literature.**

The Executive Function and Mobility Link. Traditionally, walking has been thought of as an automatic motor function with little higher mental input. However, walking and mobility are becoming increasing linked to cognitive functioning, specifically, executive functions. For example, gait impairment and executive function problems are common in aging and often coincide [12]. Further, having a gait disorder increases the chance of developing non-Alzheimer dementia threefold [13]. Lastly, older adults with poor executive functions walk slower, have increased gait variability, fall more often, and perform poorly on complex mobility tasks such as rising from a chair and dual tasking.

Significantly, pharmacological and cognitive training that targets the executive functions have been demonstrated to improve gait [14]. For example, utilizing a randomized single-blind controlled design, Verghese and colleagues studied cognitive training to improve gait velocity in 24 sedentary older adults. The cognitive training utilized a computer-based program which focused on training attention, visual spatial skills and executive functions. Of significance, participants randomized to cognitive training improved their gait velocity from baseline during a normal walking condition and on a walking while talking condition. It was concluded that improvement in walking speed was due to the cognitive training modules that targeted tasks relevant to gait (e.g. executive functions) [13].

Neuroimaging and Aerobic Exercise. To date the neural mechanisms underlying the improvements associated with cardiovascular fitness and aerobic exercise in aging adults has not been well studied in human populations [9]. However, recently Colcombe and colleagues demonstrated, both cross-sectionally and longitudinally following an

aerobic intervention, that increased cardiovascular fitness resulted in increased task-related activation in the frontal attentional circuitry thought to be necessary for executive functions [9]. For example, after an aerobic exercise intervention older adults showed significantly greater level of activity in both the medial frontal gyrus and superior frontal gyrus during a version of the flanker task than non-exercising controls. Successful completion of this task requires participants to invoke selective spatial attention through the frontal circuitry, which in turn should bias regions of visual cortex to isolate the central (target) cue, and inhibit the peripheral flanking cues. This is one of the few studies that has attempted to identify specific improvements in the plasticity of the aging human brain following aerobic exercise.

Work from the Co-PI's lab has consistently demonstrated that during verbal fluency tasks, older adults show a bilateral pattern of recruitment in the frontal cortex when compared to the strongly left lateralized activity pattern of young adults. Consistent with our pilot data (see preliminary studies), we hypothesize that aerobic exercise will result in a "younger looking" pattern of lateralization in older adults during a verbal fluency task.

4. Objectives:

Cognitive decline is a significant health concern among older adults, with limited treatment options [15]. Cognitive decline greatly hinders quality of life as it often coincides with mobility disability [16]. Despite growing fiscal resources, cognitive decline and mobility disability rates continue to rise in older adults. As such, interventions that can target these vital aspects of independence are desperately needed. Recent studies have shown that computer-based cognitive training strategies may improve performance on cognitive measures but the generalizability of the benefit is lacking [10]. Interestingly previous research demonstrates that aerobic exercise can improve cognitive performance in older adults in an array of age-susceptible frontally-mediated cognitive-executive functions [4]. Importantly, these outcomes are in addition to the robust, well documented benefits of physical activity. While it is plausible that aerobic exercise can potentiate and increase generalizability of cognitive training while concurrently benefiting mobility, this intervention approach has not been adequately studied in sedentary older adults at-risk for mobility disability.

Therefore, the **short term objective** of this proposal is to take the next logical step and identify the combined/synergists effects of cognitive training in conjunction with aerobic exercise on executive functions and mobility in older adults at risk for mobility disability. To address this research question 60 adults (age 18-89) will be randomized to one of two 12 week intervention groups: 1) Cognitive Training alone (CT) or 2) Aerobic Exercise + Cognitive Training (AE+CT). The aerobic exercise arm of the study will follow the same format shown to improve a broad range of executive functions in older adults in previous research [7, 17]. The cognitive training arm will consists of a popular commercially-available brain fitness program that has demonstrated specific cognitive

improvements and high adherence [18, 19]. Groups will be equalized for contact and monitoring. The following specific aims and hypotheses are proposed:

1. To assess the relative effect of the interventions on frontally-mediated executive function tasks.

Consistent with the demonstrated executive function improvement following aerobic exercise we hypothesize that compared to CT alone, those randomized to AE+CT will demonstrate greater improvements on frontally-mediated cognitive tasks.

2. To assess the relative effect of the interventions on changes in brain activity (assessed through functional MRI) and blood derived neurotrophic factor (BDNF)..

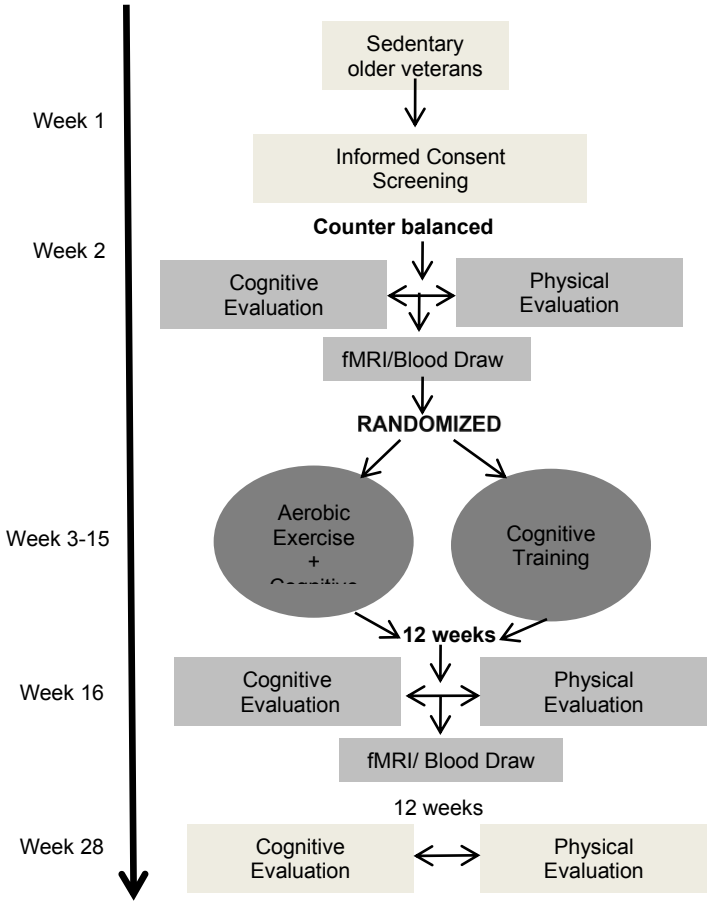
Consistent with our pilot data, we hypothesize more unilateral (left) pattern of frontal recruitment in the AE+CT group, suggesting a more efficient recruitment of the frontal cortex. Additionally we hypothesize an increase in BDNF in the AE+CT group.

3. To assess the relative effect of the interventions on changes in mobility (walking speed) and perceived health status (self-reported physical function and quality of life).

We hypothesize that compared to CT alone, those randomized to AE+CT will demonstrate greater improvements on mobility and physical function.

5. Study design and methods:

The study will enroll between 60 and 80 older, sedentary adults. Participants will be randomly assigned to one of two 12-week interventions conditions: AE+CT or CT alone.



Specific Aim 1: To assess the relative effect of the interventions on frontally-mediated executive function tasks.

The primary outcome will be changes in the battery of executive function tests which target verbal fluency, response inhibition, and working memory. Additionally, to strengthen our methodology, we will examine tasks that are not expected to change because of their higher reliance on peripheral memory systems or crystallized word knowledge. The following experimental measures will be given in the standard manner, using different stimuli when possible in each of the conditions (i.e. pre, post-intervention, 12 week follow-up). The order of stimulus lists will be counterbalanced across participants. We will also look at retention of benefits as all participants will be followed-up 12 weeks after completion of the interventions.

Measures for Specific Aim 1.

Verbal Fluency

1. *Letter Verbal Fluency*: Participants produce as many words (F, A, S, or P, R, W) as they can that begin with that letter in 60 seconds. Clinical findings are in line with numerous functional magnetic resonance imaging studies, demonstrating that letter verbal fluency is associated with extensive activation in the left frontal cortex. Further, age-related decline in letter fluency have been demonstrated .

2. *Semantic Verbal Fluency:* Participants produce as many words as they can that fit a particular category (animals or fruits/vegetables) in 60 seconds. This is generally considered a measure of a verbal component of executive function and has been demonstrated to decline with age.

3. *Switching Verbal Fluency:* The switching condition evaluates the examinee's ability to generate exemplars while alternating between two different semantic categories

(i.e. fruits and furniture) in 60 seconds. Switching requires the ability to engage in strategic search processes such as initiation, cognitive flexibility and mental set shifting. Because it is related to fronto-executive functioning, impaired performance is seen among patients with frontal lobe lesions and older adults.

Response Inhibition

1. *Color-word Interference Test*: Participants say the color in which another color word is printed in (e.g., for BLUE printed in red ink, the answer is 'red'). This is a commonly-used executive function task. Additionally, the interference effect, caused by difficulty inhibiting over-learned word reading, is often more pronounced in older adults.

Working Memory

1. *N-back task:* Both the verbal and visual working memory tasks will be evaluated in the versions of the n-back task. Versions of n-back tasks have been shown to be sensitive to working memory. During the n-back tasks, verbal or visual stimuli are shown one at a time using a laptop computer. For each stimulus, participants click the left mouse button of the computer if the stimulus is a target and the right mouse button if the stimulus is a non-target. Targets are stimuli that occur n stimuli previously (i.e., n stimuli back). For each task, 1-back, 2-back, and 3-back runs are given. Each run has 100 stimulus presentations, with 20 targets and 80 non-targets. Stimuli are presented for 500 ms with inter-stimulus intervals of 3000 ms. A 60 sec break occurs after the first 50 stimuli of each run. Both accuracy and latency of response are recorded. Verbal and visual stimuli are as follows: (1) Verbal stimuli are common, highly imageable English words 4 to 6 letters in length. 3 stimuli are chosen from each of 3 categories (animals, tools, vehicles). Targets are trials in which an animal name is presented when an animal name is presented *n* stimuli back (e.g., in the following 3-back sequence, the target is in bold: "dog, horse, hammer, **cat**, car"). (2) The visual task are 9 non-sense shapes. Targets are any trial on which a shape is presented when the identical shape is presented n stimuli back.

2. *Digit Span forward/backward*: Participants must recall increasingly long strings of digits in order (forward) or reverse order (backwards) of presentation. Central executive component of working memory has been demonstrated to play a key role in digit backward span performance and age-related decline has been reported.

3. Hopkins Verbal Learning Test- Assess verbal learning and memory (immediate recall, delayed recall, delayed recognition).

4. Digit Symbol-Coding- a neuropsychological test sensitive to brain damage, dementia, age and depression. It consists of digit-symbol pairs followed by a list of digits. Under each digit the subject should write down the corresponding symbol as fast as possible. The number of correct symbols within the allowed time (e.g. 90 or 120 sec) is measured.

Non-Verbal Executive Function

1. *Sorting Test:* Participant must sort cards on one of three possible dimensions, examines problem solving and spatial concept formation.

2. *Trail Making Test A and B:* Easily administered tests measure attention, visual searching, mental processing speed, and the ability to mentally control simultaneous stimulus patterns.

Further, to measure if the AE+CT group demonstrated greater generalizability than the CT alone group we will utilize the cognitive evaluation that is part of the MindFit software. The MindFit will be utilize for the cognitive training arm of this study and is further described in the "Interventions" section of the methods. The Mindfit cognitive evaluation was designed to evaluate cognitive functioning in older adults and describe progress rates of participants utilizing the software. It is anticipated that the CT training group will only improve on measures specific to the MindFit cognitive evaluation. In contrast, those randomized to the AE+CT will have improvements on executive function tests not specific to the MindFit cognitive training program. Test within the MindFit cognitive evaluation include (as described in the software):

1. Naming: This is a naming task that involves visual information processing and matching objects, words, and letters in a fast-response condition. The software measures accuracy percentage of correct responses and response time.

2. Psychomotor skills (i.e., tracking): In this test, the user is asked to track a moving ball with the computer mouse cursor. This task requires eye-hand coordination, spatial perception, and focused attention. The software measures the accuracy of the tracking—the percentage of time that the computer cursor is in the middle of the ball.

3. Sustained attention in tasks involving allocated attention to a target—with and without distractor: In this test, the user is asked to click on a ball appearing in different locations on the screen. When a (distracting) yellow hoop also appears, the user is instructed to ignore the hoop and continue clicking on the ball. The software measures the accuracy—percentage of correct clicks and response time.

4. Time estimation, both auditory and visual: In this test, pictures are presented, sounds are turned on, and the task is to estimate the length of each stimulus presentation. The software measures deviation (under- and overestimation of time).

5. Maze test—planning is tested in a maze task: In this test, the users must make their way through three mazes, one after another. The level of complexity increases with each subsequent maze. The software measures the number of errors made per maze and execution time (the time to complete each maze).

All cognitive testing will take place in the primary laboratory of the PI/Co-PI at the Atlanta VA Medical Center. All evaluations will be done by the PI or appropriate study staff. Additionally to evaluate efficacy of interventions participants will self report on:

1. Mac-Q- 6 item questionnaire to asking participants to describe their ability to perform memory task as compared to when they were in high school.
2. Behavior Rating Inventory of Executive Function (BRIEF)- Assesses self reported impairment of executive function task related to activities of daily living.
3. Self Efficacy for exercise scale - a 13-item instrument that measures self-efficacy barriers to exercise,
4. Epworth Sleepiness Scale- self report used to determine the level of daytime sleepiness.
5. Apathy Index (MAI)- 14 item self report instrument that measures overall apathy and participants interest in new things.
6. Pittsburgh Sleep Quality Index- 10 item questionnaire examining participants sleep habits over the past month.

The final neurocognitive assessment test is the the Visual Paired Comparison [VPC] task. This task was developed at Emory and has already been used for studies approved through the Emory IRB (IRB#00001272). This testing will take place at Wesley Woods, where previous testing with the VPC task has taken place, on the same day that subjects undergo their MRI. Thus, subjects will not have to undergo a separate visit to complete this testing.

Task: The VPC task does not require any significant computer skills, thus the task should not pose operational problems for patient groups or the control subjects. Eye-tracking data will be gathered concurrently with the administration of the behavioral task. The eye-tracking apparatus and procedures are described below.

The VPC Task: Subjects view a new picture and a recently presented picture side-by-side on the computer monitor and the subject's spontaneous tendency to look at the new picture is measured. Eye movements will be recorded while subjects view pairs of black and white images. Each trial of this task consists of two phases, a familiarization phase and a test phase. Familiarization phase. In this phase the subject is presented with two identical black and white line drawings, side by side on the computer screen. After a delay interval (2 sec, or 2 min) during which the screen is blank, the test phase begins. Test phase. After the delay, subjects are presented with a picture of the old stimulus and a picture of the novel stimulus, side by side. This task requires about 30 minutes.

Specific Aim 2: To assess the relative effect of the interventions on changes in brain activity (assessed through functional MRI), visual outcomes, and blood derived neurotrophic factor (BDNF).

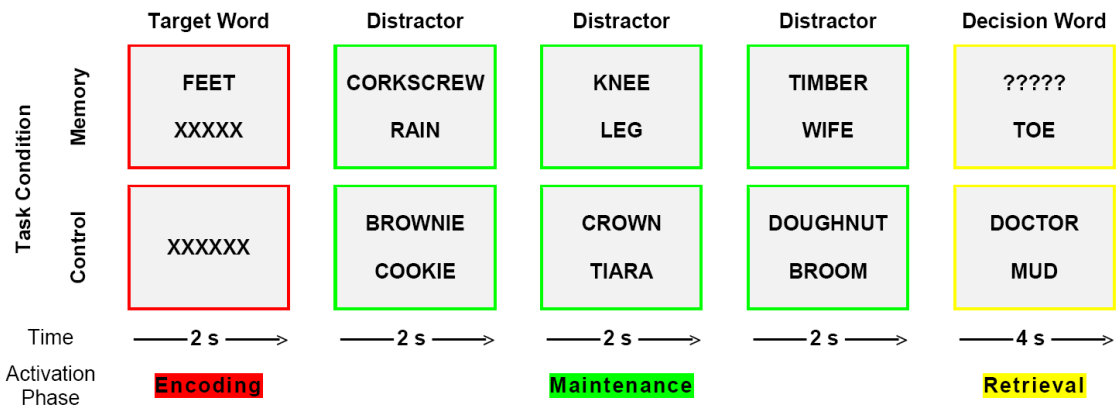
Research Design: Our overarching goal for aim 2 is to examine the effects of AE+CT versus CT alone on changes in the hemodynamic response during two fMRI tasks: verbal fluency and verbal working memory.

MRI. Structural images using T-1 weighted and diffusion imaging (DTI) sequences will be acquired while the participant is at rest within the scanner. During this time (15 minutes) participants are given the option to view a movie to alleviate potential boredom.

FMRI Tasks. For the verbal fluency task in the scanner, participants will see different categories at the center of a video screen while in the scanner. The participant's task will be to generate different exemplars of the respective category (semantic) or as many words that begin with a particular letter (phonemic). The fMRI fluency task will consists of two blocked conditions of category or phonemic generation, which will alternate with a control condition (reading the word "rest" aloud). A baseline condition will be used to control for activity associated with (a) basic visual processing, (b) articulation and, (c) hearing of the subject's own voice. A total of 4 blocks for each condition will be collected (i.e., 40 trials for category and phonemic fluency).

For the verbal working memory task, both memory and control trials will be presented in a pseudorandom order across five runs, each containing 14 trials. This will produced 35 memory trials and 35 control trials over the course of the experiment. For a memory trial, the first slide will depict a single lexical target. Participants will be instructed to read and remember this "target word" with the knowledge that sometime later they will be shown a second word, referred to here as the "decision word". Upon seeing the decision word, participants will initiate a button press indicating whether the target and decision words are semantically related. The time in-between presentation of the target and decision words will be filled with additional slides, referred to here as "distractor slides". Each distractor slide will present two words. Participants will be asked to make a semantic relatedness judgment for each of these distractor word pairs. A single memory trial is depicted in the diagram below. The timing parameters will be identical for both the memory and control trials. Specifically, slides 1-4 will present for 2 seconds each, and slide 5 will be shown for 4 seconds. These parameters were established through pilot testing in the lab of a colleague (Dr. Anna Moore) of the Primary Mentor and were chosen to allow sufficient time for the button-press response and to elicit satisfactory task performance. A 10-second blank screen inter-trial interval will used to allow hemodynamic response recovery.

Diagram of the experimental task, showing a single memory trial and a single control trial. Each trial will last 12 seconds for both task conditions (control & memory) and will be separated from the subsequent trial by a 10-second blank screen. Color-coding indicates the phases delineated for the functional task activation analysis.



The fMRI acquisition for semantic will follow protocols currently utilized in the investigators' laboratory and therefore will employ a sparse-temporal sampling design in which the response is assessed in the scanner during an off-phase, and the hemodynamic response is acquired after a short time delay; thereby movement artifacts due to the articulation process are avoided. Since the working memory task involves no overt speech, a continuous sampling acquisition will be used to allow for temporal resolution of the different phases of the working memory task. A T2*-weighted Fast-Field Echo, Echo-Planer-Imaging (FFE-EPI) sequence utilizing a parallel imaging technique will be used. Prior to the first scan, a training session outside of the scanner will be performed to familiarize the participants with experimental tasks. Participants will be scanned prior to and following the 12 week intervention but not at 12 week follow-up. Additionally, for fluency both semantic and phonemic stimulus presentation will be different but equalized for difficulty from pre to post.

The MRI/FMRI will be conducted at Emory University's Center for Systems Imaging (CSI). The CSI houses a 3 T Siemens Trio scanner with the total imaging matrix (TIM) suite. The scanner is equipped with a 32-channel Siemens head coil, which allows for rapid acquisition of high-resolution functional images (e.g., 2 X 2 X 2 mm voxels with a whole brain acquisition every second). It has the latest Siemens VB17 software and has a number of advanced Siemens product sequences including Blood Oxygenation Level Dependent (BOLD) imaging and in-line analysis suite with 3D PACE realtime motion correction.

Blood Draws and Biomarker Evaluation

Whole blood will be collected, by a certified phlebotomist, via venipuncture or peripheral venous line into standard plasma tubes or serum separator tubes (~40 ml per visit). Following collection, tubes will be transferred in accordance with blood borne pathogens training standards to the Molecular Core facility (for which Dr. Boatright is Director) of the Atlanta VAMC. Serum and plasma aliquots will be analyzed for biomarkers (e.g., BDNF) by ELISA, western immunoblotting, and other standard molecular biology assays.

The procedure below describes the blood collection timeline during the 12-week intervention period and follow up:

Baseline: 4 measurements over 1 hour, taken every 15 minutes, to demonstrate reliability of the measures.

Week 1: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. These time points will be mimicked in the non-exercised CT group.

Week 4: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post. These time points will be mimicked in the non-exercised CT group.

Week 8: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post.
These time points will be mimicked in the non-exercised CT group.

Week 12: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post.
These time points will be mimicked in the non-exercised CT group.

Follow-up: 1 measurement taken at 12-week follow-up.

All blood draws will be conducted at the Atlanta VA Medical Center. Tubes will be coded and thus have no identifiable patient information. It is expected that all blood sample processing and storing will be conducted at the Atlanta VAMC in the Molecular Core facility. However, in the event that equipment or other resources are needed for completing sample analysis, samples will be analyzed at Dr. Boatright's laboratory at Emory University (5th Floor, Emory Eye Center, Clinic Building B, 1365-B Clifton Road, Atlanta, GA). Sample labels will be de-identified by having only Subject #, Visit #, Draw #. The master code list will be maintained in a locked file cabinet in the Atlanta VAMC office of the PI or Co-investigator. Deidentified sample remainders will be kept at the Atlanta VA Core facility or, if needed, the Emory facility, while the study remains open.

Visual Assessment
Visual assessment will include retinal function (electroretinogram; ERG*)*, retinal morphology (scanning laser ophthalmoscope/spectral domain-optical coherence tomography; SLO/SD-OCT), and other selected measures of visual function. All methods are currently routinely conducted in the laboratories of the investigators [5, 15].

ERG: Following 30 minutes of dark-adaptation, a DTL fiber electrode (0.5% proparacaine hydrochloride in the eye receiving the DTL fiber) will be connected to the subject with a cup electrode attached to the temple serving as a reference for the DTL fiber. A dim flash (-1.43 cd s/m^2 or 1.5 Td•s) is then presented (10 flashes with 10 second interstimulus interval, sampling interval of 256 seconds, filter settings of 0.3 to 300 Hz). Analysis of ERG waveforms will include amplitude and implicit times of a- and b-waves and filtered OPs. Following dark-adaptation, the subject will be light-adapted for 10 minutes and 28 Hz flicker will be presented (average of 25 flashes at 3.0 cd s/m^2 or 128 Td•s). Amplitude and implicit time of the flicker waveform will be analyzed. Analysis of the response from the different subject cohorts will consist of two-way

repeated ANOVA for each ERG parameter with Holm-Sidak post-hoc comparisons to evaluate differences between subjects groups across time for each flash stimulus.

SLO/SD-OCT: Following placement of dilating drops (2.5% phenylephrinehydrochloride,1% tropicamide), the subject is asked to place their head in a chin rest in front of a SLO/SD-OCT instrument. The subject looks at a fixation light and the back of the eye is non-invasively imaged using a laser. This is a clinically-approved instrument that requires no contact with the patient's eye. The imaging will take no longer than 10 minutes. Dilation of the pupils lasts for 3-5 hours. Subjects will be instructed to bring sunglasses to testing to wear after the exam when leaving the building.

Visual Function:
Contrast sensitivity (CS) will be determined using the CSV1000E Contrast Chart and/or the Pelli-Robson Contrast Sensitivity Chart [27]. For the CSV1000E Contrast Chart, the subject will view the self-illuminated (85 cd/m^2) screen monocularly while wearing their normal correction while a drifting grating is presented. CS thresholds will be assessed for both eyes individually. The Pelli-Robson chart is viewed at a distance of 1 m. Subjects will be encouraged to give a response for each letter until 2 of 3 letters in a triplet are incorrectly answered.

Visual acuity (VA) is measured using the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart [28] and the Smith-Kettlewell Institute Low Luminance (SKILL) [29] chart. For the ETDRS chart, subjects will be placed at a distance of 3 m, if at least the top line can be read correctly, and encouraged to give a response for each letter until 5 successive incorrect answers are recorded. This test will take 10 minutes to complete. The SKILL test is designed to assess vision under conditions of low contrast and lighting, through a test performed under standard office lighting. The SKILL card has a standard high-contrast near acuity chart on one side, and a chart of gray letters on a dark background on the other. The low reflectance of the dark side of the card effectively simulates testing in a dim environment. The SKILL score is the acuity loss (number of letters) between the light and dark sides. This test will take 12 minutes to complete.

Visual field (VF) is measured by the Humphrey Visual Field Analyzer (HVFA). The subject will look into a half-dome and fixate on a light in the middle while the perimetry targets are flashed onto the dome at predetermined points within a pattern (3-zone screening protocol) to determine the threshold light sensitivity at each point in the pattern. The two eyes are tested separately. This test will take 30 minutes to complete.

Comparisons across subject groups will be performed using two-way repeated ANOVA with Holm Sidak post hoc comparisons to evaluate differences in CS and VA between subject groups across time.

Specific Aim 3: To assess the relative effect of the interventions on changes on mobility and physical function.

Research Design. Our overarching goal for aim 3 is to examine the effects of AE+CT versus CT alone on changes in mobility and physical function. To examine the effects of the interventions on mobility we will examine walking speed as our primary outcome. We chose walking speed as it has been demonstrated to highly represent overall health status in older persons [18]. Additionally, to strengthen the relationship with the interventions and activities of daily living we will include a dual-task measure of gait speed (walking while talking) as well as measures of upper extremity function and measures of visual and spatial cognition. Self reported falls as well as physical function and quality of life will be evaluated as secondary endpoints. All measures for Aim 3 will be done at pre, post and at 12 week follow-up. Significantly, the Godin Leisure-Time Exercise Questionnaire will be used to monitor the participant's physical activity levels during the 12-week follow-up period.

Measures for Specific Aim 3.

1. 400 Meter Walk. Participants will be asked to walk at their usual pace, without over-exerting. They can stop for up to 1 min for fatigue or other symptoms. A time limit of 15 minutes to perform the test has been established based on the following considerations. First, individuals who complete the walk in >15 minutes have an extremely slow pace (<0.44 m/sec), which would make their walking capacity of little utility in daily life. Second, selecting a higher cut-point, such as 30 or 60 minutes makes the objective assessment impractical and does not add to the clinical significance of the outcome. Participants will be allowed to use a cane, but not a walker, to complete the 400 m walk. Procedurally, we will first request that participants attempt the walk without the use of a cane. Those who feel unsafe will be allowed to attempt the walk with their cane.

2. Walking and Walking While Talking. Participants will be asked to walk across an electronic walk way which captures walking speed and foot placement in a quiet well-lit hallway wearing comfortable footwear. Start and stop points 10 meters apart will be marked by lines on the floor and include 3 feet from the walkway edge for initial acceleration and terminal deceleration. Eight walking trials will be randomly conducted; 4 single task walking only trials and 4 dual-task walking while talking trials. For the walking while talking trials participants will be asked to recite alternating letters of the alphabet. The order of the initial letter will randomly vary between "A" and "B" to minimize practice effects and/or count backwards by 3's. [64].

3. Short Physical Performance Battery (SPPB). The SPPB is based on a timed short distance walk, repeated chair stands and a balance test (as described by Guralnik et al.[17]). The battery will be administered by a trained and certified examiner.

4. Tinetti Balance Test. This test measures balance and gait while performing typical daily activities. The activities are graded as normal, adaptive, or abnormal

to determine the severity of balance impairment.

5. The Activities-specific Balance Confidence (ABC) Scale- indicates self report of participants' level of confidence in doing daily activity without losing their balance or becoming unsteady. Self-Report Function Questionnaire. We will use a modified version of the disability instrument that was used in LIFE-P, now called the Pepper Assessment Tool for Disability (PAT-D) [19]. The questionnaire, inquires about perceived difficulties in general activities of daily living during the last month. For each item, the response categories include: 1) no difficulty, 2) a little difficulty, 3) some difficulty, 4) a lot of difficulty, or 5) unable to do. Answers are averaged across the items, in order to better assess the overall perceived disability burden by a person. The questionnaire consists of 5 subscales: mobility, transferring, upper extremity, instrumental and basic ADLs. In addition to being a valid measure, the disability questionnaire has been shown to be responsive to change in previous exercise intervention studies among various disease populations [20].

6. Weekly Fall Diary. All participants will track falls over the course of each week while in the intervention at up until the final 12 week follow-up. In addition to the fall date and time, events surrounding the fall will be described.

7. Purdue Peg Board Task: The Purdue Pegboard measures unimanual motor dexterity. The test consists of two parts: 1) placing pins in a column of holes and 2) an assembly task using three components (pin, washer, collar). The participant is asked to place as many items as possible in 30 or 60 seconds, respectively.

8. Nine Hole Peg Board Task: A standard dexterity assessment, this task ask participants to place and remove pegs on a nine-hole pegboard as quickly as possible.

9. Pinch and Grip Strength: FDI and hand strength will be assessed using standard dynamometer (JAMAR) squeeze tests.

10. Coin rotation task: Another dexterity assessment, this task asks participants to rotate a coin (U.S. nickel) as quickly as possible for 20 rotations.

11. Halstead finger tapping: The Halstead finger tapping test is a standard test for testing psychomotor speed. The participant is asked to press a lever attached to a counter as many times as possible in 10 second trials.

12. Target force matching: The target force matching task is similar to the anisometric FDI muscle training used in the behavioral intervention. A computer presents a target force marker and the participant's task is to match the cued lift load target and hold for a set period of time. EMG is of the FDI taken during this assessment.

13. Motor tracking: The motor tracking task is a computer based presentation of a stimulus (vertical bar) moving laterally. The participant is instructed to track the movement of the stimulus using a computer cursor controlled by a touchpad input device.

14. Sleep Profiler: These monitors will provide pre and post-intervention objective data on sleep quality, patterns, and continuity that will supplement the Pittsburg Sleep Quality Index, Epworth Sleepiness Scale. They are non-invasive wearable recorders with 3 EEG electrodes housed with a headband placed just below the hairline that provide study-validated information including total sleep time, REM sleep, sleep efficiency, snoring intensity and frequency, and arousal. Participants will be provided a brief orientation and instruction on how to properly wear the device. The device requires minimum setup, and the wearable headband is similar to a headband that might be worn at the gym. It is battery powered, with up to 16 hours of recording between charges, and data is stored and easily downloaded via USB from the device.

Additional Measures of Physical Function for Aim 3.

To confirm the cardiovascular effect of the aerobic training participants will also perform a sub-maximal exercise test on a cycle ergometer before and after the intervention. This test called the VO2 sub-maximal fitness assessment will be supervised by the Principal Investigator, Dr. Joe Nocera in the Movement Studies Laboratory of the Rehabilitation Research & Development Center in the Atlanta VA**.** During this test we will measure ventilation and oxygen and carbon dioxide concentration of the inhaled and exhaled air. We will adhere to the YMCA sub-maximal test protocol. This sub-maximal test will be used to estimate the participant's maximal oxygen uptake (VO_2max) prior to, after and 12 weeks following the interventions. VO_2max is the most appropriate test as it accounts for initial fitness level of the participant. This test uses and "extrapolation" method in which heart rate workload values are obtained at 2-4 points and extrapolated to predict workload at the estimated maximum heart rate (MHR) (e.g. 220-age). VO_2max is then calculated from the predicted maximum workload. Participants will be asked to ride a stationary bicycle for 4 three-minute stages (Table 3). The first stage will be a warm-up at 50 revolutions per minute (RPM) at a power level of 25 watts. During all testing stages, heart rate will be continuously monitored and will not exceed 85% of age-predicted maximum heart rate. For the analysis, average heart rate during the final 30 seconds of the 2nd and 3rd minutes will be plotted against workload. Three minute trial workloads, shown in Table 4 below will be chosen based on the participants' heart rate at the end of the warm-up period. The fourth 3-minute stage will be a cool down period added to the end of the test. Participants will be allowed to stop the test at anytime if they feel faint, dizzy, short of breath, or for any other reason. Heart rate will be monitored using the Polar Tracker heart rate monitor. This will also be used to monitor heart rate during the exercise sessions.

Table 3. YMCA Sub-maximal Cycling Protocol

	< 80 bpm*	80-90 bpm*	90-100 bpm*	> 100 bpm*
Stage 1	125 watts	100 watts	75 watts	50 watts
Stage 2	150 watts	125 watts	100 watts	75 watts

* beats per minute

Participants will not undergo exercise stress testing. This decision may be perceived as a potential weakness of the proposed study. We considered adding an exercise stress test prior to randomization. An advantage of this would be that it would provide an additional opportunity to detect severe cardiac disease that might increase the risk of an acute event during our aerobic exercise intervention. After extensive deliberations, our research team decided with unanimous vote that including an exercise stress was not necessary and would not add additional information for the study or protection to the human subjects. This decision was based on the following considerations:

- The recommendations published in JAMA by Gill et al. [21] advised that a screening protocol based on a simple cardiovascular reserve test, similar to the one described above is more suitable for screening older adults than a protocol based on stress exercise testing.
- The American Heart Association (AHA) and the American College Sports Medicine (ACSM) joint position statement advised that “apparently healthy persons of all ages and asymptomatic persons at increased risk may participate in moderate-intensity exercise without first undergoing a medical examination or a medically supervised, symptom-limited exercise test” [22].
- The majority of older persons (>75%) are unable to satisfactorily complete a treadmill exercise test, [23] which makes its utility as a screening tool in the elderly population questionable.
- Older persons have a high prevalence of ECG abnormalities, which diminish the diagnostic accuracy of treadmill exercise testing [24].
- Participants with potential cardiac contraindications to the proposed exercise program will be identified and excluded by means of the screening process.
- Exercise of will be conducted in a supervised environment as described below in interventions section.
- We have found that a maximal or near maximal exercise test on a treadmill is an unpleasant, if not frightening experience, for sedentary and unfit adults. Requiring an exercise stress test may deter older persons from participating in the trial as assessed in the LIFE-P ancillary study on maximal exercise testing conducted at the Cooper Field Center.
- Regular exercise and physical activity may actually reduce the overall risk of **myocardial infarction** and death among older persons, possibly through improvements in cardiac risk factors and overall fitness; ACSM joint position statement advised [22].

In summary, we concluded that exercise stress testing would provide little additional information, was not necessary to protect the safety of participants, and that it is disliked

by sedentary and unfit participants. Our protocol for the aerobic exercise intervention also requires that the sessions carefully monitored for cardiac and other signs and symptoms by trained staff.

Interventions.

Aerobic Exercise + Cognitive Training Group. For this arm of intervention, randomized participants will attend both exercise training sessions and cognitive training sessions demonstrated to facilitate physiological changes. For the **aerobic exercise component** of this group, participants will follow the guidelines provided by the American College of Sports Medicine for optimizing cardiovascular fitness. Thus, participants will exercise 3 times a week on a stationary ergometer. Exercise intensity will begin at low levels (50% of maximal heart rate reserve) calculated utilizing the Karvonen method. Briefly, target exercise HR is calculated by subtracting the persons age from 220. Resting heart rate is then subtracted from this number. The answer is then multiplied by the target percent (50% for example) and the product is added back to resting heart rate to provide the target exercise session heart rate. Intensity will be increased by 5% every week (as tolerated by the participant) to a maximum of 90% of maximal heart rate. Exercise time will progress from an initial 20 minutes per session to a maximum of 60 minutes by increasing 5 minutes each week. Each session will be monitored by a certified CPR and fitness specialist. Further, the intervention site is located in the Atlanta VA Health Care System and portable defibrillators are available at the intervention site.

The **cognitive training component** for this group will take place immediately after the exercise session (15 minutes following the exercise cool down). This time frame allows for the cognitive training to be done within the 15-60 minute window in which the neurotrophic exercise mediated response is elevated. The commercially available Mindfit program will be utilized for the cognitive training. The Mindfit was selected based on its mainstream popularity and its demonstrated effectiveness and adherence (99.2%) in previous studies [14]. Each training session includes a mixture of 21 visual, auditory and cross modality tasks aimed at attention and executive functions as well as other cognitive processes. Each training session lasts 20 minutes and can progress on three levels of difficulty- easy, moderate and hard. These levels can be further adjusted according to the participant's progress.

Cognitive Training Only Group. For this arm of the intervention, randomized participants will follow the same guidelines as the cognitive component of the AE+CT group but will not partake in aerobic exercise. To equalize contact/monitoring of the groups this group will meet for the same total duration time as the AE+CT group; however, instead of aerobic exercise, progressive whole body stretching and toning exercises designed for individuals 65 and older will be done prior to their cognitive training. "Stretching" controls groups have been utilized in previous studies examining cognition and aerobic exercise and have not been shown to result in improvements in cognitive function [8].

Age-Related Macular Degeneration Group. Participants who are at least 70 years old with a diagnosis of AMD will complete 6 months of the aerobic exercise program or 6 months of the stretching and toning exercises. These participants will complete the cognitive assessments described above. This group will complete the following visual assessments:

-Contrast Sensitivity-Visual Acuity, Amsler grid: Participants will look at an illuminated screen and/or chart, one eye at a time while wearing their normal eyeglasses/contacts. We will test how pale of a target they can see, how small a target they can see, and the appearance of a grid pattern. These tests will take about 1 1/2 hours.

-Low Luminance Questionnaire: Participants will be asked to complete a Low Luminance Questionnaire that asks several questions about their ability to see in various lighting conditions.

-Dark Adaptometry: Dark adaptation or night vision is known to be affected with increasing stages of age-related macular degeneration (AMD). This test will probe the ability of the eye to dark adapt, and it will require for the pupils to be dilated. They will be asked to place their head in a chin rest in front of the machine. Each eye will be tested individually, and the opposing eye will be covered with an eye patch. After dilation, while fixating on a light at the back of the machine, a bright flash will be presented to bleach the photoreceptors in the eye and then you will be presented a series of dimmer circular spots that you will be asked to identify whether you saw or not by pushing a response button. The duration of the test is about 5 minutes per eye. Corrective lenses may be put in the machine in front of the eye to correct for blur if necessary.

-Eye Photography: A series of photographs will be taken of the eyes. For this test, the pupils will be already dilated from the previous exam. They will be asked to place their head in a chin rest in front of a special camera called a scanning laser ophthalmoscope/optical coherence tomography machine (SLO/OCT). They will look at a point of light and the back of their eye will be imaged. The SLO/OCT is used routinely in eye doctor's offices and vision clinics. It is safe and requires no contact with the eye. The imaging will take no longer than 30 minutes.

The AMD group will also complete the Physical Function Assessments. Those who are able will complete a Maximal Treadmill Exercise Test to determine peak oxygen uptake (VO2 max). They will wear a breathing apparatus to measure their oxygen, carbon dioxide, and ventilation. A registered nurse will be present for the entire test and manually monitor blood pressure at baseline and during each stage of exercise, as well as monitor continuous EKG recordings. Those who are unable to complete the maximal treadmill test will complete the submaximal test described above.

This group will complete blood draws at three time points: at the start of the exercise intervention, at 3 months, and 6 months.

Exercise Lab Space: The exercise classes and cognitive training will be held in the Movement Studies Laboratory in the Atlanta VA Rehab R&D Center. The Movement Studies Laboratory is a 41 X 34 ft work out area that will serve as the venue for the group exercise. The cognitive training arm of the study, which must be done within 15 minutes of completion of the aerobic exercise in the AE+CT group, will take place in a nearby lab space. Interventionists will have offices located on site with access to computers and phones for monitoring adherence of participants. Because the lab space is housed in the Atlanta VAMC, emergency procedures are done in accordance with emergency preparedness plans. A phone is located in the facility which can be used to contact emergency services (911) in the event of a medical emergency. CPR and the AED will be utilized if needed until Emergency Services assumes responsibility. All staff are certified in basic life support and first aid and the facility houses AED devices.

Risks/Discomforts

Cognitive Testing. Patients may experience some frustration if they have trouble with cognitive testing. Researchers will be trained on how to handle frustration by taking breaks, allowing patients to express frustration, and offering encouragement. As noted above, we will also include items that the participants can answer correctly to minimize frustration. In the vast majority of patients, these techniques are adequate to deal with frustrations.

Magnetic Resonance Imaging (MRI). More than 150 million diagnostic magnetic resonance studies have been performed worldwide. The vast majority of these procedures were completed with no sign of patient injury [25]. There is a high degree of patient safety with an fMRI because of the miniscule value of magnetic susceptibility and lack of ferromagnetic components of human tissue. Studies have ranged in magnetic field intensities from 1.5 to 8T. No negative cardiac, physiological, or cognitive effects were noted [26]. Therefore, long-term effects on human health from magnetic resonance imaging are unlikely. Those at risk for injury include those with indwelling ferromagnetic material (e.g. foreign object in eye, surgical implant) or an implanted bioengineering device (e.g. pacemaker, infusion pump), due to the possible interaction with a magnetic field. Subjects identified as at risk from the screening checklist will be excluded from the study.

Another potential hazardous effect is related to the high level of noise produced by the machinery during imaging. Unprotected, patients can experience hearing loss. For this reason, individuals will be given foam earplugs to wear to minimize this risk.

Additionally, some individuals are perceptible to experiencing distress during the fMRI process. The small, closed-in space may trigger anxiety. Participants will be screened

for claustrophobia, generalized anxiety disorder, post-traumatic stress disorder, or obsessive-compulsive disorder.

Further, persons who are pregnant (or could be pregnant) or those with a seizure disorder should not undergo magnetic resonance imaging, and will be excluded.

Subjects may have early stages of disease, not previously diagnosed, detected through the use of MRI. A trained neuroradiologist is available to the investigators to evaluate the findings and determine if there is pathology present or a normal variant should the investigators suspect that the imaging shows abnormalities. If pathology is present or suspected, subjects will be counseled about what the findings are and what should be the next steps for clarification of ambiguous findings or seeking help with pathological findings. This is not a risk in the conventional sense of physical harm or disease, but does pose a potential psychological risk to the patient.

Participants ages 70 years and older with Age-Related Macular Degeneration will not complete the fMRI.

Blood Draw. The risks of drawing blood from a vein include discomfort at the site of injection; possible bruising and swelling around the injection site; rarely an infection; and, uncommonly fainting from the procedure. A trained phlebotomist will collect all blood samples following standard protocol.

Visual Assessments.

Physical function and Exercise Intervention. While the risk of a cardiac emergency is increased when a person is exercising, these events are rare and usually occur during high-intensity activities. The cardiovascular benefits of exercise have been consistently shown to outweigh the acute cardiovascular risk during the act of exercising. Moreover, a person beginning a moderately intense exercise program is actually at a lower overall risk of sudden death than their sedentary peer. Neither an ECG nor a stress electrocardiogram will be included in the screening process due to the lack of evidence to support their usage in detecting those who will have an adverse exercise outcome.

Some participants will experience exercise-related injuries and possibly muscle soreness and fatigue as a result of the testing and the intervention. There is a small risk for loss of balance and injury from a fall while walking and during balance measurements. However, walking and balance measurements will only be a few minutes at a time and research personnel will be with the participant at all times. Most injuries will be self-limited, though it is possible for permanent injuries to occur, including broken bones or joint problems. There are minor risks of musculoskeletal problems associated with the performance evaluation measures of the study. The potential risks of loss of confidentiality will be minimized through assigning all data collection instruments a unique code without individual identifying information. All HIPAA regulations pertaining to protection of participants and eliminating identification will be followed.

Potential Benefits

There are substantial potential benefits of participation in this study. All participants may benefit from the pre intervention evaluation, which may detect unknown or inadequately treated medical problems. Potential benefits for adults who exercise include:

- improvement of physiologic indicators of health (e.g., balance, blood pressure);
- full or partial elimination of impairments in ability to do daily activities;
- improvement in mental and/or social health status;
- reduction of risk of falls and fall-related injuries;
- reduction in risk of mortality; and
- reduction in medical care costs.

Potential benefits for adults who participate in the exercise testing include:

- improved awareness of their cardiovascular fitness level;
- greater confidence in the knowledge of their cardiovascular fitness level;
- greater knowledge of their fitness changes and the relationship between those measures and their ADLs.
- improvement in mental and/or social health status;
- reduction of risk of falls and fall-related injuries;
- reduction in risk of mortality; and
- reduction in medical care costs

Participants may be compensated ($200) for their participation in the study. The AMD group may be compensated up to $150 for their participation in the study.

6. Participant selection:

The study will enroll between 60 and 80 older, sedentary adults. Participants will be randomly assigned to one of two 12-week interventions conditions: AE+CT or CT alone. The pre and post evaluations will be conducted over 3 days during a 1-week period. For example, Monday-Cognitive Evaluation, Wednesday-fMRI, Friday-Physical Evaluation. Each day will last no more than 2 hours. The investigators expect a dropout rate of anywhere from 10-15% of participants. To ensure completion of study by 60 participants, as many as 80 subjects may be enrolled. Importantly, all participants will be evaluated on

Inclusion/Exclusion Criteria. We will complete a *Health Screening for Exercise* with all potential participants. If the participant answers *no* to all the screening questions, study

staff will proceed with scheduling the participant for enrollment. If a participant answers *yes* to any question, the study staff must receive a physician's written medical approval for participation in the fitness assessment and/or exercise intervention. We will provide the physician a letter to sign if the participant is cleared explaining the study protocol. The key inclusion criteria and final participant pool will consist of right handed English speaking individuals aged 18 to 89. Participants will be sedentary as defined by < 120 min/week of aerobic exercise over prior 3 months and a summary score on the SPPB between 7 – 10 [17]. Additionally, participants will be non-demented (MMSE ≥ 24) and will have no cognitive-executive function deficit (MoCA ≥ 26). Further, those with severe diabetes requiring insulin will also be excluded. However, those with less severe, controlled diabetes that meet our physical and cognitive function inclusion criteria will be allowed to participant. Additionally, those with early staged Parkinson's disease (H&Y 1-3) will be allowed to participate.

Recruitment. Individuals will be recruited from the Atlanta VA Rehab R&D Subject Registry (IRB00000159) based on the inclusion and exclusion criteria. The Subject Registry study staff will query the data base of all patients meeting the inclusion and exclusion criteria who have consented to be contacted for research purposes and will print out their contact information. The Principal Investigator, or his research colleagues, will contact these individuals and will summarize the study procedures. If the potential participant is interested we will mail them a copy of the informed consent and will schedule an orientation session that describes the study goals and provides an opportunity to go over the informed consent.

Healthy subjects will also be recruited via the Emory University Alzheimer Disease Research Center (ADRC) Registry. Written permission from all patients is recorded on a signed informed consent form before inclusion in the ADRC Registry. This consent requires that the patients be willing to complete detailed histories, undergo comprehensive neurological and neuropsychological evaluations on an annual basis; submit blood for ascertainment of genetic information and establishment of lymphoblastoid cell lines; and agree to be contacted regarding participation in research projects of Emory investigators.

The PI and the Research Coordinator(s) will be added to the ADRC Registry protocol (IRB #133-98). Following this, the ADRC request form will be filled out in order to identify an appropriate list of potential participants.

The VA Data Manager will query medical records within the VISN 7 Corporate Data Warehouse to identify potentially eligible patients with upcoming appointments at the VA Medical Center. Once patients are identified, the Research Coordinator will discuss the patients and the study with the providers. Those patients determined to be appropriate for the study will be introduced to the study team by the provider.

We propose to use the VA Informatics and Computing Infrastructure (VINCI) databases to identify patients at the Atlanta VA Medical Center who may be eligible for participation in the research study. We will ask the VINCI data managers to pull

requested data from the Corporate Data Warehouse (CDW). The data requested includes patient names, dates of birth, phone numbers, addresses, social security numbers, visit and hospital information, laboratory tests, medication use, eye exams, and smoking history.

This identified data will be directly transferred electronically from the VINCI environment to the secured research server VHAATGFPC10 located in the OIT server room at the facility. This will be done by Christine Jasien, Atlanta VAMC data manager. The servers are managed by the Atlanta and Region 3 OIT offices and security is managed by the Information Security Officers at that facility. The server room has appropriate security controls in place (including a locked room, password protection, and encryption), and the drive that the data will reside at will be controlled so that only the research team has access to the data. Once the data has been transferred to the secured research server Antonio Laracuente, Director, Atlanta VAMC Research Operations will create a folder specific to the study under said secure drive. Only approved individuals listed on eIRB for the study will be granted permission and access to this folder.

Medical records housed in VINCI will be used to ensure that patients who are not eligible for participation are not approached. Patients approved for contact will be sent an IRB approved letter with a brief summary of the research and study contact information. The study team will discuss the study with interested patients by telephone, providing further information about the study.

Additionally, participants may be recruited from word of mouth.

Individuals will be provided a copy of the informed consent and will be scheduled for an orientation session that describes the study goals and provides an opportunity to go over the informed consent. The PI/Co-PI or their trained and qualified colleagues will go over and describe the consent in a private office of the PI/Co-PI.

7. Statistical Analysis:

Statistical Analysis for Specific Aim 1. We will model each of the outcome measures in Aim 1 (verbal fluency, non-verbal executive function and crystallized word knowledge, as well as the MindFit cognitive evaluation) jointly using a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. We expect that modeling the outcomes jointly, using subject as a random factor, will allow for some gains in power. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall

effects of time and treatment, and most importantly, how the effects of treatment differ with time.

Statistical Analysis for Specific Aim 2. For each of the outcome measures in Aim 2 (changes in fMRI activity during verbal fluency and verbal working memory as well as changes in BDNF) we will use a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall effects of time and treatment, and most importantly, how the effects of treatment differ with time.

Statistical Analysis for Specific Aim 3. For each of the outcome measures in Aim 3 we will use a mixed linear model to determine whether the mean responses of the groups are significantly different across the time points. Our fixed factors will be time, treatment group and their interaction. This model will enable us to estimate the overall effects of time and treatment, and most importantly, how the effects of treatment differ with time.

Power Analysis and Sample Size. We powered this study on the executive function task of verbal fluency because of the high importance of word retrieval for everyday function and quality of life. For example, people over the age of 65 frequently have difficulty recalling words they wish to use to describe objects, actions, and concepts thus rendering them unable to effectively communicate. In aging, difficulties with fluency may result from a deterioration of frontal substrates that underlie the ability to access and/or retrieve word forms. Importantly, the cortical areas required for fluency are precisely those affected by aerobic exercise. While there currently is little understanding of the effects of exercise plus cognitive training on verbal fluency in older adults, data from our ongoing CDA-1 pilot study has shown an average improvement pre to post in an exercise-alone group (n=10) of 7 words. In our non-exercise control group (n=2), the average improvement was 1 word. While this data does not provide enough information to enable us to estimate a required sample size under the mixed model that will be used in the final analysis, we can conservatively estimate the sample size assuming a two-sample t-test would be performed on the post-pre differences for each group. Under this simpler model, a sample size of 30 per group will give us approximately 80% power to detect a true difference between groups of 4.5 words, and 90% power to detect a difference of 5.2 words. While these effect sizes are relatively large (0.75 and 0.87, respectively), we will have more power under the mixed model that we actually will use to analyze the data. Importantly, we feel these improvements are clinically relevant as longitudinal research from a sample of cognitively normal adults (n=96; avg. age 75) followed for 2.3 to 5.9 years demonstrated an average rate of change -0.4 ± 1.6 annually.

The research staff will use REDCap issued through the Atlanta Veterans Health Care System. Only deidentified data will be entered into REDCap by VA credentialed research personnel to run data analysis.

8. Adverse event reporting:

In the case of a reportable event, the Principal Investigator will complete the form "Reportable Protocol Event Form for VA sites," attach supporting documentation, and provide it to the VA Science Information Office. The SIO will then route the report to the Emory IRB. The Emory IRB will be notified within five days of any adverse event occurring.

9. Data and safety monitoring plan (DSMP)

This is a minimal risk protocol and therefore data will not be reviewed by a Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC).

This study will only recruit healthy older adults. Prior to beginning any portion of this study we must receive a physician's written medical approval for participation in physical activity. We will contact the physician and inform him/her of their patient's interest in participating in this study and explain the study criteria for participation detailing the intervention and testing procedures. Additionally, the pre/post testing carries minimal risks due to the sub-maximal nature of the fitness testing. As per the YMCA protocol, "physician supervision is not necessary with sub-maximal testing in low to moderate risk adults." (ACSM's Health Related Physical Assessment Manual, 2007). Although any exercise program carries with some possibility of exercise-induced cardiovascular events, we will minimize this by including those who have been cleared by a health care professional. While the risk of a cardiac emergency is increased when a person is exercising, these events are rare and usually occur during high-intensity activities. This study will utilize a moderately intense exercise regiment for all participants. The cardiovascular benefits of exercise have been consistently shown to outweigh the acute cardiovascular risk during the act of exercising. Moreover, a person beginning a moderately intense exercise program is actually at a lower overall risk of sudden death than their sedentary peer.

Protection Against Risk

Several precautions will be made to reduce the risk of exercise-related injuries:

- The exercises are carefully designed with warm-up, stretching and cooling down components to minimize injuries;
- Written instructions highlighting some fundamental guidelines all study participants should do to ensure that they are exercising safely will be provided to all study participants;
- For those individuals who are extremely de-conditioned, frequent rest periods and exercise from a sitting position will be offered at the beginning of the training period;
- At each exercise session, participants will be questioned about the presence of musculoskeletal symptoms;

- If a participant experiences a significant illness requiring mobility restriction, surgery or hospitalization, the project coordinator will contact the participant's primary physician for clearance to return to the intervention classes.
- If adverse symptoms develop a 1-week hiatus from the exercise session will be enforced, and during this time the participant will perform static stretching exercises. A slow reintroduction of the exercise program will follow all hiatuses;
- The instructors/evaluators will be instructed to be alert for the emergence of symptoms of angina and shortness of breath. Participants will be instructed to discontinue exercise if there is significant pain, weakness, or joint swelling after exercise.
- We will ensure that that the site has immediate access to a phone and that provision of interventions and evaluations are undertaken in the immediate vicinity of a phone. All trainers and evaluators will also be encouraged to carry cell phones;
- All activities will occur inside an air-conditioned facility because environmental extremes are poorly tolerated;
- Every precaution will be taken to provide fluids, rest and other measures to insure each participant is comfortable, safe and secure in the testing environment.

Confidentiality of data is maintained by using research identification numbers that uniquely identify each individual. Safeguards are established to ensure the security and privacy of participants' study records. The information collected from participants in this study has a low potential for abuse, since the data do not address sensitive issues. Nevertheless, appropriate measures are taken to prevent unauthorized use of study information. The research ID number is used. The research records are kept in a locked cabinet in the locked office of the PI. The files matching participants' names and demographic information with research ID numbers are kept in a separate locked room and are stored in a locked file that uses a different key from that of all other files. Only study personnel have access to these files. Electronic data will be stored in a password-protected file on a secure network. After the study is completed, procedures for long-term storage of VA data will be followed.

FMRI data will be stored in a secure electronic environment in a locked office (6205 A&B) at the Woodruff Memorial Research Building, 1639 Pierce Dr. Atlanta, GA 30322, without identifiers.

10. Pharmaceutical, biologic, and device information:

N/A

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