Protocol

1. Project Title

Graded Intensity Aerobic Exercise to Improve Cerebrovascular Function and Performance in Aged Veterans

(Short title: Graded Intensity in Aerobic Exercise)

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2. Abstract:

This study will examine cerebrovascular function in older Veterans before and after a proven aerobic exercise intervention, allowing us to better understand the robust impact of exercise on brain function and health. Task-based fMRI and the resultant BOLD map is currently a dominant method of neurocognitive investigation, particularly related to documenting the beneficial changes demonstrated with rehabilitation. No single imaging technique can resolve the complexities of imaging neural plasticity in brain systems, therefore we propose a solution of combining imaging techniques and modeling the impact of perfusion and CVR on the BOLD response. We will model the hypothesized change in perfusion and CVR to the BOLD response following the intervention to quantify the percentage of variance both perfusion and CVR account for in the taskinduced BOLD signal. This will allow us to understand the degree to which change in perfusion and CVR impacts the well-documented beneficial change in task-induced BOLD following exercise.

3. Background:

The US Census Bureau reports that, of the 18.8 million Veterans of the U.S. armed forces, 9.3 million are age 65 or older. Another 7.7 million fall between the ages of 35 and 65 years of age. Currently the median age for Veterans is around 64 years. As the Veteran population continues to age, the characteristics and comorbidities associated with aging will place a significant financial burden on the healthcare system. According to the Alzheimer's Association, one in three of Veterans over the age of 65 will die with Alzheimer's disease (AD) or a related dementia.[1] Intertwined with aging-related pathologies are physiological changes in cerebrovascular function.[2,3] In fact, recent epidemiological and clinicopathological data indicate considerable overlap between cerebrovascular dysfunction in aging and AD.[4,5] While AD is an overt pathology, its time in a "sub-clinical" stage appears be years or even decades before the crossing of a diagnostic threshold.[6] This sub-clinical stage spans a significant amount of time and provides a significant opportunity to initiate targeted interventions for limiting vascular dysfunction in older, at-risk Veterans.

It has been demonstrated that of all modifiable risk factors for AD, decreasing sedentary behavior is the most statistically significant and effective measure to counter the disease processes and associated cognitive decline in older adults.[7] Nearly a quarter million cases of AD would be prevented in the US alone by improving the cardiovascular fitness profile in older adults.[7] This staggering number is an exciting target not only for prevention but also as a means to better understand aging processes and underlying mechanisms malleable to exercise. Equally significant, this number probably underestimates the total impact of increasing cardiovascular fitness due to its effects on other risk factors in aging, including hypertension and obesity.[8]

Two significant goals that are critical to VA RR&D and the aging Veteran population will be achieved with this study. First, this study will define the impact of aerobic exercise on cerebrovascular health in aging Veterans. Decreased vascular health because of aging, and particularly sedentary aging, fosters both cognitive and motor dysfunction[9] and, as noted, has implications for neurodegenerative disease. We do know that older adults who are physically active have improved vascular health but we do not know the impact of an exercise intervention on cerebrovascular health. We will fill this gap by examining changes in basal cerebral perfusion and CVR in older Veterans following a proven cardiovascular fitness intervention. If our hypotheses of improved perfusion and CVR is supported it would inform current intervention strategies and would add important new information about the potential of exercise to improve brain health in aging. This would have immediate implications for aging Veterans at risk for neurodegenerative disease brought on by cerebrovascular dysfunction.

Secondly, this study will advance the current state of literature regarding the beneficial impact of exercise on brain health and function. Research over the last few decades have driven the continual promotion of exercise as one of the most impactful interventions of central nervous system health and function.[10-12] At the forefront of much of this research is the use of task-based fMRI BOLD to quantify beneficial changes in cortical function following aerobic exercise.[13-15] While transformative, the true impact of this research is limited in scope until we can define the influence of cerebrovascular function on the well-documented beneficial change in BOLD response. Because the BOLD signal reflects the health and function of the cerebrovasculature,[16,17] we believe the changes brought on by exercise are at least partially mediated by improved perfusion and CVR. Our statistical model in Aim 3 will allow us to quantify the impact of perfusion and CVR on the BOLD signal during task.

Decreased Cerebral Perfusion in Aging

The brain uses approximately 20% of available oxygen for normal function, making tight regulation of blood flow and oxygen delivery critical for survival.[18] This high demand coupled with a lack of energy stores within the cortex necessitates that cerebral perfusion be constantly and consistently maintained. In a normal physiological state, total blood flow to the brain is remarkably constant, however, with advancing age basal perfusion declines by roughly 4 mL/min per year.[19] It is presumed reactive oxygen species in the cerebrovasculature decreases NO bioavailability leading to the gradual

decline in endothelial function and subsequently perforating arteries, arterioles and capillaries. Ultimately, extracellular matrix components lose elasticity causing decreased vessel function and decreased blood flow.[9,20]

Morphological studies demonstrate age-related degradation in perfusion throughout the intracranial vessels, however deceased cerebral perfusion is most notably amplified in the frontal cortex.[2,19] While age-related decline in cerebral perfusion is not great enough to cause major ischemic injury, it results in hypoperfusion that prevents sufficient nutrients from reaching areas of high-metabolic demand such as the frontal cortex.[21] Our own data, presented below, demonstrate a decline in basal cerebral perfusion in the inferior frontal gyrus (IFG) and motor cortex in older adults when compared to younger adults. Of note, the decreased blood supply and inadequate delivery of nutrients is instrumental in age-related cognitive decline, specifically with cognitiveexecutive processes partially mediated by the frontal cortex.[3]

Decreased Cerebrovascular Reactivity in Aging

In addition to blood flow, the dynamic parameter of CVR has a critical role in cerebrovascular health and function. The cerebrovasculature must maintain blood flow within precise limits during rest, activity, disease or injury. In healthy individuals, perfusion is tightly regulated to meet the metabolic demands of the brain via vasoconstriction or vasodilation. Thus, the ability of cerebrovasculature to dilate in the face of increased demand is vital for cerebral function. However, the vasoreactive properties and vascular response to demand have repeatedly been demonstrated to degrade with age and neuropathology.[9,22,23] To quantify the efficiency of cerebrovascular response, the degree of vasodilation in response to CO2, a vasoactive agent, is measured.[24,25] Decreased responsiveness to CO2 is an accepted marker of CVR dysfunction and has been demonstrated to be sensitive to changes brought on by aging.[2]

While the physiologic mechanisms that take place in the human cerebrovasculature resulting in neural decline are as yet unexplained, extensive work in the periphery demonstrates that age-related arterial stiffening and endothelia dysfunction play critical roles in the development of CVR dysfunction.[9,20] Impairment of the oxidative stress response and dysfunctional inflammatory pathways feed a cascade of age-related CVR malfunction causing increased reactive oxygen species and reduced NO. Together the increase in reactive oxygen species levels and decreased NO suppresses the response of immune cells causing systemic vascular endothelia dysfunction.[26,27,28] Behaviorally, decreased CVR has been observed in cognitive decline demonstrated in aging as well as dementia.[23]

Impact of Exercise on Cerebrovascular Reactivity

Aerobic exercise has consistently been associated with enhanced flow-mediated dilatation in the brachial artery of older adults.[29] In the cortex, vasodilatation of the cerebrovascular in response to a CO2 challenge is associated with maximal aerobic

capacity in both young and old adults.[30] Thus, in both the periphery and in the cortex, those with higher levels of cardiovascular fitness have improved CVR to stimulus. CVR was also shown to increases among stroke survivors after six months of aerobic training.[31] Our own pilot data demonstrate marked decline in CVR with age. However, the degree of CVR decay is lessened in older adults that have high levels of selfreported aerobic activity.

Link Between Cerebral Perfusion, CVR and Task-Based fMRI BOLD Response
Seminal studies have continually demonstrated that sedentary individuals who undergo exercise intervention demonstrate BOLD activity that coincides with improvements in an array of tasks in both motor and cognitive domains.[13-15] While the accessibility and ease of implementation have made BOLD a valuable tool, its interpretation is not straightforward.[16] BOLD is a convoluted neurovascular signal that arises due to the interplay between changes in blood flow and metabolism in response to inhibitory/excitatory causing stimulus. Task-induced BOLD change (ΔBOLD) is measured relative to baseline BOLD signal, which means that baseline physiology (blood flow and perfusion) affects the task-induced ΔBOLD particularly in magnitude. We believe improvements in these baseline physiological factors are at least partially responsible for the well-documented, exercise-induced BOLD change. As noted, precise levels of blood flow depend on the health of the blood vessels that carry them. The blood carries important nutrient such as glucose and oxygen to support cellular energy demands, thus the amount of baseline perfusion is an indirect measure of tissue baseline metabolic demand. It should be noted that perfusion is a proxy for baseline neural activity, as increased neural activity results in increased demand for blood flow.[32] Further, the blood vessels must dilate in response to an increased demand for blood flow.[32] CVR is an index of baseline vascular health that is necessary to support efficient cerebral perfusion. Thus, to isolate the issue of vascular 'plumbing' versus lower baseline neural activity, we would need to quantify both perfusion and CVR. Taken together, for a better interpretation of task-induced ΔBOLD, it is important to understand the relationship between baseline cerebral physiology (perfusion and CVR) and taskinduced ΔBOLD. To better understand the literature highlighting BOLD alterations following exercise, it is critical to quantify the impact of baseline physiologic measures, including perfusion and CVR on task-induced BOLD changes. We plan to accomplish this task by modeling the contributions of perfusion and CVR to ΔBOLD response both before and after the intervention.

Mechanisms of Improved Cerebrovascular Function with Aerobic Exercise
Arterial stiffening and cerebral endothelia dysfunction associated with aging results in decreased basal perfusion and CVR. These changes are known to contribute to agerelated declines in both cognitive and motor functions.[3,9] Our preliminary work demonstrates that age-related vascular dysfunction is diminished in those that report regular engagement in aerobic exercise.

Importantly, the mechanisms behind aerobic exercise and its influence on the peripheral vasculature have been extensively studied and provide strong support for the beneficial

role of exercise in cerebrovascular health and function. Aerobic exercise is associated with less large-artery stiffness and increased arterial compliance and elasticity.[9] Decreased arterial stiffness is a result of functional changes in vascular smooth muscle tone and structural changes to the atrial wall and extracellular matrix components.[43]

This is critical as it supports the concept that aerobic exercise not only prevents arterial stiffness but also reverses any stiffening that occurs with age. Similarly, repeated studies demonstrate that aerobic exercise protects against the development of microvascular endothelia dysfunction.[33,34] These studies, in both young and older aerobically-trained adults demonstrate greater NO bioavailability as the primary driver of a healthier endothelial profile. Both rodent and human studies support the use of aerobic exercise to lessen oxidative stress as well.[35,36] Collectively, these systemic changes result in alteration to the vessel formation, which appears to be the mechanism at play for increased perfusion via increased cardiovascular fitness.

In aging, there is increased risk of cerebrovascular dysfunction due to stiffening of the large arteries and impaired endothelia function. These physiological changes result in decreased perfusion and CVR. Much of the research done in the peripheral vasculature and early cross-section work provide evidence that cerebrovascular targets may be adaptable to an aerobic exercise intervention and serves as the guide for our mechanistic framework. A major gap in our understanding of exercise is how the cerebrovasculature responds to improved cardiovascular fitness in sedentary older adults. We have established an effective exercise intervention that has improved both motor and cognitive function with a concurrent and correlated change in BOLD signal during related tasks. These robust changes are hypothesized to be due, in part, to improved vascular function. As a next step, we propose to examine changes in cortical perfusion as well as changes in CVR following a proven aerobic exercise intervention.

4. Specific Aims:

The study will enroll 134 older, sedentary adults. Participants will be pseudorandomized to one of two 12-week intervention conditions: Aerobic Exercise or Stretching.

Specific Aim 1: To determine the effect of a 12-week aerobic exercise intervention on changes in cerebral perfusion using arterial spin labeling in older Veterans.

Hypothesis: Compared to a non-aerobic intervention Control group, the aerobicallytrained group will have increased basal perfusion in areas that demonstrate age-related decline and are malleable to aerobic exercise (inferior frontal and motor cortices).

Specific Aim 2: To determine the effect of a 12-week aerobic exercise intervention on changes in cerebrovascular reactivity using a hypercapnic CO2 response test in older Veterans.

Hypothesis: Following the interventions, aerobically-trained older adults will have increased CVR to CO2 in the inferior frontal and motor cortices when compared to controls.

Specific Aim 3: To quantify the impact of cerebrovascular perfusion and cerebrovascular reactivity on the BOLD response during task-based (cognitive executive and motor) fMRI.

Hypothesis: Perfusion and CVR will account for a significant portion of variability in the BOLD response during task-based fMRI both before and after the intervention.
Measures for Specific Aim 3.

5. Design and Methods:

Video and Telephone Consent Process

To limit face-to-face interactions with participants, the study will be using Emory and VA approved video or audio communication to screen and consent. Some VA-approved virtual methods include Microsoft Teams Version 1.3+, Zoom 5.0 or greater, and Cisco WebEx version 40+. Recordings of the audio or visual communication will not be permitted. If the participant meets initial eligibility criteria, we will mail or email through VA Outlook using encryption two unsigned copies of the ICF/HIPAA. We will ensure the individual has enough time after receiving the document to read it before scheduled phone/video call. Trained staff will perform consenting process including speaking with the individual to discuss the study and highlighting each section of the consent form, allowing the participant an opportunity to ask questions before providing consent, and giving the participant enough time to consider being in the study. Study team will inform the individual that if they would like to take more time to consider the study, another telephone call can be scheduled. If the individual would like to participate, the participant will sign and date the document and return it to study team via mail or via email to the VA Outlook email address. If the participant chooses to return the signed consent via email, they are required to send the consent as a reply to the original VA encrypted message sent by study staff. The study team will write a “Note to File” documenting everything about the interaction including:

1. When and how the consent form was sent
2. When the video/telephone call was made
3. What was discussed during the call
4. When the signed consent form was received
5. When the signed consent form was signed by the person obtaining consent
6. When a copy of the consent form signed by both subject and study team was given to the participant
7. A description of why signature dates are different (if applicable)

Once the study team receives the signed consent form, the person obtaining consent should sign the form and date it for the day it was signed. Study procedures will begin

once the signed copy is received. A copy of the fully signed consent form will be given to the participant via mail or in person at the next scheduled visit.

If consent is virtual, the option for DocuSign will be offered to the potential participant. A DocuSign envelope (email containing the links to study documents) with a reminder to NOT sign the documents prior to the scheduled contact time to review the documents with study staff and have questions answered. At the scheduled time the study team member consenting will contact the potential participant via VA and Emory-approved video or audio communication to guide the patient through the opening the DocuSign envelope and will review the documents contained.

As per standard review of consent, the study team member will review the study consent. Trained staff will perform consenting process including speaking with the individual to discuss the study and highlighting each section of the consent form, allowing the participant an opportunity to ask questions before providing consent, and giving the participant enough time to consider being in the study. Study team will inform the individual that if they would like to take more time to consider the study, another telephone call can be scheduled. If the individual would like to participate, the study team member will guide the participant through completion of the fields in the study documents to sign them and finalize the documents. The study team member will then receive an email from DocuSign while still in the consent session with the participant and will verify it is completed correctly and accurately. The study team member will then guide the participant through downloading a copy of the signed consent documents to their local computer for their records. Thank them and conclude the call. The study team member will download a signed copy to the study folder on the VA network drive as the study record and a copy will be provided to Health Information Systems for upload to the medical record. The study team will document the consenting process in a "Research Consent Progress Note" in the VA medical record (CPRS or new system when we transition) everything about the interaction
including:
1. When and how the consent form was sent
2. When the video/telephone call was made
3. What was discussed during the call
4. When consent was reviewed virtually and signed via DocuSign with a copy downloaded to their local computer for their records.

Study procedures can begin once the DocuSign copy is received and documented

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. *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.

2. *Hopkins Verbal Learning Test*: Assess verbal learning and memory (immediate recall, delayed recall, delayed recognition).

3. *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.

4. *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.

Non-Verbal Executive Function

1. *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.

Crystallized Word Knowledge

1. *American National Adult Reading Test (ANART)*: reading test used to help assess intelligence.

All cognitive testing will take place in the primary laboratory of the 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.
7. Patient Health Questionnaire (PHQ-9) – 9-item instrument that screens for signs and symptoms of depression.

8. Pelvic Floor Distress Inventory Questionnaire (PFDI-20) – 20 item questionnaire that screens for bowel, bladder, or pelvic symptoms.

Video Conferencing for Assessments

To limit face-to-face interactions with participants, the study will be using Emory and VA-approved video or audio communication to screen and consent. Some VA-approved virtual methods include Microsoft Teams Version 1.3+, Zoom 5.0 or greater, and Cisco

WebEx version 40+. Recordings of the audio or visual communication will be permitted as described previously to facilitate scoring of the assessment data in an off-line manner. During appointment scheduling, the participant will be asked if they have access to a computer with video, microphone, and speaker capabilities with access to high-speed internet that supports this level of communication. Once equipment access is confirmed the participant will be eligible to participate in tele-assessment, instructions for accessing the tele-assessment session will be sent via encrypted email through the VA outlook server. Any test that can be administered via video should be administered at this time. If an assessment cannot be accomplished remotely, it may be administered on the day of an in-person visit.

Physical Function

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 400m 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 walkway 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 singles tasks 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.[37]

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.[38]). The battery will be administered by a trained and certified examiner.

4. *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)[44]. The questionnaire inquiries 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 [45].

5. *Sit and Reach Test:* This test assesses the flexibility of the lower back and hamstrings. Participants will sit on the floor and reach as far they can towards their toes. The test will be performed three times while measuring the distance reached.

6. *Functional Reach Test:* This test will assess participants' balance and functional mobility by having them stand perpendicular to a wall and reach out as far as possible.

7. *Leg Press:* An assessment of bilateral leg movement function will be completed using the Keiser Leg press.

8. To confirm the cardiovascular effect of the aerobic training participants will also perform a Maximal Treadmill Exercise Test or a Submaximal Treadmill Exercise Test.

Submaximal Treadmill Exercise Test: (For participants unable to complete a maximal test or if maximal testing is not indicated…) Submaximal exercise testing will be performed using an exercise protocol validated for this purpose (e.g., Naughton, modified Bruce, or similar). For graded protocols where participants progress through a series of stages in which speed, incline, or both are increased in a stepwise manner, the progression will be such that it allows the participant to adapt to a specific workload before advancing onto a more difficult stage. Termination of the test occurs if the participant requests to stop, the submaximal threshold is reached, other symptoms prohibit further exercise, or there is an absolute indication for termination of exercise testing.

Maximal Treadmill Exercise Test: Unless contraindicated, participants will undergo maximal exercise testing using an exercise protocol validated for this purpose (e.g., Bruce, Balke, or similar, or using a ramp protocol). Participants will progress through a gradual increase in work rate based on the specific protocol being used until they either become fatigued and decide to stop, develop other symptoms that prohibit further exercise (e.g., musculoskeletal pain), or there is an absolute indication for termination of exercise testing. Vital signs, including blood pressure

and heart rate, will be recorded at regular intervals throughout the test, and participants will be monitored with continuous electrocardiography. Participants may also be asked to report their rate of perceived exertion (RPE) at various points during the test. After testing, participants will be monitored until their heart rate and perceived exertion have normalized. A registered nurse (RN) or exercise physiologist (EP) will be present for the test duration.

9. *Electronic Activity Data*: Electronic activity data will be generated by Apple Watches functioning in two possible modes. Each mode has unique requirements for data security. The Watch will collect the participants' daily total activity level.

   Stand-alone: Some Apple Watches loaned to participants will be paired with VA furnished iPhones. When not loaned out to participants, these iPhones will remain at the VA in a locked and secure environment (12C-173b). Because the watches will be functioning at a remote location, they will be unable to sync the data they generate to an iPhone or a network cloud. Instead, our team has written code to preserve this data in formatted files on the watch until the watch is returned to our possession. The data stored on passcode protected Apple Watches is secured with AES-256 encryption.

   Once the watch is returned to the VA, it will be synced to the VA-issued iPhone. (iCloud backup will be disabled for apps handling Apple Watch generated data.) The data transferred will be devoid of participant identifiers except participant study ID, year of birth, timestamps, and Apple Watch device identifiers. It should be noted that although the timestamps contain dates, these dates are not directly related to significant life events (e.g. birth, death, hospital admission) but rather are continuously catalogued while the watches are on and not charging. Also, Apple Watch device identifiers are not perpetually unique to a participant because these watches are VA property and will be worn by numerous participants over time. Regardless, data stored on passcode-protected watches and phones will remain AES 256-bit encrypted.

   Once data files are located on a VA iPhone, they will be backed up to a study controlled Apple iTunes account that is linked to a study-provided Apple User identification (UID) and transferred to a disk-encrypted, password-protected VA laptop. (Local iTunes encryption is optional by default but will be enabled for our purposes.)

   Deployed with VA-issued iPhone: Some Apple Watches will be loaned to participants along with the iPhones to which they are paired. This mode is preferable to the Stand-alone mode; however, Stand-alone mode may be necessary if access to VA-issued iPhones is limited. Most data handling

procedures and precautions used for Stand-alone mode apply to this use-case as well, including use of participant Study ID, AES 256-bit encryption, and disabled iCloud backup. One exception is that data generated by the Apple Watch will be synced directly to the paired VA-issued iPhone (i.e. - our code writing files to the Apple Watch will not be used). The iPhones loaned to participants will not have data plans. Therefore, unless participants have Wi-Fi access, data will be retrieved from the iPhones as described for Stand-alone mode after the phones are returned to the study team (i.e. study-controlled iTunes encrypted backup, and ultimately, transfer to a VA password-protected computer). If participants have Wi-Fi access, study data will be transferred to a remote server (hosted by Emory University in a secure data center under a VA Data Usage Agreement) via SSL encrypted connections. Terms specified in the handling of research data in the Data Usage Agreement will apply.

To increase data security, de-identified user data will be encrypted using Apple's Secure Enclave coprocessor embedded in Apple's A9 series processors (in the iPhone SE and Apple Watch). A FIPS-140-2 FISMA High environment, the Secure Enclave runs the L4 microkernel which is signed by an ephemeral key and cnonce entangled to the unique ID (UID) of the user with antireplay initiated at login. Antireplay revokes all data back transformation of encrypted data without express sign-on through direct passcode authentication. This means that all data is encrypted throughout transmission until authenticated by the UID on an iOS device. The encryption scheme uses a dedicated AES-256-XNT engine. No software or firmware can read this data directly. Only after decryption by the silicon based cryptographic engine using the UID key can the data be accessed, and this is further restricted to devices sharing a group id (GID). This can only be completed on another iOS device that has the UID (which is controlled by the study coordinator) and the Secure Enclave decryption engine on a device with at least an A7 series encryption coprocessor. Apple synchronizes this encrypted, de-identified data on its secure Health environment located on cloud services. These data cannot be decrypted or read by any party that does not have access to the UID, generated at the outset of the study and resides with the study staff. Only study staff can read files from the Health environment due to this encryption. Even if the physical chips are removed from the device to access the data, the device UID that created would maintain encryption of the data rendering the files inaccessible. Apple's cryptographic architecture received NIST validation as FIPS-140-2 validated with Certificate #3148 (3/8/2018 - 3/9/2023).

For all modes, filenames contain dates and times indicating when the files were created for transmission/export. These dates/times do not necessarily reflect date and times that the data were generated.

Motor Testing

1. *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.

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

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

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

5. *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.

MRI

Structural Imaging: High-resolution T1-weighted and T2-weighted structural imaging will be obtained to assess Tissue Integrity Gradation via T2w T1w Ratio (TIGR) and will also serve as an anatomical reference for functional data. We will also collect Diffusion Weighted Images (DWI) to assess white matter integrity. During this time participants are given the option to view a movie to alleviate potential boredom.

Metabolic Imaging: Cerebral Blood Flow will be assessed with MRI scans that separate pure blood signal from surrounding tissue (e.g. pCASL or VSASL). If arterial transit time is a factor based on the imaging sequence, we will remove this confound by either using longer post-labeling delays or measuring multiple post-labeling delays. For a few minutes of scanning, we will ask participants to breath via a mask air richer in CO_2 than normal atmospheric air (5-8%). CO2 will be administered by study staff. The CO_2 reactivity procedures are tolerated by normal, elderly subjects and patients with neurological diseases such as history of strokes and have been performed numerous times in our imaging center without complications.

Functional Imaging: Integrity of functional brain networks will be assessed while the participant is lying still inside the scanner, and Blood Oxygen Level Dependent (BOLD) fMRI signals are collected. Alternatively, the participant may be asked to perform either a language task or motor task while BOLD fMRI data is collected, depending on the question of interest.

Magnetic Resonance Spectroscopy: Magnetic resonance spectroscopy will be performed on selected large (e.g. 4-27 mL) voxels to estimate quantities of various neurochemicals. γ-aminobutyric acid (GABA) and glutamate are the primary neurochemicals of interest, though other substances (e.g., N-acetylaspartate, creatine) may also be measured.

Regions of interest include, but are not limited to, the DMN (medial and lateral structures), motor cortex (M1), inferior frontal gyrus, and fusiform gyrus.

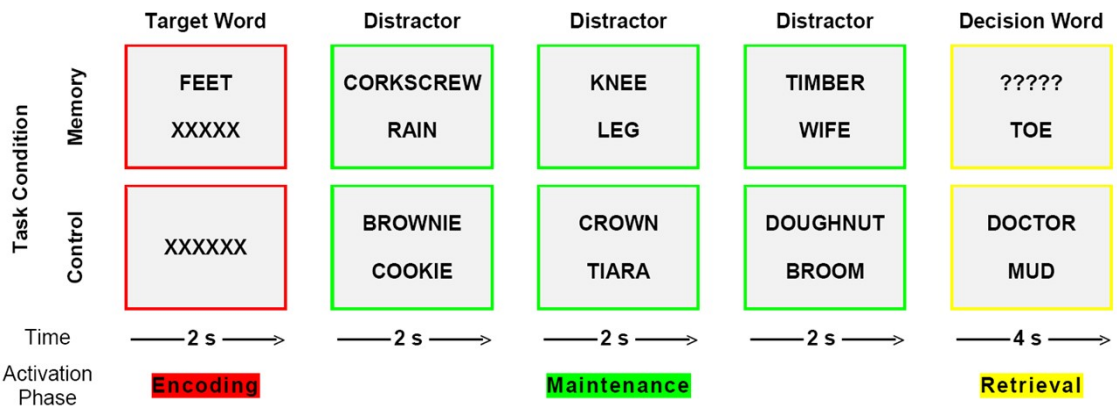
No exogenous contrast agent will be used during any of the MRI and MRS protocols.

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 consist 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 produce 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. A 10-second blank screen inter-trial interval will be 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 investigator's 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 FastField 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. 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 Hospital (EUH). EUH 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 real time motion correction.

Blood Draws and Biomarker Evaluation

Whole blood will be collected, by a certified phlebotomist, through one venous port 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 of the Atlanta VAHCS. Serum and plasma aliquots will be analyzed for biomarkers (e.g., BDNF) by ELISA, western immunoblotting, and other standard molecular biology assays.

Saliva will be collected at study assessment visits in a 15mL sterilized tube; roughly 3mL. Once collected, saliva will be immediately placed on ice and stored at -80 degrees until subsequent analysis.

The procedure below describes the blood and saliva collection timeline during the 12-week intervention period for both intervention groups:

> *Week 1*: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post.
>
> *Week 6*: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post.
>
> *Week 12*: Taken immediately prior to exercise, immediately post, 15 min post, 30 min post.

All blood draws will be conducted at the Atlanta VA Health Care System. 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 VAHCS 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 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 VAHCS office of the PI or Coinvestigator. Deidentified sample remainders will be kept at the Atlanta VA Core facility or, if needed, the Emory facility, while the study remains open.

Finger prick blood measurements
Similar to a glucose meter, a hematocrit (Hct) meter requires a very small droplet of blood to measure hemoglobin and Hct using a photometer with broad spectrum. We will use an analyzer called the HemoPoint H2 from StanBio Laboratory. This device is a palm-sized blood hematocrit test meter that quickly measures hemoglobin and Hct from a small blood sampling (0.3µl). Measurements are completed in 30 sec, so this measurement can be completed rapidly and repeatedly within a single session. Prior to each puncture, we will cleanse the target skin site with an alcohol swab. We will use a 28 or 30G lancet to draw a droplet of blood into a measurement strip for each measurement. After recording the data, we will discard the lancet and the lactate strip into a Sharps container for safe disposal. We will apply direct pressure to the site of puncture with a sterile gauze pad. The puncture procedure will attempt to minimize pain and discomfort for the participants. The Hct measures will be utilized along with MRI-based pCASL measures to accurately quantify the subject's CBF.

Interventions
Aerobic Exercise Group: For this arm of intervention, randomized participants will attend spin exercise 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 25 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.

Interval Exercise Group: For 12-weeks participants will engage in progressive whole body stretching, toning and balance exercises designed for individuals 65 years and older. Participants will meet for the same total duration time as the aerobic exercise group. Heart rate will be monitored to ensure participants maintain exercise levels below aerobic training zone.

Remote Exercise Monitoring: As an alternative, participants that are uncomfortable with receiving face-to-face training within the hospital will be given the option to continue their exercise intervention visits remotely. For participants that have been randomly assigned to the 'spin' exercise training program, will be provided a stationary cycle ergometer that has been sanitized properly to use during the 12-week period. All subjects will be monitored and trained through VA and Emory approved remote access sites.

Control Group: A random subset of participants will be in a 12-week wait-list control. These participants will undergo the same scanning procedures as described above without cognitive or physical function testing. After 12-weeks these participants will come in and undergo the pre-assessment, 12-week exercise intervention and post assessment as described above.

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. Because the lab space is housed in the Atlanta VAHCS, 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. A second location for exercise classes is the Geriatric Research

Education and Clinical Center (the GRECC), an offsite Atlanta VA affiliate facility, that follows the same rules and regulations as the rest of the Atlanta VA Healthcare System.

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.[39] 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.[40] 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.

The reported potential side effects of the CO_2 mask may include a feeling of dizziness, faintness, or anxiety during CO_2 inhalation.

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.

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.

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

6. Participant selection:
The study will enroll sedentary older adults and young adults as a comparison group for the MRI scans. Older adult participants will be pseudo-randomized to one of three 12week intervention conditions: Aerobic Exercise, Interval Exercise, or Control. The pre and post evaluations will be conducted over 2 days during a 1-week period. Each day will last no more than 2 hours. The investigators expect a dropout rate of anywhere from 15-20% of participants. To ensure completion of study by 108 participants, as many as 134 subjects may be enrolled.

Inclusion/Exclusion Criteria
Prior to beginning any portion of this study, we must receive a physician, physician assistant, or nurse practitioner's written medical approval for participation in the fitness assessment and/or exercise intervention. We will provide a letter to sign explaining the study protocol. The key inclusion criteria and final participant pool will consist of right-handed English-speaking individuals ages 18 to 80. Older participants will be sedentary as defined by <40 min/week of aerobic exercise. Additionally, participants will be non-demented 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. Participants must not have conditions incompatible with MRI (including but not limited to, ferrous metal implants, cardiac pacemakers or similar devices, claustrophobia, morbid obesity). Potential participants with major psychiatric disorder (including but not limited to psychosis, major depression, bipolar disorder) by history will be excluded.

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 database 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.

In order to decrease burden of the potential recruitment pool of participants, cognitive, motor, and physical function measurements will be shared between this study Effects of Acute Exercise on Functional Magnetic Resonance Spectroscopy Measures of GABA in Aging and Chronic Stoke (IRB00001334) and Graded Intensity Aerobic Exercise to Improve Cerebrovascular Function and Performance in Aged Veterans (IRB00106626) as all studies administer the same assessments.

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.

CDW/VINCI data will be used to identify potential research participants. Veterans who are age 65 or older and who live within 20 miles of the Atlanta VA Health Care System will be identified and contacted via mail and then subsequently by phone. Addresses and phone numbers will be used for recruitment only and the information will be verified as current and accurate in CPRS prior to use.

Additionally, participants may be recruited from word of mouth, newspaper advertisement, and study flyer distribution within the Atlanta VA Health Care System and surrounding community.

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-Investigator or their trained and qualified colleagues will go over and describe the consent in a private office of the PI/Coinvestigator.

7. Statistical Analysis:

Statistical Analysis for Specific Aim 1

Specific Aim 1 is to determine whether there will be a statistically significant difference in the cerebral perfusion data between the two groups (Spin and Control) following the intervention. We will assess the perfusion values jointly using a linear mixed-effects model to determine whether the mean change in perfusion between the groups is significantly different. For the model the fixed effect will be group (Spin vs Control) and the random effect will be time (Pre vs Post). We expect that modeling the outcomes jointly will increase the statistical power. This model will enable us to estimate the

overall effects of time and the interventions, and most importantly, how the effects of the interventions differ with time. Additional analyses will use multiple regressions to describe the effect of participant demographics such as gender, race, age, hypertensive status as well as MoCA score. A false discovery rate (FDR) of q = 0.05 will be calculated and used to correct for multiple tests and effect sizes will be computed.

Statistical Analysis for Specific Aim 2

Specific Aim 2 is to determine the impact of an aerobic intervention on CVR. Similar to Aim 1, we will test each BOLD change value jointly using a linear mixed-effects model to determine whether the mean change of the groups is significantly different across the time points (Pre and Post). Fixed effect will be group (Spin vs Control) and the random effect will be time (Pre vs Post). Additional analyses will use multiple regressions to describe the effect by participant demographics (gender, race, age, and hypertensive status) or by MoCA score. An FDR of q = 0.05 will be calculated and used to correct for multiple test and effect sizes will be computed.

Statistical Analysis for Specific Aim 3

The task fMRI BOLD images will be processed for slice-timing correction, and each image volume will be corrected for rigid-body head motion. The volume registered images will be inspected for artifacts induced by motion, cardiac pulsation and respiratory cycles and removed spatio-temporally using Independent Component Analysis (ICA). These artifact-free images will then be spatially transformed to MNI space using linear and non-linear co-registration algorithms. Following this spatial transformation, the images will be smoothed within the brain to increase the signal-tonoise ratio. Then the images will be scaled to accurately determine the relative percent signal change in BOLD induced by the task. Finally, task-induced BOLD changes (ΔBOLD) from pre- to post-aerobic intervention and corresponding hemodynamic response functions are quantified using deconvolution technique.

Since the goal of this aim is to quantify the impact of baseline physiologic measures (ie, resting blood flow and CVR) on task-induced BOLD changes, we plan to accomplish this task by modeling the contributions of perfusion and CVR to ΔBOLD responses. The equations below describe our simple modeling approach using linear regression:

$$\Delta BOLDi,ROI = A + B \cdot CBFi,ROI + C \cdot CVRi,ROI + D \cdot (CBFi,ROI \times CVRi,ROI)$$

Where subscript i=pre or post session, ROI refers to the regions of interest, and coefficients A is the modeled intercept, B and C are modeled slopes for perfusion and CVR contributions, and D is modeled slope for the interaction between perfusion and CVR respectively. We will compare the slopes obtained for pre and post exercise to explore (a) how each of the baseline measures (ie, perfusion and CVR) change in response to exercise, and (b) within each condition (pre or post), disentangle perfusion and vascular influence on task-induced BOLD change. Also, modeling for the interaction will account for the coupling effect which by itself can provide insightful information in terms of exercise-induced perfusion-CVR coupled effects. We plan to carry out the

above processing and modeling at the ROI level as it renders more detection power and is computationally less intensive. From a statistical standpoint, we will compute the F score to test the significance of overall fitting of the model, and we will also obtain the *t* score for each predictor (perfusion and CVR) to test the significance of their association with change in measured variable (ΔBOLD). To evaluate responsiveness to training and its impact on the model, post hoc analysis will include stepwise multiple regression to evaluate delta VO2 on perfusion and CVR in a-priori ROIs as well as behavioral outcomes.

Cardiovascular Fitness Assessment

The YMCA test uses an "extrapolation" method in which heart rate workload values are obtained at two to four points and extrapolated to predict workload at estimated maximum heart rate (220 - age). VO_2 max is then calculated from predicted maximum workload. For YMCA submax, *R*=0.86, SEE=10% of the predicted when compared to $V0_2$ max protocols.[37] In addition to this calculation, inspired and expired gases will be sampled during the YMCA test by a COSMED metabolic CPET via a facemask. Oxygen and carbon dioxide excretion will be measured allowing for $\%VO_2$ throughout the test as well as a measurement anaerobic threshold.

Behavioral and Self-Report Data

The analysis plan for behavioral performance will include the following dependent variables: semantic fluency performance and the motor tests, MOCA, and $V0_2$ submax, and each self-report outcome. Paired t-tests will be performed to test for a pre- to post effect on fluency and motor skills. Similarly, subscale scores and total scores will be evaluated pre to post intervention for each self-report measure. Spearman's correlations (rs) will be used to correlate behavioral performance to primary outcomes and a mixed regression will be used to assess the influence of session (pre and post) on outcomes.

REDCap

This study will utilize REDCap issued through the Atlanta Veterans Health Care System. Only deidentified data will be entered in REDCap by VA credentialed research personnel to run data analysis.

Power Analysis and Sample Size

For Aim 1, we used preliminary data comparing older active adults to older sedentary adults. Effect size for difference in inferior frontal perfusion for a small sample (n=10) was d=-2.740. If this effect size is taken as an estimate of the effect size for change in the Spin group with n=44 and α FDR-corrected to 0.0261, then power approaches 1.00. A more conservative estimated change in perfusion being half the effect, with the standard deviation remaining constant, would produce an effect size of 1.370. With the same n and α as above, the power still approaches 1.00. The required effect size to achieve a power of 0.80 would be d=0.5, which would seem quite achievable given our preliminary data. Hence, the power should be more than adequate and seems plausible based on what we know. Because we want to maintain sensitivity at this level, the study is powered mainly to this Aim, while power for Aims 2 and 3 is more than adequate. For

example, for Aim 3, with α < .05, minimum power of 0.80 and 88 subjects, we have the power to detect an increase in R^2 as small as 0.111 in each ROI. We have detected significant correlations between BOLD response in M1[41] and IFG[42] with smaller sample sizes (n=36 and n=41, respectively). Accounting for much less than 11% of the variance in criterion variables with the predictors would be too little variance to provide information useful for the design of future clinical trials. Hence, we should have the power to detect effects large enough to impact future clinical trials.

For behavioral data, we estimate the effect size for semantic fluency from 2 sources of data using the same treatment we will use for this study. For our lab the effect size for change in the Spin group was d=1.804, and for the preliminary data from an ongoing study the effect size for change in semantic fluency was d=1.410[13] Using the more conservative of the two estimates, n=44 and a FDR corrected α=0.0261, power approaches 1.00. Again, an effect size of d=0.5 would achieve a power of 0.80, which seems quite achievable given the large effect sizes in two independent samples. Use of the Control in this instance is problematic because of a large negative outlier, yielding a large negative mean (-2.000, indicating a loss of semantic fluency) and a large standard deviation. Even so, these data yield an effect size of d=0.886, but these data are not stable enough to yield a good estimate of between-group effect size. With n=44 per group and α=.05 (FDR corrected), an effect size of d=0.64 would yield a power of 0.80. Based on the strong effect size for Spin, we believe this is an achievable effect size for a between-group analysis based on what we know about the Spin intervention.

8. Adverse event reporting:
In the case of a reportable event, the Principal Investigator will follow the current Atlanta VA Research and Development operating procedure for reporting. This includes notifying the Emory IRB within the appropriate time period of any adverse event occurring.

All study staff will be trained on proper reporting procedures of adverse events (i.e.- contact emergency medical services for immediate medical emergency and later the Principal Investigator, if not present). The PI will notify the IRB and VA SIO of any reportable events.

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).**

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;
- 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).
- 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, physician assistant, or nurse practitioner 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 ensure 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. A research ID number will be used. The research records will be 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. Identifiers might be removed from the identifiable

private information that are collected. After that removal, the information could be used for future research studies. 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.

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