

Graded Intensity Aerobic Exercise to Improve Cerebrovascular Function and Performance in Aged Veterans: Protocol for a Randomized Controlled Trial

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Submitted to: JMIR Research Protocols
on: March 15, 2024

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Abstract

Background: The growing healthcare challenges from a rapidly expanding aging population necessitates examination of effective rehabilitation techniques that mitigate age-related comorbidity and improve quality of life. To date, exercise is one of a few proven interventions known to attenuate age-related declines in cognitive and sensorimotor functions critical to sustained independence. This work aims to implement a multi-modal imaging approach to better understand the mechanistic underpinnings of the beneficial exercise induced adaptations to brain and behavior in sedentary older adults. Due to the complex cerebral and vascular dynamics that encompass neuroplastic change with aging and exercise, we propose an imaging protocol that will model exercise induced changes to cerebral perfusion, cerebral vascular reactivity (CVR) and cognitive & sensorimotor task-dependent fMRI after prescribed exercise.

Objective: Our hypothesis is that the 12-week aerobic exercise intervention will increase basal perfusion and improve CVR as measure by increased magnitude of reactivity in areas susceptible to neural and vascular decline (inferior frontal and motor cortices) in previously sedentary older adults. To better understand the neural versus vascular adaptations in the motor and inferior frontal cortices, we will map changes in basal perfusion and CVR over target regions of interest (inferior frontal and the motor cortices) that we have demonstrated to be beneficially altered during fMRI BOLD (verbal fluency and motor tapping) by increased cardiovascular fitness.

Methods: Sedentary adults (aged 65-80) will be randomly assigned to either a 12-week aerobic-based, interval-based cycling intervention or a 12-week balance and stretching intervention. Assessments of cardiovascular fitness using the YMCA submaximal VO₂ test, basal cerebral perfusion using arterial spin labeling (ASL), CVR using hypercapnic fMRI, cortical activation using fMRI during verbal fluency and motor tapping tasks, and a battery of cognitive-executive and motor function tasks outside of the scanning environment will be performed before and after the interventions.

Results: Our own studies and others show that improved cardiovascular fitness in older adults' results in improved outcomes related to both physical and cognitive health as well as quality of life. A consistent but unexplained finding in many of these studies is a change in cortical activation patterns during task-based fMRI that corresponds with improved task performance (cognitive-executive and motor).

Conclusions: To date, exercise is one of the most impactful interventions aimed to improve physical and cognitive health in aging. This study aims to better understand the mechanistic underpinnings of improved health and function of the cerebrovascular system. If our hypothesis of improved perfusion and cerebrovascular reactivity following a 12-week aerobic exercise intervention are supported, it would add critically important insight about the potential of exercise to improve brain

health in aging and could inform exercise prescription for older adults at risk for neurodegenerative disease brought on by cerebrovascular dysfunction.

(JMIR Preprints 15/03/2024:58316)

DOI: <https://doi.org/10.2196/preprints.58316>

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Medina Bello; Kevin Mammino; Mark Vernon; Daniel Wakeman; Chanse Denmon; Lisa Krishnamurthy; Venkatagiri Krishnamurthy; Keith McGregor; Thomas Novak; Joe R. Nocera

Background

The growing healthcare challenges from a rapidly expanding aging population necessitates examination of effective rehabilitation techniques that mitigate age-related comorbidity and improve quality of life. To date, exercise is one of a few proven interventions known to attenuate age-related declines in cognitive and sensorimotor functions critical to sustained independence.

Objective

This work aims to implement a multi-modal imaging approach to better understand the mechanistic underpinnings of the beneficial exercise induced adaptations to brain and behavior in sedentary older adults. Due to the complex cerebral and vascular dynamics that encompass neuroplastic change with aging and exercise, we propose an imaging protocol that will model exercise induced changes to cerebral perfusion, cerebral vascular reactivity (CVR) and cognitive & sensorimotor task-dependent fMRI, functional magnetic resonance imaging, after prescribed exercise.

Methods

Sedentary adults (aged 65-80) were randomly assigned to either a 12-week aerobic-based, interval-based cycling intervention or a 12-week balance and stretching intervention. Assessments of cardiovascular fitness used the YMCA submaximal VO₂ test, basal cerebral perfusion using arterial spin labeling (ASL), CVR using hypercapnic fMRI, cortical activation using fMRI during verbal fluency and motor tapping tasks, and a battery of cognitive-executive and motor function tasks outside of the scanning environment will be performed before and after the interventions.

Results

Our own studies and others show that improved cardiovascular fitness in older adults' results in improved outcomes related to both physical and cognitive health as well as quality of life. A consistent but unexplained finding in many of these studies is a change in cortical activation patterns during task-based fMRI that corresponds with improved task performance (cognitive-executive and motor). Our hypothesis is that the 12-week aerobic exercise intervention will increase basal perfusion and improve CVR as measure by increased magnitude of reactivity in areas susceptible to neural and vascular decline (inferior frontal and motor cortices) in previously sedentary older adults. To better understand the neural versus vascular adaptations in the motor and inferior frontal cortices, we will map changes in basal perfusion and CVR over target regions of interest (inferior frontal and the motor cortices) that we have demonstrated to be beneficially altered during fMRI BOLD, Blood Oxygen Level Dependent, (verbal fluency and motor tapping) by increased cardiovascular fitness.

Conclusion

Exercise is one of the most impactful interventions aimed to improve physical and cognitive health in aging. This study aims to better understand the mechanistic underpinnings of improved health and function of the cerebrovascular system. If our hypothesis of improved perfusion and cerebrovascular reactivity following a 12-week aerobic exercise intervention are supported, it would add critically important insight about the potential of exercise to improve brain health in aging and could inform exercise prescription for older adults at risk

for neurodegenerative disease brought on by cerebrovascular dysfunction (Registration: ClinicalTrials.gov NCT05932069, <https://clinicaltrials.gov/study/NCT05932069>).

Introduction

The rapid growth in an aging global population presents significant healthcare and fiscal challenges due to increased prevalence of age-related neurodegenerative disease and disability. For example, of the 60 million Americans over 65 years of age, it is estimated that one in three of these older adults will be diagnosed with Alzheimer's disease (AD) or a related dementia in their lifetime. Intertwined with aging-related pathologies are physiological changes in cerebrovascular function [1]. In fact, recent epidemiological and clinico-pathological data indicate considerable overlap between cerebrovascular dysfunction in aging and AD [2-3]. While AD is an overt pathology, its "sub-clinical" progression appears to take decades before crossing a diagnostic threshold [4]. This sub-clinical stage spans a significant timeframe for targeted interventions aimed at limiting vascular dysfunction in high-risk older adults.

It has been demonstrated that of all modifiable risk factors for dementing illness, decreasing sedentary behavior is the most statistically significant and effective measure to counter disease progression and associated cognitive decline in older adults [5]. For example, nearly a quarter million cases of AD could be prevented in the US alone by improving the cardiovascular fitness profile in older adults. This staggering number is an exciting target not only for prevention during aging 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 [6].

This study will explore the impact of aerobic exercise on cerebrovascular health in older adults. Research over the last few decades has driven the continual promotion of exercise as one of the most impactful interventions of central nervous system health and function [7-9]. We know that older adults who are physically active have improved peripheral

vascular health but the impact of an exercise intervention on cerebrovascular health is less known. We will fill this gap by examining changes in basal cerebral perfusion and CVR in older adults following a proven cardiovascular fitness intervention [10-13]. If our hypotheses of improved perfusion and CVR are supported, it would inform 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 older adults at risk for neurodegenerative disease brought on by cerebrovascular dysfunction.

Methods

This study design followed a parallel group design where participants engaged in exercise for 12-weeks.

Participants

Older adult participants were pseudo-randomized to one of two 12-week interventions: Interval-based, aerobic 'Spin' cycling exercise, or non-aerobic, balance and stretching exercise to serve as an active control condition. Each intervention was group based and in person with trained personnel leading each class. Prior to beginning any portion of this study, we must receive written approval by a qualified physician for participation in the fitness assessment and exercise intervention.

The key inclusion criteria and final participant pool will consist of right-handed, English-speaking individuals aged 65 to 80. Participants must also self-report a sedentary lifestyle defined as not participating in at least 30 minutes of moderate-intensity physical activity on at least three days/week for at least three months. Additionally, participants will be non-demented and have no deficits in cognitive-executive function (MoCA \geq 26). Those with severe diabetes requiring insulin will also be excluded, however, individuals with controlled diabetes that meet our inclusion criteria for sedentariness and cognitive function will be allowed to participate. Individuals with any conditions contraindicated by MRI acquisition (including but not limited to, ferrous metal implants, cardiac pacemakers or similar devices, claustrophobia, morbid obesity), and those with any history of major psychiatric disorder (including but not limited to psychosis, major depression, bipolar disorder) will also be excluded.

Measures

Pre and post evaluations of cardiovascular fitness, cognitive & motor function, and brain imaging will be conducted over 2 days (< 3 hours per day) within a 2-week timeframe.

Day 1: Cognitive/Motor and Physical Function Assessments

Table 1 highlights each of the physical, cognitive, and motor assessments performed in the study. Each of the indicated outcomes will be assessed before and after the 12-week interventions. This pre/post repeated measures design will be conducted on all randomized sedentary, older adult (65-80yo) participants.

System(s)	Assessment	Description	Outcomes	Component Function	Relevant Cortices
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Cardiovascular, Motor	Modified Balke VO2 test	Treadmill test where participants walk at a constant 3mph, and treadmill grade is increased by 2.5% every 2 minutes until 90% of estimated heart rate max ((220-age)*0.90) is achieved.	Estimated VO2 max, Peak VO2	Cardiovascular fitness, gross movement economy	Whole brain (gray matter)
Cognitive-Executive	Montreal Cognitive Assessment (MoCA)	This brief instrument asks a series of questions to screen for cognitive impairment.	Scaled score (0-30). < 26 indicates cognitive impairment and is a study disqualifier.	Visual/Executive, naming, memory, attention, abstraction, orientation	Frontal, Temporal, Parietal
	Digit span forward/backward	Participants are provided a sequence of digits and prompted to immediately repeat the sequence as heard (forward), or in reverse order (backwards). If repeated correctly, the next trial is presented with a longer sequence. The task ends with 3 incorrect response attempts at a given digit span.	Longest digit span repeated correctly	Working memory	Frontal, Temporal, Parietal
	D-KEFS: Verbal fluency	Letter fluency- participants recall words beginning with a specified letter as quickly as possible. Category fluency- recall words belonging to designated semantic category.	# of words within 60s	Letter fluency, semantic fluency	Frontal (Inferior Frontal Gyrus (IFG))
	D-KEFS: Color word interference (Stroop)	Inhibition- Participants are presented with the words "red", "green", "blue" printed incongruently in red, green, or blue font. They are instructed to state the font color as quickly as possible while minimizing mistakes. Switching- The same words with incongruent font color are presented to participants, however, some words will be presented with a box outlining them. Participants are instructed to state the font color in non-outlined text conditions, and instead read each word aloud (as opposed to font color) when the word is outlined by a box.	Total time + (total time/100) x # uncorrected errors	Response Inhibition, response switching	Frontal, Temporal, Parietal
Cognitive, Motor	D-KEFS: Trail making test	Participants are timed as they connect numbers and letters in ordered sequences	Time to completion (seconds)	Cognitive flexibility, visual sequence tracking	Visual, Primary Motor, Frontal
	Computerized N-back	Participants are provided a target letter before this task. They are then presented with a series of individual letters on a computer screen. They are instructed to denote, via keyboard response (1-"No", 2-"Yes"), whether a presented letter corresponds with the target letter. 0-back condition requires response based on the letter immediately presented to them, and 2-back condition requires response based on the letter presented 2 steps behind the immediate letter presented.	Response accuracy (% correct), Reaction Time (ms)	Working memory, processing speed, manual dexterity	Visual, Primary Motor, Frontal

Day 2: MR Environment

MRI Acquisition. MRI data will be collected on a 3T Siemens Prisma fit (Erlangen, Germany). This will consist of an anatomical T1-weighted MPRAGE scan (TE = 3 ms, TR = 2500 ms, flip angle = 7°) and seven fMRI scans for each subject: One fMRI scan collected during dual-echo pseudo-continuous pCASL (TE1/TE2 = 10/25ms, TR = 4000ms, one fMRI scan collected during CVR (TE = 21 ms, TR = 1000 ms, voxel size = 3mm x 3mm x 3mm, flip angle = 50°, number of volumes = 420), three runs of a language based task (TE = 25 ms, TR = 5800 ms, voxel size = 3mm x 3mm x 3mm, flip angle = 70°, number of volumes = 71) and two runs of a motor based task (TE = 25 ms, TR = 2000 ms, voxel size = 3mm x 3mm x 3mm, flip angle = 70°, number of volumes = 154).

Preprocessing. Preprocessing of anatomical T1w and fMRI data will be conducted utilizing fMRIPrep 22.0.22 (RRID:SCR_016216), which is based on Nipype 1.8.5 (RRID:SCR_002502). The pipeline included brain extraction, tissue segmentation and spatial normalization to MNI template space for the T1w. Regarding the fMRI data, preprocessing involved estimation of BOLD image and head-motion, slice timing correction, registration to the T1w image and resampling to MNI space (see Supplementary Materials for full preprocessing pipeline details).

Semantic Fluency: During the MRI session, participants will perform 3 blocks of a verbal

fluency task programmed using via E-Prime 3.0 software (Psychology Software Tools, Pittsburgh, PA) [14] (Figure 1). At the start of each block, the phrase “please wait” is projected to participants via a 20-inch HP computer display for 20s. The task is initiated when this phrase is replaced by a word corresponding to a specific semantic category. This category disappears and reappears 8 separate times, and participants are instructed to verbalize a single response corresponding to this category each time it reappears on the screen. Participants are also instructed to avoid repeating any responses from the same category. A single task block consists of 6 separate categories (requiring 8 individuated/non-repeating responses per category). Interleaved between each semantic category is a “reading” condition. Here, participants are presented with the word “Rest” 4 separate times and instructed to verbally respond with the word “Rest” each time it appears. During these scans, verbal response will be manually recorded by an investigator, and subsequently scored based on the total number of correct (and non-repeating) responses divided by the number of response windows provided (i.e. Block Score: $(\# \text{ correct} / 48 \text{ response windows}) * 100$, Session Score: $(\# \text{ correct} / 144 \text{ response windows}) * 100$).

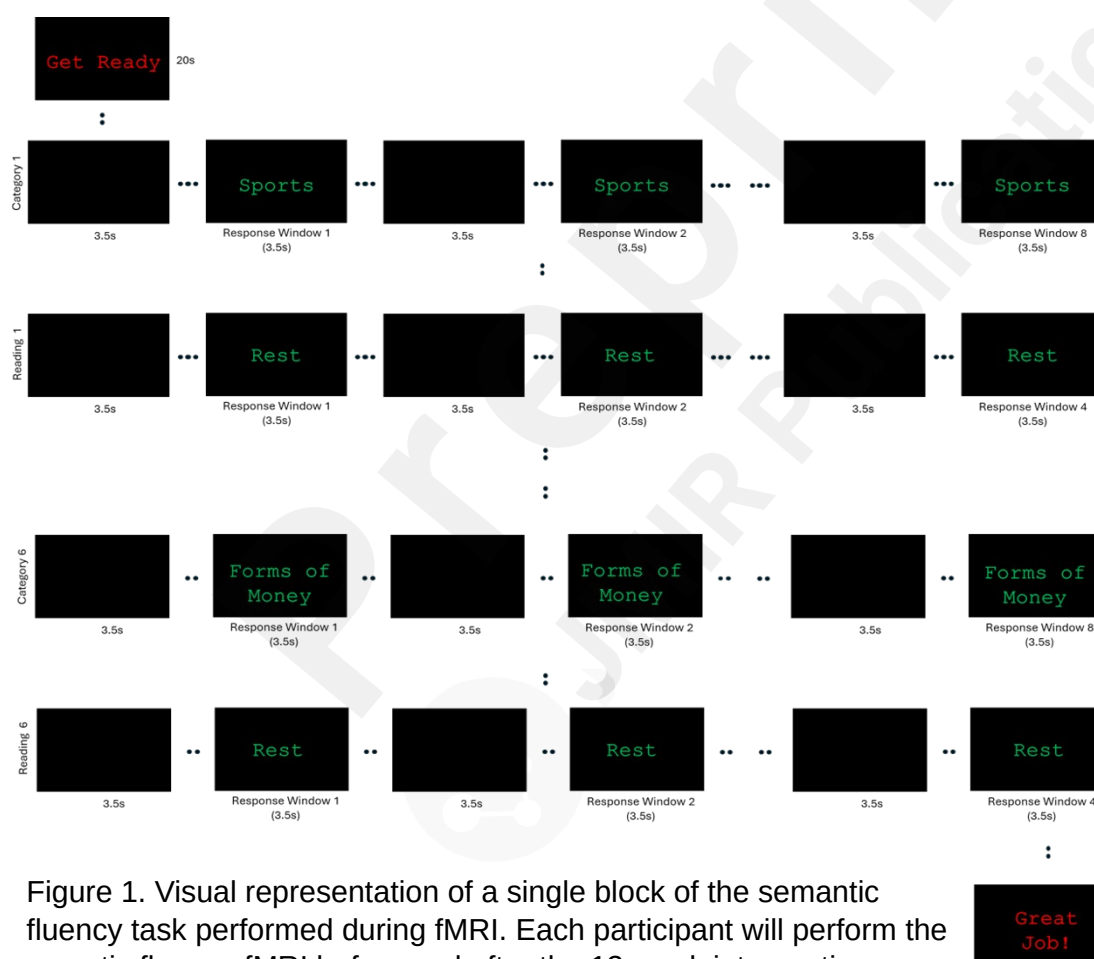


Figure 1. Visual representation of a single block of the semantic fluency task performed during fMRI. Each participant will perform the semantic fluency fMRI before and after the 12-week interventions. This pre/post repeated measures design will be conducted on all randomized sedentary, older adult (65-80yo) participants.

Motor Tapping: Participants will also perform 2 blocks of a motor tapping task programmed via PsychoPy [15] and using an MR compatible 4-button box (MagConcept response unit) (Figure 2.). Briefly, individuals are presented with a red dot in the center of the projected screen for 15s. The task will commence when this dot turns green, and subsequently alternates between red and green at a frequency of 1 Hz. Participants are instructed to press the button oriented to their index finger at the pace of the flashing green dot. Each block consists of five 28s tapping intervals separated by six 28s rest intervals. During these rest intervals, a solid red dot is projected, and participants are instructed to do nothing until they are presented with the next sequence of green dots.

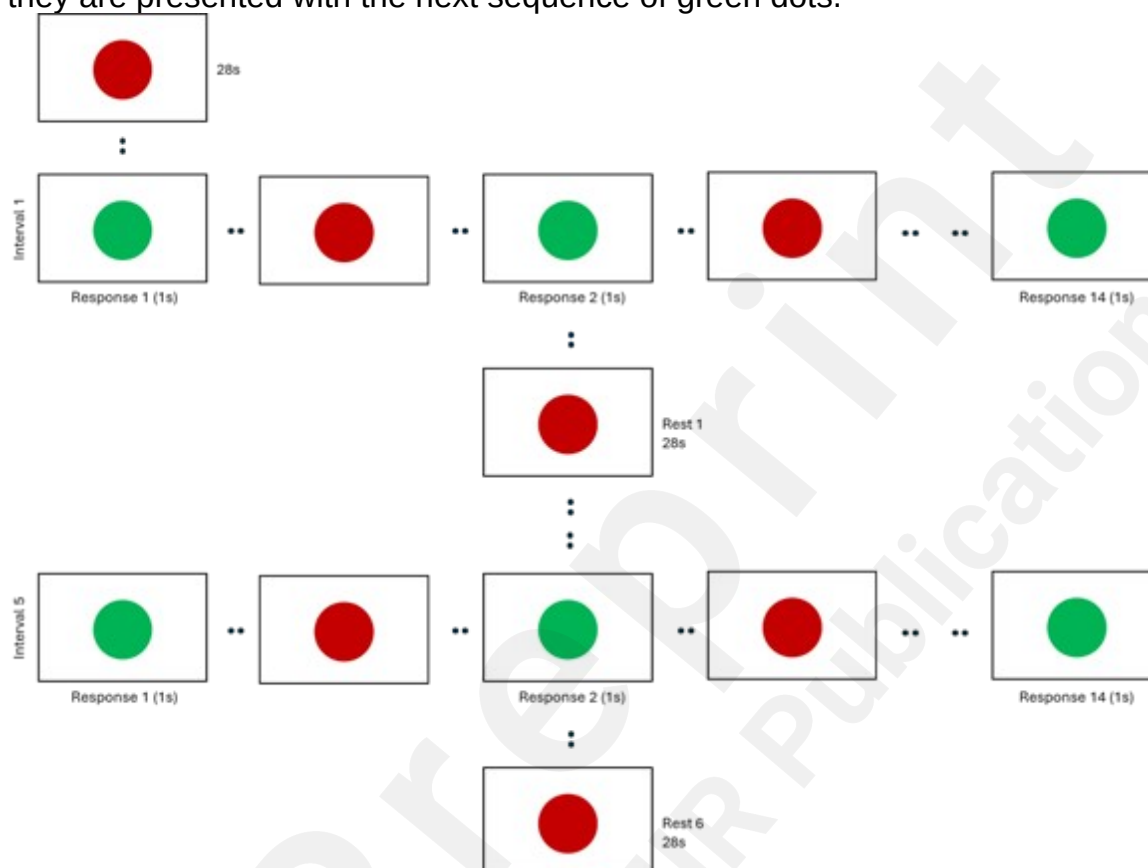


Figure 2. Visual representation of a single block of the motor tapping task performed during fMRI. Each participant will perform the semantic fluency fMRI before and after the 12-week interventions. This pre/post repeated measures design will be conducted on all randomized sedentary, older adult (65-80yo) participants.

The ASL sequence acquisition first magnetically labels blood water at the region of interest by applying a 180-degree radiofrequency inversion pulse. The labeled water exchanges with tissue water, which alters the tissues magnetization and the image intensity creating a "tag" image. This process is repeated without labeling blood to create a "control" image. Subtracting the control and tag images produces perfusion imaging which reflects the amount of arterial blood delivered to voxels within a given region of interest. This difference signal is directly proportional to cerebral blood flow and can be mapped on a voxel-wise basis to obtain regional blood flow information. To convert the difference signal (=control-label) into physiological units, a single compartment model is utilized to obtain units of mL/100g/min. The ASL scan lasts approximately 7 minutes.

The aforementioned sequence parameters to be used for ASL acquisition is standard for assessing blood flow maps of the whole brain along with our regions of interest (inferior frontal and the motor cortices). The Harvard-Oxford cortical atlas developed in MNI 152

atlas space will be the starting point for the frontal region of interest. The resulting ASL with corresponding T1-weighted images will be registered into MNI 152 atlas space using the nonlinear algorithms from FMRIB Software Library (FSL) Images will be imported into AFNI for analysis.

CVR will be assessed using a block-design hypercapnic response paradigm where participants alternate between breathing room air and a 5% CO₂ gas mixture during fMRI (see a detailed description in [16]). Participants will be fitted with a nose clip and mouthpiece attached to a two-way breathing valve that affords inhalation of normal room air, or CO₂ mixture filled in a 100L Douglas bag. Additional physiological parameters, including end tidal (Et) CO₂ and breathing rate will be continuously monitored and recorded using a Philips NM3 Capnograph. At the onset of this scan, participants will inhale normal room air for 50s. A research staff member will then manually switch the valve to CO₂ air for 50s, and subsequently alternate between breathing conditions until three 50s blocks of CO₂ breathing, and four 50s blocks of room air breathing have been collected (total scan duration ~7min).

Provided Et-CO₂ is essentially the input function to the brain vasculature, it is critical to measure this trace as it is a quantitative metric for the degree of stimulation the blood vessels receive. As such, the primary outcome from this measure is BOLD change per mmHg of inhaled CO₂ in our regions of interest.

Ethical Considerations

Informed consent will be provided to all participants before any testing commences. All study protocols will be in compliance with the Declaration of Helsinki and have been approved by the Emory University Internal Review Board and Atlanta VA Research and Development Office (Grant: RX002825-01). All interventions and assessments will take place at Emory University and Atlanta VA. Participants will be compensated \$150 for completing all pre/post assessments and the intervention. To ensure that all personal information collected during this study is kept strictly confidential, identifiable data will be stored securely and accessible only to authorized personnel involved in the research. Any published results will be presented in aggregate form, ensuring individual participants cannot be identified. Participants' identities and sensitive information will be protected throughout and beyond the duration of this study, in accordance with ethical guidelines and legal requirements.

Data Analyses

For data analysis we aim to determine whether the two groups (Spin and Control) have different responses of cerebral perfusion in the regions of interests following the intervention. Our *a priori* hypothesis is that individuals in the aerobic group will show increased perfusion in both the right and left inferior frontal gyri and motor cortices when compared to those in the Control. The primary outcome is cerebral blood flow in units of mL/100g/min within each region of interest. We will test each mL/100g/min region of interest value jointly using a mixed effects linear 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). We expect that modeling the outcomes jointly will allow for some gains in 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 (gender, race, age, and hypertensive status) or by pre-intervention cognitive status (MoCA). 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.

Next, we aim to determine the impact of an aerobic intervention on CVR. Our *a priori* hypothesis is that individuals in the aerobic group will show increased CVR in both the right and left inferior frontal gyri and motor cortices when compared to those in the control condition. Similar to our analyses specific to perfusion, we will test each region of interest BOLD change value jointly using a mixed effects linear 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 participant demographics (gender, race, age, and hypertensive status) or by pre-intervention cognitive (MoCA). A FDR of $q=0.05$ will be calculated and used to correct for multiple tests and effect sizes will be computed.

After the aforementioned preprocessing steps are performed on ASL and CVR, 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 (i.e. resting blood flow and CVR) on task-induced BOLD changes, we plan to accomplish this task by modeling the contributions of CBF and CVR to ΔBOLD responses. The equations below describe our simple modeling approach using linear regression:

$$\Delta\text{BOLD}_{i,\text{ROI}} = A + B \cdot \text{CBF}_{i,\text{ROI}} + C \cdot \text{CVR}_{i,\text{ROI}} + D \cdot (\text{CBF}_{i,\text{ROI}} \times \text{CVR}_{i,\text{ROI}})$$

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 (i.e. CBF 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 statistics 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).

Average AUC will be taken from the right and left inferior frontal gyri ROI's during semantic fluency for both pre- and post-sessions. AUC will be averaged from the right and left inferior frontal gyri and treated as a single ROI for this analysis. The base analyses for this aim will be 2 analyses of variance (ANOVAs), 2 groups X 2 times (pre- vs. post-AE).

The analysis plan for behavioral performance will include the following dependent

variables: VO₂, in-scanner verbal fluency performance, the motor task, as described above as well as our cognitive battery. Paired t-tests will be performed to test for a pre to post effect. Spearman's correlations (r) 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.

Potential Outcomes

Decreased vascular health because of aging, and particularly sedentary aging, fosters both cognitive and motor dysfunction [17] which has implications for neurodegenerative disease. Task-based fMRI and the resultant BOLD map is a dominant method of neurocognitive investigation, particularly related to documenting the beneficial changes demonstrated with rehabilitation. 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 [11,18-19]. 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 [20-21], we believe the changes brought on by exercise are at least partially mediated by improved perfusion and CVR. 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 task-induced 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.

Results

Final data are expected to be collected in early 2024, and the primary results of the study are expected to be published by late 2024. We expect that the research will provide better understanding into the mechanistic underpinnings of improved health and function of the cerebrovascular system following aerobic exercise in older adults. We believe this will inform exercise prescription for older adults at risk for neurodegenerative disease and increased rationale for large-scale implementation and increased access to a low cost intervention.

Discussion

The purpose of this study is to explore the impact of aerobic exercise on cerebrovascular health in older adults. We know that older adults who are physically active have improved peripheral vascular health but the impact of an exercise intervention on cerebrovascular health is less known. Therefore, we will fill this gap by examining changes in basal cerebral perfusion and CVR in older adults following a proven exercise intervention. If the hypotheses of improved perfusion and CVR are supported, it would inform intervention strategies and have implications for age-related disease brought on by cerebrovascular dysfunction.

The brain uses ~20% of available oxygen for normal function, making tight regulation

of blood flow and oxygen delivery critical for survival [26]. This high demand coupled with a lack of energy stores within the cortex necessitates that cerebral blood flow be constantly and consistently maintained. In a normal physiological state, total blood flow to the brain is remarkably constant, however, with advancing age basal cerebral blood flow declines by roughly 4 mL min per year [27]. It is presumed reactive oxygen species in the cerebral vasculature decreases NO bioavailability leading to the gradual decline in endothelial function and subsequently perforating arteries, arterioles and capillaries (Santos). Ultimately, extracellular matrix components lose elasticity causing decreased vessel function and decreased blood flow [28].

Morphological studies demonstrate age-related degradation throughout the intracranial vessels. However, studies have demonstrated age-related decreases in perfusion are notably amplified in the frontal cortex [16]. It has been highlighted that the age-related decline in cerebral perfusion is not great enough to cause major ischemic injury, however, it results in hypoperfusion that prevents sufficient nutrients from reaching highly metabolically demanding areas (e.g frontal cortex) [29]. Of note, the decreased blood supply and inadequate delivery of nutrients is noted as a key physiologic process in age-related cognitive decline and specifically cognitive-executive processes partially mediated by the frontal cortex [30].

Impact of Exercise on Cerebral Blood Flow. Studies have focused on the beneficial effects of exercise on age-related changes in blood flow in the periphery but less is known about aging and exercise on cerebral perfusion. In the periphery exercise with an aerobic component is associated with improved perfusion in older adults when compared to their sedentary peers [19]. Specific to the cerebral cortex, a cross-sectional study showed blood flow velocity in the middle cerebral artery, probed using Doppler, was significantly higher in adults with high levels of cardiovascular fitness [31]. Of note, the decrease in basal cerebral blood flow is tapered in those that report higher levels of physical activity [32]. Although these data give us an indication that aerobic exercise can enhance cerebral perfusion, it is currently unknown if a proven aerobic intervention in previous sedentary older adults can benefit basal cerebral perfusion.

Decreased Cerebrovascular Reactivity in Aging. In addition to the static parameter of blood flow, the dynamic parameter of CVR has a critical role in cerebrovascular health and function. As noted, the cerebral vasculature must maintain blood flow within precise limits during rest, activity, disease or injury. In healthy individuals, cerebral blood flow 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 vaso-properties and vascular response to demand have repeatedly been demonstrated to degrade with age and neuropathology [6,19]. Most often, to quantify the efficiency of cerebrovascular response, the degree of vasodilation in response to vasoactive agent (CO₂) is measured. Decreased responsiveness to CO₂ is a proven marker of CVR dysfunction and has been demonstrated to be sensitive to changes brought on by aging and neural disease [16,32].

While the mechanisms are difficult to directly probe in human cerebrovasculature, extensive work in the periphery demonstrates that age-related arterial stiffening and endothelial dysfunction play critical roles in the development of CVR dysfunction [19]. Impairment of the oxidative stress response and dysfunctional inflammatory pathways feed a cascade of age-related CVR malfunction. Dysfunction in the stress response and inflammatory pathways causes increased reactive oxygen species and reduced NO. Together the increase in reactive oxygen species and decrease NO suppresses the response of immune cell causing systemic vascular endothelial dysfunction [33-35].

Behaviorally, decreased CVR has been observed in cognitive decline demonstrated in aging as well as dementia [36].

Impact of Exercise on Cerebral Blood Flow. Aerobic exercise has consistently been associated with enhanced flow mediated dilatation in the brachial artery of older adults [37]. 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 [5-6,38]. 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 increase among stroke survivors after 6-months of aerobic training³¹. Our own pilot data, described in greater detail below, demonstrate marked decline in CVR with age. However, the degree of CVR decay is lessened in older adults that have high levels of self-reported aerobic activity. Thus, the purpose of this study is a key next step towards quantifying CVR change following a proven aerobic intervention in previous sedentary older adults.

Link Between Cerebral Perfusion, CVR and task-based fMRI BOLD Response. Seminal studies have continually demonstrated that previously sedentary individuals who undergo exercise intervention show BOLD activity that coincide with improvements in an array of tasks in both motor and cognitive domains [7,11,39]. While the accessibility and ease of implementation have made BOLD a valuable tool, its interpretation is not straightforward [40]. 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 (Figure 2). Task-induced BOLD change (Δ BOLD) is measured relative to baseline BOLD signal, which means that baseline physiology (blood flow and perfusion; Figure 2-Red box) affects the task-induced Δ BOLD (especially 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 cerebral blood flow 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 [41]. Further, the blood vessels must dilate in response to an increased demand for blood flow. CVR is an index of baseline vascular health that is necessary to support efficient cerebral perfusion. Thus, in order to tease out 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 Δ BOLD, it is important to understand the relationship between baseline cerebral physiology (perfusion and CVR) and task-induced Δ BOLD. 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 Δ BOLD response both before and after the intervention.

Limitations

We have considered the following potential weaknesses. First, the length of the intervention (12-weeks) is not sufficient to induce change in cerebrovascular function. Of note, we encourage all of our research participants to maintain a lifelong commitment to physical activity and hope that our 12-week intervention is the beginning of behavioral change. We do believe, however, that the 12-week timeline is sufficient to significantly change both perfusion and reactivity based on, 1) the significant change demonstrated in cardiovascular

fitness and BOLD response in 12-weeks utilizing the proposed Spin intervention, and 2) the numerous studies demonstrated change in perfusion and reactivity in the periphery with 12-week interventions. Further, it is known that acute bouts of moderate-intensity aerobic exercise reduces both central and peripheral femoral artery stiffness [22] while increasing endothelial function [23-24] is also anti-inflammatory.

An additional potential weakness is that older adults will not be compliant and adherent to the intervention. Compliance in our studies generally has been at or above 90% with retention consistently being above 85%; however, long-term success of interventions will depend on accessibility and sustainability. Community exercise programs that increase accessibility are obtaining good results [25], but sustainability is still an issue. Dealing with these issues in later stage clinical trials is in our long-term goals once we know the full extent of the intervention and it is consistent with our long-term goals.

Conclusion

We believe this study will impact older adults and the state of the science by defining and quantifying the positive impact of aerobic exercise on cerebrovascular health. Decreased vascular health as a result of aging, and particularly sedentary aging, lays the groundwork for both cognitive and motor dysfunction and is linked to neurodegenerative disease. Cross-sectional works and our preliminary data suggest that our aerobic exercise intervention will improve cerebrovascular health. If our hypotheses are supported it would inform current intervention strategies and would add important new information on the implications of the cerebrovasculature on the BOLD signal. These findings would advance the current state of literature regarding the beneficial impact of exercise on brain health and function.

In terms of future direction, our lab has repeatedly shown that neurologic changes brought on by aging and disease may not be inevitable or immutable. We have new evidence that indicates increased levels of physical fitness through aerobic activity may mitigate losses in interhemispheric inhibition. We would anticipate that cerebrovascular health in aging is intertwined with levels of interhemispheric inhibition and both will improve over the course of our exercise intervention. The next steps for our lab would be to further develop a continuum of research investigating the relationship between neurodegenerative disease, cerebrovascular reactivity, and physical activity which would provide a cornerstone to build on our understanding of cerebrovascular disease processes and opportunities to prevent, intervene or reverse.

Acknowledgments

This study was supported by the Center of Visual and Neurocognitive Rehabilitation at the Joseph Maxwell Cleland Atlanta VA Medical Center. Funding was provided by the Department of Veteran Affairs (grant RX004563-01A1).

Data Availability

Data will be shared in accordance with the Department of Veteran Affairs Rehabilitation Research policy.

Author Contributions

JN, LK, VK and KM conceived and designed the presented study. MB, JN, KM, MV, DW, CD and TN planned and carried out logistical and regulatory aspects of the study. MB and TN wrote the manuscript with consultation and input from all authors.

Conflicts of Interest

None declared.

Abbreviations

ASL: arterial spin labeling

BOLD: Blood Oxygen Level Dependent

CVR: cerebral vascular reactivity

FDR: false recovery rate

fMRI: functional magnetic resonance imaging

No AI was used in writing this manuscript.

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Supplementary Files

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