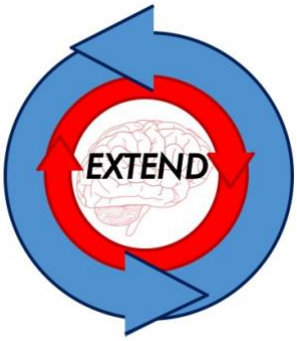
Manual of Procedures



Bike Extend

Exercise effects on brain connectivity and learning from minutes to months

Version #1.74
Last modified: Dec 11, 2024

Table of Contents

1. Title

Exercise Effects on Brain Connectivity and Learning from Minutes to Months
(Bike Extend Trial)

2. Principal Investigator and Key Staff

The trial is sponsored by the National Institute on Aging (NIA) and is funded by the US Department of Health & Human Services and the National Institutes of Health.

FWA #FWA00003007

Principal Investigator and Study Director:
Michelle Voss, PhD
Assistant Professor
Department of Psychological and Brain Sciences
University of Iowa
227 Spence Laboratories
300 Iowa Avenue
Iowa City, Iowa 52242-03522
Tel: 319-384-3821
Email: michelle-voss@uiowa.edu

Co-Investigators:
Gary Pierce, PhD
Associate Professor
Department of Health and Human Physiology
University of Iowa
412 Field House
225 South Grand Avenue
Iowa City, Iowa 52242-03522
Tel: 319-335-9487
Email: gary-pierce@uiowa.edu

Eliot Hazeltine, PhD
Professor
Department of Psychological and Brain Sciences
University of Iowa
W307 Seashore Hall
300 Iowa Avenue
Iowa City, Iowa 52242-03522
Tel: 319-335-1855
Email: eliot-hazeltine@uiowa.edu

Vincent Magnotta, PhD
Professor
Department of Radiology
0453-D John Colloton Pavilion
Iowa City, Iowa 52242
Tel: 319-356-8255
Email: vincent-magnotta@uiowa.edu

Michael Muellerleile, M.D
Clinical Assistant Professor
Cardiology, Internal Medicine
University of Iowa Carver College of Medicine
Iowa City, Iowa 52242-03522
Email: michael-muellerleile@uiowa.edu

Jeffrey Long, PhD
Professor
Departments of Biostatistics and Psychiatry
1-328 Medical Education Building
Iowa City, Iowa 52242
Tel: 319-335-8524
Email: jeffrey-long@uiowa.edu

James Mills, MED, MS
Biostatistician
1-323 Medical Education Building
Iowa City, Iowa 52242
Tel: 319-512-0268
Email: jim-mills@uiowa.edu

Key Staff:

Project Manager: Chase Hamilton, BA
Exercise Specialist: Abby O'Deen, BS
Graduate Student Leaders: Matthew Sodoma, BS

3. Study Locations

- Spence Laboratories of Psychology – Health, Brain, and Cognition Lab (G8-G13)
- University of Iowa Hospitals and Clinics (UIHC) and Iowa Institute for Biomedical Imaging – Mock MRI (room #L550A PBDB/IIBI)
- Pappajohn Biomedical Discovery Building (PBDB) (rm #L445, #L520 PBDB/IIBI)
- Field House – Exercise is Medicine Community Outreach Lab (E141 FH)

4. Overview

Although exercise is known to delay cognitive decline and decrease our risk of Alzheimer's disease, there is a lack of understanding of how exercise protects the aging brain. The overall goal of this study is to determine the effects of moderate intensity physical exercise training on relational learning processes and functional brain connectivity in healthy older adults. We examine the effect of moderate intensity exercise training on learning and connectivity with a 6-month randomized controlled trial (RCT) that compares moderate intensity stationary cycling to very light intensity cycling. We further test the mechanisms involved by determining the selective acute effects of moderate intensity exercise that would improve cardiorespiratory fitness compared to light intensity exercise, and by determining if change in cardiorespiratory fitness is a key factor for benefits on functional connectivity and learning.

5. Background and Specific Aims

Animal models robustly support that endurance exercise protects brain areas vulnerable to aging such as the hippocampus (1) and that these benefits are directly related to better *learning* (1-3). In contrast, human studies have shown mixed findings on the cognitive benefits of exercise with healthy older adults (4-9), even when the studies are well-powered and exercise benefits brain regions that deteriorate with aging including the hippocampus and prefrontal cortex (10-15). Given the importance of cognition for independence in daily life, this presents a critical need to determine how exercise works against the mechanisms driving *cognitive aging* (16, 17). Our ***objective*** with the EXTEND trial is to fill this translational gap by determining if exercise improves the same kinds of learning in older adults that have been shown to improve in animal models from exercise by improving hippocampal function. ***Our overall hypothesis is that exercise improves learning when it increases functional hippocampal-cortical communication that otherwise declines with aging.*** We test this hypothesis with 3 aims:

1. **Determine the relationship between chronic training-related effects of moderate intensity exercise on hippocampal-cortical functional connectivity (*FC)* and changes in learning from exercise.** Although we have shown that long-term moderate intensity exercise training improves hippocampal-cortical *FC*, it is unclear how these changes from exercise counteract cognitive aging. Our *hypothesis* is that training-related changes in hippocampal-cortical *FC* with the Default Network (DN) will improve learning in an array of tasks that require the hippocampus for acquisition of new relational memories, but not in tasks that do not require the hippocampus to learn such as motor or response learning. We test this with a 6-month RCT that compares the effects of moderate vs very light intensity exercise of the same modality (stationary cycling) on learning.

2. **Determine the relationship between rapid transient effects of a single session of moderate intensity exercise on hippocampal-cortical *FC* and training-related changes in hippocampal *FC* and learning from exercise.** Animal studies have shown that exercise rapidly affects hippocampal plasticity following a single exercise session, that effects last up to 1 hour, and occur in the same pathways that benefit from long-term training. These effects

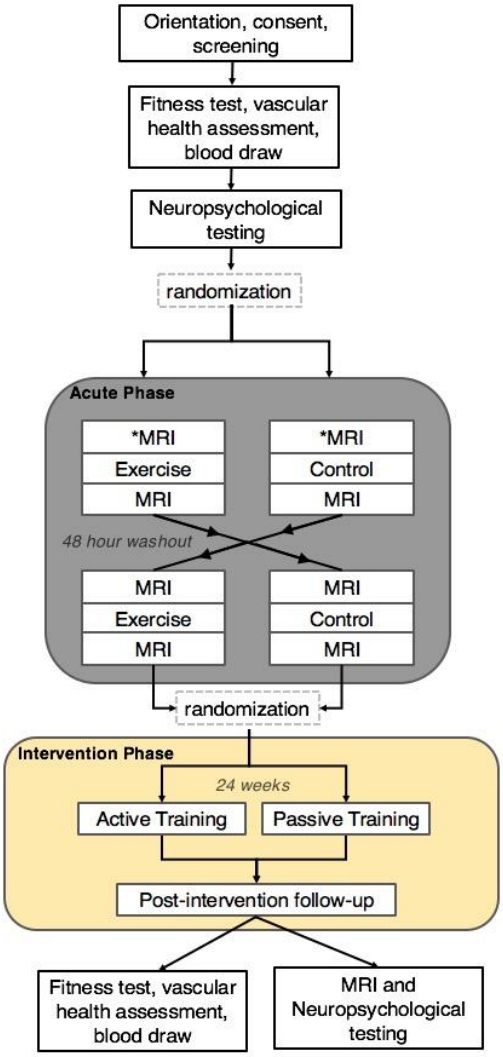
may reveal insight about the mechanisms of change from training. Our preliminary data support the *hypothesis* that we can see such rapid effects in humans with fMRI. We test this by assessing acute increases in functional hippocampal-cortical *FC* that are specific to moderate intensity exercise, and determining if this response predicts improvements in hippocampal *FC* and learning from 6 months of exercise training.

3. **Determine how moderate intensity exercise changes hippocampal-cortical *FC* and learning.** Our data indicate that cardiorespiratory fitness (CRF) is a critical factor in cross-sectional differences of hippocampal-cortical *FC* and learning. CRF reflects complex improvements in vascular, cardiac, and metabolic health from moderate intensity exercise. Given the observed rapid effects, our working *hypothesis* is that the physiological processes leading to improved CRF from moderate intensity exercise are critical to the benefits on hippocampal *FC* and learning. We test this by assessing whether individual differences in training-related changes hippocampal-cortical *FC* and learning are predicted by training-related changes in CRF.

Data from this trial will be *significant* by revealing mechanisms by which exercise could mitigate age-related cognitive decline in learning that depends on the hippocampus. There currently are no treatments that reliably reverse the course of cognitive aging associated with hippocampal deterioration. Exercise has shown promise in animal studies, but this promise has not been successfully translated to human studies of healthy aging. Data from this study could help bridge this gap. Given the hippocampus is central to Alzheimer's and related dementias, results could also lead to an understanding of the mechanisms by which exercise reduces risk of this devastating and costly disease (18-20).

6. General Study Design

The study is a standard randomized, controlled trial, single-blind design of relatively inactive but healthy older adults. A target N of 120 participants will participate and be randomized to either an experimental (moderate intensity) treatment group (N=60) or an active control (light-intensity + stretching) group (N=60). Since we are interested in the effects of exercise training on cardiorespiratory fitness and in turn cognitive performance, we will ask participants to complete assessments of cardiorespiratory fitness (CRF) and cognition before and after the training program. Following completion of pre-training screening, fitness, and cognition assessments, participants will be randomly assigned into one of two groups indicating order of acute exercise conditions and will again be randomized to determine their chronic training group (see Figure 1). Including pre- and post-training testing, participants who complete the study will be enrolled for approximately 28-30 weeks (~4 weeks pre-testing, 24 weeks training, and ~2 weeks post-testing).



Study Flow Chart:

Figure 1. Overall study timeline. Note randomization occurs once for counter-balancing the order of acute phase conditions, and then again for assignment to intervention group.

*MRI denotes that the first MRI of the first acute exercise visit will serve as the pre-test MRI for the first acute visit, and as the pre-test MRI for the 6-month training study.

7. Study Population

This study will involve recruitment and screening of approximately 240 healthy older adults between the ages of 55 and 80 years old. Of the participants that we screen, we aim to enroll 120 participants into our study. All subjects will be screened to ensure they are currently not physically active, which is defined as not participating in regular moderate intensity activity more than 60 minutes per week for the past six months. They must be capable of participating in a physical activity program without exacerbating any pre-existing condition(s). Subjects will be recruited throughout the Iowa City, Coralville, and North Liberty areas. The following table illustrates our targeted enrollment:

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			Total
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native	0	0		0	0					0
Asian	4	2		0	0					6
Native Hawaiian or Other Pacific Islander	0	0		0	0					0
Black or African American	4	2		0	0					6
White	67	35		4	2					108
More than One Race	0	0		0	0					0
Unknown or Not Reported										
Total	75	39		4	2					120

To meet our recruitment goals, we will over-represent zip codes in the surrounding area that have a relatively higher proportion of under-represented minorities.

8. Inclusion/Exclusion Criteria

Inclusion Criteria: No history of neurological incident or disease, no chronic metabolic or psychiatric disease, not currently taking steroid-based medications except for topical skin cancer treatment, eligible to participate in an aerobic exercise intervention based on the Physical Activity Readiness Questionnaire, currently exercising with their heart rate into a moderate intensity zone (self-reported breathing hard, sweating) less than 60 minutes a week for the past six months, and corrected vision of 20/40. Because our older adult sample is over the age of 40, further eligibility for the exercise intervention will be determined following approval from a physician that monitors electrocardiography (ECG) response during a maximal aerobic exercise test that is part of the second study visit described below.

Exclusion Criteria:

- Score < 20 on the Montreal Cognitive Assessment (MoCA)
- Inability to comply with experimental instructions
- Inability to complete an MRI (e.g., due to magnetic implants that would preclude MRI scanning such as an aneurysm clip or metallic stent, metallic fragments in the body, or cardiac pacemaker or other electronic implant or device)
- Qualify as “high risk” for acute cardiovascular event by published standards of the American College of Sports Medicine

- Diagnosed with any of the following: Major Depression, ADD or ADHD, Epilepsy, Meningitis, Parkinson's disease, Stroke, Brain surgery, Heart condition or other cardiovascular event, COPD, Uncontrolled Asthma (this includes anyone who has asthma and is not on medication), Cystic fibrosis, Unregulated Thyroid disorder (this includes anyone with thyroid disease that is not on medication), Renal or liver disease, Heart murmur. Smoking or living with someone who smokes in the past 3 months

Once participants are deemed eligible and do not qualify for exclusionary criteria, subjects are still allowed to withdraw at any point from the study if they are uncomfortable or simply no longer want to participate.

In this study, fetuses, neonates, pregnant women, children (under the age of 18), prisoners, institutionalized individuals, and other vulnerable populations will not be included.

9. Recruitment

The following materials/method will be used in recruiting subjects:

- Advertisements
- Posters
- Brochures
- Email
- Letters
- University of Iowa Hospitals and Clinics EPIC Database
- Lab website - http://psychology.uiowa.edu/health-brain-cognition-lab/participate
- Existing Registry/database - We will contact participants that have been in our previous studies and who indicated they would like to be contacted about participating in future studies.
- An example of the recruitment flyer and post-card can be found in Appendix I

Over email or telephone, the study coordinator will ask preliminary screening questions to rule out initial eligibility criteria.

10. Description of Informed Consent Process

The consent process will take place in the mock MRI room in the Iowa Institute of Biomedical Imaging in a private room within the Magnetic Resonance Research Facility. Only the experimenter and participant will be present during the consent, so all information will be collected in privacy.

Since a portion of our participants will be elderly adults, we will assess their decision-making capacity before signing the consent through two means. First, the experimenter will assess their ability to accurately communicate and respond to questions on the phone or via email as they are explaining the study and initial eligibility requirements. This will be done by periodically asking,

'Do you have any questions?' Secondly, once the participant has read the consent form, but before they sign it, the experimenter will complete the "Evaluation to sign informed consent document for research" that is provided by the UI IRB office.

Consent will be obtained using an informed consent document. The study coordinator or a trained research assistant will conduct the session. It will include the following:

- Consent and discussion of participant questions.
- To minimize the potential for coercion or undue influence during the consent process, after the participant has read the consent form an experimenter will ask them whether they have any questions or would like to take more time to consider their decision. During this time, the experimenter will remind the participant that participation is completely voluntary and will encourage the participant to take as much time as they need to decide.
- After any questions are answered, the experimenter will ask if they consent to participate in the study. If they do indicate their agreement to participate, they will be asked to sign the consent document. They will be given a copy of the consent document for their records. After the consent is signed, the subjects will begin the study procedures.
- Potential subjects have the chance to opt out of further contact or additional information about the study at any time during the recruitment steps described above.

A sample informed consent document can be found in Appendix II.

11. Screening Procedures

Before consent for the study, we will ask screening questions pertaining to our exclusionary criteria. This includes the following questions that will be asked on the phone or via email:

What is your date of birth? Exclude if NOT between the ages of 55 and 80 years old.

- Are you fluent in the English language? Exclude if no.
- Do you have visual acuity or corrected visual acuity of at least 20/40? Exclude if no.
- What medications are you currently taking? Exclude if taking hormone replacement therapy or other steroid-based medications (except for topical skin cancer treatments).
- Have you ever been told by a medical doctor that you cannot have an MRI? Exclude if yes.
- Do you have a cardiac pacemaker or other electronic device? Exclude if yes.

I am going to read a list of conditions. When I am through, please answer “yes” if you have had any of the conditions, and “no” if none of the conditions have applied to you. I do not want to know which condition you may have experienced, just if you have had any of them.

Depression
Anxiety disorder
ADD or ADHD
Epilepsy
Meningitis

Parkinson's disease
Stroke
Brain surgery
COPD
Uncontrolled Asthma (not on medication or inhaler for the past three months or more)
Cystic fibrosis
Unregulated Thyroid disorder (not on medication for the past 3 months or more)
Renal or liver disease
Heart murmur
Smoking or living with someone who smokes in the past 3 months

Have you ever had any of those conditions? Exclude if yes.

Have you been physically active enough to work up a sweat more than 60 minutes per week in the previous six months? If yes, ask for more details. Exclude if they exercise (brisk walk, bike rides) regularly. If unsure and are not referring to moderate intensity exercise—invite them for a screening visit.

If they are not eligible, they will be told this but asked if they would be interested in being contacted for future studies.

Additional screening procedures will take place during their **screening and orientation visit** which includes consent, cognitive screening with the MoCA, and orientation to the MRI environment. Several self-report questionnaires will also be completed. This visit is to screen out individuals that may have preclinical dementia, who are "high risk" for an acute cardiovascular event by published standards of the American College of Sports Medicine (2010), or cannot comply with instructions on our neuropsychological and cognitive tasks. This visit will also introduce participants to the mock MRI and screen out those who would not be comfortable in the MRI machine due to claustrophobia or discomfort lying on their back for one hour. Following cognitive screening, the experimenter will conduct the following tests:

- Participant demographic and Health history questionnaire
- Physical Activity Readiness Questionnaire
- Godin Leisure-Time Exercise Questionnaire
- Weekly Questionnaire addressing diet, medication, and sleep habits
- Pfeffer FAQ
- Generalized Depression Scale (GDS)
- ACSM Questionnaire (ACSM) – determines whether participants are low risk for a cardiovascular event based on the American College of Sports Medicine standards (2010).
- IPAQ
- RAVLT
- Oral trail making
- FAS word fluency

After the questionnaires have been completed, participants will undergo a mock MRI for ten minutes.

After the orientation session, participants will be informed that their performance on some of these tests is part of the current study but may also be helpful in determining their eligibility for future studies. They will be informed that the tests included as helpful for future eligibility include the initial demographic survey, the physical and mental activities questionnaire, the first test they did with memory and attention questions (MoCA), and the results of the basic health assessment. They will be asked verbally whether they agree to have us make these test scores available as eligibility criteria for future studies. The experimenter will mark their decision on the health assessment form in addition to the consent form.

Eligible participants will then return to the hospital for a **vascular health and fitness assessment** in the Translational Vascular Physiology Lab. Participants will report to the hospital between 7-10 am after an overnight fast of at least 8 hours and no caffeine or exercise for at least 20 hours. Subjects will have 4 electrodes placed on the chest for monitoring of ECG. After resting quietly for 15 min in supine position, the following measurements will be performed by Dr. Gary Pierce or his research staff and collected participant information will be recorded on their vascular data sheet:

- **Pulse wave velocity (PWV) and blood pressure**. Carotid-femoral, carotid-brachial, and carotid-radial PWV will be measured non-invasively by recording carotid, femoral, brachial and radial artery pressure waveforms sequentially with an applanation tonometer (Non-invasive Hemodynamics Workstation, Cardiovascular Engineering, Inc.). Pressure waveforms are gated to the ECG R wave in order to calculate the transit time (t) between the foot of the carotid and the respective peripheral (femoral, brachial, radial) waveforms. The carotid-femoral transit distance (CFTD) is estimated between the 2 anatomical sites as the difference between the suprasternal notch (SSN) to carotid (SSN-C) and femoral (SSN-F) sites. Thus, the CFTD is calculated as CFTD= (SSN-F)-(SSN-C) and PWV calculated as CFTD/t. This approach accounts for parallel transmission of the pulse wave up the brachiocephalic and carotid arteries, and simultaneously along the aortic arch using the SSN as a fiducial point where parallel transmission begins (e.g., bifurcation site of aortic arch and brachiocephalic artery).
- **Carotid artery compliance/stiffness**. Carotid artery compliance and Beta-stiffness index will be determined noninvasively by high-resolution ultrasonography (Logiq 7, GE Healthcare) of the right common carotid artery and contralateral assessment of carotid artery blood pressure via non-invasive carotid artery applanation tonometry, respectively. Carotid artery diameters are measured ~2 cm proximal to the carotid bulb with the transducer placed at a 90° angle to the vessel by off-line analysis of DICOM images with image analysis software (Medical Imaging Applications, LLC). Maximal diameters (i.e., systolic expansion) and minimal diameters (e.g., diastolic relaxation) are measured in sync with carotid artery blood pressure waveforms. Carotid blood pressure waveforms are calibrated using diastolic and mean brachial artery blood pressure obtained from standard brachial artery cuff blood pressure.
- **Blood sample.** A blood sample will be taken at this session to measure serum lipids, glucose, and insulin levels. We will collect three 2.0 teaspoon (10 mL) blood samples at the health assessment visit at pre-testing and at post-testing (12.0 teaspoons or 60 mL in

total). All samples will be collected by research personnel trained in phlebotomy by Dr. Pierce's lab.

The following will take place in the Exercise Testing laboratory by Dr. Gary Pierce or his research staff:

- **Maximal 12 lead ECG exercise test with oxygen uptake (VO_2) analysis**: Participants will undergo a maximal exercise test with 12-lead ECG and cardiopulmonary gas analysis (for determining VO_2max) in the E141 FH supervised by a trained exercise specialist and licensed practitioner (cardiologist). Participants with evidence of cardiovascular disease at baseline (e.g., evidence of myocardial infarction, abnormal cardiac arrhythmia, myocardial ischemia, conduction delays) or during the exercise test (>1mm ST segment depression or elevation; >3 beat ventricular tachycardia; atrial fibrillation, chest pain suggestive of angina) will be excluded from the study.
    - The goal of this maximal exercise test is to identify each participant's true VO_2max. In order to be a true maximum test, three of the following four criteria has to be met:
        - RER value of greater than 1.10
        - A plateau in VO_2 despite increased workload
        - RPE of 18 or greater
        - Maximal heart rate within 10 bpm based on predicted HR_{max} (220-age) (or attainment of 90% of predicted HR max or greater)
    - When these criteria are not met, we will document the test as a VO_2peak measurement, and this information will be evaluated for its potential usefulness as a covariate in data analyses.

During the vascular and maximum exercise test screening each participant will be given an Actigraph accelerometer and an exercise log sheet and asked to log the information required for 7-10 days. Actigraph measurements will be repeated each month throughout the intervention until post-testing.

12. Cognitive measurements

Questionnaires, written assessments, and cognitive tasks to be collected during two two-hour neuropsychological and cognitive testing sessions. Each of these assessments will be collected both pre- and post-training and tasks are counter-balanced for order as needed. Example session lists:

Neuropsychological session 1:

- Spatial Navigation Task (version A) - 50 minutes. Participants will be required to explore a virtual environment and complete tasks inside the environment. Participants will be given 7 minutes for free exploration of the environment. After the time is up, the participant will be presented a blank 2-D map and asked to place an "X" at all landmark locations (20 possible correct). This test-sequence will be repeated 5 times.
- Task switching task (version A) - ~12 minutes. Participants will initially be led through two learning phases to learn two different tasks.

- Associative learning task (version A) (~30 minutes). This requires participants to learn a response to the specific pair of faces presented rather than to learn responses to individual faces. For example, instead of responding to faces A and B when given the pair A-B, this task requires participants to respond to A-B with a unique response than what would be given for another pair including A such as A-C. This type of associative processing places more demand on relational memory than the configural motor learning task.
- Spatial Navigation Task - delay phase - 10 minutes. Following a delay (during which the task-switching task and associative learning tasks are completed), participants will be shown a picture of one landmark and asked to navigate to the landmark using the shortest path possible.
- WRAT (Wide Range Achievement Test) Reading Subtest ~ 5 minutes -This task is a short measure of IQ. Requires participants to read a list of words, one at a time, in a clear voice. Will be audio recorded and the participant will be informed of this.

Neuropsychological session 2:

- Spatial navigation task (version B) - 50 minutes. Participants will be required to explore routes within a virtual environment. Participants will be asked to follow arrows along the same route in the virtual environment for 5 minutes. The route will take about 90 seconds to successfully navigate. After a 30-minute delay, participants will then be tested by having them draw the route they learned on a 2-D map of the environment.
- Task switching task (version B) - ~12 minutes. Participants will initially be led through two learning phases to learn two different tasks.
- Associative learning task (~30 minutes). This is a configural response learning task. Participants first learn the stimulus-response mappings for a set of faces for each finger of the left and right hands. Then faces are shown in pairs with pair frequency manipulated. Configural response learning measures the extent to which participants get faster at responding to frequently practiced pairs compared to infrequently practiced pairs, despite equal exposure and practice to all individual face stimuli. This task matches the associative learning task in stimuli and response features, but requires less demand on the hippocampus for relational learning processes.
- Spatial Navigation Task (version B) - delay phase - 10 minutes. Following a 30-minute delay (during which the task-switching and associative learning tasks are completed), participants will be asked to re-trace their route to a given landmark.
- Processing speed measures (Digit symbol substitution, pattern comparison, letter comparison)

13. Randomization

Randomization will occur once to determine the order of exercise conditions for the acute cross-over design, and again to determine the training groups (see Figure 1). Eligible participants will be randomized to one of two randomization groups that indicates the order of their acute exercise conditions, a separate randomization will occur to determine their exercise training group (see

Figure 1). Therefore, *all* enrolled participants complete an acute exercise assessment with both light and moderate intensity sessions (21). Following the acute exercise session, participants will continue in one of two training groups (moderate, light intensity).

When participants express interest to participate in their exercises as a couple (dyad), co-enrollment will be allowed on a limited basis. Co-enrollment means that only one of the dyad will be randomized and the partner will be automatically assigned to the same intervention group. The data for the partner then qualifies as "missing" for all analyses that depend on assumptions of random assignment. Our power analysis accounted for losing up to 15% of data due to drop out (10%) and scanner motion (5%), which would leave the sample at about 100 participants and still at strong power (~90%, two-tailed) with allowance for covariates. Based on our pilot study and adherence so far, we predict to lose less data from drop-out and motion than we initially predicted. Therefore, we will consider non-randomized partners as another source of "missing data" and set our limit for non-randomized dyads to N=6 (5%).

For participants who drop-out after randomization, we will take the following approach to determine the best type of post-testing to be done at the time of drop-out:

- First, ask them if they are still willing to do their post-test MRI and full cognitive testing sessions.
- If they cannot do all the testing, we will ask if they could do one 2-hour cognitive testing session in the lab. This would allow us to collect our learning and memory behavioral measures that tap into hippocampal function and our abbreviated battery including the RAVLT in-person.
- However, if the drop-out participant is not willing to return to the lab for post-testing, then we will ask them if they are willing to do a brief phone survey which includes the tasks in the short battery above.

14. Neuroimaging measures

This study will utilize the 3T GE Discovery 750W MRI scanner to collect images that allow us to measure brain structure (T1, T2, diffusion), functional connectivity of brain networks sensitive to aging (resting state BOLD), neuronal metabolism (quantitative susceptibility mapping and T1ρ), and cerebrovascular reactivity (BOLD response during a breath hold paradigm). We expect to scan for one hour before each acute exercise session and 30 minutes following each acute exercise session, and for 1 hour following completion of the 6-month exercise training intervention (see outline below). In the event that a participant is uncomfortable in the scanner (e.g., wide shoulders or large head size), but would like to participate in scanning, we will prioritize T1 structural images and resting state fMRI scans which are central to testing our specific aims. Following each MRI session, images will be downloaded directly from XNAT into BIDS format. Quality control procedures will be conducted following data collection each week using mriqc.

MRI scans acquired at first baseline MRI visit:

- Pre-exercise:
    - 3 x 8min resting state fMRI scans (eyes open)
    - Breath-hold task

  - T1 weighted anatomical image (MPRAGE)
  - T2 FLAIR anatomical image
- Post-exercise:
  - 3 x 8min resting state fMRI scans (eyes open)

MRI scans acquired at second baseline MRI visit:
- Pre-exercise:
  - 3 x 8min resting state fMRI scans (eyes open)
  - 64 Directional DTI scan to assess white matter integrity
  - T1 ρ
- Post-exercise:
  - 3 x 8min resting state fMRI scans (eyes open)

MRI scans acquired at post-training MRI visit:
  - 3 x 8min resting state fMRI scans (eyes open)
  - Breath-hold task
  - T1 weighted anatomical image (MPRAGE)
  - T2 FLAIR anatomical image
  - 64 Directional DTI scan to assess white matter integrity
  - T1 ρ

*Note the post-test MRI will begin in the mock MRI room with assessment of the RAVLT, oral trail-making, and the FAS word fluency tasks.

15. Research Interventions

There are two phases of exercise interventions, including an acute exercise assessment and a long-term training assessment. Following completion of a neuropsychological session, we will provide a brief orientation to our recumbent bicycles and self-report surveys administered during exercise. The purpose of this is to calibrate the exercise protocol for the experimental acute exercise sessions and to minimize novelty effects on acute testing days. The exercise orientation includes the following:
- Learn and practice the scale measurement that will be used during the exercise testing sessions (5-min)
  - Scale Measurements:
    - Rating of Perceived Exertion (scale 6-20), Feeling Scale, Felt Arousal Scale
  - Learn heart-rate monitor and exercise bike procedures
    - Teach participants the proper strap placement and what their target heart rate is for different exercise intensities. Introduce the exercise bicycle and fit participants to the bike. Finally, we will indicate to the participant that the experimenters will be refraining from engaging in social conversation during the exercise, but that any questions should be welcomed by the participant.
    - During this interactive practice session, the research assistant will be engaging with the participant until their ideal speed and resistance is identified (i.e. RPE of 12 – 14, or perceived moderate exertion).

All participants will then complete both conditions (moderate, very light intensity) of the acute exercise intervention (within-subjects) with MRI scans before and after each exercise session:

- Acute exercise assessments will be a minimum of 48-hours apart and counter-balanced for order of exercise intensity.
- Order of conditions will be counter-balanced based on randomization.
- We will compare 20 minutes of supervised (a) light intensity cycling (<40% HRR, "light" intensity, ACSM) on a stationary motorized cycle ergometer, and (b) volitional cycling at a moderate intensity (50 to 60% HRR) which will be at the same pedaling speed as the light cycling (i.e., pedaling resistance will increase to obtain desired heart rate) (22).
- Resting state functional MRI scans will be acquired immediately before and after exercise (see Figure 1). Blood pressure and HR will be measured throughout exercise and scanning. Self-reported affect, arousal, and RPE will be measured during exercise at 5-minute intervals.

Following the acute exercise session, participants will continue to the second phase by entering one of two training groups, as determined by their initial randomization:

- **Moderate intensity exercise training** will be a 24-week supervised cycling program, with supervision directly from our research team**.** Participants will start in waves of ~5 per wave. All participants will first receive a one-on-one orientation with an exercise training specialist that has been trained by Dr. Gary Pierce in monitoring an exercise program for healthy older adults. Since participants will initially be relatively inactive, the exercise group will start with a 5 minute-warm-up, 20-minutes moderate intensity cycling at 50-60% Heart Rate Reserve (HHR) and 20 minutes passive cycling, and 5 minute cool-down per session, for 3 sessions/week. In each additional week, we will replace 5 minutes of passive cycling with 5 minutes of moderate intensity cycling per session, until the total time for moderate intensity is 40 minutes per session by the start of week 5 (with additional 5 minute warm-up and 5 minute cool-down). Starting at week 6 the exercise group will increase their moderate intensity cycling to 60-70% HHR with a similar build up to the first 5 weeks. Starting at week 13 the exercise group will increase their moderate intensity cycling to 70-80% HHR with a similar build up to the first 5 weeks. This program allows for a gradual build-up of endurance while including training for approximately 135 minutes of moderate-to-vigorous intensity exercise per week.

- **Functional fitness training** will be a 24-week supervised exercise program designed to focus on functional flexibility and mobility, with supervision directly from our research team. All participants will first receive a one-on-one orientation with an exercise training specialist that has been trained by Dr. Gary Pierce in monitoring an exercise program for healthy older adults. Training will start with a 15 minute-warm-up of stretching, 20 minutes of light intensity cycling and 15 minutes of dynamic stretching to increase range of motion and functional fitness, for 3 sessions/week. In each additional week, additional stretches will be added to maintain variety and improve flexibility of all major muscle groups. Our goal is to maintain HR at or below 40% HRR during training sessions for this group.

Both groups will wear a Heart Rate Monitor (Polar wrist watch and chest-band) during their exercise sessions. We will download HR monitor data following each session and this data will be used to give feedback on their target HR for the following session. Target HR for each condition will be based on the baseline maximal exercise test. Ratings of perceived exertion (RPE) will also be collected during the baseline max test, and in cases where there was a discrepancy between expected RPE and HR during this test, RPE will be used in conjunction with HR in developing a target HR for moderate and passive cycling sessions.

All training sessions in the first two weeks are scheduled to be in our lab. After the first two weeks, participants can do up to two sessions per week outside the lab. At the conclusion of each training session in the lab, participants are given instructions on how to complete their training at home including use of the HR monitor. In order to maintain blinding to intensity prescriptions, participants are guided to mimic the heart rate zones they are targeting in their lab sessions. For participants that live outside of a 30-mile radius, we have an additional option for more frequent home training sessions, provided that participants demonstrate they can charge and upload their HR data to Polar Flow (online) for trainer review and feedback at least once per week.

16. General Data Management/Quality Assurance

Paper/hard copy records (hard copy surveys, questionnaires, case report forms, pictures, etc.)

- Standardized procedures will be used to collect and maintain basic demographic and health data on all participants, including: (1) collecting names, email addresses, and phone numbers; (2) contacting participants with personalized letters and cards via email; (3) maintaining phone contact with participants throughout the study; (4) setting up a (secure and access-restricted) tracking file with names, email addresses, phone numbers, projected session times.
- All contact information will be stored on a secure file server and will be kept separate from study data. Once a participant has been contacted and screened with our screening session, and qualifies for the study, they will be assigned a subject number and all testing materials beyond that point will contain only the subject number.
- The participant consent forms and demographic forms (which will have names) will be stored in separate, locked locations. Behavioral data that is administered via paper-and-pencil questionnaires will be kept in a folder identifying the subject only through their participant number, which will be kept in a locked cabinet in Room G8 at Spence Laboratories.

Electronic records (computer files, electronic databases, etc.)

- All lab computers are password-protected and restricted to only lab members based on their Iowa hawkid and password. Data collected and recorded on a computer will be identified only through the participant's subject number.
- The participant database that houses participant names and contact information will include information about the participant's subject ID during the study for scheduling purposes. Following completion of the study, the participant's subject number will be

erased from the database and linked to study data only through a unique lab-id that will be used to maintain record of participants across studies.

- The master electronic database will be within a secure electronic data capture system (RedCap).

The following is a list of study participant confidentiality safeguards:

- **Electronic files** – data identifying participants that are stored electronically will be maintained in an encrypted form or in a separate file.
- **Forms** - forms or pages containing personal identifying information will be separated from other pages of the data forms and retained in a secure location.
- **Data listings** - participant name, name code, hospital chart, record number, Social Security Number, or other unique identifiers will not be included in any published data listing.
- **Data distribution** - data listings that contain participant name, name code, or other identifiers easily associated with a specific participant will not be distributed.
- **Data disposal** - computer listings that contain participant-identifying information will be disposed of in an appropriate manner.
- **Access** - participant records will not be accessible to persons outside the site without the express written consent of the participant.
- **Storage** - study forms and related documents retained both during and after study completion will be stored in a secure location.
- **Blood samples –** blood samples will be processed and stored in dedicated freezer space within Dr. Pierce's lab with study ID and session labels.

Computers will be used to store and analyze clinical data and we will address elements of computer security to ensure that the data remain confidential.

17. Overall Data Analysis Approach

The primary analysis concerns the difference in functional connectivity among the chronic treatment groups of moderate intensity (M) and light intensity (L) at the single terminus (last visit) of the 6-month RCT. Multiple regression will be used to regress functional connectivity (FC) on dummy coded group membership, and the stratification variables of age and sex. Suppose that FC for the i^{th} participant at the end of the study is denoted as y_i ($i = 1, \dots, N$) and group is coded as grp_i = 1 if M and grp_i = 0 if L. Then the multiple regression model for the primary analysis is

$$y_i = \beta_0 + \beta_1 grp_i + \beta_2 sex_i + \beta_3 age_i + e_i.$$

Under the typical assumption that e_i is normally distributed, the coefficients can be estimated using ordinary least squares. The null hypothesis of interest is that there is no group difference, $H_0: \beta_1 = 0$, which can be evaluated with a *t*-test. In order to account for missing data, multiple imputation will be used, and the null hypothesis of interest will be evaluated based on pooled estimates using Rubin's rules (23). In order to ensure maximal statistical power for the primary aim, the first analysis will have a single outcome, which is the average FC between the posterior hippocampus, posterior cingulate cortex, and the ventral medial prefrontal cortex. Additional exploratory analysis will be performed on individual connections with adjustment for multiple testing (i.e., adjustment for false discovery rate within a region of interest).

We will use a similar approach to examine intervention effects on learning rate in the hippocampal-dependent learning tasks compared to the non-hippocampal tasks. The final analysis of *Aim 1* will examine the relationship between changes in hippocampal-cortical FC and learning rate by adding hippocampal-cortical FC as an independent variable to the regression predicting learning rate. Secondary analysis for chronic effects on learning will evaluate mediation models that treat change in CRF as a continuous variable. The purpose of this analysis is to test the model proposed in *Aim 3* whereby change in CRF acts as a critical mediator leading to change in hippocampal-cortical FC and hippocampal-dependent learning. The α-adjustment for the secondary analysis will be more stringent than the primary analysis.

The secondary aim examines whether acute increases in FC that are specific to moderate intensity exercise are related to improvement in FC and learning at the end of the 6-month RCT. Specifically, we will test the prediction proposed in *Aim 2* that greater acute increases in FC to the M compared to L condition will be associated with a greater effect in the chronic M group. Acute increases will be computed for each participant based on a fitted linear mixed model (LMM) from the acute phase. The LMM models change from M to L accounting for the cross-over in conditions. A type of difference score will be computed for each participant based on the fixed and random effects estimates representing the acute M – L difference (24). Positive values indicate an increase in FC for M compared to L (and negative values indicate a decrease; 0 indicates no change). A multiple regression model will be used to regress FC on chronic group, the acute M – L difference, and their interaction. Suppose that the acute M – L difference for the i^{th} participant is denoted as $diff_i$. Then the regression model for the second aim is

$$y_i = \gamma_0 + \gamma_1 grp_i + \gamma_2 diff_i + \gamma_3 (grp_i)(diff_i) + e_i.$$

The interaction term allows the effect of the acute M – L difference to vary by chronic group. When $\gamma_3 \neq 0$, the acute difference has a different effect for the chronic M group. Therefore, the null hypothesis of interest for the second aim is $H_0: \gamma_3 = 0$. Missing data will again be handled with multiple imputation. The first test will consist of the same hippocampal-cortical outcome described above, and additional exploratory analysis will be performed adjusting for multiple testing.

The main analysis for all three aims will be conducted under the intent-to-treat (ITT) principle. A participant will be counted as a member of their group at the time of re-randomization in the chronic phase. Participants will be analyzed in their initial group assignments in a blinded manner, regardless of dropout or adherence. Fidelity will be assessed by separate regression models in which CRF is the outcome with the goal of examining if the treatment caused a sufficient difference in CRF. A small number of participants will be allowed to enroll as a couple with the couple being the unit for random assignment. One member of the couple will be randomly assigned for analysis and the other member's data will not be considered for analysis in the primary aim (data might be inspected for exploratory purposes).

Due to its importance in achieving our objectives, the power analysis is based on preliminary data for the relationship between aerobic exercise training change in learning rate on one of the learning tasks. Based on the effect size observed from a multiple linear regression of training group and additional covariates as predictors, our power analysis maintains an allowance of 10 predictor variables including training group. Based on these considerations, a sample size of 120 older adults, randomized to one of two training groups (N=60 per group), would ensure 95% power. If we further account for up to ~15% (N=18) missing data due to factors such as (a) motion or drop-out during scanning, (b) missing post-test due to drop-out during training, or (c) co-enrollment with spouse, we would still have a final sample size of ~N=100, which would achieve ~90% two-tailed power and still result in larger group sizes than our published results of hippocampal-cortical FC following exercise training. We do not have plans for formal interim analyses and there is no predefined interim statistical analysis or result that would cause termination of the trial.

Dealing with missing data: The primary analysis will be conducted according to the ITT principle, in which participants are analyzed in their assigned group at randomization. Multiple imputation will be used for pooled estimation, which provides unbiased estimates under the ignorable mechanism. It is not possible to determine if a missing data mechanism is ignorable or non-ignorable. In order to address the possibility of a non-ignorable mechanism, a sensitivity analysis using pattern mixture modeling with multiple imputation will be conducted under the framework discussed by Little and colleagues (25).

Assessing effects of adherence and training context: ITT analysis is recognized as the best approach for making sound inferences regarding the treatment effect (26). However, ITT focuses on the effect of treatment assignment rather than on the effect of the treatment for participants who experienced the treatment as defined in the protocol. For example, ITT analysis does not adjust for potential non-adherence (variations in session attendance) or treatment cross-over

(exposure to the treatment intended for another group). In exercise trials both of these issues are theoretically important to examine because (a) mechanistically exercise effects are expected to be strongest in a dose-response manner relative to the prescribed exercise program, and (b) there is significant variability in the extent to which participants achieve the prescribed exercise intensity during their training. For example, the latter issue can occur if participants in the M group have difficulty consistently getting their heart rate up to higher intensities due to physical or motivational constraints; or, in contrast, if participants in the L group enrolled in the study with expectations to work harder and in turn get their heart rate up above the prescribed lighter intensity zone when they are exercising at their home sessions.

Therefore, in a series of un-blinded exploratory per-protocol analyses (27), we will test the extent to which adherence and training context affects training-induced change in primary outcomes of hippocampal-cortical FC and learning. Based on the issues outlined above, analyses will initially be based on pre-planned definitions of context and adherence. First, we will test the extent to which training heart rate (HR) differed in the lab compared to home sessions as a function of intervention group. Based on preliminary descriptive data, we predict that HR will be higher during home sessions for both groups, but there will be no average group differences in this context effect. Second, we will test all training group effects described above with an additional continuous interaction term for %sessions completed. We predict that greater sessions attended will have a weak to moderate effect on the benefit of M compared to L intensity training. Third, we will test the same group interactions with a continuous interaction term for %sessions in the prescribed HR zone. We predict this will have a moderate to strong effect on primary outcomes, as adhering to the intensity prescription is predicted to have a stronger effect than attendance alone. A final analysis will further unpack the direction of intensity adherence, with negative values indicating the percent of sessions below the prescribed HR zone and positive values indicating the percent of sessions above the prescribed HR zone. This final intensity adherence analysis will test both *whether* and *how* gains in benefits were associated with variations in adherence to prescribed HR intensity.

18. Safety

There are no known risks to participating in the questionnaire assessments or cognitive tests. There may be risks involved in exercising on a motorized bicycle: participants may become fatigued or uncomfortable while exercising. Additionally, there may be a small risk of loss of confidentiality associated with study participation. Listed below are potential risks associated with assessments to be completed throughout the trial:

- Maximal ECG Stress Test with oxygen (VO2) monitoring: Potential risks of a stress test are fatigue, dry mouth, heart palpitations, muscle strain, shortness of breath and chest pain. There is a small risk of heart attack (0.04%) and death (0.01%) in middle-aged and older adults. The risk of heart attack or death in young healthy adults is significantly lower. A physician will be present to monitor the subject's 12 lead ECG, blood pressure, and any symptoms. American Heart Association/American College of Sports Medicine guidelines for contraindications to begin the test, and indications to stop the test will be

followed. After the ECG Stress/VO2 test, the subjects will be monitored for 15 minutes, will be symptom free, and the ECG will be back to baseline before released.

- Subjects may feel uncomfortable answering questions.
- Pulse wave velocity: There are no known or foreseeable risks associated with the use of applanation tonometry for pulse wave analysis. ECG electrodes may cause minor irritation to the skin.
- Fasting for 8 hours: The most common risk when fasting is dehydration, therefore subjects will be encouraged to drink plenty of water. Subjects may experience hunger and irritability and if they experience fainting, nausea, or vomiting they will be instructed to stop fasting.
- Some participants feel anxiety in the MRI scanner due to feelings associated with claustrophobia
- Some participants experience discomfort from lying down for an extended period of time
- The MRI scanner produces a loud hammering noise, which has produced hearing loss in a very small number of patients.
- A metal object flying through the air toward the magnet and hitting the participant presents the greatest risk associated with MRI
- Potential risks associated with obtaining blood samples are minimal but include slight bruising, pain, a temporary feeling of faintness, and/or a small risk of infection.

What have we done to minimize risks?

ECG ergometer stress test with VO2 monitoring: A physician with training in cardiology will be present for all pre- and post-intervention maximal exercise tests that are conducted at the Exercise Physiology Lab at the Field House. The physician will monitor the subject's 12 lead ECG, blood pressure, and any symptoms. American Heart Association/American College of Sports Medicine guidelines for contraindications to begin the test, and indications to stop the test will be followed. Our trained exercise specialist administers the exercise test, with supervision by a physician. Dr. Schmid or Dr. Muellerleile will supervise our pre- and post-intervention maximal exercise tests.

After the ECG Stress/VO2 test, participants will be monitored for 15 minutes to ensure they are symptom free and that the ECG is back to baseline before the participant is released. Personnel involved in exercise testing will be required to be certified in CPR and First Aid. In addition, the EPL is equipped with an AED, phone to call 911, and a fully supplied crash cart maintained by the University of Iowa Hospitals and Clinics. The pre-intervention exercise test serves as a baseline measure of cardiorespiratory fitness as well as a screening tool to ensure no cardiovascular abnormalities are present during moderate-to-vigorous aerobic exercise. Following this screening session, the physician's role is to indicate whether the participant is now eligible to continue with participation in an exercise training program with minimal risk of an adverse event. Participants with evidence of cardiovascular disease at baseline (e.g., evidence of myocardial infarction, abnormal cardiac arrhythmia, myocardial ischemia, conduction delays) or during the exercise test (>1mm ST segment depression or elevation; >3 beat ventricular tachycardia; atrial fibrillation, chest pain suggestive of angina) will be excluded from the study because of the associated increase in risk for an adverse event during training.

Once a participant passes screening during their maximal exercise test as described above, exercise training sessions are each supervised in our lab at Spence Labs by a trained exercise specialist. Personnel involved in exercise training will be required to be certified in CPR and First Aid. Lead exercise trainers will be Clinical Exercise Physiology Master's students recruited from the Health and Human Physiology Department at the University of Iowa. In addition, we require that trainers have had previous experience in training older adults.

To protect against potential adverse events during exercise training, the exercise training lab is equipped with an AED, a phone to call 911, and during all exercise sessions we monitor heart rate, blood pressure, and perceived exertion to ensure we are aware of any discomfort that would be a warning sign for an adverse event. All training sessions include a cool-down that is designed to ensure participants' heart rate and blood pressure are back to near resting levels before leaving our training site. In the event that a participant expresses symptoms of a potential adverse event to exercise (e.g., light headed or an unusually high spike in heart rate or blood pressure), exercise trainers will immediately stop exercise and have the participants rest with the opportunity to drink water. If there is any sign that the symptoms are not resolving from a break in exercise, the trainer will call 911 to ensure timely arrival of an emergency medical team to our training site. Because our training site is a 10-minute drive and 20-minute walk from the hospital, and our team doctors could also be working at clinics up to a 25-minute drive away, the safest response to any potential medical event will be to call 911.

Following any level of potential adverse event, our staff will document the events preceding, during, and following the onset of symptoms in a detailed post-mortem. This will be sent to the PI, co-Investigator Dr. Pierce, and our local supervising doctors within 24-48 hours of the event. The adverse event will be reported to the IRB if it is unanticipated, serious, or would have implications for the study protocol to protect participant safety. The level of feedback necessary will be dictated by the assessment of the local supervising doctors (Schmid and Muellerleile). If the event is potentially serious, the post-mortem will be sent to the PI, Dr. Pierce, our local supervising doctors, the IRB, our safety officer Dr. Burns, and Dr. Wagster within 24 hours of the event. In all cases, feedback from the medical team will be used to improve any training procedures that may have been associated with the symptoms for that participant and to protect against future events if necessary. To address the potential risk of boredom, fatigue or frustration during the behavioral tasks, we will provide frequent breaks to participants, including opportunity to get water and stretch their legs.

To address the risk that subjects could feel uncomfortable answering questions, we will remind participants that all questions are voluntary. It may be the case that they are ineligible if they are not comfortable answering some questions that are critical to ensuring their safety and meeting the goals of the study. In this case participants will be reminded that participation is voluntary and we would be happy to contact them for future studies that they may find more comfortable participating in.

To address the potential for anxiety in the MRI scanner, we will conduct a "Mock MRI" before the actual MRI. This session allows the participant to experience what it is like to lie in the magnet but without the pressure and time-constraints of the actual scanner session. Importantly,

this allows us to anticipate and prevent participant anxiety as much as possible during the real MRI.

To address the potential for discomfort during the MRI session, we will work with the participant during the Mock MRI to arrive at a comfortable lying position (e.g., with or without a pillow under their legs and how much cushioning they prefer if they would like a pillow). We will also utilize the Mock MRI session to screen out anyone who does not fit comfortably in the bore of the scanner, such as their shoulders or stomach pressing against the walls of the bore. During the MRI itself, we will regularly check in with the participant about their comfort, so that we can make adjustments in their positioning when needed. Participants who are uncomfortable with the MRI scan do not have to complete the MRI visit if they fit all other criteria to participate in the rest of the study.

To address any potential for hearing loss, participants will wear earplugs during all scanning.

To address the risk of flying metal objects, we require that all people involved with the study remove all metal from their clothing and pockets. No metal objects will be brought into the magnet room while participants are inside the room. In addition, the door to the room remains closed throughout the study so that no one can accidentally bring a metal object into the room.

To address potential risks associated with the blood sample, all blood draws will be performed by a research team member trained and certified in drawing blood, or a nurse. To reduce anxiety associated with lab draws, the lab draw will be done by experienced research nurse or staff to ensure correct technique and minimize risk of multiple needle pricks to obtain a blood sample.

To address potential for loss of confidentiality, we will:

- Store paper/pencil data in secure, locked file cabinets, accessible only to lab personnel
- Computer data will be stored on lab testing PCs that are password protected and only accessible to lab personnel
- All data will be stored with only subject ID's attached, in a separate location from the consent and demographic forms
- Subject ID's will be assigned following informed consent
- Subject ID's will be linked to a unique lab ID that will coexist with identifying information in our secure database
- Any lists linking study ID with the unique lab ID will be made by querying the secure database and will be stored only locally on our password protected computer and our password protected back-up server in the psychology department
- All personnel involved in the study will be required to complete the training courses available for education on the protection of human research subjects provided by the University of Iowa Human Subjects Office
- All study staff will be trained in confidentiality procedures and will receive the required University of Iowa data security training.

Research procedures will collect only the minimum private information necessary to conduct the study. Measures will be taken to protect privacy throughout the study, including:

- Consent, demographic, and fitness assessment will be done in a private room
- All computerized testing will occur in either a private room or a room with computers separated by dividers
- There is no risk of loss of privacy in computerized sessions when more than one person are in the room at once, no data or performance scores are read out verbally or appear on the screen to the participant or for others to see

19. Adverse and Serious Adverse Events

Reporting all adverse events is an integral part of conducting this clinical trial. This will allow the researchers to track any occurrences of serious events and ensure they can be reviewed by the Institutional Review Board (IRB), the Data Safety Monitoring Board (DSMB), and the National Institute on Aging (NIA). The purpose of reporting adverse events is the protection and safety of the study participants. Additionally, it will provide a description and contrast of the major side effects associated with both the intervention and control groups.

Defining Adverse Events
All adverse events will be classified as either an adverse (AE) or a serious adverse event (SAE). They may occur during screening, testing, or during the administration of the intervention. Furthermore, an adverse event may or may not be related to the testing or intervention procedures.

All adverse events shall be defined as any occurrence of an adverse effect (undesirable and unintended, although not necessarily unexpected, result of the intervention); any adverse finding including a sign, symptom, or abnormal assessment or any other unfavorable outcome affects a human subject detrimentally, or that worsens, as a result of their participation in the study.

Defining Serious Adverse Events
Serious adverse events are defined as events that may be harmful to the participant and/or may be serious enough to warrant either temporary or permanent discontinuation of the study intervention because they are either intolerable or they are judged to be potentially harmful to the study participant.

Criteria and Definitions for Serious Adverse Events
Any adverse event will be classified as a serious adverse event when it satisfies any one of the following criteria (standard criteria as defined in the Code of Federal Regulations, 21CFR 312.32):

- It results in death
- It is life threatening. Life threatening events are those that pose the immediate risk of death from the event.
- It results in inpatient hospitalization or prolongation of existing hospitalization. Hospitalization will be defined as at least a 24-hour inpatient hospitalization or

prolongation on an existing hospitalization. In general, all other hospitalizations are to be considered adverse events. Examples include outpatient and less than 24-hour hospitalizations. However, an outpatient hospitalization or one less than 24 hours could be considered a serious adverse event if it were life threatening or an important medical event.

- It results in a persistent or significant disability. Significant disability is one that results in a permanent or severe disruption of the participant's ability to conduct normal life activities. If the disability is related to the normal progression of an already present disease or condition, it will not be considered a serious adverse event. The decision to classify a disability as permanent or significant will be based on the clinical judgment of the study's medical director.
- It is a pregnancy resulting in a congenital abnormality or birth defect. A congenital anomaly is an adverse outcome in a child or fetus of a participant who has participated in the testing and/or the intervention prior to conception or during pregnancy.
- It is an important medical event. An important medical event is one that may jeopardize the study participant and may require medical and/or surgical intervention to prevent one of the above listed outcomes. The decision to classify a medical event as a serious adverse event will be based on the clinical judgment of the study's medical director.

Adverse and Serious Adverse Events
The most likely way in which an adverse event is identified would be when the participant reports an event to the exercise interventionists, research assistants, or research coordinator during contact time (i.e. during intervention sessions or at testing appointments).

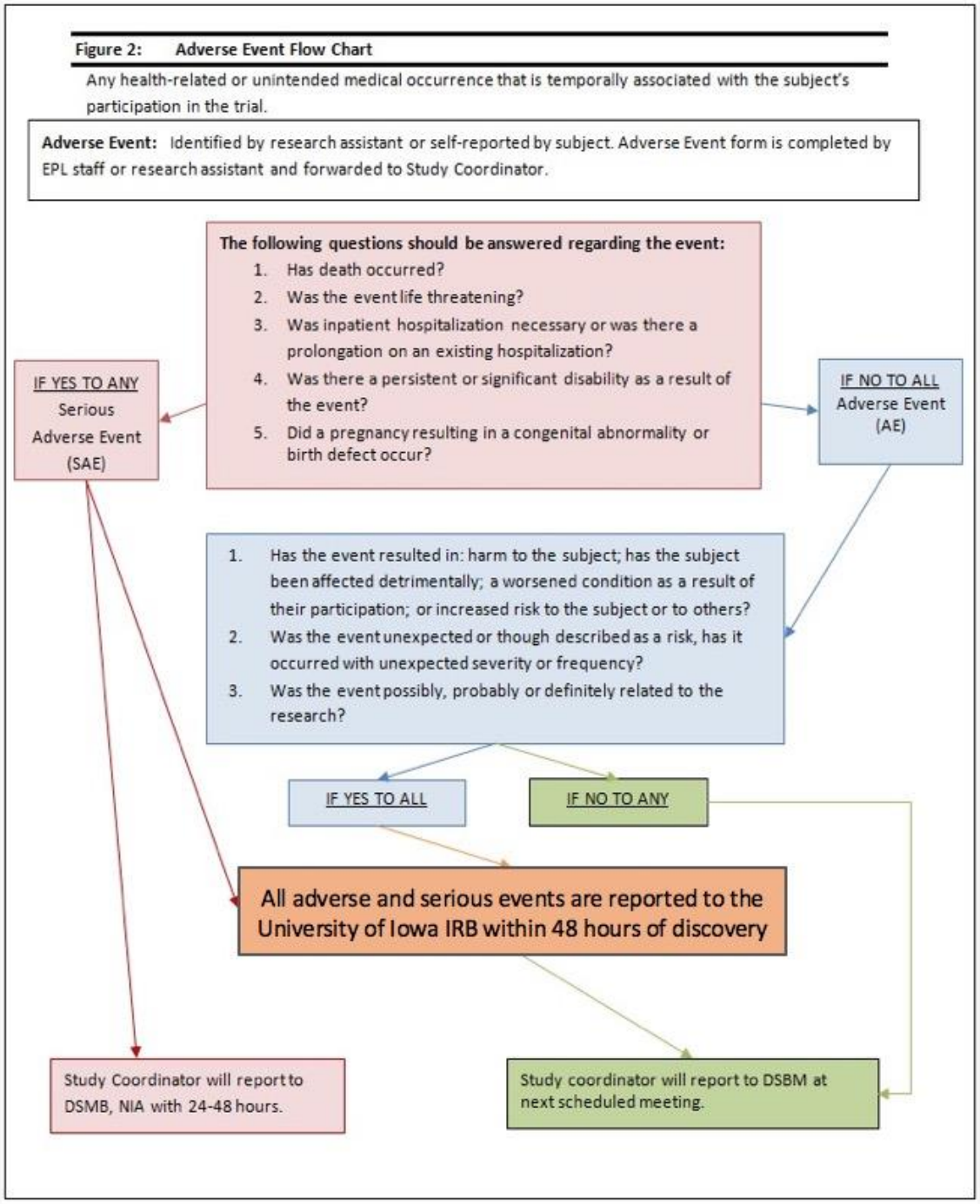
Reporting of Adverse Events
When an adverse event is identified, the staff member identifying the adverse event will report it to the study coordinator and the principal investigator. The study coordinator will then contact the participant and an Adverse Event Report Form (Appendix III) will be completed. All adverse events that are unexpected, serious, or could affect our protocol will be electronically reported to the University of Iowa Institutional Review Board within 24 hours. If the event is not classified as a serious adverse event, it will be reported to the DSMB at the next scheduled conference call. If an event has been determined serious, reports of serious adverse events will be forwarded to the University of Iowa IRB, the DSMB, and the NIA project officer within 24-48 hours of occurrence. All adverse event documents will be stored in a separate secure cabinet by the project coordinator.

Adverse Event Flowchart

Template forms for reporting adverse events can be found in Appendix III.

Figure 2: Adverse Event Flow Chart

Any health-related or unintended medical occurrence that is temporally associated with the subject's participation in the trial.



20. Anticipated Problems

Based on our strong preliminary data and by the PI's own recent research we believe the hypotheses will be supported. However, should this not be the case, examination of moderators and additional brain networks that interact with hippocampal-cortical systems will provide alternative approaches to testing our aims.

Recruitment:

Recruitment of older healthy adults can be a challenge. However, we are confident we can accomplish our recruitment goals by 1) advertising throughout the community with mass mailings, 2) contacting age-relevant individuals through the University of Iowa mass email system, and advertisements in the 3) daily Noon News distributed to UIHC employees, and 4) the UIHC Epic Database.

Subject Retention:

A related potential barrier is participant dropout. We will minimize dropout through careful screening, by providing ample rest and personal support between study sessions, providing personable and professional personal exercise training, and monetary compensation.

Injuries:

Extreme care will be taken to avoid any exercise-related injuries. Participants will be provided proper demonstration and instruction for the bike exercise intervention and educated about any risks and benefits prior to the intervention. Five minute warm-up periods will be included and followed by gradual progression of intensity until the duration is complete. Five minute cool down periods will also be included. If the participant is not feeling well the day of their intervention session, they will be instructed to reschedule so as to not invoke any potential injuries related to illness. If the participant does report an injury or injury occurrence to the staff, they will be referred to their primary care physician for treatment and be instructed not to participate in the intervention until they receive consent from their physician.

21. DSMB Meetings and Membership

The Data Safety and Monitoring Board (DSMB) will meet twice a year to review all active protocols. For all monitoring activities, the DSMB will follow the guidelines established by the National Center for Research Resources which include:

- Monitor the progress of the intervention trial (e.g., review subject recruitment, attrition, and minority involvement), and the safety of research participants (e.g., reviewing un-blinded data for safety)
- Assure compliance with requirements regarding the reporting of adverse events

- Assure that any action that results in the temporary or permanent suspension of the protocol is reported to all the appropriate monitoring bodies
- Assure data accuracy, confidentiality, and protocol compliance

The DSMB is comprised of individuals without a stake in the study outcomes, who will oversee subject safety. The DSMB consists of:

- A person with expertise in clinical trials and has experience in exercise in cognitive aging: Dr. Michael Marsiske from the University of Florida (Chair)
- An expert who reviews any adverse events or serious AEs and who has DSMB experience: Dr. Jeffrey Burns from the University of Kansas
- A biostatistician or someone who has skills in that area and who has DSMB experience: Dr. Charles Hall from the Albert Einstein College of Medicine

Although the NIH Institute/Center appointed Program Officer (PO) (Dr. Molly Wagster) is not formally engaged within the monitoring process, the PO and designated University of Iowa Institutional Review Board representative will be kept abreast of any and all issues which arise and will receive all required and requested information as required by policy, regulation, and/or law.

22. References

1. Voss M, Vivar C, Kramer A, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn Sci. 2013;17(10):525-44. doi: 10.1016/j.tics.2013.08.001. PubMed PMID: 24029446.

2. van Praag H, Christie B, Sejnowski T, Gage F. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999;96(23):13427-31. PubMed PMID: 10557337.

3. van Praag H, Shubert T, Zhao C, Gage F. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci. 2005;25(38):8680-5. doi: 10.1523/JNEUROSCI.1731-05.2005. PubMed PMID: 16177036.

4. Barnes D, Santos-Modesitt W, Poelke G, Kramer A, Castro C, Middleton L, Yaffe K. The Mental Activity and eXercise (MAX) trial: a randomized controlled trial to enhance cognitive function in older adults. JAMA Intern Med. 2013;173(9):797-804. doi: 10.1001/jamainternmed.2013.189. PubMed PMID: 23545598.

5. Angevaren M, Aufdemkampe G, Verhaar H, Aleman A, Vanhees L. Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive

impairment. Cochrane Database Syst Rev. 2008(3):CD005381. doi: 10.1002/14651858.CD005381.pub3. PubMed PMID: 18646126.

6. Nagamatsu L, Flicker L, Kramer A, Voss M, Erickson K, Hsu C, Liu-Ambrose T. Exercise is medicine, for the body and the brain. Br J Sports Med. 2014;48(12):943-4. doi: 10.1136/bjsports-2013-093224. PubMed PMID: 24659507.

7. Naqvi R, Liberman D, Rosenberg J, Alston J, Straus S. Preventing cognitive decline in healthy older adults. CMAJ. 2013;185(10):881-5. doi: 10.1503/cmaj.121448. PubMed PMID: 23589432.

8. Young J, Angevaren M, Rusted… J. Aerobic exercise to improve cognitive function in older people without known cognitive impairment. The Cochrane …. 2015;4:CD005381. doi: 10.1002/14651858.CD005381.pub4. PubMed PMID: 25900537.

9. Sink K, Espeland M, Castro C, Church T, Cohen R, Dodson J, Guralnik J, Hendrie H, Jennings J, Katula J, Lopez O, McDermott M, Pahor M, Reid K, Rushing J, Verghese J, Rapp S, Williamson J, LIFE SI. Effect of a 24-Month Physical Activity Intervention vs Health Education on Cognitive Outcomes in Sedentary Older Adults: The LIFE Randomized Trial. JAMA. 2015;314(8):781-90. Epub 2015/08/26. doi: 10.1001/jama.2015.9617. PubMed PMID: 26305648; PMCID: PMC4698980.

10. Erickson K, Voss M, Prakash R, Basak C, Szabo A, Chaddock L, Kim J, Heo S, Alves H, White S, Wojcicki T, Mailey E, Vieira V, Martin S, Pence B, Woods J, McAuley E, Kramer A. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A. 2011;108(7):3017-22. doi: 10.1073/pnas.1015950108. PubMed PMID: 21282661.

11. Voss M, Prakash R, Erickson K, Basak C, Chaddock L, Kim J, Alves H, Heo S, Szabo A, White S, Wojcicki T, Mailey E, Gothe N, Olson E, McAuley E, Kramer A. Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. Front Aging Neurosci. 2010;2. doi: 10.3389/fnagi.2010.00032. PubMed PMID: 20890449.

12. Voss M, Weng T, Burzynska A, Wong C, Cooke G, Clark R, Fanning J, Awick E, Gothe N, Olson E, McAuley E, Kramer A. Fitness, but not physical activity, is related to functional integrity of brain networks associated with aging. NeuroImage. 2016;131:113-25. doi: 10.1016/j.neuroimage.2015.10.044. PubMed PMID: 26493108.

13. Colcombe S, Kramer A, Erickson K, Scalf P, McAuley E, Cohen N, Webb A, Jerome G, Marquez D, Elavsky S. Cardiovascular fitness, cortical plasticity, and aging. Proc Natl Acad Sci U S A. 2004;101(9):3316-21. doi: 10.1073/pnas.0400266101. PubMed PMID: 14978288.

14. Colcombe S, Erickson K, Scalf P, Kim J, Prakash R, McAuley E, Elavsky S, Marquez D, Hu L, Kramer A. Aerobic exercise training increases brain volume in aging humans. J Gerontol A Biol Sci Med Sci. 2006;61(11):1166-70. PubMed PMID: 17167157.

15. Gordon B, Rykhlevskaia E, Brumback C, Lee Y, Elavsky S, Konopack J, McAuley E, Kramer A, Colcombe S, Gratton G, Fabiani M. Neuroanatomical correlates of aging,

cardiopulmonary fitness level, and education. Psychophysiology. 2008;45(5):825-38. doi: 10.1111/j.1469-8986.2008.00676.x. PubMed PMID: 18627534.

16. Lindenberger U. Human cognitive aging: corriger la fortune? Science. 2014;346(6209):572-8. doi: 10.1126/science.1254403. PubMed PMID: 25359964.

17. Hertzog C, Kramer AF, Wilson RS, Lindenberger U. Enrichment effects on adult cognitive development can the functional capacity of older adults be preserved and enhanced? Psychological Science in the Public Interest. 2009;9(1):1-65. doi: 10.1111/j.1539-6053.2009.01036.x. PubMed PMID: 20865146.

18. Lautenschlager N, Cox K. Can participation in mental and physical activity protect cognition in old age?: Comment on "The Mental Activity and eXercise (MAX) trial: a randomized controlled trial to enhance cognitive function in older adults". JAMA Intern Med2013. p. 805-6.

19. Lautenschlager N, Cox K, Flicker L, Foster J, van Bockxmeer F, Xiao J, Greenop K, Almeida O. Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. JAMA. 2008;300(9):1027-37. doi: 10.1001/jama.300.9.1027. PubMed PMID: 18768414.

20. Norton S, Matthews F, Barnes D, Yaffe K, Brayne C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 2014;13(8):788-94. doi: 10.1016/S1474-4422(14)70136-X. PubMed PMID: 25030513.

21. Weng TB, Pierce GL, Darling WG, Falk D, Magnotta VA, Voss MW. The Acute Effects of Aerobic Exercise on the Functional Connectivity of Human Brain Networks. Brain Plasticity. 2017;2(2):171-90. Epub 2017/03/28. doi: 10.3233/bpl-160039. PubMed PMID: 29765855; PMCID: PMC5928541.

22. ACSM. ACSM's Guidelines for Exercise Testing and Prescription. 9th Edition ed. Medicine ACoS, editor: Lippincott Williams & Wilkins; 2013.

23. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York: John Wiley & Sons, Inc.; 1987.

24. Voss MW, Weng TB, Narayana-Kumanan K, Cole RC, Wharff C, Reist L, DuBose L, Sigurdsson G, Mills JA, Long JD, Magnotta VA, Pierce GL. Acute Exercise Effects Predict Training Change in Cognition and Connectivity. Med Sci Sports Exerc. 2019. Epub 2019/08/07. doi: 10.1249/MSS.0000000000002115. PubMed PMID: 31385912.

25. Little RJ, D'Agostino R, Cohen ML, Dickersin K, Emerson SS, Farrar JT, Frangakis C, Hogan JW, Molenberghs G, Murphy SA, Neaton JD, Rotnitzky A, Scharfstein D, Shih WJ, Siegel JP, Stern H. The prevention and treatment of missing data in clinical trials. N Engl J Med. 2012;367(14):1355-60. Epub 2012/10/05. doi: 10.1056/NEJMsr1203730. PubMed PMID: 23034025; PMCID: PMC3771340.

26. Cook TD, DeMets DL. Introduction to Statistical Methods for Clinical Trials. Cook TD, DeMets DL, editors: Chapman and Hall/CRC; 2007.

27. Sussman JB, Hayward RA. An IV for the RCT: using instrumental variables to adjust for treatment contamination in randomised controlled trials. BMJ. 2010;340:c2073. Epub 2010/05/06. doi: 10.1136/bmj.c2073. PubMed PMID: 20442226; PMCID: PMC3230230.

Appendix I. Sample Recruitment Flyer/Letter/Postcard

Exercise Effects on Brain Connectivity and Learning From Minutes to Months (BIKE Extend)

The University of Iowa BIKE research team would like to invite you to see if you qualify for a free exercise training program designed for adults 55-80 years of age.

Participants will complete 6 months of exercise training guided by an exercise specialist. Participation will also involve 3 MRI scanning sessions and 4 neuropsychological assessment days. All program costs are free to participants and parking is provided.

Your participation in the BIKE EXCEL research study will contribute to the knowledge of how exercise training may benefit cognitive abilities, brain functionality, functional status and quality of life in older adults. Participants who complete the study will be compensated $295 + parking.

Principal Investigator:
Dr. Michelle Voss

Project Coordinator:
Conner Wharff

Funding Agency:
National In

In Collab ty
of Iowa De
Human Phy

54

For More Information Contact:
Conner Wharff: BIKE Project Coordinator

Health, Brain and Cognition Lab
W311 Seashore Hall
Iowa City, IA 52241-1407

Would you like to be part of an active and fun program geared toward becoming more physically fit and improving your memory? We invite all healthy older adults aged 55-80 to contact us about taking part in our upcoming exercise training program and research study. Participants should have no history of neurological or psychiatric disorder. Compensation is provided. For more information, please reply to this email: pbs-hbclab@uiowa.edu

Fill out our eligibility questionnaire at this link: bit.ly/BikeExtend

FOR IRB USE ONLY
APPROVED BY: IRB-01
IRB ID #: 201705800
APPROVAL DATE: 06/05/19
EXPIRATION DATE:
05/15/20

Appendix II. Sample Informed Consent

INFORMED CONSENT DOCUMENT

Project Title: **Exercise Effects on Brain Health and Learning From Minutes to Months**

Principal Investigator: **Michelle Voss**

Research Team Contact: **Michelle W. Voss**

Office phone: 319-335-2057

Email: michelle-voss@uiowa.edu

This consent form describes the research study to help you decide if you want to participate. This form provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research subject.

- If you have any questions about or do not understand something in this form, you should ask the research team for more information.
- You should discuss your participation with anyone you choose such as family or friends.
- Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

WHAT IS THE PURPOSE OF THIS STUDY?

This is a research study. We are inviting you to participate in this research study because you are a healthy adult between the ages of 55 and 80 years old.

The purpose of this research study is to examine the relationship between physical activity and cognitive (brain) health.

The long-term goal of this research is to gain knowledge that will help doctors make recommendations for healthy life choices for adults.

HOW MANY PEOPLE WILL PARTICIPATE?

Approximately 120 people will take part in this study conducted by investigators at the University of Iowa.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study, your involvement will last for about 28 weeks.

There will be 83 visits total, which includes 72 exercise sessions, as well as other visits, which include assessments of fitness, brain function, and MRI scans.

You will have on average 3 visits per week and no single visit will last more than 2.5 hours.

WHAT WILL HAPPEN DURING THIS STUDY?

This study is a 24-week exercise intervention. The following table summarizes what is included in the study:

Appointment	Time	Location	Brief description
Visit 1: Orientation, cognitive screening, paper and pencil tasks, and mock MRI	2 hrs	PBDB	Orientation to the study, cognitive screening, paper and pencil tasks, and mock MRI. After this visit, if you are eligible to continue in the study, you will return for further sessions.
* Visit 2: Health Assessment VO2 Max Aerobic Fitness Test	1 hr.	Field House	Maximal exercise test on a cycle ergometer, supervised by a physician and trained staff. After this visit, if you are eligible to continue in the study, you will return for further sessions.
* Visit 3: Health assessment Vascular Measurements	1 hr.	UIHC	Test of arterial stiffness, vascular health assessment and blood draw – details on this can be found in the "Health Assessment" section on the following page.
Visit 4: Neuropsychological	2 hrs.	Spence Labs	Computer
Visit 5: Neuropsychological	2 hrs.	Spence Labs	Computer
Visit 6: MRI	2 hrs.	PBDB	MRI scans and exercise

Visit 7: MRI	2 hrs.	PBDB	MRI scans and exercise
24 week exercise intervention	3 per week for 50 min each session	Spence Labs	You will be guided through an exercise intervention
Visit 8: Health Assessment VO2 Max Aerobic Fitness Test	1 hr.	Field House	Maximal exercise test on a cycle ergometer, supervised by a physician and trained staff.
* Visit 9: Health Assessment Vascular Measurements	1 hr.	UIHC	Test of arterial stiffness, vascular health assessment and blood draw – details on this can be found in the "Health Assessment" section on the following page.
Visit 10: MRI	1 hr.	PBDB	MRI scan
Visit 11: Neuropsychological	2 hrs.	Spence Labs	Computer
Visit 12: Neuropsychological	2 hrs.	Spence Labs	Computer

*** The vascular measurement will not specifically be the second visit, it can occur at any time after visit 1 but before the exercise intervention. Scheduling this visit will be dependent on staff availability and nurse availability.**

Each of the appointments in the table are described in more detail below:

Orientation and cognitive screening- During this screening and orientation session, we will introduce you to the study and ask you to complete a number of tests and surveys to assess whether you qualify to continue in the study. The questionnaires will ask about your demographic information (such as date of birth, race/ethnicity, sex, native language, educational background, occupation),

questions about physical characteristics (handedness, hearing, color blindness), your medications, hospitalizations, medical conditions or medical diagnosis, and about your history of falls.

We will also ask for your contact information and whether you agree to have your demographic and health history information stored in a database in order to contact you about future research studies in this lab.

You will then be asked to complete some tests by answering questions from a lab researcher to test your cognitive processing (how you think about information) and your ability to follow instructions. You will also answer surveys about your health, your feelings, and your activities over the past month. We will collect some basic health information, such as height, weight, resting heart rate, resting blood pressure, waist-to-hip assessment, and neck circumference.

Your participation is completely voluntary and you are free to skip questions in the surveys or tests that you do not wish to answer. Based on the information we collect from you, your participation in the study may be complete after this session.

The last part of this session will be a brief "Mock MRI." Since a later session of this experiment includes a scan of your brain in a Magnetic Resonance Imaging scanner, we would like to take some time in your first visit to make sure you will be comfortable in an MRI scanner. The Mock MRI will give you a chance to experience what an actual MRI will be like, and to ask any questions you have before deciding whether you are comfortable participating in the MRI part of the study. We will also introduce you to a breath holding task.

Health assessment- The maximal aerobic exercise test will take place in E137 in the Field House on a separate day from your vascular measurements. Since the vascular assessment involves a blood draw, we will ask you to complete this session on a separate day from that session in order to minimize the likelihood you may experience dizziness/light-headedness for the exercise test. During the exercise test, you will have electrodes placed on your chest for monitoring the electric activity of your heart. To assess your aerobic fitness, you will be asked to cycle on a stationary bike with chest electrodes and a face mask that analyzes the gas in your breath. This procedure will be performed by Dr. Pierce and our staff. The amount of time you will be on the bicycle will vary but most people cycle between 8 and 15 minutes. Before your test, we will measure your height and weight. In the event that Dr. Pierce or his staff are concerned about the safety of your participation in additional exercise sessions associated with the study, we will contact you to describe the specific concern and provide guidance on whether to seek additional medical advice. If this occurs, and for your safety, you will not be eligible to continue in the study.*

*Aerobic fitness tests will **not** be completed without a physician or cardiologist present.

You will be asked to complete a health assessment visit that involves obtaining some measurements of vascular health and an exercise testing visit once before the intervention and once after the intervention. On the day of your vascular assessment appointment we will ask you to arrive at the Hospital between 7-10am after an overnight fast of at least 8 hours and no caffeine or exercise for at least 20 hours. We encourage you to drink plenty of water while you are fasting from food. To measure your blood pressure and the stiffness of your blood vessels, Dr. Pierce and his staff will ultrasound your right upper arm while using an arm cuff. Additionally, Dr. Pierce and his staff will obtain a blood sample at this session to measure serum lipids, glucose, and insulin levels. They will collect three 2.0 teaspoon (10 mL) blood samples at the health assessment visit at pre-testing and at post-testing (12.0 teaspoons or 60 mL in total). All samples will be collected by research personnel trained in phlebotomy by Dr. Pierce's lab.

If blood values come back outside of the reference ranges that are given by the University of Iowa Diagnostic Laboratories (UIDL), the project coordinator will present these values to a licensed practitioner (physician) to determine if values are statistically significant. If values are significant, the project coordinator will inform the participant about the value. The participant would still be eligible to continue with their participation.

Neuropsychological assessment- You will be asked to complete two neuropsychological testing sessions before the intervention and two after the intervention. You will be asked to perform a variety of computerized tasks and paper/pencil tasks that measure learning and motor skills. For example, you will be asked to learn specific responses on a keyboard to stimuli such as faces on the computer screen. Another example of these tasks is remembering the placement of different objects on a screen. You will be given breaks between tests. Following the completion of your first neuropsychological assessment day, we will introduce you to the exercise bike you will ride at the MRI sessions/intervention sessions and introduce you to some scales that assess how you are feeling while you ride the bike during the MRI sessions/intervention sessions.

Actigraph accelerometer use - We will show you how to use a wrist-worn motion sensor to wear on your non-dominant arm until you return to our lab, for at least 7 days but no more than 10. The device is not waterproof and has a two-month battery life, so you must remove it for bathing, swimming, and other water contact, but not for battery charging. The accelerometer should be worn at all times, including sleeping. We would like you to keep the device on at all times to minimize the risk of losing it or forgetting to put it back on. You will also be asked to fill out an activity log to indicate what hours of the day were generally active or sleeping. We will ask you to do this once during the pre-testing phase

and once during the post-testing phase, in addition to one week/month during the exercise intervention.

MRI Scan- For each acute exercise session, you will complete a baseline MRI session that includes scans for high-resolution anatomy and resting cerebral blood flow, resting state scans to assess functional neural synchrony and vasoreactivity.

Directly following initial scans, you will begin a 20-minute exercise session on a recumbent (laying back rather than sitting), stationary motorized bicycle. Blood pressure and heart rate will be measured before, during, and after exercise. Ratings of perceived exertion (RPE) will be measured throughout exercise at 5-minute intervals. Immediately after completing the exercise session, you will enter the MRI scanner for 30 minutes of post-exercise scans, including resting state fMRIs. Physiological arousal and heart rate will be measured throughout all scanning sessions, and blood pressure will be taken after each post- exercise scan.

In the course of this session, we will give you water to drink and we can give you a towel and point a fan at you if you request it.

Details: Upon arrival, you will be greeted by an HBC lab researcher. They will escort you to the waiting room and guide you through the MRI screening procedures and familiarization with the tasks you will do in the MRI scanner. Once ready to start the experiment, you will be asked to remove all metal objects. If your clothes have zippers or metal buttons (or similar) that would interfere with the pictures of your brain, we may ask you to change into hospital clothing that does not contain any metal.

An MRI scanner takes images of your brain by sending out a magnetic field and radio waves. Because the MRI scanner contains a very strong magnet, you may not be able to have the MRI if you have certain kinds of metal in your body (for example, a heart pacemaker, a metal plate, and certain types of heart valves or brain aneurysm clips). Someone will ask you questions about this before you have the MRI. Let us know if you have had surgery of any kind.

In particular, you should not participate in this study if you have any of the following in your body:

Pacemaker

Coronary Stent

Defibrillator

Neurostimulator

There are a number of conditions that will prevent you from having the MRI, we will review these with you to see if you can have the MRI.

Once cleared to have an MRI in this study, you will enter the MRI room with your HBC experimenter and MRI technicians. The MRI scanner is a large machine that contains a hollow tube. You will be asked to lie on your back on a special table that slides into the tube. For this study a coil will be positioned around your head. The head coil is similar in shape to a helmet. The coil is the part of the machine that receives the MR signal and allows images to be captured. The sides of the tube will be fairly close to your body and the scanner makes a loud hammering noise while you are inside. You will be able to talk to people in the room through a speaker system. We will monitor you closely while you are inside the scanner. You will also be given headphones to wear. The headphones reduce the sharpness of the banging noise the MRI machine makes. However, you will still be able to hear us talk to you, and you will also be able to talk to us at any time. We will also give you a squeeze ball to press in case of an emergency. This sets off an alarm that notifies the technologist that you need help.

Next, the bed will move into the magnet. The MRI technician will talk to you throughout the study, ask you how you are doing and tell you what to expect next. You will be in the magnet for approximately 50 minutes. We will use a mirror mounted on the head coil so that you can see visual stimuli and special noise-canceling headphones to hear audio sounds. You will be able to communicate with the technologist using a microphone built into the headphones. During some scans, you will wear a monitoring device on your finger or a respiratory belt around your chest to collect basic information about your body, such as your heart rate and breathing. You will be told before the scan of the exact procedure that will occur during the MRI. If you become uncomfortable and wish to stop the examination at any time, you may tell the technologist, and the study will be ended and you will be moved out of the magnet. The technologist will also let you know when the images are being taken, because you will need to hold very still during that time. You should hold relatively still in between images as well. For most of this time you will not have to do anything except lie quietly while we collect images of your brain. When the study is complete, you will be moved out of the magnet. You should get up slowly to allow your body to get use to moving and being vertical again.

After the completion of your MRI scan, a member of the research team will electronically move the images that were taken to another secure computer. Any information that could be used to identify you as the subject of these images will be replaced with a unique ID code. The images and information about how they were taken will be stored in a central database so that other researchers can use them to evaluate how MR imaging could be used for their research projects (whether they are located at the University of Iowa or at another site). These investigators must submit a formal written request to the research team for approval to have access to the data. The data may then be copied electronically to their laboratory's computers. Your name and corresponding code will be kept in a locked cabinet that can only be accessed by the research team. The other investigators who have requested to use the images will not have access to the correspondence between the unique ID code and your name.

The MRI images for this study are not being used to evaluate your health. The images obtained for this study are for specific research purposes and are not being used to find medical abnormalities. These images will not routinely be reviewed by a radiology physician to diagnose existing abnormalities.

There is a possibility that we will discover that you have an abnormal image. We cannot make a determination from the images that we are collecting if this abnormal image is associated with disease. If we notice an abnormality, we will show the image to a diagnostic radiologist who will then advise us on how to proceed. If the abnormal image presents a medical concern, a physician will contact you to explain that concern to you. In order to reduce the risk that we unnecessarily upset someone about an abnormal image, we will not allow you or anyone with you to see the pictures at the time of the study. If you would like to see your images at a later time, you may set up an appointment. This appointment must be at least one week after your study date.

Following completion of the final pre-intervention MRI session, we will send you some secure links to four surveys and ask that you complete these surveys prior to your first intervention session. In addition, we'll give you a paper copy of one other survey that we would like you to complete and return at your first intervention session.

24-week exercise intervention- You will be asked to complete a 24-week exercise intervention program 3 times a week for 50 minutes at each session. At the end of each week of exercise training you will complete two questionnaires that ask about changes in medication usage, diet, sleep habits, and leisure time activity. We will require you to attend all training sessions in the lab for the first two weeks. For the remaining weeks in the 6 month intervention you will complete one session per week in the lab, and do the remaining two sessions on your own at your home or a gym of your choice. If you are uncomfortable doing home sessions after two weeks, you may come to the lab for more than one training session a week as needed until you are comfortable with doing sessions at home. For all home sessions, you will be instructed on how to use a wrist-worn heart rate monitor and this data will be used to give feedback for your next home sessions. Each week you will practice your session in the lab with our exercise specialist and will receive refresher instructions to take home with you. All exercise sessions at Spence Labs will be supervised by a trained exercise specialist, and all research assistants involved in the study will be trained in CPR, First Aid, and use of an automated external defibrillator (AED) that we have on-site. You will be randomly assigned to receive one of the 2 study treatments, which are two types of exercise programs that are designed to improve your physical health. This means that whichever study treatment you receive will be determined purely by chance, like flipping a coin. You will have a 1 out of 2 chance of receiving any one of the study treatments. Throughout the exercise sessions, you will wear a wrist-worn heart rate monitor and we will ask you to

report how you feel physically and mentally. This data will be used to individualize your training throughout the intervention.

For participants who will receive gas compensation (participants who live 30+ miles away from Spence Labs), we will require that you come in to Spence Labs for two of the three sessions for the first week of the exercise intervention. For the second week, we will require that participants come into our lab for 1-2 sessions. We will make sure that these individuals feel comfortable using the heart rate watch and completing the exercise sessions on their own. After the first two weeks we will have you come into lab once every 3 weeks. Each week that participants do all of their sessions at home we will have them call the exercise specialist to check in (M-F between 9 am - 5 pm), make sure that all of the sessions have been running smoothly, answer any questions, and to check your watch (online via Polar Flow**) to hold you accountable for completing the sessions.

We will give you instructions on how to download Polar FlowSync** to your own personal computers. We will give you the charging/connecting cable so that you can connect your watch to the computer. Participants will be required to download the watch after each session they complete at home. Usernames and Passwords will not be given out to the participants. All you will have to do is download the watch. The exercise specialist will then log in to their account on Polar Flow** to see your heart rate data.

**Polar Flow and Polar FlowSync are online workout log that stores heart rate data from the watches that we use for exercise sessions.

MRI scan sessions – All study participants will complete a 1 hour MRI scan at post-testing. This will take place at the end of the study.

Audio Recording

One aspect of this study involves making audio recordings of you. These recordings help us analyze performance on certain tests. The audio can be stopped at any moment if you wish, and will be immediately erased if so indicated.

No portion of the audio recording will be heard outside of the research team without first obtaining your explicit, written permission. We will keep these recordings indefinitely unless you ask us to destroy them.

Please initial your choice below:

[ ] Yes [ ] No I give you permission to record the audio for one neuropsychological task (once during pre-testing and once during post-testing).

Participant initials: _____

Access to medical records- While a main goal of the study is to understand how changes in the brain related to getting older are related to changes in learning and memory, we are also

interested in how your physical health relates to your cognitive function. One way to assess physical health is by measuring "biomarkers" of healthy aging that are typically measured with bloodwork during annual check-ups at the doctor. We are interested in trends from appointments/measurements over the last 10-20 years. This will help us study the relationship between blood-based markers of biological aging and brain and cognitive health.

This part of the study is OPTIONAL, your decision will not affect your ability to participate in the main part of study. We will look for test results that indicate any of the following:

- HgA1c- a test for hemoglobin A1c, which is a marker of blood glucose levels and indicates metabolic health
- BUN - stands for blood urea nitrogen, this is a marker of kidney health
- Cholesterol levels (LDL, HDL)
- Triglycerides, a type of fat (lipid) in your blood
- hsCRP - which stands for high sensitivity C-reactive protein, is a marker of risk for cardiovascular disease (CVD)

Please initial your choice below:

[ ] Yes [ ] No I give you permission to access information from my medical records through ICTS.

Participant initials: _____

WHAT ARE THE RISKS OF THIS STUDY?

You may experience one or more of the risks indicated below from being in this study. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study.

Fitness Assessment: Potential risks are fatigue, dry mouth, heart palpitations, muscle strain, shortness of breath and chest pain. There is a small risk of heart attack (0.04%) and death (0.01%) in middle-aged and older adults. The risk of heart attack or death in young healthy adults is significantly lower.

Vascular health assessment: There are no known or foreseeable risks associated with this assessment. ECG electrodes may cause minor irritation to the skin.

Blood sample: Potential risks associated with obtaining blood samples are minimal but include slight bruising, pain, a temporary feeling of faintness, and/or a small risk of infection. To reduce the risks associated with blood draws, all blood draws will be performed by a research team member trained and certified in drawing blood, or a nurse. To reduce anxiety associated with lab draws, the lab draw will be done by experienced research nurse or staff to ensure correct technique and minimize risk of multiple needle pricks to obtain a blood sample.

You may be uncomfortable inside the MRI scanner if you do not like to be in closed spaces ("claustrophobia"). During the procedure, you will be able to talk with the MRI staff through a speaker system. You can tell them to stop the scan at any time.

The MRI scanner produces a loud hammering noise, which has produced hearing loss in a very small number of patients. You will be given noise cancelling headphones to reduce this risk.

A metal object flying through the air toward the magnet and hitting you presents the greatest risk associated with MRI. To reduce this risk, we require that all people involved with the study remove all metal from their clothing and pockets. No metal objects will be brought into the magnet room while you are inside the room. In addition, the door to the room remains closed throughout the study so that no one can accidentally bring a metal object into the room.

There are no known risks associated with limited exposure to magnetic fields. Magnets of this strength have been in use for medical imaging for over 15 years. However, we will keep a record of the length of time you were in the magnet as well as the amount of radio waves used during that time.

There is no known risk associated with MRI to the unborn child. In fact, MRI is often used to look at problems in unborn children. However, we cannot rule out the possibility that such a risk will be discovered in the future. Its effect on the unborn child is not known. Therefore, if you are a woman of childbearing age, you should not participate if you are pregnant, trying to become pregnant, or currently breastfeeding. If you are not sure of your status, we can schedule a serum pregnancy test for you.

You may become fatigued or uncomfortable during the exercise sessions. We will provide ample water and a fan directed toward you. At the first cognitive session we will orient you to the stationary bike and ensure that we select a speed at which you are comfortable performing the exercise, and we will adjust as necessary. You may request a break at any time.

Fasting for 8 hours: The most common risk when fasting is dehydration, therefore you are encouraged to drink plenty of water. You may experience hunger and irritability and if you experience fainting, nausea, or vomiting you are instructed to stop fasting.

There is a risk of loss of confidentiality. Measures in place to protect confidentiality are indicated in the 'What About Confidentiality' section later in this document.

Less Likely / Less Common (10% - 35%)

Mild

- Muscle strain
- Chest pain
- Fainting or nausea

Rare (less than 10%)

Life Threatening

- Heart attack (0.04%)
- Metal objects flying through the air in the MRI room

WHAT ARE THE BENEFITS OF THIS STUDY?

We don't know if you will benefit from being in this study.

However, we hope that, in the future, other people might benefit from this study because we may learn more about how exercise affects cognitive and cardiovascular health, which will provide implications for prescribing exercise as a behavioral treatment for improved quality of life.

WILL IT COST ME ANYTHING TO BE IN THIS STUDY?

You will not have any costs for being in this research study.

WILL I BE PAID FOR PARTICIPATING?

You will be paid for being in this research study. You will need to provide your social security number (SSN) in order for us to pay you if your compensation totals over $75.

You may choose to participate without being paid if you do not wish to provide your social security number (SSN) for this purpose. You may also need to provide your address if a check will be mailed to you. If your social security number is obtained for payment purposes only, it will not be retained for research purposes.

You will be reimbursed for all sessions except for the cognitive screening session Each of the training participants are estimated to receive $333 for participation plus $61 for parking ($394 total). Gas compensation is available for certain participants (details under the *).

Visit type and payment:

Orientation (No compensation + $3 parking; 1 sessions completed)

Fitness testing/Health Assessment ($24 compensation + 1$ parking per session; 2 sessions completed)

Cognitive testing ($24 compensation + $1.50 parking per session; 4 sessions completed)

Acute imaging pre-test MRIs ($81 compensation + $3 parking per session; 2 sessions completed)

Exercise training ($42, payment for about $1.50 per 28 training sessions plus incidental parking)

Post-testing MRI ($27 compensation + $2 parking; 1 session completed)

The above parking prices are just estimates, all parking costs will be reimbursed fully.

If you must withdraw from the study at any time, we will compensate you for the sessions completed.

You will not be paid for the initial orientation visit except for parking expenses.

If you do not complete the initial assessment sessions or the doctor supervising your exercise test recommends you do not participate in the intervention, you will be compensated for your time based on number of hours of testing completed.

If you do not complete the intervention, you will not be paid for the intervention sessions.

* We will compensate for gas for certain participants. For participants who are within a 30 mile radius there will be no gas compensation. Participants who have to travel between 30-60 miles to Spence Laboratories, we will compensate up to $100 for their gas if they complete majority of the sessions (more detail in next paragraph). Participants who travel above 60 miles will be compensated up to $200 for their gas if they complete majority of the sessions.

We will compensate for gas even if participants do not complete the study. We will pay them based on quarterly increments. Payment for gas will be received in a lump sum at the end of participation. If a participant only makes it a quarter of the way through the study, we will pay them a quarter of what they would have received if they completed the entire study. The graph below breaks down all percentages of reimbursement.

25% completion = Completing week 5 of the exercise intervention

50% completion = Completing week 11 of the exercise intervention

75% completion = Completing week 18 of the exercise intervention

100% completion = Completing the entire study

	25% completion	50% completion	75% completion	100% completion
30-60 miles away	$25	$50	$75	$100
60+ miles away	$50	$100	$150	$200

WHO IS FUNDING THIS STUDY?

The National Institutes of Health (NIH) is funding this research study. This means that the University of Iowa is receiving payments from the NIH to support the activities that are required to conduct the study. No one on the research team will receive a direct payment or increase in salary from NIH for conducting this study.

WHAT IF I AM INJURED AS A RESULT OF THIS STUDY?

If you are injured or become ill from taking part in this study, medical treatment is available at the University of Iowa Hospitals and Clinics.

The University of Iowa does not plan to provide free medical care or payment for treatment of any illness or injury resulting from this study unless it is the direct result of proven negligence by a University employee.

If you experience a research-related illness or injury, you and/or your medical or hospital insurance carrier will be responsible for the cost of treatment.

WHAT ABOUT CONFIDENTIALITY?

We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

- federal government regulatory agencies,
- auditing departments of the University of Iowa, and
- the University of Iowa Institutional Review Board (a committee that reviews and approves research studies)

To help protect your confidentiality, we will use a study ID code (without your name) to identify all of your study data. The following will be stored in a secure location: the list matching your study ID code and your name, your consent forms, and demographic information forms. All hardcopies of study data will be kept in a secure file cabinet in Spence Laboratory in Psychology, accessible only by lab personnel trained in confidentiality procedures. All computerized data will be kept on a secure server that is password protected, with access limited to: study investigators, the project coordinator, data analysts, and data manager. All study staff will be trained in confidentiality procedures and will receive the required University of Iowa data security training. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time

WILL MY HEALTH INFORMATION BE USED DURING THIS STUDY? (Note: this section only applies to subjects who have agreed to allow the study access to medical record information)

The Federal Health Insurance Portability and Accountability Act (HIPAA) requires your health care provider to obtain your permission for the research team to access or create "protected health information" about you for purposes of this research study. Protected health information

is information that personally identifies you and relates to your past, present, or future physical or mental health condition or care. We will access or create health information about you, as described in this document, for purposes of this research study. Once your health care provider has disclosed your protected health information to us, it may no longer be protected by the Federal HIPAA privacy regulations, but we will continue to protect your confidentiality as described under "Confidentiality."

We may share your health information related to this study with other parties including federal government regulatory agencies, the University of Iowa Institutional Review Boards and support staff.

You cannot participate in this study unless you permit us to use your protected health information. If you choose not to allow us to use your protected health information, we will discuss any non-research alternatives available to you. Your decision will not affect your right to medical care that is not research-related. Your signature on this consent document authorizes your health care provider to give us permission to use or create health information for you.

Although you may not be allowed to see study information until after this study is over, you may be given access to your health care records by contacting your health care provider. Your permission for us to access or create protected health information about you for purposes of this study has no expiration date. You may withdraw your permission for us to use your health information for this research study by sending a written notice to **Michelle Voss, W322 Seashore Hall, 301 E Jefferson St., Iowa City, IA 52242.** However, we may still use your health information that was collected before withdrawing your permission. Also, if we have sent your health information to a third party, such as the study sponsor, or we have removed your identifying information, it may not be possible to prevent its future use. You will receive a copy of this signed document.

IS BEING IN THIS STUDY VOLUNTARY?

Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won't be penalized or lose any benefits for which you otherwise qualify.

Can Someone Else End my Participation in this Study?

Under certain circumstances, the researchers might decide to end your participation in this research study earlier than planned. This might happen because you are unable to follow instructions for cognitive testing or unable to perform the aerobic exercise that is required of the study.

In the event that participants are to fall below a 50% adherence rate for exercise sessions (both home sessions and in lab sessions) across any 3 week continuous segment during the exercise intervention, their participation will be terminated. If the participant is under the adherence rate of 50% during a 2 week period, the

exercise specialist and project coordinator will inform the participant that their adherence rate needs to rise to or above 50% or their participation will be ended. If participation is ended, the subject will still be paid for the sessions that they attended and the parking costs will be covered also.

What if I Decide to Drop Out of the Study?

If you decide to leave the study early, we will ask you to come to a close out visit to discuss your reasons for withdrawing. We would like to use this information to help us improve future studies.

WHAT IF I HAVE QUESTIONS?

We encourage you to ask questions. If you have any questions about the research study itself, please contact: **Michelle Voss at 319-335-2057.** If you experience a research-related injury, please contact: **Chase Hamilton our project coordinator at 319-353-2278**.

If you have questions, concerns, or complaints about your rights as a research subject or about research related injury, please contact the Human Subjects Office, 105 Hardin Library for the Health Sciences, 600 Newton Rd, The University of Iowa, Iowa City, IA 52242-1098, (319) 335-6564, or e-mail irb@uiowa.edu. General information about being a research subject can be found by clicking "Info for Public" on the Human Subjects Office web site, http://hso.research.uiowa.edu/. To offer input about your experiences as a research subject or to speak to someone other than the research staff, call the Human Subjects Office at the number above.

This Informed Consent Document is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by signing this Informed Consent Document. Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a copy of this form.

Subject's Name (printed): _____

Do not sign this form if today's date is on or after

_____ _____

(Signature of Subject) (Date)

Statement of Person Who Obtained Consent

I have discussed the above points with the subject or, where appropriate, with the subject's legally authorized representative. It is my opinion that the subject understands the risks, benefits, and procedures involved with participation in this research study.

_____ _____

(Signature of Person who Obtained Consent) (Date)

Appendix III. Example Adverse Event/Serious Adverse Event Forms

Adverse Event Form

BIKE

Site Number: _____

Pt_ID: _____

Has the participant had any Adverse Events during this study? ☐ **Yes** ☐ **No** ***(If yes, please list all Adverse Events below)***

Severity	Study Intervention Relationship	Action Taken Regarding Study Intervention	Outcome of AE	Expected	Serious
1 = Mild 2 = Moderate 3 = Severe	1 = Definitely related 2 = Possibly related 3 = Not related	1 = None 2 = Discontinued permanently 3 = Discontinued temporarily 4 = Reduced Dose 5 = Increased Dose 6 = Delayed Dose	1 = Resolved, No Sequel 2 = AE still present- no treatment 3 = AE still present-being treated 4 = Residual effects present-not treated 5 = Residual effects present- treated 6 = Death 7 = Unknown	1 = Yes 2 = No	1 = Yes 2 = No (If yes, complete SAE form)

Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken	Outcome of AE	Expected?	Serious Adverse Event?	Initials
1.									
2.									
3.									

Adverse Event Form Version 1.0

Serious Adverse Event (SAE) Report Form

Protocol Title: BIKE
Protocol Number: [Enter protocol number] **Site Number:** [Enter site number] **Pt_ID:** [Enter participant id]

1. SAE Onset Date: [enter SAE onset date] (dd/mmm/yyyy)
2. SAE Stop Date: [enter SAE stop date] (dd/mmm/yyyy)
3. Location of serious adverse event (e.g. at study site or elsewhere):

   [Enter location of SAE]
4. Was this an unexpected adverse event?

   ☐ Yes ☐ No
5. Brief description of participant with no personal identifiers:

   Sex: ☐ Female ☐ Male Age: [Enter participant age]
6. Adverse Event Term(s):

   [Enter adverse event terms]
7. Brief description of the nature of the serious adverse event (attach description if more space needed):

   [Enter brief description of the nature of the SAE]
8. Category of the serious adverse event:

   ☐ death – date [Enter death date](dd/mmm/yyyy)
   ☐ life-threatening
   ☐ hospitalization - initial or prolonged
   ☐ disability / incapacity
   ☐ congenital anomaly / birth defect
   ☐ required intervention to prevent permanent impairment
   ☐ other: [other category of SAE]

Serious Adverse Event (SAE) Report Form

9. Intervention type:
   - ☐ Medication or Nutritional Supplement: specify [specify text]
   - ☐ Device: Specify: [specify text]
   - ☐ Surgery: Specify: [specify text]
   - ☐ Behavioral/Life Style: Specify: [specify text]

10. Relationship of event to intervention:
    - ☐ Unrelated (clearly not related to the intervention)
    - ☐ Possible (may be related to intervention)
    - ☐ Definite (clearly related to intervention)

11. Was study intervention discontinued due to event? ☐ Yes ☐ No

12. What medications or other steps were taken to treat serious adverse event?

    [Medications or other steps were taken to treat SAE]

13. List any relevant tests, laboratory data, history, including preexisting medical conditions

    [List any relevant tests, lab data, history, including preexisting medical conditions]

14. Type of report:
    - ☐ Initial
    - ☐ Follow-up
    - ☐ Final

Signature of Principal Investigator: [Signature of PI] Date: [sign date] (dd/mmm/yyyy)