BMJ Open

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023231
Article Type:	Protocol
Date Submitted by the Author:	27-Mar-2018
Complete List of Authors:	Sandroff, Brian; University of Alabama at Birmingham, Physical Therapy Motl, Robert; University of Alabama at Birmingham, Department of Physical Therapy Bamman, Marcus; University of Alabama at Birmingham Cutter, Gary; University of Alabama at Birmingham, Department of Biostatistics Bolding, Mark; University of Alabama at Birmingham Rinker, John; University of Alabama at Birmingham Wylie, Glenn; Kessler Foundation Genova, Helen; Kessler Foundation DeLuca, John; Kessler Foundation
Keywords:	Multiple sclerosis < NEUROLOGY, exercise, cognition, memory, MRI

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Brian M. Sandroff[1], Robert W. Motl[1], Marcas M. Bamman[2], Gary R. Cutter[3], Mark Bolding[4], John R. Rinker[5], Glenn R. Wylie[6], Helen Genova[6], John DeLuca[6]

[1] University of Alabama at Birmingham, Department of Physical Therapy, Birmingham, AL, USA
[2] University of Alabama at Birmingham, Departments of Cell, Developmental, & Integrative Biology; Medicine; and Neurology, Birmingham, AL, USA
[3] University of Alabama at Birmingham, Department of Biostatistics, Birmingham, AL, USA
[4] University of Alabama at Birmingham, Department of Radiology, Birmingham, AL, USA
[5] University of Alabama at Birmingham, Department of Neurology, Birmingham, AL, USA
[6] Kessler Foundation, Neuropsychology and Neuroscience Research, West Orange, NJ, USA

Correspondence to Brian M. Sandroff, PhD, University of Alabama at Birmingham, Department of Physical Therapy, SHP 389, 1720 2nd Ave S, Birmingham, AL, 35294, *phone* (205) 934-5972, *email* sandroff@uab.edu

Word Count: 7,228

Abstract

Introduction: This randomized controlled trial (RCT) examines treadmill walking exercise training effects on learning and memory performance, hippocampal volume, and hippocampal resting-state functional connectivity in persons with multiple sclerosis (MS) who have objective impairments in learning new information.

Methods and Analysis: Forty fully-ambulatory persons with MS who demonstrate objective learning and memory impairments will be randomly assigned into either the intervention or active control study conditions. The intervention condition involves supervised, progressive treadmill walking exercise training 3 times/week for a 3-month period. The active control condition involves supervised, progressive low-intensity resistive exercise that will be delivered at the same frequency as the intervention condition. The primary outcome will involve composite performance on neuropsychological learning and memory tests, and the secondary outcomes involve MRI measures of hippocampal volume and resting-state functional connectivity administered before and after the 3-month study period. Outcomes will be administered by treatment-blinded assessors using alternate test forms to minimize practice effects, and MRI data processing will be performed by blinded data analysts.

Ethics and Dissemination: This study has been approved by a University Institutional Review Board and further is registered at clinicaltrials.gov: NCT03319771. If successful, the results from this study will eventually inform subsequent RCTs for developing physical rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in larger samples of cognitively-impaired persons with MS. The results from this early-phase RCT will further lay preliminary groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. This approach is paramount as learning and memory impairment is common, burdensome, and poorly-managed in MS.

Keywords: *multiple sclerosis; exercise; cognition; memory; MRI*

Strengths and Limitations of This Study

- The current randomized controlled trial applies a highly novel, systematically-developed exercise training intervention for improving learning and memory and hippocampal structure and function among persons with MS who present with objective learning and memory impairment.
- This study is adequately powered and involves the inclusion of an active control comparison condition (i.e., low intensity resistive exercise) as well as blinded MRI data analysts.
- However, this efficacy study does include a relatively short intervention period (i.e., 3-months) and will not examine the sustainability and durability of the intervention effects on cognitive and hippocampal neuroimaging outcomes.
- This study involves fully-ambulatory persons with MS and will not involve persons with substantial ambulatory disability.
- Finally, this study will not directly compare the effects of treadmill walking exercise training with cognitive rehabilitation as a control comparison condition on learning and memory and hippocampal neuroimaging outcomes.

Introduction

The impairment of learning and memory, particularly with learning new information, is a common, burdensome, and poorly-managed manifestation of multiple sclerosis (MS).[1] Over 50% of MS patients demonstrate impaired performance on neuropsychological tests of verbal and/or visuospatial learning and memory[1] that is associated with hippocampal lesions, atrophy, and altered resting-state functional connectivity based on neuroimaging (i.e., MRI/fMRI) studies.[2-4] MS-related learning and memory impairment and decline have been associated with depression,[5] unemployment,[6,7] loss of independence, and social isolation.[8] Currently, there are no FDA-approved pharmacological treatments for learning and memory dysfunction in MS. The lack of FDA-approved pharmacological treatments is based on results from randomized controlled trials (RCTs) of disease-modifying (e.g., interferon beta-1a, interferon beta-1b, glatiramer acetate, and natalizumab) and symptomatic (e.g., L-amphetamine sulfate, donepezil) agents. Results from both pharmacotherapeutic approaches have been conflicting and disappointing based on methodological limitations.[9] Cognitive rehabilitation is currently the best-characterized behavioral approach for improving MS-related learning and memory impairment and is seemingly mediated through changes in brain function (i.e., increased hippocampal activation and resting-state functional connectivity;[10-14]). However, cognitive rehabilitation is difficult to apply outside the clinical setting (i.e., low ecological validity) and does not generally result in physical health benefits beyond improved cognition. This underscores the consideration of more ecologically valid behavioral approaches for managing learning and memory impairment and its potential association with hippocampally-mediated functional brain outcomes in MS that can result in many physical health benefits; one such approach includes exercise training.[15]

The consideration of exercise for improving learning and memory in persons with MS is based, in part, on the body of literature in the general population that documents robust, beneficial effects of exercise training on memory and hippocampal structure/function.[16] By comparison, there have been

few well-designed, targeted exercise training RCTs on learning and memory and hippocampal neuroimaging outcomes in MS, although the data are promising.[17] For example, one recent cross-sectional study described statistically significant, moderate-sized correlations between cardiorespiratory fitness (i.e., a presumed surrogate of aerobic exercise training) and hippocampal volume in 35 persons with MS.[18] Another noteworthy case study involving two memory-impaired persons with MS reported that 3-months of aerobic exercise training (stationary cycling) resulted in a > 50% increase in learning and memory performance, 16.5% increase in hippocampal volume, as well as increased hippocampal resting-state functional connectivity.[19] By comparison, the non-aerobic exercise condition demonstrated minimal changes in those outcomes.[19] One systematically-developed[20] pilot RCT examined the effects of aerobic treadmill walking exercise training compared with a waitlist control condition on learning and memory performance and hippocampal viscoelasticity using non-conventional MRI (i.e., magnetic resonance elastography) in 8 fully-ambulatory persons with MS.[21] Overall, there were small-to-moderate intervention effects on verbal learning and memory performance (d=0.34), and large intervention effects on hippocampal viscoelastic properties (i.e., increased hippocampal shear stiffness) (d>0.94). The change in verbal learning and memory was strongly associated with change in hippocampal viscoelasticity (r>.93, p<.01). Collectively, such preliminary observations suggest that aerobic exercise training might improve learning and memory through neuroplasticity in MS and warrant the development of an early-phase RCT for examining the effects of aerobic exercise training on learning and memory, and hippocampal structure/function in a larger sample of persons with MS.

The present RCT aims to provide the first Class I evidence for treadmill walking exercise training effects on learning and memory, hippocampal volume, and hippocampal resting-state functional connectivity in an adequately-powered sample of persons with MS who have objective impairment in learning new information. This will provide the first evidence for exercise training as a possible *treatment* for MS-related learning and memory impairment (i.e., beyond merely improving learning and

memory performance), given that previous trials of exercise, cognition, and neuroimaging have not recruited persons with objective MS-related learning and memory impairment *a priori.*[e.g., 21] The current RCT will further provide the first high-quality evidence of potential mechanisms of aerobic exercise-related effects on hippocampal outcomes in MS, whereas previous studies have been limited by experimental design[e.g., 18] or sample size.[e.g., 19,21] This study will lay the foundation for a definitive Phase III RCT by providing effect sizes of treadmill walking exercise training versus an active control condition on the outcomes that can be used in power analyses for determining the appropriate sample size for such a trial. The present study will allow for a better understanding of the potential mechanisms (i.e., improved hippocampal structure and function) of how treadmill walking exercise improves learning and memory in this population.

Methods and Analysis

Experimental Overview and Hypotheses.

The study protocol was drafted in accordance with the SPIRIT statement[22] and further has been approved by the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB). This study is registered at clinicaltrials.gov: NCT03319771. All potential protocol modifications will be approved by the UAB IRB and will be reported at clinicaltrials.gov. The proposed study, data collection, and intervention will take place at UAB in Birmingham, AL, USA. This study involves a single-blind, early Phase II RCT on the effects of supervised treadmill walking exercise training compared with an active control condition (i.e., low intensity resistive exercise) on learning and memory, hippocampal structure/function, and cardiorespiratory fitness outcomes in 40 fully-ambulatory (i.e., Expanded Disability Status Scale (EDSS) ≤ 4.0) persons with MS who have impairment in learning new information. Composite performance on neuropsychological tests of learning and memory represents the primary outcome, whereas neuroimaging outcomes of hippocampal volume and hippocampal resting-state

functional connectivity represent the secondary outcomes. Cardiorespiratory fitness changes will be included as a manipulation check for documenting the success of the intervention.

During baseline, participants will first complete a battery of neuropsychological tests addressing verbal and visuospatial learning and memory; a maximal, graded exercise test on a motor-driven treadmill to measure cardiorespiratory fitness; and an MRI scan for measurement of hippocampal volume and resting-state functional connectivity. Those outcomes will be measured by assessors who are uninvolved in the exercise training (i.e., treatment blinded assessors). After baseline testing, participants will be randomly assigned to either the intervention or active control conditions using concealment. Participants further will be masked to condition (i.e., unaware that the treadmill walking exercise training condition represents the intervention condition and the low intensity resistive exercise condition represents the active control condition).

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise, and will be based on American College of Sports Medicine (ACSM) guidelines for exercise prescription[23] and pilot work.[21,24-26] The exercise training itself will take place 3 times per week over 3 months, and will be facilitated by trained exercise leaders. The exercise prescription will initially consist of 15 minutes of light-to-moderate intensity treadmill walking exercise and eventually progress to 40 minutes of vigorous intensity exercise by month 3 (Table 1). The active control condition will involve low intensity resistive exercise that will be delivered using the same frequency and duration of the treadmill walking exercise condition and facilitated by trained exercise leaders. Regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising) over the duration of the study. The cognitive, cardiorespiratory fitness, and MRI outcomes will be assessed again following the 3-month study period by treatment blinded assessors.

The primary hypothesis is that those who undergo treadmill walking exercise training will demonstrate larger improvements in composite learning and memory performance than those who undergo low intensity resistive exercise. We further hypothesize that those who undergo treadmill walking exercise training condition will demonstrate greater increases in hippocampal volume and hippocampal resting-state functional connectivity (i.e., adaptive increases) and improvements in cardiorespiratory fitness than those in the low intensity resistive exercise condition. We lastly hypothesize that (1) the treadmill walking exercise-induced improvements in learning and memory will be accounted for by hippocampal volume and hippocampal resting-state functional connectivity (i.e., partial mediation) and (2) those with the largest improvements in cardiorespiratory fitness will demonstrate the largest improvements in the primary and secondary end-points.

Participants.

Sample Size. We plan to enroll 40 fully-ambulatory persons with MS (i.e., 20 per condition) who have impairment in learning new information (see below); this is based on a power analysis and presumed 15% attrition. The minimal sample size of 34 persons with MS (i.e., 17 per condition) was determined based on power analysis using standard assumptions of alpha (0.05) and beta (0.80) for detecting moderate-sized effects (i.e., η_p^2=0.06) based on Cohen's guidelines[27] for a time by condition interaction in mixed-factor ANOVA on composite learning and memory performance. Our previous pilot RCT of treadmill walking exercise training on cognition yielded a large time by condition interaction on composite learning and memory performance (i.e., η_p^2=0.11) in fully-ambulatory persons with MS.[21] Another study on aerobic exercise effects on hippocampal outcomes in persons with schizophrenia reported large intervention effects on hippocampal volume (i.e., η_p^2=0.50).[28] Using those effect sizes as a guide, the proposed sample size of 34 and planned sample size of 40 will be more than adequate for detecting moderate or larger effects on learning and memory and hippocampal outcomes in persons with MS. The required sample size of 34 also adequately powers the secondary study hypotheses based

on previous cross-sectional data in MS that indicates moderate-sized correlations (i.e., ρ = .42) between cardiorespiratory fitness and hippocampal volume.[18]

Recruitment. Subjects will be recruited directly through the UAB MS Center, the Alabama-Mississippi Chapter of the National MS Society (NMSS), and our laboratory database of previous participants with MS who have inquired about participating in exercise studies. Advertisements for the study will be distributed through the UAB MS Center, facilitators of local MS support groups, *MS Connection* publications, and e-mail distributions. As a backup plan, we may recruit prospective participants with MS through the North American Research Committee on Multiple Sclerosis (NARCOMS) patient registry or iConquerMS if enrollment is slow.

Inclusion/Exclusion Criteria. All participants will be between the ages of 18-54, have a clinically definite MS diagnosis based on established criteria,[i.e., 29] and be fully-ambulatory based on EDSS[30] scores between 0-4.0. All participants will demonstrate impairment in learning new information based on open-trial Selective Reminding Task (SRT) scores at least 1.5 *SD's* below the normative score for healthy controls.[31] Participants will be relapse-free for at least 30 days (i.e., relative neurologic stability), and will not have a history of schizophrenia, bipolar disorder I or II, or substance-abuse disorders. Participants further will not be taking medications that can affect cognition (e.g., antipsychotics, benzodiazepines), and all participants will be on a stable FDA-approved disease-modifying therapy (e.g., interferon beta-1a; interferon beta-1b; glatiramer acetate; natalizumab; dimethyl fumarate, etc.) regimen for at least 6 months. Participants will be right-handed (to control for organization of the brain) and will have a low risk for contraindications for maximal exercise testing based on a "no" response on all items of the Physical Activity Readiness Questionnaire (PAR-Q;[32]) or a single "yes" response along with a physician's approval. Participants further will have a low risk for contraindications for MRI based on: (a) not having metal (e.g., non-MRI compatible aneurysm clips, metal shards in the body or eyes, or recently placed surgical hardware) or electronic devices (e.g.,

pacemaker, cochlear implant) within the body. Lastly, participants will not be meeting public health guidelines for participating in physical activity (i.e., at least 150 minutes per week of moderate-to-vigorous aerobic activity). This will be based on health contribution scores on the Godin Leisure-Time Exercise Questionnaire (GLTEQ) that fall within the 'insufficiently active' classification (i.e., less than 14 arbitrary units on the summed 'strenuous' and 'moderate' sections of the GLTEQ).[33]

Outcome Measures.

To minimize threats to internal validity and maximize the rigor and reproducibility of the present RCT, the outcome measures will be assessed by personnel who are uninvolved with the intervention or control conditions (i.e., treatment blinded assessors). The outcome assessments further will occur at a different UAB laboratory than the intervention or control conditions to prevent possible contamination.

Learning and Memory: Participants will undertake several neuropsychological tests addressing various aspects of learning and memory as the primary study outcomes. Neuropsychological testing will occur in a quiet, sound-dampened room in the Exercise Neuroscience Research Laboratory (ENRL) at UAB. These tests include the California Verbal Learning Test-II[CVLT-II;34] and SRT[35] as measures of verbal learning and memory, and the Brief Visuospatial Memory Test-Revised[BVMT-R;36] and 10/36 Spatial Recall Test[SPART;37] as measures of visuospatial learning and memory. These neuropsychological tests have strong psychometric properties,[e.g., 38] and are widely-used to document the efficacy of cognitive rehabilitation interventions in persons with MS.[12,39,40]

Briefly, the CVLT-II involves the examiner reading a list of 16 words, with four items belonging to four categories (e.g., vegetables, animals, furniture, modes of transportation) that are randomly arranged. The list is read aloud five times in the same order, with each word voiced at a rate of approximately one word per second. Participants are instructed to recall as many items as possible, in any order, following each list reading. The primary outcome of the CVLT-II is the total number of correct words identified over the five trials (i.e., raw score).[34] The SRT involves the examiner reading a list of

12 unrelated words. Participants are asked to recall as many words as possible following the presentation of the list. After the first trial, only the words that participants did not recall are given as the new list. However, participants are instructed to recall as many words as possible from the original (i.e., entire) list for each of 5 total trials. The primary outcome is the total number of correctly recalled words across the 5 trials.[35] For both the CVLT-II and SRT, higher scores indicate better verbal learning and memory.

The BVMT-R involves three trials of the examiner presenting a 2×3 array of abstract geometric figures in front of the participant for 10 seconds. Following this period, the array is removed and participants are required to draw the array as precisely as possible, with the figures in the correct location. Each drawing is scored on a 0-2 scale, based on accurately portraying each figure and its correct location. The primary outcome of the BVMT-R is the total raw score across the three trials, with higher scores indicating better visuospatial memory.[36] The 10/36 SPART involves three trials of the examiner presenting a 6×6 checkerboard with 10 pieces positioned in certain locations on the board in front of the participant for 10 seconds. Following this period, the display is removed, and participants are asked to replicate the design of the checkerboard on a blank grid. This is repeated for 2 additional trials. The primary outcome of the 10/36 SPART is the total number of correct responses across the three trials.[37]

As the primary study outcome involves composite learning and memory performance, we will first compute *z*-scores that account for age, sex, and education based on normative scores per individual learning and memory test (i.e., CVLT-II, SRT, BVMT-R, 10/36 SPART).[41,42] We will then combine the *z*-scores into a composite learning and memory measure (i.e., mean of *z*-scores for CVLT-II, SRT, BVMT-R, and 10/36 SPART).

Hippocampal Volume and Resting State Functional Connectivity: Participants will undergo neuroimaging, which will include structural imaging as well as a resting-state scan. The MR instrument

that will be used is an FDA-approved Siemens MAGNETOM Skyra 3T clinical imager housed in the Civitan International Neuroimaging Research Center at UAB. Each scan session will begin with the acquisition of high-resolution T1-weighted axial anatomical images (MP-RAGE). This 3D isotropic sequence will be acquired sagittally (TR = 11.6 ms, TE = 4.9 ms, flip angle = 8°, effective TI = 1017.6 ms, 256 x 256 matrix, FOV = 300 mm, NEX = 1, 172 slices, 1.17 mm slice thickness, 0 mm skip). Total imaging time for this sequence is 8 min 38 sec. In addition, an inversion-recovery sequence will be acquired axially (TR = 8530 ms, TE = 81 ms, flip angle = 180°, 256 x 256 matrix, FOV = 220 mm, NEX = 1, 32 slices, 4 mm slice thickness, 0 mm skip). Together, these scans will be used for volumetric analyses and for image segmentation and normalization of the resting-state fMRI scan. Functional imaging will consist of multi-slice gradient echo, T2*-weighted images acquired with echoplanar imaging (EPI) methods (TE= 60 ms; TR= 2000 ms; FOV = 24 cm; flip angle = 90°; slice thickness = 5 mm contiguous, matrix = 64×64, in-plane resolution = 2.50 mm^2). In order to provide coverage of the entire brain, a total of 32 images will be acquired in the axial plane. For the resting-state scan, 180 volumes will be acquired. Structural volumes for the hippocampus will be calculated using Freesurfer automated brain segmentation software (http://surfer.nmr.mgh.harvard.edu). Preprocessing of the resting-state functional connectivity data will be performed using AFNI software (http://afni.nimh.nih.gov/afni/). The first three volumes will be removed in order to control for saturation effects. Preprocessing steps include slice timing correction, realignment to an image exactly half-way through the acquisition run using affine transformation, co-registration to the T1 MPRAGE image for localization of activated areas, smoothing (6 mm FWHM) to minimize anatomical differences and increase the signal to noise ratio, scaling each voxel to the grand mean intensity of that voxel (across the acquisition run), high-pass filtering (150 seconds), and normalization using a nonlinear approach (3dQwarp) to a standardized T1 template from the Montreal Neurological Institute (MNI). In all cases, the data will be checked for excessive motion (a shift of more than 3.5 mm, or 1° of angular motion) and for spikes (using the Root Mean Squared Error [RMSE] of

each volume relative to a reference volume [which will be the volume half-way through the acquisition run]). Data acquisition runs with excessive motion will be discarded. Individual acquisitions with a RMSE amplitude that exceeds the 75th percentile plus the value of 150% of the interquartile range of RMSE for all volumes in a given run will be excluded from further analysis using the 'censorTR' function in 3dDeconvolve. In all cases, the motion parameters from the realignment step will be used as regressors of no interest in the deconvolution, and the residuals will be saved. The residuals for each subject in each group (exercise and control) will then be included in a probabilistic ICA, using MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components), as implemented in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The results of the ICA will be spatial maps of the resting-state networks. We will extract the connectivity map of the hippocampal network that will then be used in a dual-regression analysis.[43] In the first regression (spatial regression), the spatial hippocampal map from the ICA is regressed onto each subject's functional data, resulting in a dataset that characterizes the temporal dynamics of the hippocampal network. In the second regression (temporal regression), the dataset resulting from the spatial regression (the temporal dynamics of the hippocampal network) are regressed onto each subject's functional data. This results in a map for the hippocampal network representing each voxel's connectivity with this network. This map will then be used in the group-level analyses. Importantly, all MRI processing and analyses will be performed by scientists at Kessler Foundation (i.e., HG, GRW), who will be blinded to condition. To our knowledge, this is among the first exercise RCTs in any population to include this additional level of rigor to enhance the proposed trial's reproducibility.

Cardiorespiratory Fitness: Cardiorespiratory fitness will be measured as peak oxygen consumption (VO_{2peak}), using a maximal, graded exercise test on a motor-driven treadmill and an open-circuit spirometry system (ParvoMedics True One 2400, Sandy, UT) for analyzing expired gases using a modified Balke protocol in the ENRL at UAB. This protocol was successfully used in our previous small

pilot RCT.[21] This protocol further is commonly used for measuring cardiorespiratory fitness in older adults[44] and persons with chronic stroke[45] and is consistent with the ACSM guidelines for exercise testing of MS patients.[23] The test will be preceded by a 3-minute warm up. The initial work rate for the exercise test will be at a brisk, but submaximal pace, and the grade will continuously increase at a rate of 2.0% every 2-minutes until the participant reaches volitional fatigue. Heart rate (HR) and rating of perceived exertion will be recorded every minute during the test. VO_{2peak} will be expressed in $ml \cdot kg^{-1} \cdot min^{-1}$ based on highest recorded 20-second VO_2 value when two of four criteria are satisfied: (1) VO_2 plateau with increasing grade; (2) respiratory exchange ratio ≥1.10; (3) peak heart rate within 10 $beats \cdot minute^{-1}$ of age-predicted maximum (i.e., ~1 SD); or (4) peak rating of perceived exertion ≥17.[46] The test will be followed by a 3-minute cool-down period.

Disability Status: Participants will undergo a neurological examination by a neurologist (JRR) for generating a baseline EDSS score. All participants will be fully-ambulatory (i.e., EDSS scores ≤ 4.0).

Additional Neuropsychological Tests: To evaluate the effects of the intervention on other domains of cognition that are commonly-impaired in MS, we will apply the MACFIMS neuropsychological battery[47] as exploratory outcomes. In addition to the CVLT-II and BVMT-R, the MACFIMS includes valid and reliable tests of cognitive processing speed,[48,49] executive function,[50] verbal fluency,[51] and spatial perception.[51] Further, baseline performance on the MACFIMS neuropsychological battery will serve to characterize the overall baseline cognitive status of the sample. For all neuropsychological tests, including measures of learning and memory, alternate forms will be applied at each testing session (i.e., baseline and follow-up) to minimize the effects of practice on cognition.

Additional Neuroimaging Outcomes: The MRI protocol further will involve the collection of structural MRI data on T2-lesion volume as a potential covariate of intervention effects, given its association with MS-related cognitive dysfunction.[52]

Intervention Condition.

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise training based on ACSM guidelines for maximizing adaptations with exercise training. This will occur at a laboratory exercise research facility. Exercise intensities will be prescribed based on percent oxygen consumption reserve (% VO_2R) using values derived from the baseline graded exercise test. We note that HR reserve (HRR) and VO_2R have a 1:1 relationship.[23] The exact exercise prescription is presented in Table 1 and further represents the identical stimulus that was included in our pilot RCT that demonstrated improvements in learning and memory and hippocampal neuroimaging outcomes.[21] The exercise progression in terms of duration and intensity will involve 3 distinct stages: (a) the initiation stage; (b) the improvement stage; and (c) the maintenance stage. The initiation stage (Weeks 1-2) aims to prepare participants for more intense aerobic exercise (i.e., by accumulating small improvements in cardiorespiratory fitness with light-to-moderate intensity exercise) and develop an orthopedic tolerance for exercise stress.[23] Following this period, participants will progress to the improvement stage of exercise training. This stage provides a gradual increase in the overall aerobic exercise stimulus (i.e., moderate-to-vigorous intensity), whereby participants realize substantial improvements in cardiorespiratory fitness (Weeks 3-8).[23] The final stage of exercise progression is the maintenance stage (i.e., vigorous intensity), which aims to maintain the levels of cardiorespiratory fitness that were developed during the improvement stage over the long-term (Weeks 9-12).[23] Consistent with ACSM recommendations, the intervention will not involve progression of both intensity and duration in a single exercise session. Such a gradual progression of exercise training is advantageous for deconditioned persons to safely achieve the benefits of aerobic exercise training.[23]

The exercise training itself will be led by certified exercise leaders who are not involved in the collection of outcome assessments. At the outset of each session, participants will be fitted with a Polar HR Monitor (Oy, Finland), and HR will be monitored continuously throughout each session. Each session

will begin with a 5-10 min warm-up, followed by the exercise; the target HRR range associated with the VO_2R range will be maintained for as long as possible during each exercise period. This will be followed by a 5-10 min cool-down. Participants will complete an exercise log at the conclusion of each session for better characterizing the experience with the intervention. Log data will include perceived exertion,[53] well-being, enjoyment, and mental/physical fatigue. Throughout each session, we further will collect data on treadmill speed and grade, as well as time spent within the prescribed VO_2R/HRR range for improving the rigor and reproducibility of the intervention.

Active Control Condition.

The active control condition will involve supervised, low intensity resistive exercise in order to control for the effects of social contact and attention. This condition will take place at a laboratory exercise research facility in a space that is isolated from the intervention condition in order to avoid participant and site contamination. The low intensity resistive exercise control condition will be delivered using the same frequency and duration of the treadmill walking exercise training condition. The low intensity resistive exercises will be based on a manual provided by the NMSS[54] and sessions will be led by certified exercise leaders who are not involved in the collection of outcome assessments. Low intensity resistive exercises will target the head/neck, shoulder, elbow/forearm, hand/wrist, trunk/hip, ankle/foot. The progression of activities over the 3-month period will involve performing additional exercises and sets along with using progressively thicker elastic resistance bands (i.e., Therabands) that provide minimal resistance. The first 6 weeks of the intervention period will involve performing the activities without resistance. In weeks 7-8, the extra thin Theraband (i.e., least resistance) will be used to perform the exercises for the upper-extremities only. In weeks 9-10, the thin Theraband will be introduced and in weeks 11-12, the medium Theraband will be introduced. Such a progression is not expected to induce cardiorespiratory fitness adaptations and is designed to maintain participant interest. Each session is designed to last up to 60 minutes in total. Each session will begin

with a 5-10 min warm-up, followed by low intensity resistive exercise (following the same duration as the treadmill walking exercise training condition) activities, and a 5-10 min cool-down. Throughout each session, participants will wear a HR monitor, and we will collect data on HR and perceived exertion [53] to ensure that this condition occurs at a low intensity. This stimulus has been included as a control comparison condition in a recent exercise training RCT in persons with MS and did not result in cognitive improvements and was well-received with no increase in drop-out compared with progressive exercise training.[55]

Importantly, to minimize attrition for both the intervention and control conditions, exercise leaders will apply highly-developed principles and techniques associated with Social Cognitive Theory[56] for enhancing participant adherence and compliance throughout each session. Further, regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising).

Procedure.

Participant flow through the study is presented in Figure 1. Participant recruitment, contact, and screening will be undertaken via telephone and/or email by an ENRL project coordinator. If a participant satisfies initial inclusion/exclusion criteria, the project coordinator will then administer the open-trial SRT via telephone to ensure that all participants have impairments in learning new information; the open-trial SRT has been used as a screening tool for impairments in learning new information in previous memory rehabilitation RCTs in MS.[12] This test involves the project coordinator reading a list of words to the prospective participant over the phone. The participant then repeats as many words as they can from memory back to the project coordinator. This process is repeated until participants can remember the entire list of words. If participants demonstrate scores that are at least 1.5 *SD*'s below the normative score for healthy controls, the project coordinator will request contact information from the potential participant's neurologist, whereby they will email or fax a letter asking them to verify a definite

MS diagnosis and confirmation that the participant has been on a stable DMT regimen for at least 6-months. Upon receipt of these materials from the participant's neurologist, the project coordinator will schedule the participant for baseline testing. Baseline testing will be led by assessors who are blinded to condition, and will take place over 3 non-consecutive days to minimize cognitive and physical fatigue. On the first day, participants will initially provide written informed consent with IRB-approved ENRL personnel, followed by a neurological examination for generation of an EDSS score. Participants will then undertake several neuropsychological tests of learning and memory (i.e., CVLT-II, BVMT-R) as part of the full MACFIMS neuropsychological battery, followed by the graded exercise test. The second day of baseline testing will involve the MRI protocol, and the third day of baseline testing will involve undertaking other neuropsychological tests of learning and memory (i.e., SRT, 10/36 SPART). Participants will be remunerated $50 for completing the baseline assessments. Of note, the separation of learning and memory measures across testing sessions is necessary considering that there might be overlap between performance on the CVLT-II and SRT (i.e., learning and memory tests in the verbal domain) and between the BVMT-R and 10/36 SPART (i.e., learning and memory tests in the visuospatial domain), respectively.

After baseline testing, participants will be randomly assigned to either the treadmill walking exercise training or active control conditions using concealment (i.e., opaque, sealed envelopes) and computerization by the study biostatistician (i.e., GRC). Participants further will blinded to the intent of the condition (i.e., unaware that the treadmill walking exercise training condition represents the experimental condition and the low intensity resistive exercise condition represents the active control condition). To do this, the study will be advertised as a comparison of two different physical exercise programs on memory performance in persons with MS. We note that it is not possible for participants to be blinded to the actual condition (i.e., participating in treadmill walking exercise or low-intensity resistive exercise activities).

Participants will undertake the intervention or active control conditions as described above over a 3-month period. Participants will be remunerated $10 per treadmill walking exercise/low intensity resistive exercise visit attended (i.e., up to $360 total). This remuneration will be disbursed in weekly increments for maximizing compliance with this early stage research. Following the completion of the 3-month study period, participants will again undergo assessments of learning and memory, hippocampal volume and resting-state functional connectivity, and cardiorespiratory fitness using the same procedures as baseline testing (i.e., follow-up testing). Follow-up measures, using alternate forms where possible, will be administered by treatment blinded assessors. Participants will be remunerated $50 for completing the follow-up assessments.

Data Integrity.

All data will be entered, checked, and double-checked by UAB ENRL personnel under the direct supervision of the ENRL project coordinator and study principal investigator (BMS). All ENRL personnel have undergone extensive training in good clinical practice and laboratory procedures. Given that the current study is not a multi-site, NIH-defined Phase III RCT, we do not include a formal data monitoring committee.

Statistical Analysis.

The data analyses will be overseen by a biostatistician (i.e., GRC) and follow intent-to-treat principles (i.e., include all persons regardless of adherence and/or compliance). In the case of a drop-out, missing data will be imputed using multiple imputation and by carrying the last observed value forward. We further will perform exploratory data analyses only in those who completed follow-up testing (i.e., completer's or per protocol analysis) and in those who demonstrated good adherence (i.e., attended at least 83% of sessions) and compliance.[17] The data will be analyzed using a mixed-factor model with time (baseline and follow-up) as a within-subjects factor and condition (intervention or active control) as a between-subjects factor on composite learning and memory performance,

hippocampal, and cardiorespiratory fitness outcomes. Of note, we will require an MRI-overall alpha of .05 (corrected for multiple comparisons) for significance for hippocampal neuroimaging outcomes. The correction for multiple comparisons will be achieved by establishing a suitable voxel cluster-level threshold through Monte Carlo simulations (using the 3dClustSim program, part of the AFNI suite of image analysis programs; http://afni.nimh.nih.gov/afni/). Effect sizes will be expressed as partial eta-squared (η_p^2) and Cohen's *d*.[27]

We will examine hippocampal volume and resting-state functional connectivity outcomes as potential mediators of the effects of treadmill walking exercise training on learning and memory, consistent with the methodology of Baron and Kenny.[57] This statistical mediation approach is consistent with the proposed gold standard approach for examining exercise-related mechanisms of cognitive improvement at the brain-systems level in the general population.[16] As pre-conditions of the mediation analysis, we will first perform correlations among group (i.e., intervention or control), change in learning and memory performance, and change in hippocampal volume and resting-state functional connectivity, respectively, using Spearman's rho rank-order correlations (ρ)[58] to test if those outcomes are interrelated. Consistent with previous preliminary studies,[19,21] we expect that treadmill walking exercise training will be associated with improvements in learning and memory and increases in hippocampal volume and functional connectivity. Then, to establish mediation, we will perform hierarchical linear regression models for evaluating each of the mediators (i.e., changes in hippocampal volume and resting-state functional connectivity outcomes, respectively), separately. This will involve regressing change in learning and memory performance on group in Step 1 and then adding change in the mediator in Step 2. As such, we expect significant effects of group on change in learning and memory, and that the effect of group (i.e., intervention or control) on change in learning and memory will be attenuated, but not reach zero, when accounting for the effects of changes in hippocampal structure and function. In other words, we expect that changes in hippocampal structure and function

will be partial mediators of the effects of treadmill walking exercise training on learning and memory in persons with MS with impairments in learning new information. The effect sizes from the interaction terms from the ANOVAs and correlations will serve as effect sizes for the subsequent power analyses required for a subsequent Phase III RCT.

Ethics and Dissemination

Adverse Events.

This study has been approved by the UAB IRB, and we verify that all persons involved in the research hold current IRB certification for the protection of human subjects. Importantly, during any and all exercise activities for the current study, we will have a minimum of two researchers present who are trained in CPR, AED, First Aid, and emergency procedures. Further, we will monitor participants on a daily basis for adverse events such as musculoskeletal injuries related to exercise. In the event of an adverse event, this will be reported to the UAB Institutional Review Board within 48 hours, and the participant will cease participation until receiving physician's clearance to resume any study-related activities.

Throughout the study, we will monitor MS relapses that may affect study participation. Importantly, we expect that the overall study relapse rate will be low (i.e., < 6% for both exercise and control conditions; 27% lower for exercise conditions), based on a recent systematic review of safety of exercise training in persons with MS.[59] We further will minimize the potential for relapses by only including persons with MS with relative neurologic stability who are on a stable disease-modifying therapy regimen. Nevertheless, all decisions (i.e., inclusion/exclusion, safety of continued participation) in the event of a relapse will be made on a patient-by-patient basis, with direct consultation with the study neurologist (JRR).

Confidentiality.

This study involves several approaches to maintain confidentiality. Participant information will be coded using a study code, and study forms will not contain any individually identifying information. The study code involves only an ID number that indicates the order whereby participants enrolled in the study. For example, the first participant to enroll in the study will be ID#001, and the second participant will be ID#002, etc. There will be no human-derived elements in this code (e.g., initials, dates, etc.). The code further will not pertain to any information on random assignment to groups. The master list linking study codes to individual identities will be maintained by the investigator on a password-protected, shared drive space on the UAB server and will not be divulged to others. Paper records will be stored in a locking file cabinet in the project coordinator's locked office in the ENRL. Electronic data will be stored on UAB computers which are firewall protected, encrypted and password-restricted. The servers are monitored at all times for outages. Secured login IDs, granted on a need-to-know basis, further are required to access confidential information.

Discussion.

The current study is the first adequately-powered RCT to include an exercise training stimulus (i.e., treadmill walking exercise that progressively increases in duration and intensity) that is based on research on the acute[24,25] and chronic[19,21] effects of exercise on cognition in persons with MS that further targets hippocampal outcomes. Importantly, this study is the first RCT of exercise training to selectively recruit persons with objective impairments in learning new information in MS.[17] This is a critical methodological study component, as potential *treatment* effects (i.e., beyond simply improving cognitive performance) of exercise on MS-related cognitive dysfunction can only be assessed if participants demonstrate cognitive impairment. The current proposal will provide the first Class I evidence for the efficacy of treadmill walking exercise training as a potential rehabilitative approach to *treat* MS-related learning and memory impairment. This efficacy study will include an active control

condition to account for the effects of attention and social contact associated with supervised exercise training; no previous RCTs of exercise training on cognition in MS have adopted this approach.[17] We further will include cardiorespiratory fitness outcomes as a manipulation check for documenting the success of the intervention; this critical feature has been lacking in previous RCTs of exercise on cognition in MS.[17] The proposed study will be among the first exercise training RCTs in any population to include blinded MRI data analyses, uniquely adding another layer of rigor for improving the study's reproducibility. Such methodological features are critical for reducing threats to internal validity (i.e., Type I error) and providing efficacy evidence for chronic treadmill walking exercise training as a behavioral approach for managing learning and memory dysfunction in persons with MS who have the most need.

The current early-phase RCT will provide critical information for the development of future exercise trials on learning and memory in persons with MS. If successful, the current trial will provide effect sizes for power analyses for determining appropriate sample sizes for a subsequent Phase III RCT. Indeed, such a line of research will lay the foundation for approaches that can be eventually translated into community-based settings for rehabilitating learning and memory in persons with MS by examining the effects of a treadmill walking exercise training intervention on learning and memory and hippocampal neuroimaging outcomes. Although results from studies of cognitive rehabilitation are promising, such interventions are not easily adapted outside of the clinic, and further do not offer health benefits beyond improved cognition. On the other hand, exercise is a behavior with high ecological validity (i.e., easily adaptable in the community) and offers a myriad of physical health benefits. As such, the proposed study uniquely represents the first step in developing and optimizing a potentially generalizable exercise training intervention for improving behavioral (i.e., learning and memory), and brain (i.e., hippocampal) outcomes among cognitively-impaired persons with MS. As a whole, this line of research might result in the development of exercise training guidelines that can be

adapted by MS patients for specifically improving brain health and cognition. Importantly, there are no guidelines for persons with MS to manage cognitive impairment in the community using any approach.

If the current RCT does not result in statistically significant treadmill walking exercise-related improvements in composite learning and memory performance, we will focus on refining the exercise stimulus for specifically improving learning and memory amongst memory-impaired persons with MS for informing the development of a future RCT. This could involve adjusting the modality and intensity of exercise training. Indeed, there is preliminary evidence that other exercise modalities (e.g., aerobic cycle ergometry) and intensities (e.g., high-intensity interval training) have resulted in improvements in learning and memory in persons with MS.[60,61] If the current treadmill walking exercise training intervention does not improve learning and memory performance, another potential approach for refining the exercise stimulus involves the inclusion of additional sensory stimuli to treadmill walking exercise (i.e., treadmill walking exercise plus virtual reality) for inducing cognitive change. This approach is based, in part, on animal work that demonstrates particularly large improvements in hippocampal neurogenesis, synaptogenesis, and learning and memory performance when exercise is performed in enriched environments compared with standard environments.[62] There too is evidence in persons with traumatic brain injury whereby 4-weeks of exercise plus virtual reality improved verbal and visuospatial learning and memory.[63] Alternatively, if treadmill walking exercise training does not improve cardiorespiratory fitness outcomes (i.e., as a manipulation check), we plan to examine the effects of a longer intervention period (i.e., 6-months) on the primary and secondary study outcomes in a subsequent RCT, as has been done in older adults.[64] We do not expect this to be the case, given that the exact treadmill walking exercise training stimulus resulted in large cardiorespiratory fitness improvements in our small pilot RCT.[26]

Although the current RCT addresses a critical problem by applying a highly novel, systematically-developed exercise training intervention for improving learning and memory and hippocampal structure

and function among persons with MS who present with learning and memory impairment, there are several noteworthy limitations. First, the current RCT involves a relatively short intervention period and no long-term follow-up. Given that preliminary work directly supports the feasibility and preliminary efficacy of 3-months of treadmill walking exercise training on learning and memory and hippocampal neuroimaging outcomes, the current adequately-powered RCT seeks to rigorously examine the potential treatment effects of that exact intervention on learning and memory and its potential neural correlate(s). We believe this to be a necessary step prior to investigating the durability and sustainability of progressive treadmill walking exercise training on those outcomes in subsequent large-scale studies. To that end, the present study will not examine the effectiveness of treadmill walking exercise training on the primary outcomes in a large, national sample of persons with MS. Rather, this study will advance our systematic and rigorous line of research by providing critical efficacy data prior to the development of a subsequent RCT for examining the effectiveness of the intervention for eventual translation into a community-based program. Another limitation is that the study sample will not involve persons with MS who present with substantial ambulatory disability. Rather, this RCT only focuses on persons with MS who are fully-ambulatory based on pilot data and safety concerns associated with treadmill walking exercise training. Thus, the results of the current RCT might not be generalizable amongst all persons with MS, particularly those with severe ambulatory impairment. Finally, the present study will not directly compare the effects of treadmill walking exercise training with cognitive rehabilitation as a control comparison condition on learning and memory and hippocampal neuroimaging outcomes. Instead, this study includes an active, non-aerobic exercise training control condition in order to control for the potential effects of attention and social contact normally associated with supervised exercise training for testing the primary study hypotheses. Examinations of the comparative and combined effects of exercise training and cognitive rehabilitation on learning and memory and hippocampal neuroimaging outcomes will be performed in subsequent effectiveness trials.

Regardless of the study outcome, we plan to communicate the trial results via peer-reviewed publications. If successful, the results from this study will eventually inform RCTs for developing rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in a large sample of cognitively-impaired persons with MS. In the long term, the results from this early-phase RCT will lay the groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. Such an evidence-based approach for rehabilitation, using chronic exercise training, is paramount considering the highly prevalent, disabling, and poorly-managed nature of MS-related learning and memory impairment.

References:

1. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol* 2008;7:1139-51.

2. Guerts JJ, Bo L, Roosendaal SD, et al. Extensive hippocampal demyelination in multiple sclerosis. *J Neuropath Exp Neurol* 2007;66(9):819-27.

3. Benedict RH, Ramasamy D, Munschauer F, et al. Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. *J Neurol Neurosurg Psychiatr* 2009;80(2):201-6.

4. Hulst HE, Schoonehim MM, Van Geest Q, et al. Memory impairment in multiple sclerosis: relevance of hippocampal activation and hippocampal connectivity. *Mult Scler* 2015;21(13):1705-12.

5. Feinstein A, Magalhaes S, Richard J-F, et al. The link between multiple sclerosis and depression. *Nat Rev Neurol* 2014;10:507-17.

6. Morrow SA, Drake A, Zivadinov R, et al. Predicting loss of employment over three years in multiple sclerosis: clinically meaningful cognitive decline. *Clin Neuropsychol* 2010;24:1131-45.

7. Ruet A, Deloire M, Hamel D, et al. Cognitive impairment, health-related quality of life and vocational status at early stages of multiple sclerosis: a 7-year longitudinal study. *J Neurol* 2013;260:776-84.

8. Amato MP, Ponziani G, Siracusa G, et al. Cognitive dysfunction in early-onset multiple sclerosis: a reappraisal after 10 years. *Arch Neurol* 2001;58:1602-6.

9. Amato MP, Langdon D, Montalban X, et al. Treatment of cognitive impairment in multiple sclerosis: position paper. *J Neurol* 2013;260:1452-68.

10. Rosti-Otajarvi EM, Hamalainen PI. Neuropsychological rehabilitation for multiple sclerosis. *Cochrane Database Syst Rev* 2014;2:CD009131.

11. Chiaravalloti ND, Wylie G, Leavitt V, et al. Increased cerebral activation after behavioral treatment for memory deficits in MS. *J Neurol* 2012;259:1337-46.

12. Chiaravalloti ND, Moore NB, Nikelshpur OM, et al. An RCT to treat learning impairment in multiple sclerosis: the MEMREHAB trial. *Neurology* 2013;81:2066-72.

13. Goverover Y, Chiaravalloti ND, O'Brien A, et al. Evidence based cognitive rehabilitation for persons with multiple sclerosis: an updated review of the literature from 2007-2016. *Arch Phys Med Rehabil* 2018;99(2):390-407.

14. das Nair R, Martin KJ, Lincoln NB. Memory rehabilitation for people with multiple sclerosis. *Cochrane Database Syst Rev* 2016;3:CD008754.

15. Motl RW, Sandroff BM, Benedict RHB. Cognitive dysfunction and multiple sclerosis: developing a rationale for considering the efficacy of exercise training. *Mult Scler* 2011;17(9):1034-40.

16. Stillman CM, Cohen J, Lehman ME, et al. Mediators of physical activity on neurocognitive function: a review at multiple levels of analysis. *Front Hum Neurosci* 2016;10(626):1-17.

17. Sandroff BM, Motl RW, Scudder MR, et al. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. *Neuropsychol Rev* 2016;26(3):271-94.

18. Motl RW, Pilutti LA, Hubbard EA, et al. Cardiorespiratory fitness and its association with thalamic, hippocampal, and basal ganglia volumes in multiple sclerosis. *Neuroimage Clin* 2015;7:661-6.

19. Leavitt VM, Cirnigliaro C, Cohen A, et al. Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: preliminary findings. *Neurocase* 2014;20(6):695-7.

20. Sandroff BM. Exercise and cognition in multiple sclerosis: the importance of acute exercise for developing better interventions. *Neurosci Biobehav Rev* 2015;59:173-83.

21. Sandroff BM, Johnson CL, Motl RW. Exercise training effects on memory and hippocampal viscoelasticity in multiple sclerosis: a novel application of magnetic resonance elastography. *Neuroradiology* 2017;59(1):61-7.

22. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 Statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

23. American College of Sports Medicine. ACSM's resource manual for guidelines for exercise testing and prescription, seventh edition. Philadelphia, PA: Lippincott Williams & Wilkins 2013.

24. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of walking, cycling, and yoga exercise on cognition in persons with relapsing-remitting multiple sclerosis without impaired cognitive processing speed. *J Clin Exp Neuropsychol* 2015;37(2):209-19.

25. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of varying intensities of treadmill walking exercise on inhibitory control in persons with multiple sclerosis: a pilot investigation. *Physiol Behav* 2016;154:20-7.

26. Sandroff BM, Balto JM, Klaren RE, et al. Systematically-developed pilot randomized controlled trial of exercise and cognition in persons with multiple sclerosis. *Neurocase* 2016;22(5):443-50.

27. Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates 1988.

28. Pajonk FG, Wobrock T, Gruber O, et al. Hippocampal plasticity in response to exercise in schizophrenia. *Arch Gen Psychiatry* 2010;67(2):133-43.

29. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;50(1):121-7.

30. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-52.

31. Chiaravalloti ND, Balzano J, Moore NB, et al. The open-trial selective reminding test (OT-SRT) as a tool for the assessment of learning and memory. *Clin Neuropsychol* 2009;23:231-54.

32. Thomas S, Reading J, Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 1992;17(4):338–45.

33. Motl RW, Bollaert RE, Sandroff BM. Validation of the Godin Leisure-Time Exercise Questionnaire classification coding system using accelerometry in multiple sclerosis. *Rehabil Psychol* 2018;*in press*:doi: 10.1037/rep0000162 [Epub ahead of print].

34. Delis DC, Kramer JH, Kaplan E, et al. California Verbal Learning Test, Second Edition (CVLT-II). San Antonio, TX: Psychological Corporation 2000.

35. Buschke F, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 1974;24:1019-25.

36. Benedict RHB. The Brief Visuospatial Memory Test Revised (BVMT-R). Lutz, FL: Psychosocial Assessment Resources 1997.

37. Rao S. A manual for the brief repeatable battery of neuropsychological tests in multiple sclerosis. Milwaukee, WI: Medical College of Wisconsin 1990.

38. Strober L, Englert J, Munschauer F, et al. Sensitivity of conventional memory tests in multiple sclerosis: comparing the Rao Brief Repeatable Neuropsychological Battery and the Minimal Assessment of Cognitive Function in MS. *Mult Scler* 2009;15(9):1077-84.

39. Amato MP, Goretti B, Viterbo RG, et al. Computer-assisted rehabilitation of attention in patients with multiple sclerosis: results of a randomized, double-blind trial. *Mult Scler* 2014;20(1):91-8.

40. Mattioli F, Stampatori C, Bellomi F, et al. A RCT comparing specific intensive cognitive training to aspecific psychological intervention in RRMS: the SMICT study. *Front Neurol* 2015;5(278):1-8.

41. Boringa JB, Lazeron RH, Reuling IE, et al. The brief repeatable battery of neuropsychological tests: normative values allow application in multiple sclerosis clinical practice. *Mult Scler* 2001;7(4):263-7.

42. Parmenter BA, Testa SM, Schretlen DJ, et al. The utility of regression-based norms in interpreting the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *J Int Neuropsychol Soc* 2010;16(1):6-16.

43. Smith DV, Utevsky AV, Bland AR, et al. Characterizing individual differences in functional connectivity using dual-regression and seed-based approaches. *Neuroimage* 2014;15:1-12.

44. Colcombe S, Kramer AF, Erickson KI, et al. Cardiovascular fitness, cortical plasticity and aging. *Proc Natl Acad Sci USA* 2004;101(9):3316-21.

45. Macko RF, Katzel LI, Yataco A, et al. Low-velocity graded treadmill stress testing in hemiparetic stroke patients. *Stroke* 1997;28(5):988-92.

46. Motl RW, Fernhall B. Accurate prediction of cardiorespiratory fitness using cycle ergometry in minimally disabled persons with relapsing-remitting multiple sclerosis. *Arch Phys Med Rehabil* 2012;93(3):490–5.

47. Benedict RHB, Fischer JS, Archibald CJ, et al. Minimal neuropsychological assessment of MS patients: a consensus approach. *Clin Neuropsychol* 2002;16:381-97.

48. Smith A. Symbol digit modalities test: Manual. Los Angeles, CA: Western Psychological Services 1982.

49. Gronwall DMA. Paced auditory serial addition task: a measure of recovery from concussion. *Percept Mot Skills* 1977;44:367-73.

50. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan executive function system: Technical manual. San Antonio, TX: Psychological Corporation 2001.

51. Benton AL, Sivan AB, Hamsher K, et al. Contributions to Neuropsychological Assessment, Second Edition. New York, NY: Oxford University Press 1994.

52. Patti F, De Stefano M, Lavorgna L, et al. Lesion load may predict long-term cognitive dysfunction in multiple sclerosis patients. *PLoS One* 2015;10(3):e0120754.

53. Borg G. Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics 1998.

54. National Multiple Sclerosis Society. Stretching for people with MS: an illustrated manual. New York, NY: National Multiple Sclerosis Society 2004.

55. Sandroff BM, Bollaert RE, Pilutti LA, et al. Multimodal exercise training in multiple sclerosis: a randomized controlled trial in persons with substantial mobility disability. *Contemp Clin Trials* 2017;61:39-47.

56. Bandura A. Health promotion by social cognitive means. *Health Educ Behav* 2004;31:143-64.

57. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986;51(6):1173-82.

58. Rousselet GA, Pernet CR. Improving standards in brain-behavior correlation analyses. *Front Hum Neurosci* 2012;6:119.

59. Pilutti LA, Platta ME, Motl RW, et al. The safety of exercise training in multiple sclerosis: a systematic review. *J Neurol Sci* 2014;343(1-2):3-7.

60. Briken S, Gold SM, Patra S, et al. Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial. *Mult Scler* 2014;20(3):382-90.

61. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: a randomized controlled trial. *Mult Scler* 2017;*in press*:doi: 10.1177/1352458517728342 [Epub ahead of print].

62. van Praag H. Neurogenesis and exercise: past and future directions. *Neuromolecular Med* 2008;10(2):128-40.

63. Grealy MA, Johnson DA, Rushton SK. Improving cognitive function after brain injury: the use of exercise and virtual reality. *Arch Phys Med Rehabil* 1999;80:661-7.

64. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci USA* 2011;108(7):3017-22.

Author Contributions

BMS: Study concept and design, study registration, study principal investigator, obtained IRB approval, drafting of the manuscript, critical revision of the manuscript

RWM, MMB, GRC, MB, JRR, GRW, HG, JDL: Study concept and design, critical revision of the manuscript

Funding Statement

This paper was supported by an investigator-initiated grant from EMD Serono, Inc. The principal investigator (BMS) will have access to all data from the study, complete freedom to direct the data analyses and reporting without influence of sponsors, and affirm that there is no editorial direction or censorship from sponsors.

Competing Interest Statement

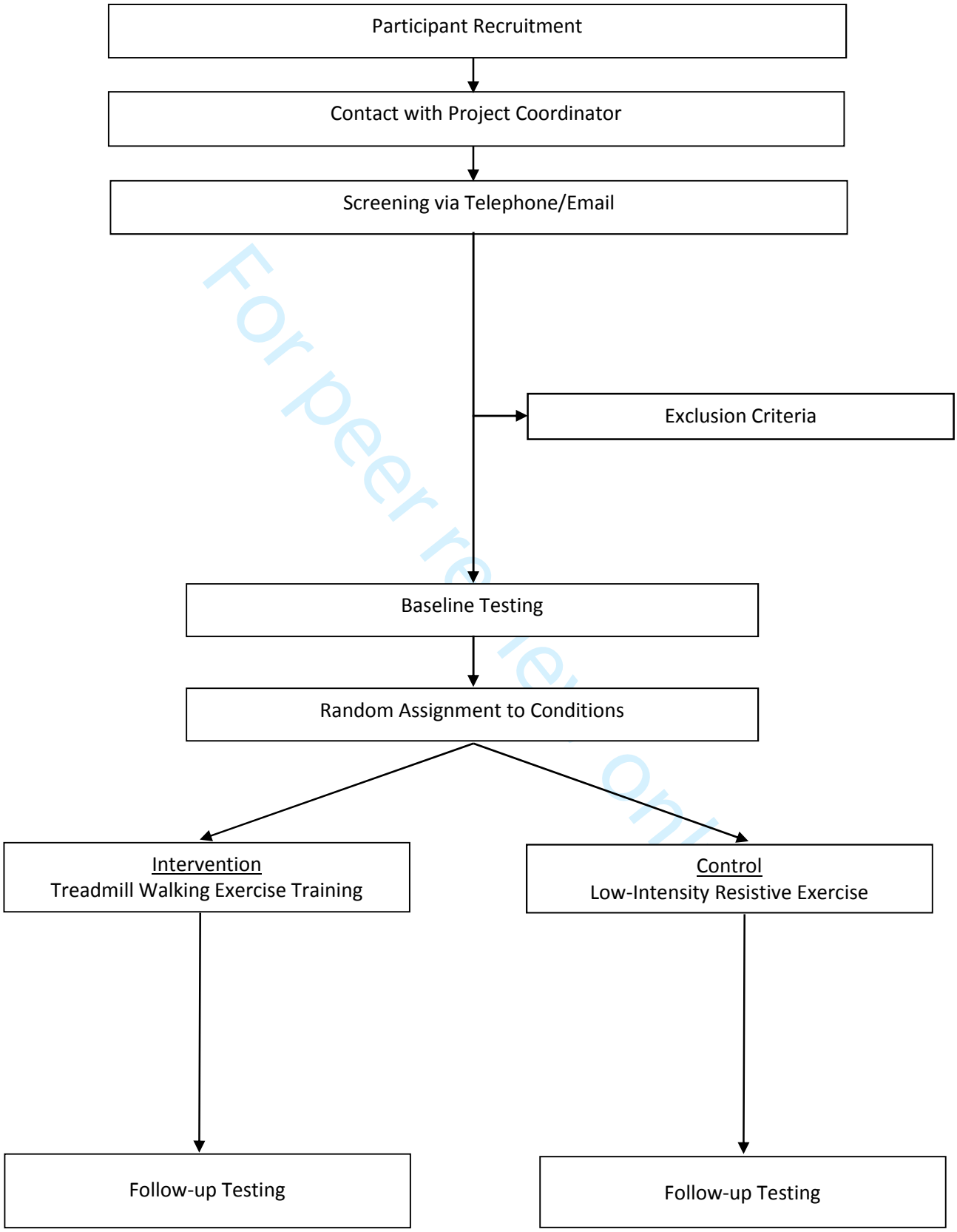
All authors declare no conflicts of interest.

Table 1: Exercise prescription and progression over the 3-month period for treadmill walking exercise training condition based on pilot work and ACSM guidelines

Week	Sessions	Exercise Intensity	Exercise Duration	Training Stage
Baseline Testing				
1	1-3	40-50% VO_2R/HRR	15-20 min	Initiation
2	4-6	40-50% VO_2R/HRR	20-25 min	Initiation
3	7-9	50-60% VO_2R/HRR	20-25 min	Improvement
4	10-12	50-60% VO_2R/HRR	25-30 min	Improvement
5-6	13-18	60-70% VO_2R/HRR	25-30 min	Improvement
7-8	19-24	60-70% VO_2R/HRR	30-35 min	Improvement
9-10	25-30	70-80% VO_2R/HRR	30-35 min	Maintenance
11-12	31-36	70-80% VO_2R/HRR	35-40 min	Maintenance
Follow-up Testing				

Note: VO_2R=oxygen consumption reserve; HRR=heart rate reserve

Figure 1: Participant flow through the study.



BMJ Open

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023231.R1
Article Type:	Protocol
Date Submitted by the Author:	02-Jul-2018
Complete List of Authors:	Sandroff, Brian; University of Alabama at Birmingham, Physical Therapy Motl, Robert; University of Alabama at Birmingham, Department of Physical Therapy Bamman, Marcus; University of Alabama at Birmingham Cutter, Gary; University of Alabama at Birmingham, Department of Biostatistics Bolding, Mark; University of Alabama at Birmingham Rinker, John; University of Alabama at Birmingham Wylie, Glenn; Kessler Foundation Genova, Helen; Kessler Foundation DeLuca, John; Kessler Foundation
Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Multiple sclerosis < NEUROLOGY, exercise, cognition, memory, MRI

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Brian M. Sandroff[1], Robert W. Motl[1], Marcas M. Bamman[2], Gary R. Cutter[3], Mark Bolding[4], John R. Rinker[5], Glenn R. Wylie[6], Helen Genova[6], John DeLuca[6]

[1] University of Alabama at Birmingham, Department of Physical Therapy, Birmingham, AL, USA
[2] University of Alabama at Birmingham, Departments of Cell, Developmental, & Integrative Biology; Medicine; and Neurology, Birmingham, AL, USA
[3] University of Alabama at Birmingham, Department of Biostatistics, Birmingham, AL, USA
[4] University of Alabama at Birmingham, Department of Radiology, Birmingham, AL, USA
[5] University of Alabama at Birmingham, Department of Neurology, Birmingham, AL, USA
[6] Kessler Foundation, Neuropsychology and Neuroscience Research, West Orange, NJ, USA

Correspondence to Brian M. Sandroff, PhD, University of Alabama at Birmingham, Department of Physical Therapy, SHP 389, 1720 2nd Ave S, Birmingham, AL, 35294, *phone* (205) 934-5972, *email* sandroff@uab.edu

Word Count: 7,641

Abstract

Introduction: This randomized controlled trial (RCT) examines treadmill walking exercise training effects on learning and memory performance, hippocampal volume, and hippocampal resting-state functional connectivity in persons with multiple sclerosis (MS) who have objective impairments in learning new information.

Methods and Analysis: Forty fully-ambulatory persons with MS who demonstrate objective learning and memory impairments will be randomly assigned into either the intervention or active control study conditions. The intervention condition involves supervised, progressive treadmill walking exercise training 3 times/week for a 3-month period. The active control condition involves supervised, progressive low-intensity resistive exercise that will be delivered at the same frequency as the intervention condition. The primary outcome will involve composite performance on neuropsychological learning and memory tests, and the secondary outcomes involve MRI measures of hippocampal volume and resting-state functional connectivity administered before and after the 3-month study period. Outcomes will be administered by treatment-blinded assessors using alternate test forms to minimize practice effects, and MRI data processing will be performed by blinded data analysts.

Ethics and Dissemination: This study has been approved by a University Institutional Review Board and further is registered at clinicaltrials.gov: NCT03319771. If successful, the results from this study will eventually inform subsequent RCTs for developing physical rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in larger samples of cognitively-impaired persons with MS. The results from this early-phase RCT will further lay preliminary groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. This approach is paramount as learning and memory impairment is common, burdensome, and poorly-managed in MS.

Keywords: *multiple sclerosis; exercise; cognition; memory; MRI*

Strengths and Limitations of This Study

- The current randomized controlled trial applies a systematically-developed exercise training intervention for improving learning and memory and hippocampal structure and function among persons with MS who present with objective learning and memory impairment.
- This study is adequately powered and involves the inclusion of an active control comparison condition (i.e., low intensity resistive exercise) as well as blinded MRI data analysts.
- However, this efficacy study does include a relatively short intervention period (i.e., 3-months) and will not examine the sustainability and durability of the intervention effects on cognitive and hippocampal neuroimaging outcomes.
- This study was powered based on pilot data on exercise effects on behavioral measures of learning and memory in persons with MS (i.e., the primary study outcomes) and not on pilot data on the secondary outcomes of exercise effects on hippocampal volume and functional connectivity.
- This study involves fully-ambulatory persons with MS and will not involve persons with substantial ambulatory disability.

Introduction

The impairment of learning and memory, particularly with learning new information, is a common, burdensome, and poorly-managed manifestation of multiple sclerosis (MS).[1] Over 50% of MS patients demonstrate impaired performance on neuropsychological tests of verbal and/or visuospatial learning and memory[1] that is associated with hippocampal lesions, atrophy, and altered resting-state functional connectivity based on neuroimaging (i.e., MRI/fMRI) studies.[2-4] MS-related learning and memory impairment and decline have been associated with depression,[5] unemployment,[6,7] loss of independence, and social isolation.[8] Currently, there are no FDA-approved pharmacological treatments (i.e., disease-modifying and symptomatic therapies) for learning and memory dysfunction in MS.[9] Cognitive rehabilitation is currently the best-characterized behavioral approach for improving MS-related learning and memory impairment and is seemingly mediated through changes in brain function (i.e., increased hippocampal activation and resting-state functional connectivity;[10-14]). However, cognitive rehabilitation is difficult to apply outside the clinical setting and does not generally result in physical health benefits beyond improved cognition. This underscores the consideration of other behavioral approaches for managing learning and memory impairment and its potential association with hippocampally-mediated functional brain outcomes in MS that can be easily applied outside the clinical setting and result in many physical health benefits; one such approach includes exercise training.[15]

The consideration of exercise for improving learning and memory in persons with MS is based, in part, on the body of literature in the general population that documents robust, beneficial effects of exercise training on memory and hippocampal structure/function.[16] There further is a substantial body of animal work that describes upregulation of central neuro- and vascular trophic factors with several weeks of exercise that accompanies such neuropsychological and brain-systems changes.[17,18] By comparison, there have been few well-designed, targeted exercise training RCTs on learning and

memory and hippocampal neuroimaging outcomes in MS, although the data are promising.[19] For example, one recent cross-sectional study described statistically significant, moderate-sized correlations between cardiorespiratory fitness (i.e., a presumed surrogate of aerobic exercise training) and hippocampal volume in 35 persons with MS.[20] Another noteworthy case study involving two memory-impaired persons with MS reported that 3-months of aerobic exercise training (stationary cycling) resulted in a > 50% increase in learning and memory performance, 16.5% increase in hippocampal volume, as well as increased hippocampal resting-state functional connectivity.[21] By comparison, the non-aerobic exercise condition demonstrated minimal changes in those outcomes.[21] One systematically-developed[22] pilot RCT examined the effects of aerobic treadmill walking exercise training compared with a waitlist control condition on learning and memory performance and hippocampal viscoelasticity using non-conventional MRI (i.e., magnetic resonance elastography) in 8 fully-ambulatory persons with MS.[23] Overall, there were small-to-moderate intervention effects on verbal learning and memory performance (d=0.34), and large intervention effects on hippocampal viscoelastic properties (i.e., increased hippocampal shear stiffness) (d>0.94). The change in verbal learning and memory was strongly associated with change in hippocampal viscoelasticity (r>.93, p<.01). Collectively, despite experimental design[20] and sample size[21,23] limitations, such preliminary observations suggest that aerobic exercise training might improve learning and memory through neuroplasticity in MS and warrant the development of an adequately-powered, early-phase RCT for examining the effects of aerobic exercise training on learning and memory, and hippocampal structure/function in a larger sample of persons with MS.

The present RCT aims to provide the first evidence for treadmill walking exercise training effects on learning and memory, hippocampal volume, and hippocampal resting-state functional connectivity in an adequately-powered sample of persons with MS who have objective impairment in learning new information. This will provide the first evidence for exercise training as a possible *treatment* for MS-

related learning and memory impairment (i.e., beyond merely improving learning and memory performance), given that previous trials of exercise, cognition, and neuroimaging have not recruited persons with objective MS-related learning and memory impairment *a priori.*[e.g., 23] The current RCT will further provide the first high-quality evidence of potential mechanisms of aerobic exercise-related effects on hippocampal outcomes in MS, whereas previous studies have been limited by experimental design[e.g., 20] or sample size.[e.g., 21,23] This study will lay the foundation for a definitive Phase III RCT by providing effect sizes of treadmill walking exercise training versus an active control condition on the outcomes that can be used in power analyses for determining the appropriate sample size for such a trial. The present study will provide preliminary experimental evidence of the potential mechanisms (i.e., improved hippocampal structure and function) of how treadmill walking exercise might improve learning and memory in this population.

Methods and Analysis

Experimental Overview and Hypotheses.

The study protocol was drafted in accordance with the SPIRIT statement[24] and further has been approved by the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB). This study is registered at clinicaltrials.gov: NCT03319771. All potential protocol modifications will be approved by the UAB IRB and will be reported at clinicaltrials.gov. The proposed study, data collection, and intervention will take place at UAB in Birmingham, AL, USA. This study involves a single-blind, early Phase II RCT on the effects of supervised treadmill walking exercise training compared with an active control condition (i.e., low intensity resistive exercise) on learning and memory, hippocampal structure/function, and cardiorespiratory fitness outcomes in 40 fully-ambulatory (i.e., Expanded Disability Status Scale (EDSS) ≤ 4.0) persons with MS who have impairment in learning new information. Composite performance on neuropsychological tests of learning and memory represents the primary

outcome, whereas neuroimaging outcomes of hippocampal volume and hippocampal resting-state functional connectivity represent the secondary outcomes. Cardiorespiratory fitness changes will be included as a manipulation check for documenting the success of the intervention.

During baseline, participants will first complete a battery of neuropsychological tests addressing verbal and visuospatial learning and memory; a maximal, graded exercise test on a motor-driven treadmill to measure cardiorespiratory fitness; and an MRI scan for measurement of hippocampal volume and resting-state functional connectivity. Those outcomes will be measured by assessors who are uninvolved in the exercise training (i.e., treatment blinded assessors). After baseline testing, participants will be randomly assigned to either the intervention or active control conditions using concealment. Participants further will be masked to condition (i.e., unaware that the treadmill walking exercise training condition represents the intervention condition and the low intensity resistive exercise condition represents the active control condition).

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise, and will be based on American College of Sports Medicine (ACSM) guidelines for exercise prescription[25] and pilot work.[23,26-28] The exercise training itself will take place 3 times per week over 3 months, and will be facilitated by trained exercise leaders. The exercise prescription will initially consist of 15 minutes of light-to-moderate intensity treadmill walking exercise and eventually progress to 40 minutes of vigorous intensity exercise by month 3 (Table 1). We note that this exercise stimulus is identical to that of our pilot RCT in 8 persons with MS.[23] The active control condition will involve low intensity resistive exercise that will be delivered using the same frequency and duration of the treadmill walking exercise condition and facilitated by trained exercise leaders. This is a methodological improvement over our pilot RCT that involved a waitlist (i.e., passive) control condition.[23] Regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising) over the duration of the study.

The cognitive, cardiorespiratory fitness, and MRI outcomes will be assessed again following the 3-month study period by treatment blinded assessors.

The primary hypothesis is that those who undergo treadmill walking exercise training will demonstrate larger improvements in composite learning and memory performance than those who undergo low intensity resistive exercise. We further hypothesize that those who undergo treadmill walking exercise training condition will demonstrate greater increases in hippocampal volume and hippocampal resting-state functional connectivity (i.e., adaptive increases) and improvements in cardiorespiratory fitness than those in the low intensity resistive exercise condition. We lastly hypothesize that (1) the treadmill walking exercise-induced improvements in learning and memory will be accounted for by hippocampal volume and hippocampal resting-state functional connectivity (i.e., partial mediation) and (2) those with the largest improvements in cardiorespiratory fitness will demonstrate the largest improvements in the primary and secondary end-points.

Participants.

Sample Size. We plan to enroll 40 fully-ambulatory persons with MS (i.e., 20 per condition) who have impairment in learning new information (see below); this is based on a power analysis and presumed 15% attrition. The minimal sample size of 34 persons with MS (i.e., 17 per condition) was determined based on power analysis using standard assumptions of alpha (0.05) and beta (0.80) for detecting moderate-sized effects (i.e., η_p^2=0.06) based on Cohen's guidelines[29] for a time by condition interaction in mixed-factor ANOVA on composite learning and memory performance. Our previous pilot RCT of treadmill walking exercise training on cognition yielded a large time by condition interaction on composite learning and memory performance (i.e., η_p^2=0.11) in fully-ambulatory persons with MS.[23] Another study on aerobic exercise effects on hippocampal outcomes in persons with schizophrenia reported large intervention effects on hippocampal volume (i.e., η_p^2=0.50).[30] Using those effect sizes as a guide, the proposed sample size of 34 and planned sample size of 40 will be more than adequate for

detecting moderate or larger effects on learning and memory and hippocampal outcomes in persons with MS. The required sample size of 34 also adequately powers the secondary study hypotheses based on previous cross-sectional data in MS that indicates moderate-sized correlations (i.e., ρ = .42) between cardiorespiratory fitness and hippocampal volume.[20]

Recruitment. Subjects will be recruited directly through the UAB MS Center, the Alabama-Mississippi Chapter of the National MS Society (NMSS), and our laboratory database of previous participants with MS who have inquired about participating in exercise studies. Advertisements for the study will be distributed through the UAB MS Center, facilitators of local MS support groups, *MS Connection* publications, and e-mail distributions. As a backup plan, we may recruit prospective participants with MS through the North American Research Committee on Multiple Sclerosis (NARCOMS) patient registry or iConquerMS if enrollment is slow.

Inclusion/Exclusion Criteria. All participants will be between the ages of 18-54, have a clinically definite MS diagnosis based on established criteria,[i.e., 31] and be fully-ambulatory based on EDSS[32] scores between 0-4.0. All participants will demonstrate impairment in learning new information based on open-trial Selective Reminding Task (SRT) scores at least 1.5 *SD's* below the normative score for healthy controls.[33] Participants will be relapse-free for at least 30 days (i.e., relative neurologic stability), and will not have a history of schizophrenia, bipolar disorder I or II, or substance-abuse disorders. Participants further will not be taking medications that can affect cognition (e.g., antipsychotics, benzodiazepines), and all participants will be on a stable FDA-approved disease-modifying therapy (e.g., interferon beta-1a; interferon beta-1b; glatiramer acetate; natalizumab; dimethyl fumarate, etc.) regimen for at least 6 months. Participants will be right-handed (to control for organization of the brain) and will have a low risk for contraindications for maximal exercise testing based on a "no" response on all items of the Physical Activity Readiness Questionnaire (PAR-Q;[34]) or a single "yes" response along with a physician's approval. Participants further will have a low risk for

contraindications for MRI based on: (a) not having metal (e.g., non-MRI compatible aneurysm clips, metal shards in the body or eyes, or recently placed surgical hardware) or electronic devices (e.g., pacemaker, cochlear implant) within the body. Lastly, participants will not be meeting public health guidelines for participating in physical activity (i.e., at least 150 minutes per week of moderate-to-vigorous aerobic activity). This will be based on health contribution scores on the Godin Leisure-Time Exercise Questionnaire (GLTEQ) that fall within the 'insufficiently active' classification (i.e., less than 14 arbitrary units on the summed 'strenuous' and 'moderate' sections of the GLTEQ).[35]

Outcome Measures.

To minimize threats to internal validity and maximize the rigor and reproducibility of the present RCT, the outcome measures will be assessed by personnel who are uninvolved with the intervention or control conditions (i.e., treatment blinded assessors). The outcome assessments further will occur at a different UAB laboratory than the intervention or control conditions to prevent possible contamination.

Learning and Memory: Participants will undertake several neuropsychological tests addressing various aspects of learning and memory as the primary study outcomes. Neuropsychological testing will occur in a quiet, sound-dampened room in the Exercise Neuroscience Research Laboratory (ENRL) at UAB. These tests include the California Verbal Learning Test-II[CVLT-II;36] and SRT[37] as measures of verbal learning and memory, and the Brief Visuospatial Memory Test-Revised[BVMT-R;38] and 10/36 Spatial Recall Test[SPART;39] as measures of visuospatial learning and memory. These neuropsychological tests have strong psychometric properties,[e.g., 40] and are widely-used to document the efficacy of cognitive rehabilitation interventions in persons with MS.[12,41,42]

Briefly, the CVLT-II involves the examiner reading a list of 16 words, with four items belonging to four categories (e.g., vegetables, animals, furniture, modes of transportation) that are randomly arranged. The list is read aloud five times in the same order, with each word voiced at a rate of approximately one word per second. Participants are instructed to recall as many items as possible, in

any order, following each list reading. The primary outcome of the CVLT-II is the total number of correct words identified over the five trials (i.e., raw score).[36] The SRT involves the examiner reading a list of 12 unrelated words. Participants are asked to recall as many words as possible following the presentation of the list. After the first trial, only the words that participants did not recall are given as the new list. However, participants are instructed to recall as many words as possible from the original (i.e., entire) list for each of 5 total trials. The primary outcome is the total number of correctly recalled words across the 5 trials.[37] For both the CVLT-II and SRT, higher scores indicate better verbal learning and memory.

The BVMT-R involves three trials of the examiner presenting a 2×3 array of abstract geometric figures in front of the participant for 10 seconds. Following this period, the array is removed and participants are required to draw the array as precisely as possible, with the figures in the correct location. Each drawing is scored on a 0-2 scale, based on accurately portraying each figure and its correct location. The primary outcome of the BVMT-R is the total raw score across the three trials, with higher scores indicating better visuospatial memory.[38] The 10/36 SPART involves three trials of the examiner presenting a 6×6 checkerboard with 10 pieces positioned in certain locations on the board in front of the participant for 10 seconds. Following this period, the display is removed, and participants are asked to replicate the design of the checkerboard on a blank grid. This is repeated for 2 additional trials. The primary outcome of the 10/36 SPART is the total number of correct responses across the three trials.[39]

As the primary study outcome involves composite learning and memory performance, we will first compute *z*-scores that account for age, sex, and education based on normative scores per individual learning and memory test (i.e., CVLT-II, SRT, BVMT-R, 10/36 SPART).[43,44] We will then combine the *z*-scores into a composite learning and memory measure (i.e., mean of *z*-scores for CVLT-II, SRT, BVMT-R, and 10/36 SPART). Given evidence of hippocampal lateralization of verbal and visuospatial learning and

memory in persons with MS,[45] we further will examine the effects of the intervention on those constructs separately in exploratory analyses.

Hippocampal Volume and Resting State Functional Connectivity: Participants will undergo neuroimaging, which will include structural imaging as well as a resting-state scan. The MR instrument that will be used is an FDA-approved Siemens MAGNETOM Skyra 3T clinical imager housed in the Civitan International Neuroimaging Research Center at UAB. Each scan session will begin with the acquisition of high-resolution T1-weighted axial anatomical images (MP-RAGE). This 3D isotropic sequence will be acquired sagittally (TR = 11.6 ms, TE = 4.9 ms, flip angle = 8°, effective TI = 1017.6 ms, 256 x 256 matrix, FOV = 300 mm, NEX = 1, 172 slices, 1.17 mm slice thickness, 0 mm skip). Total imaging time for this sequence is 8 min 38 sec. In addition, an inversion-recovery sequence will be acquired axially (TR = 8530 ms, TE = 81 ms, flip angle = 180°, 256 x 256 matrix, FOV = 220 mm, NEX = 1, 32 slices, 4 mm slice thickness, 0 mm skip). Together, these scans will be used for volumetric analyses and for image segmentation and normalization of the resting-state fMRI scan. Functional imaging will consist of multi-slice gradient echo, T2*-weighted images acquired with echoplanar imaging (EPI) methods (TE= 60 ms; TR= 2000 ms; FOV = 24 cm; flip angle = 90°; slice thickness = 5 mm contiguous, matrix = 64×64, in-plane resolution = 2.50 mm^2). In order to provide coverage of the entire brain, a total of 32 images will be acquired in the axial plane. For the resting-state scan, 180 volumes will be acquired. Structural volumes for the hippocampus will be calculated using Freesurfer automated brain segmentation software (http://surfer.nmr.mgh.harvard.edu). Preprocessing of the resting-state functional connectivity data will be performed using AFNI software (http://afni.nimh.nih.gov/afni/). The first three volumes will be removed in order to control for saturation effects. Preprocessing steps include slice timing correction, realignment to an image exactly half-way through the acquisition run using affine transformation, co-registration to the T1 MPRAGE image for localization of activated areas, smoothing (6 mm FWHM) to minimize anatomical differences and increase the signal to noise ratio, scaling each voxel to the grand

mean intensity of that voxel (across the acquisition run), high-pass filtering (150 seconds), and normalization using a nonlinear approach (3dQwarp) to a standardized T1 template from the Montreal Neurological Institute (MNI). In all cases, the data will be checked for excessive motion (a shift of more than 3.5 mm, or 1° of angular motion) and for spikes (using the Root Mean Squared Error [RMSE] of each volume relative to a reference volume [which will be the volume half-way through the acquisition run]). Data acquisition runs with excessive motion will be discarded. Individual acquisitions with a RMSE amplitude that exceeds the 75th percentile plus the value of 150% of the interquartile range of RMSE for all volumes in a given run will be excluded from further analysis using the 'censorTR' function in 3dDeconvolve. In all cases, the motion parameters from the realignment step will be used as regressors of no interest in the deconvolution, and the residuals will be saved. The residuals for each subject in each group (exercise and control) will then be included in a probabilistic ICA, using AROMA (ICA-based Automatic Removal Of Motion Artifacts), as implemented in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The results of the ICA will be spatial maps of the resting-state networks. We will extract the connectivity map of the hippocampal network that will then be used in a dual-regression analysis.[46] In the first regression (spatial regression), the spatial hippocampal map from the ICA is regressed onto each subject's functional data, resulting in a dataset that characterizes the temporal dynamics of the hippocampal network. In the second regression (temporal regression), the dataset resulting from the spatial regression (the temporal dynamics of the hippocampal network) are regressed onto each subject's functional data. This results in a map for the hippocampal network representing each voxel's connectivity with this network. This map will then be used in the group-level analyses. Importantly, all MRI processing and analyses will be performed by scientists at Kessler Foundation (i.e., HG, GRW), who will be blinded to condition. To our knowledge, this is among the first exercise RCTs in any population to include this additional level of rigor to enhance the proposed trial's reproducibility.

Cardiorespiratory Fitness: Cardiorespiratory fitness will be measured as peak oxygen consumption (VO_{2peak}), using a maximal, graded exercise test on a motor-driven treadmill and an open-circuit spirometry system (ParvoMedics True One 2400, Sandy, UT) for analyzing expired gases using a modified Balke protocol in the ENRL at UAB. This protocol was successfully used in our previous small pilot RCT.[23] This protocol further is commonly used for measuring cardiorespiratory fitness in older adults[47] and persons with chronic stroke[48] and is consistent with the ACSM guidelines for exercise testing of MS patients.[25] The test will be preceded by a 3-minute warm up. The initial work rate for the exercise test will be at a brisk, but submaximal pace, and the grade will continuously increase at a rate of 2.0% every 2-minutes until the participant reaches volitional fatigue. Heart rate (HR) and rating of perceived exertion will be recorded every minute during the test. VO_{2peak} will be expressed in $ml \cdot kg^{-1} \cdot min^{-1}$ based on highest recorded 20-second VO_2 value when two of four criteria are satisfied: (1) VO_2 plateau with increasing grade; (2) respiratory exchange ratio ≥1.10; (3) peak heart rate within 10 $beats \cdot minute^{-1}$ of age-predicted maximum (i.e., ~1 SD); or (4) peak rating of perceived exertion ≥17.[49] The test will be followed by a 3-minute cool-down period.

Disability Status: Participants will undergo a neurological examination by a neurologist (JRR) for generating a baseline EDSS score. All participants will be fully-ambulatory (i.e., EDSS scores ≤ 4.0).

Additional Neuropsychological Tests: To evaluate the effects of the intervention on other domains of cognition that are commonly-impaired in MS, we will apply the MACFIMS neuropsychological battery[50] as exploratory outcomes. In addition to the CVLT-II and BVMT-R, the MACFIMS includes valid and reliable tests of cognitive processing speed,[51,52] executive function,[53] verbal fluency,[54] and spatial perception.[54] Further, baseline performance on the MACFIMS neuropsychological battery will serve to characterize the overall baseline cognitive status of the sample. For all neuropsychological tests, including measures of learning and memory, alternate forms will be applied at each testing session (i.e., baseline and follow-up) to minimize the effects of practice on cognition.

Additional Neuroimaging Outcomes: The MRI protocol further will involve the collection of structural MRI data on T2-lesion volume as a potential covariate of intervention effects, given its association with MS-related cognitive dysfunction.[55]

Intervention Condition.

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise training based on ACSM guidelines for maximizing adaptations with exercise training. This will occur at a laboratory exercise research facility. Exercise intensities will be prescribed based on percent oxygen consumption reserve (% VO_2R) using values derived from the baseline graded exercise test. We note that HR reserve (HRR) and VO_2R have a 1:1 relationship.[25] The exact exercise prescription is presented in Table 1 and further represents the identical stimulus that was included in our pilot RCT that demonstrated improvements in learning and memory and hippocampal neuroimaging outcomes.[23] The exercise progression in terms of duration and intensity will involve 3 distinct stages: (a) the initiation stage; (b) the improvement stage; and (c) the maintenance stage. The initiation stage (Weeks 1-2) aims to prepare participants for more intense aerobic exercise (i.e., by accumulating small improvements in cardiorespiratory fitness with light-to-moderate intensity exercise) and develop an orthopedic tolerance for exercise stress.[25] Following this period, participants will progress to the improvement stage of exercise training. This stage provides a gradual increase in the overall aerobic exercise stimulus (i.e., moderate-to-vigorous intensity), whereby participants realize substantial improvements in cardiorespiratory fitness (Weeks 3-8).[25] The final stage of exercise progression is the maintenance stage (i.e., vigorous intensity), which aims to maintain the levels of cardiorespiratory fitness that were developed during the improvement stage over the long-term (Weeks 9-12).[25] Consistent with ACSM recommendations, the intervention will not involve progression of both intensity and duration in a single exercise session. Such a gradual progression of

exercise training is advantageous for deconditioned persons to safely achieve the benefits of aerobic exercise training.[25]

The exercise training itself will be led by certified exercise leaders who are not involved in the collection of outcome assessments. At the outset of each session, participants will be fitted with a Polar HR Monitor (Oy, Finland), and HR will be monitored continuously throughout each session. Each session will begin with a 5-10 min warm-up, followed by the exercise; the target HRR range associated with the VO_2R range will be maintained for as long as possible during each exercise period. This will be followed by a 5-10 min cool-down. Participants will complete an exercise log at the conclusion of each session for better characterizing the experience with the intervention. Log data will include perceived exertion,[56] well-being, enjoyment, and mental/physical fatigue. Throughout each session, we further will collect data on treadmill speed and grade, as well as time spent within the prescribed VO_2R/HRR range for improving the rigor and reproducibility of the intervention.

Active Control Condition.

The active control condition will involve supervised, low intensity resistive exercise in order to control for the effects of social contact and attention. This condition will take place at a laboratory exercise research facility in a space that is isolated from the intervention condition in order to avoid participant and site contamination. The low intensity resistive exercise control condition will be delivered using the same frequency and duration of the treadmill walking exercise training condition. The low intensity resistive exercises will be based on a manual provided by the NMSS[57] and sessions will be led by certified exercise leaders who are not involved in the collection of outcome assessments. Low intensity resistive exercises will target the head/neck, shoulder, elbow/forearm, hand/wrist, trunk/hip, ankle/foot. The progression of activities over the 3-month period will involve performing additional exercises and sets along with using progressively thicker elastic resistance bands (i.e., Therabands) that provide minimal resistance. The first 6 weeks of the intervention period will involve

performing the activities without resistance. In weeks 7-8, the extra thin Theraband (i.e., least resistance) will be used to perform the exercises for the upper-extremities only. In weeks 9-10, the thin Theraband will be introduced and in weeks 11-12, the medium Theraband will be introduced. Such a progression is not expected to induce cardiorespiratory fitness adaptations and is designed to maintain participant interest. Each session is designed to last up to 60 minutes in total. Each session will begin with a 5-10 min warm-up, followed by low intensity resistive exercise (following the same duration as the treadmill walking exercise training condition) activities, and a 5-10 min cool-down. Throughout each session, participants will wear a HR monitor, and we will collect data on HR and perceived exertion[56] to ensure that this condition occurs at a low intensity. This stimulus has been included as a control comparison condition in a recent exercise training RCT in persons with MS and did not result in cognitive improvements and was well-received with no increase in drop-out compared with progressive exercise training.[58]

Importantly, to minimize attrition for both the intervention and control conditions, exercise leaders will apply highly-developed principles and techniques associated with Social Cognitive Theory[59] for enhancing participant adherence and compliance throughout each session. Further, regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising).

Procedure.

Participant flow through the study is presented in Figure 1. Participant recruitment, contact, and screening will be undertaken via telephone and/or email by an ENRL project coordinator. If a participant satisfies initial inclusion/exclusion criteria, the project coordinator will then administer the open-trial SRT via telephone to ensure that all participants have impairments in learning new information; the open-trial SRT has been used as a screening tool for impairments in learning new information in previous memory rehabilitation RCTs in MS.[12] This test involves the project coordinator reading a list

of words to the prospective participant over the phone. The participant then repeats as many words as they can from memory back to the project coordinator. This process is repeated until participants can remember the entire list of words. If participants demonstrate scores that are at least 1.5 *SD*'s below the normative score for healthy controls, the project coordinator will request contact information from the potential participant's neurologist, whereby they will email or fax a letter asking them to verify a definite MS diagnosis and confirmation that the participant has been on a stable DMT regimen for at least 6-months. Upon receipt of these materials from the participant's neurologist, the project coordinator will schedule the participant for baseline testing. Baseline testing will be led by assessors who are blinded to condition, and will take place over 3 non-consecutive days to minimize cognitive and physical fatigue. On the first day, participants will initially provide written informed consent with IRB-approved ENRL personnel, followed by a neurological examination for generation of an EDSS score. Participants will then undertake several neuropsychological tests of learning and memory (i.e., CVLT-II, BVMT-R) as part of the full MACFIMS neuropsychological battery, followed by the graded exercise test. The second day of baseline testing will involve the MRI protocol, and the third day of baseline testing will involve undertaking other neuropsychological tests of learning and memory (i.e., SRT, 10/36 SPART). Participants will be remunerated $50 for completing the baseline assessments. Of note, the separation of learning and memory measures across testing sessions is necessary considering that there might be overlap (interference) between performance on the CVLT-II and SRT (i.e., learning and memory tests in the verbal domain) and between the BVMT-R and 10/36 SPART (i.e., learning and memory tests in the visuospatial domain), respectively.

After baseline testing, participants will be randomly assigned to either the treadmill walking exercise training or active control conditions using concealment (i.e., opaque, sealed envelopes) and computerization by the study biostatistician (i.e., GRC). Participants further will blinded to the intent of the condition (i.e., unaware that the treadmill walking exercise training condition represents the

experimental condition and the low intensity resistive exercise condition represents the active control condition). To do this, the study will be advertised as a comparison of two different physical exercise programs on memory performance in persons with MS. We note that it is not possible for participants to be blinded to the actual condition (i.e., participating in treadmill walking exercise or low-intensity resistive exercise activities).

Participants will undertake the intervention or active control conditions as described above over a 3-month period. Participants will be remunerated $10 per treadmill walking exercise/low intensity resistive exercise visit attended (i.e., up to $360 total). This remuneration will be disbursed in weekly increments for maximizing compliance with this early stage research. Following the completion of the 3-month study period, participants will again undergo assessments of learning and memory, hippocampal volume and resting-state functional connectivity, and cardiorespiratory fitness using the same procedures as baseline testing (i.e., follow-up testing). Follow-up measures, using alternate forms where possible, will be administered by treatment blinded assessors. Participants will be remunerated $50 for completing the follow-up assessments.

Data Integrity.

All data will be entered, checked, and double-checked by UAB ENRL personnel under the direct supervision of the ENRL project coordinator and study principal investigator (BMS). All ENRL personnel have undergone extensive training in good clinical practice and laboratory procedures. Given that the current study is not a multi-site, NIH-defined Phase III RCT, we do not include a formal data monitoring committee.

Statistical Analysis.

The data analyses will be overseen by a biostatistician (i.e., GRC) and follow intent-to-treat principles (i.e., include all persons regardless of adherence and/or compliance). In the case of a drop-out, missing data will be imputed using multiple imputation and by carrying the last observed value

forward. We further will perform exploratory data analyses only in those who completed follow-up testing (i.e., completer's or per protocol analysis) and in those who demonstrated good adherence (i.e., attended at least 83% of sessions) and compliance.[19] The data will be analyzed using a mixed-factor model with time (baseline and follow-up) as a within-subjects factor and condition (intervention or active control) as a between-subjects factor on composite learning and memory performance, hippocampal, and cardiorespiratory fitness outcomes. Of note, we will require an MRI-overall alpha of .05 (corrected for multiple comparisons) for significance for hippocampal neuroimaging outcomes. The correction for multiple comparisons will be achieved by establishing a suitable voxel cluster-level threshold through Monte Carlo simulations (using the 3dClustSim program, part of the AFNI suite of image analysis programs; http://afni.nimh.nih.gov/afni/). Effect sizes will be expressed as partial eta-squared (η_p^2) and Cohen's *d*.[29]

We will examine hippocampal volume and resting-state functional connectivity outcomes as potential mediators of the effects of treadmill walking exercise training on learning and memory, consistent with the methodology of Baron and Kenny.[60] This statistical mediation approach is consistent with the proposed gold standard approach for examining exercise-related mechanisms of cognitive improvement at the brain-systems level in the general population.[16] As pre-conditions of the mediation analysis, we will first perform correlations among group (i.e., intervention or control), change in learning and memory performance, and change in hippocampal volume and resting-state functional connectivity, respectively, using Spearman's rho rank-order correlations (ρ)[61] to test if those outcomes are interrelated. Consistent with previous preliminary studies,[21,23] we expect that treadmill walking exercise training will be associated with improvements in learning and memory and increases in hippocampal volume and functional connectivity. Then, to establish mediation, we will perform hierarchical linear regression models for evaluating each of the mediators (i.e., changes in hippocampal volume and resting-state functional connectivity outcomes, respectively), separately. This will involve

regressing change in learning and memory performance on group in Step 1 and then adding change in the mediator in Step 2. As such, we expect significant effects of group on change in learning and memory, and that the effect of group (i.e., intervention or control) on change in learning and memory will be attenuated, but not reach zero, when accounting for the effects of changes in hippocampal structure and function. In other words, we expect that changes in hippocampal structure and function will be partial mediators of the effects of treadmill walking exercise training on learning and memory in persons with MS with impairments in learning new information. The effect sizes from the interaction terms from the ANOVAs and correlations will serve as effect sizes for the subsequent power analyses required for a subsequent Phase III RCT.

Patient and Public Involvement.

Patients and the public were not involved in the development of the research question, experimental design, selection of outcome measures, and conduct of the study. Once the data are collected and analyzed, we will send lay-language newsletters summarizing the results of the study to each study participant via postal mail. We further plan to disseminate the results of the study via publication in peer-reviewed journals, and will send the appropriate links to study participants once the results are published.

Ethics and Dissemination

Adverse Events.

This study has been approved by the UAB IRB, and we verify that all persons involved in the research hold current IRB certification for the protection of human subjects. Importantly, during any and all exercise activities for the current study, we will have a minimum of two researchers present who are trained in CPR, AED, First Aid, and emergency procedures. Further, we will monitor participants on a daily basis for adverse events such as musculoskeletal injuries related to exercise. In the event of an adverse event, this will be reported to the UAB Institutional Review Board within 48 hours, and the

participant will cease participation until receiving physician's clearance to resume any study-related activities.

Throughout the study, we will monitor and record MS relapses that may affect study participation. Importantly, we expect that the overall study relapse rate will be low (i.e., < 6% for both exercise and control conditions; 27% lower for exercise conditions), based on a recent systematic review of safety of exercise training in persons with MS.[62] We further will minimize the potential for relapses by only including persons with MS with relative neurologic stability who are on a stable disease-modifying therapy regimen. Nevertheless, all decisions (i.e., inclusion/exclusion, safety of continued participation) in the event of a relapse will be made on a patient-by-patient basis, with direct consultation with the study neurologist (JRR). We will report any relapses with the final study results. In accordance with the policies and procedures of the UAB IRB, if any harm occurs to participants as a result of the trial, treatment will be provided; however, this treatment will not be provided free of charge.

Confidentiality.

This study involves several approaches to maintain confidentiality. Participant information will be coded using a study code, and study forms will not contain any individually identifying information. The study code involves only an ID number that indicates the order whereby participants enrolled in the study. For example, the first participant to enroll in the study will be ID#001, and the second participant will be ID#002, etc. There will be no human-derived elements in this code (e.g., initials, dates, etc.). The code further will not pertain to any information on random assignment to groups. The master list linking study codes to individual identities will be maintained by the investigator on a password-protected, shared drive space on the UAB server and will not be divulged to others. Paper records will be stored in a locking file cabinet in the project coordinator's locked office in the ENRL. Electronic data will be stored on UAB computers which are firewall protected, encrypted and password-restricted. The servers are

monitored at all times for outages. Secured login IDs, granted on a need-to-know basis, further are required to access confidential information.

Discussion.

The current study is the first adequately-powered RCT to include an exercise training stimulus (i.e., treadmill walking exercise that progressively increases in duration and intensity) that is based on research on the acute[26,27] and chronic[21,23] effects of exercise on cognition in persons with MS that further targets hippocampal outcomes. Importantly, this study is the first RCT of exercise training to selectively recruit persons with objective impairments in learning new information in MS.[19] This is a critical methodological study component, as potential *treatment* effects (i.e., beyond simply improving cognitive performance) of exercise on MS-related cognitive dysfunction can only be assessed if participants demonstrate cognitive impairment. The current proposal will provide the first evidence for the efficacy of treadmill walking exercise training as a potential rehabilitative approach to *treat* MS-related learning and memory impairment. This efficacy study will include an active control condition to account for the effects of attention and social contact associated with supervised exercise training; no previous RCTs of exercise training on cognition in MS have adopted this approach.[19] We further will include cardiorespiratory fitness outcomes as a manipulation check for documenting the success of the intervention; this critical feature has been lacking in previous RCTs of exercise on cognition in MS.[19] The proposed study will be among the first exercise training RCTs in any population to include blinded MRI data analyses, uniquely adding another layer of rigor for improving the study's reproducibility. Such methodological features are critical for reducing threats to internal validity (i.e., Type I error) and providing efficacy evidence for chronic treadmill walking exercise training as a behavioral approach for managing learning and memory dysfunction in persons with MS who have the most need.

The current early-phase RCT will provide critical information for the development of future exercise trials on learning and memory in persons with MS. If successful, the current trial will provide effect sizes for power analyses for determining appropriate sample sizes for a subsequent Phase III RCT. Indeed, such a line of research will lay the foundation for approaches that can be eventually translated into community-based settings for rehabilitating learning and memory in persons with MS by examining the effects of a treadmill walking exercise training intervention on learning and memory and hippocampal neuroimaging outcomes. Although results from studies of cognitive rehabilitation are promising, such interventions are not easily adapted outside of the clinic, and further do not offer health benefits beyond improved cognition. On the other hand, exercise is a behavior that is easily adaptable in the community and offers a myriad of physical health benefits. As such, the proposed study uniquely represents the first step in developing and optimizing a potentially generalizable exercise training intervention for improving behavioral (i.e., learning and memory), and brain (i.e., hippocampal) outcomes among cognitively-impaired persons with MS. As a whole, this line of research might result in the development of exercise training guidelines that can be adapted by MS patients for specifically improving brain health and cognition. Importantly, there are no guidelines for persons with MS to manage cognitive impairment in the community using any approach.

If the current RCT does not result in statistically significant treadmill walking exercise-related improvements in composite learning and memory performance, we will focus on refining the exercise stimulus for specifically improving learning and memory amongst memory-impaired persons with MS for informing the development of a future RCT. This could involve adjusting the modality and intensity of exercise training. Indeed, there is preliminary evidence that other exercise modalities (e.g., aerobic cycle ergometry) and intensities (e.g., high-intensity interval training) have resulted in improvements in learning and memory in persons with MS.[63,64] If the current treadmill walking exercise training intervention does not improve learning and memory performance, another potential approach for

refining the exercise stimulus involves the inclusion of additional sensory stimuli to treadmill walking exercise (i.e., treadmill walking exercise plus virtual reality) for inducing cognitive change. This approach is based, in part, on animal work that demonstrates particularly large improvements in hippocampal neurogenesis, synaptogenesis, and learning and memory performance when exercise is performed in enriched environments compared with standard environments over several weeks.[18] There too is evidence in persons with traumatic brain injury whereby 4-weeks of exercise plus virtual reality improved verbal and visuospatial learning and memory.[65] Alternatively, if treadmill walking exercise training does not improve cardiorespiratory fitness outcomes (i.e., as a manipulation check), we plan to examine the effects of a longer intervention period (i.e., 6-months) on the primary and secondary study outcomes in a subsequent RCT, as has been done in older adults.[66] We do not expect this to be the case, given that the exact treadmill walking exercise training stimulus resulted in large cardiorespiratory fitness improvements in our small pilot RCT.[28]

Although the current RCT addresses a critical problem by applying a systematically-developed exercise training intervention for improving learning and memory and hippocampal structure and function among persons with MS who present with learning and memory impairment, there are several noteworthy limitations. First, the current RCT was powered based on pilot data on the effects of treadmill walking exercise training on the primary outcomes of behavioral neuropsychological tests of learning and memory in persons with MS. This study was not powered based on pilot data on the effects of treadmill walking exercise training on structural or functional hippocampal neuroimaging outcomes (i.e., the secondary study outcomes), given that pilot data are not available on the present neuroimaging endpoints in persons with MS. For the current power analyses, we operationalized the effect sizes from an aerobic exercise intervention on hippocampal neuroimaging outcomes in persons with schizophrenia as a preliminary guide for powering the secondary study outcomes.[30] Therefore, it is unknown if the study is truly adequately powered for detecting effects of treadmill walking exercise training on

hippocampal structure and functional connectivity (i.e., the secondary endpoints) in persons with MS who present with impairments in learning new information. The present RCT involves a relatively short intervention period, no long-term follow-up, or a comparison group of persons with MS without deficits in learning new information for possibly examining the neuroprotective effects of the intervention over time. Given that preliminary work directly supports the feasibility and preliminary efficacy of 3-months of treadmill walking exercise training on learning and memory and hippocampal neuroimaging outcomes, the current adequately-powered RCT seeks to rigorously examine the potential treatment effects of that exact intervention on learning and memory and its potential neural correlate(s). We believe this to be a necessary step prior to investigating the durability and sustainability of progressive treadmill walking exercise training on those outcomes in subsequent large-scale studies. We note that 3-months of aerobic exercise training has resulted in improvements in learning and memory as well as hippocampal structure and function in other populations [30,67]. Relatedly, the present study will not examine the effectiveness of treadmill walking exercise training on the primary outcomes in a large, national sample of persons with MS. Rather, this study will advance our systematic and rigorous line of research by providing critical efficacy data in a highly-controlled environment prior to the development of a subsequent RCT for examining the effectiveness of the intervention for eventual translation into a community-based program. The present study will not involve the collection of exploratory serum biomarkers (e.g., brain-derived neurotrophic factor, vascular endothelial growth factor) as possible molecular/cellular mediators of the effects of treadmill walking exercise training on behavioral (i.e., neuropsychological) and brain-systems (i.e., hippocampal volume and functional connectivity) outcomes. If the current RCT is successful, the examination of such potential mechanisms will be a central focus of future mechanistic research efforts. Another limitation is that the study sample will not involve persons with MS who present with substantial ambulatory disability. Rather, this RCT only focuses on persons with MS who are fully-ambulatory based on pilot data and safety concerns

associated with treadmill walking exercise training. Thus, the results of the current RCT might not be generalizable amongst all persons with MS, particularly those with severe ambulatory impairment. Finally, the present study will not directly compare the effects of treadmill walking exercise training with cognitive rehabilitation as a control comparison condition on learning and memory and hippocampal neuroimaging outcomes. Instead, this study includes an active, non-aerobic exercise training control condition in order to control for the potential effects of attention and social contact normally associated with supervised exercise training for testing the primary study hypotheses. Examinations of the comparative and combined effects of exercise training and cognitive rehabilitation on learning and memory and hippocampal neuroimaging outcomes will be performed in subsequent effectiveness trials.

Regardless of the study outcome, we plan to communicate the trial results via peer-reviewed publications. If successful, the results from this study will eventually inform RCTs for developing rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in a large sample of cognitively-impaired persons with MS. In the long term, the results from this early-phase RCT will lay the groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. Such an evidence-based approach for rehabilitation, using chronic exercise training, is paramount considering the highly prevalent, disabling, and poorly-managed nature of MS-related learning and memory impairment.

References:

1. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol* 2008;7:1139-51.

2. Guerts JJ, Bo L, Roosendaal SD, et al. Extensive hippocampal demyelination in multiple sclerosis. *J Neuropath Exp Neurol* 2007;66(9):819-27.

3. Benedict RH, Ramasamy D, Munschauer F, et al. Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. *J Neurol Neurosurg Psychiatr* 2009;80(2):201-6.

4. Hulst HE, Schoonehim MM, Van Geest Q, et al. Memory impairment in multiple sclerosis: relevance of hippocampal activation and hippocampal connectivity. *Mult Scler* 2015;21(13):1705-12.

5. Feinstein A, Magalhaes S, Richard J-F, et al. The link between multiple sclerosis and depression. *Nat Rev Neurol* 2014;10:507-17.

6. Morrow SA, Drake A, Zivadinov R, et al. Predicting loss of employment over three years in multiple sclerosis: clinically meaningful cognitive decline. *Clin Neuropsychol* 2010;24:1131-45.

7. Ruet A, Deloire M, Hamel D, et al. Cognitive impairment, health-related quality of life and vocational status at early stages of multiple sclerosis: a 7-year longitudinal study. *J Neurol* 2013;260:776-84.

8. Amato MP, Ponziani G, Siracusa G, et al. Cognitive dysfunction in early-onset multiple sclerosis: a reappraisal after 10 years. *Arch Neurol* 2001;58:1602-6.

9. Amato MP, Langdon D, Montalban X, et al. Treatment of cognitive impairment in multiple sclerosis: position paper. *J Neurol* 2013;260:1452-68.

10. Rosti-Otajarvi EM, Hamalainen PI. Neuropsychological rehabilitation for multiple sclerosis. *Cochrane Database Syst Rev* 2014;2:CD009131.

11. Chiaravalloti ND, Wylie G, Leavitt V, et al. Increased cerebral activation after behavioral treatment for memory deficits in MS. *J Neurol* 2012;259:1337-46.

12. Chiaravalloti ND, Moore NB, Nikelshpur OM, et al. An RCT to treat learning impairment in multiple sclerosis: the MEMREHAB trial. *Neurology* 2013;81:2066-72.

13. Goverover Y, Chiaravalloti ND, O'Brien A, et al. Evidence based cognitive rehabilitation for persons with multiple sclerosis: an updated review of the literature from 2007-2016. *Arch Phys Med Rehabil* 2018;99(2):390-407.

14. das Nair R, Martin KJ, Lincoln NB. Memory rehabilitation for people with multiple sclerosis. *Cochrane Database Syst Rev* 2016;3:CD008754.

15. Motl RW, Sandroff BM, Benedict RHB. Cognitive dysfunction and multiple sclerosis: developing a rationale for considering the efficacy of exercise training. *Mult Scler* 2011;17(9):1034-40.

16. Stillman CM, Cohen J, Lehman ME, et al. Mediators of physical activity on neurocognitive function: a review at multiple levels of analysis. *Front Hum Neurosci* 2016;10(626):1-17.

17. Vivar C, van Praag H. Running changes the brain: the long and short of it. *Physiology* 2017;32:410-24.

18. van Praag H. Neurogenesis and exercise: past and future directions. *Neuromol Med* 2008;10:128-40.

19. Sandroff BM, Motl RW, Scudder MR, et al. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. *Neuropsychol Rev* 2016;26(3):271-94.

20. Motl RW, Pilutti LA, Hubbard EA, et al. Cardiorespiratory fitness and its association with thalamic, hippocampal, and basal ganglia volumes in multiple sclerosis. *Neuroimage Clin* 2015;7:661-6.

21. Leavitt VM, Cirnigliaro C, Cohen A, et al. Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: preliminary findings. *Neurocase* 2014;20(6):695-7.

22. Sandroff BM. Exercise and cognition in multiple sclerosis: the importance of acute exercise for developing better interventions. *Neurosci Biobehav Rev* 2015;59:173-83.

23. Sandroff BM, Johnson CL, Motl RW. Exercise training effects on memory and hippocampal viscoelasticity in multiple sclerosis: a novel application of magnetic resonance elastography. *Neuroradiology* 2017;59(1):61-7.

24. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 Statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

25. American College of Sports Medicine. ACSM's resource manual for guidelines for exercise testing and prescription, seventh edition. Philadelphia, PA: Lippincott Williams & Wilkins 2013.

26. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of walking, cycling, and yoga exercise on cognition in persons with relapsing-remitting multiple sclerosis without impaired cognitive processing speed. *J Clin Exp Neuropsychol* 2015;37(2):209-19.

27. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of varying intensities of treadmill walking exercise on inhibitory control in persons with multiple sclerosis: a pilot investigation. *Physiol Behav* 2016;154:20-7.

28. Sandroff BM, Balto JM, Klaren RE, et al. Systematically-developed pilot randomized controlled trial of exercise and cognition in persons with multiple sclerosis. *Neurocase* 2016;22(5):443-50.

29. Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates 1988.

30. Pajonk FG, Wobrock T, Gruber O, et al. Hippocampal plasticity in response to exercise in schizophrenia. *Arch Gen Psychiatry* 2010;67(2):133-43.

31. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;50(1):121-7.

32. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-52.

33. Chiaravalloti ND, Balzano J, Moore NB, et al. The open-trial selective reminding test (OT-SRT) as a tool for the assessment of learning and memory. *Clin Neuropsychol* 2009;23:231-54.

34. Thomas S, Reading J, Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 1992;17(4):338–45.

35. Motl RW, Bollaert RE, Sandroff BM. Validation of the Godin Leisure-Time Exercise Questionnaire classification coding system using accelerometry in multiple sclerosis. *Rehabil Psychol* 2018;*in press*:doi: 10.1037/rep0000162 [Epub ahead of print].

36. Delis DC, Kramer JH, Kaplan E, et al. California Verbal Learning Test, Second Edition (CVLT-II). San Antonio, TX: Psychological Corporation 2000.

37. Buschke F, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 1974;24:1019-25.

38. Benedict RHB. The Brief Visuospatial Memory Test Revised (BVMT-R). Lutz, FL: Psychosocial Assessment Resources 1997.

39. Rao S. A manual for the brief repeatable battery of neuropsychological tests in multiple sclerosis. Milwaukee, WI: Medical College of Wisconsin 1990.

40. Strober L, Englert J, Munschauer F, et al. Sensitivity of conventional memory tests in multiple sclerosis: comparing the Rao Brief Repeatable Neuropsychological Battery and the Minimal Assessment of Cognitive Function in MS. *Mult Scler* 2009;15(9):1077-84.

41. Amato MP, Goretti B, Viterbo RG, et al. Computer-assisted rehabilitation of attention in patients with multiple sclerosis: results of a randomized, double-blind trial. *Mult Scler* 2014;20(1):91-8.

42. Mattioli F, Stampatori C, Bellomi F, et al. A RCT comparing specific intensive cognitive training to aspecific psychological intervention in RRMS: the SMICT study. *Front Neurol* 2015;5(278):1-8.

43. Boringa JB, Lazeron RH, Reuling IE, et al. The brief repeatable battery of neuropsychological tests: normative values allow application in multiple sclerosis clinical practice. *Mult Scler* 2001;7(4):263-7.

44. Parmenter BA, Testa SM, Schretlen DJ, et al. The utility of regression-based norms in interpreting the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *J Int Neuropsychol Soc* 2010;16(1):6-16.

45. van Geest Q, Hulst HE, Meijer KA, et al. The importance of hippocampal dynamic connectivity in explaining memory function in multiple sclerosis. *Brain Behav* 2018;8(5):e00954.

46. Smith DV, Utevsky AV, Bland AR, et al. Characterizing individual differences in functional connectivity using dual-regression and seed-based approaches. *Neuroimage* 2014;15:1-12.

47. Colcombe S, Kramer AF, Erickson KI, et al. Cardiovascular fitness, cortical plasticity and aging. *Proc Natl Acad Sci USA* 2004;101(9):3316-21.

48. Macko RF, Katzel LI, Yataco A, et al. Low-velocity graded treadmill stress testing in hemiparetic stroke patients. *Stroke* 1997;28(5):988-92.

49. Motl RW, Fernhall B. Accurate prediction of cardiorespiratory fitness using cycle ergometry in minimally disabled persons with relapsing-remitting multiple sclerosis. *Arch Phys Med Rehabil* 2012;93(3):490–5.

50. Benedict RHB, Fischer JS, Archibald CJ, et al. Minimal neuropsychological assessment of MS patients: a consensus approach. *Clin Neuropsychol* 2002;16:381-97.

51. Smith A. Symbol digit modalities test: Manual. Los Angeles, CA: Western Psychological Services 1982.

52. Gronwall DMA. Paced auditory serial addition task: a measure of recovery from concussion. *Percept Mot Skills* 1977;44:367-73.

53. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan executive function system: Technical manual. San Antonio, TX: Psychological Corporation 2001.

54. Benton AL, Sivan AB, Hamsher K, et al. Contributions to Neuropsychological Assessment, Second Edition. New York, NY: Oxford University Press 1994.

55. Patti F, De Stefano M, Lavorgna L, et al. Lesion load may predict long-term cognitive dysfunction in multiple sclerosis patients. *PLoS One* 2015;10(3):e0120754.

56. Borg G. Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics 1998.

57. National Multiple Sclerosis Society. Stretching for people with MS: an illustrated manual. New York, NY: National Multiple Sclerosis Society 2004.

58. Sandroff BM, Bollaert RE, Pilutti LA, et al. Multimodal exercise training in multiple sclerosis: a randomized controlled trial in persons with substantial mobility disability. *Contemp Clin Trials* 2017;61:39-47.

59. Bandura A. Health promotion by social cognitive means. *Health Educ Behav* 2004;31:143-64.

60. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986;51(6):1173-82.

61. Rousselet GA, Pernet CR. Improving standards in brain-behavior correlation analyses. *Front Hum Neurosci* 2012;6:119.

62. Pilutti LA, Platta ME, Motl RW, et al. The safety of exercise training in multiple sclerosis: a systematic review. *J Neurol Sci* 2014;343(1-2):3-7.

63. Briken S, Gold SM, Patra S, et al. Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial. *Mult Scler* 2014;20(3):382-90.

64. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: a randomized controlled trial. *Mult Scler* 2017;*in press*:doi: 10.1177/1352458517728342 [Epub ahead of print].

65. Grealy MA, Johnson DA, Rushton SK. Improving cognitive function after brain injury: the use of exercise and virtual reality. *Arch Phys Med Rehabil* 1999;80:661-7.

66. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci USA* 2011;108(7):3017-22.

67. Pereira AC, Huddleston DE, Brickman AM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA* 2007;104(13):5638-43.

Author Contributions

BMS: Study concept and design, study registration, study principal investigator, obtained IRB approval, drafting of the manuscript, critical revision of the manuscript

RWM, MMB, GRC, MB, JRR, GRW, HG, JDL: Study concept and design, critical revision of the manuscript

Funding Statement

This paper was supported by an investigator-initiated grant from EMD Serono, Inc (One Technology Place, Rockland, MA 02370). The principal investigator (BMS) will have access to all data from the study, complete freedom to direct the data analyses and reporting without influence of sponsors, and affirm that there is no editorial direction or censorship from sponsors.

Competing Interest Statement

All authors declare no conflicts of interest.

Table 1: Exercise prescription and progression over the 3-month period for treadmill walking exercise training condition based on pilot work and ACSM guidelines

Week	Sessions	Exercise Intensity	Exercise Duration	Training Stage
Baseline Testing				
1	1-3	40-50% VO_2R/HRR	15-20 min	Initiation
2	4-6	40-50% VO_2R/HRR	20-25 min	Initiation
3	7-9	50-60% VO_2R/HRR	20-25 min	Improvement
4	10-12	50-60% VO_2R/HRR	25-30 min	Improvement
5-6	13-18	60-70% VO_2R/HRR	25-30 min	Improvement
7-8	19-24	60-70% VO_2R/HRR	30-35 min	Improvement
9-10	25-30	70-80% VO_2R/HRR	30-35 min	Maintenance
11-12	31-36	70-80% VO_2R/HRR	35-40 min	Maintenance
Follow-up Testing				

Note: VO_2R=oxygen consumption reserve; HRR=heart rate reserve

Figure 1: Participant flow through the study.

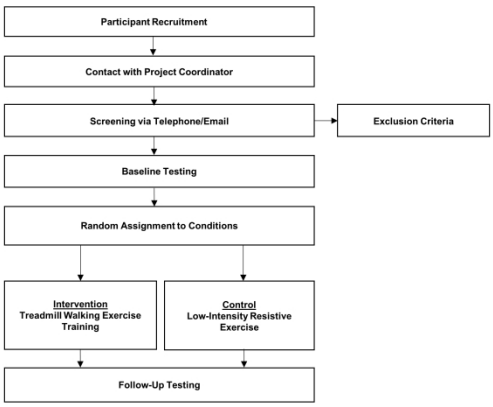


Figure 1

338x190mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page 1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page 2, 6)
	2b	All items from the World Health Organization Trial Registration Data Set (Page 6)
Protocol version	3	Date and version identifier (Page 6)
Funding	4	Sources and types of financial, material, and other support (Page 33)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page 1)
	5b	Name and contact information for the trial sponsor (Page 33)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities (Page 33)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) (Pages 19, 21-22)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (Pages 4-6)
	6b	Explanation for choice of comparators (Pages 4-6)
Objectives	7	Specific objectives or hypotheses (Pages 6-8)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Pages 6-8)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (Page 6)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (Pages 9-10)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (Pages 15-17)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (Pages 21-22)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (Page 17)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Page 17)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (Pages 10-14)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Pages 17-19;Figure 1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (Pages 8-9)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (Page 9)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (Pages 18-19)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (Page 18)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (Pages 17-19)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Pages 18-19)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (Pages 18-19)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (Pages 10-14)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (Pages 17,19-20)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (Page 19)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Pages 19-21)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Pages 19-21)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Pages 19-20)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Page 19)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Page 19)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Pages 21-22)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Pages 21-22)

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page 21)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (Page 6)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) (Pages 17-19)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable (Pages 17-19)
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (Page 22)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page 33)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Page 33)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation (Page 22)

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (Page 21)
	31b	Authorship eligibility guidelines and any intended use of professional writers (Page 33)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code (Pages 6, 21)
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (N/A)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (N/A)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023231.R2
Article Type:	Protocol
Date Submitted by the Author:	24-Oct-2018
Complete List of Authors:	Sandroff, Brian; University of Alabama at Birmingham, Physical Therapy Motl, Robert; University of Alabama at Birmingham, Department of Physical Therapy Bamman, Marcus; University of Alabama at Birmingham Cutter, Gary; University of Alabama at Birmingham, Department of Biostatistics Bolding, Mark; University of Alabama at Birmingham Rinker, John; University of Alabama at Birmingham Wylie, Glenn; Kessler Foundation Genova, Helen; Kessler Foundation DeLuca, John; Kessler Foundation
Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	Multiple sclerosis < NEUROLOGY, exercise, cognition, memory, MRI

Rationale and Design of a Single-Blind, Randomized Controlled Trial of Exercise Training for Managing Learning and Memory Impairment in Persons with Multiple Sclerosis

Brian M. Sandroff[1], Robert W. Motl[1], Marcus Bamman[2], Gary R. Cutter[3], Mark Bolding[4], John R. Rinker[5], Glenn R. Wylie[6], Helen Genova[6], John DeLuca[6]

[1] University of Alabama at Birmingham, Department of Physical Therapy, Birmingham, AL, USA
[2] University of Alabama at Birmingham, Departments of Cell, Developmental, & Integrative Biology; Medicine; and Neurology, Birmingham, AL, USA
[3] University of Alabama at Birmingham, Department of Biostatistics, Birmingham, AL, USA
[4] University of Alabama at Birmingham, Department of Radiology, Birmingham, AL, USA
[5] University of Alabama at Birmingham, Department of Neurology, Birmingham, AL, USA
[6] Kessler Foundation, Neuropsychology and Neuroscience Research, West Orange, NJ, USA

Correspondence to Brian M. Sandroff, PhD, University of Alabama at Birmingham, Department of Physical Therapy, SHP 389, 1720 2nd Ave S, Birmingham, AL, 35294, *phone* (205) 934-5972, *email* sandroff@uab.edu

Word Count: 7,641

Abstract

Introduction: This randomized controlled trial (RCT) examines treadmill walking exercise training effects on learning and memory performance, hippocampal volume, and hippocampal resting-state functional connectivity in persons with multiple sclerosis (MS) who have objective impairments in learning new information.

Methods and Analysis: Forty fully-ambulatory persons with MS who demonstrate objective learning and memory impairments will be randomly assigned into either the intervention or active control study conditions. The intervention condition involves supervised, progressive treadmill walking exercise training 3 times/week for a 3-month period. The active control condition involves supervised, progressive low-intensity resistive exercise that will be delivered at the same frequency as the intervention condition. The primary outcome will involve composite performance on neuropsychological learning and memory tests, and the secondary outcomes involve MRI measures of hippocampal volume and resting-state functional connectivity administered before and after the 3-month study period. Outcomes will be administered by treatment-blinded assessors using alternate test forms to minimize practice effects, and MRI data processing will be performed by blinded data analysts.

Ethics and Dissemination: This study has been approved by a University Institutional Review Board and further is registered at clinicaltrials.gov: NCT03319771. The primary results will be disseminated via peer-reviewed publications and the final data will be made available to third parties in applicable data repositories. If successful, the results from this study will eventually inform subsequent RCTs for developing physical rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in larger samples of cognitively-impaired persons with MS. The results from this early-phase RCT will further lay preliminary groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. This approach is paramount as learning and memory impairment is common, burdensome, and poorly-managed in MS.

Keywords: *multiple sclerosis; exercise; cognition; memory; MRI*

Strengths and Limitations of This Study

- The current randomized controlled trial applies a systematically-developed exercise training intervention for improving learning and memory and hippocampal structure and function among persons with MS who present with objective learning and memory impairment.
- This study is adequately powered and involves the inclusion of an active control comparison condition (i.e., low intensity resistive exercise) as well as blinded MRI data analysts.
- However, this efficacy study does include a relatively short intervention period (i.e., 3-months) and will not examine the sustainability and durability of the intervention effects on cognitive and hippocampal neuroimaging outcomes.
- This study was powered based on pilot data on exercise effects on behavioral measures of learning and memory in persons with MS (i.e., the primary study outcomes) and not on pilot data on the secondary outcomes of exercise effects on hippocampal volume and functional connectivity.
- This study involves fully-ambulatory persons with MS and will not involve persons with substantial ambulatory disability.

Introduction

The impairment of learning and memory, particularly with learning new information, is a common, burdensome, and poorly-managed manifestation of multiple sclerosis (MS).[1] Over 50% of MS patients demonstrate impaired performance on neuropsychological tests of verbal and/or visuospatial learning and memory[1] that is associated with hippocampal lesions, atrophy, and altered resting-state functional connectivity based on neuroimaging (i.e., MRI/fMRI) studies.[2-4] MS-related learning and memory impairment and decline have been associated with depression,[5] unemployment,[6,7] loss of independence, and social isolation.[8] Currently, there are no FDA-approved pharmacological treatments (i.e., disease-modifying and symptomatic therapies) for learning and memory dysfunction in MS.[9] Cognitive rehabilitation is currently the best-characterized behavioral approach for improving MS-related learning and memory impairment and is seemingly mediated through changes in brain function (i.e., increased hippocampal activation and resting-state functional connectivity;[10-14]). However, cognitive rehabilitation is difficult to apply outside the clinical setting and does not generally result in physical health benefits beyond improved cognition. This underscores the consideration of other behavioral approaches for managing learning and memory impairment and its potential association with hippocampally-mediated functional brain outcomes in MS that can be easily applied outside the clinical setting and result in many physical health benefits; one such approach includes exercise training.[15]

The consideration of exercise for improving learning and memory in persons with MS is based, in part, on the body of literature in the general population that documents robust, beneficial effects of exercise training on memory and hippocampal structure/function.[16] There further is a substantial body of animal work that describes upregulation of central neuro- and vascular trophic factors with several weeks of exercise that accompanies such neuropsychological and brain-systems changes.[17,18] By comparison, there have been few well-designed, targeted exercise training RCTs on learning and

memory and hippocampal neuroimaging outcomes in MS, although the data are promising.[19] For example, one recent cross-sectional study described statistically significant, moderate-sized correlations between cardiorespiratory fitness (i.e., a presumed surrogate of aerobic exercise training) and hippocampal volume in 35 persons with MS.[20] Another noteworthy case study involving two memory-impaired persons with MS reported that 3-months of aerobic exercise training (stationary cycling) resulted in a > 50% increase in learning and memory performance, 16.5% increase in hippocampal volume, as well as increased hippocampal resting-state functional connectivity.[21] By comparison, the non-aerobic exercise condition demonstrated minimal changes in those outcomes.[21] One systematically-developed[22] pilot RCT examined the effects of aerobic treadmill walking exercise training compared with a waitlist control condition on learning and memory performance and hippocampal viscoelasticity using non-conventional MRI (i.e., magnetic resonance elastography) in 8 fully-ambulatory persons with MS.[23] Overall, there were small-to-moderate intervention effects on verbal learning and memory performance (d=0.34), and large intervention effects on hippocampal viscoelastic properties (i.e., increased hippocampal shear stiffness) (d>0.94). The change in verbal learning and memory was strongly associated with change in hippocampal viscoelasticity (r>.93, p<.01). Collectively, despite experimental design[20] and sample size[21,23] limitations, such preliminary observations suggest that aerobic exercise training might improve learning and memory through neuroplasticity in MS and warrant the development of an adequately-powered, early-phase RCT for examining the effects of aerobic exercise training on learning and memory, and hippocampal structure/function in a larger sample of persons with MS.

The present RCT aims to provide the first evidence for treadmill walking exercise training effects on learning and memory, hippocampal volume, and hippocampal resting-state functional connectivity in an adequately-powered sample of persons with MS who have objective impairment in learning new information. This will provide the first evidence for exercise training as a possible *treatment* for MS-

related learning and memory impairment (i.e., beyond merely improving learning and memory performance), given that previous trials of exercise, cognition, and neuroimaging have not recruited persons with objective MS-related learning and memory impairment *a priori.*[e.g., 23] The current RCT will further provide the first high-quality evidence of potential mechanisms of aerobic exercise-related effects on hippocampal outcomes in MS, whereas previous studies have been limited by experimental design[e.g., 20] or sample size.[e.g., 21,23] This study will lay the foundation for a definitive Phase III RCT by providing effect sizes of treadmill walking exercise training versus an active control condition on the outcomes that can be used in power analyses for determining the appropriate sample size for such a trial. The present study will provide preliminary experimental evidence of the potential mechanisms (i.e., improved hippocampal structure and function) of how treadmill walking exercise might improve learning and memory in this population.

Methods and Analysis

Experimental Overview and Hypotheses.

The study protocol was drafted in accordance with the SPIRIT statement[24] and further has been approved by the University of Alabama at Birmingham (UAB) Institutional Review Board (IRB). This study is registered at clinicaltrials.gov: NCT03319771. All potential protocol modifications will be approved by the UAB IRB and will be reported at clinicaltrials.gov. The proposed study, data collection, and intervention will take place at UAB in Birmingham, AL, USA. This study involves a single-blind, early Phase II RCT on the effects of supervised treadmill walking exercise training compared with an active control condition (i.e., low intensity resistive exercise) on learning and memory, hippocampal structure/function, and cardiorespiratory fitness outcomes in 40 fully-ambulatory (i.e., Expanded Disability Status Scale (EDSS) ≤ 4.0) persons with MS who have impairment in learning new information. Composite performance on neuropsychological tests of learning and memory represents the primary

outcome, whereas neuroimaging outcomes of hippocampal volume and hippocampal resting-state functional connectivity represent the secondary outcomes. Cardiorespiratory fitness changes will be included as a manipulation check for documenting the success of the intervention.

During baseline, participants will first complete a battery of neuropsychological tests addressing verbal and visuospatial learning and memory; a maximal, graded exercise test on a motor-driven treadmill to measure cardiorespiratory fitness; and an MRI scan for measurement of hippocampal volume and resting-state functional connectivity. Those outcomes will be measured by assessors who are uninvolved in the exercise training (i.e., treatment blinded assessors). After baseline testing, participants will be randomly assigned to either the intervention or active control conditions using concealment. Participants further will be masked to condition (i.e., unaware that the treadmill walking exercise training condition represents the intervention condition and the low intensity resistive exercise condition represents the active control condition).

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise, and will be based on American College of Sports Medicine (ACSM) guidelines for exercise prescription[25] and pilot work.[23,26-28] The exercise training itself will take place 3 times per week over 3 months, and will be facilitated by trained exercise leaders. The exercise prescription will initially consist of 15 minutes of light-to-moderate intensity treadmill walking exercise and eventually progress to 40 minutes of vigorous intensity exercise by month 3 (Table 1). We note that this exercise stimulus is identical to that of our pilot RCT in 8 persons with MS.[23] The active control condition will involve low intensity resistive exercise that will be delivered using the same frequency and duration of the treadmill walking exercise condition and facilitated by trained exercise leaders. This is a methodological improvement over our pilot RCT that involved a waitlist (i.e., passive) control condition.[23] Regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising) over the duration of the study.

The cognitive, cardiorespiratory fitness, and MRI outcomes will be assessed again following the 3-month study period by treatment blinded assessors.

The primary hypothesis is that those who undergo treadmill walking exercise training will demonstrate larger improvements in composite learning and memory performance than those who undergo low intensity resistive exercise. We further hypothesize that those who undergo treadmill walking exercise training condition will demonstrate greater increases in hippocampal volume and hippocampal resting-state functional connectivity (i.e., adaptive increases) and improvements in cardiorespiratory fitness than those in the low intensity resistive exercise condition. We lastly hypothesize that (1) the treadmill walking exercise-induced improvements in learning and memory will be accounted for by hippocampal volume and hippocampal resting-state functional connectivity (i.e., partial mediation) and (2) those with the largest improvements in cardiorespiratory fitness will demonstrate the largest improvements in the primary and secondary end-points.

Participants.

Sample Size. We plan to enroll 40 fully-ambulatory persons with MS (i.e., 20 per condition) who have impairment in learning new information (see below); this is based on a power analysis and presumed 15% attrition. The minimal sample size of 34 persons with MS (i.e., 17 per condition) was determined based on power analysis using standard assumptions of alpha (0.05) and beta (0.80) for detecting moderate-sized effects (i.e., η_p^2=0.06) based on Cohen's guidelines[29] for a time by condition interaction in mixed-factor ANOVA on composite learning and memory performance. Our previous pilot RCT of treadmill walking exercise training on cognition yielded a large time by condition interaction on composite learning and memory performance (i.e., η_p^2=0.11) in fully-ambulatory persons with MS.[23] Another study on aerobic exercise effects on hippocampal outcomes in persons with schizophrenia reported large intervention effects on hippocampal volume (i.e., η_p^2=0.50).[30] Using those effect sizes as a guide, the proposed sample size of 34 and planned sample size of 40 will be more than adequate for

detecting moderate or larger effects on learning and memory and hippocampal outcomes in persons with MS. The required sample size of 34 also adequately powers the secondary study hypotheses based on previous cross-sectional data in MS that indicates moderate-sized correlations (i.e., ρ = .42) between cardiorespiratory fitness and hippocampal volume.[20]

Recruitment. Subjects will be recruited directly through the UAB MS Center, the Alabama-Mississippi Chapter of the National MS Society (NMSS), and our laboratory database of previous participants with MS who have inquired about participating in exercise studies. Advertisements for the study will be distributed through the UAB MS Center, facilitators of local MS support groups, *MS Connection* publications, and e-mail distributions. As a backup plan, we may recruit prospective participants with MS through the North American Research Committee on Multiple Sclerosis (NARCOMS) patient registry or iConquerMS if enrollment is slow.

Inclusion/Exclusion Criteria. All participants will be between the ages of 18-54, have a clinically definite MS diagnosis based on established criteria,[i.e., 31] and be fully-ambulatory based on EDSS[32] scores between 0-4.0. All participants will demonstrate impairment in learning new information based on open-trial Selective Reminding Task (SRT) scores at least 1.5 *SD's* below the normative score for healthy controls.[33] Participants will be relapse-free for at least 30 days (i.e., relative neurologic stability), and will not have a history of schizophrenia, bipolar disorder I or II, or substance-abuse disorders. Participants further will not be taking medications that can affect cognition (e.g., antipsychotics, benzodiazepines), and all participants will be on a stable FDA-approved disease-modifying therapy (e.g., interferon beta-1a; interferon beta-1b; glatiramer acetate; natalizumab; dimethyl fumarate, etc.) regimen for at least 6 months. Participants will be right-handed (to control for organization of the brain) and will have a low risk for contraindications for maximal exercise testing based on a "no" response on all items of the Physical Activity Readiness Questionnaire (PAR-Q;[34]) or a single "yes" response along with a physician's approval. Participants further will have a low risk for

contraindications for MRI based on: (a) not having metal (e.g., non-MRI compatible aneurysm clips, metal shards in the body or eyes, or recently placed surgical hardware) or electronic devices (e.g., pacemaker, cochlear implant) within the body. Lastly, participants will not be meeting public health guidelines for participating in physical activity (i.e., at least 150 minutes per week of moderate-to-vigorous aerobic activity). This will be based on health contribution scores on the Godin Leisure-Time Exercise Questionnaire (GLTEQ) that fall within the 'insufficiently active' classification (i.e., less than 14 arbitrary units on the summed 'strenuous' and 'moderate' sections of the GLTEQ).[35]

Outcome Measures.

To minimize threats to internal validity and maximize the rigor and reproducibility of the present RCT, the outcome measures will be assessed by personnel who are uninvolved with the intervention or control conditions (i.e., treatment blinded assessors). The outcome assessments further will occur at a different UAB laboratory than the intervention or control conditions to prevent possible contamination.

Learning and Memory: Participants will undertake several neuropsychological tests addressing various aspects of learning and memory as the primary study outcomes. Neuropsychological testing will occur in a quiet, sound-dampened room in the Exercise Neuroscience Research Laboratory (ENRL) at UAB. These tests include the California Verbal Learning Test-II[CVLT-II;36] and SRT[37] as measures of verbal learning and memory, and the Brief Visuospatial Memory Test-Revised[BVMT-R;38] and 10/36 Spatial Recall Test[SPART;39] as measures of visuospatial learning and memory. These neuropsychological tests have strong psychometric properties,[e.g., 40] and are widely-used to document the efficacy of cognitive rehabilitation interventions in persons with MS.[12,41,42]

Briefly, the CVLT-II involves the examiner reading a list of 16 words, with four items belonging to four categories (e.g., vegetables, animals, furniture, modes of transportation) that are randomly arranged. The list is read aloud five times in the same order, with each word voiced at a rate of approximately one word per second. Participants are instructed to recall as many items as possible, in

any order, following each list reading. The primary outcome of the CVLT-II is the total number of correct words identified over the five trials (i.e., raw score).[36] The SRT involves the examiner reading a list of 12 unrelated words. Participants are asked to recall as many words as possible following the presentation of the list. After the first trial, only the words that participants did not recall are given as the new list. However, participants are instructed to recall as many words as possible from the original (i.e., entire) list for each of 5 total trials. The primary outcome is the total number of correctly recalled words across the 5 trials.[37] For both the CVLT-II and SRT, higher scores indicate better verbal learning and memory.

The BVMT-R involves three trials of the examiner presenting a 2×3 array of abstract geometric figures in front of the participant for 10 seconds. Following this period, the array is removed and participants are required to draw the array as precisely as possible, with the figures in the correct location. Each drawing is scored on a 0-2 scale, based on accurately portraying each figure and its correct location. The primary outcome of the BVMT-R is the total raw score across the three trials, with higher scores indicating better visuospatial memory.[38] The 10/36 SPART involves three trials of the examiner presenting a 6×6 checkerboard with 10 pieces positioned in certain locations on the board in front of the participant for 10 seconds. Following this period, the display is removed, and participants are asked to replicate the design of the checkerboard on a blank grid. This is repeated for 2 additional trials. The primary outcome of the 10/36 SPART is the total number of correct responses across the three trials.[39]

As the primary study outcome involves composite learning and memory performance, we will first compute *z*-scores that account for age, sex, and education based on normative scores per individual learning and memory test (i.e., CVLT-II, SRT, BVMT-R, 10/36 SPART).[43,44] We will then combine the *z*-scores into a composite learning and memory measure (i.e., mean of *z*-scores for CVLT-II, SRT, BVMT-R, and 10/36 SPART). Given evidence of hippocampal lateralization of verbal and visuospatial learning and

memory in persons with MS,[45] we further will examine the effects of the intervention on those constructs separately in exploratory analyses.

Hippocampal Volume and Resting State Functional Connectivity: Participants will undergo neuroimaging, which will include structural imaging as well as a resting-state scan. The MR instrument that will be used is an FDA-approved Siemens MAGNETOM Skyra 3T clinical imager housed in the Civitan International Neuroimaging Research Center at UAB. Each scan session will begin with the acquisition of high-resolution T1-weighted axial anatomical images (MP-RAGE). This 3D isotropic sequence will be acquired sagittally (TR = 11.6 ms, TE = 4.9 ms, flip angle = 8°, effective TI = 1017.6 ms, 256 x 256 matrix, FOV = 300 mm, NEX = 1, 172 slices, 1.17 mm slice thickness, 0 mm skip). Total imaging time for this sequence is 8 min 38 sec. In addition, an inversion-recovery sequence will be acquired axially (TR = 8530 ms, TE = 81 ms, flip angle = 180°, 256 x 256 matrix, FOV = 220 mm, NEX = 1, 32 slices, 4 mm slice thickness, 0 mm skip). Together, these scans will be used for volumetric analyses and for image segmentation and normalization of the resting-state fMRI scan. Functional imaging will consist of multi-slice gradient echo, T2*-weighted images acquired with echoplanar imaging (EPI) methods (TE= 60 ms; TR= 2000 ms; FOV = 24 cm; flip angle = 90°; slice thickness = 5 mm contiguous, matrix = 64×64, in-plane resolution = 2.50 mm^2). In order to provide coverage of the entire brain, a total of 32 images will be acquired in the axial plane. For the resting-state scan, 180 volumes will be acquired. Structural volumes for the hippocampus will be calculated using Freesurfer automated brain segmentation software (http://surfer.nmr.mgh.harvard.edu). Preprocessing of the resting-state functional connectivity data will be performed using AFNI software (http://afni.nimh.nih.gov/afni/). The first three volumes will be removed in order to control for saturation effects. Preprocessing steps include slice timing correction, realignment to an image exactly half-way through the acquisition run using affine transformation, co-registration to the T1 MPRAGE image for localization of activated areas, smoothing (6 mm FWHM) to minimize anatomical differences and increase the signal to noise ratio, scaling each voxel to the grand

mean intensity of that voxel (across the acquisition run), high-pass filtering (150 seconds), and normalization using a nonlinear approach (3dQwarp) to a standardized T1 template from the Montreal Neurological Institute (MNI). In all cases, the data will be checked for excessive motion (a shift of more than 3.5 mm, or 1° of angular motion) and for spikes (using the Root Mean Squared Error [RMSE] of each volume relative to a reference volume [which will be the volume half-way through the acquisition run]). Data acquisition runs with excessive motion will be discarded. Individual acquisitions with a RMSE amplitude that exceeds the 75th percentile plus the value of 150% of the interquartile range of RMSE for all volumes in a given run will be excluded from further analysis using the 'censorTR' function in 3dDeconvolve. In all cases, the motion parameters from the realignment step will be used as regressors of no interest in the deconvolution, and the residuals will be saved. The residuals for each subject in each group (exercise and control) will then be included in a probabilistic ICA, using AROMA (ICA-based Automatic Removal Of Motion Artifacts), as implemented in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The results of the ICA will be spatial maps of the resting-state networks. We will extract the connectivity map of the hippocampal network that will then be used in a dual-regression analysis.[46] In the first regression (spatial regression), the spatial hippocampal map from the ICA is regressed onto each subject's functional data, resulting in a dataset that characterizes the temporal dynamics of the hippocampal network. In the second regression (temporal regression), the dataset resulting from the spatial regression (the temporal dynamics of the hippocampal network) are regressed onto each subject's functional data. This results in a map for the hippocampal network representing each voxel's connectivity with this network. This map will then be used in the group-level analyses. Importantly, all MRI processing and analyses will be performed by scientists at Kessler Foundation (i.e., HG, GRW), who will be blinded to condition. To our knowledge, this is among the first exercise RCTs in any population to include this additional level of rigor to enhance the proposed trial's reproducibility.

Cardiorespiratory Fitness: Cardiorespiratory fitness will be measured as peak oxygen consumption (VO_{2peak}), using a maximal, graded exercise test on a motor-driven treadmill and an open-circuit spirometry system (ParvoMedics True One 2400, Sandy, UT) for analyzing expired gases using a modified Balke protocol in the ENRL at UAB. This protocol was successfully used in our previous small pilot RCT.[23] This protocol further is commonly used for measuring cardiorespiratory fitness in older adults[47] and persons with chronic stroke[48] and is consistent with the ACSM guidelines for exercise testing of MS patients.[25] The test will be preceded by a 3-minute warm up. The initial work rate for the exercise test will be at a brisk, but submaximal pace, and the grade will continuously increase at a rate of 2.0% every 2-minutes until the participant reaches volitional fatigue. Heart rate (HR) and rating of perceived exertion will be recorded every minute during the test. VO_{2peak} will be expressed in $ml \cdot kg^{-1} \cdot min^{-1}$ based on highest recorded 20-second VO_2 value when two of four criteria are satisfied: (1) VO_2 plateau with increasing grade; (2) respiratory exchange ratio ≥1.10; (3) peak heart rate within 10 $beats \cdot minute^{-1}$ of age-predicted maximum (i.e., ~1 SD); or (4) peak rating of perceived exertion ≥17.[49] The test will be followed by a 3-minute cool-down period.

Disability Status: Participants will undergo a neurological examination by a neurologist (JRR) for generating a baseline EDSS score. All participants will be fully-ambulatory (i.e., EDSS scores ≤ 4.0).

Additional Neuropsychological Tests: To evaluate the effects of the intervention on other domains of cognition that are commonly-impaired in MS, we will apply the MACFIMS neuropsychological battery[50] as exploratory outcomes. In addition to the CVLT-II and BVMT-R, the MACFIMS includes valid and reliable tests of cognitive processing speed,[51,52] executive function,[53] verbal fluency,[54] and spatial perception.[54] Further, baseline performance on the MACFIMS neuropsychological battery will serve to characterize the overall baseline cognitive status of the sample. For all neuropsychological tests, including measures of learning and memory, alternate forms will be applied at each testing session (i.e., baseline and follow-up) to minimize the effects of practice on cognition.

Additional Neuroimaging Outcomes: The MRI protocol further will involve the collection of structural MRI data on T2-lesion volume as a potential covariate of intervention effects, given its association with MS-related cognitive dysfunction.[55]

Intervention Condition.

The intervention condition will include 3-months of supervised, progressive light, moderate, and vigorous intensity treadmill walking exercise training based on ACSM guidelines for maximizing adaptations with exercise training. This will occur at a laboratory exercise research facility. Exercise intensities will be prescribed based on percent oxygen consumption reserve (% VO_2R) using values derived from the baseline graded exercise test. We note that HR reserve (HRR) and VO_2R have a 1:1 relationship.[25] The exact exercise prescription is presented in Table 1 and further represents the identical stimulus that was included in our pilot RCT that demonstrated improvements in learning and memory and hippocampal neuroimaging outcomes.[23] The exercise progression in terms of duration and intensity will involve 3 distinct stages: (a) the initiation stage; (b) the improvement stage; and (c) the maintenance stage. The initiation stage (Weeks 1-2) aims to prepare participants for more intense aerobic exercise (i.e., by accumulating small improvements in cardiorespiratory fitness with light-to-moderate intensity exercise) and develop an orthopedic tolerance for exercise stress.[25] Following this period, participants will progress to the improvement stage of exercise training. This stage provides a gradual increase in the overall aerobic exercise stimulus (i.e., moderate-to-vigorous intensity), whereby participants realize substantial improvements in cardiorespiratory fitness (Weeks 3-8).[25] The final stage of exercise progression is the maintenance stage (i.e., vigorous intensity), which aims to maintain the levels of cardiorespiratory fitness that were developed during the improvement stage over the long-term (Weeks 9-12).[25] Consistent with ACSM recommendations, the intervention will not involve progression of both intensity and duration in a single exercise session. Such a gradual progression of

exercise training is advantageous for deconditioned persons to safely achieve the benefits of aerobic exercise training.[25]

The exercise training itself will be led by certified exercise leaders who are not involved in the collection of outcome assessments. At the outset of each session, participants will be fitted with a Polar HR Monitor (Oy, Finland), and HR will be monitored continuously throughout each session. Each session will begin with a 5-10 min warm-up, followed by the exercise; the target HRR range associated with the VO_2R range will be maintained for as long as possible during each exercise period. This will be followed by a 5-10 min cool-down. Participants will complete an exercise log at the conclusion of each session for better characterizing the experience with the intervention. Log data will include perceived exertion,[56] well-being, enjoyment, and mental/physical fatigue. Throughout each session, we further will collect data on treadmill speed and grade, as well as time spent within the prescribed VO_2R/HRR range for improving the rigor and reproducibility of the intervention.

Active Control Condition.

The active control condition will involve supervised, low intensity resistive exercise in order to control for the effects of social contact and attention. This condition will take place at a laboratory exercise research facility in a space that is isolated from the intervention condition in order to avoid participant and site contamination. The low intensity resistive exercise control condition will be delivered using the same frequency and duration of the treadmill walking exercise training condition. The low intensity resistive exercises will be based on a manual provided by the NMSS[57] and sessions will be led by certified exercise leaders who are not involved in the collection of outcome assessments. Low intensity resistive exercises will target the head/neck, shoulder, elbow/forearm, hand/wrist, trunk/hip, ankle/foot. The progression of activities over the 3-month period will involve performing additional exercises and sets along with using progressively thicker elastic resistance bands (i.e., Therabands) that provide minimal resistance. The first 6 weeks of the intervention period will involve

performing the activities without resistance. In weeks 7-8, the extra thin Theraband (i.e., least resistance) will be used to perform the exercises for the upper-extremities only. In weeks 9-10, the thin Theraband will be introduced and in weeks 11-12, the medium Theraband will be introduced. Such a progression is not expected to induce cardiorespiratory fitness adaptations and is designed to maintain participant interest. Each session is designed to last up to 60 minutes in total. Each session will begin with a 5-10 min warm-up, followed by low intensity resistive exercise (following the same duration as the treadmill walking exercise training condition) activities, and a 5-10 min cool-down. Throughout each session, participants will wear a HR monitor, and we will collect data on HR and perceived exertion[56] to ensure that this condition occurs at a low intensity. This stimulus has been included as a control comparison condition in a recent exercise training RCT in persons with MS and did not result in cognitive improvements and was well-received with no increase in drop-out compared with progressive exercise training.[58]

Importantly, to minimize attrition for both the intervention and control conditions, exercise leaders will apply highly-developed principles and techniques associated with Social Cognitive Theory[59] for enhancing participant adherence and compliance throughout each session. Further, regardless of the assigned condition, all participants will be asked to not undertake additional exercise (i.e., not join a gym and begin exercising).

Procedure.

Participant flow through the study is presented in Figure 1. Participant recruitment, contact, and screening will be undertaken via telephone and/or email by an ENRL project coordinator. If a participant satisfies initial inclusion/exclusion criteria, the project coordinator will then administer the open-trial SRT via telephone to ensure that all participants have impairments in learning new information; the open-trial SRT has been used as a screening tool for impairments in learning new information in previous memory rehabilitation RCTs in MS.[12] This test involves the project coordinator reading a list

of words to the prospective participant over the phone. The participant then repeats as many words as they can from memory back to the project coordinator. This process is repeated until participants can remember the entire list of words. If participants demonstrate scores that are at least 1.5 *SD*'s below the normative score for healthy controls, the project coordinator will request contact information from the potential participant's neurologist, whereby they will email or fax a letter asking them to verify a definite MS diagnosis and confirmation that the participant has been on a stable DMT regimen for at least 6-months. Upon receipt of these materials from the participant's neurologist, the project coordinator will schedule the participant for baseline testing. Baseline testing will be led by assessors who are blinded to condition, and will take place over 3 non-consecutive days to minimize cognitive and physical fatigue. On the first day, participants will initially provide written informed consent (see Supplementary File 1 for sample informed consent document) with IRB-approved ENRL personnel, followed by a neurological examination for generation of an EDSS score. Participants will then undertake several neuropsychological tests of learning and memory (i.e., CVLT-II, BVMT-R) as part of the full MACFIMS neuropsychological battery, followed by the graded exercise test. The second day of baseline testing will involve the MRI protocol, and the third day of baseline testing will involve undertaking other neuropsychological tests of learning and memory (i.e., SRT, 10/36 SPART). Participants will be remunerated $50 for completing the baseline assessments. Of note, the separation of learning and memory measures across testing sessions is necessary considering that there might be overlap (interference) between performance on the CVLT-II and SRT (i.e., learning and memory tests in the verbal domain) and between the BVMT-R and 10/36 SPART (i.e., learning and memory tests in the visuospatial domain), respectively.

After baseline testing, participants will be randomly assigned to either the treadmill walking exercise training or active control conditions using concealment (i.e., opaque, sealed envelopes) and computerization by the study biostatistician (i.e., GRC). Participants further will blinded to the intent of

the condition (i.e., unaware that the treadmill walking exercise training condition represents the experimental condition and the low intensity resistive exercise condition represents the active control condition). To do this, the study will be advertised as a comparison of two different physical exercise programs on memory performance in persons with MS. We note that it is not possible for participants to be blinded to the actual condition (i.e., participating in treadmill walking exercise or low-intensity resistive exercise activities).

Participants will undertake the intervention or active control conditions as described above over a 3-month period. Participants will be remunerated $10 per treadmill walking exercise/low intensity resistive exercise visit attended (i.e., up to $360 total). This remuneration will be disbursed in weekly increments for maximizing compliance with this early stage research. Following the completion of the 3-month study period, participants will again undergo assessments of learning and memory, hippocampal volume and resting-state functional connectivity, and cardiorespiratory fitness using the same procedures as baseline testing (i.e., follow-up testing). Follow-up measures, using alternate forms where possible, will be administered by treatment blinded assessors. Participants will be remunerated $50 for completing the follow-up assessments.

Data Integrity.

All data will be entered, checked, and double-checked by UAB ENRL personnel under the direct supervision of the ENRL project coordinator and study principal investigator (BMS). All ENRL personnel have undergone extensive training in good clinical practice and laboratory procedures. Given that the current study is not a multi-site, NIH-defined Phase III RCT, we do not include a formal data monitoring committee.

Statistical Analysis.

The data analyses will be overseen by a biostatistician (i.e., GRC) and follow intent-to-treat principles (i.e., include all persons regardless of adherence and/or compliance). In the case of a drop-

out, missing data will be imputed using multiple imputation and by carrying the last observed value forward. We further will perform exploratory data analyses only in those who completed follow-up testing (i.e., completer's or per protocol analysis) and in those who demonstrated good adherence (i.e., attended at least 83% of sessions) and compliance.[19] The data will be analyzed using a mixed-factor model with time (baseline and follow-up) as a within-subjects factor and condition (intervention or active control) as a between-subjects factor on composite learning and memory performance, hippocampal, and cardiorespiratory fitness outcomes. Of note, we will require an MRI-overall alpha of .05 (corrected for multiple comparisons) for significance for hippocampal neuroimaging outcomes. The correction for multiple comparisons will be achieved by establishing a suitable voxel cluster-level threshold through Monte Carlo simulations (using the 3dClustSim program, part of the AFNI suite of image analysis programs; http://afni.nimh.nih.gov/afni/). Effect sizes will be expressed as partial eta-squared (η_p^2) and Cohen's *d.*[29]

We will examine hippocampal volume and resting-state functional connectivity outcomes as potential mediators of the effects of treadmill walking exercise training on learning and memory, consistent with the methodology of Baron and Kenny.[60] This statistical mediation approach is consistent with the proposed gold standard approach for examining exercise-related mechanisms of cognitive improvement at the brain-systems level in the general population.[16] As pre-conditions of the mediation analysis, we will first perform correlations among group (i.e., intervention or control), change in learning and memory performance, and change in hippocampal volume and resting-state functional connectivity, respectively, using Spearman's rho rank-order correlations (ρ)[61] to test if those outcomes are interrelated. Consistent with previous preliminary studies,[21,23] we expect that treadmill walking exercise training will be associated with improvements in learning and memory and increases in hippocampal volume and functional connectivity. Then, to establish mediation, we will perform hierarchical linear regression models for evaluating each of the mediators (i.e., changes in hippocampal

volume and resting-state functional connectivity outcomes, respectively), separately. This will involve regressing change in learning and memory performance on group in Step 1 and then adding change in the mediator in Step 2. As such, we expect significant effects of group on change in learning and memory, and that the effect of group (i.e., intervention or control) on change in learning and memory will be attenuated, but not reach zero, when accounting for the effects of changes in hippocampal structure and function. In other words, we expect that changes in hippocampal structure and function will be partial mediators of the effects of treadmill walking exercise training on learning and memory in persons with MS with impairments in learning new information. The effect sizes from the interaction terms from the ANOVAs and correlations will serve as effect sizes for the subsequent power analyses required for a subsequent Phase III RCT.

Patient and Public Involvement.

Patients and the public were not involved in the development of the research question, experimental design, selection of outcome measures, and conduct of the study. Once the data are collected and analyzed, we will send lay-language newsletters summarizing the results of the study to each study participant via postal mail. We further plan to disseminate the results of the study via publication in peer-reviewed journals, and will send the appropriate links to study participants once the results are published.

Ethics and Dissemination

Adverse Events.

This study has been approved by the UAB IRB, and we verify that all persons involved in the research hold current IRB certification for the protection of human subjects. Importantly, during any and all exercise activities for the current study, we will have a minimum of two researchers present who are trained in CPR, AED, First Aid, and emergency procedures. Further, we will monitor participants on a daily basis for adverse events such as musculoskeletal injuries related to exercise. In the event of an

adverse event, this will be reported to the UAB Institutional Review Board within 48 hours, and the participant will cease participation until receiving physician's clearance to resume any study-related activities.

Throughout the study, we will monitor and record MS relapses that may affect study participation. Importantly, we expect that the overall study relapse rate will be low (i.e., < 6% for both exercise and control conditions; 27% lower for exercise conditions), based on a recent systematic review of safety of exercise training in persons with MS.[62] We further will minimize the potential for relapses by only including persons with MS with relative neurologic stability who are on a stable disease-modifying therapy regimen. Nevertheless, all decisions (i.e., inclusion/exclusion, safety of continued participation) in the event of a relapse will be made on a patient-by-patient basis, with direct consultation with the study neurologist (JRR). We will report any relapses with the final study results. In accordance with the policies and procedures of the UAB IRB, if any harm occurs to participants as a result of the trial, treatment will be provided; however, this treatment will not be provided free of charge.

Confidentiality.

This study involves several approaches to maintain confidentiality. Participant information will be coded using a study code, and study forms will not contain any individually identifying information. The study code involves only an ID number that indicates the order whereby participants enrolled in the study. For example, the first participant to enroll in the study will be ID#001, and the second participant will be ID#002, etc. There will be no human-derived elements in this code (e.g., initials, dates, etc.). The code further will not pertain to any information on random assignment to groups. The master list linking study codes to individual identities will be maintained by the investigator on a password-protected, shared drive space on the UAB server and will not be divulged to others. Paper records will be stored in a locking file cabinet in the project coordinator's locked office in the ENRL. Electronic data will be stored

on UAB computers which are firewall protected, encrypted and password-restricted. The servers are monitored at all times for outages. Secured login IDs, granted on a need-to-know basis, further are required to access confidential information.

Dissemination.

The primary study results will be made available to scientists interested in MS, neuroimaging, cognition, and/or exercise as a treatment for cognitive deficits in order to avoid unintentional duplication of research. Specifically, the primary results will be disseminated via peer-reviewed publications, and replication of the protocol will be encouraged. The primary results will further be disseminated via conference presentation. The data upon which final summary statistics are based will be made available to third parties in applicable data repositories.

Discussion.

The current study is the first adequately-powered RCT to include an exercise training stimulus (i.e., treadmill walking exercise that progressively increases in duration and intensity) that is based on research on the acute[26,27] and chronic[21,23] effects of exercise on cognition in persons with MS that further targets hippocampal outcomes. Importantly, this study is the first RCT of exercise training to selectively recruit persons with objective impairments in learning new information in MS.[19] This is a critical methodological study component, as potential *treatment* effects (i.e., beyond simply improving cognitive performance) of exercise on MS-related cognitive dysfunction can only be assessed if participants demonstrate cognitive impairment. The current proposal will provide the first evidence for the efficacy of treadmill walking exercise training as a potential rehabilitative approach to *treat* MS-related learning and memory impairment. This efficacy study will include an active control condition to account for the effects of attention and social contact associated with supervised exercise training; no

previous RCTs of exercise training on cognition in MS have adopted this approach.[19] We further will include cardiorespiratory fitness outcomes as a manipulation check for documenting the success of the intervention; this critical feature has been lacking in previous RCTs of exercise on cognition in MS.[19] The proposed study will be among the first exercise training RCTs in any population to include blinded MRI data analyses, uniquely adding another layer of rigor for improving the study's reproducibility. Such methodological features are critical for reducing threats to internal validity (i.e., Type I error) and providing efficacy evidence for chronic treadmill walking exercise training as a behavioral approach for managing learning and memory dysfunction in persons with MS who have the most need.

The current early-phase RCT will provide critical information for the development of future exercise trials on learning and memory in persons with MS. If successful, the current trial will provide effect sizes for power analyses for determining appropriate sample sizes for a subsequent Phase III RCT. Indeed, such a line of research will lay the foundation for approaches that can be eventually translated into community-based settings for rehabilitating learning and memory in persons with MS by examining the effects of a treadmill walking exercise training intervention on learning and memory and hippocampal neuroimaging outcomes. Although results from studies of cognitive rehabilitation are promising, such interventions are not easily adapted outside of the clinic, and further do not offer health benefits beyond improved cognition. On the other hand, exercise is a behavior that is easily adaptable in the community and offers a myriad of physical health benefits. As such, the proposed study uniquely represents the first step in developing and optimizing a potentially generalizable exercise training intervention for improving behavioral (i.e., learning and memory), and brain (i.e., hippocampal) outcomes among cognitively-impaired persons with MS. As a whole, this line of research might result in the development of exercise training guidelines that can be adapted by MS patients for specifically improving brain health and cognition. Importantly, there are no guidelines for persons with MS to manage cognitive impairment in the community using any approach.

If the current RCT does not result in statistically significant treadmill walking exercise-related improvements in composite learning and memory performance, we will focus on refining the exercise stimulus for specifically improving learning and memory amongst memory-impaired persons with MS for informing the development of a future RCT. This could involve adjusting the modality and intensity of exercise training. Indeed, there is preliminary evidence that other exercise modalities (e.g., aerobic cycle ergometry) and intensities (e.g., high-intensity interval training) have resulted in improvements in learning and memory in persons with MS.[63,64] If the current treadmill walking exercise training intervention does not improve learning and memory performance, another potential approach for refining the exercise stimulus involves the inclusion of additional sensory stimuli to treadmill walking exercise (i.e., treadmill walking exercise plus virtual reality) for inducing cognitive change. This approach is based, in part, on animal work that demonstrates particularly large improvements in hippocampal neurogenesis, synaptogenesis, and learning and memory performance when exercise is performed in enriched environments compared with standard environments over several weeks.[18] There too is evidence in persons with traumatic brain injury whereby 4-weeks of exercise plus virtual reality improved verbal and visuospatial learning and memory.[65] Alternatively, if treadmill walking exercise training does not improve cardiorespiratory fitness outcomes (i.e., as a manipulation check), we plan to examine the effects of a longer intervention period (i.e., 6-months) on the primary and secondary study outcomes in a subsequent RCT, as has been done in older adults.[66] We do not expect this to be the case, given that the exact treadmill walking exercise training stimulus resulted in large cardiorespiratory fitness improvements in our small pilot RCT.[28]

Although the current RCT addresses a critical problem by applying a systematically-developed exercise training intervention for improving learning and memory and hippocampal structure and function among persons with MS who present with learning and memory impairment, there are several noteworthy limitations. First, the current RCT was powered based on pilot data on the effects of

treadmill walking exercise training on the primary outcomes of behavioral neuropsychological tests of learning and memory in persons with MS. This study was not powered based on pilot data on the effects of treadmill walking exercise training on structural or functional hippocampal neuroimaging outcomes (i.e., the secondary study outcomes), given that pilot data are not available on the present neuroimaging endpoints in persons with MS. For the current power analyses, we operationalized the effect sizes from an aerobic exercise intervention on hippocampal neuroimaging outcomes in persons with schizophrenia as a preliminary guide for powering the secondary study outcomes.[30] Therefore, it is unknown if the study is truly adequately powered for detecting effects of treadmill walking exercise training on hippocampal structure and functional connectivity (i.e., the secondary endpoints) in persons with MS who present with impairments in learning new information. The present RCT involves a relatively short intervention period, no long-term follow-up, or a comparison group of persons with MS without deficits in learning new information for possibly examining the neuroprotective effects of the intervention over time. Given that preliminary work directly supports the feasibility and preliminary efficacy of 3-months of treadmill walking exercise training on learning and memory and hippocampal neuroimaging outcomes, the current adequately-powered RCT seeks to rigorously examine the potential treatment effects of that exact intervention on learning and memory and its potential neural correlate(s). We believe this to be a necessary step prior to investigating the durability and sustainability of progressive treadmill walking exercise training on those outcomes in subsequent large-scale studies. We note that 3-months of aerobic exercise training has resulted in improvements in learning and memory as well as hippocampal structure and function in other populations [30,67]. Relatedly, the present study will not examine the effectiveness of treadmill walking exercise training on the primary outcomes in a large, national sample of persons with MS. Rather, this study will advance our systematic and rigorous line of research by providing critical efficacy data in a highly-controlled environment prior to the development of a subsequent RCT for examining the effectiveness of the intervention for eventual translation into a

community-based program. The present study will not involve the collection of exploratory serum biomarkers (e.g., brain-derived neurotrophic factor, vascular endothelial growth factor) as possible molecular/cellular mediators of the effects of treadmill walking exercise training on behavioral (i.e., neuropsychological) and brain-systems (i.e., hippocampal volume and functional connectivity) outcomes. If the current RCT is successful, the examination of such potential mechanisms will be a central focus of future mechanistic research efforts. Another limitation is that the study sample will not involve persons with MS who present with substantial ambulatory disability. Rather, this RCT only focuses on persons with MS who are fully-ambulatory based on pilot data and safety concerns associated with treadmill walking exercise training. Thus, the results of the current RCT might not be generalizable amongst all persons with MS, particularly those with severe ambulatory impairment. Finally, the present study will not directly compare the effects of treadmill walking exercise training with cognitive rehabilitation as a control comparison condition on learning and memory and hippocampal neuroimaging outcomes. Instead, this study includes an active, non-aerobic exercise training control condition in order to control for the potential effects of attention and social contact normally associated with supervised exercise training for testing the primary study hypotheses. Examinations of the comparative and combined effects of exercise training and cognitive rehabilitation on learning and memory and hippocampal neuroimaging outcomes will be performed in subsequent effectiveness trials.

Regardless of the study outcome, we plan to communicate the trial results via peer-reviewed publications. If successful, the results from this study will eventually inform RCTs for developing rehabilitation interventions (i.e., treadmill walking exercise training) for improving learning and memory and its relationship with hippocampal outcomes in a large sample of cognitively-impaired persons with MS. In the long term, the results from this early-phase RCT will lay the groundwork for ultimately providing clinicians and patients with guidelines for better using chronic treadmill walking exercise for improving cognition and brain health. Such an evidence-based approach for rehabilitation, using chronic

exercise training, is paramount considering the highly prevalent, disabling, and poorly-managed nature of MS-related learning and memory impairment.

References:

1. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. *Lancet Neurol* 2008;7:1139-51.

2. Guerts JJ, Bo L, Roosendaal SD, et al. Extensive hippocampal demyelination in multiple sclerosis. *J Neuropath Exp Neurol* 2007;66(9):819-27.

3. Benedict RH, Ramasamy D, Munschauer F, et al. Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. *J Neurol Neurosurg Psychiatr* 2009;80(2):201-6.

4. Hulst HE, Schoonehim MM, Van Geest Q, et al. Memory impairment in multiple sclerosis: relevance of hippocampal activation and hippocampal connectivity. *Mult Scler* 2015;21(13):1705-12.

5. Feinstein A, Magalhaes S, Richard J-F, et al. The link between multiple sclerosis and depression. *Nat Rev Neurol* 2014;10:507-17.

6. Morrow SA, Drake A, Zivadinov R, et al. Predicting loss of employment over three years in multiple sclerosis: clinically meaningful cognitive decline. *Clin Neuropsychol* 2010;24:1131-45.

7. Ruet A, Deloire M, Hamel D, et al. Cognitive impairment, health-related quality of life and vocational status at early stages of multiple sclerosis: a 7-year longitudinal study. *J Neurol* 2013;260:776-84.

8. Amato MP, Ponziani G, Siracusa G, et al. Cognitive dysfunction in early-onset multiple sclerosis: a reappraisal after 10 years. *Arch Neurol* 2001;58:1602-6.

9. Amato MP, Langdon D, Montalban X, et al. Treatment of cognitive impairment in multiple sclerosis: position paper. *J Neurol* 2013;260:1452-68.

10. Rosti-Otajarvi EM, Hamalainen PI. Neuropsychological rehabilitation for multiple sclerosis. *Cochrane Database Syst Rev* 2014;2:CD009131.

11. Chiaravalloti ND, Wylie G, Leavitt V, et al. Increased cerebral activation after behavioral treatment for memory deficits in MS. *J Neurol* 2012;259:1337-46.

12. Chiaravalloti ND, Moore NB, Nikelshpur OM, et al. An RCT to treat learning impairment in multiple sclerosis: the MEMREHAB trial. *Neurology* 2013;81:2066-72.

13. Goverover Y, Chiaravalloti ND, O'Brien A, et al. Evidence based cognitive rehabilitation for persons with multiple sclerosis: an updated review of the literature from 2007-2016. *Arch Phys Med Rehabil* 2018;99(2):390-407.

14. das Nair R, Martin KJ, Lincoln NB. Memory rehabilitation for people with multiple sclerosis. *Cochrane Database Syst Rev* 2016;3:CD008754.

15. Motl RW, Sandroff BM, Benedict RHB. Cognitive dysfunction and multiple sclerosis: developing a rationale for considering the efficacy of exercise training. *Mult Scler* 2011;17(9):1034-40.

16. Stillman CM, Cohen J, Lehman ME, et al. Mediators of physical activity on neurocognitive function: a review at multiple levels of analysis. *Front Hum Neurosci* 2016;10(626):1-17.

17. Vivar C, van Praag H. Running changes the brain: the long and short of it. *Physiology* 2017;32:410-24.

18. van Praag H. Neurogenesis and exercise: past and future directions. *Neuromol Med* 2008;10:128-40.

19. Sandroff BM, Motl RW, Scudder MR, et al. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. *Neuropsychol Rev* 2016;26(3):271-94.

20. Motl RW, Pilutti LA, Hubbard EA, et al. Cardiorespiratory fitness and its association with thalamic, hippocampal, and basal ganglia volumes in multiple sclerosis. *Neuroimage Clin* 2015;7:661-6.

21. Leavitt VM, Cirnigliaro C, Cohen A, et al. Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: preliminary findings. *Neurocase* 2014;20(6):695-7.

22. Sandroff BM. Exercise and cognition in multiple sclerosis: the importance of acute exercise for developing better interventions. *Neurosci Biobehav Rev* 2015;59:173-83.

23. Sandroff BM, Johnson CL, Motl RW. Exercise training effects on memory and hippocampal viscoelasticity in multiple sclerosis: a novel application of magnetic resonance elastography. *Neuroradiology* 2017;59(1):61-7.

24. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 Statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.

25. American College of Sports Medicine. ACSM's resource manual for guidelines for exercise testing and prescription, seventh edition. Philadelphia, PA: Lippincott Williams & Wilkins 2013.

26. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of walking, cycling, and yoga exercise on cognition in persons with relapsing-remitting multiple sclerosis without impaired cognitive processing speed. *J Clin Exp Neuropsychol* 2015;37(2):209-19.

27. Sandroff BM, Hillman CH, Benedict RHB, et al. Acute effects of varying intensities of treadmill walking exercise on inhibitory control in persons with multiple sclerosis: a pilot investigation. *Physiol Behav* 2016;154:20-7.

28. Sandroff BM, Balto JM, Klaren RE, et al. Systematically-developed pilot randomized controlled trial of exercise and cognition in persons with multiple sclerosis. *Neurocase* 2016;22(5):443-50.

29. Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates 1988.

30. Pajonk FG, Wobrock T, Gruber O, et al. Hippocampal plasticity in response to exercise in schizophrenia. *Arch Gen Psychiatry* 2010;67(2):133-43.

31. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001;50(1):121-7.

32. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-52.

33. Chiaravalloti ND, Balzano J, Moore NB, et al. The open-trial selective reminding test (OT-SRT) as a tool for the assessment of learning and memory. *Clin Neuropsychol* 2009;23:231-54.

34. Thomas S, Reading J, Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 1992;17(4):338–45.

35. Motl RW, Bollaert RE, Sandroff BM. Validation of the Godin Leisure-Time Exercise Questionnaire classification coding system using accelerometry in multiple sclerosis. *Rehabil Psychol* 2018;*in press*:doi: 10.1037/rep0000162 [Epub ahead of print].

36. Delis DC, Kramer JH, Kaplan E, et al. California Verbal Learning Test, Second Edition (CVLT-II). San Antonio, TX: Psychological Corporation 2000.

37. Buschke F, Fuld PA. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 1974;24:1019-25.

38. Benedict RHB. The Brief Visuospatial Memory Test Revised (BVMT-R). Lutz, FL: Psychosocial Assessment Resources 1997.

39. Rao S. A manual for the brief repeatable battery of neuropsychological tests in multiple sclerosis. Milwaukee, WI: Medical College of Wisconsin 1990.

40. Strober L, Englert J, Munschauer F, et al. Sensitivity of conventional memory tests in multiple sclerosis: comparing the Rao Brief Repeatable Neuropsychological Battery and the Minimal Assessment of Cognitive Function in MS. *Mult Scler* 2009;15(9):1077-84.

41. Amato MP, Goretti B, Viterbo RG, et al. Computer-assisted rehabilitation of attention in patients with multiple sclerosis: results of a randomized, double-blind trial. *Mult Scler* 2014;20(1):91-8.

42. Mattioli F, Stampatori C, Bellomi F, et al. A RCT comparing specific intensive cognitive training to aspecific psychological intervention in RRMS: the SMICT study. *Front Neurol* 2015;5(278):1-8.

43. Boringa JB, Lazeron RH, Reuling IE, et al. The brief repeatable battery of neuropsychological tests: normative values allow application in multiple sclerosis clinical practice. *Mult Scler* 2001;7(4):263-7.

44. Parmenter BA, Testa SM, Schretlen DJ, et al. The utility of regression-based norms in interpreting the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). *J Int Neuropsychol Soc* 2010;16(1):6-16.

45. van Geest Q, Hulst HE, Meijer KA, et al. The importance of hippocampal dynamic connectivity in explaining memory function in multiple sclerosis. *Brain Behav* 2018;8(5):e00954.

46. Smith DV, Utevsky AV, Bland AR, et al. Characterizing individual differences in functional connectivity using dual-regression and seed-based approaches. *Neuroimage* 2014;15:1-12.

47. Colcombe S, Kramer AF, Erickson KI, et al. Cardiovascular fitness, cortical plasticity and aging. *Proc Natl Acad Sci USA* 2004;101(9):3316-21.

48. Macko RF, Katzel LI, Yataco A, et al. Low-velocity graded treadmill stress testing in hemiparetic stroke patients. *Stroke* 1997;28(5):988-92.

49. Motl RW, Fernhall B. Accurate prediction of cardiorespiratory fitness using cycle ergometry in minimally disabled persons with relapsing-remitting multiple sclerosis. *Arch Phys Med Rehabil* 2012;93(3):490–5.

50. Benedict RHB, Fischer JS, Archibald CJ, et al. Minimal neuropsychological assessment of MS patients: a consensus approach. *Clin Neuropsychol* 2002;16:381-97.

51. Smith A. Symbol digit modalities test: Manual. Los Angeles, CA: Western Psychological Services 1982.

52. Gronwall DMA. Paced auditory serial addition task: a measure of recovery from concussion. *Percept Mot Skills* 1977;44:367-73.

53. Delis DC, Kaplan E, Kramer JH. Delis-Kaplan executive function system: Technical manual. San Antonio, TX: Psychological Corporation 2001.

54. Benton AL, Sivan AB, Hamsher K, et al. Contributions to Neuropsychological Assessment, Second Edition. New York, NY: Oxford University Press 1994.

55. Patti F, De Stefano M, Lavorgna L, et al. Lesion load may predict long-term cognitive dysfunction in multiple sclerosis patients. *PLoS One* 2015;10(3):e0120754.

56. Borg G. Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics 1998.

57. National Multiple Sclerosis Society. Stretching for people with MS: an illustrated manual. New York, NY: National Multiple Sclerosis Society 2004.

58. Sandroff BM, Bollaert RE, Pilutti LA, et al. Multimodal exercise training in multiple sclerosis: a randomized controlled trial in persons with substantial mobility disability. *Contemp Clin Trials* 2017;61:39-47.

59. Bandura A. Health promotion by social cognitive means. *Health Educ Behav* 2004;31:143-64.

60. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986;51(6):1173-82.

61. Rousselet GA, Pernet CR. Improving standards in brain-behavior correlation analyses. *Front Hum Neurosci* 2012;6:119.

62. Pilutti LA, Platta ME, Motl RW, et al. The safety of exercise training in multiple sclerosis: a systematic review. *J Neurol Sci* 2014;343(1-2):3-7.

63. Briken S, Gold SM, Patra S, et al. Effects of exercise on fitness and cognition in progressive MS: a randomized, controlled pilot trial. *Mult Scler* 2014;20(3):382-90.

64. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: a randomized controlled trial. *Mult Scler* 2017;*in press*:doi: 10.1177/1352458517728342 [Epub ahead of print].

65. Grealy MA, Johnson DA, Rushton SK. Improving cognitive function after brain injury: the use of exercise and virtual reality. *Arch Phys Med Rehabil* 1999;80:661-7.

66. Erickson KI, Voss MW, Prakash RS, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci USA* 2011;108(7):3017-22.

67. Pereira AC, Huddleston DE, Brickman AM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA* 2007;104(13):5638-43.

Author Contributions

BMS: Study concept and design, study registration, study principal investigator, obtained IRB approval, drafting of the manuscript, critical revision of the manuscript

RWM, MB, GRC, MB, JRR, GRW, HG, JDL: Study concept and design, critical revision of the manuscript

Funding Statement

This paper was supported by an investigator-initiated grant from EMD Serono, Inc (One Technology Place, Rockland, MA 02370). The principal investigator (BMS) will have access to all data from the study, complete freedom to direct the data analyses and reporting without influence of sponsors, and affirm that there is no editorial direction or censorship from sponsors.

Competing Interest Statement

All authors declare no conflicts of interest.

Table 1: Exercise prescription and progression over the 3-month period for treadmill walking exercise training condition based on pilot work and ACSM guidelines

Week	Sessions	Exercise Intensity	Exercise Duration	Training Stage
Baseline Testing				
1	1-3	40-50% VO_2R/HRR	15-20 min	Initiation
2	4-6	40-50% VO_2R/HRR	20-25 min	Initiation
3	7-9	50-60% VO_2R/HRR	20-25 min	Improvement
4	10-12	50-60% VO_2R/HRR	25-30 min	Improvement
5-6	13-18	60-70% VO_2R/HRR	25-30 min	Improvement
7-8	19-24	60-70% VO_2R/HRR	30-35 min	Improvement
9-10	25-30	70-80% VO_2R/HRR	30-35 min	Maintenance
11-12	31-36	70-80% VO_2R/HRR	35-40 min	Maintenance
Follow-up Testing				

Note: VO_2R=oxygen consumption reserve; HRR=heart rate reserve

Figure 1: Participant flow through the study.

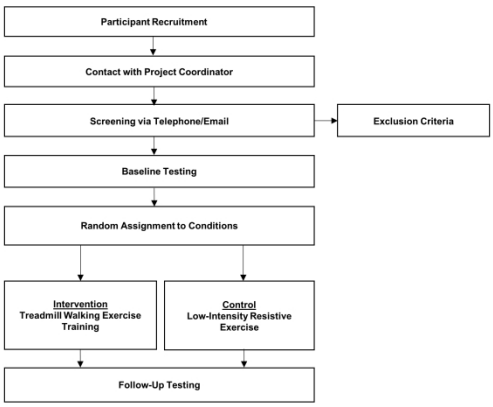


Figure 1

338x190mm (300 x 300 DPI)

CONSENT FORM

Title of Research: Effects of Exercise Training on Learning and Memory Outcomes in MS

IRB Protocol #: **XXX-XXXXXXXXX**

Principal Investigator: Brian M. Sandroff, Ph.D.

Purpose of the Research

We are asking you to take part in a research study. The purpose of this research study is to compare the effects of two different 12-week long exercise programs (treadmill walking or stretching-and-toning) on cognitive (thinking) performance in persons with multiple sclerosis (MS). The study principal investigator will discuss with you your responsibilities as a participant. As a participant, you will complete one of these 12-week long exercise programs. This is important because exercise has been identified as a possible way to improve thinking performance in people with MS. There will be 40 participants enrolled at UAB.

Explanation of Procedures

If you agree to participate, during the course of this study, the following research procedures will occur during 40 separate visits to the laboratory over the course of 3 months:

The first visit will last about 3 hours in total and will occur at the UAB/Lakeshore Collaborative Research Center, located at 3810 Ridgeway Drive, Birmingham, AL 35209.

- On the first visit, you will first take a paper-and-pencil thinking test of how well you can remember a list of words.
    - Depending on your score on this test, you may be ineligible to participate in this study.
        - If this is the case, you will be paid $50 in the form of a check for your time.

- If you are eligible to participate based on that test, you will then undergo a brief examination, where a researcher will measure your reflexes, how well you can feel a light touch, your muscle strength, vision, and ask you some questions concerning your bladder/bowel function and thinking ability.

- You will then complete a brief questionnaire on whether or not it would be safe for you to undergo an MRI scan.
    - If it is unsafe for you to undergo an MRI scan, you will be ineligible to participate in this study, but you will be paid $50 in the form of a check for your time.
- You will then have your blood pressure measured when you are seated comfortably in a chair using an automated blood pressure cuff in order to ensure your safety in participating in the study.

- If your blood pressure is too high (i.e., greater than 200/110), you will be ineligible to participate in this study, but you will be paid $50 for your time.

- If your blood pressure is below 200/110 at rest, you will then complete several computerized and paper-and-pencil thinking tasks that will take approximately 1 hour and will include the following:
  - A test of how well you can remember a list of words that is read to you out loud
  - A test of how well you can draw shapes from memory
  - A test of how quickly you can match shapes with single-digit numbers
  - A test of how quickly and accurately you can add up 2 numbers in a row, out loud, for 10 minutes
  - A test of how quickly and accurately you can recognize complicated images on a computer
  - A test measuring how well you can tell the difference between several arrows that look alike
  - A test of how many words beginning with a certain letter that you can name

- You will then undergo a six-minute long walking test which will be performed indoors, on a flat surface with no obstacles.
  - You will be asked to walk laps around a circle of cones and continue for 6 minutes.
  - You will be allowed to stop and rest during the test; however, the clock will not stop.
  - For your safety, study staff will stay close to you during walking trials.

- You will then undergo a brief test measuring how fast you can place pegs in and out of holes on a plastic peg board with each hand.

- You will then complete a maximal exercise test to determine your aerobic fitness level.
  - This test will involve fitting you with a mouthpiece to monitor your breathing and a heart rate monitor to assess your heart rate during exercise.
  - The test will require you to walk on a motor-driven treadmill at zero incline (flat), and after a 3-minute warm-up, the incline will continually increase until you can no longer continue to exercise.
  - The test should take approximately 10-15 minutes.
  - For your safety, two researchers will be present within an arm's reach at all times during this test.
  - The researcher will end the exercise session early if you begin to feel uncomfortable.

Three (3) days later, you will visit the Civitan Neuroimaging Laboratory in UAB Highlands Hospital (1201 11th Ave S, Birmingham, AL 35205) to complete more tests of your thinking ability, walking speed, physical function, and an MRI/fMRI scan. This visit will last approximately 3 hours.

- First, you will complete several computerized and paper-and-pencil thinking tasks that will take approximately 1 hour and will include the following:
  - A test examining your language skills
  - A test examining how many different ways you can sort a set of cards

- A test examining how quickly and accurately you can draw a line between dots
- A test examining how well you can purposely remember some information while ignoring distracting information
- A test examining how quickly you can analyze patterns of shapes and letters

- Then, you will complete a short walking test measuring how fast you can walk over a 25-foot distance.

- You will then undertake a short series of tests measuring how fast you normally walk, your ability to stand up and sit down in a chair, and your ability to balance with your feet together.

- This will be followed by a short test measuring how flexible you are when sitting down.

- You will then undertake an MRI scan.
  - Magnetic resonance imaging (MRI) uses a strong magnetic field and radio waves to take pictures of the brain.
    - fMRI allows he researchers to see what parts of the brain are used when you perform certain thinking tasks.
    - The MRI scanner is a metal cylinder surrounded by a strong magnetic field.

  - During the MRI, you will lie on a table that can slide in and out of the cylinder.
    - A device called a "coil" will be placed over your head.
    - Before the scan, you will be told about the thinking tasks that you will do during the scan and you may have the opportunity to practice.
    - There is a computer screen that you will be able to see when you are inside the scanner.
    - The screen will show you the thinking tasks that you will do in the scanner.
      - These tasks include matching shapes and single-digit numbers, remembering a list of words, and recognizing complicated shapes as quickly and accurately as possible.

  - You will be in the scanner for about 60 minutes.

  - During the scan, you will undertake the 3 thinking tasks.
    - You will respond by pressing a button on a button box that will be attached to your hand with a Velcro strap.

  - You may be asked to do these tasks, or you may be asked to lie still for up to 10 minutes at a time.

  - While in the scanner, you will hear loud knocking noises and you will be fitted with earplugs or earmuffs to muffle the sound. You will be able to communicate with the MRI staff at all times during your scan and you may ask to be moved out of the machine at any time.

- It is very important for the experiment that you do not move your head or body inside the scanner.
  - Padding, a vacuum bag, or expanding foam will be placed around your head to help keep it in position.
  - You may also be asked to bite down on a mouth bar to help keep your head still.

- For the next 12 weeks (i.e., 3 months), you will then be randomly picked (like the flip of a coin) by a computer to participate in one of two exercise groups.
  - One group will complete an in-person treadmill walking exercise program, and the other group will complete an in-person stretching-and-toning exercise program
    - Both programs are based on activity guidelines for people with MS and will be led by trained exercise leaders.
  - You will have a 50/50 chance of being placed in either group.

- No matter which group you are assigned to, you will be asked to complete 36 exercise training visits (treadmill walking or stretching-and-toning) that will take place at the UAB/Lakeshore Collaborative Research Center.
  - This facility is located at 3810 Ridgeway Drive, Birmingham, AL 35209.

- These visits will take place 3 days per week for 12 weeks and will each last approximately 1 hour in total.

- These will be individual visits led by a trained exercise leader and will initially consist of 15 minutes of actual exercise and progress up to 40 minutes of actual exercise.
  - Each visit will begin and end with a 5-minute warm-up/cool-down period.

- During the initial visit, you will learn how to safely perform the exercises.
  - You will be given a log book to monitor and record your progress.

- For each visit, you will also be given individualized feedback and changes will be made to your program if needed.

- Before you begin the first training session, you will complete two additional tests of how well you can remember information, and you will also complete a number of questionnaires that ask you about your everyday functioning, how you feel about your life, and your mood.
  - You will then complete the first exercise session of either treadmill walking or stretching-and-toning exercise.

- At the end of your 12-week training program, you will return to the UAB/Lakeshore Collaborative Research Center for a follow-up visit.

- This visit will last 3 hours where you will repeat the tests you completed during your initial visit.

- Three (3) days later, you will return to UAB Highlands Hospital for a final follow-up visit.
  - This visit will also last 3 hours where you will repeat the tests you completed during your second overall visit, and will also involve another MRI/fMRI scan.

- We are performing imaging solely for the research purposes described above. It is not a clinical scan intended for diagnostic or therapeutic purposes. The MRI scans that will be performed in connection with the study you are participating in are not necessarily equivalent to the type of MRI scans more commonly used to diagnose medical problems. Many potentially serious medical problems may be undetectable on the scans performed in the study.

- Under no circumstance will the investigator, research staff, or imaging staff interpret the scan as normal or abnormal. They are unable to make any medical comments about your scan. The scan will not be looked at or read for any healthcare treatment or diagnostic purpose. If you are experiencing physical symptoms or otherwise have concerns about your health, you should see your primary care physician or specialist physician. If you want your scan to be reviewed by a physician so the physician can look for medical issues, you can request a copy of your scan. We will provide an electronic copy at no charge.

Risks and Discomforts

The study described above may involve the following risks and/or discomforts:

Exercise:

- With the completion of the maximal exercise test and exercise training visits, there are always risks of death, heart attack, arrhythmia, difficulty breathing, and complications that require hospitalization.

- All lab personnel in attendance are trained in CPR, AED, and First Aid.

- Importantly, it is still possible that you will experience some fatigue, sprains, cramps, and muscle soreness after the completion of the maximal exercise test and exercise training visits. Those responses can be temporary (i.e., for a few hours afterwards).

- Any possible symptoms associated with an increase in body temperature will be reduced by controlling the room temperature with air conditioning and using multiple fans.

- There is a small risk of falling, injury, head trauma, and death when performing a walking exercise on a treadmill. To minimize this risk, you will be encouraged to hold on to the handrails on the treadmill, as well as having a gait belt (i.e., a belt with handles on it where

spotters can provide physical support in case of a slip) around your waist, and a research assistant within arm's reach to quickly assist if you lose your balance.

- During the maximal exercise test, there may be some discomfort when wearing the mouthpiece.

MRI:

- People are at risk for injury from the MRI magnet if they have:
  - Pacemakers or other implanted electrical devices
  - Brain stimulators
  - Particular types of dental implants
  - Aneurysm clips (metal clips on the wall of a large artery)
  - Metallic prostheses (including metal pins and rods, heart valves, and internal hearing aids [cochlear implants])
  - Permanent eyeliner
  - Implanted delivery pumps
  - Shrapnel fragments
  - Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware.

- You will be screened for these conditions before having any scan, and if you have any, you will not receive an MRI scan.

- If you have a question about any metal objects being present in your body, you should inform the study personnel.

- In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scanning room.

- People with fear of confined spaces may become anxious during an MRI.

- Those with back problems may have back pain or discomfort from lying in the scanner.

- Some may experience dizziness or paresthesia (tingling or numbness).

- The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss.
  - Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, you should let the study personnel know right away.

  - You will notify the investigators if you have hearing or ear problems.

- You will be asked to complete an MRI screening form for each MRI scan you have. There are no known long-term risks of MRI scans.

- It is not known if MRI is completely safe for a developing fetus. If you are a woman, you will have a pregnancy test before the MRI scan. Therefore, if you are pregnant, you will not be eligible to participate in the study.

Thinking tasks:

- You may also feel tired or experience mild frustration during the tasks designed to measure your thinking ability. This is because the tasks are often difficult. You will be allowed to take short breaks as necessary.

Walking tests:

- There is also a small risk of falling or injury during the six-minute or 25-foot walking test as well as the Short Physical Performance Battery. For your safety, study staff will stay close to you during the walking trials.

- There is also a risk of dizziness or fatigue. You will be permitted to take breaks as needed to minimize these effects.

- All study procedures and testing will be performed under supervision of qualified personnel.

Questionnaires:

- There is a small psychosocial risk associated with the completion of questionnaires such as embarrassment or anxiety in responding to some questions.

You will be assigned to an exercise group by chance, which may prove to be less effective or slightly more fatiguing than the other exercise group.

There also may be risks and discomforts that cannot be foreseen. You will be given more information if other risks are found.

Benefits

You may not benefit directly from taking part in this study. However, the beneficial effects of exercise training on general health are very well known. Further, this study may help researchers better understand the effects of exercise in persons with MS. The results of this research may also lead to more effective ways to treat individuals with MS.

Alternatives

The study principal investigator will discuss with you the alternatives to participation and their risks and benefits. The alternative is to not participate in the study.

Confidentiality

Information obtained about you for this study will be kept confidential to the extent allowed by law. However, research information that identifies you may be shared with people or organizations for quality assurance or data analysis, or with those responsible for ensuring compliance with laws and regulations related to research. They include:

- the UAB Institutional Review Board (IRB). An IRB is a group that reviews the study to protect the rights and welfare of research participants.
- the Office for Human Research Protections (OHRP).

The information from the research may be published for scientific purposes; however, your identity will not be given out.

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.

Voluntary Participation and Withdrawal

Whether or not you take part in this study is your choice. There will be no penalty if you decide not to be in the study. If you decide not to be in the study, you will not lose any benefits you are otherwise owed.

You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution. Contact the Principal Investigator if you want to withdraw from the study.

You may be removed from the study without your consent if the sponsor ends the study or if the Principal Investigator decides it is not in the best interest of your health.

If you are a UAB student or employee, taking part in this research is not a part of your UAB class work or duties. You can refuse to enroll, or withdraw after enrolling at any time before the study is over, with no effect on your class standing, grades, or job at UAB. You will not be offered or receive any special consideration if you take part in this research.

Cost of Participation

There will be no cost to you for taking part in this study. All tests, exams, and exercise related to this study will be provided to you at no cost during the 3-month study period. However, attending visits can involve some transportation costs (i.e., the cost of gas). But, parking will be free for each of the 40 visits you attend. The costs of your standard medical care will be billed to you and/or your insurance company in the usual manner.

Payment for Participation in Research

You will receive the following compensation: $50 after the baseline evaluation and MRI scan (i.e., the first 2 study visits); up to $360 at the conclusion of the 3-month exercise program (i.e., $5 for attending each exercise visit [up to 36 visits] plus an additional $5 for travel expenses per exercise visit; and $50 for after completion of the follow-up evaluation and MRI scan. This will total up to $460. Please ask the study staff about the method of payment that will be used for this study (e.g., check). The payment is prorated per visit in the event that you stop participating in the study or the investigator terminates the study.

Payment for Research-Related Injuries

UAB and EMD Serono, Inc. have not provided for any payment if you are harmed as a result of taking part in this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

Significant New Findings

You will be told by the Principal Investigator or the study staff if new information becomes available that might affect your choice to stay in the study.

Questions

If you have any questions, concerns, or complaints about the research or a research-related injury including available treatments, please contact the study doctor. You may contact Prof. Brian Sandroff, PhD by phone at 205-934-5972 or by email at sandroff@uab.edu.

If you have questions about your rights as a research participant, or concerns or complaints about the research, you may contact the UAB Office of the IRB (OIRB) at (205) 934-3789 or toll free at 1-855-860-3789. Regular hours for the OIRB are 8:00 a.m. to 5:00 p.m. CT, Monday through Friday.

Legal Rights

You are not waiving any of your legal rights by signing this consent form.

Signatures

Your signature below indicates that you have read (or been read) the information provided above, including your responsibilities as a participant, the alternatives to participation, and the approximate number of subjects involved in the trial. Your signature below further indicates that you agree to participate in this study. You will receive a copy of this signed consent form.

Signature of Participant Date

Signature of Person Obtaining Consent Date

University of Alabama at Birmingham

AUTHORIZATION FOR USE/DISCLOSURE OF PROTECTED HEALTH INFORMATION (PHI) FOR RESEARCH

Participant Name: _____
Research Protocol: Effects of Exercise Training on Learning and Memory Outcomes in MS

UAB IRB Protocol Number: XXX-XXXXXXXXX
Principal Investigator: Brian M. Sandroff, Ph.D.

What is the purpose of this form? You are being asked to sign this form so that UAB may use and release your protected health information for research. Participation in research is voluntary. If you choose to participate in the research, you must sign this form so that your protected health information may be used for the research.

Why do the researchers want my protected health information? The researchers want to use your protected health information as part of the research protocol listed above and as described to you in the informed consent.

What protected health information do the researchers want to use? All medical information, including but not limited to information and/or records of any diagnosis or treatment of disease or condition, which may include sexually transmitted diseases (e.g., HIV, etc.) or communicable diseases, drug/alcohol dependency, etc.; all personal identifiers, including but not limited to your name, social security number, medical record number, date of birth, dates of service, etc.; any past, present, and future history, examinations, laboratory results, imaging studies and reports and treatments of whatever kind, including but not limited to drug/alcohol treatment, psychiatric/psychological treatment; financial/billing information, including but not limited to copies of your medical bills, and any other information related to or collected for use in the research protocol, regardless of whether the information was collected for research or non-research (e.g., treatment) purposes.

Who will disclose, use and/or receive my protected health information? All Individuals/entities listed in the informed consent documents, including but not limited to, the physicians, nurses and staff and others performing services related to the research (whether at UAB or elsewhere); other operating units of UAB, HSF, UAB Highlands, Children's of Alabama, Eye Foundation Hospital, and the Jefferson County Department of Health, as necessary for their operations; the IRB and its staff; the sponsor of the research and its employees and agents, including any CRO; and any outside regulatory agencies, such as the Food and Drug Administration, providing oversight or performing other legal and/or regulatory functions for which access to participant information is required.

How will my protected health information be protected once it is given to others? Your protected health information that is given to the study sponsor will remain private to the extent possible, even though the study sponsor is not required to follow the federal privacy laws. However, once your information is given to other organizations that are not required to follow federal privacy laws, we cannot assure that the information will remain protected.

How long will this Authorization last? Your authorization for the uses and disclosures described in this Authorization does not have an expiration date.

Can I cancel this Authorization? You may cancel this Authorization at any time by notifying the Principal Investigator, in writing, referencing the research protocol and IRB Protocol Number. If you cancel this Authorization, the study doctor and staff will not use any new health information for research. However, researchers may continue to use the protected health information that was provided before you cancelled your authorization.

Can I see my protected health information? You have a right to request to see your protected health information. However, to ensure the scientific integrity of the research, you will not be able to review the research information until after the research protocol has been completed.

Signature of participant: _____ Date: _____
or participant's legally authorized representative: _____ Date: _____
Printed Name of participant's representative: _____
Relationship to the participant: _____

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page 1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page 2, 6)
	2b	All items from the World Health Organization Trial Registration Data Set (Page 6)
Protocol version	3	Date and version identifier (Page 6)
Funding	4	Sources and types of financial, material, and other support (Page 33)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page 1)
	5b	Name and contact information for the trial sponsor (Page 33)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities (Page 33)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) (Pages 19, 21-22)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (Pages 4-6)
	6b	Explanation for choice of comparators (Pages 4-6)
Objectives	7	Specific objectives or hypotheses (Pages 6-8)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Pages 6-8)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (Page 6)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (Pages 9-10)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (Pages 15-17)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) (Pages 21-22)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (Page 17)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Page 17)
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (Pages 10-14)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Pages 17-19;Figure 1)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (Pages 8-9)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (Page 9)
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions (Pages 18-19)
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned (Page 18)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (Pages 17-19)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Pages 18-19)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (Pages 18-19)
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol (Pages 10-14)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols (Pages 17,19-20)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol (Page 19)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Pages 19-21)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Pages 19-21)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) (Pages 19-20)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed (Page 19)
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial (Page 19)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct (Pages 21-22)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (Pages 21-22)

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page 21)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) (Page 6)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) (Pages 17-19)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable (Pages 17-19)
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial (Page 22)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page 33)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators (Page 33)
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation (Page 22)

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions (Page 21)
	31b	Authorship eligibility guidelines and any intended use of professional writers (Page 33)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code (Pages 6, 21)
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (N/A)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable (N/A)

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.