STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Study Title: Exercise Training Effects on Cognition and Brain Function in Multiple Sclerosis: A Systematically-Developed Randomized Controlled Trial

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Study Protocol

1. Abstract

Slowed cognitive processing speed (CPS) is a common and debilitating consequence of multiple sclerosis (MS) that is notoriously difficult to treat. As such, we undertook a systematic line of research that indicated that supervised, progressive treadmill walking exercise (TMWX) training might improve CPS and brain functioning among fully-ambulatory persons with MS. The current study will be the first adequately-powered, single-blind randomized controlled trial (RCT) that examines the efficacy of 12-weeks of TMWX training compared with an active control condition on CPS, thalamocortical brain connectivity (based on resting-state fMRI), and exploratory functional outcomes in 88 fully-ambulatory persons with MS who present with slowed CPS. 65 of those persons will be recruited at Kessler Foundation. The intervention condition involves supervised, progressive TMWX training 3 times/week over 12-weeks; this initially involves 15-minutes of light-to-moderate intensity TMWX that progresses up to 40-minutes of vigorous intensity TMWX. The active control condition involves supervised, minimal intensity, stretching-and-resistance exercise that will be delivered on the same frequency as the intervention condition. The primary study outcomes involve Symbol Digit Modalities Test performance (i.e., CPS) and fMRI-based measures of thalamocortical resting-state functional connectivity. Exploratory study outcomes involve measures of community participation, activities of daily living, quality of life, and functional mobility. All study outcomes will be administered before and after the 12-week study period by treatment-blinded assessors. If successful, the current study will provide the first Class I evidence for the effects of TMWX training as an approach for improving CPS and its neural correlate, and possibly mitigating the impact of slowed CPS on functional outcomes in MS.

2. Study Objectives

The proposed research represents the first opportunity to investigate the efficacy, possible mechanism, and functional impact of treadmill walking exercise training on CPS and thalamocortical RSFC in fully-ambulatory persons with MS with slowed CPS using a highly-innovative and rigorous approach. If successful, the proposed study will provide the first Class I evidence for the efficacy of treadmill walking exercise training as a rehabilitative approach to improve CPS, its neural correlate, and possibly mitigate the impact of slowed CPS on functional outcomes in persons with MS who need such an intervention the most.

Specific Aim 1: Examine the efficacy of 3-months of progressive treadmill walking exercise training compared with a social contact, attention control condition (i.e., stretching-and-toning activities) on CPS and thalamocortical RSFC outcomes in fully-ambulatory persons with MS who have slowed CPS.

Hypothesis 1: Those who are randomly assigned to the treadmill walking exercise condition will demonstrate improvements in CPS and increased thalamocortical RSFC relative to those in the control condition.

Specific Aim 2: Examine thalamocortical RSFC as a potential mechanism of treadmill walking exercise training effects on CPS in fully-ambulatory persons with MS who have slowed CPS.

Hypothesis 2: Thalamocortical RSFC will mediate the effect of treadmill walking exercise training on CPS, such that increases in thalamocortical RSFC will explain exercise-related improvements in CPS.

Exploratory Aim 3: Examine the potential impact of 3-months of progressive treadmill walking exercise training on functional outcomes that are commonly associated with MS-related CPS impairment in fully-ambulatory persons with MS who have slowed CPS.

Exploratory Hypothesis 3: Those who are randomly assigned to the treadmill walking exercise training condition will demonstrate improvements in community participation, ability to perform activities of daily living, QOL, and functional mobility relative to those in the control condition.

3. Study Significance

Cognitive impairment is highly prevalent, poorly-managed, and disabling in persons with MS and exercise training might represent a promising approach to manage this symptom of the disease. The proposed study aims to examine the effects of 3-months of supervised, progressive (both intensity and duration) treadmill walking exercise training (designed based on pilot work and American College of Sports Medicine guidelines) compared with an active control condition (i.e., stretching-and-toning activities) on cognitive processing speed and functional MRI outcomes in 88 cognitively-impaired persons with MS. This study is critical for providing evidence supporting treadmill walking exercise training as a behavioral approach for managing slowed cognitive processing speed (i.e., the most common MS-related cognitive impairment) and improving brain health in persons with MS.

4. Literature Review

Cognitive impairment is prevalent, impactful, and poorly-managed in multiple sclerosis (MS). Upwards of 67% of patients demonstrate cognitive impairment based on neuropsychological testing [1]. Such cognitive impairment primarily manifests as slowed cognitive processing speed (CPS). Importantly, MS-related CPS impairment contributes to reduced community participation, ability to perform activities of daily living, quality of life (QOL), and functional mobility [1]. There are no FDA-approved pharmacological treatments (i.e., disease modifying therapies (DMT)/symptomatic treatments) for CPS impairment in MS. There have been no published cognitive rehabilitation studies directly targeting CPS in persons with MS who have objective CPS impairment [2]. This collectively underscores the critical importance of identifying new approaches for managing cognitive impairment in MS, particularly those that can result in secondary health and functional benefits. One particularly promising approach is exercise training [3,4].

There is an exceptionally strong and consistent body of literature supporting exercise training effects on cognition across the lifespan (i.e., healthy children through older adults) [5]. By comparison, there have been 5 RCTs of exercise training and cognition in MS, and the results of those studies are conflicting and equivocal [6-10]. This is based on methodological flaws (e.g., lack of inclusion of a physical fitness outcome measure as a manipulation check for documenting the success of an exercise training intervention), lack of inclusion of cognitively-impaired samples, and inconsistent focus on cognition as a primary outcome [11]. Relatedly, none of the exercise training RCTs in MS included a systematically developed exercise stimulus for optimally improving cognition nor considered the role of brain systems subserving exercise training effects on CPS.

We have spent the past 5 years systematically delineating an *optimal* exercise training intervention for improving cognition in persons with MS. This involved a stepwise process of identifying the optimal domain(s) of fitness (i.e., cardiorespiratory fitness or muscular strength) for improving cognition; the cognitive domain(s) that would be most sensitive to exercise; MS subsamples who would be likely to demonstrate cognitive benefits with exercise; and the optimal exercise stimulus itself (i.e., modality [type] and intensity) for improving cognition [12-20]. Collectively, the results from our rigorous, systematic line of research indicate that an optimal exercise intervention involves progressive (i.e., both intensity and duration) treadmill walking exercise training for improving CPS, in particular, among fully-ambulatory persons with MS. This optimal intervention is highly promising for improving CPS based on systematic preliminary data and pilot testing, but it has not yet been delivered in persons with MS who have slowed CPS. This is the critical next step for research on exercise and cognition in MS, as no prior RCTs of exercise have specifically recruited persons with MS-related cognitive impairment [11]. This methodological aspect is especially noteworthy, as the proposed study will be the *first* to examine exercise training as a potential *treatment* for slowed CPS in fully-ambulatory persons with MS [11]. We further highlight that existing MS RCTs have not included an active control condition that accounts for attention and social contact nor outcome assessors who are treatment-blinded for appropriately and rigorously testing the effects of exercise training on cognition [11]. The proposed study will be delivered as an adequately-powered, single-blind RCT, using an active control condition, and further will include cardiorespiratory fitness as a manipulation check for documenting the success of the intervention in persons with MS who have impaired CPS.

One significant advance in research on exercise and cognition across the lifespan has been the focus on neuroimaging outcomes [5], yet no published RCTs of exercise training and cognition in MS have included neuroimaging approaches for measuring changes in brain function. The inclusion of functional neuroimaging outcomes is critical for providing information on the potential neural mechanism(s) of exercise-related improvements in cognition in MS (e.g., [21]). We proposed that research should examine the effects of progressive treadmill walking exercise training on resting-state functional connectivity (RSFC) of the thalamus with other cortical regions (e.g., frontal cortex) for better understanding the effects of exercise on CPS [19,22]. This is based on evidence that thalamic dysfunction is consistently linked with MS-related CPS-impairment [23,24]. Such potential relationships in MS are supported by preliminary cross-sectional data that suggest that frontal [25] and thalamic [26] areas might be important regions of interest for examining aerobic exercise effects on the brain in this population. Data from a case study by members of our research team suggest that aerobic exercise training can improve cognition and RSFC in related neural networks in a cognitively-impaired person with MS [27]. The aforementioned conceptual arguments and empirical evidence support the inclusion of neuroimaging outcomes of thalamocortical RSFC in this proposal as a possible neural mechanism subserving the effect of treadmill walking exercise training on CPS in those with MS. Importantly, impaired CPS is a major influence of functional outcomes in MS [1]. The present proposal will further examine the effects of treadmill walking exercise training on community participation, ability to perform activities of daily living, QOL, and functional mobility. The inclusion of secondary functional outcomes will broaden the impact of this intervention for persons with MS-related CPS impairment.

5. Methodology

5.1 Participants.

5.1.1. Sample Size. We plan to enroll 88 fully-ambulatory persons with MS (i.e., 44 per condition) who have objective impairment in CPS; this is based on a power analysis and presumed 25% attrition. Of the 88 total participants, 65 will be recruited at Kessler Foundation, as this study is currently ongoing and was partially completed at the University of Alabama at Birmingham. The minimal sample size of 66 persons with MS (i.e., 33 per condition) was determined based on power analysis using previous effect sizes from our preliminary RCT of TMWX training on CPS outcomes (i.e., η_p^2=0.11) and standard assumptions of Type I error (0.05) and power (0.80) based on analysis of covariance (ANCOVA) on follow-up SDMT scores, controlling for baseline SDMT score [19]. We note that this rate of attrition exceeds that of the overall average of exercise training studies among persons with MS based on a systematic review [28] making our calculations conservative.

5.1.2. Recruitment. Prospective participants will be recruited directly through the community, local chapter of the National MS Society (NMSS), and our laboratory database of previous participants with MS who have inquired about participating in future studies. Advertisements for the study will be distributed through local MS centers, 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.

5.1.3. Screening. The screening process for potential participants will first involve a phone screen, followed by an in-person screen. Via telephone, the project coordinator will obtain the following information from potential participants:

- Age between 18-59 years
- Definite diagnosis of MS
- Relapse-free for 30 days, not acutely taking corticosteroids
- No major depressive disorder, schizophrenia, bipolar disorder I or II, substance abuse disorder
- Not currently taking medications that can affect cognition (i.e., benzodiazepines, anti-psychotics)
- Not having known/diagnosed cardiovascular, metabolic, or kidney disease
    - If the prospective participant does report having known/diagnosed cardiovascular, metabolic or kidney disease, then physician's approval will be required in order to be eligible for study enrollment.
- Confirmation of on at least 6-months of a stable disease-modifying therapy (DMT) regimen
- Right-handedness
- Not engaging in at least 150 minutes of moderate-to-vigorous physical activity behavior
- Not currently engaging in cognitive rehabilitation or participating in regular brain fitness activities
- Not currently pregnant

If a participant passes the above inclusion/exclusion criteria, the project coordinator will then administer the TICS-M over the phone to ensure that all participants are not decisionally impaired. In order to pass this inclusion criterion, participants must demonstrate TICS-M scores of 18 or higher. We further will administer the Mini Mental State Examination (MMSE) during the in-person screen to confirm a lack of decisional-impairment (see 5.2 Research Procedures below). If participants are not decisionally impaired, the project coordinal will request contact information for 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, we will perform an in-person screening visit after informed consent is provided (See 5.2 Research Procedures below). If a participant does not qualify for the study, they will be notified via telephone and asked if they would be willing to be contacted for future research opportunities from our laboratory.

5.1.4 Randomization. As this study represents a RCT, participants will be randomly assigned to either a treadmill walking exercise or active control condition. We anticipate that 44 participants will be randomly assigned to each condition. Briefly, the treadmill walking exercise condition will involve supervised treadmill walking exercise that progresses in terms of duration and intensity over the course of 3-months. Participants will attend 36 sessions that will each last approximately 60 minutes, and will take place 3-times per week. The active control condition will involve supervised stretching-and-toning activities that will occur over the course of 3-months. Participants will attend 36 sessions that will each last approximately 60 minutes, and will take place 3-times per week.

5.1.5 Participant Payment. Participants are eligible to be remunerated up to $380 if enrolled (if excluded after the in-person screening, $50 for attending the screening). The disbursements will be distributed such that for enrolled participants, $100 will be paid after completion of Baseline Visit 2, up to $180 after Exercise Visit 36 (prorated at a rate of $5/session if participants do not attend all 36 sessions), and $100 after completion of follow-up visit two. The payments will be in the form of a check mailed to the participant's home.

5.1.6. Risks and Minimization of Risks.

Exercise*:* The completion of maximal exercise always involves risks of death, acute myocardial infarction, and complications that require hospitalization. Importantly, exhaustion, fatigue, and muscle soreness are associated with maximal exercise, but are temporary symptoms. Additionally, there is a risk of falling when performing walking exercise on a treadmill and when performing the timed 25-foot walk (T25FW) and six-minute walk (6MW) tests. Other risks of completing treadmill walking exercise, the T25FW test, and the 6MW test include arrhythmia, musculoskeletal injury, and difficulty breathing. Some MS patients are thermosensitive with symptoms temporarily increasing or worsening with a large rise in core body temperature. We note that MS patients are able to complete a maximal exercise in a room temperature laboratory without any adverse symptoms arising [29,30]. Risks associated with the stretching and toning activities include strains, cramps, and musculoskeletal injury; however, these risks are small as the stretching and toning activities are performed at minimal exercise intensity, and derive from the NMSS guidelines for stretching with MS.

The risks of exercise will be minimized by screening for individuals with factors placing them at increased risk for complications. This includes an initial health risk screening to minimize the risk of cardiovascular complications during maximal and submaximal exercise [31,32]. This procedure further is consistent with American Heart Association recommendations for exercise testing [33]. Importantly, although increasing age is a risk factor for cardiovascular disease, there is no evidence that age *per se* is a strong predictor of exercise-associated cardiovascular complications, and the referral of individuals for medical clearance solely based on age is associated with unnecessary health care referrals [32]. Additionally, participants with MS will be ambulatory without assistance, relapse free for 30 days, and will not have acutely taken steroids in that time period. These criteria will allow for healthy samples of MS patients. Further, all personnel are trained in CPR and emergency lab procedures. To minimize the risks of exhaustion, fatigue, and muscle soreness associated with aerobic exercise, participants will be encouraged to warm-up and/or stretch before any assessment of exercise. To minimize the risk of falling when walking on the treadmill, we will encourage the use of handrails on the treadmill as well as having a gait belt around the participant's waist and two research assistants within

arm's reach. To minimize the risk of overheating, given that some persons with MS are thermosensitive, the testing environment will be controlled with an air conditioner (i.e., manipulated by keeping the ambient air temperature below normal room temperature) and there will be fans that blow air directly on the participant. Participants will further wear clothing appropriate for exercise. Such environmental regulation will keep the participant cool during the sessions and avoid symptom exacerbation.

MRI*:* Regarding the MRI/fMRI, the main risk is related to the presence of metal within the body (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). The risks of MRI/fMRI to fetuses are believed to be minimal, but are nevertheless not known. In general, participants may experience fatigue, anxiety, or claustrophobia while confined in the small space of the magnet. The MRI/fMRI scanner is very loud and may cause auditory discomfort. MRI does not involve ionizing radiation and static magnetic fields of up to 8T are considered as non-significant risk.

Participants will be screened thoroughly to determine whether there could be any known or unknown metal in the body. Because the risks of MRI/fMRI to fetuses are believed to be minimal, but are nevertheless not known, a pregnancy test will be administered to all females on the day of the MRI/fMRI scan, and participation of pregnant women in this study will not be allowed. In general, participants may experience fatigue, anxiety, or claustrophobia while confined in the small space of the magnet. Participants will be briefed in detail about what is experienced during the scanning session. This should reduce problems related to anxiety or claustrophobia. The scanner is very loud, so participants will be given headphones and earplugs. Participants will be able to be heard by the scanner operator at all times, and will be given a button to push if at any time they want to be removed from the scanner. Thus, subjects will have control over their presence in the magnet, which in turn tends to minimize feelings of claustrophobia.

Neuropsychological Testing*:* There are potential risks of becoming frustrated when completing the neuropsychological assessments. Fatigue is another risk associated with neuropsychological assessment.

To minimize the risks of frustration during neuropsychological testing, we will provide clear instructions and practice tests. Further, in the event that a participant has difficulty comprehending any assessment, we will verbally summarize the objective in lay language, and if necessary, will read the questionnaire instructions out loud. To minimize the risks associated with fatigue during neuropsychological testing, we will provide small breaks if necessary. Alternatively, if a participant is too fatigued to continue testing, we will divide the assessments over a 2-day period.

Functional Outcomes*:* There exists some minor but unlikely psychosocial risk associated with the completion of psychological questionnaires. For example, some participants might experience embarrassment or anxiety in responding to some questions. The assessment of ability to perform activities of daily living and functional mobility (i.e., Life-Space Mobility Assessment) involve activities that are common in everyday life. Thus, a risk for a fall during these activities is no greater than what these individuals would experience in a normal day.

In the context of functional testing, a research assistant will be standing next to the participant to provide the subject with support if they were to lose their balance. This will provide an added measure of safety that is not present in their normal activities. In the event that a participant has difficulty comprehending any assessment, we will verbally summarize the objective in lay language, and if necessary, will read the questionnaire instructions out loud. To minimize the risks associated with the T25FW test, we will have multiple research assistants within arm's reach at all times, per standardized protocol [34]. In the event that a participant has

difficulty comprehending any assessment, we will verbally summarize the objective in lay language, and if necessary, will read the questionnaire instructions out loud.

5.1.7 Special Populations. Pregnant women, human fetuses and neonates will not be included in the study. Additionally, children and prisoners will not be included in the study. Adverse events will be immediately reported to the study Statistician and Safety Officer, in addition to the Kessler Foundation IRB.

5.1.8 Informed Consent. The PI, Project Coordinator, or Research Assistants will present the study at the in-person screening visit and ample time will be allotted for potential participants to ask questions and have them answered by study personnel. The consent form will be reviewed page by page, and again time will be allotted for questions and answers. Finally, when the study protocol and consent have been reviewed and all questions have been addressed, individuals agreeing to participate will sign the consent form. Each participant will be given a copy of her signed consent form (by email). All conversations regarding the study will be held in a private setting (i.e., a testing room within Kessler Foundation's Neuroscience and Neuropsychology Laboratory).

5.1.9 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 a locked office at Kessler Foundation. Electronic data will be stored on Kessler Foundation's 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, are required to access confidential information.

5.2 Research Procedures

5.2.1 Outcomes.

Cognition*:* This study will involve several neuropsychological tests of cognitive functioning. Based on our pilot work, the primary neuropsychological outcome for measuring cognitive processing speed (CPS) in the current proposal will be the oral version of the Symbol Digit Modalities Test (SDMT) [35]. This test is considered a more pure measure of CPS, as it relies less on working memory (i.e., the central executive) than other neuropsychological measures that are commonly used in MS research (i.e., the Paced Auditory Serial Addition Test; PASAT) [36]. The SDMT further is a better predictor of whole-brain atrophy and T2-lesion volume than the PASAT [36]. Indeed, the SDMT has emerged as the best predictor of future cognitive decline in persons with relapsing-remitting MS [37]. As such, it is often considered to be a proxy of generalized cognitive

impairment in this population [38]. The current proposal further will include other exploratory neuropsychological measures of CPS. Those measures include the PASAT [39], modified flanker task [40], Pattern Comparison Test [41], and DKEFS Trail-Making and Color-Word Interference Tests [42]. Such measures will be included to examine the generalizability of the effects of treadmill walking exercise training on the overall construct of CPS. Those tests have strong psychometric properties (e.g., [43]), and are commonly-used to document the efficacy of cognitive rehabilitation interventions in persons with MS (e.g., [44]).

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 [45] as exploratory outcomes. This includes valid and reliable tests of learning and memory [46,47], executive function [42], verbal fluency [48], and spatial perception [48]; the SDMT and PASAT further are included in the MACFIMS. As has been recommended for administration of MS-specific neuropsychological batteries, we will apply a near-vision test (e.g., Rosenbaum Pocket Vision Screener) during screening to ensure that visual acuity is adequate (i.e., corrected vision better than 20/80) to perform the tests as intended [45]. This inclusion criterion is not expected to greatly affect enrollment. We further will apply a short neuropsychological test of IQ (i.e., WTAR). For all neuropsychological tests, alternate forms will be applied at each testing session to minimize the effects of practice on cognition.

Brain Function (thalamocortical 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 Skyra 3T clinical imager housed in the Rocco Ortenzio Neuroimaging Center. The structural imaging scan will be performed to measure thalamic and frontal cortex volume, and the resting-state scan will be performed in order to measure functional connectivity between the thalamus and frontal cortex. These two scans will collectively last approximately 30 minutes. Importantly, all MRI processing and analyses will be performed by Dr. Glenn Wylie, who will be blinded to condition. To our knowledge, this is among the first exercise randomized controlled trials 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 graded exercise test to exhaustion 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. We have successfully used this method of assessing cardiorespiratory fitness in persons with MS in several pilot studies [49]. This protocol further is commonly used for measuring cardiorespiratory fitness in older adults [50] and persons with chronic stroke [51] and is consistent with the American College of Sports Medicine (ACSM) guidelines for exercise testing of MS patients [31]. 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 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. The test will be followed by a 3-minute cool-down period.

Functional Outcomes: To measure community participation, we will administer the Community Integration Questionnaire (CIQ) [52]. This measure is particularly sensitive to the effects of MS-related CPS impairment

[53]. We will administer the Lawton-Brody Instrumental Activities of Daily Living (IADL) Scale [54] as a measure of ability to perform activities of daily living; worse IADL performance has been associated with lower SDMT scores in persons with MS [55]. We will administer the Multiple Sclerosis Impact Scale-29 (MSIS-29) [56] as a self-report measure of MS-specific QOL; scores on this questionnaire have been strongly associated with performance on the SDMT [57]. Lastly, we will measure functional mobility using T25FW test and Life-Space Mobility Assessment (LSMA). The T25FW is considered the best-characterized measure of MS-related mobility and has been associated with MS-related cognitive impairment [34]. The LSMA is a measure of real-world mobility. This is a well-validated, composite measure of a person's frequency of movement in geographically defined life-space zones [58].

Disability Status*:* Neurological impairment will be assessed with the Expanded Disability Status Scale (EDSS) [59]. The EDSS is a standard neurological exam for persons with MS and will be supervised by Dr. Sandroff, who is a Neurostatus-certified examiner.

Walking Endurance: We further will include the 6MW as a test of walking endurance, as this is the best characterized test of walking endurance in persons with MS based on its psychometric properties [60].

Questionnaires: We will include several questionnaires in the study that will assess psychological variables. Those questionnaires include the Exercise Self-Efficacy Scale (EXSE [61]), Functional Assessment of Multiple Sclerosis Scale (FAMS [62]), Physical Activity Self-Regulation Scale (PASR-12 [63]), NEO-Five Factor Inventory (NEO-FFI [64]), Beck Depression Scale Fast-Screen [65], and Hospital Anxiety and Depression Scale (HADS [66]).

Functional MRI Tasks: During the fMRI procedure, we will include 3 runs of a behavioral task. This will include a modified Letter Comparison Task [mLC; 41]. Each mLC run will last approximately 5 minutes.

5.2.2 Protocol Overview. The proposed study involves a single-blind, randomized controlled trial (RCT) design. Participants will undertake baseline assessments, and will then be randomly assigned into either the 3-month intervention or 3-month active control condition. Following the 3-month study period, participants will return to the laboratory and complete follow-up outcome measures (i.e., the same measures as baseline, using alternate forms where applicable) by assessors who are unaware of which condition participants were randomly assigned to.

5.2.3 Baseline Testing. The baseline outcome assessments will be spread out across 2 visits for minimizing fatigue and interference from cognitive tests. There will be approximately 2 days separating each of these testing visits. Collectively, baseline testing will involve completion of the in-person screening measures, neuropsychological tests, measures of walking, physical function tests, questionnaires, cardiorespiratory fitness test, and MRI scan. Importantly, all data collection will be done in-person, and will not involve collection of data from medical records.

5.2.3.1 Baseline Visit 1: In-Person Screening Session. Once the project coordinator receives the verification of the participant's definite MS diagnosis and confirmation of being on at least 6-months of a stable DMT regimen, the project coordinator will schedule the participant for the first baseline testing visit via telephone—this will also include several in-person screening measures that will take place at the outset of this session. Baseline Visit 1 (i.e., including the in-person screening component) will occur at Kessler Foundation. Participants will complete the following procedures during the in-person screening session within the first baseline testing visit:

- Participants will first provide written informed consent
- Participants will then undertake the MMSE
    - In order to participate in the remainder of the study, participants will demonstrate scores of 21 or higher
- Participants will then undertake the SDMT
    - The SDMT involves pairing 9 abstract geometric symbols with single-digit numbers as quickly and accurately as possible over 90 seconds
        - In order to participate in the remainder of the study, participants will demonstrate slowed CPS based on SDMT scores at least 1 *SD* below the regression-based normative score for healthy controls (i.e., 16th percentile)
- A neurological examination by Dr. Rinker, the study neurologist, for confirming that all participants are fully-ambulatory based on EDSS scores between 0-4.0
    - During this neurological examination, participants will need to demonstrate corrected visual acuity better than 20/80 in order to participate in the study.
- Participants will then complete a detailed screening for MRI contraindications to ensure that participants will have a low risk for contraindications for MRI based on:
    - 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.
- The final in-person screening item involves the measurement of resting blood pressure using an automated sphygmomanometer (i.e., MedQuip BP2400).
    - Participants who demonstrate systolic blood pressure values of > 200 mmHg or diastolic blood pressure values > 110 mmHg will not be eligible to participate in the study, based on the American College of Cardiology/American Heart Association 2002 Guideline Update for Exercise Testing.
- Importantly, persons who are excluded from the study based on this in-person screening component of the baseline session, will be remunerated $50 for participating in the baseline testing session, and will not undertake the remaining baseline outcome assessments.

5.2.3.2 Baseline Visit 1: Other Outcome Measures. Following the completion of the in-person screening measures, participants will undertake the remainder of the baseline outcome assessments as described in Item 14.b above. For the remainder of Baseline Testing Visit 1, participants will complete the following outcome measures:

- Cognitive testing:
    - CPS Tests:
        - PASAT: Single digit numbers are played on an audio recording and participants add the last two numbers together out loud that they heard on the recording
        - Modified Flanker Task: Participants respond to simple and complex visual stimuli (i.e., arrows) presented on a laptop, as quickly and accurately as possible.
        - Pattern and Letter Comparison Test: Participants view two patterns and two sets of letters on a page and indicate as quickly as possible whether or not the two patterns or sets of letters are the same or not the same
        - DKEFS Trail-Making Test: Participants connect circles containing numbers and letters using paper and pencil as quickly and accurately as possible

        - DKEFS Color-Word Interference Test: Participants read words of colors that are written in a certain color ink, and then participants are instructed to name the color ink that the word is written in, as quickly and accurately as possible
    - Spatial perception test:
        - Judgement of Line Orientation Test: Participants are shown two different lines and are asked to tell the examiner whether the angles of the two lines are the same
    - Executive functioning test:
        - DKEFS Sorting Test: Participants are asked to sort 6 different cards in as many ways as possible in a 4-minute period
    - IQ Test:
        - Weschler Test of Adult Reading: Participants are asked to pronounce a series of words correctly
- 6MW Test:
    - Participants are asked to walk as fast and as far as possible for 6 minutes
    - During the 6MW test, a researcher will be within an arm's reach at all times

- T25FW Test:
    - Participants walk as quickly and as safely as possible over a 25-foot distance; 2 trials are performed. During the T25FW, a researcher will be within an arm's reach at all times

- Sit and Reach Test:
    - Participants will be seated on a mat, with their legs extended. Participants will be asked to bend at the waist and try to touch their toes (reaching as far as possible without bending the knees).

- Graded exercise test to exhaustion:
    - This will be performed on a motor-driven treadmill and an open-circuit spirometry system, using a modified Balke protocol (see above for the full procedure).

5.2.3.3 Baseline Visit 2. 2-days following the completion of Baseline Visit 1, participants will complete Baseline Visit 2, which will take place at the Rocco Ortenzio Neuroimaging Center at Kessler. During Baseline Visit 2, participants will complete the following procedures, as described above:

- Cognitive Tests:
    - Learning and memory tests:
        - California Verbal Learning Test-II: Participants are read a list of 16 words and are instructed to remember as many words as possible, and repeat the list back to the examiner
        - Brief Visuospatial Memory Test-Revised: Participants are shown a page with 6 abstract geometric figures, and then after a 10 second study period, are asked to draw the figures on a blank sheet of paper from memory
    - Verbal fluency test:
        - Controlled Oral Word Association Test: Participants are asked to name as many words beginning with the letters F, A, and S as possible in a 60 second period
- Questionnaires: EXSE, FAMS, PASR-12, NEO-FFI, BDI, HADS (These items are attached)
- Functional tests:
    - Community participation:
        - CIQ: See attached questionnaire
    - Activities of daily living:
        - Lawton-Brody IADL Scale: See attached questionnaire
    - Quality of life:
        - MSIS-29: See attached questionnaire
    - Walking Mobility:
        - LSMA: See attached questionnaire

- MRI behavioral task practice:
    - Participants will sit at a laptop outside of the scanner and complete 1 trial of the mLC (a version of the Letter Comparison Task wherein participants indicate if two strings of letters are the same or different).
- MRI protocol:
    - Participants will undergo functional, diffusion-weighted, and structural magnetic resonance images of the brain. The MR instrument that will be used is an FDA-approved Siemens Skyra 3 T clinical imager.
    - The MRI is for research purposes only, and not part of a participant's clinical care
    - The MRI will not involve any dye/contrast
    - Data are acquired using an FDA-approved head coil.
    - Data are acquired with acquisition strategies, or pulse sequences, that include both standard product sequences by Siemens and customized sequences designed by Dr. Glenn Wylie at Kessler Foundation through the manufacturer-provided, and FDA approved, sequence development environment.
        - Pulse sequences are computer code and tell the MRI scanner when to emit radio frequency energy and how to sample the data space in order to form an image.
            - Examples of different data space sampling strategies include evenly-spaced checkerboard grid sampling and sampling data along a spiraling trajectory.
    - Subjects will wear disposable earplugs and full-ear headphones to reduce scanner noise during all scans.
    - A series of functional, diffusion-weighted, and structural MRI images will be acquired, which are necessary for comparing functional connectivity findings with brain structure.
    - Participants will complete the modified Letter Comparison task while in the scanner via a back-projection screen located at the head of the MRI scanner.
    - Each MRI session will take approximately 1 hour
    - The subject will be free to quit the study at any time, either by indicating their desire to do so directly to the investigator or by pressing a button while in the MRI machine that alerts the technologist who is running the scanner

Participants will be remunerated $100 in the form of a check following the completion of Baseline Visit 2.

5.2.4 Randomization. Following baseline testing, participants will be randomly assigned to either the exercise training or active control condition using concealment and a computerization. Participants further will blinded to condition (i.e., unaware that the treadmill walking exercise training condition represents the experimental condition and the stretching-and-toning condition represents the active control condition).

5.2.5 Experimental Condition: Treadmill Walking Exercise Training. The experimental 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 Kessler Foundation. Exercise intensities will be prescribed based on percent oxygen consumption reserve (% VO_2R) using values derived from the baseline graded exercise test. See the table below for the exact exercise prescription. 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 [31]. 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 [31] (Weeks 3-8). 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 [31] (Weeks 9-12). 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 [31].

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				

The exercise training itself will be led by trained exercise leaders (i.e., research assistants) who are not involved in the collection of outcome assessments at Kessler Foundation under the supervision of Dr. Sandroff. 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 heart rate reserve (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. Importantly, under the supervision of Dr. Sandroff, with guidance from Prof. Robert Motl at the University of Alabama at Birmingham, we will apply highly developed principles and techniques associated with Social Cognitive Theory to maximize adherence and compliance with the intervention. This will involve certified exercise leaders delivering topics for enhancing adherence and compliance (i.e., self-efficacy, goal setting, overcoming barriers to exercise, monitoring of performance feedback, promotion of realistic outcome expectations, importance of social support) during actual exercise training sessions. 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 [67], well-being, enjoyment, and mental/physical fatigue.

At the conclusion of exercise sessions 1, 7, 13, 19, 25, and 31, participants will be invited to engage in discussions with the exercise leaders regarding adherence and compliance that will last approximately 30 minutes following those sessions. These sessions will be led by the exercise leader under the supervision of Dr. Sandroff, and with guidance from Prof. Motl, will involve the delivery of information based on Social

Cognitive Theory principles for enhancing adherence and compliance [68]. Specifically, this involves the provision of newsletters and opportunities to ask questions on topics such as outcome expectations, facilitators and barriers to exercise, goal setting, social support, and self-efficacy. An example newsletter on outcome expectations is attached in this application.

5.2.6 Control Condition: Stretching-and-toning activities. The active, non-aerobic exercise control condition will involve stretching-and-toning activities in order to control for the effects of social contact and attention. This condition will take place at Kessler Foundation. The stretching-and-toning control condition will be delivered using the same frequency and duration of the treadmill walking exercise condition. The stretching-and-toning activities will be based on a manual provided by the NMSS and sessions will be led by trained exercise leaders who are not involved in the collection of outcome assessments at Kessler Foundation under the supervision of Dr. Sandroff. This further will occur in a laboratory environment within Kessler Foundation that is isolated from the intervention condition in order to avoid contamination. Stretching-and-toning activities 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 stretches 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 CRF 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 warm-up of up to 10 minutes, followed by stretching-and-toning (following the same duration as the treadmill walking exercise training condition) activities, and a cool-down of up to 10 minutes.

As is the case for the experimental condition, HR will be monitored throughout each session. To minimize attrition, as is the case for the intervention condition, under the supervision of Dr. Sandroff, with guidance from Prof. Motl, certified exercise leaders will apply highly developed principles and techniques associated with Social Cognitive Theory for enhancing participant adherence and compliance to the stretching-and-toning prescription during each session. Participants will complete a stretching-and-toning log at the conclusion of each session for better characterizing experience with the intervention. Log data will include perceived exertion [67], well-being, enjoyment, and mental/physical fatigue. All participants will further be asked to not undertake additional exercise (i.e., not join a gym and begin exercising) or engage in cognitive rehabilitation over the study period.

At the conclusion of stretching-and-toning sessions 1, 7, 13, 19, 25, and 31, participants will be invited to engage in discussions with the exercise leaders regarding adherence and compliance that will last approximately 30 minutes following those sessions. These sessions will be led by the exercise leader under the supervision of Dr. Sandroff, and with guidance from Prof. Motl, will involve the delivery of information based on Social Cognitive Theory principles for enhancing adherence and compliance [68]. Specifically, this involves the provision of newsletters and opportunities to ask questions on topics such as outcome expectations, facilitators and barriers to exercise, goal setting, social support, and self-efficacy. An example newsletter on outcome expectations is attached in this application.

Participants will be remunerated $5 per treadmill walking exercise/stretching-and-toning visit attended (i.e., up to $180). The final remunerated amount will be delivered to participants after attending the last visit in the form of a check.

5.2.7 Follow-up Testing. Within 48 hours following the completion of the last treadmill walking exercise training/stretching-and-toning session, the Project Coordinator will contact the participant and schedule them for follow-up testing. As is the case for baseline, this will occur across 2 visits that will be separated by a 2-day period. The first Follow-Up Visit will occur at the Kessler Foundation, and the second Follow-Up Visit will occur at the Rocco Ortenzio Neuroimaging Center at Kessler Foundation. Procedures for Follow-Up Visits 1 and 2 will be identical to that of Baseline Visits 1 and 2. Participants will be remunerated $100 at the conclusion of Follow-Up Visit 2 in the form of a gift card.

5.3 Analysis. The data analyses will be overseen by a biostatistician (i.e., Dr. Gary Cutter at Pythagoras, LLC) and follow intent-to-treat principles (i.e., include all persons once randomized regardless of adherence and/or compliance). In the case of a drop-out, missing data will be imputed by carrying the last observed value forward. We further will perform exploratory data analyses in only 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 [69]. The analytic plan will account for several potential confounders of the effects of progressive TMWX training on the outcome measures; these confounders include MS duration, BMI, age, sex, T_2-lesion volume, and relapse rate, if those variables differ between groups. Further, all study data are entered and analyzed in SPSS version 25 (IBM Inc., Armonk, NY).

The primary analysis will involve a statistically efficient, hierarchical step-down testing approach using ANCOVA models to examine differences in post-test outcomes of CPS and thalamocortical RSFC (i.e., examining group differences in follow-up outcomes, adjusting for baseline as covariate). The hierarchical step-down approach presents a series of conditional hypothesis tests after the primary hypothesis is assessed. This *a priori* specifies the importance and order of the testing so that each hypothesis is tested in order, until the first non-significant result occurs. Once a null-hypothesis is not rejected, all formal testing will stop, and all subsequent results will be reported as *post-hoc* and/or descriptive. This protects against Type I error as it assumes that the outcomes are correlated, and the overall *p*-value is protected by reporting the largest *p*-value for all the tests. As the primary study outcome involves CPS based on SDMT scores, we will first perform an ANCOVA model on follow-up SDMT score, with group (intervention or control) as a between-subjects factor and baseline SDMT score as the covariate. If statistically significant (i.e., $p<.05$), we will then assess the effects of the intervention on the general construct of CPS using MANCOVA and on the individual CPS tests using separate, univariate ANCOVA models.

Regarding thalamocortical RSFC, the next set of analyses will involve performing ANCOVA models on follow-up RSFC between the thalamus and frontal cortical areas (e.g., SFG, MFG [21]), respectively, with group (intervention or control) as a between-subjects factor and baseline RSFC between the thalamus and frontal cortical areas, as the covariates. We will require an alpha of .05 (corrected for multiple comparisons) for significance for thalamocortical RSFC 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). Based on our pilot work [19,21], we expect significant group differences in SDMT score and RSFC between the thalamus and frontal cortical areas, respectively, at follow-up after controlling for baseline scores, whereby participants who are randomly assigned to the intervention group will demonstrate greater SDMT scores and stronger thalamocortical RSFC at follow-up compared with those who are randomly assigned to the control group.

As there are multiple, interrelated functional outcomes that are included as exploratory study outcomes, we will perform a multivariate analysis of covariance (MANCOVA) on follow-up scores for the functional outcomes controlling for baseline values as an approach for examining the consistency of the potential intervention effects (i.e., intervention vs. control as the between-subjects factor) on this overall construct (i.e., how functional consequences of CPS impairment may change together with TMWX training). This will involve clustering outcomes into like sets of variables. We further will explore the effects of the intervention on the individual outcomes using separate exploratory, descriptive, univariate ANCOVA models on follow-up CIQ scores (i.e., community participation), Lawton-Brody IADL scores (i.e., ability to perform activities of daily living), MSIS-29 scores (i.e., QOL), 6MW, T25FW, and LSMA performance (i.e., functional mobility), respectively, with group (intervention or control) as a between-subjects factor and baseline CIQ, Lawton-Brody IADL, MSIS-29, 6MW, T25FW, and LSMA scores, respectively, as the covariates. We expect significant and consistent group differences in those functional outcomes at follow-up after controlling for baseline scores, whereby participants who are randomly assigned to the intervention condition will demonstrate better follow-up scores on functional outcomes compared with those who are randomly assigned to the control condition.

As a manipulation check, we will perform similar ANCOVA models to examine differences in post-test CRF [VO_{2peak}] (i.e., examining group differences in follow-up VO_{2peak}, adjusting for baseline as covariate). We expect statistically significant group differences in VO_{2peak} at follow-up, after controlling for baseline VO_{2peak}, such that those who underwent the TMWX training intervention will have greater CRF relative to those who underwent the active control condition.

Effect sizes will primarily be expressed as partial eta-squared (η_p^2) [70]. Effect sizes further will be reported as Cohen's *d* in order to characterize the standardized mean difference of the effects of TMWX training on the outcomes [70] and for ease of inclusion in subsequent meta-analyses [70]. The effect sizes from the interaction terms from the ANCOVAs on CPS, thalamocortical RSFC, functional outcomes, and CRF will serve as effect sizes for the subsequent power analyses required for informing the development of a multi-site, Phase II/III RCT.

6. Work Schedule

	Year 1		Year 2				Year 3				Year 4			
Objective	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4	Quarter 1	Quarter 2	Quarter 3	Quarter 4
Hire personnel	x	x												
Train personnel		x												
Purchase equipment and supplies	x													
IRB Approval	x													
Recruit participants			x	x	x	x	x	x	x	x	x	x		
Data collection and entry			x	x	x	x	x	x	x	x	x	x		
Data analysis											x	x		
Present data at scientific meetings											x	x	x	x
Manuscript preparation and publication											x	x	x	x

7. Cost analysis

See attached budget.

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Statistical Design and Power
The proposed Phase-I/II clinical trial will use a parallel group, randomized controlled trial (RCT) design. The primary study outcomes involve the oral Symbol Digit Modalities Test (SDMT) as a measure of cognitive processing speed (CPS) and thalamocortical resting-state functional connectivity (RSFC) measured using fMRI. The exploratory study outcomes involve functional outcomes that are associated with CPS in persons with MS (i.e., community integration, ability to perform activities of daily living, quality of life, and functional mobility).

Sample Size Considerations. As described in the original application (i.e., R01HD091155-A1), we conducted a conventional power analysis using G*Power (Version 3.1.9.5) and estimated the appropriate sample size for detecting differences in follow-up SDMT scores, controlling for baseline score (Specific Aim 1). Using G*Power, this was accomplished using the 'F test' selection within the 'Test' family menu, and the Statistical Test of 'ANCOVA: Fixed effects, main effects and interactions'. The effect size (η_p^2=0.11) for the power analysis was computed from our pilot RCT[12] of TMWX training compared with a waitlist control condition on SDMT scores in persons with MS who had mild ambulatory disability, but not CPS impairment. That effect size was smaller than the effect sizes for thalamocortical RSFC outcomes, and thus was included in the main power analysis as a conservative approach. The power analysis included standard assumptions of alpha (0.05) and beta (0.80). The power analysis indicated that the minimal total sample size for testing group differences on follow-up SDMT scores, controlling for baseline scores should be 66 participants (33 per group). This supports our goal of recruiting 88 participants (44 per condition) as yielding adequate power, based on retention of 75% of the enrolled participants. The current application proposes a change of grantee organization whereby the final 72 participants will be enrolled at KF. We will explore the possible influence of study site on the outcomes in *post-hoc* sensitivity analyses. We expect that the effects of study site are likely negligible given the constants of the study team and standardized manuals of operating procedures that guide all study aspects.

Statistical Analysis. The data analyses will be overseen by Dr. Cutter and follow intent-to-treat principles (i.e., include all persons regardless of dropout). In the case of a drop-out, missing data will be imputed 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. The analytic plan will account for several potential confounders of the effects of progressive TMWX training on the outcome measures. These include MS duration, BMI, age, sex, cognitive reserve, T2-lesion volume, and relapse rate. If these variables differ between groups, they will be included as additional covariates in the following analyses.

Specific Aim 1. The primary analysis will involve a statistically efficient step-down testing approach using ANCOVA models to examine changes in CPS and thalamocortical RSFC (i.e., examining group differences in follow-up outcomes, adjusting for baseline). As the primary study outcome involves CPS based on SDMT scores, we will first perform an ANCOVA model on follow-up SDMT score, with group (exercise or control) as a between-subjects factor and baseline SDMT score as the covariate. If statistically significant (i.e., p<.05), we will then assess the effects of the intervention on thalamocortical RSFC. This second step will involve performing two separate ANCOVA models on follow-up RSFC between the thalamus and right MFG and ACC, respectively, with group (exercise or control) as a between-subjects factor and baseline RSFC between the thalamus and right MFG and ACC, respectively, as the covariates. We will require an alpha of .05 (corrected for multiple comparisons) for significance for thalamocortical RSFC 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). Based on our pilot work[12], we expect significant group differences in SDMT score and RSFC between the thalamus and right MFG and ACC, respectively, at follow-up after controlling for baseline scores, whereby participants who are randomly assigned to the exercise group will demonstrate greater SDMT scores and stronger thalamocortical RSFC at follow-up compared with those who are randomly assigned to the control group.

Specific Aim 2. We will examine RSFC between thalamus/right MFG, and RSFC between thalamus/ACC, respectively, as two potential mediators of the effects of TMWX training on CPS, consistent with the methodologies of Baron and Kenny[89] and MacKinnon et al[90]. 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[28]. As pre-conditions of the mediation analysis, we will first perform correlations among group (i.e., exercise or control), change in SDMT scores, and change in RSFC between the thalamus and right MFG and ACC, respectively, using Spearman's rho rank-order correlations (ρ)[91] to test if those outcomes are interrelated. Consistent with our pilot work[12], we expect that TMWX training will be associated with improvements in CPS and increases in thalamocortical RSFC. Moreover, we expect that improvements in CPS will be associated with increases in thalamocortical RSFC. Then, to establish mediation, we will perform hierarchical linear regression models for evaluating each of the two mediators (i.e., changes in RSFC between thalamus/right MFG, and RSFC between thalamus/ACC, respectively), separately. This will involve regressing change in SDMT score on group in Step 1 and then adding change in the mediator in Step 2. In order to establish mediation, if the pre-conditions are met, group must influence change in SDMT in Step 1, and the mediator must influence change in SDMT score in Step 2 of the regression as well as attenuate the effect of group on SDMT scores (i.e., the effect of group on SDMT should be reduced and become non-significant between Step 1 and Step 2)[89]. As such, we expect significant effects of group on change in CPS, and that the effect of group (i.e., exercise or control) on change in CPS will be attenuated, but not reach zero, when accounting for the effects of changes in thalamocortical RSFC. In other words, we expect that changes in thalamocortical RSFC will be partial mediators of the effects of TMWX training on CPS in persons with MS with impaired CPS.

Exploratory Aim 3. Considering that MS-related CPS impairment substantially limits daily functioning, we now include exploratory functional outcomes in this proposal, as suggested by reviewers. As there are multiple, interrelated outcomes within this Exploratory Aim, we will perform a multivariate analysis of covariance (MANCOVA) on follow-up scores for the functional outcomes controlling for baseline values as an approach for examining the consistency of the potential intervention effects (i.e., exercise vs. control as the between-subjects factor) on this overall construct (i.e., how functional consequences of CPS impairment may change together with TMWX training). We further will explore the effects of the intervention on the individual outcomes using separate exploratory, descriptive, univariate ANCOVA models on follow-up CIQ scores (i.e., community participation), TIADL scores (i.e., ability to perform activities of daily living), MSIS-29 scores (i.e., QOL), T25FW and LSPA performance (i.e., functional mobility), respectively, with group (exercise or control) as a between-subjects factor and baseline CIQ, TIADL, MSIS-29, T25FW, and LSPA scores, respectively, as the covariates. We expect significant and consistent group differences in those functional outcomes at follow-up after controlling for baseline scores, whereby participants who are randomly assigned to the exercise group will demonstrate better follow-up scores on functional outcomes compared with those who are randomly assigned to the control group.

As MS-related cognitive impairment involves reduced performance in other cognitive domains beyond CPS, we now include exploratory cognitive outcomes beyond the SDMT in the current proposal, as suggested by reviewers. We will adopt a similar approach (*see Exploratory Aim 3 above*) to examine the effects of the intervention on the general construct of cognitive performance using MANCOVA and on the individual cognitive tests using separate univariate ANCOVA models. Effect sizes will be expressed as partial eta-squared (η_p^2) and Cohen's d[92]. The effect sizes from the interaction terms from the ANCOVAs and correlations will serve as effect sizes for the subsequent power analyses required for informing the development of a home-based rehabilitation program using this intervention approach.

Analysis by Race/Gender. We anticipate that 70% of participants will be female and 70% will be Caucasian. We will test the effects of gender and race on the intervention effects, and if there are interactions, we will separately analyze by gender/race to explore the specific effects of the variables on the outcomes. We do recognize that we will not likely enroll sufficient numbers of male participants or participants of Hispanic ethnicity and or other races to make meaningful comparisons.

Treatment of Missing Data. We conservatively plan for approximately 25% attrition based on a previous large RCT of 6-months (i.e., a longer intervention period than the proposed study) of multimodal exercise training versus a stretching-and-toning control condition on mobility outcomes in persons with moderate-to-severe MS-related ambulatory disability in our laboratory[56]. In that study, the adherence rates for both the exercise and stretching-and-toning conditions were upwards of 75%, despite a significant barrier to participation being the inclusion of persons with substantial ambulatory disability. Of note, those adherence rates are *lower* than the overall average adherence rates for exercise trials involving persons with MS, based on a recent systematic review (i.e., upwards of 84% for both exercise and control conditions)[57]. Nevertheless we have plans for

exploring and examining the impact of such losses, as we could have upwards of 25% drop-out, given that this sample includes persons with objective CPS impairment. Initially, analyses will be done on the data set using last observation carried forward and then on the actual data collected (case-wise deletion), followed by multiple imputation for missing data. All analyses at present are planned using SPSS software (version 25 or higher), although as new options continue to evolve with SAS or R; these will be considered if there are new tools for imputation that will help understand the impact of missing data. We believe that these imputations should involve fewer than 15% of the participants, which then should have minimal impact on the analyses. However, it is important to assess this from a best case and worst case scenario and if the results are consistent, then we can be more confident of our findings. If these analyses yield differing results – more exploratory analyses are required to understand if the differences in the outcomes are coming from the control condition or the intervention condition. If the control condition, the implications can be examined in one way and if the intervention condition, it is likely to add cautionary notes to the findings.

Interim and Final Analysis. We do not plan any interim efficacy or futility analyses. The study team will work together for publications on the protocol, fidelity monitoring plan, as well as the primary study outcomes.