BMJ Open

Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis: The ASPIRE trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039691
Article Type:	Protocol
Date Submitted by the Author:	22-Apr-2020
Complete List of Authors:	Kever, Anne; Columbia University Irving Medical Center, Neurology Nelson, Katherine; Columbia University Irving Medical Center Aguerre, Ines; Columbia University Irving Medical Center Riley, Claire; Columbia University Irving Medical Center Boehme, Amelia; Columbia University Medical Center, Lee, Nancy; Columbia University Irving Medical Center Strauss Farber, Rebecca; Columbia University Irving Medical Center Levin , Seth; Columbia University Irving Medical Center Stein, Joel; Columbia University Irving Medical Center Leavitt, Victoria; Columbia University Irving Medical Center
Keywords:	Multiple sclerosis < NEUROLOGY, Clinical trials < THERAPEUTICS, SPORTS MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis: The ASPIRE trial

Anne Kever, PhD[1], Katherine E. Nelson[1,2,3], Ines M. Aguerre[1,2,3], Claire S. Riley, MD[3], Amelia K. Boehme, PhD[4], Nancy W. Lee[5], Rebecca Straus Farber, MD[3], Seth N. Levin, MD[3], Joel Stein, MD[5], Victoria M. Leavitt, PhD[1,2,3]

[1] Translational Cognitive Neuroscience Laboratory, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[2] Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[3] Multiple Sclerosis Center, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[4] Department of Neurology and Epidemiology, Columbia University Irving Medical Center, New York, New York
[5] Department of Rehabilitation and Regenerative Medicine, Columbia University Vangelos College of Physicians and Surgeons, New York, New York

Submission type: Protocol paper
Abstract Word Count: 217
Manuscript Word Count (excluding abstract, figures, references, and footnotes): 3039
Number of Figures: 2
Number of References: 51

Corresponding author:

Victoria M. Leavitt, Ph.D., FAAN
Assistant Professor of Neuropsychology
Department of Neurology, Columbia University Irving Medical Center
630 W. 168th Street, New York, NY 10032
email:VL3227@cumc.columbia.edu

ABSTRACT

Introduction The many benefits of exercise for persons with multiple sclerosis (MS) are well established, yet patients often refrain from exercise due to overheating and exhaustion. The present randomized controlled trial tests aspirin (acetylsalicylic acid, ASA) as a convenient method to prevent overheating and improve exercise performance in persons with MS.

Methods and analysis Participants are seen for a laboratory maximal exercise test on three separate days separated by at least one week. At each session, body temperature is measured before oral administration of a standard adult dose (650 mg) of aspirin, acetaminophen (APAP), or placebo. Participants then perform a maximal ramp test on a cycle ergometer. Primary outcomes are (a) time to exhaustion (TTE, i.e., time spent cycling to peak exertion) and (b) body temperature change. Cross-over analyses will include tests for effects of treatment, period, treatment–period interaction (carryover effect) and sequence.

Ethics and dissemination Ethical approval was granted by a local Institutional Review Board (IRB; reference: AAAS2529). Results of the trial will be published in peer-reviewed scientific journals and presented at national and international conferences. Neurologists, physiatrists, primary care physicians, and physiotherapists are important stakeholders and will be targeted during dissemination. Positive trial results have the potential to promote aspirin therapy, an inexpensive and readily-available treatment targeting overheating to enhance everyday exercise performance in persons with MS.

Trial registration identifier NCT03824938 (approved February 2019)

Strengths and limitations of the study

- The rigorous double-blind, placebo-controlled, crossover design will expose all participants to three conditions, ensuring robust results and reducing the influence of confounding covariates.
- Positive findings from this trial will provide an effective, inexpensive, readily available, unobtrusive treatment that will avail more persons with MS the benefits of exercise.
- For this initial trial, our sample is limited to patients with relapsing-remitting MS (RRMS) although the potential benefits of aspirin should also be tested in persons with progressive forms of MS in future studies.
- The selected dose of aspirin (650 mg) was chosen based on two prior trials in MS as well as our own pilot trial, but future trials employing a lower dose are warranted.

Introduction

Exercise holds numerous benefits for persons with MS.[1,2] Regular exercise has been shown to increase muscle strength[3], improve balance[4], decrease fatigue and depressive symptoms[5–7], and enhance overall quality of life for persons with MS (pwMS).[8] Furthermore, a growing body of research supports exercise as potentially disease modifying. MS patients enrolled in a 24-week program of exercise showed an increase in serum levels of circulating BDNF[9] and reduced pro-inflammatory circulating cytokines (i.e., IL-22) relative to those who maintained a sedentary lifestyle over the same period of time.[5] In addition, just 12-weeks of aerobic exercise resulted in increased hippocampal volume and hippocampal functional connectivity, as well as improved memory in pwMS (compared to a stretching condition).[10]

Studies, however, show that pwMS are less physically active than age-matched healthy adults[11] regardless of disability,[12] and engage in less activity than their physical capacity allows.[13] In fact, fewer than 20% of pwMS achieve adequate amounts of daily physical activity,[14] and activity levels further decrease over time as the disease develops.[15]

Despite its clear benefits, many individuals with MS avoid exercise because of overheating and exhaustion.[16] This well-described elicitation of heat sensitivity in MS is known as "Uhthoff's phenomenon," an observation presented in the MS literature over 125 years ago that has spawned a vast literature describing heat-related changes.[17–22] Importantly, several studies provide evidence that exercise is a safe intervention for pwMS without serious adverse effects and no increased relapse risk.[8,23,24] However, patients themselves may refrain from exercising due to transient discomforts presented by overheating.[25]

In everyone, exercise triggers the conversion of metabolic to mechanical energy to produce muscle contractions, liberating approximately 30-70% of the body's total energy as heat and causing a subsequent increase in core temperature.[26] As internal body temperature rises, physical fatigue increases and exercise performance worsens.[27] In pwMS, this slight increase in body temperature can lead to prohibitive levels of exhaustion during exercise, perhaps due to increased basal body temperature previously noted in this population.[19,28] Cooling methods (e.g., ice baths, cooling

contraptions or garments, cooled environments) have effectively improved exercise performance for pwMS in prior studies (none of which were double blind), [29–33] although such methods can be cumbersome, expensive, or unavailable.

Our recent double-blind, crossover design pilot RCT of aspirin (compared to placebo) significantly improved exercise performance and reduced exercise-induced overheating in pwMS.[34] Here, we present a detailed protocol of our large-scale trial of aspirin for exercise, ASPIRE: a double-blind, crossover RCT comparing the effects of aspirin and placebo on exercise performance and exercise-induced body temperature increase in pwMS. We also incorporate a third arm, acetaminophen (APAP) to examine a potential alternative treatment for exercise in pwMS. We hypothesize that compared to placebo, ASA and APAP will result in improved exercise performance (i.e., longer TTE) and a reduction in exercise-induced body temperature increase, through their antipyretic mechanism.

Methods and analysis

Study design

This is a double-blind, crossover, placebo-controlled, randomized intervention design to examine the efficacy of an oral antipyretic to improve exercise performance and reduce exercise-induced body temperature in persons with MS. The trial comprises the following three arms: Aspirin (ASA) was selected based on our prior exercise pilot as well as two prior trials showing its efficacy for reducing fatigue in non-exercising pwMS.[35,36] ASA has a favorable safety profile, with no adverse events noted in these prior trials, nor more than minimal side effects (i.e., no different than placebo). Acetaminophen (APAP) was included to investigate mechanism: whereas aspirin is pleiotropic, APAP is an antipyretic with much weaker/absent anti-inflammatory properties. Furthermore, if APAP is found to be as effective as aspirin, it will provide another option that may be preferable for some pwMS. The third arm is a placebo drug.

Participants are randomized to one of six sequences (see figure 1) with double-blind maintained by the study biostatistician (AKB) until data collection for the full sample is complete. Each

participant engages in three exercise sessions held at the same time (within ±1 hour) on separate days with ≥ 1-week intervals. All sessions are scheduled between 10 am - 5 pm to reduce the influence of circadian rhythm variability. Duration of each session is ~2 hours total (details in Procedure).

---------- insert Figure 1 here----------

Participants

Inclusion criteria. Participants are included if they meet the following criteria: (1) Diagnosis of relapsing-remitting MS based on the 2017 McDonald criteria,[37] (2) aged between 18- 65 (upper limit is set to reduce effects of age-related activity level decreases and to ensure that our sample is representative of the relapse-onset MS population); (3) answer yes to the question: Is overheating during exercise a problem for you; (4) low to moderate physical disability (i.e., Expanded Disability Status Scale (EDSS) total score ≤ 6.0); (5) relapse-free for 6 weeks prior; (6) BMI ≤ 35 (to reduce health-related confounds of obesity).

Exclusion criteria. Patients are excluded if they have any prior history of head injury, stroke, or other neurological disease/disorder; are currently taking antipyretics or pain medication daily; are currently in a depressive episode (screened with the BDI-FS, excluded if total score ≥ 9)[38], or present other psychiatric diagnoses; were formally diagnosed with sleep disorder; pulmonary disease, heart disease or other heart problem; suffer from vascular disease of the legs, high blood pressure; currently take medications for high blood pressure or any heart problem; have diabetes mellitus or problems with blood sugar levels; present any contraindications to ASA use, i.e., history of confirmed peptic ulcer, gastrointestinal or severe gynecological bleeding; have tarry stool or fecal occult blood; syndrome of asthma, rhinitis, or nasal polyps; present any contraindications to APAP use, i.e., severe active hepatic disease, Hepatitis C Virus.

Sample size determination. In a pilot trial with 12 participants, effect sizes for both outcomes (TTE and body temperature) were very large (Cohen's d = 1.45). Here, we conservatively estimated medium sized effects (0.5) to calculate a power estimate for the proposed trial. With significance set at $\alpha = 0.05$ for 2-tailed paired t-test, a crossover sample of 54 will yield 0.95 power to detect differences between interventions. Our plan to enroll 60 participants allows for 10% attrition.

Recruitment and consent. Based on a-priori power analysis, 60 patients with a diagnosis of RRMS will be enrolled. The sample is projected to comprise 66% females and 33% males, consistent with disease demographics. All participants will be recruited from the Multiple Sclerosis Center at the Columbia University Irving Medical Center (CUIMC). One hundred patients will be screened, conservatively estimating that 60% will meet eligibility. The Medical Director of the MS Center (CSR) and a dedicated research coordinator (KN) lead patient recruitment and confirm the disease-specific inclusion criteria. To avoid coercion, participants are not invited to participate by their treating neurologist. Instead, a research coordinator provides participants with an IRB-approved recruitment advertisement for the study, with instructions to contact the research coordinator if she/he is interested in participating. Participants receive weekly reminder emails and phone calls to promote patient retention and completion of follow-up visits. Data from participants who discontinue or deviate from intervention protocols are excluded from final analyses. Participants are expressly informed that participation in this study is voluntary, not part of their medical care, affords no advantage to their health, and that refusal to participate results in no negative consequences. Before commencement of the study, participants sign a consent form approved by the Institutional Review Board at CUIMC. The latter also details the provisions taken in case of injury or harm as a result of participating in the study, i.e., care provided through CUIMC or any other health provider. Participants receive $100 at the completion of their third exercise session as compensation for time and travel expenses.

Allocation, randomization, blinding, and data protection. Using a random number generator, each participant is assigned to one of six randomization schedules: placebo-APAP-ASA, placebo-ASA-APAP, ASA-APAP-placebo, ASA-placebo-APAP, APAP-ASA-placebo, APAP-placebo-ASA. The research pharmacy (providing all study drug) receives a randomization schedule to ensure that necessary procedures for maintaining the double-blind are in place. The key containing participant

ID and randomization schedule assignment is kept by the study biostatistician for the duration of the active period of the trial and will be provided to the study PI only after all participants have been tested. To test effectiveness of blinding, participants are asked which condition they think they were in at the end of each session. In order to safeguard confidentiality, participants are identified by a number and the master key is password protected, and accessible only to personnel directly involved in the study.

Intervention and procedure

Pharmaceutical pretreatment. Room temperature is held constant to within ±5 degrees Fahrenheit and recorded at the start of each session. Participants are instructed to refrain from eating two hours prior to their session. Upon arrival to the lab, written informed consent is obtained from all participants. Body temperature is measured with a tympanic thermometer (right ear; Braun Thermoscan IRT), the most accurate method for external measurement of body temperature.[39] Participants receive one of three treatments (ASA, APAP, placebo) at each session prior to the exercise test. Order of treatment is randomized and counter-balanced, with each participant serving as his/her own control. Treatment is administered as one capsule containing 650 mg of ASA, APAP, or placebo. ASA dose was selected on the basis of prior successful RCTs of ASA to reduce fatigue in non-exercising patients with MS.[35,36] Overencapsulation of ASA and manufacture of placebo is handled by the CUMC research pharmacy. Time of drug administration is recorded, and exercise does not begin until 1 hour later to allow for peak serum level to be reached.[40] During this time, participants complete a series of questionnaires to characterize the sample (for details, see Behavioral Measures).

Exercise test. Exercise tests are conducted by a certified exercise physiologist (NL). All exercise testing equipment is current and certified for use with human subjects. Cardiopulmonary exercise testing is performed using Vmax Encore Metabolic Cart (CareFusion Corp, San Diego, CA) and Electrordiogram (EKG) monitoring is done with a twelve-lead system attached to the Vmax Encore MetabolicCart with cardiosoft software (CareFusion Corp). Peak ventilatory capacity (Maximum Voluntary Ventilation, MVV) is determined before the exercise test via Vmax Encore

System (CareFusion Corp). Maximum aerobic fitness (VO2max) is measured during the graded maximal exercise test on a VIAsprint 150P electronic-braked cycle ergometer (CareFusion Corp). Participants complete a 5-minute resting phase followed by a 3-minute warm up and then start the progressive ramped exercise. An individualized ramping protocol is determined by a certified exercise physiologist based on the exercise frequency and intensity that each patient self-reports. For participants who exercise irregularly and maximum 0-2 times/week, resistance increases by 5W/minute, those who exercise regularly 2-4 times/week follow a 10W/minute protocol, while those who exercise more than 4 times/week are assigned to a 15W/minute protocol. However, depending on the type of exercise, ramping protocols can be adapted (e.g., 4 yoga sessions/week vs 4 high intensity cycling sessions/week). Participants are instructed to maintain a cadence of 50-60 revolutions per minute (RPM) for as long as possible. The test is terminated when cadence drops below 40 RPM for ≥ 5 seconds, or when the participant reaches volitional exhaustion in accordance with American Thoracic Society (ATS) standard test termination criteria.[41]

The following biophysiological data are collected during the exercise session: heart rate (HR) (every 60 seconds), blood pressure (BP) and tympanic temperature (every 120 seconds from minute 2 onwards), respiratory exchange ratio (RER), minute ventilation (i.e., total sum of volume delivered over a minute), expired oxygen (O2), carbon dioxide output (CO2), and maximum watts unleashed during the test. At exercise termination, total time to exhaustion (TTE; i.e., time before the given work rate cannot be maintained and there is cessation of the exercise), our main outcome variable, is recorded.

Behavioral Measures

Pre-exercise. During the 1h period between drug administration and start of the exercise test, participants complete the following questionnaires: the Hospital Anxiety and Depression Scale (HADS)[42] and the Fatigue Severity Scale (FSS)[43] to characterize the sample; the Paffenbarger Physical Activity Scale[44] for post-hoc evaluation of physical activity levels; the day of last menstrual cycle (if female), number of hours slept during prior night, any physical/outdoor activities engaged in today, presence of illness in self or family members today; and 10-point visual analog scales (VAS) of pain, fatigue, and sadness.

During exercise. Borg's ratings of perceived exertion scale (RPE) for breathing (RPEbr) and muscle fatigue (RPEleg) [45,46] and the thermal sensation scale (ASHRAE scale)[47] are completed at 60-second intervals throughout the session (consistent with Collett et al. 2016).[48]

Post-exercise. At termination, participants provide a last RPEbr and RPEleg, complete a 10-point VAS of fatigue, mood, and pain, and rate their Global Fatigue Change (GFC).[35]

---------- insert Figure 2 here----------

Trial status

Participant recruitment commenced on February 20th, 2019, and data collection completion is anticipated for February 2021. Currently, 34 participants have been enrolled in the study, and 16 have completed all sessions. The diversity of our sample is notable: 26% of participants are male, 9% are black, 6% are Hispanic. Recruitment has been facilitated by the appeal of this study to individuals with MS, for whom the idea of a possible treatment to reduce the discomfort of overheating during exercise resonates. Participants in the trial are uniquely motivated; upon completing a session, one patient recently stated: "I always feel so much better after these study visits!"

Data analysis

Statistical methods recommended for the proper analysis of cross-over trial data will be used (for details, see Wellek and Blettner, 2012).[49] Our primary aims consist in examining differences between ASA, APAP, and placebo with respect to (a) total time to exhaustion (TTE, i.e., length of time spend exercising) and (b) increase in exercise-induced body temperature (degrees Fahrenheit).

Differences between pretreatment conditions (APA, APAP, placebo) will be analyzed separately by sequence group (i.e., stepwise assessment of outcome differences between ASA and placebo/APAP and placebo/ ASA, APAP and placebo). Because ASA and APAP have short-term effects and the 1-week wash-out period is more than adequate, we expect no carryover effects. However, an unpaired t statistic will test within-subject differences in outcomes between Session 1 and Session 2, comparing sequence group AB (e.g., ASA -APAP) and sequence group BA (e.g., APAP-ASA). Although significant differences in outcome variables between ASA and APAP are not anticipated, we are interested in seeing how they compare in the full analyses. A mixed-effect linear model will be used to account for repeated measurements of each outcome, period effect, sequence effect, and carryover effect. Using mixed effects linear model will enable us to model differences in outcomes both within and between patients (intra- and inter-patient variability). If assumptions for mixed effects linear model are not met and log transformation is not appropriate, nonlinear mixed effects models will be used. Moreover, exploratory analyses will examine potential pretreatment differences for additional outcome measures, such as physiological indices (e.g., HR, BP) and behavioral self-reports (e.g., RPEs, fatigue, pain).

Patient and public involvement

The ASPIRE trial developed through a commitment to clinically informed and therapeutically meaningful translational research, building upon more than 125 years of observational evidence for overheating in MS coupled with anecdotal patient reports of overheating as a deterrent to exercise. Our center's MS patients have been involved throughout the development and execution of the study, providing valuable feedback to inform our selection of outcome variables and optimize our study design. Moreover, participant feedback from a previously conducted small pilot trial[34] was considered while developing the present study.

Ethics and Dissemination

This study has been approved by the Columbia University Irving Medical Center Institutional Review Board (IRB) (reference: AAAS2529) and all participants will provide informed consent prior to the study commencement. Results will be disseminated at relevant national and international meetings, conferences, and workshops. We also plan to publish our findings in peer-

reviewed, high-impact journals and aim at encouraging other research groups to investigate clinically-relevant questions rigorous experimental designs (see, e.g., Dalgas et al., 2020 for a discussion on how to bolster the quality and scope of exercise studies in MS).[50] Positive results will also be shared with medical caregivers, especially MS clinical practitioners. Furthermore, our MS Center at Columbia University Irving Medical Center distributes a bi-annual newsletter for patients that reports current research opportunities and highlights published study results to create a 360-degree feedback loop and acknowledge the essential role of patients in the success of our research. Patient feedback reveals this to be highly motivating.

Overall, the present study attempts to test the effectiveness of aspirin a treatment to reduce overheating (and associated discomfort) during exercise in pwMS. A series of cooling methods already exist but they are cost prohibitive in many instances, unavailable, or cumbersome. Exercising in an air-conditioned gym, for instance, appears at first glance to be a simple and obvious recommendation. But not everyone has the financial and practical means regularly visit a gym. Aspirin, however, is inexpensive, FDA approved, readily available, and has a favorable safety profile. If this trial is successful and confirms the finding of our pilot study[51], it will support the first widely available symptomatic treatment for MS that has the potential to make a meaningful difference. For research on exercise in MS, having a pretreatment to reduce exercise-induced overheating and exhaustion will mitigate sample bias, i.e., extant study samples likely overrepresent patients for whom overheating is not an issue, limiting generalizability. Furthermore, a positive trial outcome would set the stage for future work investigating, for example, daily aspirin use to increase free living physical activity levels.

Authors' contributions AK wrote the entire manuscript and made alterations based on co-authors comments; KEM coordinated patient recruitment and data collection, provided important input during manuscript drafting; IMA coordinated patient recruitment and reviewed the manuscript for intellectual content; CSR reviewed the manuscript for intellectual content; NWL conceptualized the exercise test and informed the choice of the physiological outcome measures; AKB provided the statistical analysis plan, SN and RSF helped with patient recruitment; JS reviewed the manuscript for intellectual content; VML, as the Principal Investigator, designed and conceptualized the study and reviewed the manuscript for intellectual content, has overall leadership of the trial. All authors reviewed draft versions and approved the final manuscript.

Funding This work was supported by a grant from the National Institutes of Health (National Center for Medical Rehabilitation Research within the National Institute of Child Health and Human Development) to VML (HD091836-01A1).

CSR reports consulting or advisory work with Biogen Idec, Celgene, Genentech/Roche, Genzyme, TG Therapeutics.

SNL has received a fellowship training grant through Genentech and honoraria for advisory work with Biogen.

VML has received consulting fees from Healios, Inc, and is the Co-Founder of eSupport Health, PBC.

References

1. Owen, N., Sparling, P. B., Healy, G. N., Dunstan, D. W. & Matthews, C. E. Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin. Proc.* **85**, 1138–41 (2010).
2. Veldhuijzen van Zanten, J. J., Pilutti, L. A., Duda, J. L. & Motl, R. W. Sedentary behaviour in people with multiple sclerosis: Is it time to stand up against MS? *Mult. Scler. J.* **22**, 1250–1256 (2016).
3. Petajan, J. H. *et al.* Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann. Neurol.* **39**, 432–41 (1996).
4. Gunn, H., Markevics, S., Haas, B., Marsden, J. & Freeman, J. Systematic Review: The Effectiveness of Interventions to Reduce Falls and Improve Balance in Adults With Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **96**, 1898–912 (2015).
5. Alvarenga-Filho, H. *et al.* Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple sclerosis patients in response to neuromediators. *J. Neuroimmunol.* **293**, 91–99 (2016).
6. Cooney, G. M. *et al.* Exercise for depression. *Cochrane Database Syst. Rev.* **9**, CD004366 (2013).
7. Pilutti, L. A., Greenlee, T. A., Motl, R. W., Nickrent, M. S. & Petruzzello, S. J. Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosom. Med.* **75**, 575–80.
8. Motl, R. W. Benefits, safety, and prescription of exercise in persons with multiple sclerosis. *Expert Rev. Neurother.* **14**, 1429–36 (2014).
9. Wens, I. *et al.* Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. *Eur. J. Neurol.* **23**, 1028–35 (2016).

10. Leavitt, V. M. *et al.* Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: Preliminary findings. *Neurocase* **20**, 695–697 (2014).
11. Casey, B., Coote, S., Galvin, R. & Donnelly, A. Objective physical activity levels in people with multiple sclerosis: Meta-analysis. *Scand. J. Med. Sci. Sports* **28**, 1960–1969 (2018).
12. Motl, R. W., McAuley, E. & Snook, E. M. Physical activity and multiple sclerosis: a meta-analysis. *Mult. Scler. J.* **11**, 459–463 (2005).
13. Marrie, R. *et al.* High frequency of adverse health behaviors in multiple sclerosis. *Mult. Scler. J.* **15**, 105–113 (2009).
14. Klaren, R. E., Motl, R. W., Dlugonski, D., Sandroff, B. M. & Pilutti, L. A. Objectively Quantified Physical Activity in Persons With Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **94**, 2342–2348 (2013).
15. Motl, R. W., McAuley, E. & Sandroff, B. M. Longitudinal Change in Physical Activity and Its Correlates in Relapsing-Remitting Multiple Sclerosis. *Phys. Ther.* **93**, 1037–1048 (2013).
16. Stroud, N., Minahan, C. & Sabapathy, S. The perceived benefits and barriers to exercise participation in persons with multiple sclerosis. *Disabil. Rehabil.* **31**, 2216–2222 (2009).
17. Flensner, G., Ek, A.-C., Söderhamn, O. & Landtblom, A.-M. Sensitivity to heat in MS patients: a factor strongly influencing symptomology - an explorative survey. *BMC Neurol.* **11**, 27 (2011).
18. Leavitt, V. M., Sumowski, J. F., Chiaravalloti, N. & Deluca, J. Warmer outdoor temperature is associated with worse cognitive status in multiple sclerosis. *Neurology* **78**, 964–968 (2012).
19. Leavitt, V. M. *et al.* Elevated body temperature is linked to fatigue in an Italian sample of relapsing–remitting multiple sclerosis patients. *J. Neurol.* **262**, 2440–2442 (2015).

20. Marino, F. E. Heat reactions in multiple sclerosis: an overlooked paradigm in the study of comparative fatigue. *Int J Hyperth.* **25**, 34–40 (2009).

21. Skjerbaek, A. G. *et al.* Heat sensitive persons with multiple sclerosis are more tolerant to resistance exercise than to endurance exercise. *Mult Scler* **19**, 932–940 (2013).

22. White, A. T., Vanhaitsma, T. A., Vener, J. & Davis, S. L. Effect of passive whole body heating on central conduction and cortical excitability in multiple sclerosis patients and healthy controls. *J Appl Physiol 1985* **114**, 1697–1704 (2013).

23. Pilutti, L. A., Platta, M. E., Motl, R. W. & Latimer-Cheung, A. E. The safety of exercise training in multiple sclerosis: A systematic review. *J. Neurol. Sci.* **343**, 3–7 (2014).

24. Dalgas, U. & Stenager, E. Progressive resistance therapy is not the best way to rehabilitate deficits due to multiple sclerosis: No. *Mult. Scler. J.* **20**, 141–142 (2014).

25. Kayes, N. M., McPherson, K. M., Taylor, D., Schlüter, P. J. & Kolt, G. S. Facilitators and barriers to engagement in physical activity for people with multiple sclerosis: a qualitative investigation. *Disabil. Rehabil.* **33**, 625–642 (2011).

26. Edwards, R. H., Hill, D. K. & Jones, D. A. Heat production and chemical changes during isometric contractions of the human quadriceps muscle. *J. Physiol.* **251**, 303–315 (1975).

27. González-Alonso, J. *et al.* Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J. Appl. Physiol.* **86**, 1032–1039 (1999).

28. Sumowski, J. F. & Leavitt, V. M. Body Temperature Is Elevated and Linked to Fatigue in Relapsing-Remitting Multiple Sclerosis, Even Without Heat Exposure. *Arch. Phys. Med. Rehabil.* **95**, 1298–1302 (2014).

29. Grahn, D. A., Murray, J. V & Heller, H. C. Cooling via one hand improves physical performance in heat-sensitive individuals with multiple sclerosis: a preliminary study. *BMC Neurol* **8**, 14 (2008).

30. Op 't Eijnde, B., Keytsman, C., Wens, I. & Hansen, D. Whole-body cooling does not compromise muscle oxidative capacity in subjects with multiple sclerosis. *NeuroRehabilitation* **35**, 805–11 (2014).

31. Grover, G. *et al.* Environmental temperature and exercise modality independently impact central and muscle fatigue among people with multiple sclerosis. *Mult. Scler. J. - Exp. Transl. Clin.* **3**, 205521731774762 (2017).

32. White, A. T., Wilson, T. E., Davis, S. L. & Petajan, J. H. Effect of precooling on physical performance in multiple sclerosis. *Mult. Scler.* **6**, 176–180 (2000).

33. Devasahayam, A. J. *et al.* Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. *BMC Neurol.* **20**, 33 (2020).

34. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* (2017) doi:10.1177/1352458517739138.

35. Wincheruk, D. M. *et al.*. A randomized controlled crossover trial of aspirin for fatigue in multiple sclerosis. Neurology [online serial]. 2005;64:1267– 1269. Accessed at: http://www.ncbi.nlm.nih.gov/pubmed/15824361. *Neurology* **64**, 1267– 1269 (2005).

36. Shaygannejad, V., Janghorbani, M., Ashtari, F. & Zakeri, H. Comparison of the effect of aspirin and amantadine for the treatment of fatigue in multiple sclerosis: a randomized, blinded, crossover study. *Neurol. Res.* **34**, 854–858 (2012).

37. Thompson, A. J. *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173 (2018).

38. Benedict, R. H., Fishman, I., McClellan, M. M., Bakshi, R. & Weinstock-Guttman, B. Validity of the Beck Depression Inventory-Fast Screen in multiple sclerosis. *Mult. Scler. J.* **9**, 393–396 (2003).

39. Chamberlain, J. *et al.* Determination of Normal Ear Temperature with an Infrared Emission Detection Thermometer. *Ann. Emerg. Med.* **25**, 15–20 (1995).

40. Aspirin Comprehensive Prescribing Information [online].

41. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med Online Ser.* 211–277 (2003).

42. Zigmond, A. S. & Snaith, R. P. The Hospital Anxiety and Depression Scale. *Acta Psychiatr. Scand.* **67**, 361–370 (1983).

43. Krupp, L. B. The Fatigue Severity Scale: Application to Patients With Multiple Sclerosis and Systemic Lupus Erythematosus. *Arch. Neurol.* **46**, 1121 (1989).

44. Washburn, R. A., Smith, K. W., Jette, A. M. & Janney, C. A. Washburn RA, Smith KW, Jette AM, Janney CA. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol* **46**, 153–162 (1993).

45. Borg, G. A. V. Psychophysiological bases of perceived exertion. *Med. Sci. Sports Exerc.* **14**, 377–381 (1982).

46. Morrison, E. H. *et al.* Ratings of Perceived Exertion During Aerobic Exercise in Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **89**, 1570–1574 (2008).

47. Humphreys, M. A. & Hancock, M. Humphreys, M. A., & Hancock, M. (2007). Do people like to feel 'neutral'?: Exploring the variation of the desired thermal sensation on the ASHRAE scale. *Energy Build.* **39**, 867–874 (2007).

48. Collett, J., Meaney, A., Howells, K. & Dawes, H. Acute recovery from exercise in people with multiple sclerosis: an exploratory study on the effect of exercise intensities. *Disabil. Rehabil.* **39**, 551–558 (2017).

49. Wellek, S. & Blettner, M. On the Proper Use of the Crossover Design in Clinical Trials. *Dtsch. Aerzteblatt Online* (2012) doi:10.3238/arztebl.2012.0276.

50. Dalgas, U. *et al.* Moving exercise research in multiple sclerosis forward (the MoXFo initiative): Developing consensus statements for research. *Mult. Scler. J.* 135245852091036 (2020) doi:10.1177/1352458520910360.

51. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* **24**, 1511–1513 (2018).

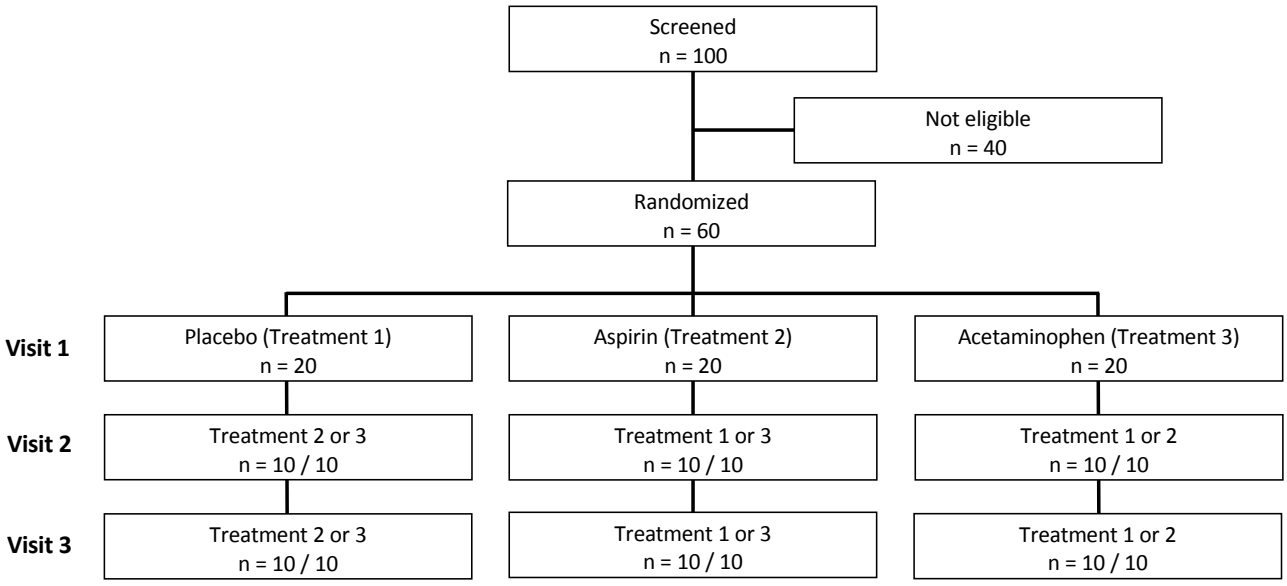

Figure 1. Design of the double-blind, cross-over, randomized and placebo-controlled trial

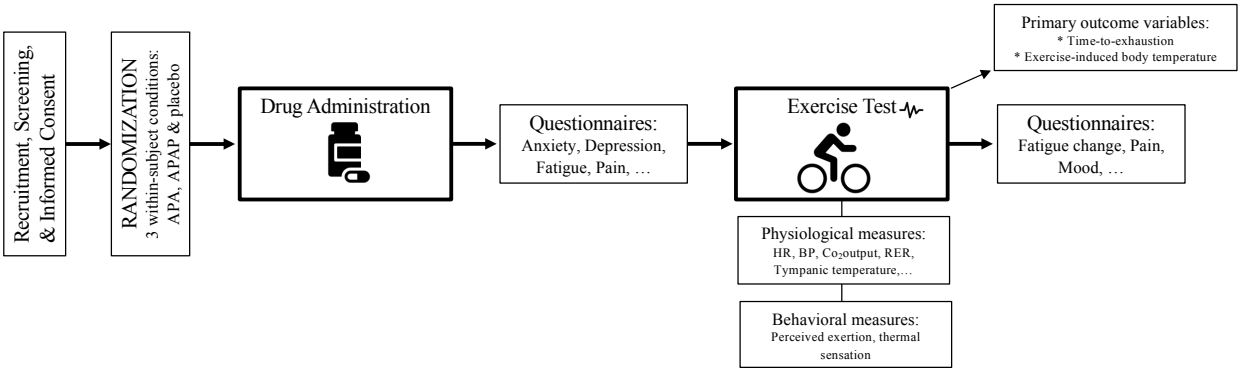

Figure 2. Overview of experimental procedure

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	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 & 12
	5b	Name and contact information for the trial sponsor	12
	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	_____NA____

	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)	NA
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	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
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)	5
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	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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7

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	9
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)	5-6
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	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7

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	7
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	7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	?

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	9
	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	7
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	10-11
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	10-11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10-11
	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)	10-11
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	NA
	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	7
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	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Detailed upon request
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	____7_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____NA_____
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	__7-8_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____1_____
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	____7-8_____
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	____7 _____
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	__11-12_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	Added to submission form
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided as additional file

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	NA

*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.

BMJ Open

The ASPIRE trial: Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039691.R1
Article Type:	Protocol
Date Submitted by the Author:	12-Aug-2020
Complete List of Authors:	Kever, Anne; Columbia University Irving Medical Center, Neurology Nelson, Katherine; Columbia University Irving Medical Center Aguerre, Ines; Columbia University Irving Medical Center Riley, Claire; Columbia University Irving Medical Center Boehme, Amelia; Columbia University Medical Center, Lee, Nancy; Columbia University Irving Medical Center Strauss Farber, Rebecca; Columbia University Irving Medical Center Levin , Seth; Columbia University Irving Medical Center Stein, Joel; Columbia University Irving Medical Center Leavitt, Victoria; Columbia University Irving Medical Center
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Multiple sclerosis < NEUROLOGY, Clinical trials < THERAPEUTICS, SPORTS MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The ASPIRE trial: Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis

Anne Kever, PhD[1], Katherine E. Nelson[1,2,3], Ines M. Aguerre[1,2,3], Claire S. Riley, MD[3], Amelia K. Boehme, PhD[4], Nancy W. Lee[5], Rebecca Strauss Farber, MD[3], Seth N. Levin, MD[3], Joel Stein, MD[5], Victoria M. Leavitt, PhD[1,3]

[1] Translational Cognitive Neuroscience Laboratory, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[2] Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[3] Multiple Sclerosis Center, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[4] Department of Neurology and Epidemiology, Columbia University Irving Medical Center, New York, New York
[5] Department of Rehabilitation and Regenerative Medicine, Columbia University Vangelos College of Physicians and Surgeons, New York, New York

Submission type: Protocol paper
Abstract Word Count: 245
Manuscript Word Count (excluding abstract, figures, and references): 3145
Number of Figures: 2
Number of References: 48

Corresponding author:

Victoria M. Leavitt, Ph.D., FAAN
Assistant Professor of Neuropsychology
Department of Neurology, Columbia University Irving Medical Center
630 W. 168th Street, New York, NY 10032
email: vl2337@cumc.columbia.edu

ABSTRACT

Introduction The many benefits of exercise for persons with multiple sclerosis (MS) are well established, yet patients often refrain from exercise due to overheating and exhaustion. The present randomized controlled trial tests aspirin (acetylsalicylic acid, ASA) as a convenient method to prevent overheating and improve exercise performance in persons with MS. The effects of ASA are compared to those of acetaminophen (APAP), and placebo.

Methods and analysis Participants are seen for a laboratory maximal exercise test on three separate days separated by at least one week. At each session, body temperature is measured before oral administration of a standard adult dose (650 mg) of ASA, APAP, or placebo. One hour after drug administration, participants perform a maximal ramp test on a cycle ergometer. Primary outcomes are (a) time to exhaustion (TTE, i.e., time spent cycling to peak exertion) and (b) body temperature change. Cross-over analyses will include tests for effects of treatment, period, treatment–period interaction (carryover effect) and sequence.

Ethics and dissemination Ethical approval was granted by a local Institutional Review Board (IRB; reference: AAAS2529). Results of the trial will be published in peer-reviewed scientific journals and presented at national and international conferences. Neurologists, physiatrists, primary care physicians, and physiotherapists are important stakeholders and will be targeted during dissemination. Positive trial results have the potential to promote aspirin therapy, an inexpensive and readily-available treatment to reduce overheating and allow more persons with MS to benefit from exercise.

Trial registration identifier NCT03824938 (approved February 2019)

Strengths and limitations of the study

- The rigorous double-blind, placebo-controlled, crossover design will expose all participants to three conditions, ensuring robust results and reducing the influence of confounding covariates.
- Positive findings from this trial will provide an effective, inexpensive, readily available, unobtrusive treatment that will avail more persons with MS the benefits of exercise.
- For this initial trial, our sample is limited to patients with relapsing-remitting MS (RRMS) although the potential benefits of aspirin should also be tested in persons with progressive forms of MS in future studies.
- The selected dose of aspirin (650 mg) was chosen based on two prior trials in MS as well as our own pilot trial, but future trials employing a lower dose are warranted.

Introduction

Exercise holds numerous benefits for persons with multiple sclerosis (pwMS)[1,2] including increased muscle strength,[3] improved balance,[4] decreased fatigue and depressive symptoms,[5–7] and enhanced overall quality of life.[8] Furthermore, a growing body of research supports exercise as potentially disease modifying. In one study, MS patients enrolled in a 24-week program of exercise demonstrated increased serum levels of circulating brain-derived neurotrophic factor (BDNF, critically involved in counteracting inflammatory processes of MS)[9] and reduced pro-inflammatory circulating cytokines (often elevated during exacerbations of MS disease activity) relative to those who maintained a sedentary lifestyle over the same period of time.[5] In addition, another study showed that just 12-weeks of aerobic exercise resulted in increased hippocampal volume and hippocampal functional connectivity, as well as improved memory in pwMS (compared to a stretching condition).[10]

Despite these and other well-documented benefits of exercise, individuals with MS sometimes avoid exercise due to transient discomforts of overheating and fatigue[11]. Studies show that compared to their age-matched healthy peers, pwMS are less physically active regardless of disability and engage in less activity than their physical capacity allows.[12–14] In fact, fewer than 20% of pwMS achieve adequate amounts of daily physical activity, levels that further decrease as the disease develops.[15] The link between exercise and overheating has been known to the field for over a century: in 1890, Wilhelm Uhthoff's famous observation of the deleterious consequences of exercise for patients with MS precipitated a vast literature describing heat-related changes in MS.[16,17]

Exercise triggers the conversion of metabolic to mechanical energy, liberating approximately 30-70% of the body's total energy as heat and thus causing an increase in core temperature.[18] In everyone, as internal body temperature rises, physical fatigue increases and exercise performance worsens.[19] In pwMS, slight increases in body temperature may lead to prohibitive levels of exhaustion during exercise. As a response to this, several cooling methods (e.g., ice baths, cooling contraptions or garments, cooled environments) developed for pwMS have effectively reduced fatigue[20] and improved exercise performance,[21–25] although such methods can be cumbersome,

prohibitively expensive, or unavailable. Also, it is important to note that none of these prior trials of cooling methods in MS were conducted using double-blind study design.

Our recent double-blind, crossover pilot trial demonstrated that aspirin (compared to placebo) significantly improved exercise performance and reduced exercise-induced overheating in pwMS.[26] Here, we present a detailed protocol of our large-scale trial of aspirin for exercise, ASPIRE: a double-blind, crossover RCT comparing the effects of aspirin (ASA), acetaminophen (APAP) and placebo on exercise performance and exercise-induced body temperature increase in pwMS. The second arm, APAP, has been included for two reasons. First, if effective, APAP represents a potential alternative to ASA as a cooling treatment. Second, this third arm allows interrogation of mechanism: whereas aspirin is pleiotropic, APAP has much weaker/absent anti-inflammatory properties thereby isolating (to some degree) the antipyretic mechanism of ASA.[27]

Here, we test the hypothesis that ASA and APAP pre-treatment (compared to placebo) will improve exercise performance and reduce exercise-induced body temperature increase during a controlled, laboratory maximal exercise test in pwMS.

Methods and analysis

Study design

This is a double-blind, crossover, placebo-controlled, randomized intervention design to examine the efficacy of an oral antipyretic (i.e., ASA and APAP) to improve exercise performance and reduce exercise-induced body temperature in persons with MS. The trial comprises the following three arms: aspirin (ASA) was selected based on our prior exercise pilot as well as two prior trials showing its efficacy for reducing fatigue in non-exercising pwMS.[28,29] ASA has a generally favorable safety profile[30], with no adverse events noted in these prior trials, nor more than minimal side effects (i.e., no different than placebo).[28] As noted, acetaminophen (APAP) was included to investigate mechanism: while both ASA and APAP are antipyretic, ASA has prominent anti-inflammatory properties.[27] Furthermore, if APAP is found to be as effective as aspirin, it will provide another option that may be preferable for some pwMS. The third arm is placebo.

Participants are randomized to one of six sequences (see figure 1) with double-blind maintained by the study biostatistician (AKB) until data collection for the full sample is complete. Each participant engages in three exercise sessions held at the same time (within ±1 hour) on separate days with ≥ 1-week intervals. All sessions are scheduled between 10 am - 5 pm to reduce the influence of circadian rhythm variability. Duration of each session is ~2 hours total (details in Procedure).

---------- insert Figure 1 here ----------

Participants

Inclusion criteria. Participants are included if they meet the following criteria: (1) diagnosis of relapsing-remitting MS based on the 2017 McDonald criteria,[31] (2) aged between 18- 65 years (upper limit is set to reduce effects of age-related activity level decreases and to ensure that our sample is representative of the relapse-onset MS population); (3) answer yes to the question: Is overheating during exercise a problem for you?; (4) low to moderate physical disability (i.e., Expanded Disability Status Scale (EDSS) total score ≤ 6.0); (5) relapse-free for 6 weeks prior; (6) BMI ≤ 35 (to reduce health-related confounds of obesity).

Exclusion criteria. Patients are excluded if they have any prior history of head injury, stroke, or other neurological disease/disorder; are currently taking antipyretics or pain medication daily; are currently in a depressive episode (screened with the BDI-FS, excluded if total score ≥ 9)[32], or present other psychiatric diagnoses; were formally diagnosed with sleep disorder; pulmonary disease, heart disease or other heart problem; suffer from vascular disease of the legs, high blood pressure; currently take medications for high blood pressure or any heart problem; have diabetes mellitus or problems with blood sugar levels; present any contraindications to ASA use, i.e., history of confirmed peptic ulcer, gastrointestinal or severe gynecological bleeding; have tarry

stool or fecal occult blood; syndrome of asthma, rhinitis, or nasal polyps; present any contraindications to APAP use, i.e., severe active hepatic disease, Hepatitis C Virus.

Sample size determination. In a pilot trial with 12 participants, effect sizes for both outcomes (TTE and body temperature) were very large (Cohen's d = 1.45). Here, we conservatively estimated medium sized effects (0.5) to calculate a power estimate for the proposed trial. With significance set at $\alpha = 0.05$ for 2-tailed paired t-test, a 3-arm crossover sample of 54 will yield 0.95 power to detect differences between interventions. Our plan to enroll 60 participants allows for 10% attrition, which accounts for both participant drop-out as well as exclusion due to relapses.

Recruitment and consent. Based on a-priori power analysis, 60 patients with a diagnosis of RRMS will be enrolled. The sample is projected to comprise 66% females and 33% males, consistent with disease demographics. All participants will be recruited from the Multiple Sclerosis Center at the Columbia University Irving Medical Center (CUIMC). One hundred patients will be screened, conservatively estimating that 60% will meet eligibility based on our pilot trial.[33] The Medical Director of the MS Center (CSR) and a dedicated research coordinator (KN) lead patient recruitment and confirm the disease-specific inclusion criteria. To avoid coercion, participants are not invited to participate by their treating neurologist. Instead, a research coordinator provides participants with an IRB-approved recruitment advertisement for the study, with instructions to contact the research coordinator if s/he is interested in participating. Participants receive weekly reminder emails and phone calls to promote patient retention and completion of follow-up visits. Data from participants who discontinue or deviate from intervention protocols are excluded from final analyses but will be retained for inspection of any systematic differences. Participants are expressly informed that participation in this study is voluntary, not part of their medical care, affords no advantage to their health, and that refusal to participate results in no negative consequences. Before commencement of the study, participants sign a consent form approved by the Institutional Review Board at CUIMC. The latter also details the provisions taken in case of injury or harm as a result of participating in the study, i.e., care provided through CUIMC or any other health provider. Participants receive $100 at the completion of their third exercise session as compensation for time and travel expenses.

Allocation, randomization, blinding, and data protection. Using a random number generator, each participant is assigned to one of six randomization schedules: placebo-APAP-ASA, placebo-ASA-APAP, ASA-APAP-placebo, ASA-placebo-APAP, APAP-ASA-placebo, APAP-placebo-ASA. The research pharmacy (providing all study drug) receives a randomization schedule to ensure that necessary procedures for maintaining the double-blind are in place. The key containing participant ID and randomization schedule assignment is kept by the study biostatistician for the duration of the active period of the trial and will be provided to the study PI only after all participants have been tested. To test effectiveness of blinding, participants are asked via paper questionnaire which condition they think they were in at the end of each session. In order to safeguard confidentiality, participants are identified by a number and the master key is password protected, and accessible only to personnel directly involved in the study.

Intervention and procedure

Pharmaceutical pretreatment. Room temperature is held constant to within ±5 degrees Fahrenheit and recorded at the start of each session. Participants are instructed to not eat two hours prior to their session (to avoid subsequent increases in body temperature[34]) and to refrain from use of ASA or APAP within 24-hours of their study visit. Upon arrival to the lab, written informed consent is obtained from all participants. Body temperature is measured with a tympanic thermometer (right ear; Braun Thermoscan IRT), a standard, non-invasive method which best approximates core body temperature.[35,36] Participants receive one of three treatments (ASA, APAP, placebo) at each session prior to the exercise test. Order of treatment is randomized and counter-balanced, with each participant serving as his/her own control (see Figure 2). Treatment is administered as one capsule containing 650 mg (i.e., standard adult dose for ASA, APAP) of ASA, APAP, or placebo. ASA dose was selected on the basis of prior successful RCTs of ASA to reduce fatigue in non-exercising patients with MS.[28,29] Overencapsulation of ASA and manufacture of placebo is handled by the CUIMC research pharmacy. Time of drug administration is recorded, and exercise does not begin until 1 hour later to allow for peak serum level to be reached.[37] During this time, participants complete a series of questionnaires to characterize the sample (for details, see Behavioral Measures).

Exercise test. Exercise tests are conducted by a certified exercise physiologist (NL). All procedures are consistent with *American College of Sports Medicine (ACSM)* Guidelines [38]. All exercise testing equipment is current and certified for use with human subjects. Cardiopulmonary exercise testing is performed using Vmax Encore Metabolic Cart (CareFusion Corp, San Diego, CA) and Electrordiogram (EKG) monitoring is done with a twelve-lead system attached to the Vmax Encore Metabolic Cart with cardiosoft software (CareFusion Corp). Peak ventilatory capacity (Maximum Voluntary Ventilation, MVV) is determined before the exercise test via Vmax Encore System (CareFusion Corp). Maximum aerobic fitness (VO2max) is measured during the graded maximal exercise test on a VIAsprint 150P electronic-braked cycle ergometer (CareFusion Corp).

Participants complete a 5-minute resting phase followed by a 3-minute warm up and then start the progressive ramped exercise. An individualized ramping protocol is determined by a certified exercise physiologist based on the exercise frequency and intensity that each patient self-reports. For participants who exercise irregularly and maximum 0-2 times/week, resistance increases by 5W/minute, those who exercise regularly 2-4 times/week follow a 10W/minute protocol, while those who exercise more than 4 times/week are assigned to a 15W/minute protocol. However, depending on the type of exercise, ramping protocols can be adapted (e.g., 4 yoga sessions/week vs 4 high intensity cycling sessions/week). Participants are instructed to maintain a cadence of 50-60 revolutions per minute (RPM) for as long as possible. The test is terminated when cadence drops below 40 RPM for ≥ 5 seconds, or when the participant reaches volitional exhaustion in accordance with American Thoracic Society (ATS) standard test termination criteria.[39]

The following biophysiological data are collected during the exercise session (to ensure participant safety,[38] and for exploratory analyses): heart rate (HR) (every 60 seconds), blood pressure (BP) and tympanic temperature (every 120 seconds from minute 2 onwards), respiratory exchange ratio (RER), minute ventilation (i.e., total sum of volume delivered over a minute), expired oxygen (O2), carbon dioxide output (CO2), and maximum watts unleashed during the test. At exercise termination, total time to exhaustion (TTE; i.e., time before the given work rate cannot be maintained and there is cessation of the exercise), our main outcome variable, is recorded.

Behavioral Measures

Pre-exercise. During the 1h period between drug administration and start of the exercise test, participants complete the following questionnaires: the 14-item Hospital Anxiety and Depression Scale (HADS, frequency score from 0-3)[40] and the 9-item Fatigue Severity Scale (FSS, agreement score from 1 to 7)[41] to characterize the sample; the Paffenbarger Physical Activity Scale[42] for post-hoc evaluation of physical activity levels; the day of last menstrual cycle (if female), number of hours slept during prior night, any physical/outdoor activities engaged in today, presence of illness in self or family members today; and 10-point visual analog scales (VAS) of pain, fatigue, and sadness. These variables are collected to enable future exploratory analyses.

During exercise. The Borg Ratings of Perceived Exertion scale (RPE) for breathing (RPEbr) and muscle fatigue (RPEleg) [43,44] and the Thermal Sensation scale (ASHRAE scale)[45] are completed at 60-second intervals throughout the session (consistent with Collett et al. 2016).[46]

Post-exercise. At termination, participants provide a last RPEbr and RPEleg, complete a 10-point VAS of fatigue, mood, and pain, and rate their Global Fatigue Change (GFC).[28]

---------- insert Figure 2 here ----------

Trial status

Participant recruitment commenced on February 20th, 2019, and data collection completion is anticipated for February 2021. Currently, 34 participants have been enrolled in the study, and 16 have completed all sessions. The diversity of our sample is notable: 26% male; race/ethnicity: 9% Black, 6% Hispanic/Latinx. Recruitment has been facilitated by the appeal of this study to individuals with MS, for whom the idea of a possible treatment to reduce the discomfort of overheating during exercise resonates. Participants in the trial are uniquely motivated; upon

completing a session, one patient recently stated: "I always feel so much better after these study visits!"

Data analysis

Statistical methods recommended for the proper analysis of cross-over trial data will be used (for details, see Wellek and Blettner, 2012).[47] Our primary outcomes evaluate differences between treatment condition (ASA, APAP, and placebo) with respect to (a) total time to exhaustion (TTE, i.e., length of time spent exercising) and (b) increase in exercise-induced body temperature (degrees Fahrenheit).

Differences between pretreatment conditions (APA, APAP, placebo) will be analyzed separately by sequence group to account for possible order effects (i.e., stepwise assessment of outcome differences across 6 possible sequences). Although significant differences in outcome variables between ASA and APAP are not anticipated, we are interested in seeing how they compare in the full analyses. A mixed-effect linear model will be used to account for repeated measurements of each outcome, period effect, sequence effect, and carryover effect. Using mixed effects linear model will enable us to model differences in outcomes both within and between patients (intra- and inter-patient variability). If assumptions for mixed effects linear model are not met and log transformation is not appropriate, nonlinear mixed effects models will be used. Moreover, exploratory analyses will examine potential pretreatment differences for additional outcome measures, such as physiological indices (e.g., HR, BP) and behavioral self-reports (e.g., RPEs, fatigue, pain).

Patient and public involvement

The ASPIRE trial was developed through a commitment to clinically informed and therapeutically meaningful translational research, building upon more than 125 years of observational evidence for the detrimental effects of exercise-induced overheating in MS, coupled with anecdotal patient reports of overheating as a deterrent to exercise. Our center's MS patients have been involved throughout the development and execution of the study, providing valuable feedback to inform our

selection of outcome variables and optimize our study design. Moreover, participant feedback from a previously conducted small pilot trial[26] was considered while developing the present study.

Ethics and Dissemination

This study has been approved by the Columbia University Irving Medical Center Institutional Review Board (IRB) (reference: AAAS2529) and all participants will provide informed consent prior to the study commencement. Results will be disseminated at relevant national and international meetings, conferences, and workshops. We also plan to publish our findings in peer-reviewed, high-impact journals and aim to encourage other research groups to investigate clinically-relevant questions utilizing rigorous experimental designs (see, e.g., Dalgas et al., 2020, for a cogent discussion on how to bolster the quality and scope of exercise studies in MS).[48] Positive results will also be shared with medical caregivers, especially MS clinical practitioners. Furthermore, our MS Center at Columbia University Irving Medical Center distributes a bi-annual newsletter for patients that reports current research opportunities and highlights published study results to create a 360-degree feedback loop and acknowledge the essential role of patients in the success of our research. Patient feedback reveals this to be highly motivating.

Overall, the present study attempts to test the effectiveness of aspirin as a treatment to reduce overheating (and associated discomfort) during exercise in pwMS. Several cooling methods already exist but they are cost prohibitive in many instances, unavailable, or cumbersome. Exercising in an air-conditioned gym, for instance, appears at first glance to be a simple and obvious recommendation. But not everyone has the financial and practical means to regularly visit a gym. Aspirin, however, is inexpensive, FDA-approved, readily available, and has a favorable safety profile.[30] If this trial is successful and confirms the finding of our pilot study[33], it will support the first widely available symptomatic treatment to facilitate exercise in MS with the potential to make a meaningful difference. For research on exercise in MS, having a pretreatment to reduce exercise-induced overheating and exhaustion will mitigate sample bias, i.e., we can speculate that the extant literature may be biased toward study samples that overrepresent patients for whom overheating is not an issue, therefore limiting generalizability. Furthermore, a positive outcome of the ASPIRE trial would set the stage for future work investigating daily aspirin use to increase free

living physical activity levels or feasibility of aspirin pretreatment for individuals with progressive forms of MS.

Figure 1. Design of the double-blind, cross-over, randomized and placebo-controlled trial

Figure 2. Overview of experimental procedure

Authors' contributions AK and VML wrote the manuscript and made alterations based on co-authors comments; KEM coordinated patient recruitment and data collection, provided important input during manuscript drafting; IMA coordinated patient recruitment and reviewed the manuscript for intellectual content; CSR reviewed the manuscript for intellectual content; NWL conceptualized the exercise test and informed the choice of the physiological outcome measures; AKB provided the statistical analysis plan, SN and RSF helped with patient recruitment; JS reviewed the manuscript for intellectual content; VML, as the Principal Investigator, designed and conceptualized the study, and has overall leadership of the trial. All authors reviewed draft versions and approved the final manuscript.

Funding This work was supported by a grant from the National Institutes of Health (National Center for Medical Rehabilitation Research within the National Institute of Child Health and Human Development) to VML (HD091836-01A1).

CSR reports consulting or advisory work with Biogen Idec, Celgene, Genentech/Roche, Genzyme, TG Therapeutics.

SNL has received a fellowship training grant through Genentech and honoraria for advisory work with Biogen.

VML has received consulting fees from Healios, Inc, and is the Co-Founder of eSupport Health, PBC.

Competing interests None declared

References

1. Owen, N., Sparling, P. B., Healy, G. N., Dunstan, D. W. & Matthews, C. E. Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin. Proc.* **85**, 1138–41 (2010).
2. Veldhuijzen van Zanten, J. J., Pilutti, L. A., Duda, J. L. & Motl, R. W. Sedentary behaviour in people with multiple sclerosis: Is it time to stand up against MS? *Mult. Scler. J.* **22**, 1250–1256 (2016).
3. Petajan, J. H. *et al.* Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann. Neurol.* **39**, 432–41 (1996).
4. Gunn, H., Markevics, S., Haas, B., Marsden, J. & Freeman, J. Systematic Review: The Effectiveness of Interventions to Reduce Falls and Improve Balance in Adults With Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **96**, 1898–912 (2015).
5. Alvarenga-Filho, H. *et al.* Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple sclerosis patients in response to neuromediators. *J. Neuroimmunol.* **293**, 91–99 (2016).
6. Cooney, G. M. *et al.* Exercise for depression. *Cochrane Database Syst. Rev.* **9**, CD004366 (2013).
7. Pilutti, L. A., Greenlee, T. A., Motl, R. W., Nickrent, M. S. & Petruzzello, S. J. Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosom. Med.* **75**, 575–80.
8. Motl, R. W. Benefits, safety, and prescription of exercise in persons with multiple sclerosis. *Expert Rev. Neurother.* **14**, 1429–36 (2014).
9. Wens, I. *et al.* Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. *Eur. J. Neurol.* **23**, 1028–35 (2016).

10. Leavitt, V. M. *et al.* Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: Preliminary findings. *Neurocase* **20**, 695–697 (2014).
11. Christensen, M. E., Brincks, J., Schnieber, A. & Soerensen, D. The intention to exercise and the execution of exercise among persons with multiple sclerosis – a qualitative metasynthesis. *Disabil. Rehabil.* **38**, 1023–1033 (2016).
12. Marrie, R. *et al.* High frequency of adverse health behaviors in multiple sclerosis. *Mult. Scler. J.* **15**, 105–113 (2009).
13. Casey, B., Coote, S., Galvin, R. & Donnelly, A. Objective physical activity levels in people with multiple sclerosis: Meta-analysis. *Scand. J. Med. Sci. Sports* **28**, 1960–1969 (2018).
14. Motl, R. W., McAuley, E. & Snook, E. M. Physical activity and multiple sclerosis: a meta-analysis. *Mult. Scler. J.* **11**, 459–463 (2005).
15. Motl, R. W., McAuley, E. & Sandroff, B. M. Longitudinal Change in Physical Activity and Its Correlates in Relapsing-Remitting Multiple Sclerosis. *Phys. Ther.* **93**, 1037–1048 (2013).
16. Opara, J. A., Brola, W., Wylegala, A. A. & Wylegala, E. Uhthoff's phenomenon 125 years later - what do we know today? *J. Med. Life* **9**, 101–105 (2016).
17. Panginikkod, S. & Rukmangadachar, L. *Uhthoff Phenomenon*. (StatPearls Publishing, 2019).
18. Edwards, R. H., Hill, D. K. & Jones, D. A. Heat production and chemical changes during isometric contractions of the human quadriceps muscle. *J. Physiol.* **251**, 303–315 (1975).
19. González-Alonso, J. *et al.* Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J. Appl. Physiol.* **86**, 1032–1039 (1999).
20. NASA/MS Cooling group. A randomized controlled study of the acute and chronic effects of cooling therapy for MS. *Neurology* **60**, 1955–1960 (2003).

21. Grahn, D. A., Murray, J. V & Heller, H. C. Cooling via one hand improves physical performance in heat-sensitive individuals with multiple sclerosis: a preliminary study. *BMC Neurol* **8**, 14 (2008).

22. Op 't Eijnde, B., Keytsman, C., Wens, I. & Hansen, D. Whole-body cooling does not compromise muscle oxidative capacity in subjects with multiple sclerosis. *NeuroRehabilitation* **35**, 805–11 (2014).

23. Grover, G. *et al.* Environmental temperature and exercise modality independently impact central and muscle fatigue among people with multiple sclerosis. *Mult. Scler. J. - Exp. Transl. Clin.* **3**, 205521731774762 (2017).

24. White, A. T., Wilson, T. E., Davis, S. L. & Petajan, J. H. Effect of precooling on physical performance in multiple sclerosis. *Mult. Scler.* **6**, 176–180 (2000).

25. Devasahayam, A. J. *et al.* Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. *BMC Neurol.* **20**, 33 (2020).

26. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* (2017) doi:10.1177/1352458517739138.

27. Hersh, E. V., Moore, P. A. & Ross, G. L. Over-the-counter analgesics and antipyretics: A critical assessment. *Clin. Ther.* **22**, 500–548 (2000).

28. Wingerchuk, D. M. *et al.* A randomized controlled crossover trial of aspirin for fatigue in multiple sclerosis. *Neurology* **64**, 1267–1269 (2005).

29. Shaygannejad, V., Janghorbani, M., Ashtari, F. & Zakeri, H. Comparison of the effect of aspirin and amantadine for the treatment of fatigue in multiple sclerosis: a randomized, blinded, crossover study. *Neurol. Res.* **34**, 854–858 (2012).

30. Symmons, D. Safety profile of low-dose aspirin. *The Lancet* **348**, 1394-1395. (1996).

31. Thompson, A. J. *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173 (2018).

32. Benedict, R. H., Fishman, I., McClellan, M. M., Bakshi, R. & Weinstock-Guttman, B. Validity of the Beck Depression Inventory-Fast Screen in multiple sclerosis. *Mult. Scler. J.* **9**, 393–396 (2003).

33. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* **24**, 1511–1513 (2018).

34. Booth, G. Changes in temperature of the skin following the ingestion of food. *Arch. Intern. Med.* **57**, 533–543 (1936).

35. Chamberlain, J. *et al.* Determination of Normal Ear Temperature with an Infrared Emission Detection Thermometer. *Ann. Emerg. Med.* **25**, 15–20 (1995).

36. Henker, R. & Coyne, C. Comparison of peripheral temperature measurements with core temperature. *AACN Adv. Crit. Care* **6**, 21–30 (1995).

37. Aspirin Comprehensive Prescribing Information [online].

38. Riebe, D. *et al.* Updating ACSM's Recommendations for Exercise Preparticipation Health Screening: *Med. Sci. Sports Exerc.* **47**, 2473–2479 (2015).

39. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med Online Ser.* 211–277 (2003).

40. Zigmond, A. S. & Snaith, R. P. The Hospital Anxiety and Depression Scale. *Acta Psychiatr. Scand.* **67**, 361–370 (1983).

41. Krupp, L. B. The Fatigue Severity Scale: Application to Patients With Multiple Sclerosis and Systemic Lupus Erythematosus. *Arch. Neurol.* **46**, 1121 (1989).

42. Washburn, R. A., Smith, K. W., Jette, A. M. & Janney, C. A. Washburn RA, Smith KW, Jette AM, Janney CA. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol* **46**, 153–162 (1993).

43. Borg, G. A. V. Psychophysiological bases of perceived exertion. *Med. Sci. Sports Exerc.* **14**, 377–381 (1982).

44. Morrison, E. H. *et al.* Ratings of Perceived Exertion During Aerobic Exercise in Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **89**, 1570–1574 (2008).

45. Humphreys, M. A. & Hancock, M. Humphreys, M. A., & Hancock, M. (2007). Do people like to feel 'neutral'?: Exploring the variation of the desired thermal sensation on the ASHRAE scale. *Energy Build.* **39**, 867–874 (2007).

46. Collett, J., Meaney, A., Howells, K. & Dawes, H. Acute recovery from exercise in people with multiple sclerosis: an exploratory study on the effect of exercise intensities. *Disabil. Rehabil.* **39**, 551–558 (2017).

47. Wellek, S. & Blettner, M. On the Proper Use of the Crossover Design in Clinical Trials. *Dtsch. Aerzteblatt Online* (2012) doi:10.3238/arztebl.2012.0276.

48. Dalgas, U. *et al.* Moving exercise research in multiple sclerosis forward (the MoXFo initiative): Developing consensus statements for research. *Mult. Scler. J.* 135245852091036 (2020) doi:10.1177/1352458520910360.

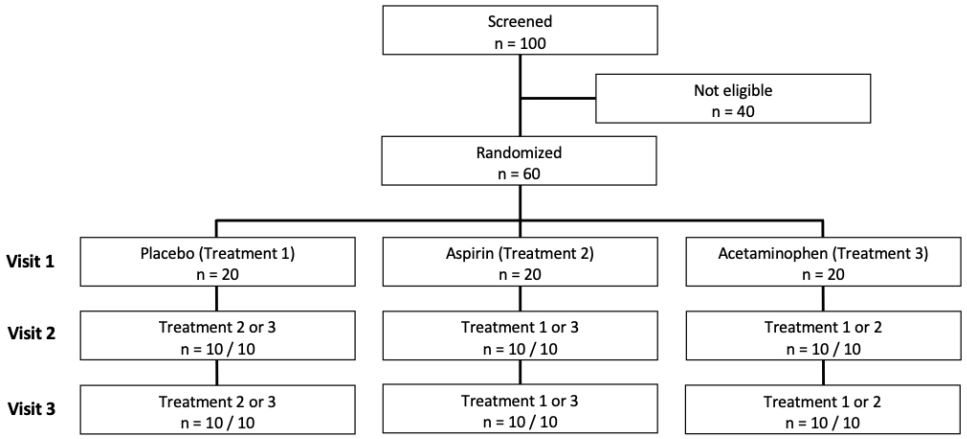

Figure 1. Design of the double-blind, cross-over, randomized and placebo-controlled trial

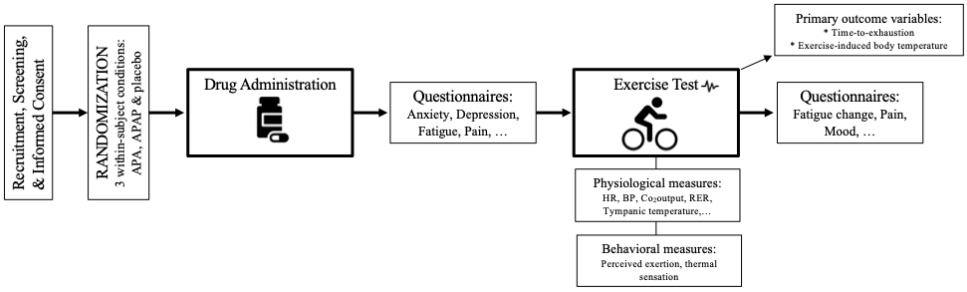

Figure 2. Overview of experimental procedure

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	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 & 12
	5b	Name and contact information for the trial sponsor	12
	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	_____NA____

	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)	NA
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	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
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)	5
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	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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7

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	9
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)	5-6
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	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7

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	7
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	7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	?

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	9
	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	7
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	10-11
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	10-11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10-11
	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)	10-11
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	NA
	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	7
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	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Detailed upon request
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	____7_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____NA_____
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	__7-8_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____1_____
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	____7-8_____
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	____7 _____
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	__11-12_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	Added to submission form
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided as additional file

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	NA

*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.

BMJ Open

The ASPIRE trial: Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039691.R2
Article Type:	Protocol
Date Submitted by the Author:	28-Sep-2020
Complete List of Authors:	Kever, Anne; Columbia University Irving Medical Center, Neurology Nelson, Katherine; Columbia University Irving Medical Center Aguerre, Ines; Columbia University Irving Medical Center Riley, Claire; Columbia University Irving Medical Center Boehme, Amelia; Columbia University Medical Center, Lee, Nancy; Columbia University Irving Medical Center Strauss Farber, Rebecca; Columbia University Irving Medical Center Levin , Seth; Columbia University Irving Medical Center Stein, Joel; Columbia University Irving Medical Center Leavitt, Victoria; Columbia University Irving Medical Center
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Multiple sclerosis < NEUROLOGY, Clinical trials < THERAPEUTICS, SPORTS MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The ASPIRE trial: Study protocol for a double-blind randomized controlled trial of aspirin for overheating during exercise in multiple sclerosis

Anne Kever, PhD[1], Katherine E. Nelson[1,2,3], Ines M. Aguerre[1,2,3], Claire S. Riley, MD[3], Amelia K. Boehme, PhD[4], Nancy W. Lee[5], Rebecca Strauss Farber, MD[3], Seth N. Levin, MD[3], Joel Stein, MD[5], Victoria M. Leavitt, PhD[1,3]

[1] Translational Cognitive Neuroscience Laboratory, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[2] Center for Translational & Computational Neuroimmunology, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[3] Multiple Sclerosis Center, Department of Neurology, Columbia University Irving Medical Center, New York, New York
[4] Department of Neurology and Epidemiology, Columbia University Irving Medical Center, New York, New York
[5] Department of Rehabilitation and Regenerative Medicine, Columbia University Vangelos College of Physicians and Surgeons, New York, New York

Submission type: Protocol paper
Abstract Word Count: 245
Manuscript Word Count (excluding abstract, figures, and references): 3162
Number of Figures: 2
Number of References: 48

Corresponding author:

Victoria M. Leavitt, Ph.D., FAAN
Assistant Professor of Neuropsychology
Department of Neurology, Columbia University Irving Medical Center
630 W. 168th Street, New York, NY 10032
email: vl2337@cumc.columbia.edu

ABSTRACT

Introduction The many benefits of exercise for persons with multiple sclerosis (MS) are well established, yet patients often refrain from exercise due to overheating and exhaustion. The present randomized controlled trial tests aspirin (acetylsalicylic acid, ASA) as a convenient method to prevent overheating and improve exercise performance in persons with MS. The effects of ASA are compared to those of acetaminophen (APAP), and placebo.

Methods and analysis Participants are seen for a laboratory maximal exercise test on three separate days separated by at least one week. At each session, body temperature is measured before oral administration of a standard adult dose (650 mg) of ASA, APAP, or placebo. One hour after drug administration, participants perform a maximal ramp test on a cycle ergometer. Primary outcomes are (a) time to exhaustion (TTE, i.e., time spent cycling to peak exertion) and (b) body temperature change. Cross-over analyses will include tests for effects of treatment, period, treatment–period interaction (carryover effect) and sequence.

Ethics and dissemination Ethical approval was granted by the institutional review board at Columbia University Irving Medical Center (reference: AAAS2529). Results of the trial will be published in peer-reviewed scientific journals and presented at national and international conferences. Neurologists, physiatrists, primary care physicians, and physiotherapists are important stakeholders and will be targeted during dissemination. Positive trial results have the potential to promote aspirin therapy, an inexpensive and readily-available treatment to reduce overheating and allow more persons with MS to benefit from exercise.

Trial registration identifier NCT03824938 (approved February 2019)

Strengths and limitations of the study

- The rigorous double-blind, placebo-controlled, crossover design will expose all participants to three conditions, ensuring robust results and reducing the influence of confounding covariates.
- Positive findings from this trial will provide an effective, inexpensive, readily available, unobtrusive treatment that will avail more persons with MS the benefits of exercise.
- For this initial trial, our sample is limited to patients with relapsing-remitting MS (RRMS) although the potential benefits of aspirin should also be tested in persons with progressive forms of MS in future studies.
- The selected dose of aspirin (650 mg) was chosen based on two prior trials in MS as well as our own pilot trial, but future trials employing a lower dose are warranted.

Introduction

Exercise holds numerous benefits for persons with multiple sclerosis (pwMS)[1,2] including increased muscle strength,[3] improved balance,[4] decreased fatigue and depressive symptoms,[5–7] and enhanced overall quality of life.[8] Furthermore, a growing body of research supports exercise as potentially disease modifying. In one study, MS patients enrolled in a 24-week program of exercise demonstrated increased serum levels of circulating brain-derived neurotrophic factor (BDNF, critically involved in counteracting inflammatory processes of MS)[9] and reduced pro-inflammatory circulating cytokines (often elevated during exacerbations of MS disease activity) relative to those who maintained a sedentary lifestyle over the same period of time.[5] In addition, another study showed that just 12-weeks of aerobic exercise resulted in increased hippocampal volume and hippocampal functional connectivity, as well as improved memory in pwMS (compared to a stretching condition).[10]

Despite these and other well-documented benefits of exercise, individuals with MS sometimes avoid exercise due to transient discomforts of overheating and fatigue[11]. Studies show that compared to their age-matched healthy peers, pwMS are less physically active regardless of disability and engage in less activity than their physical capacity allows.[12–14] In fact, fewer than 20% of pwMS achieve adequate amounts of daily physical activity, levels that further decrease as the disease develops.[15] The link between exercise and overheating has been known to the field for over a century: in 1890, Wilhelm Uhthoff's famous observation of the deleterious consequences of exercise for patients with MS precipitated a vast literature describing heat-related changes in MS.[16,17]

Exercise triggers the conversion of metabolic to mechanical energy, liberating approximately 30-70% of the body's total energy as heat and thus causing an increase in core temperature.[18] In everyone, as internal body temperature rises, physical fatigue increases and exercise performance worsens.[19] In pwMS, slight increases in body temperature may lead to prohibitive levels of exhaustion during exercise. As a response to this, several cooling methods (e.g., ice baths, cooling contraptions or garments, cooled environments) developed for pwMS have effectively reduced fatigue[20] and improved exercise performance,[21–25] although such methods can be cumbersome,

prohibitively expensive, or unavailable. Also, it is important to note that none of these prior trials of cooling methods in MS were conducted using double-blind study design.

Our recent double-blind, crossover pilot trial demonstrated that aspirin (compared to placebo) significantly improved exercise performance and reduced exercise-induced overheating in pwMS.[26] Here, we present a detailed protocol of our large-scale trial of aspirin for exercise, ASPIRE: a double-blind, crossover RCT comparing the effects of aspirin (ASA), acetaminophen (APAP) and placebo on exercise performance and exercise-induced body temperature increase in pwMS. The second arm, APAP, has been included for two reasons. First, if effective, APAP represents a potential alternative to ASA as a cooling treatment. Second, this third arm allows interrogation of mechanism: whereas aspirin is pleiotropic, APAP has much weaker/absent anti-inflammatory properties thereby isolating (to some degree) the antipyretic mechanism of ASA.[27]

Here, we test the hypothesis that ASA and APAP pre-treatment (compared to placebo) will improve exercise performance and reduce exercise-induced body temperature increase during a controlled, laboratory maximal exercise test in pwMS.

Methods and analysis

Study design

This is a double-blind, crossover, placebo-controlled, randomized intervention design to examine the efficacy of an oral antipyretic (i.e., ASA and APAP) to improve exercise performance and reduce exercise-induced body temperature in persons with MS. The trial comprises the following three arms: aspirin (ASA) was selected based on our prior exercise pilot as well as two prior trials showing its efficacy for reducing fatigue in non-exercising pwMS.[28,29] ASA has a generally favorable safety profile[30], with no adverse events noted in these prior trials, nor more than minimal side effects (i.e., no different than placebo).[28] As noted, acetaminophen (APAP) was included to investigate mechanism: while both ASA and APAP are antipyretic, ASA has prominent anti-inflammatory properties.[27] Furthermore, if APAP is found to be as effective as aspirin, it will provide another option that may be preferable for some pwMS. The third arm is placebo.

Participants are randomized to one of six sequences (see figure 1) with double-blind maintained by the study biostatistician (AKB) until data collection for the full sample is complete. Each participant engages in three exercise sessions held at the same time (within ±1 hour) on separate days with ≥ 1-week intervals. All sessions are scheduled between 10 am - 5 pm to reduce the influence of circadian rhythm variability. Duration of each session is ~2 hours total (details in Procedure).

---------- insert Figure 1 here ----------

Participants

Inclusion criteria. Participants are included if they meet the following criteria: (1) diagnosis of relapsing-remitting MS based on the 2017 McDonald criteria,[31] (2) aged between 18- 65 years (upper limit is set to reduce effects of age-related activity level decreases and to ensure that our sample is representative of the relapse-onset MS population); (3) answer yes to the question: Is overheating during exercise a problem for you?; (4) low to moderate physical disability (i.e., Expanded Disability Status Scale (EDSS) total score ≤ 6.0); (5) relapse-free for 6 weeks prior; (6) BMI ≤ 35 (to reduce health-related confounds of obesity).

Exclusion criteria. Patients are excluded if they have any prior history of head injury, stroke, or other neurological disease/disorder; are currently taking antipyretics or pain medication daily; are currently in a depressive episode (screened with the BDI-FS, excluded if total score ≥ 9)[32], or present other psychiatric diagnoses; were formally diagnosed with sleep disorder; pulmonary disease, heart disease or other heart problem; suffer from vascular disease of the legs, high blood pressure; currently take medications for high blood pressure or any heart problem; have diabetes mellitus or problems with blood sugar levels; present any contraindications to ASA use, i.e., history of confirmed peptic ulcer, gastrointestinal or severe gynecological bleeding; have tarry

stool or fecal occult blood; syndrome of asthma, rhinitis, or nasal polyps; present any contraindications to APAP use, i.e., severe active hepatic disease, Hepatitis C Virus.

Sample size determination. In a pilot trial with 12 participants, effect sizes for both outcomes (TTE and body temperature) were very large (Cohen's d = 1.45). Here, we conservatively estimated medium sized effects (0.5) to calculate a power estimate for the proposed trial. With significance set at $\alpha = 0.05$ for 2-tailed paired t-test, a 3-arm crossover sample of 54 will yield 0.95 power to detect differences between interventions. Our plan to enroll 60 participants allows for 10% attrition, which accounts for both participant drop-out as well as exclusion due to relapses.

Recruitment and consent. Based on a-priori power analysis, 60 patients with a diagnosis of RRMS will be enrolled. The sample is projected to comprise 66% females and 33% males, consistent with disease demographics. All participants will be recruited from the Multiple Sclerosis Center at the Columbia University Irving Medical Center (CUIMC). One hundred patients will be screened, conservatively estimating that 60% will meet eligibility based on our pilot trial.[33] The Medical Director of the MS Center (CSR) and a dedicated research coordinator (KN) lead patient recruitment and confirm the disease-specific inclusion criteria. To avoid coercion, participants are not invited to participate by their treating neurologist. Instead, a research coordinator provides participants with an IRB-approved recruitment advertisement for the study, with instructions to contact the research coordinator if s/he is interested in participating. Participants receive weekly reminder emails and phone calls to promote patient retention and completion of follow-up visits. Data from participants who discontinue or deviate from intervention protocols are excluded from final analyses but will be retained for inspection of any systematic differences. Participants are expressly informed that participation in this study is voluntary, not part of their medical care, affords no advantage to their health, and that refusal to participate results in no negative consequences. Before commencement of the study, participants sign a consent form approved by the institutional review board at CUIMC (for a model, please see Supplementary Materials). The latter also details the provisions taken in case of injury or harm as a result of participating in the study, i.e., care provided through CUIMC or any other health provider. Participants receive $100 at the completion of their third exercise session as compensation for time and travel expenses.

Allocation, randomization, blinding, and data protection. Using a random number generator, each participant is assigned to one of six randomization schedules: placebo-APAP-ASA, placebo-ASA-APAP, ASA-APAP-placebo, ASA-placebo-APAP, APAP-ASA-placebo, APAP-placebo-ASA. The research pharmacy (providing all study drug) receives a randomization schedule to ensure that necessary procedures for maintaining the double-blind are in place. The key containing participant ID and randomization schedule assignment is kept by the study biostatistician for the duration of the active period of the trial and will be provided to the study PI only after all participants have been tested. To test effectiveness of blinding, participants are asked via paper questionnaire which condition they think they were in at the end of each session. In order to safeguard confidentiality, participants are identified by a number and the master key is password protected, and accessible only to personnel directly involved in the study.

Intervention and procedure

Pharmaceutical pretreatment. Room temperature is held constant to within ±5 degrees Fahrenheit and recorded at the start of each session. Participants are instructed to not eat two hours prior to their session (to avoid subsequent increases in body temperature[34]) and to refrain from use of ASA or APAP within 24-hours of their study visit. Upon arrival to the lab, written informed consent is obtained from all participants. Body temperature is measured with a tympanic thermometer (right ear; Braun Thermoscan IRT), a standard, non-invasive method which best approximates core body temperature.[35,36] Participants receive one of three treatments (ASA, APAP, placebo) at each session prior to the exercise test. Order of treatment is randomized and counter-balanced, with each participant serving as his/her own control (see Figure 2). Treatment is administered as one capsule containing 650 mg (i.e., standard adult dose for ASA, APAP) of ASA, APAP, or placebo. ASA dose was selected on the basis of prior successful RCTs of ASA to reduce fatigue in non-exercising patients with MS.[28,29] Overencapsulation of ASA and manufacture of placebo is handled by the CUIMC research pharmacy. Time of drug administration is recorded, and exercise does not begin until 1 hour later to allow for peak serum level to be reached.[37] During this time, participants complete a series of questionnaires to characterize the sample (for details, see Behavioral Measures).

Exercise test. Exercise tests are conducted by a certified exercise physiologist (NL). All procedures are consistent with *American College of Sports Medicine (ACSM)* Guidelines [38]. All exercise testing equipment is current and certified for use with human subjects. Cardiopulmonary exercise testing is performed using Vmax Encore Metabolic Cart (CareFusion Corp, San Diego, CA) and Electrordiogram (EKG) monitoring is done with a twelve-lead system attached to the Vmax Encore Metabolic Cart with cardiosoft software (CareFusion Corp). Peak ventilatory capacity (Maximum Voluntary Ventilation, MVV) is determined before the exercise test via Vmax Encore System (CareFusion Corp). Maximum aerobic fitness (VO2max) is measured during the graded maximal exercise test on a VIAsprint 150P electronic-braked cycle ergometer (CareFusion Corp).

Participants complete a 5-minute resting phase followed by a 3-minute warm up and then start the progressive ramped exercise. An individualized ramping protocol is determined by a certified exercise physiologist based on the exercise frequency and intensity that each patient self-reports. For participants who exercise irregularly and maximum 0-2 times/week, resistance increases by 5W/minute, those who exercise regularly 2-4 times/week follow a 10W/minute protocol, while those who exercise more than 4 times/week are assigned to a 15W/minute protocol. However, depending on the type of exercise, ramping protocols can be adapted (e.g., 4 yoga sessions/week vs 4 high intensity cycling sessions/week). Participants are instructed to maintain a cadence of 50-60 revolutions per minute (RPM) for as long as possible. The test is terminated when cadence drops below 40 RPM for ≥ 5 seconds, or when the participant reaches volitional exhaustion in accordance with American Thoracic Society (ATS) standard test termination criteria.[39]

The following biophysiological data are collected during the exercise session (to ensure participant safety,[38] and for exploratory analyses): heart rate (HR) (every 60 seconds), blood pressure (BP) and tympanic temperature (every 120 seconds from minute 2 onwards), respiratory exchange ratio (RER), minute ventilation (i.e., total sum of volume delivered over a minute), expired oxygen (O2), carbon dioxide output (CO2), and maximum watts unleashed during the test. At exercise termination, total time to exhaustion (TTE; i.e., time before the given work rate cannot be maintained and there is cessation of the exercise), our main outcome variable, is recorded.

Behavioral Measures

Pre-exercise. During the 1h period between drug administration and start of the exercise test, participants complete the following questionnaires: the 14-item Hospital Anxiety and Depression Scale (HADS, frequency score from 0-3)[40] and the 9-item Fatigue Severity Scale (FSS, agreement score from 1 to 7)[41] to characterize the sample; the Paffenbarger Physical Activity Scale[42] for post-hoc evaluation of physical activity levels; the day of last menstrual cycle (if female), number of hours slept during prior night, any physical/outdoor activities engaged in today, presence of illness in self or family members today; and 10-point visual analog scales (VAS) of pain, fatigue, and sadness. These variables are collected to enable future exploratory analyses.

During exercise. The Borg Ratings of Perceived Exertion scale (RPE) for breathing (RPEbr) and muscle fatigue (RPEleg) [43,44] and the Thermal Sensation scale (ASHRAE scale)[45] are completed at 60-second intervals throughout the session (consistent with Collett et al. 2016).[46]

Post-exercise. At termination, participants provide a last RPEbr and RPEleg, complete a 10-point VAS of fatigue, mood, and pain, and rate their Global Fatigue Change (GFC).[28]

---------- insert Figure 2 here ----------

Trial status

Participant recruitment commenced on February 20th, 2019, and data collection completion is anticipated for February 2021. Currently, 34 participants have been enrolled in the study, and 16 have completed all sessions. Data collected until now will not be reported in this paper. The diversity of our sample is notable: 26% male; race/ethnicity: 9% Black, 6% Hispanic/Latinx. Recruitment has been facilitated by the appeal of this study to individuals with MS, for whom the idea of a possible treatment to reduce the discomfort of overheating during exercise resonates.

Participants in the trial are uniquely motivated; upon completing a session, one patient recently stated: "I always feel so much better after these study visits!"

Data analysis

Statistical methods recommended for the proper analysis of cross-over trial data will be used (for details, see Wellek and Blettner, 2012).[47] Our primary outcomes evaluate differences between treatment condition (ASA, APAP, and placebo) with respect to (a) total time to exhaustion (TTE, i.e., length of time spent exercising) and (b) increase in exercise-induced body temperature (degrees Fahrenheit).

Differences between pretreatment conditions (APA, APAP, placebo) will be analyzed separately by sequence group to account for possible order effects (i.e., stepwise assessment of outcome differences across 6 possible sequences). Although significant differences in outcome variables between ASA and APAP are not anticipated, we are interested in seeing how they compare in the full analyses. A mixed-effect linear model will be used to account for repeated measurements of each outcome, period effect, sequence effect, and carryover effect. Using mixed effects linear model will enable us to model differences in outcomes both within and between patients (intra- and inter-patient variability). If assumptions for mixed effects linear model are not met and log transformation is not appropriate, nonlinear mixed effects models will be used. Moreover, exploratory analyses will examine potential pretreatment differences for additional outcome measures, such as physiological indices (e.g., HR, BP) and behavioral self-reports (e.g., RPEs, fatigue, pain).

Patient and public involvement

The ASPIRE trial was developed through a commitment to clinically informed and therapeutically meaningful translational research, building upon more than 125 years of observational evidence for the detrimental effects of exercise-induced overheating in MS, coupled with anecdotal patient reports of overheating as a deterrent to exercise. Our center's MS patients have been involved throughout the development and execution of the study, providing valuable feedback to inform our

selection of outcome variables and optimize our study design. Moreover, participant feedback from a previously conducted small pilot trial[26] was considered while developing the present study.

Ethics and Dissemination

This study has been approved by the Columbia University Irving Medical Center Institutional Review Board (IRB) (reference: AAAS2529) and all participants will provide informed consent prior to the study commencement. Results will be disseminated at relevant national and international meetings, conferences, and workshops. We also plan to publish our findings in peer-reviewed, high-impact journals and aim to encourage other research groups to investigate clinically-relevant questions utilizing rigorous experimental designs (see, e.g., Dalgas et al., 2020, for a cogent discussion on how to bolster the quality and scope of exercise studies in MS).[48] Positive results will also be shared with medical caregivers, especially MS clinical practitioners. Furthermore, our MS Center at Columbia University Irving Medical Center distributes a bi-annual newsletter for patients that reports current research opportunities and highlights published study results to create a 360-degree feedback loop and acknowledge the essential role of patients in the success of our research. Patient feedback reveals this to be highly motivating.

Discussion

Overall, the present study attempts to test the effectiveness of aspirin as a treatment to reduce overheating (and associated discomfort) during exercise in pwMS. Several cooling methods already exist but they are cost prohibitive in many instances, unavailable, or cumbersome. Exercising in an air-conditioned gym, for instance, appears at first glance to be a simple and obvious recommendation. But not everyone has the financial and practical means to regularly visit a gym. Aspirin, however, is inexpensive, FDA-approved, readily available, and has a favorable safety profile.[30] If this trial is successful and confirms the finding of our pilot study[33], it will support the first widely available symptomatic treatment to facilitate exercise in MS with the potential to make a meaningful difference. For research on exercise in MS, having a pretreatment to reduce exercise-induced overheating and exhaustion will mitigate sample bias, i.e., we can speculate that the extant literature may be biased toward study samples that overrepresent patients for whom

overheating is not an issue, therefore limiting generalizability. Furthermore, a positive outcome of the ASPIRE trial would set the stage for future work investigating daily aspirin use to increase free living physical activity levels or feasibility of aspirin pretreatment for individuals with progressive forms of MS.

Figure 1. Design of the double-blind, cross-over, randomized and placebo-controlled trial

Figure 2. Overview of experimental procedure

Authors' contributions AK and VML wrote the manuscript and made alterations based on co-authors comments; KEN coordinated patient recruitment and data collection, provided important input during manuscript drafting; IMA coordinated patient recruitment and reviewed the manuscript for intellectual content; CSR reviewed the manuscript for intellectual content; NWL conceptualized the exercise test and informed the choice of the physiological outcome measures; AKB provided the statistical analysis plan, SNL and RSF helped with patient recruitment; JS reviewed the manuscript for intellectual content; VML, as the Principal Investigator, designed and conceptualized the study, and has overall leadership of the trial. All authors reviewed draft versions and approved the final manuscript.

Funding This work was supported by a grant from the National Institutes of Health (National Center for Medical Rehabilitation Research within the National Institute of Child Health and Human Development) to VML (HD091836-01A1).

CSR reports consulting or advisory work with Biogen Idec, Celgene, Genentech/Roche, Genzyme, TG Therapeutics.

SNL has received a fellowship training grant through Genentech and honoraria for advisory work with Biogen.

VML has received consulting fees from Healios, Inc, and is the Co-Founder of eSupport Health, PBC.

Competing interests None declared

References

1. Owen, N., Sparling, P. B., Healy, G. N., Dunstan, D. W. & Matthews, C. E. Sedentary behavior: emerging evidence for a new health risk. *Mayo Clin. Proc.* **85**, 1138–41 (2010).
2. Veldhuijzen van Zanten, J. J., Pilutti, L. A., Duda, J. L. & Motl, R. W. Sedentary behaviour in people with multiple sclerosis: Is it time to stand up against MS? *Mult. Scler. J.* **22**, 1250–1256 (2016).
3. Petajan, J. H. *et al.* Impact of aerobic training on fitness and quality of life in multiple sclerosis. *Ann. Neurol.* **39**, 432–41 (1996).
4. Gunn, H., Markevics, S., Haas, B., Marsden, J. & Freeman, J. Systematic Review: The Effectiveness of Interventions to Reduce Falls and Improve Balance in Adults With Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **96**, 1898–912 (2015).
5. Alvarenga-Filho, H. *et al.* Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple sclerosis patients in response to neuromediators. *J. Neuroimmunol.* **293**, 91–99 (2016).
6. Cooney, G. M. *et al.* Exercise for depression. *Cochrane Database Syst. Rev.* **9**, CD004366 (2013).
7. Pilutti, L. A., Greenlee, T. A., Motl, R. W., Nickrent, M. S. & Petruzzello, S. J. Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. *Psychosom. Med.* **75**, 575–80.
8. Motl, R. W. Benefits, safety, and prescription of exercise in persons with multiple sclerosis. *Expert Rev. Neurother.* **14**, 1429–36 (2014).
9. Wens, I. *et al.* Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. *Eur. J. Neurol.* **23**, 1028–35 (2016).

10. Leavitt, V. M. *et al.* Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: Preliminary findings. *Neurocase* **20**, 695–697 (2014).
11. Christensen, M. E., Brincks, J., Schnieber, A. & Soerensen, D. The intention to exercise and the execution of exercise among persons with multiple sclerosis – a qualitative metasynthesis. *Disabil. Rehabil.* **38**, 1023–1033 (2016).
12. Marrie, R. *et al.* High frequency of adverse health behaviors in multiple sclerosis. *Mult. Scler. J.* **15**, 105–113 (2009).
13. Casey, B., Coote, S., Galvin, R. & Donnelly, A. Objective physical activity levels in people with multiple sclerosis: Meta-analysis. *Scand. J. Med. Sci. Sports* **28**, 1960–1969 (2018).
14. Motl, R. W., McAuley, E. & Snook, E. M. Physical activity and multiple sclerosis: a meta-analysis. *Mult. Scler. J.* **11**, 459–463 (2005).
15. Motl, R. W., McAuley, E. & Sandroff, B. M. Longitudinal Change in Physical Activity and Its Correlates in Relapsing-Remitting Multiple Sclerosis. *Phys. Ther.* **93**, 1037–1048 (2013).
16. Opara, J. A., Brola, W., Wylegala, A. A. & Wylegala, E. Uhthoff's phenomenon 125 years later - what do we know today? *J. Med. Life* **9**, 101–105 (2016).
17. Panginikkod, S. & Rukmangadachar, L. *Uhthoff Phenomenon*. (StatPearls Publishing, 2019).
18. Edwards, R. H., Hill, D. K. & Jones, D. A. Heat production and chemical changes during isometric contractions of the human quadriceps muscle. *J. Physiol.* **251**, 303–315 (1975).
19. González-Alonso, J. *et al.* Influence of body temperature on the development of fatigue during prolonged exercise in the heat. *J. Appl. Physiol.* **86**, 1032–1039 (1999).
20. NASA/MS Cooling group. A randomized controlled study of the acute and chronic effects of cooling therapy for MS. *Neurology* **60**, 1955–1960 (2003).

21. Grahn, D. A., Murray, J. V & Heller, H. C. Cooling via one hand improves physical performance in heat-sensitive individuals with multiple sclerosis: a preliminary study. *BMC Neurol* **8**, 14 (2008).

22. Op 't Eijnde, B., Keytsman, C., Wens, I. & Hansen, D. Whole-body cooling does not compromise muscle oxidative capacity in subjects with multiple sclerosis. *NeuroRehabilitation* **35**, 805–11 (2014).

23. Grover, G. *et al.* Environmental temperature and exercise modality independently impact central and muscle fatigue among people with multiple sclerosis. *Mult. Scler. J. - Exp. Transl. Clin.* **3**, 205521731774762 (2017).

24. White, A. T., Wilson, T. E., Davis, S. L. & Petajan, J. H. Effect of precooling on physical performance in multiple sclerosis. *Mult. Scler.* **6**, 176–180 (2000).

25. Devasahayam, A. J. *et al.* Vigorous cool room treadmill training to improve walking ability in people with multiple sclerosis who use ambulatory assistive devices: a feasibility study. *BMC Neurol.* **20**, 33 (2020).

26. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* (2017) doi:10.1177/1352458517739138.

27. Hersh, E. V., Moore, P. A. & Ross, G. L. Over-the-counter analgesics and antipyretics: A critical assessment. *Clin. Ther.* **22**, 500–548 (2000).

28. Wingerchuk, D. M. *et al.* A randomized controlled crossover trial of aspirin for fatigue in multiple sclerosis. *Neurology* **64**, 1267–1269 (2005).

29. Shaygannejad, V., Janghorbani, M., Ashtari, F. & Zakeri, H. Comparison of the effect of aspirin and amantadine for the treatment of fatigue in multiple sclerosis: a randomized, blinded, crossover study. *Neurol. Res.* **34**, 854–858 (2012).

30. Symmons, D. Safety profile of low-dose aspirin. *The Lancet* **348**, 1394-1395. (1996).

31. Thompson, A. J. *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173 (2018).

32. Benedict, R. H., Fishman, I., McClellan, M. M., Bakshi, R. & Weinstock-Guttman, B. Validity of the Beck Depression Inventory-Fast Screen in multiple sclerosis. *Mult. Scler. J.* **9**, 393–396 (2003).

33. Leavitt, V. M. *et al.* Aspirin is an effective pretreatment for exercise in multiple sclerosis: A double-blind randomized controlled pilot trial. *Mult. Scler. J.* **24**, 1511–1513 (2018).

34. Booth, G. Changes in temperature of the skin following the ingestion of food. *Arch. Intern. Med.* **57**, 533–543 (1936).

35. Chamberlain, J. *et al.* Determination of Normal Ear Temperature with an Infrared Emission Detection Thermometer. *Ann. Emerg. Med.* **25**, 15–20 (1995).

36. Henker, R. & Coyne, C. Comparison of peripheral temperature measurements with core temperature. *AACN Adv. Crit. Care* **6**, 21–30 (1995).

37. Aspirin Comprehensive Prescribing Information [online].

38. Riebe, D. *et al.* Updating ACSM's Recommendations for Exercise Preparticipation Health Screening: *Med. Sci. Sports Exerc.* **47**, 2473–2479 (2015).

39. ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med Online Ser.* 211–277 (2003).

40. Zigmond, A. S. & Snaith, R. P. The Hospital Anxiety and Depression Scale. *Acta Psychiatr. Scand.* **67**, 361–370 (1983).

41. Krupp, L. B. The Fatigue Severity Scale: Application to Patients With Multiple Sclerosis and Systemic Lupus Erythematosus. *Arch. Neurol.* **46**, 1121 (1989).

42. Washburn, R. A., Smith, K. W., Jette, A. M. & Janney, C. A. Washburn RA, Smith KW, Jette AM, Janney CA. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol* **46**, 153–162 (1993).

43. Borg, G. A. V. Psychophysiological bases of perceived exertion. *Med. Sci. Sports Exerc.* **14**, 377–381 (1982).

44. Morrison, E. H. *et al.* Ratings of Perceived Exertion During Aerobic Exercise in Multiple Sclerosis. *Arch. Phys. Med. Rehabil.* **89**, 1570–1574 (2008).

45. Humphreys, M. A. & Hancock, M. Humphreys, M. A., & Hancock, M. (2007). Do people like to feel 'neutral'?: Exploring the variation of the desired thermal sensation on the ASHRAE scale. *Energy Build.* **39**, 867–874 (2007).

46. Collett, J., Meaney, A., Howells, K. & Dawes, H. Acute recovery from exercise in people with multiple sclerosis: an exploratory study on the effect of exercise intensities. *Disabil. Rehabil.* **39**, 551–558 (2017).

47. Wellek, S. & Blettner, M. On the Proper Use of the Crossover Design in Clinical Trials. *Dtsch. Aerzteblatt Online* (2012) doi:10.3238/arztebl.2012.0276.

48. Dalgas, U. *et al.* Moving exercise research in multiple sclerosis forward (the MoXFo initiative): Developing consensus statements for research. *Mult. Scler. J.* 135245852091036 (2020) doi:10.1177/1352458520910360.

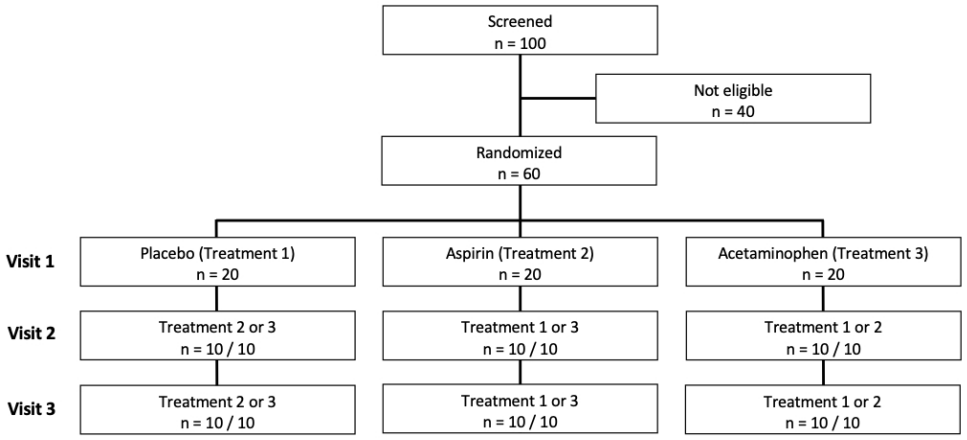

Figure 1. Design of the double-blind, cross-over, randomized and placebo-controlled trial

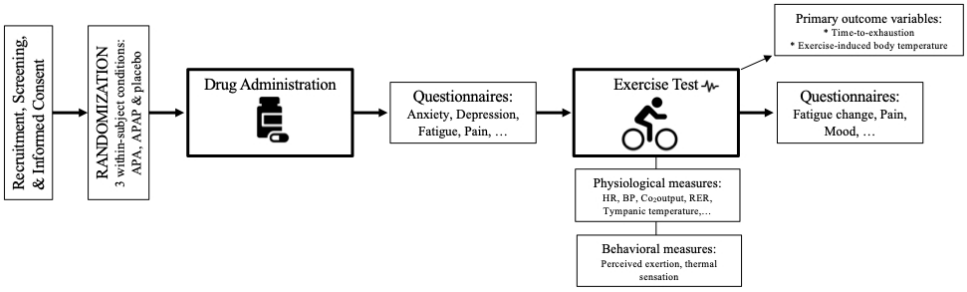

Figure 2. Overview of experimental procedure

Columbia University Consent Form

Protocol Information

Attached to Protocol: IRB-AAAS2529
Principal Investigator: Victoria Leavitt (vl2337)
IRB Protocol Title: Aspirin for Exercise in Multiple Sclerosis (ASPIRE)

General Information

Consent Number: CF-AABX0500
Participation Duration:
Anticipated Number of Subjects: 60
Research Purpose: The purpose of this study is to investigate the relationship between body temperature, fatigue and multiple sclerosis.
This study examines the effect of aspirin and acetaminophen on body temperature in people with MS, and the effect it may have on exercising.

Information on Research

You are being asked to join a research study funded by the National Institute of Health (NIH) because you have a diagnosis of Relapsing Remitting MS and have reported heat sensitivity. This consent form explains the research study and your part in the study. Please read it carefully and take as much time as you need.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time. This trial is identified by its clinical trial number: NCT03824938.

Please ask questions at any time about anything you do not understand.

Study Procedures: If you consent to participate, you will undergo the following during the study visit:

Screening for the study will include a brief depression screener. You will complete 3 study visits, separated by at least 1 week. Study visit sessions will be scheduled between 9 am - 5 pm at the same time of day (+/- 1 hour). At each of the three study visits, the following study procedures will be repeated the exact same way.

You must first refrain from eating for two hours prior to the scheduled session. You will be randomized (similar to flipping a coin, you will have an equal, random chance of being assigned to any of the three groups) to receive either aspirin (standard dose, 650mg, provided in the form of one capsule), acetaminophen (standard dose, 650mg provided, in the form of one capsule), or a placebo (one capsule with no therapeutic effect). After having your ear temperature taken and completing brief questionnaires about your current level of pain, mood, and fatigue, you will complete a grip strength test with a study coordinator using a Dynanometer. You will then be given a pill to take. Neither you nor the investigators will know which pill you have been given until after the study is completed. You will receive a different study treatment at each visit, ensuring that you will take all three study drugs over the course of the three separate study visits. After being administered the pill, you will relax for one hour (the estimated time for aspirin and acetaminophen to reach the highest concentration in the bloodstream), before once again having ear temperature taken and completing the grip strength test again.

During the waiting period after taking the study drug, the study team will collect data and administer questionnaires. Study personnel will familiarize you with a scale that will be used during your exercise session to measure how tired you are feeling (your level of exertion). Once that has been completed, an exercise physiologist will give you an exercise test on a stationary cycle. You will wear a face mask so that we can measure your respiration during exercise. We will also continuously monitor your heart rate with EKG monitoring that will have 12-leads attached to your body, as is the same with any clinical EKG. Your blood pressure, ear temperature and feelings of exertion will be recorded every 60 seconds during exercise. The exercise test will begin with a 3-minute warm up phase and then a progressed ramped increase, which will involve cycling until you feel too tired to continue. For most people, this is not longer than about 8-12 minutes. After you finish, blood pressure, ear temperature, and exertion will be recorded again, and we will have you fill out some questionnaires on your current level of pain, mood and fatigue, as well as complete the grip strength test with the Dynanometer one last time. At the end of each session, we will ask you whether you think you were given aspirin, acetaminophen, or a placebo.

Future Use of Data

We will use your data for the research described in this form and for other future research. We will label your data with a code instead of your name. The key to the code connects your name to your samples and health information. The study doctor will keep the key to the code in a password protected computer and locked file.

Identifiers will be removed from all identifiable private information and, after such removal, the information could be used for future research studies without additional consent from you.

Any clinically relevant research results will be disclosed to you upon completion of the study.

Risks

Exercise causes exertion. To ensure your safety if you participate in the exercise condition, we will consult with your physician before enrolling you to make sure you meet all study criteria. The one-on-one exercise sessions you take part in will be overseen by a certified exercise physiologist with experience working with patients who have a variety of medical conditions. In addition to the exercise physiologist, a physician will be available for all three of your sessions.

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or serious, and can even result in death in some cases. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing.

The risks of a single dose of aspirin are minimal, and because we will carefully screen for any/all conditions that may counter indicate the use of aspirin before enrollment, we do not expect any issues to arise as a result of pills taken for use in this study. Furthermore, the dosage being given is the standard dose taken for, e.g., a headache (650mg). Aspirin may increase the risk of bleeding.

The risks of a single dose of acetaminophen are also minimal, and because we will carefully screen for any/all conditions that may counter indicate the use of acetaminophen before enrollment, we do not expect any issues to arise as a result of pills taken for use in this study. Furthermore, the dosage being given is the standard dose taken for, e.g., a headache (650mg).

The risks of a stress test such as the one being conducted in this study on a stationary cycle are:
1. Feel moderate to severe chest pain.
2. Get too out of breath to continue.
3. Develop abnormally high or low blood pressure or an arrhythmia (an irregular heartbeat)
4. Become dizzy.

As heart rate, blood pressure and temperature are all being accounted for, all of these risks are minimized.

Loss of confidentiality

A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The study team plans to protect your confidentiality. Their plans for keeping your information private are described in the 'confidentiality' section of this consent form.

Benefits

You are not expected to benefit directly from participation in this study. The ultimate benefit of this research is that we better understand how changes in body temperature affect fatigue associated with multiple sclerosis.

Alternative Procedures

The alternative is to not participate.

Confidentiality

Any information obtained during this study and identified with you will remain confidential. Any information that may be of value to your physician for your personal treatment will be shared with your physician, unless you object to this. All information will be stored in locked files and all information in computer data bases will not have your name or any other identifying information associated with it.

The following individuals and/or agencies will be able to look at and copy your research records:
- The investigator, study staff and other medical professionals who may be evaluating the study
- Authorities from Columbia University and New York Presbyterian Hospital, including the Institutional Review Board ('IRB')
- The United States Food and Drug Administration ('FDA') and/or the Office of Human Research Protections ('OHRP')
- If this study is sponsored (money or supplies are being provided), the sponsor of this study, the National Institute of Health (NIH), including persons or organizations working with or owned by the sponsor
- Other government regulatory agencies (including agencies in other countries) if the sponsor is seeking marketing approval for new products resulting from this research.

Columbia University Irving Medical Center has recently implemented a new electronic medical record (EMR) system, which will be shared with Weill Cornell Medical Center and New-York Presbyterian Hospital and its affiliated institutions.

Your participation in this research study will be documented in our new EMR system. Medical records in this system can be viewed by authorized personnel from these institutions. Study monitors and others who provide oversight of the study may also need to access this record.

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

There are some important things that you need to know. The Certificate DOES NOT stop reporting that federal, state or local laws require. Some examples are laws that require reporting of child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate CANNOT BE USED to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate DOES NOT stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also DOES NOT prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

Research Related Injuries

Taking part in this research study may result in injury or harm to you. In the event of an injury resulting from your participation in this study, you should seek appropriate medical care and inform the study doctor. In the event of an emergency you should go to an emergency room.

If you are injured or harmed as a result of participating in the study and receive medical care through the New York Presbyterian Hospital (NYPH), a Columbia doctor, or any other health provider, you will be sent a bill for whatever medical care you receive. All or part of your bill may be paid by your health insurance.

Columbia University and New York-Presbyterian Hospital (NYPH) are not offering to pay you for pain, worry, lost income, the cost of your medical care or non-medical care costs that might occur as a result of your taking part in this study. However, you do not waive any of your legal rights in signing this form.

Compensation

You will be compensated $100 after the completion of all three study visits. If we pay you by check, we will ask you for your SSN or TIN number. This information will not be disclosed to any collaborators. There is no cost to you for participating in this study. Reportable payments: According to IRS regulations, compensation payments totaling more than $600 in a calendar year must be reported to the Internal Revenue Service (IRS). We will need to obtain your Social Security Number for this purpose. Reimbursement for travel or other study-related expenses are not considered compensation for tax purposes.

Voluntary Participation

Your participation in this study is completely voluntary. You can refuse to participate or withdraw at any time and such a decision will not affect your medical care at NewYork Presbyterian Hospital, now or in the future. Signing this form does not waive any of your legal rights.

Additional Information

If you have any questions or concerns about the study, you may contact Dr. Victoria Leavitt at 212 342 1351.

If you have any questions about your rights as a subject, you may contact:

Institutional Review Board
Columbia University Medical Center
154 Haven Avenue, 1st Floor
New York, NY 10032
Telephone: (212) 305-5883

Agreement to be Contacted

May we contact you in the future for taking part in a new study related to MS or other inflammatory and neurologic diseases?

YES:_____ NO:_____
Initial Initial

Statement of Consent

I have read the consent form and talked about this research study, including the purpose, procedures, risks, benefits and alternatives with the researcher. Any questions I had were answered to my satisfaction. I am aware that by signing below, I am agreeing to take part in this research study and that I can stop being in the study at any time. I am not waiving (giving up) any of my legal rights by signing this consent form. I will be given a copy of this consent form to keep for my records.

Signatures

Participant Signature Lines

Study Participant

Print Name _____ Signature _____
Date _____

Research Signature Lines

Person Obtaining Consent

Print Name _____ Signature _____
Date _____

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	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	1
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 & 12
	5b	Name and contact information for the trial sponsor	12
	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	_____NA____

	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)	NA
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	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
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)	5
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	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)	6
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	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)	7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7

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	9
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)	5-6
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	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7

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	7
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	7
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	?

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	9
	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	7
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	10-11
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	10-11
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10-11
	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)	10-11
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	NA
	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	7
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	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Detailed upon request
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)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	____7_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____NA_____
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	__7-8_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____1_____
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	____7-8_____
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	____7 _____
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	__11-12_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	Added to submission form
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Provided as additional file

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	NA

*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.