Effects of physical exercise on cognition and glymphatic system of patients with mild cognitive impairment or Alzheimer’s disease – study protocol

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Objective: To evaluate the impact of a multicomponent physical exercise program on clinical variables associated with the glymphatic clearance system, sleep-awake patterns, and cognitive function in individuals with mild cognitive impairment or mild Alzheimer’s disease.

Methods: This is a single-center parallel randomized controlled trial involving pre- and post-intervention assessments. The intervention consists of a 12 (±3)-week multicomponent aerobic and resistance physical exercise program of moderate intensity divided into 2 groups: an experimental group (undergoing multicomponent training) and a control group (no intervention). Eligible participants are those diagnosed with probable mild cognitive impairment or mild Alzheimer’s disease.

Expected results: Anticipated outcomes suggest that the multicomponent training protocol, incorporating both aerobic and resistance physical exercises at a moderate intensity, will yield improvements in glymphatic clearance dynamics, sleep-awake parameters, and performance on cognitive, functional, and behavioral tasks among eligible patients.

Relevance: The need to move beyond cognitive clinical testing justifies our trial, which proposes an assessment employing neuroimaging techniques and the analysis of biomarkers present in cerebrospinal fluid in conjunction with clinical tests for physical and cognitive assessment.

Keywords: glymphatic system; physical exercise; dementia; cerebrospinal fluid; sleep; cognition.
INTRODUCTION

Background and rationale
Alzheimer’s disease (AD), a prevalent cause of dementia in older adults, is characterized by neurodegeneration leading to cognitive decline attributable to multiple pathological pathways, such as beta-amyloid (Aβ) deposition and hyperphosphorylated tau protein. Over 25 million people worldwide have dementia, with AD as the leading type, and about 5 million new cases emerge annually, underscoring the urgent need for better understanding and treatment. For individuals already diagnosed with AD and those at risk of developing the disease, incorporating appropriate physical exercise into their daily routine can offer benefits such as enhancing overall physical health and potentially slowing down cognitive decline to some extent.

Notably, the existing literature suggests that the pathogenesis of AD is not solely linked to the accumulation of tau protein and Aβ but also involves their compromised clearance. The discovery of the glymphatic system in 2012 by Iliff et al. introduced a previously unrecognized macroscopic pathway responsible for the clearance of waste products from the brain, including extracellular proteins. This system operates as a brain-wide perivascular pathway facilitated by aquaporin-4 (AQP4) on astrocytic end-feet, delivering nutrients and neuroactive substances while removing metabolic waste through specific pathways.

The efficacy of the glymphatic system appears closely tied to sleep, with insufficient sleep potentially contributing to Aβ aggregation. During slow-wave sleep, the brain may efficiently eliminate metabolic waste, emphasizing the role of sleep in glymphatic clearance. Moreover, the glymphatic system continuously filters toxins from the brain, but during wakefulness, this system is mainly disengaged. Although the predominant clinical symptom of older patients with AD is a steady loss of cognitive function, proper glymphatic function may also protect older adults against cognitive decline.

Recognizing the limitations of relying solely on clinical cognitive testing, our research seeks to employ a comprehensive approach. We propose an evaluation that incorporates neuroimaging techniques, biomarker analysis in cerebrospinal fluid (CSF), and clinical assessments for both physical and cognitive functions. By integrating outcomes related to the glymphatic system into human experimental models, we aim to contribute to the rapidly expanding field of research in this novel area. The trial’s inclusion of pre- and post-intervention assessments for cognitive and sleep-awake patterns further enhances its potential to make distinctive contributions to the global research landscape.

Research objectives
The primary objective is to evaluate the effect of aerobic and resistance physical exercise on clinical variables related to the dynamics of the glymphatic clearance system in patients with mild cognitive impairment (MCI) or mild AD.

The secondary objectives are:

a) To evaluate the effect of aerobic and resistance physical exercise, compared with the control group, on parameters related to the dynamics of the glymphatic clearance system using diffusion magnetic resonance imaging (d-MRI);

b) To evaluate the effect of aerobic and resistance physical exercise compared with control on parameters related to sleep–awake patterns;

c) To evaluate the effect of aerobic and resistance physical exercise compared with control on cognitive function.

METHODS

Design and setting
The current study adopts a single-center parallel randomized controlled trial design, adhering to the Consolidated Standards of Reporting Trials – Harms 2022 extension and the Standard Protocol Items: Recommendations for Interventional Trials – Outcomes 2022 extension. The assessment will be conducted both before and after intervention. In experimental terms, the protocol entails a 12-week multicomponent aerobic and resistance physical exercise program of moderate intensity, categorized into 2 groups: an experimental group undergoing multicomponent training and a control group with no intervention. The independent variable is the multicomponent physical exercise protocol, while the dependent variables encompass the dynamics of the glymphatic clearance system as well as the parameters associated with sleep–awake patterns and cognitive function. Patients will be recruited from the geriatric outpatient clinic where the study will take place.

We have implemented protocols to mitigate participant dropout (ie, regular contact, flexible scheduling, and supportive environment) and to uphold transparency, disclosing a predetermined sample size of 32 individuals. Figure 1 illustrates the methodological processes for each stage of the research in a logically sequenced order.

Randomization and sample eligibility
The participants will be randomly assigned to either the experimental or control group in a 1:1 ratio through a simple computer-generated randomization sequence using the
open-source software R (version 4.2.2) integrated into the R Studio (desktop) interface. To ensure unbiased assessments, outcome assessors will be blind to the allocation. In the event of any challenges in maintaining this blinding, different trained assessors will be appointed. Participants must fulfill the following inclusion criteria:

- Acceptance of the informed consent form;  
- Diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association and the Diagnostic and Statistical Manual of Mental Disorders 5 – The major neurocognitive disorder;  
- Hachinski ischemic score ≤ 4;  
- Mini-Mental State Examination (MMSE) score between 0 and 24;  
- Brain computed tomography and/or MRI in the 12 months preceding screening consistent with a diagnosis of probable AD without other clinically significant comorbidities;  
- Physically able to use the actigraph during the study period;  
- Functionally able to participate in physical and cognitive interventions;  
- Residence in the community with no anticipated changes during the study period;  
- Co-residence with a family member or caregiver available overnight, capable of assuming the role of primary caregiver. This includes accompanying the patient to the study center, providing details about the patient’s experiences, administering medication, completing the sleep diary, and reporting any adverse events.

**Intervention scheme**

Participants allocated to the experimental group will undergo a 12 (±3)-week protocol involving moderate-intensity aerobic and resistance physical exercises. This regimen, scheduled 3 times a week on non-consecutive days, will last 55 minutes per session, totaling 36 sessions. The multicomponent physical exercise intervention will be supervised by medical researchers and seasoned fitness trainers within a dedicated and suitable environment. Rigorous recording of adverse events will take place during each training session, and the correlation between exercise intervention and these events will be carefully examined. Adequate medical services will be provided as needed.

To ensure the prescribed intensity range, adjustments (overload application and activity replacement) to both aerobic and resistance exercises will occur at the conclusion of every 2-week period. Participant heart rates will be monitored during sessions using Polar’s A4 frequency meter, ensuring that the training aligns with individual conditions and adheres to the American College of Sports Medicine’s recommendation of an exercise intensity between 60% and 80% of maximum heart rate. Prior to the intervention, a familiarization period with the protocol and proposed activities will be determined by instructor-researchers.

Conversely, participants in the control group will not partake in the multicomponent training. However, they will undergo evaluations both before and after the intervention, except for lumbar puncture, which will be performed only before the intervention for both experimental and control groups.
Outcomes and measurement scheme

Primary outcomes
The anticipated primary outcomes of our trial will revolve around the assessment of the dynamics of glymphatic clearance, primarily assessed by MRI. The d-MRI will play a crucial role in this assessment by enabling the calculation of the fractional volume of free water in the brain parenchyma, using a bi-tensor diffusion tensor imaging model, and the calculation of the diffusivity along the perivascular space index, around the deep medullary vein. Together, they will enable indirect assessment of the glymphatic system. Participants will undergo this imaging procedure both before and after the intervention. Skilled procedures will be performed by a specialist neuroradiologist.

In addition to imaging, biochemical analysis of CSF will be conducted to assess the levels of Aβ, tau, and AQP4. The CSF will be collected by lumbar puncture, performed by a specialist neurologist with the assistance of a nursing technician.

These comprehensive assessments aim to provide a thorough understanding of the impact of the multicomponent physical exercise program on the glymphatic clearance system and associated biochemical markers, contributing valuable insights to our trial.

Secondary outcomes
The expected secondary outcomes of our trial will focus on changes in sleep-awake patterns and cognitive function. Importantly, there will be no cost to the participant for undergoing the secondary outcome procedures, and the control group will also participate in these assessments despite not engaging in the intervention.

For the analysis of sleep-awake patterns, actigraphy assessments and a comprehensive clinical evaluation will be conducted both before and after the intervention. An expert geriatrician will oversee the procedures.

Cognitive function will be assessed using the Alzheimer’s Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) – standardized version for the Brazilian population and the MMSE. Participants will undergo this neurocognitive assessment before the experimental phase, with procedures being conducted by an expert neuropsychologist.

These secondary outcomes aim to provide a holistic understanding of the impact of the multicomponent physical exercise program on sleep-awake patterns and cognitive function, enhancing the overall insights gained from our trial.

Instruments
The selection of instruments for outcome measurement in our study is motivated by the need for an evaluation that extends beyond traditional cognitive clinical testing. Given the complex nature of AD and MCI, our aim is to employ a multi-faceted approach that encompasses neuroimaging techniques, CSF biomarker analysis, and clinical assessments for both physical and cognitive functions. This approach will allow us to capture a broad spectrum of variables relevant to the glymphatic clearance system, sleep-awake patterns, and cognitive function.

The use of d-MRI will allow us to indirectly assess the dynamics of the glymphatic system by examining parameters such as the fractional volume of free water in the brain parenchyma and the diffusivity along the perivascular space index. Complementing this, biochemical analysis of CSF will provide insights into the levels of key biomarkers, such as Aβ, tau, and AQP4, further elucidating the impact of physical exercise on glymphatic function. Additionally, actigraphy assessments and neurocognitive tests will allow us to evaluate changes in sleep-awake patterns and cognitive function, respectively.

Data collection and statistical analysis
Outcome assessors will collect research data, which will be transcribed into an electronic database. Standard statistical methods will be used to tabulate and calculate means and SDs. Statistical analyses will be performed using the open-source software R (version 4.2.2) with the R Studio (Desktop) interface, with a 95% CI for type I error (p < 0.05). This rigorous statistical approach will ensure the robustness and reliability of the analyses performed within our trial.

Ethical considerations and clinical registration
This research has been approved by the local medical research ethics committee under the code CAAE [74252323.7.0000.5558] and is registered with the Open Science Framework as a project protocol (https://doi.org/10.17605/OSF.IO/SA8Z3). Available at https://osf.io/sa8z3/.

EXPECTED RESULTS AND RELEVANCE
Dementia conditions have multifactorial characteristics and involve several different etiopathogenic mechanisms. Among the non-pharmacological therapies recurrently mentioned in the medical literature, physical exercise stands out as the most efficient in prevention and treatment, as well as in improving the characteristic cognitive and physiological decline of AD. As a result, there is a growing demand for interventions that can improve the quality of life of the geriatric population, and this is a pressing issue in some countries.
(eg, Brazil, China, Saudi Arabia), where estimates indicate that the inversion of the age pyramid will occur more quickly than in countries where this process has already been completed (eg, Japan, Finland, Italy). Therefore, intervention practices need to be developed in the field of health and public policies to mitigate the unwanted effects of this process. Our intervention proposal will not only contribute to aging research but also generate positive impacts for this population, allowing health promotion given the nature of the investigation and impacting the quality of life of these patients.

Theoretical framework of our proposal is based on previously published studies focused on our target population (patients with MCI and AD) that used multiple experimental methodologies (ie, animal and human experimental research models). In experimental animal models, the study by He et al. demonstrated that physical exercise accelerated glymphatic clearance, improved astrocytic AQP4 expression and polarization, attenuated the accumulation of amyloid plaques and neuroinflammation, and ultimately protected mice against synaptic dysfunction and a decline in spatial cognition. The study by Liu et al. revealed that long-term voluntary exercise promotes the removal of Aβ, attenuating its aggregation and subsequently reducing astrocyte activation, which is conducive to the maintenance of AQP4 polarity. In experimental human models, meta-analyses have provided evidence that physical exercise interventions improve cognition in humans for patients with MCI (eg, Zhou et al., physical exercise improved MMSE scores [p < 0.00001, I² = 95%]) and for patients with AD (eg, Liu et al., physical exercise improved MMSE scores [p < 0.00001; I² = 47%] and ADAS-Cog performance [p = 0.04; I² = 57%]).

ACKNOWLEDGEMENTS

The authors wish to express their sincere appreciation to the editor and 3 anonymous reviewers whose insightful suggestions have greatly enriched the quality of the present protocol. Special recognition is extended to the Brazilian government research-funding agencies: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF).

REFERENCES


Physical exercise, cognition and glymphatic system


