AGUEDA Project

Active Gains in brain Using Exercise During Aging

Statistical Analysis Plan

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1. General information of AGUEDA project

1.1 Background and rationale

Cognitive decline associated with dementia, particularly in Alzheimer's disease (AD) (1), initiates over two decades before clinical symptoms, with pathological accumulation of beta-amyloid in the brain as an early marker (2,3). Executive function and memory are the first cognitive domains affected (4). While memory decline is typical of AD, executive dysfunction may precede it (5) and predict further cognitive decline, mild cognitive impairment (MCI), and AD, making it a potential target for lifestyle interventions (6,7).

Dementia is not inherent to aging, and exercise stands out as a non-pharmacological preventive and efficient treatment for age-related cognitive decline (8). Systematic reviews have highlighted the benefits of exercise interventions in older adults, impacting executive function, memory, language, and general cognition (9–12). Although aerobic exercise has been extensively studied (13,14), resistance exercise, despite being included in physical activity guidelines, has received less attention (15,16). Recent reviews suggest significant cognitive benefits from resistance exercise (12,17,18), even at low doses (18). However, variations in study parameters make it challenging to determine the specific dose and type of resistance exercise needed for cognitive improvement (19). Therefore, well-designed randomized controlled trials (RCTs) are essential to clarify the effects of resistance exercise on cognition in older adults.

Potential mechanisms underlying exercise's cognitive benefits span molecular, brain structural/functional, and behavioral levels. Most evidence is derived from aerobic exercise studies, but resistance exercise may involve distinct pathways. Animal studies suggest inflammatory and metabolic changes, while human studies highlight neurochemical markers. Understanding these mechanisms, especially in cognitively normal older adults, requires well-designed RCTs with clear reporting of exercise characteristics.

1.2 Objective

The main objectives are:

1. The primary aim of the study is to investigate the effects of a 24-week resistance exercise program on executive function in cognitively normal older adults.
2. The secondary aims are (i) to examine the effects of resistance exercise on brain structural and functional markers (e.g., grey and white matter measures, functional connectivity or cerebral blood flow), brain Aβ deposition, peripheral molecular markers (e.g., BDNF, IGF-1, and Aβ), and other cognitive outcomes (e.g., memory and language) and (ii) to investigate mediators and moderators of the potential exercise-derived improvements observed in executive function and brain markers.
3. The tertiary aim is to investigate the effect effects of a 24-week resistance exercise program on other outcomes including changes in muscular strength, cardiorespiratory fitness, physical function, gait parameters, mental health, quality of life, diet, body composition, blood pressure, microbiome, among others.

2. Study methods

2.1 Trail design

The AGUEDA (Active Gains in brain Using Exercise During Aging) trial is a single-centre, two-arm, single-blind RCT in which 90 cognitively normal older adults, aged 65–80 years old are randomized into a resistance exercise group (n = 45) or a wait-list control group (n = 45). Measurements are performed at baseline, at midpoint (12 weeks) and post intervention (24 weeks). A visual representation ofthe participant flow during the study can be seen in **Figure 1**. More detailed information is described in the study protocol paper (20). The study has been designed following the Standard Protocol Items for Randomized Interventional Trials (SPIRIT)(21).

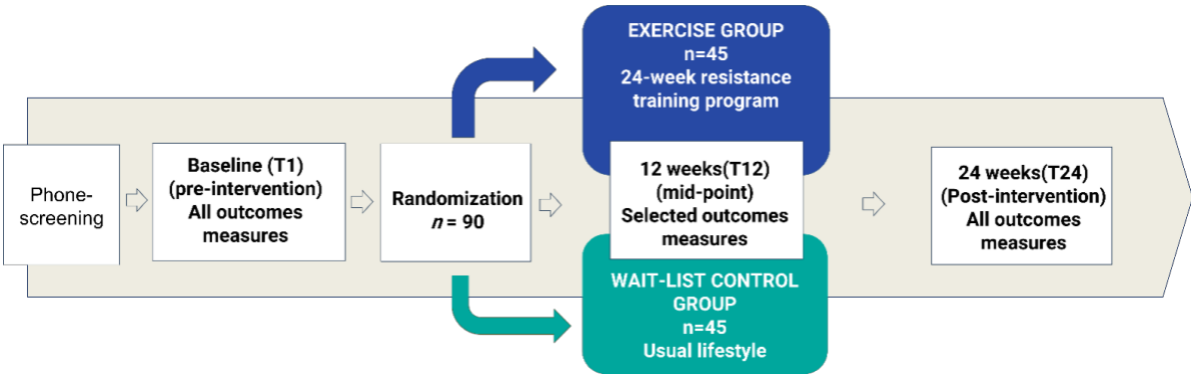

Figure 1. Overview of the AGUEDA project

2.2 Randomization

Randomization (1:1) occurs on a rolling basis and only after the completion of all baseline assessment sessions by the participant to reduce the risk of bias during the assessment. We use a computerized randomization protocol (stratified by age and sex) through the REDCap Software (22) that incorporate a checklist to make sure all assessments have been completed and the data have been adequately entered into the database.

2.3 Sample size

We based our estimates on the AGUEDA primary outcome, i.e., change on the executive function composite score over a 24-weeks resistance exercise period. The power analysis is based on a meta-analysis which showed that the effect size of exercise intervention on executive function in older adults was 0.34 (95% Confidence interval = 0.22 to 0.47) (17) with a two-tailed alpha at 0.05 and a power of 80%. Thus, we need a sample size of 35 participants for each group to obtain such an effect size. If the maximum dropout rate is 20% plus some residual power, we then need 45 participants for each group. After all computations, we decided that the target sample size for this study will be 90 participants. More detailed information is described in the protocol paper (20)

2.4 Framework

A superiority hypothesis testing framework is used, emphasizing the evaluation of a intervention's positive impact compared to a control group. In the main analysis, we compare

resistance exercise group versus control group (no exercise) in 3-time points (Baseline, 12-week and 24-week).

2.5 Statistical interim analyses and stopping guidance

No pre-specified interim analyses are performed, and therefore, a stopping guidance is not applicable.

2.5 Timing of the final analyses

The main final analyses will be performed after the finalization of the data processing of the primary outcome.

2.6 Timing of outcome assessment

The primary outcome is assessed at baseline, midpoint (12 weeks) and post intervention (24 weeks). In addition, there are other secondary outcomes only assessed at baseline and post intervention. The time frame of each outcome is explained in section *5.1 Outcome definitions*.

3. Statistical principles

3.1 Confidence and P values

All statistical tests are two-tailed. *P* for significance will be set at 0.05 and 95% confidence intervals will be estimated.

3.2 Adherence and protocol deviations

Adherence to the exercise program is measured by session attendance which is the proportion of sessions attended out of the total offered sessions.

All protocol deviations made to the protocol (e.g. change in pre-defined inclusion/exclusion criteria, baseline and post assessments, data cleaning/processing) will be reported and described in the analysis.

3.3 Analysis populations

Three analytic datasets will be defined for the statistical analyses:

1. Intention-to-treat: This dataset will be used for our primary analyses and includes all randomly assigned study participants. With this approach, all randomized participants are included in the main analysis.
2. Per protocol: This dataset will be used for our secondary analyses and includes all participants who followed the attendance rate. As such, as the 100% of the session corresponding to a total of 72 sessions (3 session per week for 24 weeks), 80% attendance is required for be included per protocol analyses, corresponding to >= 57 exercise sessions.

4. Trail population

4.1 Recruitment

We began recruitment of community-dwelling older adults in Granada (Spain) city and surrounding areas in March 2021. Strategies to achieve the targeted sample size mainly include mass mailings and social media advertisements, word of mouth, with augmentation of the recruitment strategy by using advertisements in newspapers (TV, radio, and internet).

Information necessary for the CONSORT flow diagram is collected. For the enrollment phase, we note the number of participants that were assessed for eligibility by the research team, the number of excluded participants (plus reason for exclusion), and the number of randomized participants. For the allocation, the number of participants allocated to the intervention and control group is noted. For follow-up, the number of participants lost to follow-up (12-week and 24-week) and the number who discontinued the intervention (plus reasons) are counted. Finally, the number of participants included in the analyses using the intention-to-treat and per-protocol databases is described, along with the reasons for exclusion.

4.2 Initial phone screening

Following recruitment strategies and after the participant's first contact for general information, a phone screening is performed with those individuals who are potentially interestedin participating. Then, participants meeting the phone criteria are invited to the on-site screening, and those who met the on-site criteria are enrolled in the study.

4.3 Eligibility

Inclusion and exclusion criteria is defined as follows: (i) older adults between 65 - 80 years, (ii) physically inactive (i.e., defined as not participating in any resistance exercise program in the last 6 months or accumulating less than 600 METs-Min/week by the International Physical Activity Questionnaire (IPAQ) (23), (iii) classified as cognitively normal according to the Spanish version of the modified Telephone Interview of Cognitive Status (STICS-m) (≥ 26 points) (24), Mini-Mental State Examination (MMSE) (25/30) (25) and Montreal Cognitive Assessment (MoCA) (<71 years, 24/30, 71-75 22/30, >75, 21/30) (26); and (iv) without significant depressive symptoms at baseline according to the Geriatric Depression Scale (GDS) (15) (27). Detailed information about the study design is available elsewhere(20).

4.4 Withdrawal/follow-up

The number, timing, and reasons (e.g. adverse events, withdraw consent, lost to follow-up) for withdrawal is noted and described.

4.5 Baseline patient characteristics

A baseline data table is created to describe the characteristics of the study population. The main characteristics include age, sex, education, body mass index and the outcomes variables. The characteristics of the total study population and each study arm is summarized using mean (SD) or median (interquartile range) for normally and not normally distributed continuous variables, respectively, and as number (percentage) for categorical variables.

5. Analysis

5.1 Outcome definitions

The primary outcome is assessed at baseline (before randomization), at mid-point (12-week) and after the intervention (24-week) while some of secondary outcomes are assessed only at baseline and post intervention (24-week, Table 1).

Table 1. Time frame and specific outcomes of cognitive tests.

Executive function test	Baseline	12-week	24-week	Specific outcome
Trail making test A and B	x	x	x	Interference score
Digit symbol substitution test	x	x	x	Total number of correctly paired symbols
Dimensional card sort task	x		x	Inverse efficiency score
Spatial working memory	x	x	x	Inverse efficiency score
Picture sequence	x		x	Theta score
List sort working memory	x		x	Raw score corrects trials
Flanker	x		x	Incongruent trials inverse efficiency score
Stroop task	x	x	x	Interference score
Task-switching	x	x	x	Global Cost
Other cognitive test	**Baseline**	**12-week**	**24-week**	**Specific outcome**
Rey auditory verbal learning test	x		x	Total recorded words
Rey-Osterrieth complex figure	x		x	Correct drawn elements
Boston Naming Test	x		x	Correct responses
Wechsler adult intelligence scale IV ï Similarities ï Vocabulary ï Information ï Block design ï Matrix reasoning ï Visual puzzles	x		x	Correct responses
Verbal and semantic fluency test	x	x	x	Correct responses

Primary outcome

The primary outcome of the AGUEDA project is the change on the executive function Executive function composite score (Z-score) which will be created by performed confirmatory factor analyses (CFA). CFA will include the executive function variables and the final model will be choose based on the fit of the model considering χ^2 p-value ≥ .05, Bentler Comparative Fit Index (CFI) > .95, Steiger-Lind Root Mean Squared Error of Approximation (RMSEA) < .08 as a good model fit (28).

The executive function tests included to perform the CFA will be:

- Trail Making Test (29). The difference in the time between trail A and trail B (TMT B-A) is calculated and the inverse variable will be used.
- Design symbol substitution test (30). The variable to use is the total numbers of correct symbols score.
- Dimensional Change Card Sort Test (31). The variable used will be the inverse efficiency score of color trials (Reaction time/ Accuracy).
- Spatial working memory (13). Performance is measured based on accuracy, response times, and errors. The variable used will be the inverse efficiency score (Reaction time/ Accuracy) of all trials (2,3,4 items).

- Picture Sequence Memory Test (31). The participant's score is derived from the cumulative number of adjacent pairs of pictures remembered correctly over three learning trials, the theta score.
- List Sort Memory Test (31). Scores are calculated by summing the total number of correct responses across both lists.
- Flanker (31). The interference from reporting the direction of the middle arrow and not the external arrows will be calculated by subtracting the average incongruent accuracy from that of the congruent trials.
- Stroop test. The interference score from the incongruent trials will be calculated.
- Task Switching (32). The difference in mean reaction time for the mixed block of trials, including both the repeat and switch trials, is calculated as a global cost score.

Secondary outcomes

Other cognitive outcomes. Cognitive outcomes are assessed such as general cognition, language, verbal memory, visuospatial memory and crystallized and fluid intelligence.

- Rey auditory verbal learning test (33). The Total Learning measure (RAVLT-AT) will be the capacity to recall and to accumulate words through the 5 learning trials.
- Rey-Osterrieth complex figure (ROCF) (34). The study variables that will be used are: (a) copy—overall sum of correctly drawn elements when copying the model; (b) immediate recall—total sum of correctly drawn items after 3 min.
- Boston Naming Test (BNT) (35). The variable that we will use will be the number of correct responses generated requested to the name of each figure.
- Wechsler adult intelligence scale IV (36). That tests represent a different cognitive domain: Verbal Comprehension, Working Memory Index, Perceptual Reasoning Index and Processing Speed Index. The variable that we will use will be the number of correct responses generated in each test:
    - Similarities
    - Vocabulary
    - Information
    - Block design
    - Matrix reasoning
    - Visual puzzles
- Verbal and semantic fluency test(37). The verbal fluency assessment tests letter fluency (recall as many words beginning with a specific letter) and semantic fluency (recall as many members of a category as possible). The variable that we will use will be the number of words generated by the participant.

Brain structure and function (Time frame: Baseline and 24-week). Brain structure and function is assessed by a Siemens Magnetom PRISMA Fit 3T scanner with a 64-channel head coil. Briefly, the following sequences are performed:

- T1-weighted Magnetization-Prepared Rapid Acquisition with Gradient Echo (T1-weighted MPRAGE structural): Volume, surface, thickness and shape.
- High resolution hippocampus: High resolution Hippocampal subfield segmentation.

- Resting State Echo-Planar Imaging (EPI): resting state Blood Oxygen Level Dependent (BOLD) fluctuations, activation and functional connectivity.
- Functional Magnetic Resonance Imaging n-back Task (fMRI n-back task): Task-evoked BOLD fluctuations, activation and functional connectivity.
- Diffusion weighted acquisition: White matter integrity indicators such as fractional anisotropy, mean diffusivity, radial diffusivity, and axial diffusivity.
- Three-Dimensional T2 Turbo Spin Echo Fluid Attenuated Inversion Recovery (3D T2 TSE FLAIR): White matter lesions.
- Pseudo-Continuous Arterial Spin Labeling Turbo Gradient Spin Echo (pCASL TGSE): Blood flow estimates and arterial transit time estimates.

*Brain amyloid beta deposition (*Aβ) (Time frame: Baseline and 24-week). The brain Aβ deposition is assess using a PET scan Biograph-Vision 600 Edge Positron emission tomography/Computed tomography (PET/CT) digital scanner (Siemens, Erlangen, Germany). [18F]Florbetaben is injected intravenously into each participant in accordance with the applicable regulatory guidelines. PET images are acquired from participants 90 to 110 min after intravenous injection of 300 megabecquerel (MBq) ± 20% (38). The Standardized Uptake Value Ratio (SUVR) and centiloid values will be used as outcomes.

Other outcomes

Biological samples (Time frame: Baseline and 24-week). Several biomarkers using blood, saliva, and fecal samples are determined . Blood samples are used to determine traditional blood markers and genotyping and novel blood brain-based biomarkers. In addition, fecal and saliva samples are used to determine microbiome composition and others potential biomarkers.

Physical Function (Time frame: Baseline, 12-week and 24-week; gait parameters and 2-km walking test not performed at 12-week). The Senior Fitness Test (SFT) (39) and the Short Physical Performance Battery (SPPB) (40) ,the 2-km test (only baseline and 24-week) and the Optogait are performed. Briefly, we measure (i) aerobic capacity [i.e., 6-minute walk test (m/6 min) (41), Two-minute step test (n/2 min), Up and Go test (sec) and 2-km walking test (seconds) (42), Chair Sit and Reach Test (cm)], (ii) balance [i.e., Balance test (score of Balance test)], (iii) flexibility [i.e., Chair Sit and Reach test (cm) and the Back Scratch test (cm)] and (iv) gait parameters [i.e., Stride length (cm), Stride time (sec), Step length (cm), Step time (sec))] by the optical Opto Gaitsystem (Microgate Slr; Bolzano, Italy) (43).

Muscular strength (Time frame: Baseline, 12-week and 24-week; Gymmex Iso-2 dynamometer not performed at 12-week). Measurements are taken to assess both lower and upper muscular strength. Lower body strength was evaluated using the Chair Stand test from SFT (44) and the Gymmex Iso-2 dynamometer (EASYTRCH s.r.l., Italy) (45). Upper body strength was assessed through the Arm Curl test from SFT (44), Hand dynamometer (TKK 5101 Grip D, Takey, Tokyo Japan) and the Gymmex Iso-2 dynamometer (EASYTRCH s.r.l., Italy) according to the previous published protocol (45).

Physical activity monitoring (Time frame: Baseline and 12-week). Movement behaviors are monitored using the Actigraph GT3X + accelerometry device (Pensacola, FL, USA). Participants wear the device on their non-dominant wrist for a continuous span of 9 days, with removal only during activities such as showering. Alongside the device, participants maintained a diary to record in-bed and out-of-bed times, naps, and periods when the device is not in use.

Anthropometric, body composition and blood pressure measurements (Time frame: Baseline and 24-week). Body weight (kg) measured in triplicate with an electronic scale (SECA 861, Hamburg, Germany). Height (cm) assess with a precision stadiometer (SECA 225, Hamburg, Germany). Head, neck, waist and hip circumference are measured. Body composition is measure using dual energy X-ray absorptiometry (DXA, Discovery Densitometer from Hologic). A total body scan as well as specific assessment of both hips and lumbar spine are performed to determine body composition and bone density parameters. Validated automated blood pressure monitor (Omron M3, Intellisense) is used to take blood pressure measurements.

Psychosocial, mental health and other questionnaires. A battery of questionnaires is administered including the following dimensions: mental health, psychological health, functional ability, medical information, lifestyle behaviors, andothers. Exercise self-efficacy, social provisions and groups. mood and anxiety, personality traits, social networks, pain, fatigue, etc. As such, we carefully selected a battery of tests that would considerably extend the knowledge on how exercise influences cognitive and brain health. The time frame of each questionnaire is present in table 2.

Table 2. Time frame of questionnaires

Questionnaire	Baseline	12-week	24-week
Mental health			
Geriatric Depression Scale (GDS)	x		x
Hospital Anxiety and Depression Scale	x	x	x
Perceived Stress Scale (PSS)	x		x
Rosenberg Self-Esteem Scale (RSE)	x		x
Satisfaction With Life Scale (SWLS)	x		x
UCLA Loneliness Scale (UCLA-L)	x		x
Psychological variables			
Social media use	x		x
Social Support Questionnaire	x		x
Big Five Inventory (BFI)	x		x
Short Form 36 Health questionnaire (SF-36)	x	x	x
Functional ability variables			
Instrumental activities ofdaily living	x		x
International Fitness Scale (IFIS)	x		x
Mobility and agility questionnaire	x	x	x
Questions about energy and frailty	x	x	x
McGill Pain Questionnaire	x	x	x
Medical history			
Health history	x		x
Medication list	x		x
Oral health questionnaire	x		x

COVID questionnaire	x		x
Adverse event report	x		x
Lifestyle behaviors			
Drinks questionnaire	x		x
Food-frequency Questionnaire (FFQ)	x		x
MEDAS-14	x	x	x
Smoking questions	x		x
Sitting time questionnaire	x		x
Physical activity assessment	x		x
Pittsburgh Sleep Quality Index (PSQI)	x		x
Others			
Demographic questions	x		x
Edinburgh Handedness Inventory (EHI)	x		x
Mental health	x		x
Geriatric Depression Scale (GDS)	x		x
Hospital Anxiety and Depression Scale	x		x
Perceived Stress Scale (PSS)	x		x
Rosenberg Self-Esteem Scale (RSE)	x		x
Satisfaction With Life Scale (SWLS)	x		x
UCLA Loneliness Scale (UCLA-L)	x		x
Psychological variables	x		x
Social media use	x		x
Social Support Questionnaire	x		x
Big Five Inventory (BFI)	x		x
Short Form 36 Health questionnaire (SF-36)	x		x
Functional ability variables	x		x

5.2 Analysis methods

The main analyses will consist of the intention-to-treat analyses for the primary and secondary outcomes using a constrained linear mixed model (cLMM). The model will include fixed effects for time (three levels or two levels, depending of the outcome) and treatment (two levels) as well as the unique participant identifier as a random effect. Model assumptions will be checked. In case we detect model violations, we will take appropriated measures by, for example, conducting data transformations. The treatment effects will be presented by estimated marginal means and standard errors for changes. The specific differences between analysis are described in the following's points divided into primary analysis, secondary and others outcomes.

5.2.1 Primary analysis.

Effects on primary outcomes

To investigate the overall effect of the resistance exercise intervention on the change from baseline to post-intervention in the executive function composite score, cLMM approach with repeated measures over time (baseline, 12-week and 24-week outcomes) will be used using an intention-to-treat principle. This statistical linear mixed model will include both a random intercept for each participant and a group-by- time interaction as a fixed effect.

5.2.2. Secondary analyses

Effects on secondary outcomes

To investigate the effect of the resistance exercise intervention on the secondary outcomes (other cognitive outcomes, brain structure and function and AB), the same analyses as the primary analysis using an intention-to-treat principle and cLMM analysis will be performed. The preprocessing analysis of brain structure and function measured with MRI will be carried out as secondary analyses using different programs (Freesurfer, FSL, CON, fMRIprep).

Effects on other outcomes

To investigate the effect of the resistance exercise intervention on other outcomes (i.e., biological biomarkers, physical function, muscular strength, physical activity, Anthropometric, body composition and blood pressure and Psychosocial, mental health and other questionnaires*)* the same analyses as the primary analysis using an intention-to-treat principle and cLMM analysis will be performed.

Furthermore, analyses per protocol will be followed of the executive function variables (ie., Executive function score, Trail making test, Digit symbol substitution score, Dimensional change card sort test and Spatial working memory).

Moderators and mediators

In addition, mediation and moderation analysis will be performed following AGReMA (A Guideline for Reporting Mediation Analyses) recommendations (46), using the group as independent variable (intervention group vs control group), executive function as outcome, and mediating and moderating variables.

Moderators include will be sex, age, education, apolipoprotein E (APOE) status, and Aβ status. Additionally, baseline data of various cognitive measures, including the composite executive function score, Trail Making Test, Digit Symbol Substitution Score, Dimensional Change Card Sort Test, and Spatial Working Memory, will be incorporated. The baseline values of strength measures and mental health variables, specifically depression and self-esteem, will be also analyzed. We will also assess the potential mediators, aiming to understand the mechanisms through which the intervention could exert its effects. No interim analysis will be performed.

5.2.3 Sensitivity analyses

A sensitivity analysis will be performed using an intention-to-treat principle with all randomly assigned participants, but excluding participants who encountered adverse events that may have impacted the primary outcomes. Adverse events considered for exclusion is conducted to identify and categorize adverse events based on predefined criteria using common terminology criteria for adverse events (i.e., mild, moderate, severe) (47).

5.3 Missing data

The number of missing data will be reported and patterns of missing data will be explored. Based on previous experience, we expect that missing data will be assumed as missing at random. Therefore, the linear mixed model analyses will handle our missing data. However,

once the data processing is finalized, we reconsider this expectation. In case we believe this assumption does not hold, we will explore appropriate measures for the data analyses.

5.4 Additional analyses/Compensatory analyses

A set of additional analyses will be performed to confirm the robustness of main the analyses. We will perform a 1-dimension curve analysis using an Statistical Parametric Mapping 1 package (SPM1D) available for MATLAB (http://www.spm1d.org) (33) to study whether acceleration values (i.e., expressed as ENMO [mg]) identify a significant increase in physical activity during the exercise program in comparison with the physical activity pattern at baseline for the control and exercise groups.

In-depth analysis of intervention compliance is going to be conducted to gauge the intensity as exploratory analysis. Changes in others outcomes will be also analyzed using a similar protocol as described by '5.2 Analysis methods' by following the intention to treat and per protocol principles. Furthermore, cross-sectional analyses will be performed using the baseline data of the RCT.

5.5 Harms

Any situation that may occur, such as injury, emergency or scheduled surgery was reported as adverse events categorize based on predefined criteria using common terminology criteria (i.e., mild, moderate, severe) (6). Sensitivity analyses will be done with all randomly assigned study participants who encountered adverse events that may have impacted the primary outcomes of the analyses.

5.6 Statistical software

The analyses of the primary outcomes will be performed using R. For the main analyses, we will use the 'LMMstar', lmer, nlme and lavaan packages. The use of specific packages will be reported in each manuscript. In addition, specific software's are used for secondary brain outcomes.

6. References

1. Yang Y, Wang D, Hou W, Li H. Cognitive Decline Associated with Aging. In: Advances in Experimental Medicine and Biology [Internet]. 2023. p. 25–46. Available from: https://link.springer.com/10.1007/978-981-99-1627-6_3
2. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. New England Journal of Medicine. 2012 Aug 30;367(9):795–804.
3. Jansen WJ, Janssen O, Tijms BM, Vos SJB, Ossenkoppele R, Visser PJ, et al. Prevalence Estimates of Amyloid Abnormality Across the Alzheimer Disease Clinical Spectrum. JAMA Neurol. 2022 Mar 1;79(3):228–43.
4. Kirova AM, Bays RB, Lagalwar S. Working Memory and Executive Function Decline across Normal Aging, Mild Cognitive Impairment, and Alzheimer's Disease. Vol. 2015, BioMed Research International. Hindawi Limited; 2015.
5. Harrington MG, Chiang J, Pogoda JM, Gomez M, Thomas K, Marion SDB, et al. Executive function changes before memory in preclinical Alzheimer's pathology: A prospective, cross-sectional, case control study. PLoS One. 2013 Nov 18;8(11).
6. Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, et al. A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. Brain Imaging Behav. 2012 Dec 1;6(4):517–27.
7. Erickson KI, Donofry SD, Sewell KR, Brown BM, Stillman CM. Cognitive Aging and the Promise of Physical Activity. Annu Rev Clin Psychol [Internet]. 2022 May 9;18:417–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/35044793
8. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Vol. 396, The Lancet. Lancet Publishing Group; 2020. p. 413–46.
9. Hindin SB, Zelinski EM. Extended practice and aerobic exercise interventions benefit untrained cognitive outcomes in older adults: A meta-analysis. J Am Geriatr Soc. 2012 Jan;60(1):136–41.
10. Zheng G, Xia R, Zhou W, Tao J, Chen L. Aerobic exercise ameliorates cognitive function in older adults with mild cognitive impairment: a systematic review and meta-analysis of randomised controlled trials. Br J Sports Med [Internet]. 2016 Dec;50(23):1443–50. Available from: https://bjsm.bmj.com/lookup/doi/10.1136/bjsports-2015-095699
11. Chen FT, Etnier JL, Chan KH, Chiu PK, Hung TM, Chang YK. Effects of Exercise Training Interventions on Executive Function in Older Adults: A Systematic Review and Meta-Analysis. Vol. 50, Sports Medicine. Springer; 2020. p. 1451–67.
12. Li Z, Peng X, Xiang W, Han J, Li K. The effect of resistance training on cognitive function in the older adults: a systematic review of randomized clinical trials. Vol. 30, Aging Clinical and Experimental Research. Springer International Publishing; 2018. p. 1259–73.
13. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A. 2011 Feb 15;108(7):3017–22.
14. Stillman CM, Esteban-Cornejo I, Brown B, Bender CM, Erickson KI. Effects of Exercise on Brain and Cognition Across Age Groups and Health States. Vol. 43, Trends in Neurosciences. Elsevier Ltd; 2020. p. 533–43.

15. Yoon DH, Lee JY, Song W. Effects of Resistance Exercise Training on Cognitive Function and Physical Performance in Cognitive Frailty: A Randomized Controlled Trial. Journal of Nutrition, Health and Aging. 2018 Oct 1;22(8):944–51.
16. Liu-Ambrose T, Donaldson MG. Exercise and cognition in older adults: Is there a role for resistance training programmes? Vol. 43, British Journal of Sports Medicine. 2009. p. 25–7.
17. Northey JM, Cherbuin N, Pumpa KL, Smee DJ, Rattray B. Exercise interventions for cognitive function in adults older than 50: A systematic review with meta-Analysis. Br J Sports Med. 2018;52(3):154–60.
18. Gallardo-Gómez D, del Pozo-Cruz J, Noetel M, Álvarez-Barbosa F, Alfonso-Rosa RM, del Pozo Cruz B. Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of RCTs. Ageing Res Rev [Internet]. 2022 Apr;76:101591. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1568163722000332
19. Hansford HJ, Wewege MA, Cashin AG, Hagstrom AD, Clifford BK, McAuley JH, et al. If exercise is medicine, why don't we know the dose? An overview of systematic reviews assessing reporting quality of exercise interventions in health and disease. Br J Sports Med. 2022;bjsports-2021-104977.
20. Solis-Urra P, Molina-Hidalgo C, García-Rivero Y, Costa-Rodriguez C, Mora-Gonzalez J, Fernandez-Gamez B, et al. Active Gains in brain Using Exercise During Aging (AGUEDA): protocol for a randomized controlled trial. Front Hum Neurosci [Internet]. 2023 May 22;17. Available from: https://www.frontiersin.org/articles/10.3389/fnhum.2023.1168549/full
21. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, et al. SPIRIT 2013 statement: Defining standard protocol items for clinical trials. Vol. 158, Annals of Internal Medicine. American College of Physicians; 2013. p. 200–7.
22. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform [Internet]. 2019 Jul 1;95:103208. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31078660
23. Tomioka K, Iwamoto J, Saeki K, Okamoto N. Reliability and validity of the international physical activity questionnaire (IPAQ) in elderly adults: The Fujiwara-kyo study. J Epidemiol. 2011;21(6):459–65.
24. Muñoz-García M, Cervantes S, Razquin C, Guillén-Grima F, Toledo JB, Martínez-González MÁ, et al. Validation study of a Spanish version of the modified Telephone Interview for Cognitive Status (STICS-m). Gac Sanit. 2019 Sep 1;33(5):415–20.
25. Beaman SR de, Beaman PE, Garcia-Peña C, Villa MA, Heres J, Córdova A, et al. Validation of a Modified Version of the Mini-Mental State Examination (MMSE) in Spanish. Aging, Neuropsychology, and Cognition [Internet]. 2004 Mar 9;11(1):1–11. Available from: https://www.tandfonline.com/doi/full/10.1076/anec.11.1.1.29366
26. Ojeda N, del Pino R, Ibarretxe-Bilbao N, Schretlen DJ, Pena J. [Montreal Cognitive Assessment Test: normalization and standardization for Spanish population]. Rev Neurol [Internet]. 2016 Dec 1;63(11):488–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27874165
27. Fernández-San Martín MI, Andrade C, Molina J, Muñoz PE, Carretero B, Rodríguez M, et al. Validation of the Spanish version of the geriatric depression scale (GDS) in primary care. Int J Geriatr Psychiatry. 2002;17(3):279–87.
28. Hu LT, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. Structural Equation Modeling. 1999;6(1):1–55.

29. Reitan RM. Validity of the Trail Making Test as an Indicator of Organic Brain Damage. Percept Mot Skills [Internet]. 1958 Dec 31;8(3):271–6. Available from: http://journals.sagepub.com/doi/10.2466/pms.1958.8.3.271
30. Wechsler D. Wechsler Adult Intelligence Scale—Fourth Edition Administration and Scoring Manual. 2008 Sep 18; Available from: http://journals.sagepub.com/doi/10.1177/073428298300100310
31. Zelazo PD, Anderson JE, Richler J, Wallner-Allen K, Beaumont JL, Weintraub S. NIH toolbox cognition battery (CB): Measuring executive function and attention. Monogr Soc Res Child Dev. 2013 Aug;78(4):16–33.
32. Kramer AF, Hahn S, Cohen NJ, Banich MT, McAuley E, Harrison CR, et al. Ageing, fitness and neurocognitive function. Nature [Internet]. 1999 Jul 29;400(6743):418–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10440369
33. Arrieta H, Rezola-Pardo C, Echeverria I, Iturburu M, Gil SM, Yanguas JJ, et al. Physical activity and fitness are associated with verbal memory, quality of life and depression among nursing home residents: Preliminary data of a randomized controlled trial. BMC Geriatr. 2018 Mar 27;18(1).
34. Calderón-Rubio E, Oltra-Cucarella J, Bonete-López B, Iñesta C, Sitges-Maciá E. Regression-based normative data for independent and cognitively active spanish older adults: Free and cued selective reminding test, rey–osterrieth complex figure test and judgement of line orientation. Int J Environ Res Public Health. 2021 Dec 1;18(24).
35. Pedraza O, Graff-Radford NR, Smith GE, Ivnik RJ, Willis FB, Petersen RC, et al. Differential item functioning of the Boston Naming Test in cognitively normal African American and Caucasian older adults. Journal of the International Neuropsychological Society. 2009;15(5):758–68.
36. Benson N, Hulac DM, Kranzler JH. Independent Examination of the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV): What Does the WAIS-IV Measure? Psychol Assess. 2010 Mar;22(1):121–30.
37. Nocera JR, Mammino K, Kommula Y, Wharton W, Crosson B, McGregor KM. Effects of Combined Aerobic Exercise and Cognitive Training on Verbal Fluency in Older Adults. Gerontol Geriatr Med [Internet]. 2020 Jan;6:2333721419896884. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31950073
38. Villemagne VL, Pike KE, Chételat G, Ellis KA, Mulligan RS, Bourgeat P, et al. Longitudinal assessment of Aβ and cognition in aging and Alzheimer disease. Ann Neurol. 2011 Jan;69(1):181–92.
39. Rikli RE, Jones JC. Senior fitness test manual. 2nd ed. Champaign, Il. [etc: Human Kinetics; 2013.
40. Pavasini R, Guralnik J, Brown JC, di Bari M, Cesari M, Landi F, et al. Short Physical Performance Battery and all-cause mortality: Systematic review and meta-analysis. BMC Med. 2016 Dec 22;14(1).
41. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med [Internet]. 2002 Jul 1;166(1):111–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12091180
42. Skantz H, Rantanen T, Palmberg L, Rantalainen T, Aartolahti E, Portegijs E, et al. Outdoor mobility and use of adaptive or maladaptive walking modifications among older people. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2020 Mar 9;75(4):806–12.
43. Gomez Bernal. A, Becerro-de-Bengoa-Vallejo R, Losa-Iglesias ME. Reliability of the OptoGait portable photoelectric cell system for the quantification of spatial-temporal parameters of gait in young adults. Gait Posture. 2016 Oct 1;50:196–200.

44. Langhammer B, Stanghelle JK. The Senior Fitness Test. Vol. 61, Journal of Physiotherapy. Australian Physiotherapy Association; 2015. p. 163.
45. Castro-Piñero J, Artero EG, España-Romero V, Ortega FB, Sjöström M, Suni J, et al. Criterion-related validity of field-based fitness tests in youth: A systematic review. Vol. 44, British Journal of Sports Medicine. 2010. p. 934–43.
46. Li G, Taljaard M, Van Den Heuvel ER, Levine MAH, Cook DJ, Wells GA, et al. An introduction to multiplicity issues in clinical trials: The what, why, when and how. Int J Epidemiol. 2017;46(2):746–56.
47. Freites-Martinez A, Santana N, Arias-Santiago S, Viera A. CTCAE versión 5.0. Evaluación de la gravedad de los eventos adversos dermatológicos de las terapias antineoplásicas. Actas Dermosifiliogr [Internet]. 2021 Jan 1;112(1):90–2. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0001731020302866