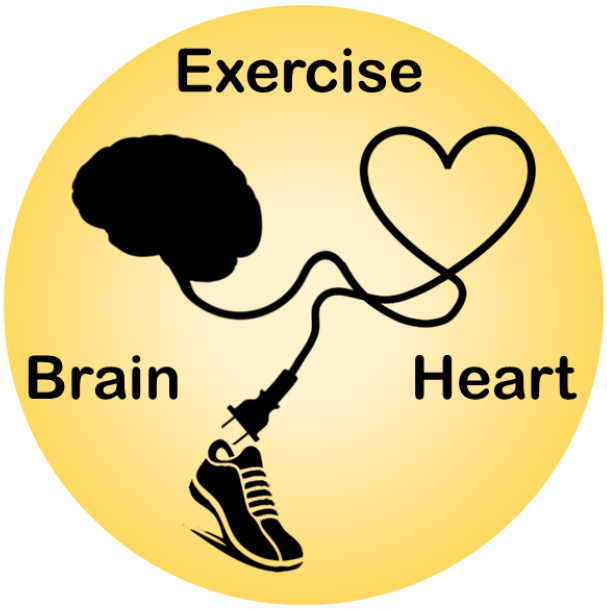
Heart-Brain Project



Statistical Analysis Plan

University of Granada, Spain

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Roles and responsibility:

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Table of contents

1. General information of Hearty-Brain study

1.1 Background and rationale

Patients with coronary heart disease (CHD) have a higher risk of developing dementia, cognitive impairment, and mental disorders. Therefore, there is a need to identify effective and sustainable initiatives to avoid or attenuate cognitive and mental health decline in these patients. Physical exercise could play a major role to prevent or reduce these declines.

1.2 Objective

The overall objective is to investigate the effects of exercise on brain health outcomes in CHD patients. The primary objective is to examine the effect of aerobic high intensity interval training combined with resistance training (HIIT+R) and aerobic high intensity interval training (HIIT) only compared to usual care on cerebral blood flow.

Secondary aims are to:

i. Examine the effect of HIIT+R and HIIT compared to usual care on cerebral vascularization.
ii. Examine the effect of HIIT+R and HIIT compared to usual care on executive function and general cognition.
iii. Examine the effect of HIIT+R and HIIT compared to usual care on cardiorespiratory fitness (treadmill time-to-exhaustion and VO_2peak).

2. Study methods

2.1 Trail design

The Hearty-Brain project is a single-blinded, exercise-based randomized controlled trial (RCT). The RCT will include three-arms containing a waiting-list control group (usual care) and two intervention groups that will receive two different 12-week supervised exercise programs (**Figure 1**): i) HIIT+R and ii) HIIT only. The study will be conducted in 90 patients with CHD who meet the eligibility criteria. Measurement will be performed at baseline, at midpoint (6 weeks) and post intervention (12 weeks). More detailed information is described in the study protocol.

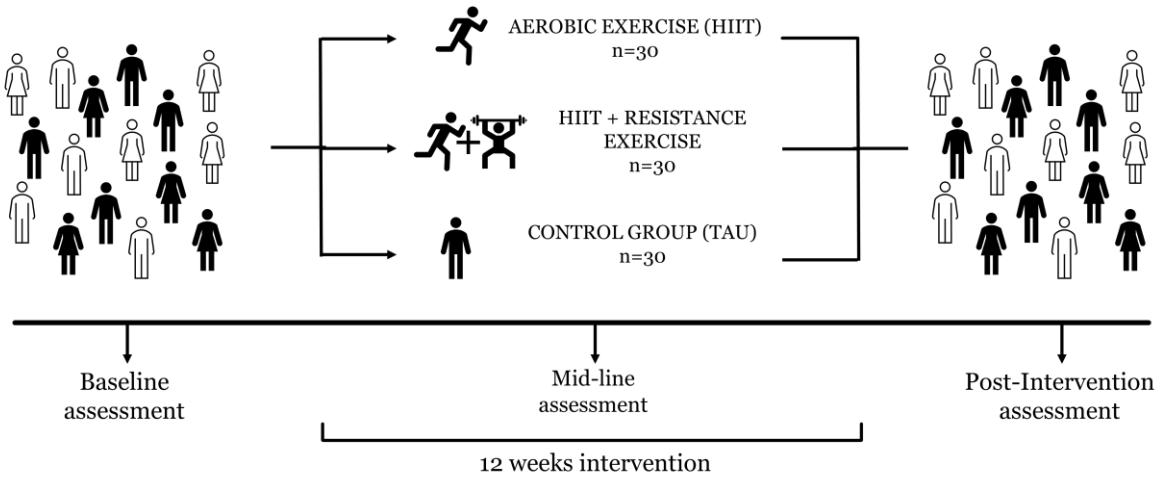

Figure 1. Overview of the Hearty-Brain Study. HIIT= high intensity interval training; R=resistance training

The Hearty-Brain research team will perform the data quality control, data processing, writing analyses scripts/programs and statistical analysis.

2.2 Randomization

Participants were randomized using a 1:1:1 ratio and stratified for age (<65 years or ≥ 65 years) and sex.

2.3 Sample size

The Heart-Brain project has been designed to detect a low-to-medium size change (i.e. effect size Cohen D=0.35) with an alpha of 0.05 and beta of 0.2 (power 0.8). The trial will be powered for 7% drop-out during the intervention (based on our previous RCTs; **Figure 2**). The estimated sample size, based on previously mentioned parameters and repeated-measures ANOVA, needed for this study was 90 participants (30 in each study arm).

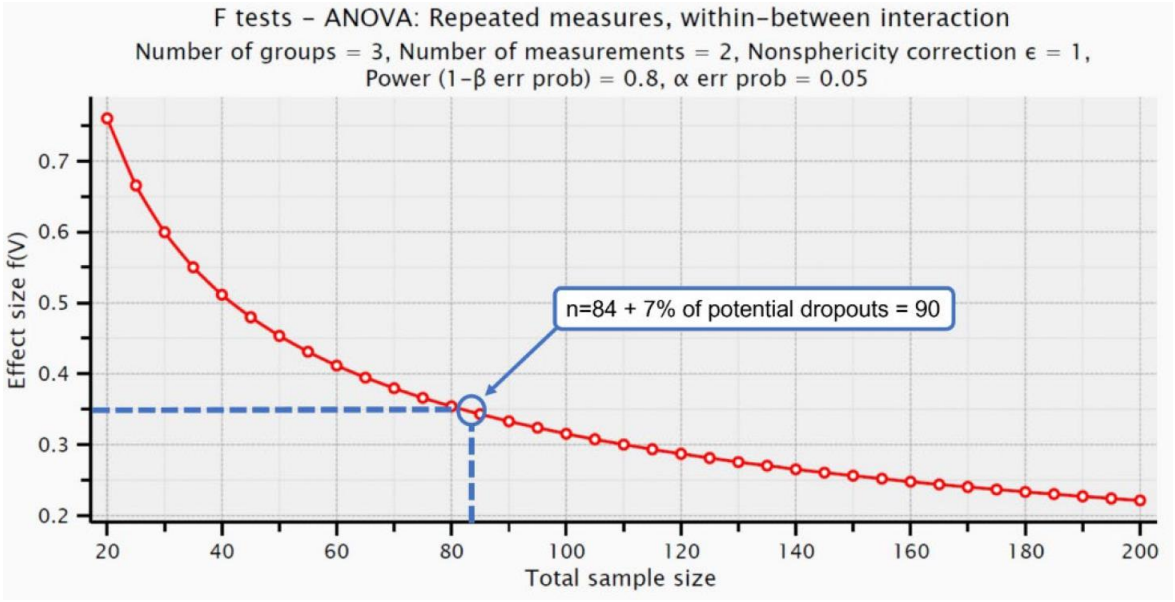

Figure 2. Sample size calculation using G*power (version 3.1).

2.4 Framework

A superiority hypothesis testing framework will be used. In the main analyses, we will compare whether both exercise interventions ((HIIT + R or HIIT only) are superior to the usual care (no exercise).

2.5 Statistical interim analyses and stopping guidance

No pre-specified interim analyses are performed, and therefore, a stopping guidance was not applicable. Only basic analyses (not including the primary outcome) were performed for the final report of the funding agency after completion of the first 58 participants. The basic analyses were obligatory to receive funding. The basic analyses were performed by an independent researcher who was not involved in any aspect of the data collection (Dr. E.A. Bakker). The results of the basic analyses were not discussed with other members of the research team involved in the evaluations or intervention to avoid any bias during the implementation of the study, data collection or processing. No protocol deviations resulted from these basic analyses.

2.5 Timing of the final analyses

The final analyses will be performed after the finalization of the data collection and processing of the primary and secondary outcomes.

2.6 Timing of outcome assessment

The primary and secondary outcomes will be assessed at baseline and post intervention (12 weeks). 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 will be two-tailed. *P* for significance will be set at 0.05 and 95% confidence intervals will be estimated. No adjustment for multiplicity were made as multiplicity adjustments may be of lesser importance in the case of distinct treatment arms.(1) Moreover, only one primary outcome was defined and other outcomes (secondary/tertiary outcomes, exploratory analyses) don't required adjustment for multiple testing. However, we included a hierarchical analytic approach for the exercise interventions tested and the primary outcome.

3.2 Adherence, attendance, compliance, and protocol deviations

Since the term adherence has been used with different meanings in the literature, in our statistical analyses plan and study, we will refer to two concepts more unequivocally used, attendance and compliance. Attendance will be defined as % of sessions attended by the participant (recorded by the trainers) divided by the actual number of exercise sessions offered (12 weeks x 3 sessions / week = 36, yet there will be slight variation among the study waves due to holidays or logistic reasons). Compliance is the % of sessions in which the amount of time (HIIT+R 4.5 min; HIIT 6.5 min) in the target heart rate intensity is reached divided by the number of exercise sessions with valid data (e.g. sessions with technical issues of the heart rate bands are not considered) and excluding the familiarization phase (i.e. first two weeks of the exercise intervention). The amount of time (HIIT+R 4.5 min; HIIT 6.5 min) in the target heart rate intensity is based on the exercise protocol and the Guidelines for HIIT Prescription and Monitoring in clinical populations (2): for the first high intensity interval, we will allow the entire 4-minute period to reach the HR target zone. For subsequent high intensity intervals (i.e. 2nd, 3rd, and/or 4th), we will allow 2-minutes (halfway) to reach the HR target zone.

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.

3.3 Analysis populations

We plan to use two analytic datasets for the statistical analyses:

1. Intention-to-treat: This dataset will be used for our primary analyses and includes all randomized participants. With this approach, all randomized participants are included in the analysis, based on the groups to which they were initially randomly assigned. This imply that some participants will have valid data in both time points, while some might have missing data at baseline or post-intervention assessment.

2. Per-protocol: This dataset will be used for our secondary analyses and includes all participants with ≥ 70% of attendance.

4. Trail population

4.1 Screening data

Screening data will be based on patients that are defined as eligible by the clinical and research team. Screening is performed in three phases:

1) Screening based on the eligibility criteria (see '*4.2 Eligibility'*) by the clinicians of the research team.
2) Screening based on phone call by the research team.
3) Screening during the baseline assessments using the cardiopulmonary exercise test (CPET and cardiac ultrasound).

More details on the eligibility criteria are provided in '*4.2 Eligibility*'. The number of patients within each phase will be reported.

4.2 Eligibility

Eligible patients were defined on the inclusion and exclusion criteria. In general, stable CHD patients between 50 and 75 years old (both included) who were physically inactive and cognitively normal were included. Specific inclusion and exclusion criteria are:

Inclusion criteria:

1. Men and women aged between 50 and 75 years old, both inclusive (*Contingency plan: increase the range to 40-75 if we have difficulties to get the study sample)
2. Must have stable coronary heart disease (phase III), proven by invasive coronary angiography or CT with at least one coronary lesion > 50%.
3. Able to speak and read fluent Spanish.
4. Live in Granada city or surrounding areas (able to come to evaluations and exercise program)
5. Living in community during the study (i.e. independent home, non-assisted living facilities)
6. Ejection fraction ≥ 45%.
7. Functional grade I-II according to the New York Heart Association (NYHA) scale.
8. Sinus rhythm.
9. Stable optimal medical treatment (3 or more drugs at the determined by a cardiologist).
10. Physically inactive, considering: 1) not meeting the WHO recommendations in both the aerobic and strength part, and 2) not to be participating in a planned and structured exercise program at least 3 days per week and for more than 3 months. Both conditions must be met to be included. Note: going for a walk will not be considered an exclusion reason.
11. Classified as cognitively normal according to Stics-m

Exclusion criteria:

1. Used of assisted walking devices.
2. Acute coronary syndrome in the last year, coronary surgery, or percutaneous intervention in the last 6 months.
3. Treatment for any type of cancer in the last 2 years.

4. Severe hospitalization in the intensive care unit in the last 6 months.
5. Current psychiatric diagnosis (visit to psychiatrist and drug treatment prescription in the last year), including major depression and history of psychiatric illness (schizophrenia, bipolar disorder, hallucinations).
6. Grade III obesity.
7. Diagnosis of neurological or cerebrovascular disorder (e.g. stroke).
8. Medical contraindication for inclusion in an exercise program.
9. Diabetes with uncontrolled glycemia.
10. Resting blood pressure > 180/110.
11. Chest pain with exertion or changes in the ST segment suggestive of severe ischemia during ergometry
12. Severe inducible ischemia
13. Functional capacity in ergometry (<5 METS).
14. Obstructive left main artery disease (significant disease > 50%)
15. Unstable angina
16. Uncontrolled cardiac arrhythmia
17. Presence of metal implants (e.g., pacemaker or implantable cardioverter-defibrillator-ICD) not compatible with MRI (reported during the phone screening)
18. Paroxysmal or persistent atrial fibrillation with episodes in the last 6 months.
19. Moderate to severe pulmonary hypertension.
20. Acute endocarditis, myocarditis, or pericarditis.
21. Moderate to severe valve disease (grade 3-4)
22. Acute pulmonary embolism, or deep vein thrombosis.
23. Aortic dissection
24. High-grade heart block or complete left bundle branch block or altered basal electrocardiogram with difficulties to interpret in exercise testing.
25. Hypertrophic obstructive cardiomyopathy.
26. Retinopathy.
27. Severe autonomic or peripheral neuropathy.
28. Acute systemic illness or fever.
29. Acute or chronic renal failure (estimated glomerular filtration rate < 30 mL/min)
30. Pulmonary fibrosis or interstitial disease (respiratory failure or severe COPD confirmed by pneumological study).
31. Recent treatment for alcohol or substance abuse.
32. Claustrophobia.
33. Any surgery or medical intervention planned during the study period.
34. Plans to participate or current participation in other studies that might interferes with this study.
35. Current pregnancy or intention to get pregnant during the study period.

4.3 Recruitment

Information necessary for the CONSORT flow diagram will be collected. For the enrolment phase, we will note the number of patients that were assessed for eligibility by the research team, the number of excluded patients (plus reason for exclusion), and the number of randomized participants. For the allocation, the number of participants allocated to the intervention, and the number of participants

who received or did not receive the intervention (plus reasons) will be noted. For follow-up, the number of participants who were lost to follow-up and the number who discontinued the intervention (plus reasons) were counted. Finally, the number of participants that were included in the analyses using the intention-to-treat and per-protocol databases will be described together with the reasons for exclusion.

4.4 Withdrawal/follow-up

The number, timing, and reasons (e.g. adverse events, lost motivation to continue with the study, withdraw consent, could not or did not want to attend to the post-intervention evaluations) for withdrawal will be noted and described.

4.5 Baseline patient characteristics

A baseline table will be created to describe the characteristics of the study population. The characteristics include general characteristics (e.g. age, sex, education, body mass index), cardiovascular health (e.g. blood pressure, resting heart rate, cardiovascular diagnosis, coronary intervention, comorbidities, medication use) and the brain health outcome. The characteristics of the total study population and each study arm will be 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

Primary outcome

1. Change in cerebral blood flow (Baseline and 12 weeks). The main outcome is the change in cerebral blood flow from baseline to 12 weeks. Cerebral blood flow (mL/100 g/min) will be measured using the magnetic resonance imaging technique of TGSE-pCASL (turbo gradient spin echo-pseudo continuous arterial spin labelling). We will analyse both the global cerebral blood flow and the regional cerebral blood flow, as determined by voxel-wise analysis to measure local perfusion.

Secondary outcomes

The secondary outcomes are:

2. Change cerebral vascularization (Baseline and 12 weeks). Cerebral vascularization will be measured using the magnetic resonance angiography TOF (Time-of-flight angiography).

3. Change in executive function and general cognition (Baseline and 12 weeks). A comprehensive neuropsychological battery will assess several domains of executive function: working memory, cognitive flexibility and inhibitory control, and an executive function score will be computed and used as main behavioural outcome. Additionally, the general cognition will be assessed by the MOCA (Montreal Cognitive Assessment) test.

4. Change in cardiorespiratory fitness (Baseline and 12 weeks). Cardiorespiratory fitness will be assessed by a cardiorespiratory exercise test in a treadmill measuring gas exchange (treadmill time-to-exhaustion and VO2peak).

Tertiary outcomes

Tertiary outcome will be:

5. Change in blood brain barrier (BBB) permeability (Baseline and 12 weeks). BBB permeability will be operationally measured by using a recently developed neuroimaging technique that measures water exchange across the BBB using 3D diffusion-prepared arterial spin labelled perfusion MRI.

6. Change in brain morphology (Baseline and 12 weeks). MRI (magnetic resonance imaging) will measure brain morphology including volume, area, cortical thickness, and shapes by a T1-weighted MPRAGE structural sequence.

7. Change in white matter structure (Baseline and 12 weeks). MRI (magnetic resonance imaging) will measure white matter structure and lesions by diffusion weighted acquisition sequence.

8. Change in brain function (Baseline and 12 weeks). MRI (magnetic resonance imaging) will measure brain function during resting state. Measures of brain activity and brain connectivity will be calculated.

9. Change in blood-based neurology biomarkers (Baseline and 12 weeks). Blood samples will be used to determine plasmatic concentration of peripheral neurology biomarkers including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and cathepsin B (CTSB), as well as novel neurodegenerative biomarkers based on new evidence up to the time of the analysis.

10. Change in saliva-based neurology biomarkers (Baseline and 12 weeks). Saliva samples will be used to determine concentration of peripheral neurology biomarkers including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and cathepsin B (CTSB), as well as novel neurodegenerative biomarkers based on new evidence up to the time of the analysis.

11. Hemodynamic vascular changes (Baseline and 12 weeks). Hemodynamic vascular parameters will be measured using ultrasound echography (i.e. carotid intima–media thickness).

12. Hemodynamic cardiac changes (Baseline and 12 weeks). Cardiac parameters will be measured using ultrasound echography (i.e. ejection fraction, cardiac volumes, and cardiac output).

13. Hemodynamic transcranial changes (Baseline and 12 weeks). Hemodynamic transcranial parameters will be measured using ultrasound echography (i.e. Doppler diastolic-systolic velocity).

14. Change in general muscular strength (Baseline and 12 weeks). The maximum isometric strength of the hand and forearm muscles measured with the handgrip test (Kg) will be used to determine general muscular strength.

15. Change in lower body muscular strength (Baseline and 12 weeks). Muscular strength in lower body will be assessed using the chair stand test (number of repetitions).

16. Change in upper body muscular strength (Baseline and 12 weeks). Muscular strength in upper body will be assessed using the arm curl test (number of repetitions).

17. Change in physical function (Baseline and 12 weeks). Senior Fitness Test (including the 6-min walking test) will assess overall physical functioning and z-scores will be calculated.

18. Change in depression (Baseline and 12 weeks). Depressive symptoms will be assessed using the Global Deterioration Scale, the Health Survey Short Form (SF-36) and the Hospital Anxiety and Depression Scale.

19. Change in anxiety (Baseline and 12 weeks). Anxiety will be assessed using the Health Survey Short Form (SF-36) and the Hospital Anxiety and Depression Scale.

20. Change in stress outcomes (Baseline and 12 weeks). Stress outcomes will be assessed using the Perceived Stress Scale.

21. Change in loneliness (Baseline and 12 weeks). Loneliness will be assessed using the UCLA Loneliness Scale.

22. Change in self-esteem outcomes (Baseline and 12 weeks). Self-esteem will be assessed using the Rosenberg Self-Esteem Scale.

23. Change in social support outcomes (Baseline and 12 weeks). Social support will be assessed using the Social Provisions Scale.

24. Change in health-related quality of life (Baseline and 12 weeks). Global, physical, and mental health-related quality of life will be self-reported using the Health Survey Short Form (SF-36), in which higher scores means a better health-related quality of life.

25. Change in physical activity (Baseline, 6 and 12 weeks). Physical activity will be measured using the accelerometer Axivity AX, and a self-reported questionnaire based on the Global Physical Activity Questionnaire.

26. Change in sedentary behaviour (Baseline, 6 and 12 weeks). Sedentary behaviour will be measured using the accelerometer Axivity AX, and a self-reported questionnaire based on the Global Physical Activity Questionnaire.

27. Change in sleep quality (Baseline, 6 and 12 weeks). Sleep quality will be measured using the accelerometer Axivity AX and using and using a self-reported questionnaire.

28. Change in diet behaviour (Baseline, 6 and 12 weeks). Diet behaviour will be self-reported using the 14-item Questionnaire of Mediterranean Diet Adherence (PREDIMED-14), and a self-reported question for supplements intake.

29. Change in body mass index and body composition (Baseline and 12 weeks). Body mass index (BMI, kg/m^2) will be computed from height and weight measured by SECA instruments, and body composition will be assessed using a dual-energy x-ray absorptiometer (DXA) and a TANITA's Bioelectrical Impedance Analysis.

30. Change in lean mass (Baseline and 12 weeks). Lean mass (kg) will be assessed using a dual-energy x-ray absorptiometer (DXA) and a TANITA's Bioelectrical Impedance Analysis.

31. Change in fat mass (Baseline and 12 weeks). Fat mass (kg) will be assessed using a dual-energy x-ray absorptiometer (DXA) and a TANITA's Bioelectrical Impedance Analysis.

32. Change in bone mineral content and density (Baseline and 12 weeks). Bone mineral content and density (z-score) will be assessed using a dual-energy x-ray absorptiometer (DXA).

33. Change in blood pressure (Baseline and 12 weeks). Systolic and diastolic blood pressure will be assessed by a blood pressure monitor. Central blood pressure will be also analysed using the SphygmoCor XCEL.

34. Change in arterial stiffness (Baseline and 12 weeks). Arterial stiffness will be assessed using the pulse wave analysis and pulse wave velocity determined by the SphygmoCor XCEL.

35. Change in blood-based inflammatory biomarkers (Baseline and 12 weeks). Blood samples will be used to determine plasmatic concentrations of inflammatory peripheral biomarkers including tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1beta), glucose, insulin, HDL and LDL cholesterol.

36. Change in saliva-based inflammatory biomarkers (Baseline and 12 weeks). Saliva samples will be used to determine saliva concentrations of inflammatory peripheral biomarkers including tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1beta).

37. Change in blood-based cardiovascular biomarkers (Baseline and 12 weeks). Blood samples will be used to determine plasmatic concentrations of cardiovascular peripheral biomarkers including glucose, insulin, HDL and LDL cholesterol.

38. Change in saliva-based cardiovascular biomarkers (Baseline and 12 weeks). Saliva samples will be used to determine plasmatic concentrations of cardiovascular peripheral biomarkers including glucose, insulin, HDL and LDL cholesterol.

39. Change in epigenetics (Baseline and 12 weeks). Blood samples will be stored for epigenetic analyses.

40. Change in gene expression (Baseline and 12 weeks). Blood samples will be stored for genetic analyses, including APOE and BDNF genotypes.

41. Change in oral and gut microbiota (Baseline and 12 weeks). Saliva and fecal samples will be used to determine oral and gut microbiota including the most representative phyla (i.e., firmicutes, bacteroidetes, and proteobacteria)

5.2 Analysis methods

The main analyses will consist of the intention-to-treat analyses for the primary and secondary outcomes using a constrained baseline (meaning baseline adjusted) linear mixed model, which accounts for baseline differences among the study groups. The model will include fixed effects for time (two levels) and treatment (three 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 e.g., conducting data transformations. If the global test of significance indicated between-group differences, pairwise comparisons will be explored. Although no adjustments for multiplicity were performed, family-wise type 1 error rate on the primary outcome was retained by using a hierarchical analytic approach. The prespecified hierarchical hypotheses were tested using the prespecified sequence: HIIT + R versus usual care, HIIT versus usual care, and HIIT + R versus HIIT. The main statistical analyses will be performed by an independent researcher (Dr. EA Bakker), who is not involved in the recruitment, evaluations, and interventions, and will be performed blinded for the treatment allocation by coding the intervention arms (e.g. A, B, C).

Secondary analyses will be performed using the per-protocol defined as attending to ≥70% of the sessions offered to the exercise groups. In addition, post hoc statistical mediation analysis will be performed to examine the extent to which the observed intervention effect on the primary and secondary outcomes was mediated by the change in cardiorespiratory fitness.

No additional adjustments for covariates will be made (except for baseline adjustments of the outcome). Although the RCT is not powered for subgroups analyses, we will perform explanatory subgroup analyses for age, sex, education level and baseline level of the study outcome. Continuous variables will be stratified on the median. Sensitivity analyses will be performed using the per-protocol database plus excluding participants who had injuries or other health problems during the post assessment evaluations. We will also analyse the effects of the intervention in individuals that in addition to attending ≥70% of the sessions, also had ≥70% compliance to the training protocol based on time heart rate target zones during the HIIT protocols.

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 assumption. In case we believe this assumption does not hold, we will take appropriate measures for the data analyses by using e.g. other multiple imputation techniques.

5.4 Additional analyses

Changes in other outcomes will be analysed using a similar protocol as described by '*5.2 Analysis methods*' unless other analyses would be more appropriate depending on the outcome (e.g. ordinal / dichotomous outcomes).

Furthermore, cross-sectional analyses will be performed using the baseline data of the RCT. For example, cross-sectional associations will be explored by bivariate and partial correlations. Then further analyses will be performed using linear and logistic requestion depending on the nature of the study variables and research questions. ANOVAs will also be used to test differences in outcomes among groups.

5.5 Harms

The number and reasons of adverse events (e.g. falls, injuries, musculoskeletal problems, major cardiovascular disease events, and any other events potentially related to the implementation of the trial protocol) at each time point will be collected, reported, and described separately for each study arm. No formal statistical testing will be undertaken.

5.6 Statistical software

The analyses will be performed using R. For the main analyses we will use the 'lme4' or 'LMMstar' package. The use of packages will be reported in the manuscript.

6. References

1. Li G, Taljaard M, Van den Heuvel ER, Levine MA, 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):746-55.

2. Taylor JL, Holland DJ, Spathis JG, Beetham KS, Wisløff U, Keating SE, et al. Guidelines for the delivery and monitoring of high intensity interval training in clinical populations. 2019;62(2):140-6.