Effects of a laboratory-based aerobic exercise intervention on brain volume and cardiovascular health markers: protocol for a randomised clinical trial

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ABSTRACT

Introduction Physical activity (PA) has beneficial effects on brain health and cardiovascular disease (CVD) risk. Yet, we know little about whether PA-induced changes to physiological mediators of CVD risk influence brain health and whether benefits to brain health may also explain PA-induced improvements to CVD risk. This study combines neurobiological and peripheral physiological methods in the context of a randomised clinical trial to better understand the links between exercise, brain health and CVD risk.

Methods and analysis In this 12-month trial, 130 healthy individuals between the ages of 26 and 58 will be randomly assigned to either: (1) moderate-intensity aerobic PA for 150 min/week or (2) a health information control group. Cardiovascular, neuroimaging and PA measurements will occur for both groups before and after the intervention. Primary outcomes include changes in (1) brain structural areas (ie, hippocampal volume); (2) systolic blood pressure (SBP) responses to functional MRI cognitive stressor tasks (3) negative and positive affect; (4) baroreflex sensitivity; (5) pulse wave velocity; (6) endothelial function and (7) daily life positive and negative affect. Our results are expected to have both mechanistic and public health implications regarding brain–body interactions in the context of cardiovascular health.

Ethics and dissemination Ethical approval has been obtained from the University of Pittsburgh Institutional Review Board (IRB ID: 19020218). This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule.

Trial registration number NCT03841669.

INTRODUCTION

Physical inactivity is estimated to account for >350,000 deaths per year in the USA alone, with an additional 3.2 million annual deaths globally.1 On the other hand, physical activity (PA) is critical for reducing risk for many chronic diseases (eg, some cancers, cardiometabolic conditions and atherosclerotic cardiovascular disease (CVD)), and it has beneficial effects on a range of proximal mediators and markers of chronic disease risk—especially CVD risk.2–5 These include blood pressure (BP), cardiac autonomic control, inflammation, glucose regulation, adiposity and lipid levels. In patient samples, clinical trials show that PA accelerates recovery from myocardial infarction,6 coronary bypass surgery and congestive heart failure.7 Yet, we know little about whether PA-induced changes in peripheral physiological mediators of CVD risk influence brain health and whether benefits to brain health may also explain PA-induced improvements to CVD risk. This study combines neurobiological and peripheral physiological methods in the context of a randomised clinical trial to better understand the links between exercise, brain health and CVD risk.
failure,\textsuperscript{7} improves quality-of-life\textsuperscript{8} and reduces risk for new CVD events and all-cause mortality.\textsuperscript{9} Further, cardiac patients who improve cardiorespiratory fitness show a 20% reduced mortality rate over an 11-year follow-up period.\textsuperscript{10} In view of clear scientific evidence on PA and health, many national and international organisations have nearly identical recommendations for people to engage in PA. The most recent was established in 2018 and 2020 by the US Physical Activity Guidelines Committee and WHO, respectively, advising >150 min of moderate intensity exercise, or 75 min of vigorous intensity aerobic exercise per week to improve cardiovascular health and to reduce risks for premature mortality.\textsuperscript{11–13}

These benefits translate into brain-based improvements in cognitive, affective, stress-buffering and self-regulatory behaviours.\textsuperscript{14–16} Indeed, human and animal evidence show that the brain exhibits appreciable structural and functional plasticity (ie, change) from PA.\textsuperscript{17–21} As a result of this evidence, many organisations (eg, National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association) have developed campaigns to educate the public and healthcare professionals on the neurocognitive benefits of PA.\textsuperscript{22} In support of these health campaigns, Barnes and Yaffe\textsuperscript{23} reported that >1.1 million Alzheimer’s disease cases in the USA are potentially attributable to physical inactivity. Noteworthy here is that Alzheimer’s disease and other forms of dementia are highly comorbid with CVD and often predicted by CVD risk factors that are affected by PA (eg, hypertension).\textsuperscript{24–29} Moreover, exercise may exert ‘stress-buffering’ effects at the level of physiology and behaviour (eg, by reducing aspects of negative affect and depressive symptoms), which may define additional pathways to reduce CVD risk.\textsuperscript{30} What is not known, however, is whether the beneficial effects of exercise on the brain extend beyond emergent cognitive, affective and stress-buffering processes to also include beneficial effects on physiological and systemic mediators of CVD risk that are regulated by the brain. If so, such effects may help to more precisely define and refine novel and brain-based targets for intervention and prevention efforts.

Accordingly, the fundamental premise of this study is that PA, in the form of moderate-intensity aerobic exercise, is beneficial for both cardiovascular health and plasticity of neural circuits that regulate peripheral physiological mediators of CVD risk.\textsuperscript{30–32} Yet, despite the well-established associations between exercise and brain plasticity, we have a limited understanding of the mechanisms by which exercise affects brain plasticity or how these changes may relate to CVD risk. Similarly, although converging lines of evidence raise the possibility that aerobic exercise may partly influence CVD risk via its effects on brain plasticity, this possibility has never been tested at multiple levels of analysis in a well-controlled exercise intervention. The Exercise, Brain, and Cardiovascular Health study combines neurobiological and peripheral physiological assessment methods in the context of an experimental manipulation of exercise to better understand the links between brain health and CVD risk.

**Project objectives**

The aim of this study is to assess whether a 12-month moderate-intensity PA intervention induces changes in peripheral markers of cardiovascular control and health (eg, cardiorespiratory fitness, peripheral vascular function), and whether those changes are associated with exercise-induced structural and functional plasticity of the hippocampus and related brain circuits. Similarly, we aim to examine whether a 12-month moderate-intensity PA intervention induces changes in visceral control circuits of the brain, and whether those changes are associated with baroreflex sensitivity (BRS), heart rate variability (HRV) and glucocorticoid control.

**METHODS AND ANALYSIS**

**Project period**

Study is planned for the period between September 2019 and May 2024.

**Setting**

The intervention and assessments will take place in Pittsburgh, Pennsylvania, USA.

**Study design**

This study is a 12-month single-blind, randomised clinical trial (RCT), in which participants will be assigned to either: (1) moderate-intensity aerobic PA for 150 min/week or (2) a health information control group. The protocol is reported according to the Standard Protocol Items: Recommendations for Interventional Trials statement.\textsuperscript{33} The trial procedures are summarised in figure 1. The current protocol includes modifications to offset disruptions caused by COVID-19 pandemic.

**Recruitment**

We will randomise 130 healthy participants between 26 and 58 years of age (50% female). Recruitment of racial and ethnic minorities will be in proportion to the demographic representation of the greater Pittsburgh community: 28% black; 1% Hispanic; 2% Asian. Recruitment strategies include advertisements via newspapers, radio and television, direct mail, research registries and online media (eg, Facebook).

**Participant selection**

Eligibility criteria were crafted to recruit individuals who can safely engage in regular moderate-to-vigorous intensity exercise for 12 months (see table 1). The goal was to create eligibility criteria that would maintain safety while maximising generalisation of the results to a broad population.

**Blinding and randomisation**

Participants will be randomised after completion of the baseline sessions and evaluation of data quality assurance...
on a web-based system through the Research Electronic Data Capture (REDCap) randomisation module. The allowable time from signing the informed consent to randomisation is 8 weeks with the goal of having all sessions completed within 30 days. The lead study biostatistician randomises participants using a stratified permuted block randomisation algorithm with equal allocation to one of the two groups: (1) 150 min/week of moderate-intensity exercise (ie, brisk walking) or (2) a health information control group. We also consider stratification by two factors: (1) age at study entry (≤40 and >40 years) and (2) sex (male, female) to ensure equal allocation of participants to each group based on these criteria. Briefly, the stratified permuted block randomisation algorithm allows flexibility in achieving balanced allocation of participants among treatment groups by age and gender to minimise differences across the different conditions by the end of the trial. All investigators and staff involved in data collection and management will be blinded to group assignment. Only staff involved in the implementation of the exercise intervention, scheduling of sessions and study coordination are unblinded to group assignment.
The PIs are only unblinded in the case of an adverse or serious adverse event.

**Calculation of sample size**
We conducted power analysis and sample size estimation for hypothesis testing based on achieving 80% power at the significance level of 0.05. The estimated effect size (Cohen’s d) based on prior 6 to 12-month PA interventions in different age ranges is between 0.30 and 0.50. Assuming a conservative effect size (d=0.30), 65 participants per group is sufficient to detect differences in brain volume at 80% power even after 20% attrition (N=52/group). Meta-analyses of prior randomised exercise interventions showed moderate-to-large effects on CVD risk markers (d=0.60) and cardiorespiratory fitness (d=0.58). Based on previous studies and meta-analyses, we are sufficiently powered with the planned sample size to test for exercise-induced changes in CVD markers.

**Outcomes**
Our primary endpoints will be 12-month change scores resulting from three different measurements: (1) hippocampal volume, (2) mean systolic blood pressure (SBP) reactivity averaged from Stroop and Multi-Source Interference Task (MSIT) stressors during functional MRI (fMRI) and (3) HRV. Our secondary outcomes will result from changes in: (1) ecological momentary assessment, (2) BP, (3) negative and positive affect, (4) BRS, (5) pulse wave velocity, (6) vasodilation, (7) brain activity, (8) connectivity, (9) cortical thickness and (10) volume.

See table 2 for a complete schedule of all study assessments.

**Primary outcomes**

**Brain MRI**
We will use a Siemens Prisma 3-T scanner with a 64-channel head coil. Screening for safety, a history of claustrophobia, and metal implants or devices will occur during the initial telephone screening.

Sequences will include a high-resolution three-dimensional magnetisation-prepared rapid acquisition with gradient echo (MPRAGE) T1-weighted anatomical scan, a hippocampal focal T2-weighted sequence, a resting state echo planar imaging (EPI) scan and several task-evoked EPI scans (see table 3).

Three tests will assess task-evoked fMRI brain activity: (1) a modified Stroop task; (2) a modified MSIT and (3) an Affective responding and regulation task. Before scanning, practice on the tasks confirms task comprehension and comfort. In the Stroop task, a series of colour words (ie, red, blue, yellow and green) are displayed on a screen in various colour fonts and the participant is asked to identify the font colour in which each word is printed by pressing a button on an MRI-compatible response system. In the MSIT, a series of four numbers appear on the screen with three of the numbers being identical and

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Men and women 26–58 years</td>
<td>Self-reported chronic psychotic illness (eg, schizophrenia, bipolar disorder) or neurological disorder or brain injury resulting in ongoing symptoms or cognitive impairment (eg, multiple sclerosis, cerebral palsy, major head injury)</td>
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<tr>
<td>Ambulatory without pain or the use of assisted walking devices</td>
<td>Self-reported prior heart attack, stroke, bypass surgery, angioplasty, congestive heart failure, arrhythmia (cardiac rhythm problems)</td>
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<tr>
<td>Able to speak and read English (&gt;10 years)</td>
<td>Hypertension (SBP/DBP greater than/or equal to 160/100 mm Hg)*</td>
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<tr>
<td>Engaging in &lt;100 min/week of exercise and &lt;75th percentile of VO2 max</td>
<td>Liver (ie, hepatitis B or C, liver failure or cirrhosis) or kidney disease</td>
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<tr>
<td>Living in community for duration of the study</td>
<td>Lung disease requiring drug treatment</td>
</tr>
<tr>
<td>Reliable means of transportation</td>
<td>Type I diabetes, insulin-dependent type 2 diabetes, uncontrolled type 2 diabetes (defined as an HbA1c level &gt;10)</td>
</tr>
<tr>
<td>Eligible to undergo MRI</td>
<td>Regular use† of all centrally active or psychotropic medications (SSRIs are permitted)</td>
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*>140 SBP/90 DBP is allowed, but feedback card and information about elevated levels will be provided to the participant.
†Regular use is defined as taking medications more than seven times in a 2-week period.
‡Defined as a period of work in which half or more of the hours worked are between midnight and 08:00 in the morning and this has occurred more than 12 times during the past year.

BP, diastolic blood pressure; MCI, mild cognitive impairment; SBP, systolic blood pressure; SSRI, selective serotonin reuptake inhibitors.
one being different (eg, 1114). The participant identifies the location of the different numbers by pressing a button. Feedback is provided after each response, and congruent and incongruent conditions are presented in both tasks; also, performance difficulty is titrated upwards over time during incongruent conditions of both the Stroop task and MSIT to increase the psychological challenge, as detailed in Sheu et al.37 38 In the Affective task, negative images from the International Affective Picture System database are presented on the screen in two conditions: (1) ‘look’, in which participants are instructed to simply observe the image, or (2) ‘decrease’, in which participants are instructed to think of something that would make them feel less negative about the image (ie, reappraise the image). Participants indicate how negative they feel after each image, using a scale ranging from 1 to 5 (‘not at all negative’ to ‘very negative’).39 After the MRI, a survey is administered to confirm comprehension of the task, and to collect information about the strategies used to feel less negative and the degree of success associated with strategy employment.

Finally, cardiovascular activity during the MRI scan is monitored via a Siemens SpO2 monitor on the left index finger, a Siemens respiration belt and a 4-lead Siemens ECG monitor. During the Stroop task and MSIT, BP is taken every 60 s (at the onset of each block) using a TeslaM3 BP machine (Siemens, Erlangen, Germany). In addition, three BP readings, 2 min apart, are taken in the practice room while the participant is seated, and three BP readings (2 min apart) are taken during the MPRAGE scan. The second and third readings are averaged and used as baseline measures of BP.

**Cardiovascular health**

Multiple non-invasive measurements will assess cardiovascular health/function, including the following sections.

**Autonomic function and arterial stiffness**

These measures will be collected using a dual impedance cardiography (ICG) method that we have previously detailed (eg, first).40 We will obtain an ECG concurrently with a basal ICG (Z0) and the first derivative of pulsatile ICG change (dZ/dt) using tetrapolar lead configurations applied to the (1) thorax assessing aortic blood flow onset and (2) calf assessing peripheral pulse transit time in line with standard ICG guidelines.41 Digitised recordings of interbeat intervals will be collected during a 5 min sitting period of both paced (11 breaths per minute) and unpaced respiration to assess HRV and pulse wave velocity (ie, arterial stiffness). Continuous beat-by-beat BP will also be monitored using cuffs placed around the brachial artery and index/middle fingers, which will assess total peripheral resistance, BRS and blood pressure variability.
**Endothelial health**

Venous occlusion plethysmography will measure endothelial function. 40 First, BP occlusion cuffs are placed around the wrist and brachial artery, as well as an indium-gallium strain gauge wrapped around the forearm to measure changes in circumference (ie, volume). During a standardised sequence of inflations/deflations in cuff pressure, forearm blood flow is measured via changes in circumference from the strain gauge. Following ‘resting/ baseline’ readings, the brachial cuff is inflated to 40 mm Hg above SBP for 5 min until the pressure is released and ‘max’ blood flow readings are recorded. This post-occlusion state is referred to as reactive hyperaemia and provides a reliable index of endothelium dependent vasodilation. Max flow readings are compared with baseline readings to derive a percentage increase, with greater values associated with better endothelial health.

**Secondary outcomes**

**Ecological momentary assessment**

Ecological momentary assessment (EMA) will assess ambulatory BP and daily life experiences. 42 EMA involves data collection in real-time and in the natural environment, usually with the assistance of mobile or wearable devices. 43 During each of the 2-week long monitoring periods (pretreatment and post-treatment, 7–10 days in duration), participants will be equipped with a mobile smart phone, an ambulatory blood pressure (ABP) monitor (Oscar 2, Suntech Medical; Morrisville, NC) and a wrist-worn accelerometer (ActiGraph GT9X Link; ActiGraph; Pensacola, FL). The accelerometer will be used for the assessment of sleep and PA. On four of the monitoring days (three weekdays and one weekend day), the ABP monitor will be worn during waking hours. The monitor will be programmed to inflate on an hourly basis throughout each waking day. During the four ABP days, participants will complete hourly questionnaires following each ABP reading. The hourly assessments are designed to assess psychosocial stress and daily life mood or affect. Participants will be trained on how to use each of these ambulatory monitoring devices. If the expected number of interviews and BP readings are insufficient, participants will be asked to complete extra day(s) of monitoring.

**Blood assays and hair strands**

Fasting blood samples for the assessment of inflammatory and metabolic measures will be collected at baseline and postintervention. A hair sample (50 mg or 100–150 hair strands) will also be collected and banked for the analysis of cortisol levels. Each blood draw collects approximately 50cc of blood. Blood will be transported and processed within 1 hour of the draw. Samples of plasma, serum, whole blood, RNA and isolated mononuclear cells will be stored at −80°C for analysis in batches with both samples collected from each participant assayed together and all samples assayed in duplicate. The main blood analyses to be assessed include glucose1, insulin1, triglycerides1, and total and HDL cholesterol1, interleukin (IL)-62, tumour necrosis factor-alpha2, IL-1ra2, IL-102, intercellular adhesion molecule-12, vascular cell adhesion molecule-12, C reactive protein1 and brain derived neurotrophic factor2. Analytes identified by 1 will be measured by the University of Pittsburgh Medical Center’s Central Laboratory Services. Analytes identified by 2 will be measured in the Behavioral Immunology Laboratory (Director: Marsland).

**Cardiorespiratory fitness testing**

Cardiorespiratory fitness will be measured from a graded exercise test (GXT) (TrueOne 2400, ParvoMedics, Salt Lake City, UT) using a modified Balke protocol. 44 After a brief warm-up session, including a resting BP reading and ECG (NASIFF CardioSuite ECG cart), the participant will walk on a motor-driven treadmill at a constant speed (2.0 to 4.5 mph—based on the ability of the participant in consultation with the exercise physiologist) and increments of an incline. The intensity is increased in 2 min stages with a 2% increase in incline at each stage. Heart rate is continuously monitored via a 12 lead ECG along with BP readings (Tango 2, SunTech Medical) and rating of perceived exertion (RPE). 45, 46 every 2 min. Cardiac impedance and HRV are also measured (MindWare Technologies). Each test will be administered by an exercise physiologist. Following the American College of Sports Medicine (ACSM) criteria, 46 a maximal test is determined when three of the four criteria are met: (1) plateau in VO2 between two or more workloads (increase less than 0.15 L/min or 2.0 mL/kg/min during the last minute of corresponding workloads); (2) respiratory exchange ratio ≥1.10; (3) heart rate within 10 beats of age predicted maximal heart rate (220-age); (4) rating of RPE ≥17. Using the 9th edition of the ACSM Guidelines for Exercise Testing and Prescription, participants scoring >75th percentile will be excluded from further continuation in the study. 47

**Stadiometer measurements**

Height and weight will be recorded before the GXT. Weight will be measured on a calibrated digital scale to the nearest 0.1 kg, wearing light clothing and without shoes. Height will be assessed using a calibrated stadiometer to the nearest 0.1 cm.

**PA monitoring**

PA monitoring will be conducted for the measurement of activity patterns throughout the trial, confirming engagement of exercise intensity during any unsupervised sessions, and to determine contamination of systematic changes in activity for the control group or the aerobic exercise group. A triaxial accelerometer (ActiGraph GT9X Link) will be worn around the non-dominant wrist at baseline, postintervention and every 6 weeks during the intervention for both groups. At each measurement point, the device is worn for a minimum of seven consecutive days, including sleep. A wear log will also be collected.
during each of these periods. The device can be worn during bathing, showering and swimming.

Psychosocial assessment
A battery of questionnaires will be administered at baseline and 12-month follow-up using REDCap surveys including social, mood and quality of life scales and instruments (>2.5 hours; see table 4). Exercise self-efficacy, social support, mood and anxiety, and personality traits have all been shown to either influence exercise adherence or brain health and might explain, mediate, or moderate some of the effects of exercise on brain or CVD outcomes.

Intervention
Aerobic exercise condition
Supervised exercise will take place two times a week for 60 min and once a week for 30 min at home. Levels of exercise intensity will be prescribed based on maximal responses during the initial GXT. As the participants will be low active at baseline, the prescribed intensity will be 50%–60% of the maximum heart rate reserve for weeks 1–6 and 70%–85% for the remainder of the programme as long as it is deemed safe by the monitoring exercise physiologist and trainer, reaching the prescribed goal of 150 min/week. Participants who feel comfortable increasing the intensity more quickly will be allowed to do so if the exercise trainer agrees that it is safe. A heart rate monitor (Polar A370 or Unite watch, Polar USA, Lake Success, NY) will be worn during all sessions to ensure that exercise is occurring within the target heart rate zone. Certified exercise instructors will monitor attendance, intensity, frequency and safety. Walking, jogging or running on a treadmill will be the encouraged mode of exercise, but other types of aerobic exercise equipment such as treadmill, bike (upright or recumbent), elliptical, stair climber or rower will be allowed. Participants can use up to two different modes at each session. All modes of activity will be recorded. Compliance to home-based exercise will be monitored by exercise diaries, heart rate monitors and actigraphy. All exercise sessions start and end with 5–10 min of stretching for the purpose of warming up and cooling down.

Health information control group
The control group will be instructed to wear an ActiGraph monitor for 7 days every 6 weeks. They will not be asked to change their behaviour or exercise patterns and encouraged to perform activities as they normally would. Any change in sedentary behaviour or PA will be monitored by the ActiGraph device and examined for change at the completion of the trial.

Statistical analysis of outcomes
Before testing the specific aims, we will conduct exploratory data analysis to summarise characteristics of the sample, outcomes distributions and clinical variables. Summary statistics (mean, SD for continuous variables, frequency for categorical variables), graphical representation (boxplots, histograms, spaghetti plots) and simple pairwise correlations will be used to describe the distribution of the data, missing data and potential multicollinearity among key variables. Furthermore, transformations will be applied as needed for all non-normal data including hepatocellular carcinoma and blood biomarkers prior to analysis.

All outcomes will be tested using an intent-to-treat framework. Sensitivity will be explored using adherence to the intervention (eg, per protocol). Dropout reasons and missing data mechanisms will be reported. We will test for completely random dropouts to examine whether missingness is unrelated to other observed and unobserved measurements (missing completely at random) or to the observed measurements only (missing at random). Additional sensitivity analyses will be conducted to evaluate informative dropouts. We will apply pattern-mixture models by stratifying our data by dropout patterns and fitting separate regression models to strata.

Before testing hypotheses about mediation, we need to test whether the intervention modifies proximal endpoints that will serve as the primary outcomes of the study. To do so, we will use a general linear mixed model (GLMM). The GLMM will include random effects (intercept and slope) for individual participants and a treatment-by-time interaction as a fixed effect. This technique will allow us to model the changes in scores as a function of both time and group while also including other potentially confounding variables in the regression model. Similarly, since we are considering individual changes over time and group, we will be able to test our hypotheses with respect to the response to the intervention (eg, responders vs non-responders to exercise). GLMM modelling is a popular approach to model both individual-specific and average (population-level) changes in longitudinal data by accounting for the within-person correlation among repeated measurements and individual heterogeneity in the change curve. Linear contrasts will be used to test for differences among intervention groups and measurement times via F-test or $\chi^2$ test accordingly.

We will also test whether exercise-induced changes to peripheral physiological pathways statistically mediate the effects of exercise on the brain and whether the effects of exercise on the brain statistically mediate any beneficial effects of exercise on peripheral mediators of CVD risk, using longitudinal mediation models in a structural equation modelling framework. We will test the mediation effect by calculating the indirect path effects. These tests will be conducted at the significance level of 0.01 to reduce the risk of inflation of type 1 error. We will also explore the role of other potential mediators (ie, sleep) and moderators (eg, sex) in the direction and strength of associations.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.
<table>
<thead>
<tr>
<th>Instrument Name / Questionnaire</th>
<th>Description</th>
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<tbody>
<tr>
<td>Barratt Impulsiveness Scale (BIS-11)51</td>
<td>30 item self-report instrument designed to assess the personality/behavioural construct of impulsiveness</td>
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<tr>
<td>Barriers Self-Efficacy Scale (BARSE)52</td>
<td>A 13-item questionnaire that assesses the degree of confidence that one could exercise despite a variety of limitations such as bad weather, while on vacation, etc</td>
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<tr>
<td>Big Five Trait Taxonomy II Questionnaire (BFI-II)53</td>
<td>A 44-item questionnaire that measures the Big Five personality dimensions including Openness-to-experience, Conscientiousness, Extraversion, Agreeableness and Neuroticism</td>
</tr>
<tr>
<td>Brief-COPE Scale54 55</td>
<td>A 28-item questionnaire to rate by the 4-point Likert scale a broad range of coping responses to stressful events</td>
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<tr>
<td>Brief Fatigue Inventory (BFI)56</td>
<td>A 9-item questionnaire that measures severity of fatigue (three items) and the interference with daily activities (six items) on numeric scales from 0 to 10</td>
</tr>
<tr>
<td>Chapman Handedness Questionnaire57</td>
<td>A 13-item questionnaire to determine the extent to which a person is left-handed, right-handed or ambidextrous</td>
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<tr>
<td>Cohen Hoberman Inventory of Physical Symptoms (CHIPS)58</td>
<td>A 33-item questionnaire to determine participant's experiences of everyday physical symptoms</td>
</tr>
<tr>
<td>Composite Scale of Morningness (CSM)59</td>
<td>A 13-item measure examining participants’ optimum times of day and quality of sleep</td>
</tr>
<tr>
<td>Emotion Regulation Inventory (ERQ)60</td>
<td>A 10-item scale designed to measure respondents’ tendency to regulate their emotions in two ways: (1) Cognitive Reappraisal and (2) Expressive Suppression on a 7-point Likert-type scale</td>
</tr>
<tr>
<td>Exercise Self-Efficacy (EXSE)61</td>
<td>An 8-item measure to assess degree of confidence in the ability to continue exercising at a moderate intensity three times per week for at least 40 min per session</td>
</tr>
<tr>
<td>Exercise Social Provisions Scale (EXSPS)62</td>
<td>A 24-item questionnaire about current relationships within the exercise programme, such as I do not think other people in the exercise group respect my skills and abilities. Adapted from its original form to be specific to exercise</td>
</tr>
<tr>
<td>Interpersonal Support Evaluation List (ISEL)63</td>
<td>A 40-item questionnaire to evaluate the impact of perceived availability of social support resources on health and well-being</td>
</tr>
<tr>
<td>Lantz’ 3-item Financial Strain Scale</td>
<td>Also known as Financial Chronic Stress Scale. It focuses on measures that capture the psychosocial distress related to insufficient financial resources (aka financial strain)</td>
</tr>
<tr>
<td>Life Events List (LEI)65</td>
<td>A 55-item questionnaire that measures the amount of change, using Life Change Units, a person experienced and adjusted to in the previous 12 months. Only 24 items were included in this study</td>
</tr>
<tr>
<td>Life Orientation Test (LOT)66</td>
<td>A 10-item questionnaire that measures optimism vs pessimism. Of the 10 items, 3 items measure optimism, 3 items measure pessimism and 4 items serve as fillers</td>
</tr>
<tr>
<td>NCI Dietary Questionnaire (NCI DHQ-II)67-69</td>
<td>A 24-hour recall questionnaire of food and nutrients intakes. The NCI method estimates the distribution of usual intake, assess the effects of non-dietary covariates on usual consumption and correct bias partially</td>
</tr>
<tr>
<td>MESA Community Neighborhood Perception Scale70 71</td>
<td>A 36-item questionnaire which includes seven neighbourhood dimensions to assess aesthetic quality, walking environment, availability of healthy foods, safety, violence, social cohesion and activities with neighbours</td>
</tr>
<tr>
<td>Mindful Attention Awareness Scale (MAAS)72</td>
<td>15-item scale designed to assess a core characteristic of dispositional mindfulness, namely, open or receptive awareness of and attention to what is taking place in the present</td>
</tr>
<tr>
<td>Multidimensional Outcome Expectations for Exercise Scale (MOEES)73</td>
<td>A 15-item questionnaire to assess participants’ beliefs or expectations about the benefits of regular exercise or physical activity</td>
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<tr>
<td>Paffenbarger Physical Activity Questionnaire74</td>
<td>This questionnaire measures leisure time PA. It includes several questions regarding the frequency and intensity of PA and is able to estimates energy expenditure by a PA index</td>
</tr>
<tr>
<td>Penn State Worry Questionnaire (PSWQ)75 76</td>
<td>16-item scale designed to measure the trait of worry in adults. This scale measures the excessiveness, generality and uncontrollable dimensions of worry</td>
</tr>
<tr>
<td>Perceived Stress Scale (PSS)77</td>
<td>A 10-items questionnaire measuring the degree of perceived stress regarding certain life events during the past month</td>
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<tr>
<td>Physical Activity Enjoyment Scale (PACES)78 79</td>
<td>An 18-item questionnaire that assesses enjoyment for physical activity using a 7-point bipolar Likert scale, from 1 (I enjoy it) to 7 (I hate it)</td>
</tr>
<tr>
<td>Physical Activity Self-Regulation Scale (PASR-12)</td>
<td>A 12-item scale that measures self-regulation strategies to support physical activity adaptations and adherence</td>
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Monitoring
Data management and quality control

All adverse events and serious adverse events will be carefully monitored, recorded and reported to the institutional Human Research and Protections Office and the Data Safety and Monitoring Board (DSMB) as necessary. Our DSMB meets approximately every 6 months to discuss study progress and safety. All adverse events will be reported in future manuscripts according to NHLBI guidelines.

Before the start of the study and data collection, all staff receive ethics training and certification of research training. In addition, to maintain consistency in data collection and intervention procedures over the course of the trial, all staff undergo annual on-site training certification.

There is an 8-week window to complete all baseline measures. Once a participant enrols, all non-electronic data are double entered into a REDCap database in which appropriate permissions are controlled by a data management team. Before randomisation, all data are accounted for, checked for quality control purposes and archived on a secure server.

At postintervention, each assessment session will be repeated. These sessions are collected over a 4-week window opening exactly 2 weeks before their 12-month anniversary and closing 2 weeks after their 12-month anniversary. It is encouraged to complete session 1 and session 2 within 2 weeks of the intervention completion.

COVID-19 adjustments

The current protocol was designed to address actual and expected challenges to conducting interventional research during the COVID-19 pandemic. The principal goal was to preserve the ability to test the primary aims while also minimising data loss and participant attrition;
conserving financial and staff resources; mitigating risks and maximising safe research interactions between staff and participants; and, reducing bottlenecks in testing and enrolment deficits during research operations. The exercise intervention group will be offered the flexibility to engage in exercise either in the laboratory setting with our exercise trainers or to exercise remotely (or a combination depending on exposure, shut-downs, illness, need for reduced capacity in the exercise setting, etc). Remote monitoring of behaviour and exercise compliance will include weekly phone calls between participants and exercise trainers, distribution of videos and routines for participants to use, purchasing and mailing heart rate monitors to monitor exercise intensity, and frequent use (every 6 weeks) of actigraphy monitoring to capture activity patterns. We intend to be as accommodating as possible while carefully recording behaviours, adherence and compliance.

**Limitations**

Because of subject burden, MRI costs and other complications, we are not able to assess participants at multiple time points over the course of the 12-month interval. We recognise that multiple measurement points would provide a higher resolution for modelling trajectories of change and temporal precedence for determining causality. We also recognise the multivariate nature of the CVD data, neuroimaging data and questionnaires and will follow procedures to reduce the number of dimensions by aggregating variables according to correlated and theoretical constructs. We also recognise that participating in an exercise research study could lead to behavioural changes in the control group condition (eg, changes in the amount of PA), which could contaminate the interpretation of the comparison between the control and exercise groups. To account for possible contamination, we have included PA monitoring, every 6 weeks, using an ActiGraph device during the intervention period to examine whether the results vary as a function of the magnitude of change in PA for the control group. Finally, in developing the inclusion and exclusion criteria, we balanced goals about generalisability to a broad population while maintaining and ensuring safety of the participants. As such, all results should be interpreted in the context of the sample characteristics.

**DISCUSSION**

Cumulative epidemiological evidence indicates that physical inactivity confers risk for CVD, and, in turn, that PA can reduce CVD morbidity and mortality. The biological and behavioural mechanisms through which PA may reduce these risks, some of which may be regulated by the brain, are still unclear. Here we describe the design of an RCT to test whether aerobic exercise induces changes in brain circuits and to investigate how these changes associate with CVD risk. We will also examine how exercise alters stress physiology and positive and negative affect and how these relate to both exercise-induced changes in brain circuits and CVD risk.

Recent work has shown the importance of brain circuits in risk for CVD, but the present study has the unique opportunity to expand this knowledge to better elucidate causal mechanisms using an experimental manipulation. Identifying the biological pathways by which exercise influences the brain and peripheral physiology could lead to new approaches (pharmaceutical and non-pharmaceutical) that target particular pathways involved in disease onset and progression. The outcomes from this study have the potential to transform our understanding of the brain’s role in transducing the effects of exercise on cardiovascular health, an effect with relevance to scientists and health professionals alike. In this study, the PA prescription aligns with globally recognised guidelines, so the effects observed are likely to be generalisable to a variety of widely used interventions, broadly informing efforts to improve both brain health and CVD risk. In sum, our trial will allow us to develop a more comprehensive and realistic model of the interrelationships between exercise, peripheral physiology, risk factors for CVD and brain plasticity.

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**Acknowledgements** Special thanks go to the staff and investigators associated with the data collection and study coordination.

**Contributors** KIE and PJG conceived the study, designed the final study protocol, provided the domain knowledge expertise and are the PIs of the study; MEC, GG, TWK, RLL, MM, SBM, ALM, MFM, MRS, TV, LW helped in the design of the final study protocol, contributed to the technical design and revised the initial manuscript; CK and JR contributed to the technical design and provided biostatistical support; MEC, MM helped in the design of the study and coordinated ethics approval; CMH drafted the initial manuscript; AMC, DV-D, KIE revised the initial manuscript draft. All authors read and approved the final manuscript.

**Funding** This study is supported by a grant from the National Institutes of Health and the National Heart, Lung, and Blood Institute (P01 HL040962) awarded to PJG (PD) and KIE (Project PI).

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Consent obtained directly from patient(s).

**Provenance and peer review** Not commissioned; externally peer reviewed.

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