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Train Smart Study: protocol for a randomised trial investigating the role of exercise training dose on markers of brain health in sedentary middle-aged adults

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ABSTRACT

Introduction Regular aerobic exercise is associated with improved cognitive function, implicating it as a strategy to reduce dementia risk. This is reinforced by the association between greater cardiorespiratory fitness and larger brain volume, superior cognitive performance and lower dementia risk. However, the optimal aerobic exercise dose, namely the intensity and mode of delivery, to improve brain health and lower dementia risk has received less attention. We aim to determine the effect of different doses of aerobic exercise training on markers of brain health in sedentary middle-aged adults, hypothesising that high-intensity interval training (HIIT) will be more beneficial than moderate-intensity continuous training (MICT).

Methods and analysis In this two-group parallel, open-label blinded endpoint randomised trial, 70 sedentary middle-aged (45–65 years) adults will be randomly allocated to one of two 12-week aerobic exercise training interventions matched for total exercise training volume: (1) MICT (n=35) or HIIT (n=35). Participants will perform ~50 min exercise training sessions, 3 days per week, for 12 weeks. The primary outcome will be measured as between-group difference in cardiorespiratory fitness (peak oxygen uptake) change from baseline to the end of training. Secondary outcomes include between-group differences in cognitive function and ultra-high field MRI (7T) measured markers of brain health (brain blood flow, cerebrovascular function, brain volume, white matter microstructural integrity and resting state functional brain activity) changes from baseline to the end of training.

Ethics and dissemination The Victoria University Human Research Ethics Committee (VUHREC) has approved this study (HRE20178), and all protocol modifications will be communicated to the relevant parties (eg, VUHREC, trial registry). Findings from this study will be disseminated via peer-review publications, conference presentations, clinical communications and both mainstream and social media.

Trial registration number ANZCTR12621000144819.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Randomised trial investigating the effects of exercise training dose on quantitative 7-Tesla MRI-derived biomarkers of brain health.
⇒ The sedentary and middle-aged cohort chosen in this study represents a critical period during which vascular risk factors appear to be most strongly associated with brain health and cognitive decline in later life.
⇒ This study is limited to relatively healthy middle-aged adults without risk factors for cognitive impairment and dementia beyond their sedentary lifestyle.
⇒ Investigators administering the exercise interventions will not be blinded for all assessments due to the difficulty blinding investigators and participants to an exercise intervention. However, the assessors conducting the cognitive testing, questionnaires and MRI analyses will be blinded to the participant group.

INTRODUCTION

Dementia is one of the greatest global public health and social care challenges facing society in the 21st century.[1] More than 50 million people are currently living with dementia worldwide, and this number is expected to triple by 2050.[2] The cost of dementia to the world economy was an estimated US$818 billion in 2015.[3] There is currently no cure for dementia; however, recent population-based studies have identified that almost half of dementia cases may be associated with modifiable risk factors,[4 5] including insufficient exercise, and this percentage increases with age.[6] Considering the enormity of this economic and public health burden, risk reduction is a major focus of current dementia research.

There is a growing evidence to suggest that regular aerobic exercise training improves cognitive function in both healthy individuals[7–13] and those with mild cognitive impairment,[14 15] suggesting it has potential as a treatment to reduce dementia risk. In concert, greater cardiorespiratory fitness is associated with lower dementia risk,[16–18] greater brain volume[19–22] and improved cognition.[22 23] The mechanisms by which exercise confers a neuroprotective effect remain to be fully established, but are hypothesised to be related to a number of haemodynamic, molecular and structural adaptations in the brain.[24] For example, regular exercise promotes functional and structural remodelling of the cerebral vasculature,[24] increases resting cerebral blood flow,[12 13 25–27] and improves cerebrovascular reactivity (CVR),[7] factors that may reduce the risk of stroke, brain atrophy (ie, lower brain volumes and/or cortical thinning[28]) and neurodegeneration.[29 30] Aerobic exercise training also promotes adult hippocampal neurogenesis[31] and synaptic plasticity,[32] increases brain volume,[11 14 33–36] and preserves white matter integrity,[37] adaptations that may be related to exercise-induced increases in the expression of neurotropic factors[38–40] and/or muscle-derived mediators of neuroprotection (myokines/exerkines).[41 42] Collectively, regular aerobic exercise preserves neuronal function, protects against brain atrophy and limits age-related cognitive decline,[32] and thus represents a potential strategy to help combat the growing dementia crisis.[29]

While the concept of dose is an important question when prescribing drugs to treat any clinical condition, including dementia, the optimal aerobic exercise dose (ie, frequency, intensity, duration and mode) to prevent and treat dementia has received limited attention. Dementia Australia currently recommends at least 30 min of moderate-intensity aerobic exercise, on most days of the week, to reduce the risk of developing dementia.[43] There is, however, a need for more research on the effects of different exercise intensities on promoting beneficial brain adaptations. High-intensity interval training (HIIT) has been reported to elicit a greater increase in cardiorespiratory fitness,[44] to promote a greater shear stress volume on arterial walls (an important stimulus for vascular adaptations)[24 45 46] and to provoke greater increases of metabolic mediators for improved brain adaptations,[47–49] when compared with moderate-intensity continuous training (MICT). Taken together, these findings raise the intriguing hypothesis that HIIT may be a more effective exercise prescription than MICT for improving markers of brain health, and that changes in these markers may be correlated with improvements in cardiorespiratory fitness.

This study aims to compare the effects of 12 weeks of HIIT or MICT on markers of brain health (ie, cognitive function, brain blood flow, cerebrovascular function, brain volume and brain function) in sedentary middle-aged (45–65 years) adults. Middle-aged adults were chosen as this age group represents a critical period during which vascular risk factors appear to be most strongly associated with brain health and cognitive decline in later life.[50 51] Reducing vascular risk factors (eg, with exercise) during midlife may play an important role in delaying or preventing the onset of dementia in old age, as evidenced by the fact that exercising more in midlife is associated with a reduced risk of dementia.[52] In this two-group parallel, open-label blinded endpoint randomised trial, we hypothesise that HIIT will promote greater improvements in cardiorespiratory fitness over a 12-week training intervention compared with MICT (primary outcome), and that greater improvements in cardiorespiratory fitness will be associated with greater improvements in cognitive function and MRI-measured markers of brain health (secondary outcomes).

METHODS AND ANALYSIS
Experimental design and overview

This randomised trial will follow a two-group parallel design. Participants will be assigned (in a randomised and counter-balanced fashion—see the Randomisation section) to one of two 12-week, work-matched, aerobic exercise training interventions (MICT or HIIT; two groups of n=35). Briefly, the experimental protocol will consist of (1) eligibility screening (t_{-1}), familiarisation of the exercise protocols and baseline testing (t_0), (2) a 12-week aerobic exercise intervention with testing at the 6-week mark (t_1), (3) post-training testing (t_2) and and (4) 12-week follow-up testing (t_3) to test the maintenance and short-term robustness of the intervention induced effects (figure 1). The trial will be reported according to the Consolidated Standards of Reporting Trials guidelines. The Standard Protocol Items: Recommendations for Intervention Trials list is detailed in table 1.[53] The first participant was recruited in November 2021, and data collection is expected to be completed by late 2023. Study assessments will be conducted over two sites in Melbourne, Australia: Victoria University and the University of Melbourne's Melbourne Brain Centre Imaging Unit.

Following screening for eligibility, participants who satisfy the selection criteria will perform three baseline testing sessions. The first session will be a familiarisation session, during which participants will perform an incremental exercise test, and practice sessions of the exercise training intervention and cerebrovascular function tests (~15 min between tests). Additionally, participants will be given a wrist-based activity monitor (GENEActiv, Activinsights) for the valid and reliable assessment of baseline physical activity levels, as previously established.[54] Participants will be required to wear the activity monitor for 7 days after the familiarisation session and before the remaining baseline testing sessions. This duration is sufficient to reliably estimate habitual physical activity in adults.[55–57] The remaining two baseline testing sessions will be separated by approximately 48 hours and will be performed in the following order: (1) cognitive function tests, mood and health questionnaires and 7T MRI brain
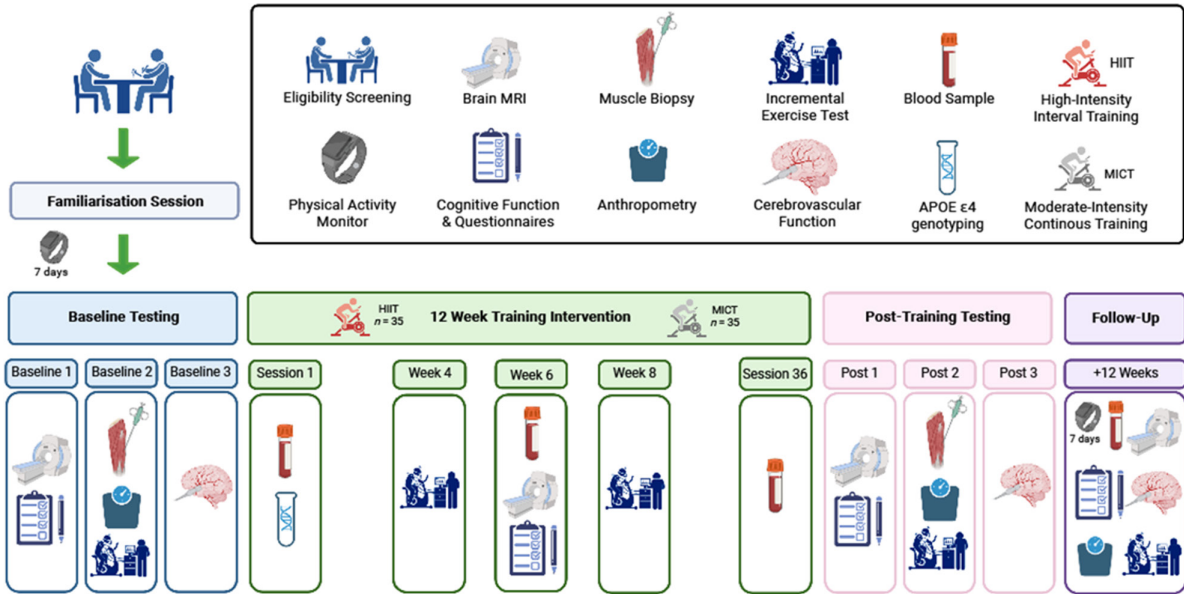
Figure 1 Graphical timeline of the Train Smart Study. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

scan; (2) resting muscle biopsy, anthropometry assessment, symptom-limited incremental exercise test and the cerebrovascular function tests.

After the initial 6 weeks of training (t_1), participants will repeat the 7T MRI brain scan and the mood and health questionnaires, while only completing the

Table 1 Standard Protocol Items for Randomised Trials: schedule of enrolment, interventions and assessments

	Enrolment	Baseline	Training (12 weeks)		Follow-up
Time point	$-t_1$	t_0	t_1 (6 weeks)	t_2 (12 weeks)	t_3 (24 weeks)
Enrolment:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
Familiarisation		X			
Interventions					
MICT			●————————————————●		
HIIT			●————————————————●		
Assessments					
Incremental exercise test		X	t_1 – 2 weeks, t_1+2 weeks and t_2		X
Physical activity monitoring		X			X
Brain MRI		X	X	X	X
Cognitive function		X	X	X	X
Mood, fatigue and QoL		X	X	X	X
Anthropometry		X		X	X
Cerebrovascular function		X		X	X
Muscle sampling		X		X	
Blood sampling		X	X	X	X
APOE ε4 genotyping		X			

APOE ε4, apolipoprotein E ε4; HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; QoL, quality of life; t_0, baseline time point; t_{-1}, screening and enrolment time point; t_1, 6 week mid-training time point; t_2, post-training time point; t_3, 12 week follow-up time point.

CogState component of the cognitive function tests. After the 12-week training intervention is complete (t_2), participants will repeat the t_0 testing sessions, replicated for order and timing. A final round of testing will be conducted 12 weeks after the end of training (t_3), during which participants will repeat the testing sessions from t_0 and t_2. Physical activity levels will also be measured for a period of 7 days prior to t_3 to give an indication of activity levels between t_2 and t_3. Every attempt will be made to ensure between-participant and within-participant assessments are performed at the same time of day (eg, between 06:00 and 09:00 hours for all blood and muscle analyses, between 09:00 and 17:00 hours for all MRI scans and cognitive function tests) to limit the effects of diurnal variation on these assessments. However, participant and equipment (eg, MRI scanner) availability will vary and ultimately take priority, and all deviations from this schedule will be reported in the final manuscript. Specific details of these protocols are described below.

Participant selection

Seventy sedentary, cognitively normal, middle-aged (45–65 years) adults will be recruited from the general population. A series of prestudy screening questionnaires will be used to ensure that participants meet entry criteria, principally that participants are sedentary and do not meet the Australian Physical Activity Guidelines for Adults (ie, the accumulation of 150 min of moderate-intensity physical activity, or 75 min of vigorous-intensity physical activity, or an equivalent combination of both moderate and vigorous activities, each week) for the previous 6 months. In addition, participants will be enrolled if they have no significant medical conditions precluding participation in an exercise intervention, have no contraindications for MRI, do not have cognitive impairment and do not present with other specified exclusions (table 2). Participants will also be required to obtain a medical clearance from their general practitioner to participate in the study.

Cognitive function will be screened by trained assessors using the Montreal Cognitive Assessment, where a score of 26–30 is validated to indicate cognitively normal.[58]

Once deemed eligible (as per table 2), participants will provide informed consent by signing a hard copy of a 'participant information and consent form'. In addition, participants will be asked to sign a hard copy of a 'consent for future use of data' form, which provides additional consent provisions for the collection and use of participant data and biological specimens in ancillary studies. JRB and NZ will be responsible for eligibility screening, as well as the collection and secure storage of consent forms.

Randomisation and blinding

Participants will be allocated into two groups by a computerised adaptive randomisation tool developed specifically for this trial using the Pocock Minimisation algorithm (R Studio),[59] and developed independently of the research team (ie, sequences are random and concealed from the research team). The randomisation tool will allocate participants via the minimisation of three covariates: cardiorespiratory fitness (above or below age-matched norms),[60] age (45–49 years, 50–59 years or 60–65 years) and sex (male, female or other). A researcher unblinded to the participant group (JRB) will be responsible for inputting covariate data into the adaptive randomisation tool prior to allocation, and randomisation will occur after the baseline sessions.

The assessors conducting the cognitive testing, questionnaires and MRI analysis will be blinded to participant group. Participants will be aware of their group allocation based on the exercise-training programme they are administered. To avoid contamination, the exercise sessions for the two groups will be conducted at different times throughout the day, and, if this is unavoidable, then participants from the two groups will be separated within the study site.

Table 2 Inclusion and exclusion criteria	
Inclusion criteria	**Exclusion criteria**
► Aged 45–65 years ► Able to participate in 36 exercise sessions over 12 weeks ► Normal cognition on screening (MoCA score ≥26) ► BMI 18–40	► Involved in regular exercise training in the previous 6 months (≥150 min of moderate-intensity physical activity or 75 min of vigorous-intensity physical activity, or an equivalent combination of both moderate and vigorous activities, per week) ► Significant medical comorbidities precluding participation in an exercise intervention (eg, severe cardiac disease) ► Contraindications to having a 7T MRI (eg, pacemaker, brain aneurysm clip). ► Cognitive impairment on screening (MoCA score ≤25) ► Recent diagnosis (<14 days), close contact or symptomatic of COVID-19 ► Underweight or morbidly obese (BMI<18 or >40) ► History of any serious traumatic brain injury (eg, ICU stay, in-patient rehabilitation required and/or a prolonged period of unconsciousness). ► High blood pressure (over 160/100 mm Hg) ► Current or ex-smoker (last 12 months) ► Any blood disorders or brain tumours ► Started any new medications (eg, hormone replacement therapy) in the previous 3 months

BMI, body mass index; ICU, Intensive-care unit; MoCA, Montreal Cognitive Assessment.

Outcomes and analyses

Primary Outcome:

► Between-group difference in cardiorespiratory fitness (peak oxygen uptake, VO_{2peak}) change from t_0 to t_2.

Secondary outcomes:

► Between-group difference in global and regional brain blood flow change from t_0 to t_2.

► Between-group difference in brain blood vessel health (eg, intracranial stenoses and pulse-wave velocities) change from t_0 to t_2.

► Between-group difference in white and grey matter volumes and hippocampal brain volume change from t_0 to t_2.

► Between-group difference in brain white matter microstructural integrity change from t_0 to t_2.

► Between-group difference in resting state functional brain activity change from t_0 to t_2.

► Between-group difference in cognitive function change (global composite score) from t_0 to t_2.

Exploratory outcomes:

► Between-group difference in all primary and secondary outcomes from t_0 to t_1, t_2 and t_3.

► Between-group difference in mood, fatigue and quality of life (QoL) measures from t_0 to t_1, t_2 and t_3.

► Between-group difference in cerebrovascular function change from t_0 to t_2 and t_3.

► Between-group difference in blood biomarkers of neurodegeneration, neurogenesis, metabolism, inflammation, blood lipids and vascular health change from t_0 to t_1, t_2 and t_3.

► Between-group difference in muscle biomarkers of neuroprotection change from t_0 to t_2.

► Between-group difference in muscle mitochondrial biomarkers (eg, respiration and biogenesis) changes from t_0 to t_2.

► APOE ε4 allele status and its effect on training-induced changes in all other outcomes.

Incremental exercise tests

Participants will perform a customised (desired time limit of 10 min) incremental exercise test to exhaustion, as previously described by our labs,[61] on six separate occasions (familiarisation, t_0, $t_1 - 2$ weeks, $t_1 + 2$ weeks, t_2 and t_3). These tests will serve to determine (VO_{2peak}; primary outcome) and peak aerobic power (W_{max}). Participants will be instructed to exercise until exhaustion, and the test will be stopped if participants reach volitional fatigue (ie, wishes to stop) and/or cannot maintain a cadence of between 60 and 70 rpm (ie, drops below 55 rpm). All incremental tests will be performed under the supervision of an accredited exercise physiologist/scientist, and will be immediately stopped in participants who experience dizziness/chest pain/severe shortness of breath/or any other pain related to (or caused by) exercise; abnormally high heart rates relative to exercise-intensity; signs of metabolic or cardiorespiratory distress; sweating responses that are inappropriate to the environmental conditions in the laboratory; and/or a systolic blood pressure of 225 mm Hg. Oxygen uptake (VO_2) will be assessed continually during the test using a gas analyser (Quark Cardiopulmonary Exercise Testing, Cosmed, Italy). In addition, heart rate (Polar Electro Oy, Kempele, Finland) and ratings of perceived exertion (RPE; Borg scale)[62] will be monitored and recorded every minute. The power at which participants cease exercise will be classified as their W_{max}, which will subsequently be used for the determination of exercise intensity during the training intervention (see the 'Exercise training interventions' section). The tests performed during the training intervention period (ie, $t_1 - 2$ weeks, $t_1 + 2$ weeks) will be used to ensure a continual progression of the training stimulus; at these time points, the incremental exercise test will be performed immediately prior to the first training session of weeks 5 and 9, thus avoiding an additional testing day in those weeks. All incremental tests will be performed on an electronically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands).

MRI

Brain imaging will be performed using a whole-body Siemens MAGNETOM 7T Plus Ultra-High Field MRI Scanner (Siemens Healthcare, Erlangen, Germany) with a combined 8-channel transmit and 32-channel receive head coil (Nova Medical, Wilmington, Massachusetts, USA). Each scan session will take approximately 60 min including the following sequences: anatomical imaging using three-dimensional T1-weighted sequence with quantitative susceptibility mapping (MEMP2RAGE), pulsed arterial-spin labelling, MR angiogram, diffusion-weighted imaging using a simultaneous multi-slice 2D spin-echo echo-planar imaging (EPI) sequence,[63] two-dimensional fluid-attenuated inversion recovery scan and resting-state functional MRI using multiband multislice gradient echo EPI sequence. These scans will be performed immediately after the cognitive function tests at t_0, t_1, t_2 and t_3. The MRI protocol can be viewed in online supplemental appendix A.

Cognitive function tests

Cognitive function will be assessed by a battery of neuropsychological tests, administered by trained research personnel who will not be involved in providing the exercise intervention. The test battery is designed to assess global cognition in addition to specific cognitive domains outlined in table 3. These domains were chosen to align with our group's previous work investigating the longitudinal effects of cerebrovascular disease (eg, stroke) or risk factors on cognitive functioning,[64 65] and the effects of cardiovascular exercise on brain and cognitive health in individuals following stroke.[66] They also align with recommendations for harmonising assessment of vascular cognitive impairment,[67] and have demonstrated sensitivity to the effects of exercise and cardiorespiratory fitness in healthy adults with and without risk of cognitive impairment or dementia.[68–70]

Table 3 Neuropsychology test battery and questionnaires

Cognitive domain	Task
Processing Speed, Attention and Working Memory	▶ CogState Detection Task (simple reaction time, milliseconds) ▶ CogState Identification Task (choice reaction time, milliseconds) ▶ CogState One-Card Learning (accuracy, %) ▶ CogState One-Back Task (accuracy, %) ▶ Digit Span Task (WAIS-IV) ▶ Trail Making Test (part A)
Executive functions	▶ Trail Making Test (part B) ▶ Controlled Oral Word Association Test & Animals Fluency ▶ Victoria Stroop Test
Verbal and Visual Memory	▶ Verbal Paired Associate Learning (WMS-IV; immediate and delayed) ▶ Rey-Osterrieth Complex Figure Test (copy and delayed) ▶ CogState One Card Learning (accuracy, %) and Continuous Paired Associate Learning subtests
Premorbid General Intellectual Function	▶ National Adult Reading Test
Mental Health/Fatigue/Quality of Life	Questionnaire
Anxiety	▶ The Generalised Anxiety Disorder-7
Depression	▶ Patient Health Questionnaire-9
Mental health	▶ Depression, Anxiety and Stress Scale 21 ▶ Short-Form 36 Questionnaire
Quality of life	▶ Assessment of Quality of Life
Fatigue	▶ Fatigue Assessment Scale

WAIS-IV - Weschler Adult Intelligence Scale, Fourth Edition[92]; WMS-IV - Weschler Memory Scale, Fourth 277 Edition[93]
WAIS-IV, Weschler Adult Intelligence Scale, Fourth Edition; WMS-IV, Weschler Memory Scale, Fourth Edition.

Testing will be performed in a quiet and private room, with participants sitting at a desk and the assessor across the other side of the desk. In accordance with the Post Ischaemic Stroke Cardiovascular Exercise Study (PISCES),[66] and the Cognition and Neocortical Volume After Stroke (CANVAS)[64] study, scores on individual cognitive tests will be calculated in accordance with published normative data, and performance in each cognitive domain will be indicated by (standardised) composite scores from the tests assessing each domain. All cognitive tests will be completed at t_0, t_2 and t_3. Only the CogState computerised tests will be performed at t_1, as these tests have demonstrated reliability and stability over multiple short test–retest intervals.[71]

Mental health, fatigue and QoL questionnaires
The Generalised Anxiety Disorder-7,[72] the Patient Health Questionnaire-9,[73] the Depression, Anxiety and Stress Scale 21,[74] the Short-Form 36 Questionnaire,[75] the Fatigue Assessment Scale[76] and the Assessment Quality of Life (AQoL)[77] questionnaires will be administered to assess general mental health, fatigue and QoL (table 3). These tests will be performed at the same time as the cognitive function tests at t_0, t_1, t_2 and t_3.

Cerebrovascular function
Cerebrovascular function will be assessed by measuring cerebral blood velocity of the middle cerebral artery (MCAv) using transcranial Doppler ultrasound (Multigon, Neurovision, Elmsford, New York, USA). After the collection of baseline resting data, MCAv will be measured during three testing conditions designed to challenge cerebrovascular responsiveness. These testing conditions include (in this order) neurovascular coupling (NVC; MCAv response to visual stimulation), dynamic cerebral autoregulation (dCA; MCAv response to changes in blood pressure) and CVR to hypercapnia (MCAv response to increased blood carbon dioxide), as described below. These tests will be conducted on the same day, with 10 min of passive rest between tests, at t_0, t_2 and t_3. In addition to MCAv, heart rate, beat-by-beat blood pressure and end tidal carbon dioxide concentration ($P_{ET}CO_2$) will be monitored during all of the cerebrovascular function tests, as previously described.[46] Participants will be required to have abstained from moderate/vigorous physical activity, caffeine and food, for at least 6 hour prior to these testing sessions.

At the start of the testing session, the right MCAv signal will be identified and tested using a 2 MHz probe over the right temporal window. Heart rate will be measured continuously using a three-electrode ECG attached to the chest (Bio-Amp, ADInstruments, Bella Vista, New South Wales, Australia). Blood pressure will be measured continuously at the left middle finger using photophlethysmography (Human NIBP, AD Instruments), ensuring the left forearm is supported at the level of the heart using a sling. Finger blood pressure will be exported to generate

beat-by-beat continuous mean arterial pressure (MAP). Expired gases will also be collected during the entire testing session, and continuously sampled (PowerLab, AD Instruments) for the determination of end-tidal carbon dioxide ($P_{ET}CO_2$). MCAv, heart rate, MAP and $P_{ET}CO_2$ will be sampled at 1000 Hz and stored (LabChart Pro, V.8 and PowerLab; ADInstruments).

NVC will be measured during repeated trials of a cognitive task (a reading exercise), as previously described.[78] Response times to these tests, and the magnitude of change in MCAv, will provide indices of brain neurovascular function. dCA will measure during repeated sit-to-stand manuvers (SSMs), as previously described.[79] Participants will perform 15 SSMs, measured as a 45° flexion of the knee (ie, shallow depth), at a frequency of 0.05 Hz (10 s standing, 10 s squatting), during which MCAv will be recorded. MCAv will also be measured during 90 s periods of standing quietly before and the SSM protocol. During SSMS, a chair will be set at the correct height behind the participant to guide the depth of each squat, and they will be instructed to not put any weight on the chair. Participants will also be asked to avoid a Valsalva-like manoeuvre (foreceful exhalation with closed mouth and nose) when in the squat positions. CVR will be measured during rebreathing of two different concentrations of carbon dioxide. Following a 5 min baseline collection period (ie, room air; 0.04% carbon dioxide, 21% oxygen and balanced nitrogen), the face mask will be connected to a Douglas bag containing 3% carbon dioxide, 21% oxygen and balanced nitrogen (4 min), and then another Douglas bag containing 6% carbon dioxide, 21% oxygen and balanced nitrogen (4 min).

Blood sampling and biochemical analysis

Venous blood samples (~20 mL each) will be taken from the participant's antecubital vein via venepuncture at six time points; before and after the first training session, before the training session at t_1, before and after the last training session and before the session at t_3. Approximately 15 mL of this sample will be split into EDTA and SST blood collection tubes, and the resultant plasma and serum will be stored in a −80°C freezer for future analyses (eg, systemic biomarkers of neurodegeneration, neurogenesis, metabolism, inflammation, blood lipids and vascular health). The resultant ~5 mL of whole blood will be split into an Eppendorf tube (~2 mL) and a Tempus Blood RNA tube (~3 mL) (Applied Biosystems, USA), and stored at −20°C for future DNA/RNA extraction and analysis. Specifically, APOE ε4 genotype will be assessed using a Taqman single-nucleotide polymorphism allelic discrimination assay. Blood lactate will also be measured before and after the first and last training sessions to help characterise the metabolic load of exercise.

Muscle biopsies

A percutaneous muscle biopsy performed with a Bergstrom needle modified with the addition of suction will be used to obtain muscle samples of ~100 to 150 mg from the vastus lateralis muscle of the quadriceps. Muscle samples will be analysed for biomarkers of neuroprotection (eg, myokine/exerkine pathways), as well as biomarkers of mitochondrial regulation (eg, mitochondrial biogenesis and mitochondrial respiratory function). Participants can elect to have none or two muscle biopsies throughout the entire study, and their choice will have no bearing on eligibility to participate in the study. If permission is given, participants will have one muscle biopsy at t_0 and another biopsy at t_2. Participants will be given the option of choosing which leg the muscle sample is taken from at t_0, and the same leg will be used for the t_2 biopsy.

Anthropometry assessment

Participant height will be measured while standing barefoot using a generic wall-mounted stature/height measuring tape, and body weight will be measured on a standard scale (PW-200-FG Patient Scale, A&D Weighing, Australia). Participants will be asked to stand wearing just light clothes.

Exercise training interventions

All training interventions will consist of 12 weeks of supervised, work-matched cycling training, performed three times per week on an electromagnetically braked cycle ergometer (Velotron, RacerMate, Seattle, USA). The training programme has been designed to ensure participants achieve the Australian Physical Activity Guidelines for Adults (ie, 150 min of moderate-intensity aerobic physical activity per week).[80] Training sessions for the MICT condition will consist of 36–48 min of continuous cycling, performed at an intensity 5 Watts above the power achieved at the ventilatory threshold during the incremental exercise test (~ 60% W_{max}), which will be determined as described previously.[81] This intensity was chosen as the ventilatory threshold represents the point at which cerebral blood flow has been reported to plateau[82] or progressively decrease[83] with further increases in exercise intensity, during continuous exercise. Training sessions for the HIIT condition will consist of between four and seven 4 min intervals, interspersed with 3 min of recovery. Interval duration (HIIT) will be set at 4 min in an attempt to maximise shear stress on cerebral vasculature during exercise (and recovery), as acute increases in cerebral blood flow are reported to peak ~3–4 min into an exercise bout.[46 84] The intensity prescribed for the HIIT group will be the power achieved at the respiratory compensation point (~ 90% W_{max}).[81] This intensity was chosen as it represents an intensity with superior cardiovascular health benefits as compared with moderate-intensity training,[85 86] without compromising on potential adherence issues. Considering the risks associated with blunted CVR in this population cohort,[87] participants in the HIIT group will be required to gradually increase exercise intensity in the last 30 s of each recovery period to 'prime and prepare' the cerebrovasculature.[88] Each training session will be preceded by 5 min (MICT) or 10 min (HIIT)

warm-up at 60% W_{max}, which is designed to ensure two groups are work-matched. To ensure adequate training progression, and in the absence of dose-limiting events (eg, a participant is unable to complete the session or an adverse event occurs during the session) training intensity will be increased by 2.5% each week in both groups. In addition, W_{max} will be reassessed every 4 weeks during the training period (ie, $t_1 - 2$ weeks, $t_1 + 2$ weeks) to recalibrate the relative intensity. If a participant experiences a dose-limiting event during training, predefined criteria are in place to modify exercise training to promote adherence and continued participation (eg, regressing the training intensity to the previous week's intensity). Make-up sessions will be offered to participants who miss training sessions (eg, due to illness or injury) to improve adherence; however, participants will be unenrolled from the study if they are unable to train for two successive weeks (due to the likelihood of detraining), or are unable to achieve a minimum of 30 training sessions over the 12-week training intervention period. To characterise and monitor exercise training load, heart rate (RS800sd; Polar Electro Oy, Kempele, Finland) will be measured and recorded throughout all training sessions for all participants. Additionally, participants will be asked to subjectively rate the perceived exertion of each training session using the session-RPE (sRPE) scale,[89] which is a valid and reliable tool for training load monitoring.[90] Participants will also be encouraged to maintain their habitual physical activity outside of the training intervention.

Sample size calculation

A total sample size of 70 (n=35 in each intervention group) will yield 80% power to detect a difference in VO_{2peak} between groups corresponding to a medium effect size (d = 0.75), assuming two-tailed significance and α=0.05. In a recent multicentre comparison of VO_{2peak} trainability between HIIT and MICT, relative VO_{2peak} was increased by 4.50±3.93 mL/kg/min following HIIT, and 1.50±3.36 mL/kg/min following MICT, in middle-aged and elderly individuals.[44] Using a mean difference between groups of 3.0 ± 4.0 mL/kg/min, this equates to a total sample size of 58 (n=29 per group). We have increased the sample size by 20% to account for attrition over the 6-month study period. Considering that we prespecified the primary analysis using an analysis of covariance (ANCOVA) model with the relevant value at baseline as a covariate, this power analysis is conservative as additional power is expected to be generated through covariance.

Statistical analyses

The primary outcome will be analysed using an ANCOVA model with the group as a factor and the baseline value of cardiorespiratory fitness (VO_{2peak}) as a covariate. Secondary analyses will be conducted using the same model for all outcomes with the baseline value of the relevant outcomes as covariates. Exploratory longitudinal analyses will be conducted within mixed effect repeated

measures framework using appropriate regression models with individual participants as random effects.

Outcome data will be analysed subject to the assumption of missingness-at-random (MAR). The sensitivity of the results to plausible departures from MAR will be explored as a part of an intention-to-treat analysis strategy.[91] According to this approach, the data and missingness are modelled jointly using a pattern-mixture model (modelling the differences between missing and observed data). Assumptions about the missing data are expressed via a parameter which measures the degree of departure from MAR and the results can be investigated over a range of assumptions expressed by different values of this parameter. All statistical analyses will be performed using Stata and R statistical software

Data management

Consistent with the International Council for Harmonisation Good Clinical Practice guidelines, the investigator site file will be located on Victoria University's internal research drive, an enterprise-grade and secure location for data storage during research and long-term retention. Participant data will be collected in a deidentifiable manner (ie, assigned a study number), and stored on REDCap, a secure web platform for managing online research databases. REDCap user rights will be overseen by the principal investigator (JRB), and assessors will only have access to the data they are unblinded to (eg, assessors conducting the cognitive testing, questionnaires and MRI analysis will have no access to the exercise data collected at Victoria University and vice versa). Personal information (eg, consent forms, risk factor questionnaire) that is collected during recruitment will be stored in a locked cabinet on site at Victoria University (accessible by JRB and NZ only), ensuring confidentiality before, during and after the trial.

Patient and public involvement

No patient involvement.

Ethics and dissemination

The Victoria University Human Research Ethics Committee (VUHREC) has approved this study (HRE20178), and all protocol modifications will be communicated to the relevant parties (eg, VUHREC, trial registry). Findings from this study will be disseminated via peer-review publications, conference presentations, clinical communications, and both mainstream and social media.

SUMMARY AND CONCLUSION

The Train Smart Study aims to discover how different doses of aerobic exercise affect markers of brain health, and to build the current evidence base in support of an improved exercise prescription to better prevent the development of dementia. The study design is robust and novel in its inclusion of an established neuropsychological

test battery typically used in clinical populations,[64 66] as well as advanced neuroimaging techniques (ie, 7T MRI) and analysis. In addition, this study will use 'gold-standard' exercise prescription practices aimed at maximising the beneficial effects of exercise on health,[61 81] and includes a 12-week follow-up for the assessment of longitudinal effects of exercise training on brain health. If our hypotheses are supported, our research has the potential to alter the current risk reduction paradigm, and help optimise exercise prescription practices for individuals at high risk of developing dementia, including those with one or more modifiable lifestyle risk factors such as mid-life sedentary behaviour.

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