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Breaking prolonged sitting with high-intensity interval training to improve cognitive and brain health in middle-aged and older adults: a protocol for the pilot feasibility HIIT2SITLess trial

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ABSTRACT

Introduction Excessive sedentary time (ST) is linked to dementia risk, poorer attentional control and episodic memory. These cognitive decrements have been associated with decreased functional connectivity (FC) in the frontoparietal network (FPN) and default mode networks (DMN) with ageing. Physical activity (PA) interventions can enhance FC in these networks, but these interventions are not designed to decrease ST among older adults. Prolonged sitting (ie, sitting continuously for ≥20 min) can acutely reduce frontoparietal brain function and attentional control, while a single PA bout lasting at least 20 min can enhance them. It has been theorised that stimulation of the cerebral norepinephrine release through peripheral increase in catecholamines may explain this effect. In contrast, the effects of shorter (<10 min) PA bouts used to interrupt prolonged sitting on neurocognitive functions remain poorly understood. This pilot randomised crossover feasibility trial capitalises on PA intensity as the major limiting factor in peripheral catecholamine increase and tests the effects of interrupting prolonged sitting every 30 min with 6 min high-intensity interval training (HIIT) compared with low-intensity interval training (LIIT) bouts. The study will address three aims: (1) to assess feasibility, acceptability, fidelity and safety of HIIT breaks to improve neurocognitive function in middle-aged and older adults; (2) to quantify the differences between conditions in the change in the amplitude and latency of the P3b component of event-related potentials (a marker for frontoparietal function) and (3) to explore the differences between conditions in attentional control, episodic memory and FC of the FPN and DMN in middle-aged and older adults.

Methods and analysis 54 healthy adults, aged 40–75 years, will be recruited from the local community and randomly assigned to a condition sequence (HIIT, LIIT vs LIIT and HIIT). Each HIIT bout comprises a 1 min warm-up, 2 min at 90% of the maximum heart rate (HR_{max}), 1 min passive rest and 2 min at 90% HR_{max}. During 2 min intervals in LIIT, participants exercise at 57%–60% of HR_{max}. The primary outcomes include the feasibility (recruitment and retention rates, percentage of valid electroencephalogram data), acceptability of time commitment, HIIT bouts and neurocognitive assessments, fidelity (the intensity of HIIT breaks, percentage of time spent sitting) and the amplitude and the latency of the P3b component of event-related brain potentials measured during the modified Eriksen flanker task at pretests, after the first and the third PA bout and at post-test. General linear mixed-effects models will be used to test the effects of the intervention on the P3b component.

Ethics and dissemination The Institutional Review Board at the University of Illinois Urbana-Champaign provided the ethical approval for the study. Findings will be disseminated in peer-reviewed journals and at scientific conferences.

Trial registration number NCT06243016.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The HIIT2SITLess study is a well-controlled randomised crossover pilot feasibility trial designed to isolate the effects of the intensity of short physical activity (PA) bouts to interrupt prolonged sitting on frontoparietal function in middle-aged and older adults.
⇒ The trial is designed based on the theory linking the activation of the locus coeruleus-norepinephrine system with high-intensity exercise to frontoparietal brain function.
⇒ The trial employs rigorous neurophysiological and cognitive measures of frontoparietal function, inhibitory control and episodic memory.
⇒ This pilot feasibility trial recruits healthy middle-aged and older adults with a limited cardiovascular risk; hence, its generalisability to populations with an increased cardiovascular risk is limited.
⇒ The study focuses on acute but not the long-term benefits of interrupting prolonged sitting with PA on brain function, attentional control and episodic memory.

INTRODUCTION

The year 2020 has marked a dramatic shift in the ageing population worldwide, when the number of older adults exceeded the number of children.[1] Most older adults aged ≥65 years experience normal age-related cognitive decline, characterised by a decreased ability to control distractions and correctly recall the details of information and events (ie, episodic memory).[2–4] These cognitive functions are indispensable for everyday functioning, learning and decision-making.[5 6] Given the ubiquity of normal age-related cognitive decline, there is an urgent need for effective approaches to improve cognitive and brain health during ageing.

Yet, effective and scalable interventions to improve cognitive and brain health in older adults are lacking. Traditional physical activity (PA) interventions (eg, a 20–40 min bout of moderate-intensity PA) show promise and can improve frontoparietal function and hippocampal-dependent episodic memory in seniors.[7] However, they have limited impact because most older adults (70%) do not engage daily in moderate-intensity PA that lasts even 10 min.[8] In contrast, the efficacy of PA interventions that use short (<10 min) but high-intensity PA to improve frontoparietal function and cognition in seniors is virtually unknown. Such interventions could boost PA adoption because they address critical barriers to PA participation in middle-aged and older adults: the lack of time and access to gyms.[9]

Traditional PA interventions designed to enhance neurocognitive function in older adults also do not reduce their excessive sedentary time (ST), amounting to 10 hours/day.[10] Epidemiological evidence suggests that remaining sedentary for 10 hours/day or more increases the risk of Alzheimer's Disease (AD) and AD-related dementias, even in physically active adults.[11] Emergent observational studies indicate that ST and prolonged sitting, such as sitting continuously for 20 min or longer, may attenuate attentional control,[12 13] episodic memory[14] and frontoparietal function.[15] For example, adults aged 21–45 years with more prolonged ST had poorer attentional control.[12] Older adults engaging in more ST had poorer episodic memory.[14] Pontifex *et al*[15] found a decrease in P3b amplitude in young adults who sat for 20 min, suggesting a decrease in frontoparietal brain function. The P3b component of event-related brain potentials (ERPs) is a stimulus-locked positive-going waveform embedded in an electroencephalogram (EEG) signal, which appears approximately 250–700 ms after stimulus onset with a maximum over parietal electrodes.[16] The amplitude of the P3b-ERP component increases proportionally with the attentional resources allocated towards the inhibition of neuronal activity extraneous to the task in order to facilitate the task-relevant attentional processing; its latency is thought to index the speed of stimulus evaluation.[16] The P3b-ERP component is considered a marker of frontoparietal function because several of its cortical generators overlap with frontoparietal regions.[17–19] Yet, it is unknown how prolonged sitting can affect frontoparietal function in middle-aged and older adults and if interrupting sitting with high-intensity PA could improve it.

Spatial patterns of coactivation between brain regions supporting cognitive performance are already observed at rest in correlated fluctuations of activity, known as intrinsic brain networks.[20] One such network, the frontoparietal network (FPN; comprising hubs in the frontal cortex and intraparietal sulcus[21]), supports cognitive control functions, including attentional control.[21–23] Higher functional connectivity (FC) at rest in this network predicts better attentional control in older adults.[22] Yet, FC in the FPN declines with age[24 25] and in AD,[26] which predicts faster cognitive decline.[25] Another network relevant to cognitive ageing is the default mode network (DMN; it comprises regions in the medial prefrontal and posterior cingulate cortices[27 28]), which supports episodic memory.[29] FC in this network also declines with age,[25 30] presaging faster cognitive decline.[25] A decline in FC within the DMN has also been related to episodic memory decline in older adults.[29] Accordingly, changes in FC in the FPN and the DMN can enhance our understanding of PA effects on brain functions that are susceptible to age-related and AD-related decline.

To be effective, PA interventions should target the mechanisms underlying the decreasing efficiency of the frontoparietal functions, attentional control and episodic memory decline during ageing. The locus coeruleus, a group of noradrenergic neurons in the pons,[31] helps maintain the structural integrity of the FPN.[32] Cerebral norepinephrine increases activation in the frontoparietal brain regions and optimises attentional control.[33–35] It also binds to β-adrenoreceptors in the hippocampus, stimulating learning and memory,[36 37] including episodic memory.[38–40] Its effects may also extend to increased FC in the DMN.[41 42] PA is thought to stimulate phasic norepinephrine release from the locus coeruleus[31 43] and enhance frontoparietal function,[44–46] attentional control[47] and episodic memory[48] via locus coeruleus projections to the prefrontal and parietal cortices[49 50] and the hippocampus.[51] Yet, the locus coeruleus-norepinephrine system (LC-NE) is highly susceptible to ageing[52] and AD.[53] Thus, PA interventions designed to stimulate the LC-NE system could significantly impact the functional integrity of the ageing brain.

High-intensity interval training (HIIT) could stimulate the LC-NE system because it uses short high-intensity intervals (interspersed with brief periods of rest), which can rapidly enhance peripheral catecholamine release[54 55] and stimulate the LC-NE system.[56 57] In confirmation, experimental studies in young adults showed that a HIIT bout lasting ≤10 min can improve frontoparietal function and attentional control at a short 15–20 min delay.[58 59] However, the effect of a single bout of HIIT on cognitive function declines after 20–30 min.[47] Thus, a single bout cannot counteract the potential adverse effect of 5 hours of prolonged sitting that adults of all ages engage in daily[60] on neurocognitive function. Whether

regularly interrupting prolonged sitting with short (<10 min) bouts of HIIT could be leveraged to improve cognitive and brain function in middle-aged and older adults over several hours is unknown.

Several previous studies tested the effect of frequent but short PA (2–5 min) breaks to prolonged sitting of primarily light intensity on cognitive function relative to sitting alone.[61–63] Yet, they were unsuccessful in improving cognitive functions. One reason for this null effect can be insufficient PA intensity (ie, light or moderate) to stimulate the LC-NE system within 2–5 min.[64–66] As discussed above, adults spend a substantial proportion of the day in prolonged sitting (~48%), which increases with age. The proposed work overcomes these limitations by leveraging short HIIT bouts at the intensity and duration sufficient to increase peripheral catecholamines[64 65] to enhance cognitive and brain functions.

STUDY AIMS AND OBJECTIVES

The lack of effective PA interventions to reduce prolonged sitting and enhance cognitive and brain function in middle-aged and older adults reflects a significant gap in our understanding of the detrimental effects of prolonged sitting on brain health and the necessary PA dose to counter its effects. The HIIT2SITLess study was designed to address this gap. The HIIT2SITLess study is a randomised crossover pilot feasibility trial designed to test three specific aims:

1. To assess the feasibility, acceptability, fidelity and safety of HIIT breaks to improve neurocognitive function.
2. To quantify the differences between conditions in a change in P3b amplitude and latency, a marker of frontoparietal function.
3. To explore the differences between conditions in attentional control, episodic memory and FC in the FPN and DMNs.

The study will test the following hypotheses:

1. HIIT interruptions to prolonged sitting will be feasible, acceptable and safe and can be implemented with fidelity to enhance neurocognitive function in middle-aged and older adults.
2. HIIT versus light intensity interval training (LIIT) bouts will result in greater changes in P3b amplitude and latency.
3. HIIT versus LIIT bouts will improve attentional control and episodic memory.
4. HIIT versus LIIT bouts will enhance FC in frontoparietal and DMNs.

Given the emergent evidence that acute responses to exercise can predict chronic adaptations in brain function and cognitive performance,[67] the findings from this study can inform future acute and chronic PA interventions to reduce prolonged sitting and enhance brain health in middle-aged and older adults.

METHODS AND ANALYSIS

Study setting and design

HIIT2SITLess is a randomised crossover trial with two interventions lasting 3.5 hours each: prolonged sitting interrupted every 30 min with 6 min HIIT bouts active condition and prolonged sitting interrupted every 30 min with 6 min LIIT bouts control condition. The study is conducted over three consecutive visits. The participants will be recruited to the trial between February 2024 and March 2026. All participants provided written informed consent in accordance with the Institutional Review Board at the University of Illinois Urbana-Champaign (see online supplemental table 1 for sponsor details).

Trial registration

The trial was registered on ClinicalTrials.gov No. NCT06243016 before the enrolment of the first participant. See online supplemental table 2 for trial registration details.

Participants

The study will enrol 54 (27 female) middle-aged (40–59 years) and older (60–75 years) cognitively healthy adults from Champaign County, IL, and the surrounding areas. This age range was chosen based on the proven safety of HIIT in similar age groups,[68] a steeper decline in physical function after the age of 75 years,[69] and previous exercise trials and cohort studies into cognitive and brain health in middle-aged[70–72] and older adults.[7 67]

Eligibility

Our inclusion and exclusion criteria have been designed to enrol individuals who are sedentary, low or moderately physically active and can safely engage in acute high-intensity exercise. The criteria were developed to emphasise safety and generalisability of study outcomes. Table 1 outlines the study's inclusion and exclusion criteria.

Blinding and randomisation

54 participants will be randomised to two condition sequences by a statistician following baseline assessments. Permuted block randomisation generated using the PROC PLAN procedure (SAS Institute)[73] is used, where sequences are randomised within a block of six participants to minimise the possibility of group imbalances due to dropout. Participants are randomised to one of two condition sequences by a study statistician: (1) X=HIIT, LIIT breaks or Y=LIIT, HIIT breaks. Generated permuted block randomisation also ensures that blocks are balanced by cognitive task (ie, flanker (F), antisaccade (A) and mnemonic similarities task (M)) sequence (FAM, MFA and AMF). The principal and coinvestigators will be blinded to the sequence allocation. The sequence will be concealed until the participant's enrolment. On enrolment, the study sequence will be verbally communicated to the study coordinator by a statistician. The coordinator will record the sequence number in Research Electronic Data Capture (REDCap). The staff implementing the

Table 1 Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 40–75 years; including premenopausal, postmenopausal and perimenopausal women regardless of hormone therapy replacement.	Physical disability or musculoskeletal disease prohibitive to vigorous exercise
BMI <40 kg/m^2	Learning disabilities
Sedentary (≥6 hours/day sitting by a survey question)	Cognitive abilities below a 26-point cut-off on a MoCA
The Canadian Society for Exercise Physiology Physical Activity and Sedentary Behaviour Questionnaire in the low and moderate physical activity range	Type 1 or 2 diabetes
Capable of exercising vigorously based on the PARQ+	Neurological condition (eg, MS, Parkinson, dementia, MCI)
Has a medical clearance for maximal exercise and HIIT from a physician	Colour blindness
Normotensive or participant's blood pressure is controlled (ie, individuals who had previously been at or above the 140/90 mm Hg threshold before the initiation of treatment but are now below this threshold)	Brain injury (eg, traumatic brain injury, stroke)
Intellectual ability no less than 1 SD relative to the population mean (ie, ≥85 where mean=100, SD=15) as measured with KBIT-2	Presence of other health conditions that may be exacerbated by exercise
No current or previous diagnosis of type 1 or type 2 diabetes confirmed by the participant's physician	History of heart disease
Fasting blood glucose <126 mg/dL or HbA1c <6.5% in the last 12 months	High cholesterol not controlled by medication
Good or corrected vision (near vision 20/30) and hearing	Signs and symptoms indicative of underlying cardiovascular disease (based on General Health History Questionnaire)
No significant abnormalities on the ECG during the maximal exercise test	A chronic pulmonary disease (eg, chronic obstructive pulmonary disease)
No signs and symptoms that suggest an underlying cardiovascular disease as recorded during the maximal exercise test by a study physician	Emphysema
No indications to prematurely stop the maximal exercise test as outlined by the ACSM's Guidelines for Exercise Testing and Prescription	Pulmonary embolus
Concussion if more than 12 months before the study screening	Asthma
History of cancer but in full remission for at least 12 months and no history of chemotherapy, signed off by the physician or an oncologist	History of renal disease
	History of seizures
	A neuropsychiatric disorder (eg, attention-deficit hyperactivity disorder, schizophrenia)
	Osteoporosis if it interferes with an individual's ability to exercise
	Severe back problems
	Severe arthritis if it interferes with an individual's ability to exercise
	Thyroid disorder not controlled by medication
	Polyneuropathy
	Sleep disorders except for obstructive sleep apnoea
	AIDS
	Hepatitis C
	History of long COVID-19
	Current or past smoking <12 months
	Corticosteroid intake <31 days before screening
	Opioids taken <6 months from screening
	Anabolic androgens taken <31 days before screening
	A serious illness or hospitalisation in the last 6 months
	Currently taking medications that can affect the central nervous system (except for antidepressants and anxiolytics)
	Current participation in an ongoing trial likely to influence exercise ability or cognitive function

ACSM, American College of Sports Medicine; BMI, body mass index; HbA1c, glycated haemoglobin; HIIT, high-intensity interval training; KBIT-2, Kaufman Brief Intelligence Test-2; MoCA, Montreal Cognitive Assessment; PARQ+, Physical Activity Readiness Questionnaire+.

trial will be unblinded. Participants will be blinded as to the intervention order until their first intervention visit.

Recruitment and retention

Recruitment of participants began in February 2024 with planned completion of enrolment by June 2025. The participant recruitment occurs via local media outlets, the local buses, the University list-serve, social media campaigns, contacts to local faith congregations, the University Extension, organisations serving older adults in Champaign County, and flyers, and individual mailouts to adults aged 40–75 years in Champaign County. Recruitment and enrolment occur continuously. The researchers will send reminders and will call to remind participants about their appointments. In case of dropout, the research coordinator will follow up with questions about reasons for withdrawal.

Study procedures

A complete schedule of study assessments is presented in online supplemental table 3.

Screening procedure

Screening call

At the beginning of the screening call, participants will sign an informed consent to the screening process (online supplemental material 1). The screening call is designed to select participants based on age, English language fluency, independent living, physical function, self-reported sitting time, PA, ability to engage in vigorous cycling, disability status, vision and hearing and to screen out individuals with a history of stroke or transient ischaemic attack, long COVID-19 and smokers. A trained researcher will then administer the Telephone Interview of Cognitive Status-modified. Only individuals with a score <32 (a cut-off for mild cognitive impairment)[74] will be included. If a participant qualifies based on these assessments, they will complete a General Health History questionnaire designed to screen out participants with an increased risk of cardiovascular disease,[75] and pre-existing conditions as listed in the exclusion criteria (table 1). An individual will also fill in the Hospital Anxiety and Depression Scale.[76] Individuals with anxiety and depression will be included due to the high prevalence of these disorders in the general population.[77 78] Hospital Anxiety and Depression Scale scores will be used to explore the potential confounding effect of these factors on the results. In addition to eligibility based on these assessments, the individual must be cleared by his/her primary care physician (PCP) for maximal and high-intensity exercise.

Physical Activity Questionnaires

The Canadian Society for Exercise Physiology Physical Activity and Sedentary Behaviour Questionnaire[79] screens out highly physically active individuals who engage in 300 min or more of moderate-to-vigorous PA per week. The Physical Activity Readiness Questionnaire+[80] is used to identify individuals who may be at a greater risk of participating in high-intensity exercise. Table 2 lists all psychosocial assessments.

Screening visit

Once the participant who qualified based on a screening call is medically cleared by his/her PCP, the participant will come to the laboratory for an in-person screening visit. Before the screening visit (as well as baseline and intervention visits), participants will be asked not to (1) exercise strenuously for 48 hours before the experimental visit, (2) drink caffeine or (3) alcohol in the 24 hours before the experimental visit. They will also come to the laboratory in the morning after the overnight fast. A trained researcher will measure their resting heart rate (HR) and blood pressure (BP). Only participants with systolic over diastolic BP (SBP/DBP) of less than 200/110 mm Hg on the screening day will undergo the maximal exercise test because higher values are a contraindication to a maximal exercise test.[75] They also must have a confirmation from their physician on a medical clearance that their BP is within a normotensive range. The anthropometric measurements will follow to ensure that the participant's body mass index (BMI) does not exceed 40 kg/m^2 due to an increased cardiovascular risk.[81] If the participant's physician cannot confirm fasting glucose levels or glycated haemoglobin levels less than below diagnostic values for type 2 diabetes in the last 12 months, a trained researcher will collect a fasting capillary blood sample using a lancet device and a point-of-care glucometer to confirm that fasting glucose levels are below 126 mg/dL. Next, participants fill in demographic information and undergo neuropsychological testing.

Neuropsychological assessments

A trained researcher administers a Montreal Cognitive Assessment to screen out individuals with scores <26 suggestive of potential cognitive impairment .[82] A standardised test of cognitive abilities (Kaufman Brief Intelligence Test-2)[79] will be administered next, and individuals with a score <85 (ie, 1 SD below the age-matched population) will be excluded.

Psychosocial assessments

A set of psychosocial questionnaires will be administered to allow for more accurate assessments of depressive symptoms (table 2).

Cardiorespiratory fitness testing

Participants will undergo a maximal exercise test on a cycle ergometer (Excalibur, Lode, Groningen, the Netherlands) using a modified Astrand protocol[83 84] with a 12-lead ECG. The test will be supervised by a study physician who is experienced in supervising graded maximal exercise tests in older adults. This test is conducted based on the recommendations from the American College of Sports Medicine (ACSM) to evaluate participants' physiological responses to exercise.[75] Its results will be used as an inclusion criterion to enhance the safety of acute high-intensity exercise. Three trained first aid and

Table 2 Psychosocial assessments

Name	Description
The Activities Collected over Time over 24-hours[139]	A 24-hour physical activity recall to measure participant's previous day physical activity, sedentary behaviours and sleep.
Beck Depression Inventory-2[140]	A 21-item inventory to assess attitudes and symptoms of depression in adults aged 18–80 years.
Cardiorespiratory Fitness Questionnaire[141]	A 5-item questionnaire to assess the level of aerobic fitness based on questions about habitual aerobic exercise.
The Epworth Sleepiness Scale[142]	An 8-item scale to assess the levels of sleepiness defined as the chance of dozing off in 8 common circumstances.
Florida Cognitive Activity Scale	A 23-item scale assessing the frequency that the participants engage in daily cognitively stimulating activities such as crossword puzzles, home repairs, playing chess etc.
FDI DIS Abbreviated[143 144]	An 8-item questionnaire to assess the frequency of engaging in common everyday activities such as visiting friends, taking care of finances, and the extent to which individuals feel limited in their ability to perform such activities.
FDI FXN Abbreviated [144 145]	A 15-item questionnaire to assess the level of difficulty an individual has with specific activities of daily living such as running and walking 0.5 mile, preparing meals, going up a flight of stairs, etc.
FXNSE without a device[146]	A 15-item scale measuring individual's confidence in completing specific functional activities unassisted.
Geriatric Depression Scale[147]	It is a 15-item scale that assesses the degree of depressive symptoms and anhedonia in older adults.
Godin-Shephard Leisure Time Physical Activity Questionnaire[148 149]	A 3-item assessment of habitual structured exercise in a typical week.
Hospital Anxiety and Depression Scale[76]	A 14-item questionnaire with questions about symptoms of depression, anxiety and psychological distress during the past week.
The Canadian Society for Exercise Physiology Physical Activity and Sedentary Behaviour Questionnaire[79]	A 7-item questionnaire assessing time spent on average in moderate-to-vigorous physical and muscle strengthening physical activity during a usual week, perceived aerobic fitness, time spent sedentary and frequency of interrupting prolonged sitting.
Kaufman Brief Intelligence Test-2[76]	A standardised and normed intelligence test for ages 4–90 years. The test comprises one verbal and two non-verbal components used to compute verbal and non-verbal IQ scores and a general IQ score.
Karolinska Sleepiness Scale[95]	A one-item assessment of individual's subjective experience of sleepiness over the past 5 min.
Montreal Cognitive Assessment (MoCA)[82 150]	MoCA is a screening tool for cognitive impairment. It comprises 13 items assessing 7 cognitive domains: visuospatial and executive function, naming, memory, language, abstraction, and orientation in time and place.
Physical Activity Readiness Questionnaire for Everyone[80]	It is a 7-item screening tool recommended as pre-participation screening before a subject begins physical activity. Questions ask about diagnosis and signs and symptoms of cardiovascular disease, medication and bone, joint and soft tissue problems that may prevent an individual from physical activity.
Pittsburgh Sleep Quality Index[80]	A 9-item tool assessing sleep quality.
Preference for Tolerance of the Intensity of Exercise Questionnaire[151]	A 16-item scale to assess individual's responses and preference for exercise intensity.
Rosenberg Sedentary Behaviour Questionnaire[152]	An 18-item questionnaire assessing the time individuals spent in various sedentary behaviours on weekdays and weekend days.
Task Engagement Scale[96–98]	A 9-item scale assessing the level of physical, emotional and cognitive engagement in a task.
Mental Effort Scale[99]	A single-item scale assessing the level of mental effort exerted during the task.

FDI DIS, Late-Life Function and Disiability Instrument -Disability Component; FDI FXN, Late-Life Function and Disability Instrument: Function Component; FXNSE, Function Self Efficacy Scale.

cardiopulmonary resuscitation (CPR)-certified experimenters will conduct the test in collaboration with the study physician. Participants' resting BP, HR and ECG readings will be collected. They will then warm up for 2 min while pedalling at the same speed of 50 revolutions per minute. Next, the workload on the cycle ergometer

will be increased depending on the participant's sex, starting at 50 watts for females and increasing every 2 min by 25 watts. Males will start at 100 watts and exercise at 50 watts increments.[83 84] The participant will cycle until volitional exhaustion.[83 84] Their HR and ECG are continuously monitored, and BP will be monitored every 2 min during exercise by a physician. Every 2 min, the study staff will record ratings of perceived exertion (RPE) using the Borg scale.[85] Relative peak oxygen consumption will be expressed in mL/kg/min and based on maximal effort as evidenced by at least two of the following criteria[75]: (1) respiratory exchange ratio ≥1.1, (2) failure of the HR to increase with increasing workload (ie, ≤10 bpm increase relative to age-predicted HR_{max}[86]) or (3) RPE>17. The test finishes with a 5 min cool-down. If there are no positive findings on the ECG as described in the indications to stopping the maximal exercise test in the ACSM's Guidelines for Exercise Testing and Prescription,[75] the individual will be cleared for participation in the study. The HR_{max} achieved during the test will be used to determine exercise intensity for each individual.

Baseline

Baseline assessments were designed to familiarise participants with the main intervention procedures, including cognitive tasks and HIIT and LITT bouts. Participants will first sign an informed study consent (online supplemental material 2). The consent includes provisions for the deidentified data for use in future studies. A trained researcher will assess their resting BP to verify that SBP/DBP is <200/110 mm Hg, which is a counterindication to exercise.[75] Participants will then practise two LIIT and two HIIT bouts every 25 min. During each 25 min block, they will complete a questionnaire battery and practice cognitive tasks described in detail under the intervention section.

LIIT bouts

Each LIIT bout will last 6 min and comprise a 1 min warm-up (cycling at 50 rpm with no resistance), followed by two low-intensity intervals, cycling at 57%–60% of their maximum HR lasting 2 min and separated by a 1 min passive recovery (sitting on a cycle ergometer). Research assistants will continuously monitor participants' HR and prompt the participants to adjust speed to elicit the prescribed exercise intensity. RPE ratings will be collected every minute.

HIIT bouts

Each HIIT bout will last 6 min and comprise a 1 min warm-up (cycling at 50 rpm with no resistance), followed by two high-intensity intervals separated by a 1 min passive recovery (sitting on a cycle ergometer). High-intensity intervals comprise cycling for 2 min at, on average, 90% of the participant's individual HR_{max} established during the maximal exercise test on the same cycle ergometer. The workload and speed will be continuously adjusted by a trained researcher to reach the 90% HR_{max}. Participants' HR will be continuously monitored by the research staff in response to exercise and 2 min after exercise to confirm the drop in HR of at least 22 beats per minute, which indicates a normal HR response after exercise.[87 88] Participant's BP is also monitored 6 min after each bout of exercise to ensure that resting BP does not exceed the <200/110 mm Hg threshold.[75] Between HIIT and LIIT bouts, participants will practice cognitive tasks (described in the intervention section) and complete questionnaires.

Cognitive task practice

Participants will complete two cognitive tasks of attentional control (a modified Eriksen flanker task and the antisaccade task) during baseline to minimise practice effects observed in previous studies.[89 90] The Mnemonic Similarity Task (MST) uses two parallel versions to control for practice effects. The cognitive tasks are described in detail in the intervention section.

Psychosocial questionnaires

A battery of questionnaires will be administered to provide a descriptive characterisation of the study sample in relation to their habitual leisure-time exercise, types of sedentary behaviours they engaged in and their habitual cognitive activities (table 2). In addition, data on sleep quality and sleeping habits will be collected. All these factors are related to cognitive and brain functions and will provide contextual descriptive information for the study sample.

Physical function questionnaires

The data on physical function, physical function self-efficacy, gait and disability will be collected to provide important characteristics of the study sample to contextualise the feasibility and acceptability data from this pilot trial.

Accelerometry

At the end of the baseline visit, participants will be provided with two activity monitors: an activPAL 4 micro (PAL Technologies, Glasgow, Scotland) to wear on their right thighs to monitor sitting and sit-to-stand transitions, and a GT9XLink (ActiGraph, Pensacola, Florida, USA) to wear on their wrists, which monitor PA and sleep continuously 24/7 over 1 week. Both devices record raw acceleration from tri-axial accelerometers. The activPAL uses accelerometer-derived information about thigh position and acceleration to determine body posture. It provides information on sitting/lying down time, sit-to-stand transitions, sedentary patterning (bouts and breaks) and stepping cadence. The raw acceleration recorded by the GT9XLink is translated to average acceleration, energy expenditure, steps and PA intensities used to estimate ST, light, moderate and vigorous PA (min/day). The device also measures sleep latency, efficiency, and total sleep time. In addition, participants will keep a sleep diary to record times in and out of bed, sleep and wake-up times and complete a 24-hour PA recall for the day preceding each intervention condition.[91] The information from

accelerometers will be used as exploratory covariates in participants' responses to the intervention. Participants will also wear the devices for 1 week preceding the second intervention day. The data from both weeks will be compared to assess consistency in free-living physical behaviours between intervention visits.

Intervention visits

Figure 1 illustrates an experimental design. At each experimental visit, participants will engage in the same protocol except for the intensity of PA. Participants are asked to come to the laboratory after an overnight fast. At the beginning of the visit, participants are outfitted with a chest HR monitor, an accelerometer and an activPAL to monitor intervention fidelity. On coming to the laboratory, participants are outfitted with a waist-worn GT9XLink accelerometer, an activPAL and an HR monitor and asked to sit quietly for 5 min. After the rest, their resting HR and BP are collected to verify that SBP/DBP is <200/110 mm Hg. Participants are then fitted with an EEG cap. During the cap preparation, participants are provided with a light, standardised meal calibrated to their recommended caloric intake based on MyPlate (https://www.myplate.gov/) recommendations specific to age group (40–50, 50–60, 60–70 and 70–75), gender and BMI and accounting for 22% of their recommended daily energy intake.[92 93] After breakfast, they complete a 24-hour PA recall (Activities Completed over Time in 24 Hours),[94] Karolinska Sleepiness Scale[95] and their HR and BP are measured. The PA recall is collected to confirm compliance with not engaging in high-intensity exercise 24 hours before the visit. Karolinska Sleepiness Scale will be used to explore any differences in intervention effects based on self-perceived levels of sleepiness. Then, participants will begin a 6 min rest while the EEG signal is collected. After the resting state EEG data collection, they will complete three cognitive tasks in a randomised order while the EEG signal is simultaneously collected. Then, participants will complete one intervention at each visit. Each intervention comprises a 3.5-hour sitting time interrupted every 30 min with a 6 min interval training bout of light (LIIT, a control condition) or high intensity (HIIT, an active condition). The same specifications for LIIT and HIIT bouts are used as during the baseline visit. Participants will complete five bouts per intervention, totaling 30 min of LIIT or HIIT, depending on the condition. The order of the intervention conditions will be randomised across participants such that each participant will serve as his/her own control. HR and BP are monitored and recorded two and 6 min after each break, respectively, to ensure that participants show a normal physiological response to exercise. Participants will also complete one a modified Eriksen flanker task with simultaneous EEG recordings twice during a 3.5-hour sitting, 15 min after the first and the third PA bout (figure 1), to assess the acute and cumulative effects of HIIT versus LIIT bouts on cognitive and brain function. In the last 15 min of sitting, participants will receive another standardised meal identical to the one received at the pretest. After they consume the meal, participants engage in the exact same neurocognitive assessments as during the pretest. After neurocognitive assessments in experimental visit 1, participants will receive two activity monitors to wear for a week preceding the second intervention visit. Participants complete two study surveys designed to assess intervention acceptability at the end of the second intervention visit.

Sedentary activities

During the 3.5-hour sitting, participants sit continuously except for HIIT/LIIT bouts and bathroom breaks. Participants are transported to the bathroom in a wheelchair. The frequency and duration of bathroom breaks are recorded. Participants sit at a table with a laptop in the same testing room as the cycle ergometer. They will complete a standardised set of home administrative tasks

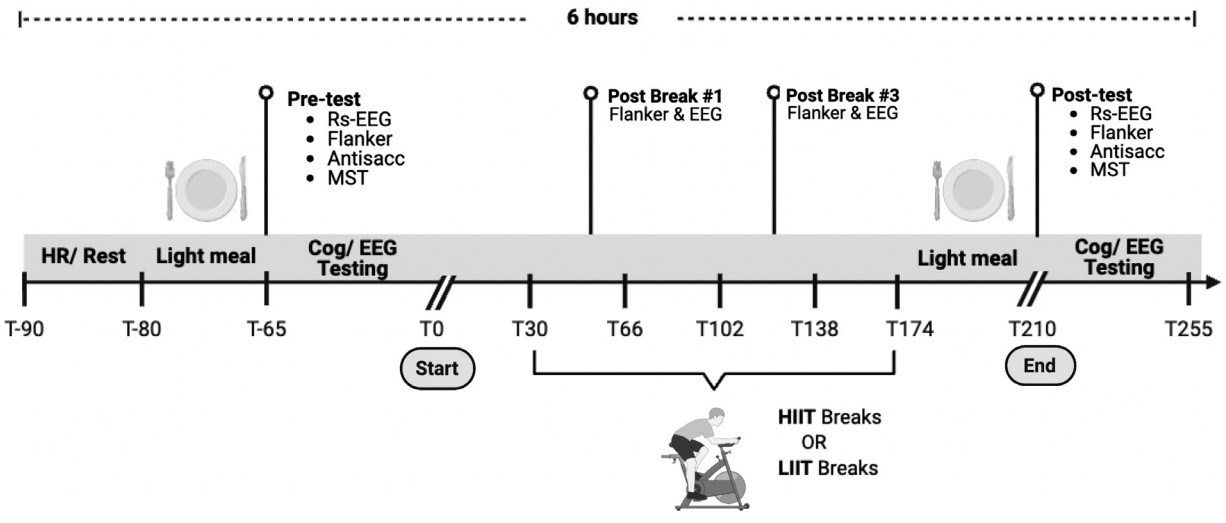

Figure 1 Study design. EEG, electroencephalogram; HIIT, high-intensity interval training; HR, heart rate; LIIT, low-intensity interval training; MST, Mnemonic Similarity Task. Created in: https://BioRender.com

(eg, planning a holiday, a birthday party) and read a standardised set of popular science articles from the New York Times. Activities will change every 30 min. Two sets of sedentary activities were developed, and their order was randomised across participants. To control for cognitive and emotional arousal, participants are asked not to use their electronic devices during the intervention. Participants are provided with plain water to drink during the 3.5-hour sitting but no food except for the two standardised meals to control for energy intake.

Mental effort, cognitive engagement and fatigue

To monitor participants' cognitive engagement and subjective task difficulty, they will fill in Task Engagement[96–98] and Cognitive Effort[99] scales before each HIIT or LIIT bout. These measures were included to control for cognitive stimulation during sedentary activities. To monitor participants' psychological arousal, we will measure the levels of perceived fatigue and vigour, they will self-report their energy, vigour and fatigue on a validated Visual Analogue Fatigue Scale before every break.[100] We will also monitor participants' perceived enjoyment of PA during each condition with Physical Activity Enjoyment Scale to inform intervention acceptability.[101]

Cognitive tasks

Modified Eriksen Flanker task

Inhibitory control is measured using a modified Eriksen flanker task before, after and twice during 3-hour sitting.[102] The modified Eriksen flanker task provides a measure of attentional control (an aspect of inhibitory control) by introducing a perceptual and response conflict. Participants are presented with a row of five 3 cm tall arrowheads appearing in the centre of the computer screen on a black background. A participant is required to respond to the directionality of the middle arrowhead, flanked by arrowheads pointing either in the same (congruent trials) or the opposite direction (incongruent trials). Incongruent flankers introduce a perceptual conflict that must be overcome to respond correctly. Congruency and directionality are random and equiprobable. Stimuli are presented for 83 ms, followed by a 1000 ms response window and a jittered inter-trial interval (ITI) of 1100, 1300 and 1500 ms. Participants will complete two blocks of 100 trials. Behavioural measures of reaction time (RT), RT variability and accuracy for each task condition will be used as secondary outcomes. This task is sensitive to modulation with acute exercise.[44] In addition, the P3b component measured during this task has shown reliable responses to a single bout of acute exercise.[103] Participants complete this task before, after and twice during the intervention (figure 1).

Antisaccade task

The antisaccade task is an accuracy-based measure of attentional control and was chosen as a complementary cognitive measure to the RT-based Eriksen flanker task. It also provides a psychometrically superior evaluation of attentional control.[104] Participants first fixate on a crosshair. Next, a tone signals the beginning of the trial. An asterisk appears to either the left or right of the crosshair, followed by a letter Q or O displayed opposite to the asterisk side. The participant has to look away from the asterisk in the direction of the letter. Then, the letter is masked. The participant must identify which letter (O or Q) appeared with a corresponding button press. The number of correctly identified letters is the secondary outcome. Participants complete 2 blocks of 76 trials with set ITI to 5000 ms and varied fixation time (1000, 2000 ms). Participants complete this task before and after each intervention.

Mnemonic Similarity Task

Episodic memory is measured with a computerised MST.[105 106] Performance on this task is a good marker of hippocampal function[105] and is sensitive to the acute effects of PA in older adults.[107] An encoding phase will be administered first. Participants study 64 coloured pictures of common objects, one at a time, for 2.0 s each with 0.5 s interstimulus interval. They then indicate whether the object was an 'indoor' or 'outdoor' item. An immediate retrieval phase follows, comprising repeats, lures (similar but new objects), and new objects. Participants will indicate if objects are old or new.[108] They complete a set of 192 objects. A lure discrimination index (probability of 'similar'/'novel' judgements in response to a lure) is another secondary outcome. Participants complete the MST task before and after 3.5-hour sitting on each intervention day.

Electroencephalogram

One of the primary outcomes of the HIIT2SITLess study is to test the effects of HIIT interruptions to prolonged sitting on the P3b-ERP component during an inhibitory control modified flanker task. Accordingly, participants are fitted with an EEG cap throughout the intervention to measure the EEG signal before and after each 3.5-hour sitting time. The EEG is recorded during a 6 min rest at pretest and post-test, followed by EEG recordings simultaneous with cognitive tasks. In addition, the EEG is recorded while the flanker task is completed twice during the 3.5-hour sitting. The EEG is measured using a 64-electrode Quik-Cap Neo Net (Compumedics, Charlotte, North Carolina, USA) with four integrated bipolar electrodes for vertical and horizontal eye movements, arranged according to the 10–10 system.

Neurofunctional measures

The P3b: The main aim of the HIIT2SITLess study is to assess the effects of HIIT interruptions to sitting on the P3b component, which is an established marker of frontoparietal brain function embedded within the stimulus-locked ERP. Both the P3b amplitude and latency have been reliably modulated by acute exercise.[103] However, its responses to prolonged sitting in older adults have not yet been investigated. Accordingly, the P3b-ERP component

will be measured during the flanker task at four time points (before, after and twice during each intervention) and twice during the antisaccade task (before and after the intervention).

N2 and error-related negativity

The effects of the intervention on other ERP components related to cognitive control will also be explored.[109] Cognitive control can be defined as a set of mental operations implicated in selection, scheduling and coordinating information processes involved in attention, memory and action in service of a goal.[110] Attentional control is part of the cognitive control system.[111] The stimulus-locked N2-ERP component[109] is thought to represent conflict processing. The N2 is a small negative-going component, which appears within 200–350 ms following stimulus onset and reaches a maximum over the frontal Fz and FCz electrodes.[109] Larger N2 amplitudes have been observed with successful conflict resolution and fewer commission errors.[112] This ERP component has been modulated by a single bout of sitting lasting 20 min in preadolescents such that a more negative N2 amplitude was observed during the flanker task (suggesting greater conflict) after a bout of sitting compared with a bout of moderate-intensity walking.[113] The error-related negativity (ERN) is a response-locked negative-going component that often appears in response to commission errors and is considered a marker of conflict monitoring mediated by the dorsal portion of the anterior cingulate cortex.[114] The ERN can be modulated by acute exercise,[115] but its response to prolonged sitting has not been investigated. Accordingly, neurofunctional responses underlying inhibitory control, which include conflict monitoring, are measured in the HIIT2SITLess study.

Frontal N400 (FN400) and late positive component

The HIIT2SITLess study will also explore the neuroelectric correlates of pattern separation (a measure of episodic memory) using the MST. Specifically, the study will explore the intervention effects on the difference waveforms in response to old and new items (an old-new effect) presented during the MST in the ERP components studied in the context of familiarity[116–118] and recollection.[119 120] For example, the anterior-central negative-going FN400 component appears approximately 400 ms after stimulus onset over frontal electrodes. The positive-going late positive component (LPC) appears posteriorly approximately 600 ms after stimulus onset.[121] Anterior-central FN400 is thought to index familiarity judgments because it varies with self-reported recognition confidence ratings.[116] In contrast, the parietal LPC is thought to index recollection because its amplitude varies with an individual's ability to identify a source of memory[120] but not with their recognition confidence.[116] Correctly identified lure items in the MST are thought to represent pattern separation, the process that reduces overlap between memory representations. This process is involved in memory recollection. In contrast, incorrectly identifying a similar item as old (lure false alarms in the MST) is thought to index pattern completion, which can rely on partial or degraded memory traces for memory retrieval, akin to recognition memory. The amplitudes of the FN400 and LPC components will be examined in response to correctly identified lures and lure false alarms during the MST.

Directional connectivity

In addition to ERPs, this study will explore changes in FC patterns during rest and task engagement in response to the HIIT interruptions to sitting. We will reconstruct cortical sources and estimate non-directional and directional (ie, effective) FC in high temporal resolution,[122 123] using the weighted Minimum Norm Estimation, a gold standard of source reconstruction, together with the Directed Transfer Function,[124 125] a technique that uses multivariate autoregressive modelling to estimate network dynamics over time. The effects of the intervention on FC between the regions of the FPN and the DMN at rest and during the flanker and the MST tasks will be explored. These networks have been chosen because FC in these networks declines with age,[126 127] but a single bout of PA can strengthen FC in both networks.[128] FC in other cognitive networks will also be explored.

End-of-study questionnaire

The HIIT2SITLess study survey

An 18-item survey developed by researchers specifically for the study. The survey includes 12 questions with answers on a 7-point Likert scale asking participants to evaluate the time commitment required for the study, engagement in HIIT and LIIT, EEG and cognitive assessments, sitting duration and sedentary behaviours that participants engaged in during the intervention. The survey also includes six open-ended questions asking participants for an explanation of their ratings and any additional comments.

The HIIT breaks survey

A 24-item survey developed specifically for the study. The survey includes 14 items measured on a 7-point Likert scale and ten open-ended items asking about the participant's experience with the HIIT breaks, including the dose (ie, duration, frequency, intensity) and how they compare to a single bout of moderate-intensity exercise. To evaluate the potential feasibility of participants adopting similar HIIT breaks at home, four questions focus on the likelihood of adopting such breaks. The remaining open-ended questions ask about participants' preferences for the type of exercise, duration, intensity and frequency.

Follow-ups

In addition to monitoring for adverse events by research staff during the study visits, adverse events will be monitored for 30 days immediately following the last intervention day. A study coordinator will call a week after the intervention and approximately 30 days after the study.

Table 3 Feasibility, missingness, fidelity and acceptability outcomes

	Description
Feasibility	
Recruitment rates	*N* randomised/*N* screened[5]
Retention rates	*N* randomised who successfully completed all conditions/*N* randomised[5]
Cognitive and EEG data	% of participants with fully completed preintervention and postintervention EEG recordings with >50% of valid, correct trials for each task and task condition.
Fidelity	
HIIT	% of high-intensity intervals at 90% HR_{max}
Sitting	% of time spent sitting during each condition (out of 180 min)
Acceptability	
Time commitment	Number and length of visits: 1-Unacceptable; 7-Fully acceptable, Number of dropouts due to time commitment.
HIIT	Duration; (2) Intensity; (3) Frequency; (4) cycling 1-Not acceptable, would not implement at home; to 7-Fully acceptable and would implement at home.
EEG/cognitive measures	1-Unacceptable; 7-Fully acceptable, Number of dropouts due to EEG measurements.

EEG, electroencephalogram; HIIT, high-intensity interval training.

If an adverse event is recorded, the research coordinator will follow up with a participant until the event is resolved.

Primary outcomes

Our primary outcomes for feasibility, fidelity and acceptability of the intervention are listed in table 3. The primary outcomes related to intervention effects on brain function are the change in task-evoked brain activity. Specifically, the amplitude and the latency of the P3b difference during the modified Eriksen flanker task over four measurements at the pretest, after the first and third PA bout, and at the post-test. We will use the area under the curve (AUC) to measure change.

Secondary outcomes

Our secondary outcomes related to aim one focus on safety. We will measure the frequency of serious adverse events and moderate severity adverse events. An adverse event in the HIIT2SITLess trial is defined as any occurrence of an undesirable and unintended, but not necessarily unexpected, result of the HIIT or LIIT intervention or study procedures. A moderate adverse event results in a low level of inconvenience or concern with the intervention or study procedures and may cause some interference with functioning. An example of a moderate adverse event is chest pain or injury with no fracture. A serious adverse event in the HIIT2SITLess trial is defined as an event that may be harmful to the participant and/or serious enough to warrant discontinuing the study due to its intolerability or potential harm to the participant. Any adverse event that meets the standard criteria outlined in the Code of Federal Regulations (21CFR 312.32) will be classified as a serious adverse event.[129] The research staff are trained on expected adverse events such as muscle soreness due to high-intensity exercise, mild discomfort or bruising due to the use of a lancet device. The staff records these events on an adverse events form. In addition, the study physician observes for any adverse events during the graded maximal exercise test. The study staff monitors participants' responses to exercise and observes for signs and symptoms of hypoglycaemia throughout each intervention session. A description of the event is recorded by attending staff and reviewed and classified by the PI.

The secondary outcomes related to aim 3 include the differences between conditions in:

1. Behavioural responses during the modified Eriksen flanker task.
2. Behavioural responses during the antisaccade task.
3. Behavioural responses during the MST task.
4. The amplitude and the latency of the N2-ERP component during the flanker task.
5. The amplitude and the latency of the P3b-ERP component during the antisaccade task.
6. The amplitude and the latency of the N2-ERP component during the antisaccade task.

All secondary outcomes will be measured at pretest and post-test except for behavioural and neuroelectric measures from the modified Eriksen flanker task, which are measured at four time points (before, after and twice during each intervention).

Exploratory outcomes

Exploratory outcomes related to aim 3 include:

1. The amplitude and the latency of the ERN component during the flanker task.

2. The amplitude and the latency of the ERN component during the antisaccade task.
3. The amplitude and the latency of the FN400 component during the MST task.
4. The amplitude and the latency of the LPC component during the MST task.
5. FC within the FPN.
6. FC within the DMN.
7. FC in other than FPN and DMN canonical brain networks.

Data monitoring

Access to person-identifiable information is restricted to a research coordinator, a graduate student and study technicians. Identifiable information is kept separate from the data and maintained on REDCap, a secure web application (capable of compliance with the Health Insurance Portability and Accountability Act, HIPPA) for building and maintaining study infrastructure, including surveys, collecting informed consent and building databases. Part of the identifiable information (medical clearance) is maintained on the HIPPA-compliant cloud storage service Box for Protected Health Information. All research records will be retained for 6 years on completion of the study based on the HIPAA (45 CFR 164.530(j)). Data quality is promoted by staff training, and data completeness is verified by a senior team leader at the end of each session. REDCap also provides outcome-specific range restrictions as an additional data quality check. Protocol amendments are listed in online supplemental table 4. Any further amendments will be approved by the IRB at the University of Illinois Urbana-Champaign. The Data Safety and Monitoring Board was deemed unnecessary due to the small scale of the trial. The trial receives safety oversight from an independent Safety Officer appointed by the National Institute on Aging.

Sample size determination

The study was powered for the effect of HIIT versus LIIT bouts on the pre-to-post-condition change in the P3b amplitude on a working memory task (which relies on attentional control) based on two dependent sample t-tests using G*Power V.3.1.[130] Our target sample of 42 adults is based on an acute effect of a single HIIT bout on the P3b amplitude relative to baseline in middle-aged and older adults reported by Tsai *et al*.[131] Using a one-tail test, an α=0.05, we will have 91% power to detect an effect size with Cohen's d=0.5 on preintervention to postintervention comparison. Kamijo *et al*[132] reported Cohen's d=1.2 for the P3b latency in older adults using a single 20 min bout of moderate intensity (expected to increase peripheral catecholamines).[65 133] We will have 80% power to detect an effect of d=0.78 based on independent samples t-test (one-tailed) comparisons. To account for 20% attrition, we will recruit 54 older adults to the study.

Statistical analyses

Missing data

We will verify whether the collected data meet the missing completely at random (MCAR) assumption using Little's test of MCAR. If this assumption holds, to account for the missing data,[134] we will fit general linear mixed-effects models.[135] (A violation of the MCAR assumption will prompt an investigation to identify its causes and appropriate statistical solutions.) Mixed-effects models assume that data are missing at random and implicitly account for the missing values by modelling weighted averages of condition effects, one for complete cases and one for singletons. To allow for the intention-to-treat analysis, we will estimate sequence (two levels: X, Y), condition (two levels: HIIT Breaks, LIIT Breaks) and time (four levels: prebreak, after break #1, after break #3 and post-test), and the two-way and three-way interactions between these factors; no interim analyses will be performed. If the analyses suggest no sequence interaction with time and condition, we will estimate the two-way condition by time interactions. All analyses of primary outcomes for aim 2 will be conducted on pretest, after the first and the third PA bout, and post-test assessments.[135] We will test intervention effects on secondary outcomes using the pretest and post-test assessments. We will also explore the intervention effects on FC at rest and during task engagement and neurofunctional correlates of attentional control, pattern separation and completion using two time points: pretest and post-test. We will include each outcome as a response variable, sequence, condition and time as fixed effects and a participant-specific random intercept. Carryover (ie, period) effects are assumed to be null based on the sufficient washout period of 1 week between treatments.[136] Results will be presented as mean differences between conditions in the AUC with one-tailed 95% CI (primary outcome) and mean differences between conditions at post-test for secondary outcomes. We will present the results as the effect sizes (Cohen's d).[137]

Data sharing

During the research period, access to the data will be restricted to the researchers directly working on the project. Data that support the conclusions of the project published in peer-reviewed scientific journals will be made available to other researchers on request. Only deidentified data that support a published manuscript will be shared. All investigators involved in the development of the trial will be coauthors of any subsequent publications resulting from the trial.

Reporting guidelines

The study was designed in accordance with Standard Protocol Items: Recommendations for Interventional Trials, and its details are provided in online supplemental material.

Publication

Publication of the results of this trial will be submitted for consideration in peer-reviewed scientific journals and will be made available to participants on request.

Patient and public involvement

Patients or the public were not involved in developing this trial protocol.

Ethics and dissemination

The study has been approved by the IRB at the University of Illinois Urbana-Champaign (IRB24-0010). All participants provide informed written consent to screening procedures and separately to the study procedures. Participants are provided with a copy of the consent document before the screening and in-person visit to allow them time to review the information (online supplemental materials 1 and 2). The data collected from participants will be used for research purposes. Deidentified data can be used for future studies and training purposes.

DISCUSSION

The HIIT2SITLess randomised crossover pilot feasibility trial was designed to assess the feasibility of HIIT as brief interruptions to prolonged sitting to enhance cognitive and brain function in middle-aged and older adults. This is a carefully designed controlled study, where PA intensity is individually tailored and carefully monitored by the research staff. The study was designed based on a theoretical premise that brief but high-intensity PA breaks to prolonged sitting can enhance function by stimulating the cerebral norepinephrine system through the abdominal vagus nerve pathway.[56 57] In contrast, light-intensity PA may not yield such improvements due to too low intensity of short (< 10 min) PA bouts. We hypothesise that implementing 6 min HIIT interruptions to prolonged sitting every 30 min will be feasible and acceptable over the 3.5-hour period to middle-aged and older adults. We also hypothesise that HIIT interruptions to prolonged sitting will enhance frontoparietal function as indicated by greater P3b amplitude and shorter P3b latency of the incongruent-congruent difference waveform during the flanker task measuring inhibitory control. Furthermore, this pilot trial will allow us to explore the intervention effects on behavioural measures of inhibitory control and episodic memory and their neuroelectric correlates. The HIIT2SITLess trial goes beyond the ERP markers of brain function and seeks to explore the effects of interrupting prolonged sitting with HIIT bouts on FC in FPN and the DMN using directional FC measures. As such, this trial is the first of its kind to test the effectiveness and feasibility of HIIT as a means to reduce prolonged sitting in the population of highly sedentary adults at risk of age-related cognitive decline.

As with every trial, this trial has several limitations. Although built on a theoretical premise, the study is not designed to test the changes in central or peripheral norepinephrine to directly test this theory. However, the P3b-ERP component is considered an index of phasic shifts in the locus coeruleus activity,[138] the main source of cerebral norepinephrine, because the locus coeruleus can exert a neuromodulatory effect on the P3b through its efferent cortical projections,[43] which overlap with cortical generators of the P3b.[17–19] HIIT is physically demanding and, therefore, entails greater risk in individuals who are at an increased risk of cardiovascular disease and those with a cardiovascular disease history. Accordingly, participating individuals must have medical clearance and show no positive findings on the graded maximal exercise test. Our study is also not designed to test the differences in other than intensity components of the PA dose (ie, PA bout frequency and duration). Nonetheless, by including two neurofunctional measures after the first and the third PA bout, we will explore the cumulative benefit of three compared with a single bout of HIIT on brain function and inhibitory control. The outcomes from the HIIT2SITLess trial will inform mechanistic models (catecholamine-driven increase in phasic locus coeruleus activity) that may underpin the effectiveness of interrupting prolonged sitting with brief PA bouts on cognitive and brain functions. The feasibility outcomes will promote the clinical applications of interrupting prolonged sitting with HIIT in highly sedentary middle-aged and older populations. The results from this study can be used to support the development of chronic interventions to test the effectiveness of reducing prolonged sitting with HIIT on brain function, structure and the underlying biological mechanisms.

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