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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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 aging. Physical activity (PA) interventions can enhance FC in these networks, but these interventions are not designed to decrease ST among older adults. Prolonged sitting (i.e., sitting continuously for ≥ 20 minutes) can acutely reduce frontoparietal brain function and attentional control, while a single PA bout lasting at least 20 minutes can enhance them. It has been theorized that stimulation of the cerebral norepinephrine release through peripheral increase in catecholamines may explain this effect. In contrast, the effects of shorter (< 10 minutes) PA bouts used to interrupt prolonged sitting on neurocognitive functions remain poorly understood. This pilot randomized crossover feasibility trial capitalizes on PA intensity as the major limiting factor in peripheral catecholamine increase and tests the effects of interrupting prolonged sitting every 30 minutes with 6-minute high-intensity interval training (HIIT) compared to low-intensity interval training (LIIT) bouts. The study will address three aims: (i) to assess feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function in middle-aged and older adults; (ii) 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); (iii) 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: Fifty-four 40-75-year-old healthy adults will be recruited from the local community and randomly assigned to a condition sequence (HIIT, LIIT versus LIIT, HIIT). Each HIIT bout comprises a 1-minute warm-up, 2 minutes at 90% of the maximum heart rate (HR_{max}), 1-minute passive rest, and 2 minutes at 90% HR_{max}. During 2-minute intervals in LIIT, participants exercise at 57-60% of HR_{max}. The primary outcomes include the feasibility (recruitment and retention rates, percent of valid EEG data), acceptability of time commitment, HIIT bouts and neurocognitive assessments, fidelity (the intensity of HIIT breaks, percent of time spent sitting) and the amplitude and the latency of the P3b component of event-related brain potentials (ERPs) measured during the modified Eriksen flanker task at pre-tests, 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.

Clinical Trial Registration: ClinicalTrials.gov No. NCT06243016

Keywords: sedentary behavior, high-intensity interval training, cognitive functions, brain function, middle age, aging

Article Summary

Strengths and limitations

- The HIIT2SITLess study is a well-controlled randomized 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.
- This is the first trial to test the acute effects of high-intensity interval training as a means of interrupting prolonged sitting and improving brain function in middle-aged to older adults (40 to 75 years old).
- 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.
- This pilot feasibility trial recruits healthy middle-aged and older adults with a limited cardiovascular risk; hence, its generalizability 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 aging 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, characterized by a decreased ability to control distractions and correctly recall the details of information and events (i.e., 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 aging.

Yet, effective and scalable interventions to improve cognitive and brain health in older adults are lacking. Traditional physical activity (PA) interventions (e.g., a 20-40-minute 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 minutes.[8] In contrast, the efficacy of PA interventions that utilize short (<10 minutes) 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 sedentary time and prolonged sitting, such as sitting continuously for 20 minutes or longer, may attenuate attentional control,[12,13] episodic memory,[14] and frontoparietal function.[15] For example, 21-45-year-old adults with more prolonged sedentary time 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 minutes, 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 electroencephalographic (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 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 aging is the 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- 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 aging. 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 optimizes 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 aging[52] and

AD.[53] Thus, PA interventions designed to stimulate the LC-NE system could significantly impact the functional integrity of the aging brain.

High-intensity interval training (HIIT) could stimulate the LC-NE system because it utilizes 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-minute delay.[58,59] However, the effect of a single bout of HIIT on cognitive function declines after 20-30 minutes.[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 minutes) 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 minutes) 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 (i.e., light or moderate) to stimulate the LC-NE system within 2-5 minutes.[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 randomized crossover pilot feasibility trial designed to test three specific aims:

(i) To assess the feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function.

(ii) To quantify the differences between conditions in a change in P3b amplitude and latency, a marker of frontoparietal function.

(iii) 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 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 default mode networks.

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 randomized crossover trial with two interventions lasting 3.5 hours each: prolonged sitting interrupted every 30 minutes with 6-minute HIIT bouts active condition and prolonged sitting interrupted every 30 minutes with 6-minute LIIT bouts control condition. The randomized crossover trial design was chosen for its efficiency in minimizing inter-individual variability to allow for a smaller sample size. The study is conducted over three consecutive visits. The participants will be recruited to the trial between February 2024 and March 2026. All participants provide written informed consent in accordance with the Institutional Review Board at the University of Illinois Urbana-Champaign (see Supplementary Table 1 for sponsor details).

Trial registration

The trial was registered on ClinicalTrials.gov No. NCT06243016 before the enrollment of the first participant. See Supplementary Table 2 for trial registration details.

Participants

The study will enroll 54 (27 female) middle-aged (40-59 years) and older (60-75 years) cognitively healthy adults from Champaign County, IL. 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.[7,73]

Eligibility

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

Blinding and randomization

Fifty-four participants will be randomized to two condition sequences by a statistician following baseline assessments. Permuted block randomization generated using the PROC PLAN procedure (SAS Institute Inc., 2023)[74] is used, where sequences are randomized within a block of six participants to minimize the possibility of group imbalances due to dropout. Participants are randomized to one of two condition sequences by a study statistician: 1) X = HIIT, LIIT breaks or Y = LIIT, HIIT breaks. Generated permuted block randomization also ensures that blocks are balanced by cognitive task (i.e., flanker, antisaccade [A], and mnemonic similarities task [M]) sequence (FAM, MFA, and AMF). The principal and co-investigators will be blinded to the sequence allocation. The sequence will be concealed until the participant's enrollment. Upon enrollment, the study sequence will be verbally communicated to the study coordinator by a statistician. Verbal communication will be video recorded. The coordinator will record the sequence number in REDCap. The trial staff, except for the principal investigator and co-investigators, will be unblinded. Participants will be blinded as to the intervention order until their first intervention visit. The PI will only be unblinded to the participant's study sequence in case of a serious adverse event or a severe AE that requires hospitalization.

Recruitment and retention

Recruitment of participants began in February 2024 with the goal of completing enrollment by June 2025. Our goal is to recruit a sample approximating 67% Caucasians (White, not Latino or Hispanic), 14% African Americans, 11% Asians, 0.4% American Indian or Alaska Native, and 6% Hispanics representative of racial and ethnic distribution in Champaign County, IL. 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, organizations serving older adults in Champaign County, and flyers. Study invitations are also mailed to individual home addresses of adults aged 40-75 years in Champaign County. In addition, the research team will give talks at local events such as Walk with a Doc, and local community meetings. Recruitment and enrollment occur continuously. To maximize compliance and retention, we will schedule baseline and intervention visits once a participant qualifies. 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 Table 2.

Screening Procedure

Screening Call

At the beginning of the screening call, participants will sign an informed consent to the screening process (Supplementary 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 ischemic attack, long COVID-19, and smokers. A trained researcher will then administer the Telephone Interview of Cognitive Status-modified (TICS-m). Only individuals with a score < 32 (a cutoff for mild cognitive impairment)[75] 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,[76] and pre-existing conditions as listed in the exclusion criteria (Table 1). An individual will also fill in the Hospital Anxiety and Depression Scale.[77] Individuals with anxiety and depression will be included due to the high prevalence of these disorders in the general

population.[78,79] 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 (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80] screens out highly physically active individuals who engage in 300 minutes or more of moderate-to-vigorous PA per week. The Physical Activity Readiness Questionnaire+[81] is used to identify individuals who may be at a greater risk of participating in high-intensity exercise. Table 3 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 visit 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: (i) exercise strenuously for 48 hours before the experimental visit, (ii) drink caffeine or (iii) 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 mmHg on the day (higher values are a contraindication to a maximal exercise test)[76] with confirmed normotensive BP by their physician. 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.[82] If the participant's physician cannot confirm fasting glucose levels or glycated hemoglobin levels (HbA1c) 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 (MoCA) to screen out individuals with scores < 26 suggestive of potential cognitive impairment scoring.[83] A

standardized test of cognitive abilities (Kaufman Brief Intelligence Test – 2; KBIT-2)[80] will be administered next, and individuals with a score < 85 (i.e., 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 3).

Cardiorespiratory fitness testing

Participants will undergo a maximal exercise test on a cycle ergometer (Excalibur, Lode, Groningen, the Netherlands) using a modified Astrand protocol[84,85] with a 12-lead electrocardiogram. 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 to evaluate participants' physiological responses to exercise.[76] Its results will be used as an inclusion criterion to enhance the safety of acute high-intensity exercise. Three trained first aid and CPR-certified experimenters will conduct the test in collaboration with the study physician. Participants' resting BP, HR, and electrocardiogram readings will be collected. They will then warm up for two minutes while pedaling 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 minutes by 25 Watts. Males will start at 100 Watts and exercise at 50 Watts increments.[84,85] The participant will cycle until volitional exhaustion.[84,85] Their HR and ECG are continuously monitored, and blood pressure will be monitored every two minutes during exercise by a physician. Every two minutes, the study staff will record ratings of perceived exertion (RPE) using the Borg scale.[86] 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[76]: (i) respiratory exchange ratio (RER) ≥ 1.1, (ii) failure of the HR to increase with increasing workload (i.e., ≤ 10 bpm increase relative to age-predicted HR_{max},[87]) or (iii) RPE > 17. The test finishes with a 5-minute cooldown. If there are no positive findings on the electrocardiogram as described in the indications to stopping the maximal exercise test in the ACSM's Guidelines for Exercise Testing and Prescription,[76] 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 familiarize participants with the main intervention procedures, including cognitive tasks and HIIT and LITT bouts. Participants will first sign an informed study consent (Supplementary Material 2). The consent includes provisions for the de-identified data for use in future studies. A trained researcher will assess their resting BP to verify that systolic over diastolic BP is < 200/110 mmHg, which is a counterindication to exercise.[76] Participants will then practice two LIIT and two HIIT bouts every 25 minutes. During each 25-minute block, they will complete a questionnaire battery and practice cognitive tasks described in detail under the intervention section.

Light-intensity-interval-training bouts (LIIT). Each LIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two low-intensity intervals, cycling at 57-60% of their maximum HR lasting two minutes and separated by a one-minute 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.

High-intensity interval-training bouts (HIIT). Each HIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two high-intensity intervals separated by a one-minute passive recovery (sitting on a cycle ergometer). High-intensity intervals comprise cycling for two minutes 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}. Participant's HR will be continuously monitored by the research staff in response to exercise and two minutes after exercise to confirm the drop in HR of at least 22 beats per minute, which indicates a normal HR response after exercise.[88,89] Participant's BP is also monitored 6 minutes after each bout of exercise to ensure that resting BP does not exceed the <200/110 mmHg threshold.[76] 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 minimize practice effects observed in

previous studies.[90,91] The mnemonic similarity task 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 characterization of the study sample in relation to their habitual leisure-time exercise, types of sedentary behaviors they engaged in, and their habitual cognitive activities (Table 3). 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 contextualize 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, FL) to wear on their wrists, which monitor PA and sleep continuously 24/7 over one 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.[92] The information from accelerometers will be used as exploratory covariates in participants' responses to the intervention. Participants will also wear the devices for one week preceding the second intervention day. The data from both weeks will be compared to assess consistency in free-living physical behaviors 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. Upon 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 minutes. After the rest, their resting HR and BP are collected to verify that SBP/DBP is <200/110 mmHg. Participants are then fitted with an EEG cap. During the cap preparation, participants are provided with a light, standardized 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, 70-75), gender and BMI and accounting for 22% of their recommended daily energy intake.[93,94] After breakfast, they complete a 24-hour PA recall (Activities Completed over Time in 24 Hours; ACT24),[95] Karolinska Sleepiness Scale,[96] 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-minute rest while the EEG signal is collected. After the resting state EEG data collection, they will complete three cognitive tasks in a randomized 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 minutes with a 6-minute 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 minutes of LIIT or HIIT, depending on the condition. The order of the intervention conditions will be randomized across participants such that each participant will serve as his/her own control. HR and BP are monitored and recorded two and six minutes after each break, respectively, to ensure that participants show a normal physiological response to exercise. Participants will also complete a modified Eriksen flanker task with simultaneous EEG recordings twice during a 3.5-hour sitting, 15 minutes 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 minutes of sitting, participants will receive another standardized meal identical to the one

received at the pre-test. After they consume the meal, participants engage in the exact same neurocognitive assessments as during the pre-test. 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 standardized set of home administrative tasks (e.g., planning a holiday, a birthday party, etc.) and read a standardized set of popular science articles from the New York Times. Activities will change every 30 minutes. Two sets of sedentary activities were developed, and their order was randomized 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 standardized 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[97–99] and Cognitive Effort[100] 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 vigor, they will self-report their energy, vigor, and fatigue on a validated Visual Analogue Fatigue Scale before every break.[101] We will also monitor participants' perceived enjoyment of PA during each condition with Physical Activity Enjoyment Scale to inform intervention acceptability.[102]

Cognitive tasks

Modified Eriksen Flanker task. Inhibitory control is measured using a modified Eriksen flanker task before, after, and twice during 3-hour sitting.[103] The modified Eriksen flanker task provides a measure of attentional control by introducing a perceptual and response conflict.

Participants are presented with a row of five 3-cm tall arrowheads appearing in the center 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. 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.[104] 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.[105] 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 two 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 (MST). Episodic memory is measured with a computerized MST.[106,107] Performance on this task is a good marker of hippocampal function[106] and is sensitive to the acute effects of PA in older adults.[108] An encoding phase will be administered first. Participants study 64 colored 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.[109] They complete a set of 192 objects. A lure discrimination index (LDI; probability of “similar”/“novel” judgments 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 (EEG)

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-minute rest at pre-test 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, NC) with four integrated bipolar electrodes for vertical and horizontal eye movements (VEOG, HEOG), 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, 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.[104] 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 (ERN). The effects of the intervention on other ERP components related to cognitive control will also be explored.[110] 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.[111] Attentional control is part of the cognitive control system.[112] The stimulus-locked N2-ERP component[110] 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.[110] Larger N2 amplitudes have been observed with successful conflict resolution and fewer commission errors.[113] This ERP component has been modulated by a single bout of sitting lasting 20 minutes in preadolescents such that a more negative N2 amplitude was observed during the flanker task (suggesting greater conflict) after a bout of sitting compared to a bout of moderate-intensity walking.[114] The 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.[115] The ERN can be modulated by acute exercise,[116] but its response to prolonged sitting has not been investigated. Accordingly, neurofunctional responses underlying inhibitory control, which includes conflict monitoring, are measured in the HIIT2SITLess study.

Frontal N400 (FN400) and Late Positive Component (LPC). The HIIT2SITLess study will also explore the neuroelectric correlates of pattern separation (a measure of episodic memory). Specifically, the study will explore the intervention effects on the difference waveforms in response to old and new items (an old-new effect) in the ERP components studied in the context of familiarity[117–119] and recollection.[120,121] 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.[122] Anterior-central FN400 is thought to index familiarity judgments because it varies with self-reported recognition confidence ratings.[117] 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[121] but not with their recognition confidence.[117] Correctly identified lure items 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) 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.

Directional functional 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 (i.e., effective) FC in high temporal resolution,[123,124] using the Minimum Norm Estimation (MNE), a gold standard of source reconstruction, together with the Directed Transfer Function (DFT),[125,126] a technique that uses multivariate autoregressive modeling 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[127,128] but a single bout of PA can strengthen FC in both networks.[129] 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 behaviors 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 (i.e., 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. If an adverse event is recorded, the research coordinator will follow up with a participant until the event is resolved.

Discontinuation

If a participant's resting BP exceeds this threshold during any of the study visits, the participant will be excluded from the study. If a participant does not comply with study protocol (e.g., does not refrain from strenuous physical activity 24 h before the intervention visit, is unable or unwilling to complete specific components of the study such as maximal exercise test on the

treadmill, physical activity monitoring or LIIT or HIIT breaks). If a participant becomes hypoglycemic during GXT, baseline or intervention sessions.

Primary Outcomes

Our primary outcomes for feasibility, fidelity, and acceptability of the intervention are listed in Table 4. The primary outcomes related to intervention effects on brain function are the amplitude and 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 posttest. 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.[130] 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 participant's responses to exercise and observes for signs and symptoms of hypoglycemia 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) Behavioral responses during the modified Eriksen flanker task

(2) Behavioral responses during the antisaccade task

(3) Behavioral 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 pre-test and post-test except for behavioral 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 Research Electronic Data Capture (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 six years upon 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. Protocol amendments are listed in Supplementary Table 3. 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 vs. 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 version 3.1.[131] 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.[132] 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 pre- to post-intervention comparison. Kamijo et al.[133] reported Cohen's d = 1.2 for the P3b latency in older adults using a single 20-minute bout of moderate intensity (expected to increase peripheral catecholamines).[65,134] 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 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,[135] we will fit general linear mixed-effects models.[136] (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 modeling 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: pre-, after break #1, after break #3, and posttest), 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 pre-, after the first and the third PA bout, and post-test assessments.[136] 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 posttest. We will include each outcome as a response variable, sequence, condition, and time as fixed effects and a participant-specific random intercept. Carryover (i.e., period) effects are assumed to be null based on the sufficient washout period of one week between treatments.[137] Results will be presented as mean differences between conditions in the Area Under the Curve 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*).[138]

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 upon request. Only de-identified data that support a published manuscript will be shared. All investigators involved in the development of the trial will be co-authors of any subsequent publications resulting from the trial.

Reporting guidelines

The study was designed in accordance with Standard Protocol Items for clinical trials (SPIRIT Statement), and its details are provided in Table 2 and the SPIRIT checklist.

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 upon 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

(Supplementary Materials 1 and 2). The data collected from participants will be used for research purposes. De-identified data can be used for future studies and training purposes.

DISCUSSION

The HIIT2SITLess randomized 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 frontoparietal 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 minutes) PA bouts. We hypothesize that implementing 6-minute HIIT interruptions to prolonged sitting every 30 minutes will be feasible and acceptable over the 3.5-hour period to middle-aged and older adults. We also hypothesize 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 behavioral 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,[139] 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 (i.e., 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 to 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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Contributors

DMP: Conceptualization, data curation, methodology, funding acquisition, project administration, resources, supervision, writing original draft; CHH, AFK, NAK: conceptualization, methodology, funding acquisition, resources; MW, TSL: methodology, software; SP, JS, JS, MK: methodology; JK: visualization. All co-authors reviewed, edited, and approved the final draft.

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Supplemental material

Supplementary Materials are available online.

Open access

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 1. Study inclusion and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
• Age 40-75 years; including pre-, post-, and peri-menopausal women regardless of hormone therapy replacement.	• Physical disability or musculoskeletal disease prohibitive to vigorous exercise
• BMI <40 kg/m^2;	• Learning disabilities
• Sedentary (≥ 6 h/day sitting by a survey question);	• Cognitive abilities below a 26-point cut-off on a MoCA
• Physically inactive adults based on the CSEP-PATH: PHYSICAL ACTIVITY AND SEDENTARY BEHAVIOUR QUESTIONNAIRE (PASB-Q) Adult (low or medium physical activity range equivalent to less than 300 min of moderate intensity physical activity per week)	• Type 1 or 2 diabetes
• Capable of exercising vigorously based on the PARQ+	• Neurological condition (e.g., MS, Parkinson, Dementia, MCI)
• Has a medical clearance for maximal exercise and HIIT from a physician	• Color blindness
• Normotensive or participant's blood pressure is controlled (i.e., 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 (e.g., traumatic brain injury, stroke)

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

• Intellectual ability no less than one standard deviation relative to the population mean (i.e., ≥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 (see General Health History, Cardiovascular History)
• No significant abnormalities on the ECG during the maximal exercise test	• A chronic pulmonary disease (e.g., chronic obstructive pulmonary disease; COPD)
• 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

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

• 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 (e.g., attention-deficit hyperactivity disorder [ADHD], schizophrenia, etc.)
	• 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 Apnea
	• Acquired immunodeficiency syndrome (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

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

	• Anabolic androgens taken <3 days before screening
	• A serious illness or hospitalization in the last six 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

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 2. Schedule of study assessments

Assessment	Screening Phone Call (Day -37 to Day -7)	Screening visit (Day-23 to Day -4)	Baseline (Day 0) – Pre-allocation	Intervention Visit 1 Day 8-21 (±2 Days)	Intervention Visit 2 Day 15-36 (±2 Days)	Follow Up Day 17-43 (± 2 Days)
	ENROLMENT		**ALLOCATION (post-baseline)**	**INTERVENTION**		**FOLLOW-UP**
TIME POINT	**-t2**	**-t1**	**t0**	**t1**		
ELIGIBILITY SCREEN						
Screening Informed Consent Form	**x**					
Screening Questionnaire	**x**					
Health & Demographics Questionnaire	**x**					
General Health History Questionnaire incl. current medications	**x**					
IPAQ Short Form	**x**					
PARQ+	**x**					
Hospital Anxiety and Depression Scale	**x**					
Medical Clearance	**x**					
Blood Sample Fasting Glucose Analysis		**x**				

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Anthropometric Assessments		x	x			
Resting Heart Rate and Blood Pressure		x	x	x	x	
Montreal Cognitive Assessment		x				
Ohio State TBI Identification Interview Form		x				
Geriatric Depression Scale		x				
Beck Depression Inventory-2						
Florida Cognitive Activity Scale		x				
KBIT-2		x				
Inclusion/Exclusion Criteria	x	x				
Graded Maximal Exercise Test (GxT)		x				
Informed Study Consent Form			x			
ENROLLMENT			x			
Cognitive Tasks			x			
HIIT and LIIT Breaks Practice			x			
Borg Rating of Perceived Exertion Scale			x	x	x	
Feeling Scale			x	x	x	
Pittsburgh Sleep Quality Index (PSQI)			x			
Godin-Shephard Leisure Time Physical Activity Questionnaire			x			

Preference for Tolerance of the Intensity of Exercise Questionnaire			x			
FDI DIS Abbreviated FDI-Disability			x			
FDI FxN Abbreviated FxN- Function			x			
FxNSE Function Self Efficacy without a device			x			
Gait Efficacy Scale (GES)			x			
Sedentary Behavior Questionnaire (SBQ)			x			
SEQUENCE ALLOCATION			x			
INTERVENTIONS						
HIIT Breaks*				x		
LIIT Breaks*					x	
INTERVENTION ASSESSMENTS						
Physical Activity & Sitting Time Monitoring in Free-Living (7 d each)			x	x		
Heart rate Monitoring (during visits)			x	x	x	
Physical Activity Monitoring (during visits – ActiGraph GT9x Link)				x	x	
Sitting Time Monitoring (activPAL) (during visits)				x	x	

Epworth Sleeping Scale (ESS)				x	x	
ACT24 Physical Activity Recall				x	x	
Mental Effort Scale				x	x	
Task Engagement Scale				x		
Vigor and Fatigue Scale				x		
Cognitive Tasks				x		
EEG Recordings				x		
HIIT Breaks Surveys						
HIIT2SITLess Study Survey						
AEs		x	x	x		x
FOLLOW-UP						
Phone call						x

Note. ACT24: Activities Completed over Time in 24 Hours; AE: adverse event; HIIT: high-intensity interval training; IPAQ: International Physical Activity Questionnaire Short Form; KBIT-2: Kaufman Brief Intelligence Test 2; LIIT: low-intensity interval training; PARQ+: Physical Activity Readiness Questionnaire for Everyone; *The order of the interventions is randomized across participants.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 3. Psychosocial assessments

Name	Description
The Activities Collected over Time over 24-hours (ACT24)[140]	A 24-hour physical activity recall to measure participant's previous day physical activity, sedentary behaviors and sleep.
Beck Depression Inventory-2[141]	A 21-item inventory to assess attitudes and symptoms of depression in adults aged 18-80 years.
Cardiorespiratory Fitness Questionnaire[142]	A 5-item questionnaire to assess the level of aerobic fitness based on questions about habitual aerobic exercise.
The Epworth Sleepiness Scale[143]	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 FDI-Disability[144,145]	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 FXN- Function[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 Function Self Efficacy 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 assess 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[77]	A 14-item questionnaire with questions about symptoms of depression, anxiety and psychological distress during the past week.
The Canadian Society for Exercise Physiology (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80]	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[77]	A standardized and normed intelligence test for ages 4-90 years. The test comprises one verbal and two non-verbal components used to compute verbal and nonverbal IQ scores and a general IQ score.
Karolinska Sleepiness Scale (KSS)[96]	A one-item assessment of individual's subjective experience of sleepiness over the past 5 minutes.
Montreal Cognitive Assessment (MoCA)[83,150]	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[81]	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.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Pittsburgh Sleep Quality Index (PSQI)[81]	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 Behavior Questionnaire (SBQ)[152]	An 18-item questionnaire assessing the time individuals spent in various sedentary behaviors on weekdays and weekend days.
Task Engagement Scale[97–99]	A 9-item scale assessing the level of physical, emotional and cognitive engagement in a task.
Mental Effort Scale[100]	A single item scale assessing the level of mental effort exerted during the task.

Table 4. Feasibility, missingness, fidelity, and acceptability outcomes

	Description
FEASIBILITY	
Recruitment rates*	*N* randomized/*N* screened[5]
Retention rates*	*N* randomized who successfully completed all conditions/*N* randomized[5]
Cognitive and EEG data	% of participants with fully completed pre- and post-intervention 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,* 2. Number of dropouts due to time commitment.
HIIT	(1) 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.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Figure captions

Figure 1. Study design.

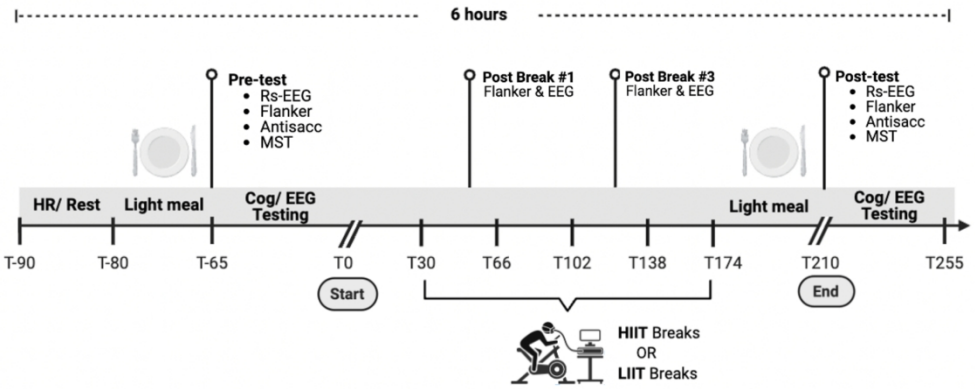

Figure 1. Study design.

238x98mm (300 x 300 DPI)

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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

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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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Dates of the study: 05/07/2024 – 07/31/2025

Supplementary Material 1. Screening Consent

Consent and Authorization Document

Consent to Screening Procedures

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT2SITLess TRIAL

You have indicated an interest in participating in a research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the utility of short physical activity breaks to sitting to reduce sitting and enhance cognitive and brain function in the short term (over several hours). This is a short-term study. If you qualify, you will be asked to visit our laboratory three times over approximately five to seven weeks and wear activity monitors for two weeks between visits.

KEY INFORMATION ABOUT THE SCREENING PROCESS

The screening procedures aim to assess your eligibility for the study. Today, a researcher will ask you questions about your age, physical activity, physical function, sitting habits, smoking, history of stroke or a transient ischemic attack, long COVID-19, your vision and hearing. The researcher will ask you questions and give you small tasks to measure how you think and how well you remember things. You will also complete a questionnaire about your general medical history, and questionnaires about your physical activity and how you feel. If you qualify based on this phone call, you will complete a medical clearance release form allowing the research team to contact your physician to determine if you can participate in high-intensity exercise. Today's call will last approximately 1 hour 15 minutes. We will also invite you to an in-person screening visit. During the screening visit, we will measure your height and weight, blood pressure, and heart rate. You will also complete several cognitive tests, a health and demographics questionnaire, and cycle at a maximal intensity on a stationary bike. The total screening time commitment for this visit is about **2.5 hrs**.

Risks of screening: Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. However, the risks include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise are known to outweigh the risks. As preventive measures, all participants need a medical clearance from their physician to participate in this research. Furthermore, our study physician will monitor you during a maximal exercise test and all our research staff are CPR and First Aid certified. The physician will observe the electric activity of your heart using an electrocardiogram. He will also measure your blood pressure throughout exercise. If you are taking beta-blocker medication, we will ask for your consent to discontinue the medication for 24 hours upon your physician's clearance

to do so, before the exercise test to ensure that the test can accurately assess your heart responses to the test. **Benefits of screening:** There are no direct benefits to you that come from screening. However, if you are eligible and agree to take part in this study, there may or may not be a transient health benefit to you. Specifically, breaking long sitting with short exercise breaks has been shown to improve sugar metabolism over several hours. We do anticipate that participation in this research may also result in transient (over several hours) benefit to cognitive and brain function.

BACKGROUND

You are being asked to take part in a research study. Before you decide, it is important for you to understand why the screening for this research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this screening process.

This screening process is being done to evaluate your eligibility to participate in research. The research study will evaluate brief high-intensity exercise breaks as a means to reduce a long bout of sitting. Such long bouts of sitting may negatively affect brain function and cognition. However, we do not know if interrupting long bouts of sitting with short exercise breaks could improve these functions and if exercise intensity matters. The screening is designed to evaluate if high-intensity exercise is safe for you and whether you meet our inclusion criteria based on your health history and cognitive tests.

SCREENING PROCEDURE

Your participation in this screening for a research study will include today's visit to the Physical Activity and Neurocognitive Health (PNC) laboratory. You may also need to see your primary care physician to ensure that high-intensity exercise is safe for you. If you are eligible, you will return to the laboratory for four study visits.

You have been asked to participate in this screening process because you indicated an interest in this research.

Scheduled Assessments

You will not be compensated for the screening procedures. However, if you qualify and participate in the study, you will be compensated up to $250 if you complete the entire study.

Screening Phone Call

During the phone call the researcher will ask you questions about your age, English language fluency, physical function, physical activity, sitting habits, and how you feel. S/he will also ask whether you can engage in vigorous exercise. To ensure that your participation in the study is safe, s/he will also ask questions about your health. For example, whether you had a stroke or a transient ischemic attack or a long COVID-19, and if you smoke. Next, s/he will ask you questions that let us know how you think, pay attention, know a few common facts, and remember words. If you qualify based on these questions, you will also complete a general health history questionnaire. You will answer questions about your cardiovascular history, including a history of heart disease and common signs and symptoms of cardiovascular disease. The questionnaire will ask about other health conditions that may increase

cardiovascular risk such as type 2 diabetes or increase the risks of high-intensity exercise such as pulmonary disease (e.g., COPD). Other conditions will include condition that may affect how you think such as epilepsy (i.e., having seizures) or a traumatic brain injury. Next, you will complete a Physical Activity Readiness Questionnaire. If you qualify based on these procedures, a researcher will ask if you agree for our research staff to contact your physician to ask for medical clearance for you to participate in a maximal exercise test on a stationary bicycle and high-intensity exercise during the intervention. If you are taking beta-blockers, s/he will also ask if you consent for the research staff to ask for your doctor's consent for you to discontinue your beta-blocker medication for 24 hours before the exercise test to ensure that the test accurately represents your heart responses to the maximal exercise test. If you do, you will sign the release of information form, which indicates that you are happy for us to contact your physician and include information that we collected about your heath history to help your physician decide if it is safe for you to engage in exercise test and high-intensity exercise in our study. It will also indicate that you are happy for your doctor to indicate their consent for you to discontinue beta-blockers for 24 hours before the exercise test. Your physician will be asked to confirm that you do not have type 1 or type 2 diabetes and that you did not have type 1 or 2 diabetes in the past. S/he will also confirm that your fasting glucose levels are below the threshold for diabetes in the last 12 months, and that your cholesterol levels are within normal range. If your physician is not able to confirm your blood sugar levels in the last 12 months but you otherwise qualify for an in-person screening visit based on the information provided to us today and by your physician, the research staff will measure your fasting glucose levels in the laboratory during your screening visit. Your physician will also confirm whether you are receiving or have received in the past cancer treatment, if your treatment included chemotherapy, and whether you have been cancer free for more than 12 months. If your physician clears you for participation in the maximal exercise test and study participation, we will invite you to an in-person screening visit at the PNC laboratory at Freer Hall in Urbana.

Today's phone call will last approximately **1 hour and 15 minutes**.

In-person Screening Visit 1 at the PNC Laboratory

Once we receive medical clearance from your physician, a researcher will contact you to confirm the time and date of your in-person screening visit. At the beginning of the visit, you will receive a heart rate monitor to wear around your chest. This is a strap with a single sensor that goes over your sternum and is attached snugly with an elastic belt. If your physician could not confirm your blood glucose levels in the last 12 months, a trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose. Next, the researchers will measure your height and weight. Then, you will rest for 5 minutes, and a researcher will take your blood pressure three time and your heart rate. You will also complete a questionnaire about your hand preference, about how you feel, and demographics questionnaire. Next, a trained researcher will ask you questions to measure your attention, memory, and language. You will complete patterns based on pictures, solve riddles, and tell the researcher the meaning of specific words. You will then complete two questionnaires about your exercise levels and activities you like to engage in. Then, a researcher will place electrocardiogram electrodes around your chest and on the side of your waist. They will collect electrocardiogram data while you rest for 10 s while lying down and standing. Next, you will cycle on a stationary bike for about 8 to 15 minutes until you cannot cycle any longer so that we can measure your aerobic fitness. The study physician will monitor the electrocardiogram during exercise to observe your heart's responses to exercise. He will also measure your blood pressure regularly during the test. This test will take place at Freer Hall on the University of Illinois campus. The amount of time you will cycle on the cycle ergometer will vary but most people cycle approximately 8-15 minutes. If you discontinued your beta-blockers for 24 hours before the test, a

researcher will remind you to take your medication after the test. Total time commitment for this appointment is approximately 2 hours and 30 minutes.

Assessments and Screening Requirements

Phone Screening

You will answer questions about your health, physical activity, sitting and complete a short test measuring your attention and memory.

- ***General Health History*** –You will also complete a questionnaire asking about your general health history. These questions will be kept confidential and will only be used to determine whether or not it is safe for you to participate in our study. For example, we will ask about your cardiovascular health, recent medical events (e.g., hospitalization), current medication, and lifestyle habits. All information you provide to us is strictly confidential. If you qualify for the study, we will keep this information as part of your confidential record. We will also ask about food allergies, your vision, hearing, and your medications.
- ***Questionnaires*** – You will complete a questionnaire that asks about your lifestyle and a physical activity readiness questionnaire. You will also complete questionnaires about how you feel and which hand you use for most daily tasks such as writing, brushing your teeth, etc.
- ***Physician's Release and Medical Clearance*** – To qualify for the study, you will be required to provide documentation from a physician regarding the exercise and research testing. The HIIT-2-SITLess research staff will ask for your permission to contact and send information to your primary care physician regarding your participation in this research. The physician must be willing to provide documentation indicating that you are cleared to participate in high-intensity exercise. If your physician determines that a physical examination is necessary or that you need to be seen by your oncologist (if you had the history of cancer) before clearing you for participation, then you or your insurance company will be responsible for all costs associated with such an exam or a visit to the oncologist.
- If you are taking beta-blockers we will ask for your permission to ask your physician to determine if it is safe for you to discontinue the medication for 24 hours before the exercise test.
- We will ask for your permission to share your medical history information with your primary care physician to help them determine your eligibility to participate in this research. However, you can opt out from sharing your medical history with your primary care physician. You will still need medical clearance from your primary care physician to participate in the study.

In-person Screening Visit at the PNC Laboratory

- ***Height, weight, and waist circumference***
  - A researcher will measure your height, weight, and waist circumference three times.
  - We will measure your waist in three points: the natural waist (the narrowest point), the umbilical (just above your navel), and around your hips.
- ***A finger prick***
  - A trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose using a point-of-care glucometer.
- ***Blood pressure and heart rate at rest***

- You will wear a heart rate monitor around your chest. After a 5 min rest, a researcher will measure your blood pressure and heart rate 3 times.

- ***Questionnaires*** – You will complete a demographic questionnaire. We will also ask questions about cognitive activities that you like to better understand your sitting habits, and about which hand you prefer to use for different activities to determine your hand preference.
- ***Cognitive and Neuropsychological Tests*** – You will complete a short cognitive test and a longer neuropsychological test where you will be asked about the meaning of words, you will choose patterns that best fit a picture, and solve some riddles.
- ***Graded Exercise Test*** - At this appointment, you will cycle on a stationary bike to measure your aerobic fitness. We will do that by measuring the air you exhale while you are cycling on a stationary bike. You will pedal at a constant speed while the researcher periodically increases the workload until you feel as if you cannot cycle any longer. You will be monitored by a physician and several exercise specialists certified in CPR and First Aid. We will measure oxygen through a mask that will collect your exhaled air. Your heart rate will be measured regularly through a 10-lead electrocardiogram chest monitor and your blood pressure will be taken several times throughout the test. The test including resting state electrocardiogram measures and preparation time will last about an hour. However, you will only cycle for about 8 to 15 minutes.

RISKS

Risks for blood collection: The blood sample collection is very common and involves minimal risk. There is a one in five chance of bruising in the area of sampling. This is generally not serious and will completely disappear within a few days. As with all invasive procedures, there is a slight risk of inflammation and infection. There is also risk of callus formation. This risk will be minimized by the use of sterile procedures and equipment at all times. Risk will also be minimized because a trained researcher will draw all blood samples.

Risks of exercise testing: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the maximal exercise test on the cycle ergometer is age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in the exercise test. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified. In addition, the maximal exercise test is supervised by a physician trained in supervising maximal exercise tests using the electrocardiogram, blood pressure readings, heart rate and observing how participants respond to the test.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section “How will the researchers protect my information”.

BENEFITS

Participating in the screening process is unlikely to have a direct benefit to you. We also cannot promise any direct benefit for taking part in this study if you qualify. However, previous research has shown that interrupting continuous sitting with short bouts of exercise can transiently improve sugar metabolism in adults (over several hours). We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this study may help develop a greater understanding of how interrupting sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will also help us understand if older adults are likely to use short, high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the screening procedures for HIIT2SITLess study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. The blood samples will be used only to determine the levels of fasting glucose as an inclusion criterion for the study. This identifying information will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Your primary care physician, if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Your primary care physician; with your consent, we will send a health history questionnaire to your primary care physician to assist them with medical clearance.

Participation in this screening process is voluntary, and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state, or local laws require. Some examples are laws that require reporting of

child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers, or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or screened by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, the researchers will contact you to let you know what they have found.

We will destroy the blood sample after we know your eligibility for the study. If you qualify and consent to participate in the study, we will retain all other data collected during screening. However, if you withdraw early from the screening process or choose not to participate in the study before you enroll, your data will be securely destroyed. To ensure confidentiality and anonymity during the screening, you will be assigned a numeric code and identified by this number only. We will keep the master list on the hard drive of a password-protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes, not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap compliant with the Health Insurance Portability and Accountability Act of 1996. Only the research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students, or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

If you qualify and consent to participate in the study, we will keep the information we collect about you during this screening for record keeping and for potential use in future research projects and training of junior researchers. As a research participant in this study, you consent to the use of your data for this study and future research by others. We will keep private information about you confidential to the extent allowed by laws and university policies. When researchers publicly discuss or publish the results of this research, they will not tell anyone that you were in the study. However, government or university officials who are responsible for monitoring this study and journal staff who review the research results for accuracy may see information that identifies you, including your signed consent form. If you give us your permission, we will use de-identified data from this study for use in future research studies. We will not ask for your additional informed consent for these studies. Your name and other information that can directly identify you will be stored securely and separately from the rest of the research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data. If you do not qualify or you do not consent to be enrolled in the study, we will destroy the information we collect about you during screening.

With your consent, the PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

If you have questions, complaints, or concerns about this screening process for the HIIT-2-SITLess study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached from Mondays to Fridays, 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or email OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously, or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate in this screening, you are free to withdraw your consent and discontinue participation at any time. You can start the screening process and then choose to stop the screening later.

This will not affect your relationship with the investigators. The researchers also have the right to stop your participation in this screening without your consent if they believe you do not qualify for the study, it is in your best interest, and/or if you were to object to any future changes that may be made in the screening and study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in this screening process is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination or a visit to an oncologist (if you had the history of cancer) in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will not be paid for taking part in this screening.

The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study, nor will the University of Illinoisprovide compensation for any injury sustained as a result of participation in thisresearch study, except as required by law.

If you qualify based on the screening phone call and Screening Visit 1, you will receive $30 compensation for your exercise test and information about your current levels of aerobic fitness.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.

- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
  - Limited information may also be shared with first responders from Emergency Medical Systems (EMS) to assist them with medical treatment; this information may include your name, address, emergency contact, your physician's contact details, age, information about your cardiovascular risk history, current medications, and description of the event;
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ and/or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this screening process for the HIIT-2-SITLess trial.

Optional

I consent for my General Health History questionnaire to be shared with my primary care physician to assist them with medical clearance for the study.

❑ Yes ❑ No

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Material 2. Study Consent

Consent and Authorization Document

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT-2-SITLess Trial

You have indicated an interest in participating in research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the feasibility and utility of short exercise bouts to reduce long sitting over several hours. This is a short-term study where you will be asked to visit the PNC laboratory three times over approximately five to seven weeks. You will also wear activity monitors for two weeks in between visits. *The risks* of this study include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise outweigh the risks. As preventive measures, you will need a medical clearance from your physician to participate in this research. Based on your responses to the maximal exercise test which was monitored by a study physician, you were considered to be at a lower risk of such events. Our research staff will also monitor your heart rate and physical responses to exercise (such as how hard you are working out and if you experience any unusual pain, fatigue etc.) during and after exercise. All our research staff are CPR and First Aid certified. If you agree to take part in this study, there may or may not be transient health benefit to you. Specifically, *breaking long sitting with short bouts of exercise has been shown to improve sugar metabolism* over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function.

BACKGROUND: THE HIIT-2-SITLESS TRIAL

This research is funded by the National Institute on Aging. You are being asked to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this study.

This research is being done to evaluate brief high-intensity exercise breaks to sitting. Although long bouts of sitting may attenuate brain function, we do not know if breaking long sitting with short bouts of exercise could improve brain function and if exercise intensity matters. The HIIT-2-SITLess trial will assess if short high-intensity exercise breaks to sitting are acceptable, and practical to older adults as means of reducing long periods of sitting. The trial will also compare changes in brain function and cognition after three and half hours of sitting interrupted with 6-min of high-intensity exercise breaks relative to 6-min of light-intensity exercise.

You have been asked to participate in this research because you are 40-75 years old and met inclusion criteria, we reviewed over the phone and during your screening visit, such as being right-handed, not exercising regularly, planning to be in the Urbana-Champaign area for the duration of the study, etc. Approximately 54 participants will be involved in this study at the University of Illinois.

STUDY PROCEDURE

Your participation in this study will last about eight weeks. You will come to the laboratory on four occasions and wear activity monitors for the total of two weeks between study visits.

Study Logistics

Scheduled Assessments

You will be compensated for these scheduled assessments and the compensation amounts are stated later in this document.

1. **Baseline Visit: Cognitive Tasks, HIIT, and LIIT Breaks Practice and Questionnaires**
   - ***Blood pressure and heart rate.*** Researchers will give you a heart rate monitor to wear around your chest. The monitor has a small flat electrocardiogram electrode inside a plastic casing attached to an elastic strap that goes around your chest. The monitor will sit in the center of your chest, and researchers will place electroconductive gel on the strap to enhance the connection between the electrode and the electrical signal from your heart. Researchers will also measure your resting blood pressure three times to monitor for high blood pressure that could prevent you from exercising.
   - ***HIIE and LIIE Practice Session***. During this visit, you will also practice high-intensity and low-intensity interval training breaks supervised by our research staff. This ensures that you feel comfortable with exercise and that we know the cadence (speed) and workload that can elicit your target heart rate during exercise. You will also fill in questionnaires (described below).
   - ***Questionnaires.*** To help us better understand your responses to high-intensity exercise, we will ask you to complete a set of questionnaires about your physical activity, enjoyment of physical activity, physical function, sleep, and sitting behaviors.
   - ***Cognitive tasks practice.*** To help you get used to cognitive tasks, you will practice two tasks on a computer for 12 minutes each. You will complete two tasks by pressing buttons on a response pad based on task instructions. You will see asterisks and letters come up on the computer screen one by one. You will need to look away from an asterisk to "catch" which letter just appeared. You will also see arrows on the screen and will have to press a button, which corresponds to the direction of a middle arrow. You will practice tasks in between physical exercise breaks.
   - Total time commitment for this appointment is approx. **2 hrs.**

2. ***High-Intensity Exercise Intervention***
   You will be asked to participate in a half-day intervention designed to minimize long bouts of sitting by cycling on a stationary bike at a high intensity every 30 min. You will come

to the laboratory in the morning fasted. This means not eating or drinking (plain water is ok) for 8-10 hours before your visit. Trained researchers will measure your resting blood pressure and heart rate. You will also wear a chest strap with a heart rate monitor and two activity monitors to measure your sitting and physical activity. You will then complete a 24-h dietary recalls, and questionnaires about sleepiness. Next, you will eat a light breakfast, which is nut free oats and seeds bar. You will then complete the same neurocognitive tests as during your EEG visit. You will start the intervention after these neurocognitive assessments. You will sit for three hours and a half hours while you complete light administrative tasks and read popular science articles. For example, you will plan a family vacation and read articles about how snowflakes form or how whales help cool the earth. You will answer brief questions about how engaging and difficult these activities are. You will also answer questions about your energy levels, and fatigue. Every 30 min, the researchers will measure your blood pressure and heart rate before and after you complete a 6-minute high-intensity exercise (HIIE) break. You will also complete a shorter neurocognitive assessment twice during sitting: 15 min after the first and the third HIIE break. You will sit in a wheelchair while a researcher will push the wheelchair to the cycle ergometer. The neurocognitive assessment will last 15 min. You will then complete another HIIE break. You will complete five HIEE breaks in total. During each break, researchers will ask you questions about the levels of physical effort, and about how you feel. Researchers will monitor your blood pressure and heart rate after each break. After the last break, a researcher will transport you back to the EEG equipment and fill in the electrodes with gel while you sit and eat the second light meal (a similar oats and seeds bar). You will then complete the same set of cognitive tests as before the intervention while researchers record EEG signal. If this is your first intervention visit, you will receive two activity monitors to take home. If it is your last visit, you will complete a questionnaire about your experience in the study, and your participation will be complete. Total time commitment: **approx. 6 hours.**

EEG. You will be asked to visit Freer Hall where you will undergo neurocognitive assessments. You will wear an electroencephalography (EEG) cap that looks like a swim cap with small electrodes located throughout the cap. The researchers will fill in the electrodes with electroconductive gel so that we can measure tiny electric currents produced by your brain while you rest and complete cognitive tasks. We will first record your brain activity while you rest with your eyes open and closed for six minutes. You will then perform various cognitive tests on a computer that assess attention, executive function, and memory. For example, for one of the tests you will see pictures of common objects. Next, you will see more pictures. For each picture you will have to indicate if you saw it before or not. You will complete assessments again after the intervention is finished. In addition, you will complete one of the tasks twice during the intervention.

Cognitive Tasks. In addition to the two tasks that you practiced during the baseline visit, you will also complete a task where you will see pictures of objects. You will make judgments about pictures, such as determining if they represent an indoor or an outdoor object.

High-Intensity Interval Exercise Break: First, you will cycle at the same speed as during the maximal exercise test with progressively increased resistance for 1 min to warm up. Next, we will increase resistance on the bike to the same level at which your heart rate increased to 90% of its maximum during exercise test. These short bouts are designed to be very hard and you will cycle at this speed and resistance for 2 minutes. Then, you will rest while sitting still on the bike for 1 minute. Next, you will cycle again for 2 minutes at the same high intensity. At least two CPR and First Aid certified researchers will assist you and monitor your heart rate throughout exercise and ask you to rate how tired your body feels due to exercise and how you feel overall during each HIIE break. Finally, you will sit again and continue with either administrative activities or reading. You will complete **5** HIEE **breaks** lasting **6 min each** for **a total of 30 min** of exercise.

3. ***Low-Intensity Interval Exercise Intervention***
You will complete all the same procedures and tests as during the HIIE intervention day except for high-intensity exercise breaks. Instead, you will complete 6-min low intensity interval exercise breaks (LIIE).

Light-Intensity Interval Exercise Break. You will first warm up by pedaling for 1 min with minimal workload at an intensity of about 50% of your maximum heart rate. Next, you will pedal at a higher speed and workload chosen to elicit light intensity or about 57-60% of your maximum heart rate which is considered very light to fairly light intensity. You will then rest and remain stationary on the cycle ergometer for 1 minute, followed by another 2 minutes pedaling at the speed and workload to elicit the same heart rate. Total time commitment: **approx. 6 hours.**

Randomization

We will assign the order of two exercise interventions randomly at baseline. This means that the order in which you will complete HIIE and LIIE interventions, will be chosen by chance, like flipping a coin. Neither you nor the study team will choose which intervention you will complete first. The study team will let you know which exercise intervention you will complete first on the day of your first intervention visit. All participants will be asked to participate in all testing procedures.

Additional Assessments and Study Requirements

1. ***Questionnaires*** – You will be asked to complete one packet of questionnaires related to your physical abilities, physical activity, sedentary activities, sleep, diet, attitudes, thoughts, and feelings. The packet should take approximately 45-60 minutes and you will be able to complete it during the Baseline visit while you practice HIIE and LIIE breaks. At the end of the second intervention visit, you will also fill in a brief questionnaire about your experiences of the intervention.
2. ***Accelerometers*** – You will be asked to wear two activity monitors for seven days on two occasions, a week before each intervention visit. The first device is about the size of a pocket watch, similar to a pedometer. It is worn around your waist during waking hours and sleep except for bathing and showering. You will wear a second device on your thigh. The device is called activPAL. It is small and flat, similar to a flat piece of a domino. This device will measure how much you sit and stand. It is attached to your thigh with transparent film dressing.

You can wear this device while bathing or showering. You will also fill out an activity and sleep log to indicate hours of the day you wore the devices, when you went to sleep, and when you woke up. The devices do not track GPS or geographic data. They will only record the movement and sitting.

3. ***Health and Demographics Questionnaire*** – You already completed this questionnaire during screening. It has become part of your research record.
4. ***General Health History*** - You also provided information about your health history during screening call, which has become the part of your research record.
5. **Physician's Release and Medical Clearance** – Before qualifying, you provided documentation from a physician regarding the exercise and research testing, which is also part of your research record.

Additionally, the investigators may contact you in the future regarding other research at this institution.

You may opt out of these communications and opportunities at any time.

RISKS

Risks of high-intensity exercise participation: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the exercise breaks have been designed to offer activities that are safe and age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in these activities. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified.

Risk of EEG: In rare instances, some individuals have reported some discomfort from the EEG cap. If this occurs, we will take the cap off and re-schedule the visit.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section "How will the researchers protect my information".

BENEFITS

We cannot promise any direct benefit for taking part in this study. However, previous research has shown that interrupting long sitting with short bouts of exercise can improve sugar metabolism in adults over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this

study may help develop a greater understanding of how breaking long sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will help us understand if older adults are likely to use short high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. You understand that the blood samples will be used only to determine the levels of fasting glucose as the inclusion criterion for the study. Information that could identify you will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Primary care physician if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Primary care physician (PCP) with participant's consent we will send health history questionnaire to PCP to assist them with medical clearance;

Participation in this project is voluntary and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state or local laws require. Some examples are laws that require reporting of child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or seen by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, such as your fasting glucose levels, the researchers will contact you to let you know what they have found.

We will destroy the blood sample collected during screening after we know your eligibility for the study. We will retain all other data collected in the course of the study. For example, if you withdraw early from the study, your data will be retained for the analyses. To ensure confidentiality and anonymity during the study, you will be assigned a numeric code, and identified by this number only. We will keep the master list on the hard drive (or SSD) of a password protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap, which is compliant with Health Insurance Portability and Accountability Act of 1996. Only research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

We will keep the information we collect about you during this research study for record keeping and for potential use in future research projects and training of junior researchers. Your name and other information that can directly identify you will be stored securely and separately from the rest of the

research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data.

The PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

Example: If you have questions, complaints, or concerns about this study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached during Mondays to Fridays 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or e-mail OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time. You can start the study and then choose to stop the study later. This will not affect your relationship with the investigator. The researchers also have the right to stop your participation in this study without your consent if they believe it is in your best interests, you were to object to any future changes that may be made in the study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in the study is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will be paid for taking part in the *scheduled* appointments described above. As an incentive, and appreciation for contributing your time to this study, you will be paid a stipend as indicated in the table below. Thus, you will receive up to *$250* if you complete all the study assessments. The total time commitment for these scheduled appointments is approximately 16.5 hours.

Appointment	Time	Stipend	Location
Neurocognitive tests + HIIE + LIIE Practice	2 hrs.	$50	Freer Hall
HIEE Intervention	6 hrs.	$100	Freer Hall
LIIE Intervention	6 hrs.	$100	Freer Hall

If you live more than 10 miles away from the study site, we will reimburse the cost of your travel in the amount of $0.625 per mile.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.
- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.

- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with collaborators at Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this study.

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Table 1. Name and contact information for the trial sponsor

Trial Sponsors:	University of Illinois Urbana-Champaign Sponsor's Reference: 1376000511A6 Federal Employment Identification Number Contact Name: Paul N. Ellinger, Comptroller Address: 1901 S. First Street, Suite A Telephone: 217-333-2187 Email: spa@illinois.edu

Supplementary Table 2. Trial registration data

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov No. NCT06243016
Date of registration in primary registry	2024-02-05
Secondary identifying numbers	IRB24-0010, 1R21AG080411-01A1
Source(s) of monetary or material support	National Institute on Aging
Primary sponsor	University of Illinois Urbana-Champaign
Secondary sponsor(s)	Northeastern University, National Institute on Aging
Contact for public queries	Dominika M Pindus, Ph.D.
Contact for scientific queries	Dominika M Pindus, Ph.D.
Public title	Breaking Sitting With High-intensity Interval Training for Brain Health (HIIT2SITLess)
Scientific title	Breaking prolonged sitting with high-intensity interval training to improve cognitive and brain health in older adults – a pilot feasibility trial
Countries of recruitment	USA
Health condition(s) or problem(s) studied	Prolonged sitting, high-intensity interval training bouts, frontoparietal function, inhibitory control and episodic memory
Intervention(s)	*Active comparator*: 6-minute high-intensity interval training (every 30 minutes over 3.5 hours of sitting) *Passive comparator*: 6-minute low-intensity interval training (every 30 minutes over 3.5 hours of sitting)
Key inclusion and exclusion criteria	Age 40-70 years, BMI < 40 kg/m^2, sedentary (≥ 6 hours of sitting per day), low to moderately physically active (based on IPAQ Short Form), capable to engage in vigorous exercise (PARQ+), medical clearance from primary care physician, normotensive, IQ ≥85, fasting plasma glucose < 126 mg/dL, good or corrected vision and hearing, no significant abnormalities on the ECG during a maximal exercise test, no signs or symptoms suggesting of underlying cardiovascular disease as recorded during maximal exercise test, no indications to prematurely stop the maximal exercise test as per ACSM's Guidelines for Exercise Testing and Prescription.
Study type	Interventional
Date of first enrolment	February 2024
Target sample size	54
Recruitment status	Recruiting
Primary outcome(s)	Change in task-evoked brain activity (P3b component)
Key secondary outcomes	Change in cognitive functions, change in resting state and task evoked brain activity

Notes. ECG: electrocardiogram, IPAQ Short Form: International Physical Activity Questionnaire Short Form, PARQ+: Physical Activity Readiness Questionnaire for Everyone, IQ: intelligence quotient.

Supplementary Table 3. Version history

Item	**Item Details**
Original IRB Protocol Number:	IRB24-0010
Original IRB Approval Issue Date:	02/20/2024
Protocol amendment number:	3
Protocol amendment approval date:	
Authors:	*DMP, RJS*
Revisions chronology	
Amendment 1 Approval Date:	04/20/2024
Amendment 1 changes:	Use of point-of-care glucose monitor instead of a venous blood sample and research-grade glucose reader.
Amendment 2 Approval Date:	05/28/2024
Amendment 2 Changes:	Expanding research staff who can perform a finger prick to collect a blood sample
Amendment 3 Approval Date:	09/13/2024
Amendment 3 Changes:	Age range change from 60-75 to 40-75 years. Amendment to inclusion criteria; shorter screening and baseline protocol.

BMJ Open

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

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	Health; Northeastern University - Boston Campus, Department of Psychology
Primary Subject Heading:	Sports and exercise medicine
Secondary Subject Heading:	Neurology
Keywords:	Exercise, Cognition, Neurophysiology < NEUROLOGY, Aging, Clinical Trial

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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 aging. Physical activity (PA) interventions can enhance FC in these networks, but these interventions are not designed to decrease ST among older adults. Prolonged sitting (i.e., sitting continuously for ≥ 20 minutes) can acutely reduce frontoparietal brain function and attentional control, while a single PA bout lasting at least 20 minutes can enhance them. It has been theorized that stimulation of the cerebral norepinephrine release through peripheral increase in catecholamines may explain this effect. In contrast, the effects of shorter (< 10 minutes) PA bouts used to interrupt prolonged sitting on neurocognitive functions remain poorly understood. This pilot randomized crossover feasibility trial capitalizes on PA intensity as the major limiting factor in peripheral catecholamine increase and tests the effects of interrupting prolonged sitting every 30 minutes with 6-minute high-intensity interval training (HIIT) compared to low-intensity interval training (LIIT) bouts. The study will address three aims: (i) to assess feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function in middle-aged and older adults; (ii) 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); (iii) 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: Fifty-four 40-75-year-old healthy adults will be recruited from the local community and randomly assigned to a condition sequence (HIIT, LIIT versus LIIT, HIIT). Each HIIT bout comprises a 1-minute warm-up, 2 minutes at 90% of the maximum heart rate (HR_{max}), 1-minute passive rest, and 2 minutes at 90% HR_{max}. During 2-minute intervals in LIIT, participants exercise at 57-60% of HR_{max}. The primary outcomes include the feasibility (recruitment and retention rates, percent of valid EEG data), acceptability of time commitment, HIIT bouts and neurocognitive assessments, fidelity (the intensity of HIIT breaks, percent of time spent sitting) and the amplitude and the latency of the P3b component of event-related brain potentials (ERPs) measured during the modified Eriksen flanker task at pre-tests, 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.

Clinical Trial Registration: ClinicalTrials.gov No. NCT06243016

Keywords: sedentary behavior, high-intensity interval training, cognitive functions, brain function, middle age, aging

Strengths and limitations

- The HIIT2SITLess study is a well-controlled randomized 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 episodic memory.
- This pilot feasibility trial recruits healthy middle-aged and older adults with a limited cardiovascular risk; hence, its generalizability 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 aging 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, characterized by a decreased ability to control distractions and correctly recall the details of information and events (i.e., episodic memory).[2–4] These cognitive functions are indispensable for everyday functioning and 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 aging.

Yet, effective and scalable interventions to improve cognitive and brain health in older adults are lacking. Traditional physical activity (PA) interventions (e.g., a 20-40-minute 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 minutes.[8] In contrast, the efficacy of PA interventions that utilize short (<10 minutes) 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 sedentary time and prolonged sitting, such as sitting continuously for 20 minutes or longer, may attenuate attentional control,[12,13] episodic memory,[14] and frontoparietal function.[15] For example, 21-45-year-old adults with more prolonged sedentary time 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 minutes, 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 electroencephalographic (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 aging 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- 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 aging. 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 optimizes 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 aging[52] and AD.[53] Thus, PA interventions designed to stimulate the LC-NE system could significantly impact the functional integrity of the aging brain.

High-intensity interval training (HIIT) could stimulate the LC-NE system because it utilizes 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-minute delay.[58,59] However, the effect of a single bout of HIIT on cognitive function declines after 20-30 minutes.[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 minutes) 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 minutes) 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 (i.e., light or moderate) to stimulate the LC-NE system within 2-5 minutes.[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 randomized crossover pilot feasibility trial designed to test three specific aims:

(i) To assess the feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function.

(ii) To quantify the differences between conditions in a change in P3b amplitude and latency, a marker of frontoparietal function.

(iii) 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 default mode networks.

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 randomized crossover trial with two interventions lasting 3.5 hours each: prolonged sitting interrupted every 30 minutes with 6-minute HIIT bouts active condition and prolonged sitting interrupted every 30 minutes with 6-minute 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 provide written informed consent in accordance with the Institutional Review Board at the University of Illinois Urbana-Champaign (see Supplementary Table 1 for sponsor details).

Trial registration

The trial was registered on ClinicalTrials.gov No. NCT06243016 before the enrollment of the first participant. See Supplementary Table 2 for trial registration details.

Participants

The study will enroll 54 (27 female) middle-aged (40-59 years) and older (60-75 years) cognitively healthy adults from Champaign County, I, 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.[7,73]

Eligibility

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

Blinding and randomization

Fifty-four participants will be randomized to two condition sequences by a statistician following baseline assessments. Permuted block randomization generated using the PROC PLAN procedure (SAS Institute Inc., 2023)[74] is used, where sequences are randomized within a block of six participants to minimize the possibility of group imbalances due to dropout. Participants are randomized to one of two condition sequences by a study statistician: 1) X = HIIT, LIIT breaks or Y = LIIT, HIIT breaks. Generated permuted block randomization also ensures that blocks are balanced by cognitive task (i.e., flanker [F], antisaccade [A] and mnemonic similarities task [M]) sequence (FAM, MFA and AMF). The principal and co-investigators will be blinded to the sequence allocation. The sequence will be concealed until the participant's enrollment. Upon enrollment, the study sequence will be verbally communicated to the study coordinator by a statistician. The coordinator will record the sequence number in REDCap. The staff implementing the 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 enrollment 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, organizations serving older adults in Champaign County, and flyers, and individual mailouts to adults aged 40-75 years in Champaign County. Recruitment and enrollment 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 Table 2.

Screening Procedure

Screening Call

At the beginning of the screening call, participants will sign an informed consent to the screening process (Supplementary 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 ischemic attack, long COVID-19, and smokers. A trained researcher will then administer the Telephone Interview of Cognitive Status-modified (TICS-m). Only individuals with a score < 32 (a cutoff for mild cognitive impairment)[75] 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,[76] and pre-existing conditions as listed in the exclusion criteria (Table 1). An individual will also fill in the Hospital Anxiety and Depression Scale.[77] Individuals with anxiety and depression will be included due to the high prevalence of these disorders in the general population.[78,79] 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 (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80] screens out highly physically active individuals who engage in 300 minutes or more of moderate-to-vigorous PA per week. The Physical Activity

Readiness Questionnaire+[81] 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: (i) exercise strenuously for 48 hours before the experimental visit, (ii) drink caffeine or (iii) 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 mmHg on the screening day will undergo the maximal exercise test because higher values are a contraindication to a maximal exercise test.[76] 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.[82] If the participant's physician cannot confirm fasting glucose levels or glycated hemoglobin levels (HbA1c) 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 (MoCA) to screen out individuals with scores < 26 suggestive of potential cognitive impairment .[83] A standardized test of cognitive abilities (Kaufman Brief Intelligence Test – 2; KBIT-2)[80] will be administered next, and individuals with a score < 85 (i.e., 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[84,85] with a 12-lead electrocardiogram. 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 to evaluate participants' physiological responses to exercise.[76] Its results will be used as an inclusion criterion to enhance the safety of acute high-intensity exercise. Three trained first aid and CPR-certified experimenters will conduct the test in collaboration with the study physician. Participants' resting BP, HR, and electrocardiogram readings will be collected. They will then warm up for two minutes while pedaling 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 minutes by 25 Watts. Males will start at 100 Watts and exercise at 50 Watts increments.[84,85] The participant will cycle until volitional exhaustion.[84,85] Their HR and ECG are continuously monitored, and blood pressure will be monitored every two minutes during exercise by a physician. Every two minutes, the study staff will record ratings of perceived exertion (RPE) using the Borg scale.[86] 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[76]: (i) respiratory exchange ratio (RER) ≥ 1.1, (ii) failure of the HR to increase with increasing workload (i.e., ≤ 10 bpm increase relative to age-predicted HR_{max},[87]) or (iii) RPE > 17. The test finishes with a 5-minute cooldown. If there are no positive findings on the electrocardiogram as described in the indications to stopping the maximal exercise test in the ACSM's Guidelines for Exercise Testing and Prescription,[76] 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 familiarize participants with the main intervention procedures, including cognitive tasks and HIIT and LITT bouts. Participants will first sign an informed study consent (Supplementary Material 2). The consent includes provisions for the de-identified data for use in future studies. A trained researcher will assess their resting BP to verify that systolic over diastolic BP is < 200/110 mmHg, which is a counterindication to

exercise.[76] Participants will then practice two LIIT and two HIIT bouts every 25 minutes. During each 25-minute block, they will complete a questionnaire battery and practice cognitive tasks described in detail under the intervention section.

Light-intensity-interval-training bouts (LIIT). Each LIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two low-intensity intervals, cycling at 57-60% of their maximum HR lasting two minutes and separated by a one-minute 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.

High-intensity interval-training bouts (HIIT). Each HIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two high-intensity intervals separated by a one-minute passive recovery (sitting on a cycle ergometer). High-intensity intervals comprise cycling for two minutes 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}. Participant's HR will be continuously monitored by the research staff in response to exercise and two minutes after exercise to confirm the drop in HR of at least 22 beats per minute, which indicates a normal HR response after exercise.[88,89] Participant's BP is also monitored 6 minutes after each bout of exercise to ensure that resting BP does not exceed the <200/110 mmHg threshold.[76] 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 minimize practice effects observed in previous studies.[90,91] The mnemonic similarity task 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 characterization of the study sample in relation to their habitual leisure-time exercise, types of

sedentary behaviors 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 contextualize 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, FL) to wear on their wrists, which monitor PA and sleep continuously 24/7 over one 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.[92] The information from accelerometers will be used as exploratory covariates in participants' responses to the intervention. Participants will also wear the devices for one week preceding the second intervention day. The data from both weeks will be compared to assess consistency in free-living physical behaviors 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. Upon

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 minutes. After the rest, their resting HR and BP are collected to verify that SBP/DBP is <200/110 mmHg. Participants are then fitted with an EEG cap. During the cap preparation, participants are provided with a light, standardized 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, 70-75), gender and BMI and accounting for 22% of their recommended daily energy intake.[93,94] After breakfast, they complete a 24-hour PA recall (Activities Completed over Time in 24 Hours; ACT24),[95] Karolinska Sleepiness Scale,[96] 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-minute rest while the EEG signal is collected. After the resting state EEG data collection, they will complete three cognitive tasks in a randomized 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 minutes with a 6-minute 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 minutes of LIIT or HIIT, depending on the condition. The order of the intervention conditions will be randomized across participants such that each participant will serve as his/her own control. HR and BP are monitored and recorded two and six minutes 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 minutes 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 minutes of sitting, participants will receive another standardized meal identical to the one received at the pre-test. After they consume the meal, participants engage in the exact same neurocognitive assessments as during the pre-test. 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 standardized set of home administrative tasks (e.g., planning a holiday, a birthday party, etc.) and read a standardized set of popular science articles from the New York Times. Activities will change every 30 minutes. Two sets of sedentary activities were developed, and their order was randomized 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 standardized 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[97–99] and Cognitive Effort[100] 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 vigor, they will self-report their energy, vigor, and fatigue on a validated Visual Analogue Fatigue Scale before every break.[101] We will also monitor participants' perceived enjoyment of PA during each condition with Physical Activity Enjoyment Scale to inform intervention acceptability.[102]

Cognitive tasks

Modified Eriksen Flanker task. Inhibitory control is measured using a modified Eriksen flanker task before, after, and twice during 3-hour sitting.[103] 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 center 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. Behavioral 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.[104] 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.[105] 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 two 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 (MST). Episodic memory is measured with a computerized MST.[106,107] Performance on this task is a good marker of hippocampal function[106] and is sensitive to the acute effects of PA in older adults.[108] An encoding phase will be administered first. Participants study 64 colored 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.[109] They complete a set of 192 objects. A lure discrimination index (LDI; probability of “similar”/“novel” judgments 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 (EEG)

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-minute rest at pre-test 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, NC) with four integrated bipolar electrodes for vertical and horizontal eye movements (VEOG, HEOG), 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 is an established marker of frontoparietal brain function embedded within the stimulus-locked event-related brain potential (ERP). Both the P3b amplitude and latency have been reliably modulated by acute exercise.[104] 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 (ERN). The effects of the intervention on other ERP components related to cognitive control will also be explored.[110] 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.[111] Attentional control is part of the cognitive control system.[112] The stimulus-locked N2-ERP component[110] 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.[110] Larger N2 amplitudes have been observed with successful conflict resolution and fewer commission errors.[113] This ERP component has been modulated by a single bout of sitting lasting 20 minutes in preadolescents such that a more negative N2 amplitude was observed during the flanker task (suggesting greater conflict) after a bout of sitting compared to a bout of moderate-intensity walking.[114] The 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.[115] The ERN can be modulated by acute exercise,[116] but its response to prolonged

sitting has not been investigated. Accordingly, neurofunctional responses underlying inhibitory control, which includes conflict monitoring, are measured in the HIIT2SITLess study.

Frontal N400 (FN400) and Late Positive Component (LPC). 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[117–119] and recollection.[120,121] 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.[122] Anterior-central FN400 is thought to index familiarity judgments because it varies with self-reported recognition confidence ratings.[117] 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[121] but not with their recognition confidence.[117] 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 (i.e., effective) FC in high temporal resolution,[123,124] using the weighted Minimum Norm Estimation (wMNE), a gold standard of source reconstruction, together with the Directed Transfer Function (DTF),[125,126] a technique that uses multivariate autoregressive modeling 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[127,128] but a single bout of PA can strengthen FC in both networks.[129] 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 behaviors 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 (i.e., 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. 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 is 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 posttest. 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.[130] 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 participant's responses to exercise and observes for signs and symptoms of hypoglycemia 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) Behavioral responses during the modified Eriksen flanker task

(2) Behavioral responses during the antisaccade task

(3) Behavioral 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 pre-test and post-test except for behavioral 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 Research Electronic Data Capture (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 six years upon 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 Supplementary 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 vs. 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 version 3.1.[131] 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.[132] 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 pre- to post-intervention comparison. Kamijo et al.[133] reported Cohen's d = 1.2 for the P3b latency in older adults using a single 20-minute bout of moderate intensity (expected to increase peripheral catecholamines).[65,134] 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,[135] we will fit general linear mixed-effects models.[136] (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 modeling 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: pre-, after break #1, after break #3, and posttest), 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 pre-, after the first and the third PA bout, and post-test assessments.[136] We will test intervention effects on secondary outcomes using the pre-test 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 posttest. We will include each outcome as a response variable, sequence, condition, and time as fixed effects and a participant-specific random intercept. Carryover (i.e., period) effects are assumed to be null based on the sufficient washout period of one week between treatments.[137] Results will be presented as mean differences between conditions in the Area Under the Curve 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*).[138]

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 upon request. Only de-identified data that support a published manuscript will be shared. All investigators involved in the development of the trial will be co-authors of any subsequent publications resulting from the trial.

Reporting guidelines

The study was designed in accordance with Standard Protocol Items for clinical trials (SPIRIT Statement), and its details are provided in Supplementary Table 3.

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 upon 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 (Supplementary Materials 1 and 2). The data collected from participants will be used for research purposes. De-identified data can be used for future studies and training purposes.

DISCUSSION

The HIIT2SITLess randomized 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 minutes) PA bouts. We hypothesize that implementing 6-minute HIIT interruptions to prolonged sitting every 30 minutes will be feasible and acceptable over the 3.5-hour period to middle-aged and older adults. We also hypothesize 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 behavioral 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,[139] 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 (i.e., 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 to 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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Contributorship

DMP: Conceptualization, data curation, methodology, funding acquisition, project administration, resources, supervision, writing original draft; CHH, AFK, NAK: conceptualization, methodology, funding acquisition, resources; MW, TSL: methodology, software; SP, JS, JS, MK: methodology; JK: visualization. All co-authors reviewed, edited, and approved the final draft.

Dominika M. Pindus is a guarantor of this work.

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Competing interests

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Patient consent for publication

Not applicable.

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Supplemental material

Supplementary Materials are available online.

Open access

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 1. Study inclusion and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
• Age 40-75 years; including pre-, post-, and peri-menopausal women regardless of hormone therapy replacement.	• Physical disability or musculoskeletal disease prohibitive to vigorous exercise
• BMI <40 kg/m^2;	• Learning disabilities
• Sedentary (≥ 6 h/day sitting by a survey question);	• Cognitive abilities below a 26-point cut-off on a MoCA
• IPAQ Short Form score in the low and moderate physical activity range	• Type 1 or 2 diabetes
• Capable of exercising vigorously based on the PARQ+	• Neurological condition (e.g., MS, Parkinson, Dementia, MCI)
• Has a medical clearance for maximal exercise and HIIT from a physician	• Color blindness
• Normotensive or participant's blood pressure is controlled (i.e., 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 (e.g., traumatic brain injury, stroke)
• Intellectual ability no less than one standard deviation relative to the population mean (i.e., ≥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 (see General Health History, Cardiovascular History)
• No significant abnormalities on the ECG during the maximal exercise test	• A chronic pulmonary disease (e.g., chronic obstructive pulmonary disease; COPD)
• No signs and symptoms that suggest an underlying cardiovascular disease as recorded during the maximal exercise test by a study physician	• Emphysema

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

• 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 (e.g., attention-deficit hyperactivity disorder [ADHD], schizophrenia, etc.) • 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 Apnea • Acquired immunodeficiency syndrome (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 hospitalization in the last six 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

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 2. Psychosocial assessments

Name	Description
The Activities Collected over Time over 24-hours (ACT24)[140]	A 24-hour physical activity recall to measure participant's previous day physical activity, sedentary behaviors and sleep.
Beck Depression Inventory-2[141]	A 21-item inventory to assess attitudes and symptoms of depression in adults aged 18-80 years.
Cardiorespiratory Fitness Questionnaire[142]	A 5-item questionnaire to assess the level of aerobic fitness based questions about habitual aerobic exercise.
The Epworth Sleepiness Scale[143]	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 FDI-Disability[144,145]	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 FXN-Function[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 Function Self Efficacy 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 assess 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[77]	A 14-item questionnaire with questions about symptoms of depression, anxiety and psychological distress during the past week.
The Canadian Society for Exercise Physiology (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80]	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[77]	A standardized and normed intelligence test for ages 4-90 years. The test comprises one verbal and two non-verbal components used to compute verbal and nonverbal IQ scores and a general IQ score.
Karolinska Sleepiness Scale (KSS)[96]	A one-item assessment of individual's subjective experience of sleepiness over the past 5 minutes.
Montreal Cognitive Assessment (MoCA)[83,150]	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[81]	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 (PSQI)[81]	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 Behavior Questionnaire (SBQ)[152]	An 18-item questionnaire assessing the time individuals spent in various sedentary behaviors on weekdays and weekend days.
Task Engagement Scale[97–99]	A 9-item scale assessing the level of physical, emotional and cognitive engagement in a task.
Mental Effort Scale[100]	A single item scale assessing the level of mental effort exerted during the task.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 3. Feasibility, missingness, fidelity, and acceptability outcomes

	Description
FEASIBILITY	
Recruitment rates*	*N* randomized/*N* screened[5]
Retention rates*	*N* randomized who successfully completed all conditions/*N* randomized[5]
Cognitive and EEG data	% of participants with fully completed pre- and post-intervention 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,* 2. Number of dropouts due to time commitment.
HIIT	(1) 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.

Figure captions

Figure 1. Study design.

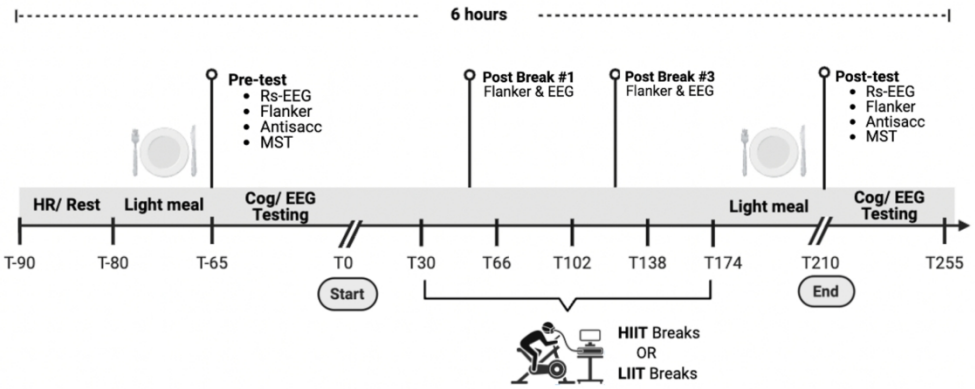

Figure 1. Study design.

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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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

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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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Supplementary Material 1. Screening Consent

Consent and Authorization Document

Consent to Screening Procedures

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT2SITLess TRIAL

You have indicated an interest in participating in a research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the utility of short physical activity breaks to sitting to reduce sitting and enhance cognitive and brain function in the short term (over several hours). This is a short-term study. If you qualify, you will be asked to visit our laboratory three times over approximately five to seven weeks and wear activity monitors for two weeks between visits.

KEY INFORMATION ABOUT THE SCREENING PROCESS

The screening procedures aim to assess your eligibility for the study. Today, a researcher will ask you questions about your age, physical activity, physical function, sitting habits, smoking, history of stroke or a transient ischemic attack, long COVID-19, your vision and hearing. The researcher will ask you questions and give you small tasks to measure how you think and how well you remember things. You will also complete a questionnaire about your general medical history, and questionnaires about your physical activity and how you feel. If you qualify based on this phone call, you will complete a medical clearance release form allowing the research team to contact your physician to determine if you can participate in high-intensity exercise. Today's call will last approximately 1 hour 15 minutes. We will also invite you to an in-person screening visit. During the screening visit, we will measure your height and weight, blood pressure, and heart rate. You will also complete several cognitive tests, a health and demographics questionnaire, and cycle at a maximal intensity on a stationary bike. The total screening time commitment for this visit is about **2.5 hrs**.

Risks of screening: Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. However, the risks include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise are known to outweigh the risks. As preventive measures, all participants need a medical clearance from their physician to participate in this research. Furthermore, our study physician will monitor you during a maximal exercise test and all our research staff are CPR and First Aid certified. The physician will observe the electric activity of your heart using an electrocardiogram. He will also measure your blood pressure throughout exercise. If you are taking beta-blocker medication, we will ask for your consent to discontinue the medication for 24 hours upon your physician's clearance

to do so, before the exercise test to ensure that the test can accurately assess your heart responses to the test. **Benefits of screening:** There are no direct benefits to you that come from screening. However, if you are eligible and agree to take part in this study, there may or may not be a transient health benefit to you. Specifically, breaking long sitting with short exercise breaks has been shown to improve sugar metabolism over several hours. We do anticipate that participation in this research may also result in transient (over several hours) benefit to cognitive and brain function.

BACKGROUND

You are being asked to take part in a research study. Before you decide, it is important for you to understand why the screening for this research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this screening process.

This screening process is being done to evaluate your eligibility to participate in research. The research study will evaluate brief high-intensity exercise breaks as a means to reduce a long bout of sitting. Such long bouts of sitting may negatively affect brain function and cognition. However, we do not know if interrupting long bouts of sitting with short exercise breaks could improve these functions and if exercise intensity matters. The screening is designed to evaluate if high-intensity exercise is safe for you and whether you meet our inclusion criteria based on your health history and cognitive tests.

SCREENING PROCEDURE

Your participation in this screening for a research study will include today's visit to the Physical Activity and Neurocognitive Health (PNC) laboratory. You may also need to see your primary care physician to ensure that high-intensity exercise is safe for you. If you are eligible, you will return to the laboratory for four study visits.

You have been asked to participate in this screening process because you indicated an interest in this research.

Scheduled Assessments

You will not be compensated for the screening procedures. However, if you qualify and participate in the study, you will be compensated up to $250 if you complete the entire study.

Screening Phone Call

During the phone call the researcher will ask you questions about your age, English language fluency, physical function, physical activity, sitting habits, and how you feel. S/he will also ask whether you can engage in vigorous exercise. To ensure that your participation in the study is safe, s/he will also ask questions about your health. For example, whether you had a stroke or a transient ischemic attack or a long COVID-19, and if you smoke. Next, s/he will ask you questions that let us know how you think, pay attention, know a few common facts, and remember words. If you qualify based on these questions, you will also complete a general health history questionnaire. You will answer questions about your cardiovascular history, including a history of heart disease and common signs and symptoms of cardiovascular disease. The questionnaire will ask about other health conditions that may increase

cardiovascular risk such as type 2 diabetes or increase the risks of high-intensity exercise such as pulmonary disease (e.g., COPD). Other conditions will include condition that may affect how you think such as epilepsy (i.e., having seizures) or a traumatic brain injury. Next, you will complete a Physical Activity Readiness Questionnaire. If you qualify based on these procedures, a researcher will ask if you agree for our research staff to contact your physician to ask for medical clearance for you to participate in a maximal exercise test on a stationary bicycle and high-intensity exercise during the intervention. If you are taking beta-blockers, s/he will also ask if you consent for the research staff to ask for your doctor's consent for you to discontinue your beta-blocker medication for 24 hours before the exercise test to ensure that the test accurately represents your heart responses to the maximal exercise test. If you do, you will sign the release of information form, which indicates that you are happy for us to contact your physician and include information that we collected about your heath history to help your physician decide if it is safe for you to engage in exercise test and high-intensity exercise in our study. It will also indicate that you are happy for your doctor to indicate their consent for you to discontinue beta-blockers for 24 hours before the exercise test. Your physician will be asked to confirm that you do not have type 1 or type 2 diabetes and that you did not have type 1 or 2 diabetes in the past. S/he will also confirm that your fasting glucose levels are below the threshold for diabetes in the last 12 months, and that your cholesterol levels are within normal range. If your physician is not able to confirm your blood sugar levels in the last 12 months but you otherwise qualify for an in-person screening visit based on the information provided to us today and by your physician, the research staff will measure your fasting glucose levels in the laboratory during your screening visit. Your physician will also confirm whether you are receiving or have received in the past cancer treatment, if your treatment included chemotherapy, and whether you have been cancer free for more than 12 months. If your physician clears you for participation in the maximal exercise test and study participation, we will invite you to an in-person screening visit at the PNC laboratory at Freer Hall in Urbana.

Today's phone call will last approximately **1 hour and 15 minutes**.

In-person Screening Visit 1 at the PNC Laboratory

Once we receive medical clearance from your physician, a researcher will contact you to confirm the time and date of your in-person screening visit. At the beginning of the visit, you will receive a heart rate monitor to wear around your chest. This is a strap with a single sensor that goes over your sternum and is attached snugly with an elastic belt. If your physician could not confirm your blood glucose levels in the last 12 months, a trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose. Next, the researchers will measure your height and weight. Then, you will rest for 5 minutes, and a researcher will take your blood pressure three time and your heart rate. You will also complete a questionnaire about your hand preference, about how you feel, and demographics questionnaire. Next, a trained researcher will ask you questions to measure your attention, memory, and language. You will complete patterns based on pictures, solve riddles, and tell the researcher the meaning of specific words. You will then complete two questionnaires about your exercise levels and activities you like to engage in. Then, a researcher will place electrocardiogram electrodes around your chest and on the side of your waist. They will collect electrocardiogram data while you rest for 10 s while lying down and standing. Next, you will cycle on a stationary bike for about 8 to 15 minutes until you cannot cycle any longer so that we can measure your aerobic fitness. The study physician will monitor the electrocardiogram during exercise to observe your heart's responses to exercise. He will also measure your blood pressure regularly during the test. This test will take place at Freer Hall on the University of Illinois campus. The amount of time you will cycle on the cycle ergometer will vary but most people cycle approximately 8-15 minutes. If you discontinued your beta-blockers for 24 hours before the test, a

researcher will remind you to take your medication after the test. Total time commitment for this appointment is approximately 2 hours and 30 minutes.

Assessments and Screening Requirements

Phone Screening

You will answer questions about your health, physical activity, sitting and complete a short test measuring your attention and memory.

- ***General Health History*** –You will also complete a questionnaire asking about your general health history. These questions will be kept confidential and will only be used to determine whether or not it is safe for you to participate in our study. For example, we will ask about your cardiovascular health, recent medical events (e.g., hospitalization), current medication, and lifestyle habits. All information you provide to us is strictly confidential. If you qualify for the study, we will keep this information as part of your confidential record. We will also ask about food allergies, your vision, hearing, and your medications.
- ***Questionnaires*** – You will complete a questionnaire that asks about your lifestyle and a physical activity readiness questionnaire. You will also complete questionnaires about how you feel and which hand you use for most daily tasks such as writing, brushing your teeth, etc.
- ***Physician's Release and Medical Clearance*** – To qualify for the study, you will be required to provide documentation from a physician regarding the exercise and research testing. The HIIT-2-SITLess research staff will ask for your permission to contact and send information to your primary care physician regarding your participation in this research. The physician must be willing to provide documentation indicating that you are cleared to participate in high-intensity exercise. If your physician determines that a physical examination is necessary or that you need to be seen by your oncologist (if you had the history of cancer) before clearing you for participation, then you or your insurance company will be responsible for all costs associated with such an exam or a visit to the oncologist.
- If you are taking beta-blockers we will ask for your permission to ask your physician to determine if it is safe for you to discontinue the medication for 24 hours before the exercise test.
- We will ask for your permission to share your medical history information with your primary care physician to help them determine your eligibility to participate in this research. However, you can opt out from sharing your medical history with your primary care physician. You will still need medical clearance from your primary care physician to participate in the study.

In-person Screening Visit at the PNC Laboratory

- ***Height, weight, and waist circumference***
  - A researcher will measure your height, weight, and waist circumference three times.
  - We will measure your waist in three points: the natural waist (the narrowest point), the umbilical (just above your navel), and around your hips.
- ***A finger prick***
  - A trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose using a point-of-care glucometer.
- ***Blood pressure and heart rate at rest***

- You will wear a heart rate monitor around your chest. After a 5 min rest, a researcher will measure your blood pressure and heart rate 3 times.

- ***Questionnaires*** – You will complete a demographic questionnaire. We will also ask questions about cognitive activities that you like to better understand your sitting habits, and about which hand you prefer to use for different activities to determine your hand preference.
- ***Cognitive and Neuropsychological Tests*** – You will complete a short cognitive test and a longer neuropsychological test where you will be asked about the meaning of words, you will choose patterns that best fit a picture, and solve some riddles.
- ***Graded Exercise Test*** - At this appointment, you will cycle on a stationary bike to measure your aerobic fitness. We will do that by measuring the air you exhale while you are cycling on a stationary bike. You will pedal at a constant speed while the researcher periodically increases the workload until you feel as if you cannot cycle any longer. You will be monitored by a physician and several exercise specialists certified in CPR and First Aid. We will measure oxygen through a mask that will collect your exhaled air. Your heart rate will be measured regularly through a 10-lead electrocardiogram chest monitor and your blood pressure will be taken several times throughout the test. The test including resting state electrocardiogram measures and preparation time will last about an hour. However, you will only cycle for about 8 to 15 minutes.

RISKS

Risks for blood collection: The blood sample collection is very common and involves minimal risk. There is a one in five chance of bruising in the area of sampling. This is generally not serious and will completely disappear within a few days. As with all invasive procedures, there is a slight risk of inflammation and infection. There is also risk of callus formation. This risk will be minimized by the use of sterile procedures and equipment at all times. Risk will also be minimized because a trained researcher will draw all blood samples.

Risks of exercise testing: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the maximal exercise test on the cycle ergometer is age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in the exercise test. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified. In addition, the maximal exercise test is supervised by a physician trained in supervising maximal exercise tests using the electrocardiogram, blood pressure readings, heart rate and observing how participants respond to the test.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section “How will the researchers protect my information”.

BENEFITS

Participating in the screening process is unlikely to have a direct benefit to you. We also cannot promise any direct benefit for taking part in this study if you qualify. However, previous research has shown that interrupting continuous sitting with short bouts of exercise can transiently improve sugar metabolism in adults (over several hours). We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this study may help develop a greater understanding of how interrupting sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will also help us understand if older adults are likely to use short, high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the screening procedures for HIIT2SITLess study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. The blood samples will be used only to determine the levels of fasting glucose as an inclusion criterion for the study. This identifying information will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Your primary care physician, if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Your primary care physician; with your consent, we will send a health history questionnaire to your primary care physician to assist them with medical clearance.

Participation in this screening process is voluntary, and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state, or local laws require. Some examples are laws that require reporting of

child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers, or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or screened by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, the researchers will contact you to let you know what they have found.

We will destroy the blood sample after we know your eligibility for the study. If you qualify and consent to participate in the study, we will retain all other data collected during screening. However, if you withdraw early from the screening process or choose not to participate in the study before you enroll, your data will be securely destroyed. To ensure confidentiality and anonymity during the screening, you will be assigned a numeric code and identified by this number only. We will keep the master list on the hard drive of a password-protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes, not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap compliant with the Health Insurance Portability and Accountability Act of 1996. Only the research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students, or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

If you qualify and consent to participate in the study, we will keep the information we collect about you during this screening for record keeping and for potential use in future research projects and training of junior researchers. As a research participant in this study, you consent to the use of your data for this study and future research by others. We will keep private information about you confidential to the extent allowed by laws and university policies. When researchers publicly discuss or publish the results of this research, they will not tell anyone that you were in the study. However, government or university officials who are responsible for monitoring this study and journal staff who review the research results for accuracy may see information that identifies you, including your signed consent form. If you give us your permission, we will use de-identified data from this study for use in future research studies. We will not ask for your additional informed consent for these studies. Your name and other information that can directly identify you will be stored securely and separately from the rest of the research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data. If you do not qualify or you do not consent to be enrolled in the study, we will destroy the information we collect about you during screening.

With your consent, the PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

If you have questions, complaints, or concerns about this screening process for the HIIT-2-SITLess study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached from Mondays to Fridays, 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or email OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously, or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate in this screening, you are free to withdraw your consent and discontinue participation at any time. You can start the screening process and then choose to stop the screening later.

This will not affect your relationship with the investigators. The researchers also have the right to stop your participation in this screening without your consent if they believe you do not qualify for the study, it is in your best interest, and/or if you were to object to any future changes that may be made in the screening and study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in this screening process is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination or a visit to an oncologist (if you had the history of cancer) in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will not be paid for taking part in this screening.

The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study, nor will the University of Illinoisprovide compensation for any injury sustained as a result of participation in thisresearch study, except as required by law.

If you qualify based on the screening phone call and Screening Visit 1, you will receive $30 compensation for your exercise test and information about your current levels of aerobic fitness.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.

- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
  - Limited information may also be shared with first responders from Emergency Medical Systems (EMS) to assist them with medical treatment; this information may include your name, address, emergency contact, your physician's contact details, age, information about your cardiovascular risk history, current medications, and description of the event;
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ and/or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this screening process for the HIIT-2-SITLess trial.

Optional

I consent for my General Health History questionnaire to be shared with my primary care physician to assist them with medical clearance for the study.

❑ Yes ❑ No

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Material 2. Study Consent

Consent and Authorization Document

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT-2-SITLess Trial

You have indicated an interest in participating in research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the feasibility and utility of short exercise bouts to reduce long sitting over several hours. This is a short-term study where you will be asked to visit the PNC laboratory three times over approximately five to seven weeks. You will also wear activity monitors for two weeks in between visits. *The risks* of this study include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise outweigh the risks. As preventive measures, you will need a medical clearance from your physician to participate in this research. Based on your responses to the maximal exercise test which was monitored by a study physician, you were considered to be at a lower risk of such events. Our research staff will also monitor your heart rate and physical responses to exercise (such as how hard you are working out and if you experience any unusual pain, fatigue etc.) during and after exercise. All our research staff are CPR and First Aid certified. If you agree to take part in this study, there may or may not be transient health benefit to you. Specifically, *breaking long sitting with short bouts of exercise has been shown to improve sugar metabolism* over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function.

BACKGROUND: THE HIIT-2-SITLESS TRIAL

This research is funded by the National Institute on Aging. You are being asked to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this study.

This research is being done to evaluate brief high-intensity exercise breaks to sitting. Although long bouts of sitting may attenuate brain function, we do not know if breaking long sitting with short bouts of exercise could improve brain function and if exercise intensity matters. The HIIT-2-SITLess trial will assess if short high-intensity exercise breaks to sitting are acceptable, and practical to older adults as means of reducing long periods of sitting. The trial will also compare changes in brain function and cognition after three and half hours of sitting interrupted with 6-min of high-intensity exercise breaks relative to 6-min of light-intensity exercise.

You have been asked to participate in this research because you are 40-75 years old and met inclusion criteria, we reviewed over the phone and during your screening visit, such as being right-handed, not exercising regularly, planning to be in the Urbana-Champaign area for the duration of the study, etc. Approximately 54 participants will be involved in this study at the University of Illinois.

STUDY PROCEDURE

Your participation in this study will last about eight weeks. You will come to the laboratory on four occasions and wear activity monitors for the total of two weeks between study visits.

Study Logistics

Scheduled Assessments

You will be compensated for these scheduled assessments and the compensation amounts are stated later in this document.

1. **Baseline Visit: Cognitive Tasks, HIIT, and LIIT Breaks Practice and Questionnaires**
   - ***Blood pressure and heart rate.*** Researchers will give you a heart rate monitor to wear around your chest. The monitor has a small flat electrocardiogram electrode inside a plastic casing attached to an elastic strap that goes around your chest. The monitor will sit in the center of your chest, and researchers will place electroconductive gel on the strap to enhance the connection between the electrode and the electrical signal from your heart. Researchers will also measure your resting blood pressure three times to monitor for high blood pressure that could prevent you from exercising.
   - ***HIIE and LIIE Practice Session***. During this visit, you will also practice high-intensity and low-intensity interval training breaks supervised by our research staff. This ensures that you feel comfortable with exercise and that we know the cadence (speed) and workload that can elicit your target heart rate during exercise. You will also fill in questionnaires (described below).
   - ***Questionnaires.*** To help us better understand your responses to high-intensity exercise, we will ask you to complete a set of questionnaires about your physical activity, enjoyment of physical activity, physical function, sleep, and sitting behaviors.
   - ***Cognitive tasks practice.*** To help you get used to cognitive tasks, you will practice two tasks on a computer for 12 minutes each. You will complete two tasks by pressing buttons on a response pad based on task instructions. You will see asterisks and letters come up on the computer screen one by one. You will need to look away from an asterisk to "catch" which letter just appeared. You will also see arrows on the screen and will have to press a button, which corresponds to the direction of a middle arrow. You will practice tasks in between physical exercise breaks.
   - Total time commitment for this appointment is approx. **2 hrs.**

2. ***High-Intensity Exercise Intervention***
   You will be asked to participate in a half-day intervention designed to minimize long bouts of sitting by cycling on a stationary bike at a high intensity every 30 min. You will come

to the laboratory in the morning fasted. This means not eating or drinking (plain water is ok) for 8-10 hours before your visit. Trained researchers will measure your resting blood pressure and heart rate. You will also wear a chest strap with a heart rate monitor and two activity monitors to measure your sitting and physical activity. You will then complete a 24-h dietary recalls, and questionnaires about sleepiness. Next, you will eat a light breakfast, which is nut free oats and seeds bar. You will then complete the same neurocognitive tests as during your EEG visit. You will start the intervention after these neurocognitive assessments. You will sit for three hours and a half hours while you complete light administrative tasks and read popular science articles. For example, you will plan a family vacation and read articles about how snowflakes form or how whales help cool the earth. You will answer brief questions about how engaging and difficult these activities are. You will also answer questions about your energy levels, and fatigue. Every 30 min, the researchers will measure your blood pressure and heart rate before and after you complete a 6-minute high-intensity exercise (HIIE) break. You will also complete a shorter neurocognitive assessment twice during sitting: 15 min after the first and the third HIIE break. You will sit in a wheelchair while a researcher will push the wheelchair to the cycle ergometer. The neurocognitive assessment will last 15 min. You will then complete another HIIE break. You will complete five HIEE breaks in total. During each break, researchers will ask you questions about the levels of physical effort, and about how you feel. Researchers will monitor your blood pressure and heart rate after each break. After the last break, a researcher will transport you back to the EEG equipment and fill in the electrodes with gel while you sit and eat the second light meal (a similar oats and seeds bar). You will then complete the same set of cognitive tests as before the intervention while researchers record EEG signal. If this is your first intervention visit, you will receive two activity monitors to take home. If it is your last visit, you will complete a questionnaire about your experience in the study, and your participation will be complete. Total time commitment: **approx. 6 hours.**

EEG. You will be asked to visit Freer Hall where you will undergo neurocognitive assessments. You will wear an electroencephalography (EEG) cap that looks like a swim cap with small electrodes located throughout the cap. The researchers will fill in the electrodes with electroconductive gel so that we can measure tiny electric currents produced by your brain while you rest and complete cognitive tasks. We will first record your brain activity while you rest with your eyes open and closed for six minutes. You will then perform various cognitive tests on a computer that assess attention, executive function, and memory. For example, for one of the tests you will see pictures of common objects. Next, you will see more pictures. For each picture you will have to indicate if you saw it before or not. You will complete assessments again after the intervention is finished. In addition, you will complete one of the tasks twice during the intervention.

Cognitive Tasks. In addition to the two tasks that you practiced during the baseline visit, you will also complete a task where you will see pictures of objects. You will make judgments about pictures, such as determining if they represent an indoor or an outdoor object.

High-Intensity Interval Exercise Break: First, you will cycle at the same speed as during the maximal exercise test with progressively increased resistance for 1 min to warm up. Next, we will increase resistance on the bike to the same level at which your heart rate increased to 90% of its maximum during exercise test. These short bouts are designed to be very hard and you will cycle at this speed and resistance for 2 minutes. Then, you will rest while sitting still on the bike for 1 minute. Next, you will cycle again for 2 minutes at the same high intensity. At least two CPR and First Aid certified researchers will assist you and monitor your heart rate throughout exercise and ask you to rate how tired your body feels due to exercise and how you feel overall during each HIIE break. Finally, you will sit again and continue with either administrative activities or reading. You will complete **5** HIEE **breaks** lasting **6 min each** for **a total of 30 min** of exercise.

3. ***Low-Intensity Interval Exercise Intervention***
You will complete all the same procedures and tests as during the HIIE intervention day except for high-intensity exercise breaks. Instead, you will complete 6-min low intensity interval exercise breaks (LIIE).

Light-Intensity Interval Exercise Break. You will first warm up by pedaling for 1 min with minimal workload at an intensity of about 50% of your maximum heart rate. Next, you will pedal at a higher speed and workload chosen to elicit light intensity or about 57-60% of your maximum heart rate which is considered very light to fairly light intensity. You will then rest and remain stationary on the cycle ergometer for 1 minute, followed by another 2 minutes pedaling at the speed and workload to elicit the same heart rate. Total time commitment: **approx. 6 hours.**

Randomization

We will assign the order of two exercise interventions randomly at baseline. This means that the order in which you will complete HIIE and LIIE interventions, will be chosen by chance, like flipping a coin. Neither you nor the study team will choose which intervention you will complete first. The study team will let you know which exercise intervention you will complete first on the day of your first intervention visit. All participants will be asked to participate in all testing procedures.

Additional Assessments and Study Requirements

1. ***Questionnaires*** – You will be asked to complete one packet of questionnaires related to your physical abilities, physical activity, sedentary activities, sleep, diet, attitudes, thoughts, and feelings. The packet should take approximately 45-60 minutes and you will be able to complete it during the Baseline visit while you practice HIIE and LIIE breaks. At the end of the second intervention visit, you will also fill in a brief questionnaire about your experiences of the intervention.
2. ***Accelerometers*** – You will be asked to wear two activity monitors for seven days on two occasions, a week before each intervention visit. The first device is about the size of a pocket watch, similar to a pedometer. It is worn around your waist during waking hours and sleep except for bathing and showering. You will wear a second device on your thigh. The device is called activPAL. It is small and flat, similar to a flat piece of a domino. This device will measure how much you sit and stand. It is attached to your thigh with transparent film dressing.

You can wear this device while bathing or showering. You will also fill out an activity and sleep log to indicate hours of the day you wore the devices, when you went to sleep, and when you woke up. The devices do not track GPS or geographic data. They will only record the movement and sitting.

3. ***Health and Demographics Questionnaire*** – You already completed this questionnaire during screening. It has become part of your research record.
4. ***General Health History*** - You also provided information about your health history during screening call, which has become the part of your research record.
5. **Physician's Release and Medical Clearance** – Before qualifying, you provided documentation from a physician regarding the exercise and research testing, which is also part of your research record.

Additionally, the investigators may contact you in the future regarding other research at this institution.

You may opt out of these communications and opportunities at any time.

RISKS

Risks of high-intensity exercise participation: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the exercise breaks have been designed to offer activities that are safe and age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in these activities. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified.

Risk of EEG: In rare instances, some individuals have reported some discomfort from the EEG cap. If this occurs, we will take the cap off and re-schedule the visit.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section "How will the researchers protect my information".

BENEFITS

We cannot promise any direct benefit for taking part in this study. However, previous research has shown that interrupting long sitting with short bouts of exercise can improve sugar metabolism in adults over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this

study may help develop a greater understanding of how breaking long sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will help us understand if older adults are likely to use short high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. You understand that the blood samples will be used only to determine the levels of fasting glucose as the inclusion criterion for the study. Information that could identify you will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Primary care physician if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Primary care physician (PCP) with participant's consent we will send health history questionnaire to PCP to assist them with medical clearance;

Participation in this project is voluntary and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state or local laws require. Some examples are laws that require reporting of child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or seen by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, such as your fasting glucose levels, the researchers will contact you to let you know what they have found.

We will destroy the blood sample collected during screening after we know your eligibility for the study. We will retain all other data collected in the course of the study. For example, if you withdraw early from the study, your data will be retained for the analyses. To ensure confidentiality and anonymity during the study, you will be assigned a numeric code, and identified by this number only. We will keep the master list on the hard drive (or SSD) of a password protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap, which is compliant with Health Insurance Portability and Accountability Act of 1996. Only research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

We will keep the information we collect about you during this research study for record keeping and for potential use in future research projects and training of junior researchers. Your name and other information that can directly identify you will be stored securely and separately from the rest of the

research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data.

The PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

Example: If you have questions, complaints, or concerns about this study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached during Mondays to Fridays 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or e-mail OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time. You can start the study and then choose to stop the study later. This will not affect your relationship with the investigator. The researchers also have the right to stop your participation in this study without your consent if they believe it is in your best interests, you were to object to any future changes that may be made in the study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in the study is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will be paid for taking part in the *scheduled* appointments described above. As an incentive, and appreciation for contributing your time to this study, you will be paid a stipend as indicated in the table below. Thus, you will receive up to *$250* if you complete all the study assessments. The total time commitment for these scheduled appointments is approximately 16.5 hours.

Appointment	Time	Stipend	Location
Neurocognitive tests + HIIE + LIIE Practice	2 hrs.	$50	Freer Hall
HIEE Intervention	6 hrs.	$100	Freer Hall
LIIE Intervention	6 hrs.	$100	Freer Hall

If you live more than 10 miles away from the study site, we will reimburse the cost of your travel in the amount of $0.625 per mile.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.
- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.

- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with collaborators at Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this study.

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Table 1. Name and contact information for the trial sponsor

Trial Sponsors:	University of Illinois Urbana-Champaign Sponsor's Reference: 1376000511A6 Federal Employment Identification Number Contact Name: Paul N. Ellinger, Comptroller Address: 1901 S. First Street, Suite A Telephone: 217-333-2187 Email: spa@illinois.edu

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Table 2. Trial registration data

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov No. NCT06243016
Date of registration in primary registry	2024-02-05
Secondary identifying numbers	IRB24-0010, 1R21AG080411-01A1
Source(s) of monetary or material support	National Institute on Aging
Primary sponsor	University of Illinois Urbana-Champaign
Secondary sponsor(s)	Northeastern University, National Institute on Aging
Contact for public queries	Dominika M Pindus, Ph.D.
Contact for scientific queries	Dominika M Pindus, Ph.D.
Public title	Breaking Sitting With High-intensity Interval Training for Brain Health (HIIT2SITLess)
Scientific title	Breaking prolonged sitting with high-intensity interval training to improve cognitive and brain health in older adults – a pilot feasibility trial
Countries of recruitment	USA
Health condition(s) or problem(s) studied	Prolonged sitting, high-intensity interval training bouts, frontoparietal function, inhibitory control and episodic memory
Intervention(s)	*Active comparator*: 6-minute high-intensity interval training (every 30 minutes over 3.5 hours of sitting) *Passive comparator*: 6-minute low-intensity interval training (every 30 minutes over 3.5 hours of sitting)
Key inclusion and exclusion criteria	Age 40-70 years, BMI < 40 kg/m^2, sedentary (≥ 6 hours of sitting per day), low to moderately physically active (based on IPAQ Short Form), capable to engage in vigorous exercise (PARQ+), medical clearance from primary care physician, normotensive, IQ ≥85, fasting plasma glucose < 126 mg/dL, good or corrected vision and hearing, no significant abnormalities on the ECG during a maximal exercise test, no signs or symptoms suggesting of underlying cardiovascular disease as recorded during maximal exercise test, no indications to prematurely stop the maximal exercise test as per ACSM's Guidelines for Exercise Testing and Prescription.
Study type	Interventional
Date of first enrolment	February 2024
Target sample size	54
Recruitment status	Recruiting
Primary outcome(s)	Change in task-evoked brain activity (P3b component)
Key secondary outcomes	Change in cognitive functions, change in resting state and task evoked brain activity

Notes. ECG: electrocardiogram, IPAQ Short Form: International Physical Activity Questionnaire Short Form, PARQ+: Physical Activity Readiness Questionnaire for Everyone, IQ: intelligence quotient.

Supplementary Table 4. Version history

Item	**Item Details**
Original IRB Protocol Number:	IRB24-0010
Original IRB Approval Issue Date:	02/20/2024
Protocol amendment number:	3
Protocol amendment approval date:	
Authors:	*DMP, RJS*
Revisions chronology	
Amendment 1 Approval Date:	04/20/2024
Amendment 1 changes:	Use of point-of-care glucose monitor instead of a venous blood sample and research-grade glucose reader.
Amendment 2 Approval Date:	05/28/2024
Amendment 2 Changes:	Expanding research staff who can perform a finger prick to collect a blood sample
Amendment 3 Approval Date:	09/13/2024
Amendment 3 Changes:	Age range change from 60-75 to 40-75 years. Amendment to inclusion criteria; shorter screening and baseline protocol.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no. / Table / Figure
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4, 9
	2b	All items from the World Health Organization Trial Registration Data Set	Suppl. Table 2
Protocol version	3	Date and version identifier	Suppl. Table 3
Funding	4	Sources and types of financial, material, and other support	27
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2
	5b	Name and contact information for the trial sponsor	Suppl. Table 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	27
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	23
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-9
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	8-9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, Table 1 (p.45-48)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	16-21, Table 2
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	21-22
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	16
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	17-20, 22-23

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	24
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10-11
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	23-24

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10-11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	23-24
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	24-25
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	24-25
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	24-25
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	23-24
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	24
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	21, 22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25-26
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	23-24, 25-26, Suppl. Table 3
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	25-26
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	23-24
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	28
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	25
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	21
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	25
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	25
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl. Material 1 and 2

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the 1 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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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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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

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 aging. Physical activity (PA) interventions can enhance FC in these networks, but these interventions are not designed to decrease ST among older adults. Prolonged sitting (i.e., sitting continuously for ≥ 20 minutes) can acutely reduce frontoparietal brain function and attentional control, while a single PA bout lasting at least 20 minutes can enhance them. It has been theorized that stimulation of the cerebral norepinephrine release through peripheral increase in catecholamines may explain this effect. In contrast, the effects of shorter (< 10 minutes) PA bouts used to interrupt prolonged sitting on neurocognitive functions remain poorly understood. This pilot randomized crossover feasibility trial capitalizes on PA intensity as the major limiting factor in peripheral catecholamine increase and tests the effects of interrupting prolonged sitting every 30 minutes with 6-minute high-intensity interval training (HIIT) compared to low-intensity interval training (LIIT) bouts. The study will address three aims: (i) to assess feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function in middle-aged and older adults; (ii) 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); (iii) 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: Fifty-four 40-75-year-old healthy adults will be recruited from the local community and randomly assigned to a condition sequence (HIIT, LIIT versus LIIT, HIIT). Each HIIT bout comprises a 1-minute warm-up, 2 minutes at 90% of the maximum heart rate (HR_{max}), 1-minute passive rest, and 2 minutes at 90% HR_{max}. During 2-minute intervals in LIIT, participants exercise at 57-60% of HR_{max}. The primary outcomes include the feasibility (recruitment and retention rates, percent of valid EEG data), acceptability of time commitment, HIIT bouts and neurocognitive assessments, fidelity (the intensity of HIIT breaks, percent of time spent sitting) and the amplitude and the latency of the P3b component of event-related brain potentials (ERPs) measured during the modified Eriksen flanker task at pre-tests, 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.

Clinical Trial Registration: ClinicalTrials.gov No. NCT06243016

Keywords: sedentary behavior, high-intensity interval training, cognitive functions, brain function, middle age, aging

Strengths and limitations

- The HIIT2SITLess study is a well-controlled randomized 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 episodic memory.
- This pilot feasibility trial recruits healthy middle-aged and older adults with a limited cardiovascular risk; hence, its generalizability 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 aging 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, characterized by a decreased ability to control distractions and correctly recall the details of information and events (i.e., episodic memory).[2–4] These cognitive functions are indispensable for everyday functioning and 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 aging.

Yet, effective and scalable interventions to improve cognitive and brain health in older adults are lacking. Traditional physical activity (PA) interventions (e.g., a 20-40-minute 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 minutes.[8] In contrast, the efficacy of PA interventions that utilize short (<10 minutes) 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 sedentary time and prolonged sitting, such as sitting continuously for 20 minutes or longer, may attenuate attentional control,[12,13] episodic memory,[14] and frontoparietal function.[15] For example, 21-45-year-old adults with more prolonged sedentary time 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 minutes, 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 electroencephalographic (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 aging 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- 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 aging. 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 optimizes 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 aging[52] and AD.[53] Thus, PA interventions designed to stimulate the LC-NE system could significantly impact the functional integrity of the aging brain.

High-intensity interval training (HIIT) could stimulate the LC-NE system because it utilizes 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-minute delay.[58,59] However, the effect of a single bout of HIIT on cognitive function declines after 20-30 minutes.[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 minutes) 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 minutes) 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 (i.e., light or moderate) to stimulate the LC-NE system within 2-5 minutes.[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 randomized crossover pilot feasibility trial designed to test three specific aims:

(i) To assess the feasibility, acceptability, fidelity, and safety of HIIT breaks to improve neurocognitive function.

(ii) To quantify the differences between conditions in a change in P3b amplitude and latency, a marker of frontoparietal function.

(iii) 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 default mode networks.

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 randomized crossover trial with two interventions lasting 3.5 hours each: prolonged sitting interrupted every 30 minutes with 6-minute HIIT bouts active condition and prolonged sitting interrupted every 30 minutes with 6-minute 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 provide written informed consent in accordance with the Institutional Review Board at the University of Illinois Urbana-Champaign (see Supplementary Table 1 for sponsor details).

Trial registration

The trial was registered on ClinicalTrials.gov No. NCT06243016 before the enrollment of the first participant. See Supplementary Table 2 for trial registration details.

Participants

The study will enroll 54 (27 female) middle-aged (40-59 years) and older (60-75 years) cognitively healthy adults from Champaign County, I, 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.[7,73]

Eligibility

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

Blinding and randomization

Fifty-four participants will be randomized to two condition sequences by a statistician following baseline assessments. Permuted block randomization generated using the PROC PLAN procedure (SAS Institute Inc., 2023)[74] is used, where sequences are randomized within a block of six participants to minimize the possibility of group imbalances due to dropout. Participants are randomized to one of two condition sequences by a study statistician: 1) X = HIIT, LIIT breaks or Y = LIIT, HIIT breaks. Generated permuted block randomization also ensures that blocks are balanced by cognitive task (i.e., flanker [F], antisaccade [A] and mnemonic similarities task [M]) sequence (FAM, MFA and AMF). The principal and co-investigators will be blinded to the sequence allocation. The sequence will be concealed until the participant's enrollment. Upon enrollment, the study sequence will be verbally communicated to the study coordinator by a statistician. The coordinator will record the sequence number in REDCap. The staff implementing the 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 enrollment 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, organizations serving older adults in Champaign County, and flyers, and individual mailouts to adults aged 40-75 years in Champaign County. Recruitment and enrollment 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 Supplementary Table 3.

Screening Procedure

Screening Call

At the beginning of the screening call, participants will sign an informed consent to the screening process (Supplementary 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 ischemic attack, long COVID-19, and smokers. A trained researcher will then administer the Telephone Interview of Cognitive Status-modified (TICS-m). Only individuals with a score < 32 (a cutoff for mild cognitive impairment)[75] 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,[76] and pre-existing conditions as listed in the exclusion criteria (Table 1). An individual will also fill in the Hospital Anxiety and Depression Scale.[77] Individuals with anxiety and depression will be included due to the high prevalence of these disorders in the general population.[78,79] 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 (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80] screens out highly physically active individuals who engage in 300 minutes or more of moderate-to-vigorous PA per week. The Physical Activity

Readiness Questionnaire+[81] 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: (i) exercise strenuously for 48 hours before the experimental visit, (ii) drink caffeine or (iii) 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 mmHg on the screening day will undergo the maximal exercise test because higher values are a contraindication to a maximal exercise test.[76] 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.[82] If the participant's physician cannot confirm fasting glucose levels or glycated hemoglobin levels (HbA1c) 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 (MoCA) to screen out individuals with scores < 26 suggestive of potential cognitive impairment .[83] A standardized test of cognitive abilities (Kaufman Brief Intelligence Test – 2; KBIT-2)[80] will be administered next, and individuals with a score < 85 (i.e., 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[84,85] with a 12-lead electrocardiogram. 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 to evaluate participants' physiological responses to exercise.[76] Its results will be used as an inclusion criterion to enhance the safety of acute high-intensity exercise. Three trained first aid and CPR-certified experimenters will conduct the test in collaboration with the study physician. Participants' resting BP, HR, and electrocardiogram readings will be collected. They will then warm up for two minutes while pedaling 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 minutes by 25 Watts. Males will start at 100 Watts and exercise at 50 Watts increments.[84,85] The participant will cycle until volitional exhaustion.[84,85] Their HR and ECG are continuously monitored, and blood pressure will be monitored every two minutes during exercise by a physician. Every two minutes, the study staff will record ratings of perceived exertion (RPE) using the Borg scale.[86] 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[76]: (i) respiratory exchange ratio (RER) ≥ 1.1, (ii) failure of the HR to increase with increasing workload (i.e., ≤ 10 bpm increase relative to age-predicted HR_{max},[87]) or (iii) RPE > 17. The test finishes with a 5-minute cooldown. If there are no positive findings on the electrocardiogram as described in the indications to stopping the maximal exercise test in the ACSM's Guidelines for Exercise Testing and Prescription,[76] 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 familiarize participants with the main intervention procedures, including cognitive tasks and HIIT and LITT bouts. Participants will first sign an informed study consent (Supplementary Material 2). The consent includes provisions for the de-identified data for use in future studies. A trained researcher will assess their resting BP to verify that systolic over diastolic BP is < 200/110 mmHg, which is a counterindication to

exercise.[76] Participants will then practice two LIIT and two HIIT bouts every 25 minutes. During each 25-minute block, they will complete a questionnaire battery and practice cognitive tasks described in detail under the intervention section.

Light-intensity-interval-training bouts (LIIT). Each LIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two low-intensity intervals, cycling at 57-60% of their maximum HR lasting two minutes and separated by a one-minute 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.

High-intensity interval-training bouts (HIIT). Each HIIT bout will last six minutes and comprise a one-minute warm-up (cycling at 50 rpm with no resistance), followed by two high-intensity intervals separated by a one-minute passive recovery (sitting on a cycle ergometer). High-intensity intervals comprise cycling for two minutes 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}. Participant's HR will be continuously monitored by the research staff in response to exercise and two minutes after exercise to confirm the drop in HR of at least 22 beats per minute, which indicates a normal HR response after exercise.[88,89] Participant's BP is also monitored 6 minutes after each bout of exercise to ensure that resting BP does not exceed the <200/110 mmHg threshold.[76] 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 minimize practice effects observed in previous studies.[90,91] The mnemonic similarity task 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 characterization of the study sample in relation to their habitual leisure-time exercise, types of

sedentary behaviors 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 contextualize 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, FL) to wear on their wrists, which monitor PA and sleep continuously 24/7 over one 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.[92] The information from accelerometers will be used as exploratory covariates in participants' responses to the intervention. Participants will also wear the devices for one week preceding the second intervention day. The data from both weeks will be compared to assess consistency in free-living physical behaviors 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. Upon

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 minutes. After the rest, their resting HR and BP are collected to verify that SBP/DBP is <200/110 mmHg. Participants are then fitted with an EEG cap. During the cap preparation, participants are provided with a light, standardized 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, 70-75), gender and BMI and accounting for 22% of their recommended daily energy intake.[93,94] After breakfast, they complete a 24-hour PA recall (Activities Completed over Time in 24 Hours; ACT24),[95] Karolinska Sleepiness Scale,[96] 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-minute rest while the EEG signal is collected. After the resting state EEG data collection, they will complete three cognitive tasks in a randomized 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 minutes with a 6-minute 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 minutes of LIIT or HIIT, depending on the condition. The order of the intervention conditions will be randomized across participants such that each participant will serve as his/her own control. HR and BP are monitored and recorded two and six minutes 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 minutes 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 minutes of sitting, participants will receive another standardized meal identical to the one received at the pre-test. After they consume the meal, participants engage in the exact same neurocognitive assessments as during the pre-test. 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 standardized set of home administrative tasks (e.g., planning a holiday, a birthday party, etc.) and read a standardized set of popular science articles from the New York Times. Activities will change every 30 minutes. Two sets of sedentary activities were developed, and their order was randomized 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 standardized 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[97–99] and Cognitive Effort[100] 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 vigor, they will self-report their energy, vigor, and fatigue on a validated Visual Analogue Fatigue Scale before every break.[101] We will also monitor participants' perceived enjoyment of PA during each condition with Physical Activity Enjoyment Scale to inform intervention acceptability.[102]

Cognitive tasks

Modified Eriksen Flanker task. Inhibitory control is measured using a modified Eriksen flanker task before, after, and twice during 3-hour sitting.[103] 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 center 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. Behavioral 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.[104] 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.[105] 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 two 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 (MST). Episodic memory is measured with a computerized MST.[106,107] Performance on this task is a good marker of hippocampal function[106] and is sensitive to the acute effects of PA in older adults.[108] An encoding phase will be administered first. Participants study 64 colored 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.[109] They complete a set of 192 objects. A lure discrimination index (LDI; probability of “similar”/“novel” judgments 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 (EEG)

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-minute rest at pre-test 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, NC) with four integrated bipolar electrodes for vertical and horizontal eye movements (VEOG, HEOG), 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 is an established marker of frontoparietal brain function embedded within the stimulus-locked event-related brain potential (ERP). Both the P3b amplitude and latency have been reliably modulated by acute exercise.[104] 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 (ERN). The effects of the intervention on other ERP components related to cognitive control will also be explored.[110] 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.[111] Attentional control is part of the cognitive control system.[112] The stimulus-locked N2-ERP component[110] 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.[110] Larger N2 amplitudes have been observed with successful conflict resolution and fewer commission errors.[113] This ERP component has been modulated by a single bout of sitting lasting 20 minutes in preadolescents such that a more negative N2 amplitude was observed during the flanker task (suggesting greater conflict) after a bout of sitting compared to a bout of moderate-intensity walking.[114] The 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.[115] The ERN can be modulated by acute exercise,[116] but its response to prolonged

sitting has not been investigated. Accordingly, neurofunctional responses underlying inhibitory control, which includes conflict monitoring, are measured in the HIIT2SITLess study.

Frontal N400 (FN400) and Late Positive Component (LPC). 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[117–119] and recollection.[120,121] 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.[122] Anterior-central FN400 is thought to index familiarity judgments because it varies with self-reported recognition confidence ratings.[117] 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[121] but not with their recognition confidence.[117] 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 (i.e., effective) FC in high temporal resolution,[123,124] using the weighted Minimum Norm Estimation (wMNE), a gold standard of source reconstruction, together with the Directed Transfer Function (DTF),[125,126] a technique that uses multivariate autoregressive modeling 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[127,128] but a single bout of PA can strengthen FC in both networks.[129] 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 behaviors 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 (i.e., 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. 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 is 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 posttest. 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.[130] 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 participant's responses to exercise and observes for signs and symptoms of hypoglycemia 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) Behavioral responses during the modified Eriksen flanker task

(2) Behavioral responses during the antisaccade task

(3) Behavioral 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 pre-test and post-test except for behavioral 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 Research Electronic Data Capture (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 six years upon 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 Supplementary 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 vs. 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 version 3.1.[131] 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.[132] 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 pre- to post-intervention comparison. Kamijo et al.[133] reported Cohen's d = 1.2 for the P3b latency in older adults using a single 20-minute bout of moderate intensity (expected to increase peripheral catecholamines).[65,134] 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,[135] we will fit general linear mixed-effects models.[136] (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 modeling 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: pre-, after break #1, after break #3, and posttest), 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 pre-, after the first and the third PA bout, and post-test assessments.[136] We will test intervention effects on secondary outcomes using the pre-test 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 posttest. We will include each outcome as a response variable, sequence, condition, and time as fixed effects and a participant-specific random intercept. Carryover (i.e., period) effects are assumed to be null based on the sufficient washout period of one week between treatments.[137] Results will be presented as mean differences between conditions in the Area Under the Curve 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*).[138]

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 upon request. Only de-identified data that support a published manuscript will be shared. All investigators involved in the development of the trial will be co-authors of any subsequent publications resulting from the trial.

Reporting guidelines

The study was designed in accordance with Standard Protocol Items for clinical trials (SPIRIT Statement), and its details are provided in Supplementary Materials.

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 upon 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 (Supplementary Materials 1 and 2). The data collected from participants will be used for research purposes. De-identified data can be used for future studies and training purposes.

DISCUSSION

The HIIT2SITLess randomized 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 minutes) PA bouts. We hypothesize that implementing 6-minute HIIT interruptions to prolonged sitting every 30 minutes will be feasible and acceptable over the 3.5-hour period to middle-aged and older adults. We also hypothesize 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 behavioral 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,[139] 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 (i.e., 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 to 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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Contributorship

DMP: Conceptualization, data curation, methodology, funding acquisition, project administration, resources, supervision, writing original draft; CHH, AFK, NAK: conceptualization, methodology, funding acquisition, resources; MW, TSL: methodology, software; SP, JS, JS, MK: methodology; JK: visualization; FBQ, RS, TS: data acquisition. All co-authors reviewed, edited, and approved the final draft.

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Supplemental material

Supplementary Materials are available online.

Open access

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HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 1. Study inclusion and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
• Age 40-75 years; including pre-, post-, and peri-menopausal women regardless of hormone therapy replacement.	• Physical disability or musculoskeletal disease prohibitive to vigorous exercise
• BMI <40 kg/m^2;	• Learning disabilities
• Sedentary (≥ 6 h/day sitting by a survey question);	• Cognitive abilities below a 26-point cut-off on a MoCA
• IPAQ Short Form score in the low and moderate physical activity range	• Type 1 or 2 diabetes
• Capable of exercising vigorously based on the PARQ+	• Neurological condition (e.g., MS, Parkinson, Dementia, MCI)
• Has a medical clearance for maximal exercise and HIIT from a physician	• Color blindness
• Normotensive or participant's blood pressure is controlled (i.e., 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 (e.g., traumatic brain injury, stroke)
• Intellectual ability no less than one standard deviation relative to the population mean (i.e., ≥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 (see General Health History, Cardiovascular History)
• No significant abnormalities on the ECG during the maximal exercise test	• A chronic pulmonary disease (e.g., chronic obstructive pulmonary disease; COPD)
• No signs and symptoms that suggest an underlying cardiovascular disease as recorded during the maximal exercise test by a study physician	• Emphysema

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

• 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 (e.g., attention-deficit hyperactivity disorder [ADHD], schizophrenia, etc.) • 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 Apnea • Acquired immunodeficiency syndrome (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 hospitalization in the last six 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

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 2. Psychosocial assessments

Name	Description
The Activities Collected over Time over 24-hours (ACT24)[140]	A 24-hour physical activity recall to measure participant's previous day physical activity, sedentary behaviors and sleep.
Beck Depression Inventory-2[141]	A 21-item inventory to assess attitudes and symptoms of depression in adults aged 18-80 years.
Cardiorespiratory Fitness Questionnaire[142]	A 5-item questionnaire to assess the level of aerobic fitness based questions about habitual aerobic exercise.
The Epworth Sleepiness Scale[143]	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 FDI-Disability[144,145]	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 FXN-Function[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 Function Self Efficacy 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 assess 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[77]	A 14-item questionnaire with questions about symptoms of depression, anxiety and psychological distress during the past week.
The Canadian Society for Exercise Physiology (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q)[80]	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[77]	A standardized and normed intelligence test for ages 4-90 years. The test comprises one verbal and two non-verbal components used to compute verbal and nonverbal IQ scores and a general IQ score.
Karolinska Sleepiness Scale (KSS)[96]	A one-item assessment of individual's subjective experience of sleepiness over the past 5 minutes.
Montreal Cognitive Assessment (MoCA)[83,150]	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[81]	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 (PSQI)[81]	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 Behavior Questionnaire (SBQ)[152]	An 18-item questionnaire assessing the time individuals spent in various sedentary behaviors on weekdays and weekend days.
Task Engagement Scale[97–99]	A 9-item scale assessing the level of physical, emotional and cognitive engagement in a task.
Mental Effort Scale[100]	A single item scale assessing the level of mental effort exerted during the task.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Table 3. Feasibility, missingness, fidelity, and acceptability outcomes

	Description
FEASIBILITY	
Recruitment rates*	*N* randomized/*N* screened[5]
Retention rates*	*N* randomized who successfully completed all conditions/*N* randomized[5]
Cognitive and EEG data	% of participants with fully completed pre- and post-intervention 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,* 2. Number of dropouts due to time commitment.
HIIT	(1) 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.

Figure captions

Figure 1. Study design.

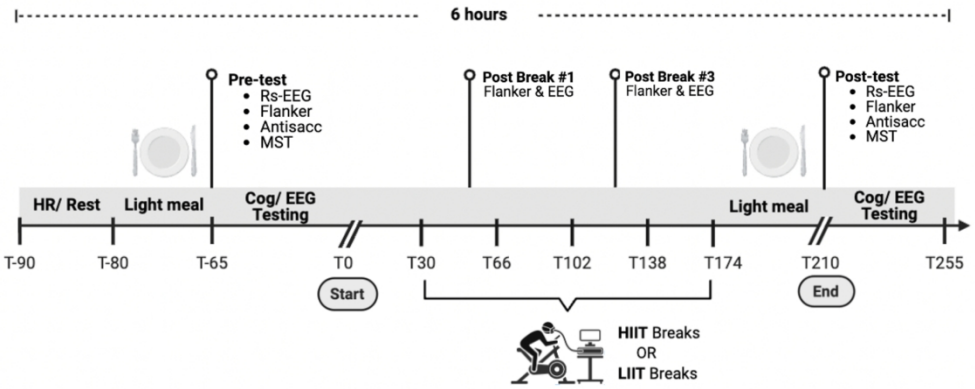

Figure 1. Study design.

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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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

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RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

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Dates of the study: 05/07/2024 – 07/31/2025

Supplementary Material 1. Screening Consent

Consent and Authorization Document

Consent to Screening Procedures

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT2SITLess TRIAL

You have indicated an interest in participating in a research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the utility of short physical activity breaks to sitting to reduce sitting and enhance cognitive and brain function in the short term (over several hours). This is a short-term study. If you qualify, you will be asked to visit our laboratory three times over approximately five to seven weeks and wear activity monitors for two weeks between visits.

KEY INFORMATION ABOUT THE SCREENING PROCESS

The screening procedures aim to assess your eligibility for the study. Today, a researcher will ask you questions about your age, physical activity, physical function, sitting habits, smoking, history of stroke or a transient ischemic attack, long COVID-19, your vision and hearing. The researcher will ask you questions and give you small tasks to measure how you think and how well you remember things. You will also complete a questionnaire about your general medical history, and questionnaires about your physical activity and how you feel. If you qualify based on this phone call, you will complete a medical clearance release form allowing the research team to contact your physician to determine if you can participate in high-intensity exercise. Today's call will last approximately 1 hour 15 minutes. We will also invite you to an in-person screening visit. During the screening visit, we will measure your height and weight, blood pressure, and heart rate. You will also complete several cognitive tests, a health and demographics questionnaire, and cycle at a maximal intensity on a stationary bike. The total screening time commitment for this visit is about **2.5 hrs**.

Risks of screening: Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. However, the risks include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise are known to outweigh the risks. As preventive measures, all participants need a medical clearance from their physician to participate in this research. Furthermore, our study physician will monitor you during a maximal exercise test and all our research staff are CPR and First Aid certified. The physician will observe the electric activity of your heart using an electrocardiogram. He will also measure your blood pressure throughout exercise. If you are taking beta-blocker medication, we will ask for your consent to discontinue the medication for 24 hours upon your physician's clearance

to do so, before the exercise test to ensure that the test can accurately assess your heart responses to the test. **Benefits of screening:** There are no direct benefits to you that come from screening. However, if you are eligible and agree to take part in this study, there may or may not be a transient health benefit to you. Specifically, breaking long sitting with short exercise breaks has been shown to improve sugar metabolism over several hours. We do anticipate that participation in this research may also result in transient (over several hours) benefit to cognitive and brain function.

BACKGROUND

You are being asked to take part in a research study. Before you decide, it is important for you to understand why the screening for this research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this screening process.

This screening process is being done to evaluate your eligibility to participate in research. The research study will evaluate brief high-intensity exercise breaks as a means to reduce a long bout of sitting. Such long bouts of sitting may negatively affect brain function and cognition. However, we do not know if interrupting long bouts of sitting with short exercise breaks could improve these functions and if exercise intensity matters. The screening is designed to evaluate if high-intensity exercise is safe for you and whether you meet our inclusion criteria based on your health history and cognitive tests.

SCREENING PROCEDURE

Your participation in this screening for a research study will include today's visit to the Physical Activity and Neurocognitive Health (PNC) laboratory. You may also need to see your primary care physician to ensure that high-intensity exercise is safe for you. If you are eligible, you will return to the laboratory for four study visits.

You have been asked to participate in this screening process because you indicated an interest in this research.

Scheduled Assessments

You will not be compensated for the screening procedures. However, if you qualify and participate in the study, you will be compensated up to $250 if you complete the entire study.

Screening Phone Call

During the phone call the researcher will ask you questions about your age, English language fluency, physical function, physical activity, sitting habits, and how you feel. S/he will also ask whether you can engage in vigorous exercise. To ensure that your participation in the study is safe, s/he will also ask questions about your health. For example, whether you had a stroke or a transient ischemic attack or a long COVID-19, and if you smoke. Next, s/he will ask you questions that let us know how you think, pay attention, know a few common facts, and remember words. If you qualify based on these questions, you will also complete a general health history questionnaire. You will answer questions about your cardiovascular history, including a history of heart disease and common signs and symptoms of cardiovascular disease. The questionnaire will ask about other health conditions that may increase

cardiovascular risk such as type 2 diabetes or increase the risks of high-intensity exercise such as pulmonary disease (e.g., COPD). Other conditions will include condition that may affect how you think such as epilepsy (i.e., having seizures) or a traumatic brain injury. Next, you will complete a Physical Activity Readiness Questionnaire. If you qualify based on these procedures, a researcher will ask if you agree for our research staff to contact your physician to ask for medical clearance for you to participate in a maximal exercise test on a stationary bicycle and high-intensity exercise during the intervention. If you are taking beta-blockers, s/he will also ask if you consent for the research staff to ask for your doctor's consent for you to discontinue your beta-blocker medication for 24 hours before the exercise test to ensure that the test accurately represents your heart responses to the maximal exercise test. If you do, you will sign the release of information form, which indicates that you are happy for us to contact your physician and include information that we collected about your heath history to help your physician decide if it is safe for you to engage in exercise test and high-intensity exercise in our study. It will also indicate that you are happy for your doctor to indicate their consent for you to discontinue beta-blockers for 24 hours before the exercise test. Your physician will be asked to confirm that you do not have type 1 or type 2 diabetes and that you did not have type 1 or 2 diabetes in the past. S/he will also confirm that your fasting glucose levels are below the threshold for diabetes in the last 12 months, and that your cholesterol levels are within normal range. If your physician is not able to confirm your blood sugar levels in the last 12 months but you otherwise qualify for an in-person screening visit based on the information provided to us today and by your physician, the research staff will measure your fasting glucose levels in the laboratory during your screening visit. Your physician will also confirm whether you are receiving or have received in the past cancer treatment, if your treatment included chemotherapy, and whether you have been cancer free for more than 12 months. If your physician clears you for participation in the maximal exercise test and study participation, we will invite you to an in-person screening visit at the PNC laboratory at Freer Hall in Urbana.

Today's phone call will last approximately **1 hour and 15 minutes**.

In-person Screening Visit 1 at the PNC Laboratory

Once we receive medical clearance from your physician, a researcher will contact you to confirm the time and date of your in-person screening visit. At the beginning of the visit, you will receive a heart rate monitor to wear around your chest. This is a strap with a single sensor that goes over your sternum and is attached snugly with an elastic belt. If your physician could not confirm your blood glucose levels in the last 12 months, a trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose. Next, the researchers will measure your height and weight. Then, you will rest for 5 minutes, and a researcher will take your blood pressure three time and your heart rate. You will also complete a questionnaire about your hand preference, about how you feel, and demographics questionnaire. Next, a trained researcher will ask you questions to measure your attention, memory, and language. You will complete patterns based on pictures, solve riddles, and tell the researcher the meaning of specific words. You will then complete two questionnaires about your exercise levels and activities you like to engage in. Then, a researcher will place electrocardiogram electrodes around your chest and on the side of your waist. They will collect electrocardiogram data while you rest for 10 s while lying down and standing. Next, you will cycle on a stationary bike for about 8 to 15 minutes until you cannot cycle any longer so that we can measure your aerobic fitness. The study physician will monitor the electrocardiogram during exercise to observe your heart's responses to exercise. He will also measure your blood pressure regularly during the test. This test will take place at Freer Hall on the University of Illinois campus. The amount of time you will cycle on the cycle ergometer will vary but most people cycle approximately 8-15 minutes. If you discontinued your beta-blockers for 24 hours before the test, a

researcher will remind you to take your medication after the test. Total time commitment for this appointment is approximately 2 hours and 30 minutes.

Assessments and Screening Requirements

Phone Screening

You will answer questions about your health, physical activity, sitting and complete a short test measuring your attention and memory.

- ***General Health History*** –You will also complete a questionnaire asking about your general health history. These questions will be kept confidential and will only be used to determine whether or not it is safe for you to participate in our study. For example, we will ask about your cardiovascular health, recent medical events (e.g., hospitalization), current medication, and lifestyle habits. All information you provide to us is strictly confidential. If you qualify for the study, we will keep this information as part of your confidential record. We will also ask about food allergies, your vision, hearing, and your medications.
- ***Questionnaires*** – You will complete a questionnaire that asks about your lifestyle and a physical activity readiness questionnaire. You will also complete questionnaires about how you feel and which hand you use for most daily tasks such as writing, brushing your teeth, etc.
- ***Physician's Release and Medical Clearance*** – To qualify for the study, you will be required to provide documentation from a physician regarding the exercise and research testing. The HIIT-2-SITLess research staff will ask for your permission to contact and send information to your primary care physician regarding your participation in this research. The physician must be willing to provide documentation indicating that you are cleared to participate in high-intensity exercise. If your physician determines that a physical examination is necessary or that you need to be seen by your oncologist (if you had the history of cancer) before clearing you for participation, then you or your insurance company will be responsible for all costs associated with such an exam or a visit to the oncologist.
- If you are taking beta-blockers we will ask for your permission to ask your physician to determine if it is safe for you to discontinue the medication for 24 hours before the exercise test.
- We will ask for your permission to share your medical history information with your primary care physician to help them determine your eligibility to participate in this research. However, you can opt out from sharing your medical history with your primary care physician. You will still need medical clearance from your primary care physician to participate in the study.

In-person Screening Visit at the PNC Laboratory

- ***Height, weight, and waist circumference***
  - A researcher will measure your height, weight, and waist circumference three times.
  - We will measure your waist in three points: the natural waist (the narrowest point), the umbilical (just above your navel), and around your hips.
- ***A finger prick***
  - A trained researcher will collect a blood sample from your fingertip to measure fasting capillary blood glucose using a point-of-care glucometer.
- ***Blood pressure and heart rate at rest***

- You will wear a heart rate monitor around your chest. After a 5 min rest, a researcher will measure your blood pressure and heart rate 3 times.

- ***Questionnaires*** – You will complete a demographic questionnaire. We will also ask questions about cognitive activities that you like to better understand your sitting habits, and about which hand you prefer to use for different activities to determine your hand preference.
- ***Cognitive and Neuropsychological Tests*** – You will complete a short cognitive test and a longer neuropsychological test where you will be asked about the meaning of words, you will choose patterns that best fit a picture, and solve some riddles.
- ***Graded Exercise Test*** - At this appointment, you will cycle on a stationary bike to measure your aerobic fitness. We will do that by measuring the air you exhale while you are cycling on a stationary bike. You will pedal at a constant speed while the researcher periodically increases the workload until you feel as if you cannot cycle any longer. You will be monitored by a physician and several exercise specialists certified in CPR and First Aid. We will measure oxygen through a mask that will collect your exhaled air. Your heart rate will be measured regularly through a 10-lead electrocardiogram chest monitor and your blood pressure will be taken several times throughout the test. The test including resting state electrocardiogram measures and preparation time will last about an hour. However, you will only cycle for about 8 to 15 minutes.

RISKS

Risks for blood collection: The blood sample collection is very common and involves minimal risk. There is a one in five chance of bruising in the area of sampling. This is generally not serious and will completely disappear within a few days. As with all invasive procedures, there is a slight risk of inflammation and infection. There is also risk of callus formation. This risk will be minimized by the use of sterile procedures and equipment at all times. Risk will also be minimized because a trained researcher will draw all blood samples.

Risks of exercise testing: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the maximal exercise test on the cycle ergometer is age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in the exercise test. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified. In addition, the maximal exercise test is supervised by a physician trained in supervising maximal exercise tests using the electrocardiogram, blood pressure readings, heart rate and observing how participants respond to the test.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section “How will the researchers protect my information”.

BENEFITS

Participating in the screening process is unlikely to have a direct benefit to you. We also cannot promise any direct benefit for taking part in this study if you qualify. However, previous research has shown that interrupting continuous sitting with short bouts of exercise can transiently improve sugar metabolism in adults (over several hours). We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this study may help develop a greater understanding of how interrupting sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will also help us understand if older adults are likely to use short, high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the screening procedures for HIIT2SITLess study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. The blood samples will be used only to determine the levels of fasting glucose as an inclusion criterion for the study. This identifying information will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Your primary care physician, if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Your primary care physician; with your consent, we will send a health history questionnaire to your primary care physician to assist them with medical clearance.

Participation in this screening process is voluntary, and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state, or local laws require. Some examples are laws that require reporting of

child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers, or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or screened by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, the researchers will contact you to let you know what they have found.

We will destroy the blood sample after we know your eligibility for the study. If you qualify and consent to participate in the study, we will retain all other data collected during screening. However, if you withdraw early from the screening process or choose not to participate in the study before you enroll, your data will be securely destroyed. To ensure confidentiality and anonymity during the screening, you will be assigned a numeric code and identified by this number only. We will keep the master list on the hard drive of a password-protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes, not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap compliant with the Health Insurance Portability and Accountability Act of 1996. Only the research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students, or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

If you qualify and consent to participate in the study, we will keep the information we collect about you during this screening for record keeping and for potential use in future research projects and training of junior researchers. As a research participant in this study, you consent to the use of your data for this study and future research by others. We will keep private information about you confidential to the extent allowed by laws and university policies. When researchers publicly discuss or publish the results of this research, they will not tell anyone that you were in the study. However, government or university officials who are responsible for monitoring this study and journal staff who review the research results for accuracy may see information that identifies you, including your signed consent form. If you give us your permission, we will use de-identified data from this study for use in future research studies. We will not ask for your additional informed consent for these studies. Your name and other information that can directly identify you will be stored securely and separately from the rest of the research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data. If you do not qualify or you do not consent to be enrolled in the study, we will destroy the information we collect about you during screening.

With your consent, the PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

If you have questions, complaints, or concerns about this screening process for the HIIT-2-SITLess study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached from Mondays to Fridays, 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or email OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously, or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate in this screening, you are free to withdraw your consent and discontinue participation at any time. You can start the screening process and then choose to stop the screening later.

This will not affect your relationship with the investigators. The researchers also have the right to stop your participation in this screening without your consent if they believe you do not qualify for the study, it is in your best interest, and/or if you were to object to any future changes that may be made in the screening and study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in this screening process is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination or a visit to an oncologist (if you had the history of cancer) in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will not be paid for taking part in this screening.

The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study, nor will the University of Illinoisprovide compensation for any injury sustained as a result of participation in thisresearch study, except as required by law.

If you qualify based on the screening phone call and Screening Visit 1, you will receive $30 compensation for your exercise test and information about your current levels of aerobic fitness.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.

- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
  - Limited information may also be shared with first responders from Emergency Medical Systems (EMS) to assist them with medical treatment; this information may include your name, address, emergency contact, your physician's contact details, age, information about your cardiovascular risk history, current medications, and description of the event;
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ and/or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this screening process for the HIIT-2-SITLess trial.

Optional

I consent for my General Health History questionnaire to be shared with my primary care physician to assist them with medical clearance for the study.

❑ Yes ❑ No

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Material 2. Study Consent

Consent and Authorization Document

Principal Investigator Name and Title: Dominika M. Pindus, Assistant Professor

Department and Institution: Kinesiology and Community Health, UIUC

Contact Information: 217-300-7317; pindus@illinois.edu

Sponsor: National Institutes of Health (specifically, National Institute on Aging), pending.

KEY INFORMATION ABOUT HIIT-2-SITLess Trial

You have indicated an interest in participating in research study conducted by Dr. Dominika M. Pindus at the University of Illinois Urbana-Champaign. The main goal of this research is to gain knowledge about the feasibility and utility of short exercise bouts to reduce long sitting over several hours. This is a short-term study where you will be asked to visit the PNC laboratory three times over approximately five to seven weeks. You will also wear activity monitors for two weeks in between visits. *The risks* of this study include a chance of incurring a minor injury and some discomfort due to intensified use of major muscle groups that have not received a great deal of use. However, no major injuries are anticipated. Cycling on a stationary bike has been shown to be a safe mode of exercise in older adults. There is also a very slim chance of serious cardiac events while exercising. This is very rare, and the benefits of exercise outweigh the risks. As preventive measures, you will need a medical clearance from your physician to participate in this research. Based on your responses to the maximal exercise test which was monitored by a study physician, you were considered to be at a lower risk of such events. Our research staff will also monitor your heart rate and physical responses to exercise (such as how hard you are working out and if you experience any unusual pain, fatigue etc.) during and after exercise. All our research staff are CPR and First Aid certified. If you agree to take part in this study, there may or may not be transient health benefit to you. Specifically, *breaking long sitting with short bouts of exercise has been shown to improve sugar metabolism* over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function.

BACKGROUND: THE HIIT-2-SITLESS TRIAL

This research is funded by the National Institute on Aging. You are being asked to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether you want to volunteer to take part in this study.

This research is being done to evaluate brief high-intensity exercise breaks to sitting. Although long bouts of sitting may attenuate brain function, we do not know if breaking long sitting with short bouts of exercise could improve brain function and if exercise intensity matters. The HIIT-2-SITLess trial will assess if short high-intensity exercise breaks to sitting are acceptable, and practical to older adults as means of reducing long periods of sitting. The trial will also compare changes in brain function and cognition after three and half hours of sitting interrupted with 6-min of high-intensity exercise breaks relative to 6-min of light-intensity exercise.

You have been asked to participate in this research because you are 40-75 years old and met inclusion criteria, we reviewed over the phone and during your screening visit, such as being right-handed, not exercising regularly, planning to be in the Urbana-Champaign area for the duration of the study, etc. Approximately 54 participants will be involved in this study at the University of Illinois.

STUDY PROCEDURE

Your participation in this study will last about eight weeks. You will come to the laboratory on four occasions and wear activity monitors for the total of two weeks between study visits.

Study Logistics

Scheduled Assessments

You will be compensated for these scheduled assessments and the compensation amounts are stated later in this document.

1. **Baseline Visit: Cognitive Tasks, HIIT, and LIIT Breaks Practice and Questionnaires**
   - ***Blood pressure and heart rate.*** Researchers will give you a heart rate monitor to wear around your chest. The monitor has a small flat electrocardiogram electrode inside a plastic casing attached to an elastic strap that goes around your chest. The monitor will sit in the center of your chest, and researchers will place electroconductive gel on the strap to enhance the connection between the electrode and the electrical signal from your heart. Researchers will also measure your resting blood pressure three times to monitor for high blood pressure that could prevent you from exercising.
   - ***HIIE and LIIE Practice Session***. During this visit, you will also practice high-intensity and low-intensity interval training breaks supervised by our research staff. This ensures that you feel comfortable with exercise and that we know the cadence (speed) and workload that can elicit your target heart rate during exercise. You will also fill in questionnaires (described below).
   - ***Questionnaires.*** To help us better understand your responses to high-intensity exercise, we will ask you to complete a set of questionnaires about your physical activity, enjoyment of physical activity, physical function, sleep, and sitting behaviors.
   - ***Cognitive tasks practice.*** To help you get used to cognitive tasks, you will practice two tasks on a computer for 12 minutes each. You will complete two tasks by pressing buttons on a response pad based on task instructions. You will see asterisks and letters come up on the computer screen one by one. You will need to look away from an asterisk to "catch" which letter just appeared. You will also see arrows on the screen and will have to press a button, which corresponds to the direction of a middle arrow. You will practice tasks in between physical exercise breaks.
   - Total time commitment for this appointment is approx. **2 hrs.**

2. ***High-Intensity Exercise Intervention***
   You will be asked to participate in a half-day intervention designed to minimize long bouts of sitting by cycling on a stationary bike at a high intensity every 30 min. You will come

to the laboratory in the morning fasted. This means not eating or drinking (plain water is ok) for 8-10 hours before your visit. Trained researchers will measure your resting blood pressure and heart rate. You will also wear a chest strap with a heart rate monitor and two activity monitors to measure your sitting and physical activity. You will then complete a 24-h dietary recalls, and questionnaires about sleepiness. Next, you will eat a light breakfast, which is nut free oats and seeds bar. You will then complete the same neurocognitive tests as during your EEG visit. You will start the intervention after these neurocognitive assessments. You will sit for three hours and a half hours while you complete light administrative tasks and read popular science articles. For example, you will plan a family vacation and read articles about how snowflakes form or how whales help cool the earth. You will answer brief questions about how engaging and difficult these activities are. You will also answer questions about your energy levels, and fatigue. Every 30 min, the researchers will measure your blood pressure and heart rate before and after you complete a 6-minute high-intensity exercise (HIIE) break. You will also complete a shorter neurocognitive assessment twice during sitting: 15 min after the first and the third HIIE break. You will sit in a wheelchair while a researcher will push the wheelchair to the cycle ergometer. The neurocognitive assessment will last 15 min. You will then complete another HIIE break. You will complete five HIEE breaks in total. During each break, researchers will ask you questions about the levels of physical effort, and about how you feel. Researchers will monitor your blood pressure and heart rate after each break. After the last break, a researcher will transport you back to the EEG equipment and fill in the electrodes with gel while you sit and eat the second light meal (a similar oats and seeds bar). You will then complete the same set of cognitive tests as before the intervention while researchers record EEG signal. If this is your first intervention visit, you will receive two activity monitors to take home. If it is your last visit, you will complete a questionnaire about your experience in the study, and your participation will be complete. Total time commitment: **approx. 6 hours.**

EEG. You will be asked to visit Freer Hall where you will undergo neurocognitive assessments. You will wear an electroencephalography (EEG) cap that looks like a swim cap with small electrodes located throughout the cap. The researchers will fill in the electrodes with electroconductive gel so that we can measure tiny electric currents produced by your brain while you rest and complete cognitive tasks. We will first record your brain activity while you rest with your eyes open and closed for six minutes. You will then perform various cognitive tests on a computer that assess attention, executive function, and memory. For example, for one of the tests you will see pictures of common objects. Next, you will see more pictures. For each picture you will have to indicate if you saw it before or not. You will complete assessments again after the intervention is finished. In addition, you will complete one of the tasks twice during the intervention.

Cognitive Tasks. In addition to the two tasks that you practiced during the baseline visit, you will also complete a task where you will see pictures of objects. You will make judgments about pictures, such as determining if they represent an indoor or an outdoor object.

High-Intensity Interval Exercise Break: First, you will cycle at the same speed as during the maximal exercise test with progressively increased resistance for 1 min to warm up. Next, we will increase resistance on the bike to the same level at which your heart rate increased to 90% of its maximum during exercise test. These short bouts are designed to be very hard and you will cycle at this speed and resistance for 2 minutes. Then, you will rest while sitting still on the bike for 1 minute. Next, you will cycle again for 2 minutes at the same high intensity. At least two CPR and First Aid certified researchers will assist you and monitor your heart rate throughout exercise and ask you to rate how tired your body feels due to exercise and how you feel overall during each HIIE break. Finally, you will sit again and continue with either administrative activities or reading. You will complete **5** HIEE **breaks** lasting **6 min each** for **a total of 30 min** of exercise.

3. ***Low-Intensity Interval Exercise Intervention***
You will complete all the same procedures and tests as during the HIIE intervention day except for high-intensity exercise breaks. Instead, you will complete 6-min low intensity interval exercise breaks (LIIE).

Light-Intensity Interval Exercise Break. You will first warm up by pedaling for 1 min with minimal workload at an intensity of about 50% of your maximum heart rate. Next, you will pedal at a higher speed and workload chosen to elicit light intensity or about 57-60% of your maximum heart rate which is considered very light to fairly light intensity. You will then rest and remain stationary on the cycle ergometer for 1 minute, followed by another 2 minutes pedaling at the speed and workload to elicit the same heart rate. Total time commitment: **approx. 6 hours.**

Randomization

We will assign the order of two exercise interventions randomly at baseline. This means that the order in which you will complete HIIE and LIIE interventions, will be chosen by chance, like flipping a coin. Neither you nor the study team will choose which intervention you will complete first. The study team will let you know which exercise intervention you will complete first on the day of your first intervention visit. All participants will be asked to participate in all testing procedures.

Additional Assessments and Study Requirements

1. ***Questionnaires*** – You will be asked to complete one packet of questionnaires related to your physical abilities, physical activity, sedentary activities, sleep, diet, attitudes, thoughts, and feelings. The packet should take approximately 45-60 minutes and you will be able to complete it during the Baseline visit while you practice HIIE and LIIE breaks. At the end of the second intervention visit, you will also fill in a brief questionnaire about your experiences of the intervention.
2. ***Accelerometers*** – You will be asked to wear two activity monitors for seven days on two occasions, a week before each intervention visit. The first device is about the size of a pocket watch, similar to a pedometer. It is worn around your waist during waking hours and sleep except for bathing and showering. You will wear a second device on your thigh. The device is called activPAL. It is small and flat, similar to a flat piece of a domino. This device will measure how much you sit and stand. It is attached to your thigh with transparent film dressing.

You can wear this device while bathing or showering. You will also fill out an activity and sleep log to indicate hours of the day you wore the devices, when you went to sleep, and when you woke up. The devices do not track GPS or geographic data. They will only record the movement and sitting.

3. ***Health and Demographics Questionnaire*** – You already completed this questionnaire during screening. It has become part of your research record.
4. ***General Health History*** - You also provided information about your health history during screening call, which has become the part of your research record.
5. **Physician's Release and Medical Clearance** – Before qualifying, you provided documentation from a physician regarding the exercise and research testing, which is also part of your research record.

Additionally, the investigators may contact you in the future regarding other research at this institution.

You may opt out of these communications and opportunities at any time.

RISKS

Risks of high-intensity exercise participation: As indicated in the introduction, it is necessary to inform you that when individuals who have been inactive engage in exercise, there is a chance of incurring minor injury, and most certainly some discomfort due to the increased use of major muscle groups that have not received a great deal of use. Although the exercise breaks have been designed to offer activities that are safe and age appropriate, it is possible that you could be injured or experience discomfort as a result of engaging in these activities. However, no major injuries are anticipated. Should you become injured as the result of these activities, we encourage you to let the exercise leader in attendance know and to consult your physician if necessary. The University of Illinois does not provide medical or hospitalization insurance coverage for participants in this research study nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. There is also a very slim chance that sudden death or cardiac irregularities can occur while exercising. As noted, this is very rare, and the benefits of exercise are known to outweigh the risks. As preventative measure, during all on-site physical assessments all staff members are First Aid and CPR certified.

Risk of EEG: In rare instances, some individuals have reported some discomfort from the EEG cap. If this occurs, we will take the cap off and re-schedule the visit.

Confidentiality: Although we will use all reasonable efforts to keep your personal information confidential, we cannot guarantee absolute confidentiality. We describe efforts taken to protect your information in the section "How will the researchers protect my information".

BENEFITS

We cannot promise any direct benefit for taking part in this study. However, previous research has shown that interrupting long sitting with short bouts of exercise can improve sugar metabolism in adults over several hours. We do anticipate that participation in this research may also result in a transient (over several hours) benefit to cognitive and brain function. We also hope the information we get from this

study may help develop a greater understanding of how breaking long sitting with exercise can enhance cognitive and brain function in older adults and if the intensity of exercise matters. The study will help us understand if older adults are likely to use short high-intensity exercise breaks to reduce sitting.

ALTERNATIVE PROCEDURES

If you do not want to participate in the study, the alternative is not to participate.

HOW WILL THE RESEARCHERS PROTECT MY INFORMATION?

Confidentiality is assured for all participants with regard to any responses and information you provide. You understand that the blood samples will be used only to determine the levels of fasting glucose as the inclusion criterion for the study. Information that could identify you will not be available to anyone outside of our research group. All data collected will be numerically coded so that no individual data will be identifiable. We will use all reasonable efforts to keep your personal information confidential, but we cannot guarantee absolute confidentiality. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your personal information may be given out only if required by law.

Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law;
- University of Illinois Urbana-Champaign Institutional Review Board;
- National Institute on Aging – the funder for this research;
- Primary care physician if the research staff, in the course of the project, learn of a medical condition that needs immediate attention;
- Primary care physician (PCP) with participant's consent we will send health history questionnaire to PCP to assist them with medical clearance;

Participation in this project is voluntary and you are free to withdraw your participation without penalty at any time.

Certificate of Confidentiality:

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. This means that the researchers cannot release or use information, documents, or samples that may identify you in any action or suit unless you say it is okay. They also cannot provide them as evidence unless you have agreed. This protection includes federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena.

Identifiable information that could still be disclosed beyond the research team: The Certificate does not stop reporting that federal, state or local laws require. Some examples are laws that require reporting of child or elder abuse, some communicable diseases, and threats to harm yourself or others. The Certificate cannot be used to stop a sponsoring United States federal or state government agency from checking records or evaluating programs. The Certificate does not stop disclosures required by the federal Food and Drug Administration (FDA). The Certificate also does not prevent your information from being used for other research if allowed by federal regulations.

Researchers may release information about you when you say it is okay. For example, you may give them permission to release information to insurers, medical providers or any other persons not connected with the research. The Certificate of Confidentiality does not stop you from willingly releasing information about your involvement in this research. It also does not prevent you from having access to your own information.

WHO WILL HAVE ACCESS TO THE INFORMATION COLLECTED DURING THIS RESEARCH STUDY?

Efforts will be made to limit the use and disclosure of your personal information, including research study records, to people who have a need to review this information. We cannot promise complete secrecy.

There are reasons why information about you may be used or seen by other people beyond the research team during or after this study. Examples include:

- ***University officials, government officials, study funders, auditors, and the Institutional Review Board may need access to the study information to make sure the study is done in a safe and appropriate manner.***
- ***Collaborating researchers at other institutions who are involved with this study.***

Most tests done in research studies are only for research and have no clear meaning for health care. If the research results have meaning for your health, such as your fasting glucose levels, the researchers will contact you to let you know what they have found.

We will destroy the blood sample collected during screening after we know your eligibility for the study. We will retain all other data collected in the course of the study. For example, if you withdraw early from the study, your data will be retained for the analyses. To ensure confidentiality and anonymity during the study, you will be assigned a numeric code, and identified by this number only. We will keep the master list on the hard drive (or SSD) of a password protected microcomputer. We will destroy this list when the study is completed.

All data will be kept on the local server at the University of Illinois. A duplicate copy of the de-identified data (only numeric codes not your name will be used) will be stored in a cloud using the University of Illinois Box account. In addition, data will be stored on a local computer and backed up to an external hard drive or SSD drive. Your consent form and most questionnaire data will be held in a secure web-based system Illinois REDCap, which is compliant with Health Insurance Portability and Accountability Act of 1996. Only research team will have access to person-identifiable data. If the data is used for future research or training (see below), only de-identified data will be made available to other researchers, students or trainees. De-identified data will be stored indefinitely.

HOW MIGHT THE INFORMATION COLLECTED IN THIS STUDY BE SHARED IN THE FUTURE?

We will keep the information we collect about you during this research study for record keeping and for potential use in future research projects and training of junior researchers. Your name and other information that can directly identify you will be stored securely and separately from the rest of the

research information we collect from you. De-identified data from this study may also be shared with the research community, with journals in which study results are published, and with databases and data repositories used for research. We will remove or code any personal information that could directly identify you before the study data are shared. This means that a number will be assigned to your record. Therefore, if any data collected about you is shared for use in future research or training, researchers or students will only see a number and not your name. Despite these measures, we cannot guarantee the anonymity of your personal data.

The PI would like to retain your contact information to contact you for future research participation. This information will not be shared with other researchers but will only be retained for potential interest in research with this PI. We will ask for your consent to do so at the end of this form.

PERSON TO CONTACT

Example: If you have questions, complaints, or concerns about this study, you can contact Dr. Dominika M. Pindus at 217-300-7317 or email: pindus@illinois.edu. If you feel you have been harmed as a result of participation, please call Dr. Dominika M. Pindus at 217-300-7317, who may be reached during Mondays to Fridays 8 am to 5 pm.

Institutional Review Board: If you have any questions about your rights as a research subject, including concerns, complaints, or to offer input, you may call the Office for the Protection of Research Subjects (OPRS) at 217-333-2670 or e-mail OPRS at irb@illinois.edu. If you would like to complete a brief survey to provide OPRS feedback about your experiences as a research participant, please follow the link here or through a link on the OPRS website: https://oprs.research.illinois.edu/. You will have the option to provide feedback or concerns anonymously or you may provide your name and contact information for follow-up purposes.

VOLUNTARY PARTICIPATION

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time. You can start the study and then choose to stop the study later. This will not affect your relationship with the investigator. The researchers also have the right to stop your participation in this study without your consent if they believe it is in your best interests, you were to object to any future changes that may be made in the study plan.

COSTS AND COMPENSATION TO PARTICIPANTS

Participation in the study is free. However, you or your health care plan or insurance company may need to pay for costs associated with obtaining medical clearance if your physician asks for a physical examination in order to clear you for participation. Some health plans will not pay these costs for people taking part in research studies. Check with your health care plan or insurance company to findout what coverage they will provide. You will be paid for taking part in the *scheduled* appointments described above. As an incentive, and appreciation for contributing your time to this study, you will be paid a stipend as indicated in the table below. Thus, you will receive up to *$250* if you complete all the study assessments. The total time commitment for these scheduled appointments is approximately 16.5 hours.

Appointment	Time	Stipend	Location
Neurocognitive tests + HIIE + LIIE Practice	2 hrs.	$50	Freer Hall
HIEE Intervention	6 hrs.	$100	Freer Hall
LIIE Intervention	6 hrs.	$100	Freer Hall

If you live more than 10 miles away from the study site, we will reimburse the cost of your travel in the amount of $0.625 per mile.

AUTHORIZATION FOR USE OF YOUR PROTECTED HEALTH INFORMATION

Signing this document means you allow us, the researchers in this study, and others working with us to use some information about your health for this research study.

This is the information we will use and include in our research records:

- Demographic and identifying information like name, address telephone number, and email address
- Related medical information about you like your medical history disclosed on the General Health History questionnaire during screening, including your family history of cardiovascular disease, current and past medications or therapies, and information from physical examinations, such as blood pressure reading, heart rate, graded maximal exercise test, and lab results, fasting glucose levels determined during screening.
- All tests and procedures that will be done in the study

How we will protect and share your information:

- We will do everything we can to keep your information private but we cannot guarantee this. Study information will be kept in a secured manner and electronic records will be password protected. Study information may be stored with other information in your medical record. Other doctors, nurses, and third parties (like insurance companies) may be able to see this information as part of the regular treatment, payment, and health care operations of the hospital. We may also need to disclose information if required by law.
- This research is covered by a Certificate of Confidentiality from the National Institutes of Health as described in the section on How Will the Researchers Protect My Information. Please refer to this section for details.

- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.
- In order to conduct this study and make sure it is conducted as described in this form, the research records may be used and reviewed by others who are working with us on this research:
  - Members of the research team at the University of Illinois Urbana-Champaign
  - The University of Illinois Urbana-Champaign Institutional Review Board (IRB), which reviews research involving people to make sure the study protects your rights;
  - Other academic research centers we are working with: Prof. Charles Hillman and Arthur Kramer at Northeastern University, who are co-investigators on the study.
  - The study sponsor: National Institute on Aging
- If we share your information with groups outside of the University of Illinois Urbana-Champaign, for example with collaborators at Northeastern University, we will not share your name or identifying information. We will label your information with a code number, so they will not know your identity.
- If you do not want us to use information about your health, you should not be part of this research. If you choose not to participate, you can still receive health care services at Carle Health, OSF Healthcare, Christie Clinic or other local healthcare providers.

What if I decide to Not Participate after I sign the Consent and Authorization Form?

You can tell us anytime that you do not want to be in this study and do not want us to use your health information. You can also tell us in writing. If you change your mind, we will not be able to collect new information about you, and you will be withdrawn from the research study. However, we can continue to use information we have already started to use in our research, as needed to maintain the integrity of the research.

This authorization does not have an expiration date.

WOULD YOU LIKE TO BE CONTACTED ABOUT FUTURE RESEARCH OPPORTUNITIES?

❑ Yes, please include your email _____ or

phone number _____

❑ No

You can be in this current research study without agreeing to future research use of your identifiable information.

CONSENT

By signing this consent form, I confirm I have read the information in this consent form and have had the opportunity to ask questions. I will be given a signed copy of this consent form. I voluntarily agree to take part in this study.

_____

Printed Name of Participant

_____ _____

Signature of Participant Date

_____

Printed Name of Person Obtaining Consent

_____ _____

Signature of Person Obtaining Consent Date

RUNNING HEAD: HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH

Supplementary Table 1. Name and contact information for the trial sponsor

Trial Sponsors:	University of Illinois Urbana-Champaign Sponsor's Reference: 1376000511A6 Federal Employment Identification Number Contact Name: Paul N. Ellinger, Comptroller Address: 1901 S. First Street, Suite A Telephone: 217-333-2187 Email: spa@illinois.edu

Supplementary Table 2. Trial registration data

Data Category	**Information**
Primary registry and trial identifying number	ClinicalTrials.gov No. NCT06243016
Date of registration in primary registry	2024-02-05
Secondary identifying numbers	IRB24-0010, 1R21AG080411-01A1
Source(s) of monetary or material support	National Institute on Aging
Primary sponsor	University of Illinois Urbana-Champaign
Secondary sponsor(s)	Northeastern University, National Institute on Aging
Contact for public queries	Dominika M Pindus, Ph.D.
Contact for scientific queries	Dominika M Pindus, Ph.D.
Public title	Breaking Sitting With High-intensity Interval Training for Brain Health (HIIT2SITLess)
Scientific title	Breaking prolonged sitting with high-intensity interval training to improve cognitive and brain health in older adults – a pilot feasibility trial
Countries of recruitment	USA
Health condition(s) or problem(s) studied	Prolonged sitting, high-intensity interval training bouts, frontoparietal function, inhibitory control and episodic memory
Intervention(s)	*Active comparator*: 6-minute high-intensity interval training (every 30 minutes over 3.5 hours of sitting) *Passive comparator*: 6-minute low-intensity interval training (every 30 minutes over 3.5 hours of sitting)
Key inclusion and exclusion criteria	Age 40-70 years, BMI < 40 kg/m^2, sedentary (≥ 6 hours of sitting per day), low to moderately physically active (based on IPAQ Short Form), capable to engage in vigorous exercise (PARQ+), medical clearance from primary care physician, normotensive, IQ ≥85, fasting plasma glucose < 126 mg/dL, good or corrected vision and hearing, no significant abnormalities on the ECG during a maximal exercise test, no signs or symptoms suggesting of underlying cardiovascular disease as recorded during maximal exercise test, no indications to prematurely stop the maximal exercise test as per ACSM's Guidelines for Exercise Testing and Prescription.
Study type	Interventional
Date of first enrolment	February 2024
Target sample size	54
Recruitment status	Recruiting
Primary outcome(s)	Change in task-evoked brain activity (P3b component)
Key secondary outcomes	Change in cognitive functions, change in resting state and task evoked brain activity

Notes. ECG: electrocardiogram, IPAQ Short Form: International Physical Activity Questionnaire Short Form, PARQ+: Physical Activity Readiness Questionnaire for Everyone, IQ: intelligence quotient.

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Supplementary Table 3. Schedule of study assessments

Assessment	Screening Phone Call (Day -37 to Day -7)	Screening visit (Day-23 to Day -4)	Baseline (Day 0) – Pre-allocation	Intervention Visit 1 Day 8-21 (±2 Days)	Intervention Visit 2 Day 16-36 (±2 Days)	Follow Up Day 17-43 (± 2 Days)
	ENROLMENT		ALLOCATION (post-baseline)	INTERVENTION		FOLLOW-UP
TIME POINT	-t2	-t1	t0	t1		
ELIGIBILITY SCREEN						
Screening Informed Consent Form	x					
Screening Questionnaire	x					
Health & Demographics Questionnaire	x					
General Health History Questionnaire incl. current medications	x					
PASB-Q	x					
PARQ+	x					
Hospital Anxiety and Depression Scale	x					
Medical Clearance	x					
Blood Sample Fasting Glucose Analysis		x				
Anthropometric Assessments		x	x			
Resting Heart Rate and Blood Pressure		x	x	x	x	
Montreal Cognitive Assessment		x				
Ohio State TBI Identification Interview Form		x				

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Geriatric Depression Scale		x				
Beck Depression Inventory-2						
Florida Cognitive Activity Scale		x				
KBIT-2		x				
Inclusion/Exclusion Criteria	x	x				
Graded Maximal Exercise Test (GxT)		x				
Informed Study Consent Form			x			
ENROLLMENT			x			
Cognitive Tasks			x			
HIIT and LIIT Breaks Practice			x			
Borg Rating of Perceived Exertion Scale			x	x	x	
Feeling Scale			x	x	x	
Pittsburgh Sleep Quality Index (PSQI)			x			
Godin-Shephard Leisure Time Physical Activity Questionnaire			x			
Preference for Tolerance of the Intensity of Exercise Questionnaire			x			
FDI DIS Abbreviated FDI-Disability			x			
FDI FxN Abbreviated FxN- Function			x			
FxNSE Function Self Efficacy without a device			x			
Gait Efficacy Scale (GES)			x			
Sedentary Behavior Questionnaire (SBQ)			x			

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

SEQUENCE ALLOCATION			**x**			
INTERVENTIONS						
HIIT Breaks*				**x**		
LIIT Breaks*					**x**	
INTERVENTION ASSESSMENTS						
Physical Activity & Sitting Time Monitoring in Free-Living (7 d each)			**x**	**x**		
Heart rate Monitoring (during visits)			**x**	**x**		
Physical Activity Monitoring (during visits – ActiGraph GT9x Link)				**x**		
Sitting Time Monitoring (activPAL) (during visits)				**x**		
Epworth Sleeping Scale (ESS)				**x**	**x**	
ACT24 Physical Activity Recall				**x**	**x**	
Mental Effort Scale				**x**	**x**	
Task Engagement Scale				**x**	**x**	
Vigor and Fatigue Scale				**x**	**x**	
Cognitive Tasks				**x**	**x**	
EEG Recordings				**x**	**x**	
HIIT Breaks Surveys					**x**	
HIIT2SITLess Study Survey					**x**	
AEs		**x**	**x**	**x**	**x**	**x**
FOLLOW-UP						
Phone call						**x**

HIIT TO BREAK PROLONGED SITTING FOR BRAIN HEALTH IN AGING - PROTOCOL

Note. ACT24: Activities Completed over Time in 24 Hours; AE: adverse event; HIIT: high-intensity interval training; KBIT-2: Kaufman Brief Intelligence Test 2; LIIT: low-intensity interval training; PARQ+: Physical Activity Readiness Questionnaire for Everyone; The Canadian Society for Exercise Physiology (CSEP) Physical Activity and Sedentary Behaviour Questionnaire (PASB-Q); *The order of the interventions is randomized across participants.

Supplementary Table 4. Version history

Item	**Item Details**
Original IRB Protocol Number:	IRB24-0010
Original IRB Approval Issue Date:	02/20/2024
Protocol amendment number:	3
Protocol amendment approval date:	
Authors:	*DMP, RJS*
Revisions chronology	
Amendment 1 Approval Date:	04/20/2024
Amendment 1 changes:	Use of point-of-care glucose monitor instead of a venous blood sample and research-grade glucose reader.
Amendment 2 Approval Date:	05/28/2024
Amendment 2 Changes:	Expanding research staff who can perform a finger prick to collect a blood sample
Amendment 3 Approval Date:	09/13/2024
Amendment 3 Changes:	Age range change from 60-75 to 40-75 years. Amendment to inclusion criteria; shorter screening and baseline protocol.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no. / Table / Figure
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4, 9
	2b	All items from the World Health Organization Trial Registration Data Set	Suppl. Table 2
Protocol version	3	Date and version identifier	Suppl. Table 3
Funding	4	Sources and types of financial, material, and other support	27
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2
	5b	Name and contact information for the trial sponsor	Suppl. Table 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	27
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	23
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-9
	6b	Explanation for choice of comparators	8
Objectives	7	Specific objectives or hypotheses	8-9
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	10
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10, Table 1 (p.45-48)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	16-21, Table 2
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	21-22
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	16
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	17-20, 22-23

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 2
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	24
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	10-11
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	23-24

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10-11
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	23-24
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	24-25
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	24-25
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	24-25
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	23-24
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	24
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	21, 22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25-26
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	23-24, 25-26, Suppl. Table 3
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	25-26
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	23-24
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	28
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	25
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	21
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	25
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	25
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl. Material 1 and 2

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the 1 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.