PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

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

Authors

Pindus, Dominika M; Paluska, Scott; So, Joseph; Wyczesany, Miroslaw; Ligeza, Tomasz S; Sarol, Jesus; Kuang, Jin; Quiroz, Flor B; Shanmugam, Ramiya; Syed, Talha; Kos, Maciej; Khan, Naiman; Hillman, Charles; Kramer, Art

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Tari, Benjamin**
Affiliation	**University College London, Surgery and Interventional Science**
Date	**25-Nov-2024**
COI	**None**

The protocol outlines an interesting pilot study to answer some important questions surrounding exercise and brain health in a vulnerable age group. I have some minor comments and questions which I hope the authors can answer.

Small Suggestions:

On page 8 the FPN and DMN are not defined as the frontoparietal network and default mode network in the first instance.

On page 10 LIIT is not defined in the first instance

Flanker on page 12 is missing [F] if on the next line FAM MFA and AMF refer to the sequence of tasks

On page 13 the sentence regarding SBP/DBP does not seem complete

Will the increase during the fitness test be on a continuous ramp (i.e., 0.21W/s for females, 0.42W/s for males)? Or will it be a step increment every 2 minutes?

Why will only HRmax be used to quantify intensity? If inspired and expired air will be collected, can the authors derive some estimate of respiratory threshold?

On page 17, will these tasks be the same as those listed earlier in the protocol?

Why are participants only completing the flanker task during the sitting period and not the other tests?

Why are home administrative tasks being introduced during the sedentary periods? Would this not be a cognitive task that might influence performance of other tasks later on? I understand there are measures to control for stimulation during these activities.

It is interesting the authors chose to modify the antisaccade task to assess attention, rather than inhibitory control or cognitive flexibility which have been shown to be affected by single bouts of exercise. As well, participants are being asked to direct their gaze toward a letter, rather than simply away from a target, they might not need to exercise the same degree of cognitive control required to control attention.

Is eye tracking being used for the antisaccade task?

Regarding FN400 and LPC, can the task be specified here as well as in exploratory outcomes?

The protocol is very detailed, however I wonder whether the number of items a participant is asked to do during these assessment days will influence the results in any way. The protocol itself is also quite verbose and might be made shorter if possible.

For statistical analyses, what will be the variables of interest for the cognitive assessments?

Can more information be provided regarding plans to share data; where the data will be stored, what variables will be made available, etc. Perhaps the variables can be in a table?

The authors aim to investigate the feasibility of this type of exercise protocol, but only use participant reports to decipher this. Are there plans to run a small scale at-home study to see the “real-world” outcomes?

Reviewer	**2**
Name	**Teo, Wei-Peng**
Affiliation	**Nanyang Technological University, National Institute of Education**
Date	**12-Dec-2024**
COI	**None**

Review for manuscript bmjopen-2024-095415

Thank you for the opportunity to review this manuscript, which reports a study protocol for

the HIIT2SITLess trial by Pindus and colleagues. This manuscript describes a study protocol designed to break-up prolonged sitting time in middle age to older adults, using 6 minutes of high vs low intensity interval training (single-session, randomised crossover design, HIIT vs LIIT) every 30 minutes, during 3.5hr of sitting. The outcomes of this study include feasibility, acceptability, neurocognitive, and EEG assessment outcomes. The authors conclude with several limitations, one of which is the acute nature of this intervention. However, they argue the benefit of acute interventions in predicting longer-term brain adaptations, and that the findings from this study will be able to inform the design on long-term intervention strategies in middle to older adults.

Overall, this paper is nicely articulated and designed. I do not have major comments here although I would like the authors to consider a few points to strengthen your arguments for this study. Please find my comments below for your consideration:

Title: The current title implies that this study pertains to only older adults (>60 years), but in fact, this project will be recruiting adults from 40-75 years. I will recommend amending the title to something along the lines of "middle age to older adults".

Introduction: I don't have major comments with the introduction, which I thought to be well articulated, and the research questions/hypotheses very clearly stated. One comment I would like to make is perhaps some age-related brain adaptations to LIIT and HIIT particular in the FPN and DMN. At the moment, the introduction seems to only suggest general adult adaptations, when in fact, the study focuses primarily on middle to older aged adults.

Methods: It states that the project will recruit participants with anxiety and depression. Are they cut-offs for the respective scales? I would imagine that those with higher severity in anxiety and/or depression would present with different neurophysiological responses, considering their condition and medical status?

Methods: It is not apparently clear to my why the authors have doubled on their neurocognitive screening with the TICS-m and MoCA? Is there a rationale for this? There is also a further use of the KBIT-2 for screening purpose, this seems like a very stringent list of exclusion criteria for your participants.

Methods: A minor point/question regarding the acceptability of this trial; It was stated in the consent form that $250 will be compensated to participants? Would this have an influence, in the sense that, participants may not drop out (to be compensated) but perceive that the trial is unacceptable? Are participants surveyed regarding the feasibility and acceptability of the trial?

VERSION 1 - AUTHOR RESPONSE

Reviewer 1: Dr. Benjamin Tari, Oxford University

General comment: The protocol outlines an interesting pilot study to answer some important questions surrounding exercise and brain health in a vulnerable age group. I have some minor comments and questions which I hope the authors can answer.

Response:

We thank Dr. Tari for his time taken to review our work, his positive feedback and thoughtful comments, which greatly contributed to enhancing the clarity of our manuscript. We respond in detail to each of his comments below.

Main Comments

1. **Will the increase during the fitness test be on a continuous ramp (i.e., 0.21W/s for females, 0.42W/s for males)? Or will it be a step increment every 2 minutes?**

Response:
The increase will be in step increments every 2 minutes. We report this detail in our methods (p. 13, lines 219-223 in the revisions):

Previous version:
"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]"

Revisions:
No change.

2. **Why will only HRmax be used to quantify intensity? If inspired and expired air will be collected, can the authors derive some estimate of respiratory threshold?**

Response:
We used HR_{max} based on reviewers' comments to our grant application who queried the significance of heart rate reserve. Since the majority of studies into acute exercise and P3b component[1] utilize HR_{max}, we adopted this metric for the trial.

3. **On page 17, will these tasks be the same as those listed earlier in the protocol? Why are participants only completing the flanker task during the sitting period and not the other tests?**

Response:
Thank you for this comment. Yes, during baseline participants will practice a modified Eriksen flanker task and the antisaccade task (p.14-15) due to established learning effect on these tasks.[2,3] Participants will complete 3 tasks at pre-test and post-test on each intervention day:

I. A modified Eriksen flanker task
II. An antisaccade task
III. Mnemonic Similarities Task

In addition, they will complete a flanker task twice during the intervention.

We could only include one task during the intervention, due to time constraints and calibrating the timing of cognitive assessment at least 11 minutes after each HIIT break, to capture the acute effect of a single exercise bout.[4] We chose the task used to assess our primary outcome, the amplitude and latency of the P3b component during the Eriksen flanker task.

4. **Why are home administrative tasks being introduced during the sedentary periods? Would this not be a cognitive task that might influence performance of other tasks later on? I understand there are measures to control for stimulation during these activities.**

Response:

These tasks have been chosen to increase external validity of the study and to limit the diversity of the sedentary tasks. There is some evidence to suggest that sedentary behavior (SB) type may moderate the chronic associations between SBs and cognitive function. (e.g., [5]) However, only one previous study tested the moderating effect of high versus low mental activity during SB on breaking prolonged sitting with physical activity (PA) bouts on executive functions and cerebral autoregulation (a crude measure of global brain function) and reported null findings.[6] We aired on the side of caution in our study design and included only SBs that could be considered cognitively engaging (i.e., with higher mental activity as opposed to passive SBs such as TV viewing). If the aims of the study are met, our design would provide stronger support for the positive effect of interrupting prolonged sitting with higher-intensity PA on inhibitory control and episodic memory beyond substituting a proportion of cognitively passive SBs with a socially engaging exercise break. As Dr. Tari noted, we also control for cognitive effort and cognitive engagement with respective scales throughout the study.

5. **It is interesting the authors chose to modify the antisaccade task to assess attention, rather than inhibitory control or cognitive flexibility which have been shown to be affected by single bouts of exercise. As well, participants are being asked to direct their gaze toward a letter, rather than simply away from a target, they might not need to exercise the same degree of cognitive control required to control attention.**
   Is eye tracking being used for the antisaccade task?

Response:

Thank you for this thoughtful comment. We employed two inhibitory control tasks: (i) a modified Eriksen flanker task and (ii) the antisaccade task. Both tasks are considered measures of attentional control (an aspect of inhibitory control requiring attention, which we now clarify on p. 17, line 352).[7] The majority of evidence on the positive effects of a single acute bout of PA on neuroelectric indices of attentional control relies on the P3b-ERP component measured during a modified Eriksen flanker task.[1] Accordingly, we chose this task to measure the P3b component as our primary outcome. The reason for including the antisaccade task is its superior psychometric properties as *a cognitive measure* of attentional control.[7] However, due to horizontal eye movements being synchronized with the stimulus in this task, the measurement of the ERP components used in the antisaccade task is challenging. Based on our internal lab testing and peer advice (Prof. Monica Fabiani, personal communication at the annual meeting of the Society for Psychophysiological Research, September 30th 2023), we established that removing horizontal eye movements would also remove signal related to cognitive performance. Hence, this task was not used to measure the primary outcome. By including both, the Eriksen flanker-based assessment of the P3b, and the antisaccade task, the study capitalizes on the superior validity of the antisaccade task as a *behavioral measure of attentional control*[7] and an established in the literature effect of acute PA bouts on *the neurophysiological measure* associated with attentional control, the P3b-ERP component during another attentional control task.[1]

Eye tracking is not being used during the antisaccade task; the task is used to measure secondary outcomes. We agree with Dr. Tari that it is an excellent avenue for future studies to explore.

Previous version

"The modified Eriksen flanker task provides a measure of attentional control by introducing a perceptual and response conflict."

Changes (p. 17, line 352-353):

"The modified Eriksen flanker task provides a measure of attentional control (an aspect of inhibitory control) by introducing a perceptual and response conflict."

6. **Regarding FN400 and LPC, can the task be specified here as well as in exploratory outcomes?**

Response:
Thank you. We added the requested detail on p. 20, lines 422, 423, 431 and 437.

Previous version
"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."

Changes (p. 20, lines 422, 423, 431 and 437):
"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."

7. **The protocol is very detailed, however I wonder whether the number of items a participant is asked to do during these assessment days will influence the results in any way. The protocol itself is also quite verbose and might be made shorter if possible.**

Response:
Thank you for this comment. The detailed protocol was designed with laboratory experimental control in mind. This study is the first step in establishing the intervention efficacy not effectiveness. We control for the effects of auxiliary assessments completed during the intervention (e.g., simple Likert scales, blood pressure and HR) in the intervention design,

where individuals act as their own controls and by randomizing participants to two intervention sequences. All assessments were deemed necessary to ensure participants' safety (heart rate and blood pressure), and to control for cognitive content of SB. Given that participants are already exposed to the modified Eriksen flanker task during baseline and pre-test, its short duration (~6 minutes) and due to high overall accuracy on this task, we do not anticipate the cognitive assessment during the intervention to exceed cognitive load beyond that required for
participant's daily activities.

We have also made several cuts to the text. The following text was deleted:

Previous version:
p. 9
"The randomized crossover trial design was chosen for its efficiency in minimizing inter-individual variability to allow for a smaller sample size."

p. 10:
Verbal communication will be video recorded.
p. 10:
"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."

p.10-11:

"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."

Revisions
p.10, lines 143-147:
"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."

p. 10-11, lines 149-169:
"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."

8. **For statistical analyses, what will be the variables of interest for the cognitive assessments?**

Response:
We will use behavioral measures of reaction time (RT), RT variability, and accuracy for each task condition (flanker), the accuracy on the antisaccade task, and lure discrimination index (probability of "similar"/"novel" judgments in response to a lure) and recognition memory index on the MST defined as percent of repetition trials correctly identified as "old"(Hits) minus the percent of novel trials endorsed as "old." We specified outcomes measured in the description of the antisaccade and the mnemonic discrimination tasks and added such description to the modified Eriksen flanker task.

Previous version:

Modified Eriksen Flanker task
"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. 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 number of correctly identified letters is the secondary outcome." (p. 18, lines 382-383 in the revised manuscript)

Mnemonic Similarity Task
"A lure discrimination index (LDI; probability of "similar"/"novel" judgments in response to a lure) is another secondary outcome." (p. 19, lines 392-393 in the revised manuscript.

Revisions:

Modified Eriksen Flanker task
"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)." (p. 18, lines 361-375 in the revised manuscript).

Antisaccade task – no change
Mnemonic Similarity Task – no change

9. **Can more information be provided regarding plans to share data; where the data will be stored, what variables will be made available, etc. Perhaps the variables can be in a table?**

Response:
We will share the final processed and de-identified quantitative data, including the amplitude and latency of the P3b component, accuracy, and reaction time (RT) on the cognitive tasks, time spent sitting, standing, and in physical activity (PA) intensities, mean heart rate during PA and sedentary breaks, and questionnaire data related to fatigue, vigor, and cognitive engagement, and participants' quantitative ratings of intervention feasibility and acceptability.

At present we do not have the data dictionary or the finalized set of variables, given that all our efforts are focused on study recruitment, testing and data quality control. Due to the time constraints on the staff involved in this pilot trial, we will work on the list of variables available for sharing and data curation closer to the completion of the study and publication of the main trial manuscript.

10. **The authors aim to investigate the feasibility of this type of exercise protocol, but only use participant reports to decipher this. Are there plans to run a small scale at-home study to see the "real-world" outcomes?**

Response:
This pilot study has a specific goal to provide initial feasibility data related to the intensity and duration of HIIT breaks as well as laboratory-based intervention. Our intervention was not designed for home use and the small at-home study was beyond the scope of the project. We agree with the Reviewer that this is an excellent goal for a future pilot study in preparation for a larger chronic intervention.

Small Suggestions:

1. **On page 8 the FPN and DMN are not defined as the frontoparietal network and default mode network in the first instance.**

Response
Thank you for noticing this omission. I have now amended the text accordingly.

Previous version:
"One such network, the FPN (comprising hubs in the frontal cortex and intraparietal sulcus[21])"
"Another network relevant to cognitive aging is the DMN (it comprises it comprises regions in the medial prefrontal and posterior cingulate cortices[27,28])"

Revision (p. 7, lines 40-41 and 45-46)
"One such network, the frontoparietal network (FPN; comprising hubs in the frontal cortex and intraparietal sulcus[21])"

"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])"

2. **On page 10 LIIT is not defined in the first instance**

Response
Thank you. The text has been amended accordingly.

Previous version:
"2. HIIT versus LIIT bouts will result in greater changes in P3b amplitude and latency."

Revision (p. 9, line 100-101):
"2. HIIT versus light intensity interval training (LIIT) bouts will result in greater changes in P3b amplitude and latency."

3. **Flanker on page 12 is missing [F] if on the next line FAM MFA and AMF refer to the sequence of tasks**

Response
Thank you. We made the correction.

4. **On page 13 the sentence regarding SBP/DBP does not seem complete**

Response
Thank you. We amended the sentence to the following:

Previous version
"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."

Revisions (p. 12, lines 196-199):
"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 medical clearance that their BP is within a normotensive range."

Reviewer 2: Dr. Wei-Peng Teo, Nanyang Technological University

Thank you for the opportunity to review this manuscript, which reports a study protocol for the HIIT2SITLess trial by Pindus and colleagues. This manuscript describes a study protocol designed to break-up prolonged sitting time in middle age to older adults, using 6 minutes of high vs low intensity interval training (single-session, randomised crossover design, HIIT vs LIIT) every 30 minutes, during 3.5hr of sitting. The outcomes of this study include feasibility, acceptability, neurocognitive, and EEG assessment outcomes. The authors conclude with several limitations, one of which is the acute nature of this intervention. However, they argue the benefit of acute interventions in predicting longer-term brain adaptations, and that the findings from this study will be able to inform the design on long-term intervention strategies in middle to older adults.

Overall, this paper is nicely articulated and designed. I do not have major comments here although I would like the authors to consider a few points to strengthen your arguments for this study. Please find my comments below for your consideration:

Response:

We thank Dr. Teo for his time, positive feedback and helpful comments, which helped us enhance the clarity of the manuscript.

1. **Title: The current title implies that this study pertains to only older adults (>60 years), but in fact, this project will be recruiting adults from 40-75 years. I will recommend amending the title to something along the lines of "middle age to older adults".**

Response:
Thank you for noticing this oversight. We have now amended the title to the include middle-aged adults.

2. **Introduction: I don't have major comments with the introduction, which I thought to be well articulated, and the research questions/hypotheses very clearly stated. One comment I would like to make is perhaps some age-related brain adaptations to LIIT and HIIT particular in the FPN and DMN. At the moment, the introduction seems to only suggest general adult adaptations, when, in fact, the study focuses primarily on middle to older-aged adults.**

Response:
Due to the novelty of the study, such evidence, to the best of our knowledge, is currently not available. We highlight this gap in the literature in our introduction:

Previous version:

"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." (p.8, lines 73-75 in the revised manuscript)

Revision:
No change.

3. **Methods: It states that the project will recruit participants with anxiety and depression. Are they cut-offs for the respective scales? I would imagine that those with higher severity in anxiety and/or depression would present with different neurophysiological responses, considering their condition and medical status?**

Response:
Thank you for this comment. We carefully considered the inclusion of participants with depression and anxiety and decided to include them because:

(i) The prevalence of depression and anxiety is relatively high in the US population of middle-aged (19/6 and 16.6% for anxiety and depression, respectively;[8]) and older adults (18.6 and 11.2%, respectively;[8]).
(ii) Antidepressant use is high in older women (24%) compared to 12% in older men, and increases with age (https://www.cdc.gov/nchs/products/databriefs/db377.htm). Hence, excluding participants based on depressive symptoms and medication use is likely to lead to selective sampling and under-representation of women and older individuals.
(iii) Recent evidence comparing cases and controls does not suggest negative cognitive associations between using anxiolytics and cognitive performance (on visuospatial ability, episodic memory).[9]
(iv) There is only a modest effect of antidepressants on executive functions.[10]
(v) Acute exercise has been shown to improve executive functions in older adults with depression,[11] and increase activation in the prefrontal cortex, which supports performance on executive function tasks, in middle-aged adults with

depressive symptoms.[12] The positive effects of a single bout of high-intensity interval training on executive functions were also observed in adolescents hospitalized for a major depressive disorder.[13] This evidence suggests that individuals with depressive symptoms can also benefit cognitively from acute bouts of exercise.

(vi) Likewise, emergent evidence suggests that acute exercise can have beneficial effects on inhibitory control in younger adults with high trait anxiety.[14]

(vii) Our randomized crossover trial design controls for inter-individual variability in cognitive performance because individuals act as their own controls.

(viii) We anticipate including descriptive information on depressive symptoms and anxiety in a final publication to provide readers with contextual details relevant to our sample.

In brief, we decided to include individuals with anxiety and depression to enhance the generalizability of our findings and based on the evidence supporting the positive effects of acute exercise on executive functions in these groups.

4. **Methods: It is not apparently clear to my why the authors have doubled on their neurocognitive screening with the TICS-m and MoCA? Is there a rationale for this? There is also a further use of the KBIT-2 for screening purpose, this seems like a very stringent list of exclusion criteria for your participants.**

Response:

Thank you for this comment. We included TICS-m as a crude screening tool to decrease the likelihood of inviting adults with potential cognitive problems to an in-person screening visit. In TICS-m shows only fair sensitivity to differentiate between cognitively unimpaired individuals and those with mild cognitive impairment,[15] while MoCA has higher sensitivity.[16] KBIT-2 is used as a fail-safe method to potentially exclude individuals with cognitive abilities below 1SD of the population in alignment with the literature in this field. We also use this measure to characterize our sample. At present none of the screened participants were excluded based on KBIT-2.

5. **Methods: A minor point/question regarding the acceptability of this trial; It was stated in the consent form that $250 will be compensated to participants? Would this have an influence, in the sense that, participants may not drop out (to be compensated) but perceive that the trial is unacceptable? Are participants surveyed regarding the feasibility and acceptability of the trial?**

Response:

Thank you for this question. Our reimbursement of $250 translates to $17.9 per hour (including a 2-hour baseline and 2 x 6-hour experimental visits), which is only slightly above the $15 minimum wage and far below the median hourly wage (~$30) in Champaign IL, where the study is based. This level of reimbursement is consistent with hourly rates paid for study participation at the University of Illinois. Hence, we do not anticipate that this level of reimbursement would coerce participants to continue the study should they find it unacceptable. Importantly, our study has been reviewed and approved by the IRB at the University of Illinois, which pays attention to the potential of reimbursement to unduly influence study participation. It has also been approved by an independent safety officer appointed by the funder (National Institute on Aging), and the level of reimbursement has not raised any concerns. Finally, participants are surveyed at the end of the study regarding the acceptability of the trial and the feasibility of adopting high-intensity physical activity breaks at home.

References

1 Kao SC, Chen FT, Moreau D, *et al.* Acute effects of exercise engagement on neurocognitive function: a systematic review and meta-analysis on P3 amplitude and latency. *Int Rev Sport Exerc Psychol*. 2022;1–43. doi: 10.1080/1750984X.2022.2155488

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VERSION 2 - REVIEW

Reviewer **1**

Name **Tari, Benjamin**

Affiliation	**University College London, Surgery and Interventional Science**
Date	**25-Feb-2025**
COI	

Thank you for your responses to my comments! Best of luck with the study.

VERSION 2 - AUTHOR RESPONSE

Reviewer 1
Dr. Benjamin Tari, University College London
Comments to the Author:
Thank you for your responses to my comments! Best of luck with the study.

Response:
We thank Dr. Tari for his kind response and are glad to learn that our responses were satisfactory.

Reviewer 2
If you have selected 'Yes' above, please provide details of any competing interests.:
Not applicable

Response:
We thank the Reviewer for their time and for pointing out an error – we have no competing interests to disclose.