Distinct effects of acute exercise and breaks in sitting on cognition in older adults: a randomised crossover trial

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ABSTRACT

Background

Sedentary behaviour has been associated with impaired cognition, whereas exercise can acutely improve cognition.

Objectives

We compared the effects of a morning bout of moderate-intensity exercise, with and without subsequent light-intensity walking breaks from sitting, on cognition in older adults.

Methods

Sedentary overweight/obese older adults with normal cognitive function (n=67; 67±7 years; 31.2±4.1 kg/m^2), completed three conditions (6-day washout): SIT: uninterrupted sitting (8hr, control); EX+SIT: sitting (1hr), moderate-intensity walking (30min), uninterrupted sitting (6.5hr); EX+BR: sitting (1hr), moderate-intensity walking (30mins), sitting interrupted every 30 minutes with 3 minutes of light-intensity walking (6.5hrs). Cognitive testing (Cogstate) was completed at 4 time points assessing: psychomotor function; attention; executive function; visual learning; and working memory. Serum brain-derived neurotrophic growth factor (BDNF) was assessed at 6 time points. The 8-hour net area under the curve (AUC) was calculated for each outcome.

Results

Working memory net AUC (z-score·hr +SEM) was improved in EX+BR (+28±28), relative to SIT (-25±28, p=0.04 vs. EX+BR). Conversely, executive function net AUC was improved in EX+SIT (-8±32) relative to SIT (-80±32, p=0.02 vs. EX+SIT). Serum BDNF net AUC

(ng·hr·mL^{-1} +SEM) was increased in both EX (+171±317) and EX+BR (+139±316), relative to SIT (-227±318; $p<0.05$ vs. EX or EX+BR).

Conclusion

A morning bout of moderate-intensity exercise increases cognitive performance and serum BDNF over 8-hours in older adults, relative to uninterrupted sitting. However, interrupting post-exercise sitting with intermittent walking may influence which aspect of cognition is improved.

Clinical Trial Registration

URL: https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=366476

Unique Identifier: ACTRN12614000737639

INTRODUCTION

Slowing cognitive decline to prevent dementia is a priority in the context of global trends of population ageing. [1] Ageing is the primary risk factor for dementia and is associated with an increased prevalence of modifiable risk factors associated with accelerated cognitive decline and progression to dementia such as obesity, hypertension, hyperlipidaemia and physical inactivity.[1–3] As older adults with cardiovascular risk factors have an increased risk for dementia, testing strategies that might improve cognitive performance and brain health in this population is a priority. Moderate-to-vigorous intensity physical activity (MVPA) is well studied in this regard for its ability to acutely improve cognitive performance, [4,5] and stimulate molecular mechanisms that enhance learning and memory such as brain derived neurotrophic growth factor (BDNF).[6] However, MVPA has been shown to occupy ~5% of the waking day in older adults.[7–9] The majority of waking time is spent in sedentary behaviour, defined by a low energy expenditure (<1.5 metabolic equivalents) in a sitting or reclining position.[10] In some studies,[11,12] sedentary time, particularly sitting accumulated in prolonged periods, is associated with all-cause mortality after adjustment for MVPA. In addition, there is emerging epidemiological evidence of an inverse association between prolonged periods of sitting and cognitive performance.[13,14]

Breaking up prolonged sitting with intermittent activity, usually of a light-intensity, can have a beneficial impact on multiple systems relevant to brain health. These include, carbohydrate and lipid metabolism,[15,16] blood pressure,[17,18] vascular function,[19,20] coagulation,[21] and sympathetic function.[18] Breaking up prolonged sitting may also acutely reduce fatigue,[22] increase cerebral blood flow velocity,[23] and improve cognitive performance.[24] Despite the potential cognitive benefits of breaking up sitting, no studies, to

our knowledge, have investigated the combined effect of an acute exercise bout with subsequent breaks in sitting.

In a randomised crossover trial, we examined the effects of acute exercise with or without breaks in sitting, on multiple aspects of cognition and concentration of serum BDNF in older adults at increased risk for developing dementia. It was hypothesized that a continuous (30 minute) bout of moderate-intensity exercise would improve cognition compared with uninterrupted sitting. It was also hypothesized that the magnitude of improvement in cognition following moderate-intensity exercise would be greater when combined with intermittent light-intensity breaks in sitting, relative to uninterrupted sitting.

METHODS

The detailed methods of this study have previously been described,[25] and full inclusion and exclusion criteria and participant medication usage are provided in the supplemental material (Table S1 and S2). Men and postmenopausal women (≥55 to ≤80 years; body mass index ≥25 $kg \cdot m^{-2}$ to <45 $kg \cdot m^{-2}$) were recruited from the local community and tested at two sites: the Physical Activity Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia; and, the Human Cardiovascular Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia (UWA), Perth, Australia. Recruitment occurred between February 2015 and July 2017. Participants gave informed consent prior to taking part.

Study Design

Participants completed three conditions, in random order, with a minimum six day washout between conditions: SIT: uninterrupted sitting (8hrs, control); EX+SIT: sitting (1hr), moderate-intensity walking (30mins), uninterrupted sitting (6.5hrs); EX+BR: sitting (1hr), moderate-intensity walking (30mins), sitting interrupted every 30 minutes with 3 minutes of light-intensity walking (6.5hrs). A familiarisation session was completed 3 to 5 days prior to testing, where participants were familiarized with cognitive testing and treadmill walking. During the 48 hours prior to testing, participants were instructed to avoid caffeine, alcohol and moderate-to-vigorous physical activity. Food was controlled from the night before testing were participants consumed a standardised dinner at home between 7pm and 9pm in place of their regular dinner. This meal was tailored for each participant to meet 33% of estimated daily energy requirements with a macronutrient profile of 55–58% carbohydrate, 29–31% fat and 12–15% protein as previously described.[25]

Exercise

Participants were instructed to avoid getting out of the chair except to void, or to complete the predetermined treadmill walking in EX+SIT and EX+BR. The 30-minute moderate-intensity exercise bout in EX+SIT and EX+BR was performed on a treadmill at the same predetermined speed and incline for both conditions. The speed was set at 3.2km·h^{-1} which was a walking pace for all participants, and the incline was tailored to induce a heart rate (HR) response indicative of moderate-intensity (HR between 65-75% of age predicted maximum HR). This incline was determined during the familiarisation session. The three-minute light intensity walking breaks were 3.2km·h^{-1} with no incline for all participants. Heart rate (Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE scale 6-20;

light intensity 9-11 RPE; moderate-intensity 12-15 RPE) were collected at 5-minute intervals during the 30-minute bout of exercise and at the end of each three minute walking break.

Experimental day protocol

Participants reported to the laboratory at ~7AM, following an overnight fast (> 10hr) and an indwelling cannula was inserted into an antecubital. The experiment began at ~8AM with a 1-hour steady state sitting period where participants completed baseline measures of cognitive performance. Thereafter, a fasting blood sample was obtained prior to administration of a standardised breakfast meal. Participants were given 20-minutes to consume breakfast and lunch, which were standardised in the same way as the standardised dinner. All meals remained the same for a given participant throughout the study. After breakfast the protocol was followed according to randomisation (Figure 1). In all conditions, blood for analysis of brain derived neurotrophic growth factor (BDNF) was collected at six time points and cognitive testing was assessed at four time points throughout the day. Blood samples were collected immediately prior to the meals, and immediately following the final cognitive testing session. While sitting, participants were instructed to read or work quietly on a laptop and avoid activities which may influence arousal levels such as watching television or making non-essential phone calls. Participants were supervised to ensure consistent behavior across each of the study conditions.

Cognitive performance

Cognitive performance is the primary outcome, and was measured on a laptop using a computerised test battery (Cogstate Ltd. Melbourne, Australia) developed for repeated testing with minimal practice effects.[26–28] The content of each task was randomised and the test battery was administered in the following order each time: Groton Maze Learning Test

(executive function); Detection Test (psychomotor function and speed of processing); Identification Test (attention); One Card Learning Test (visual learning); and a composite of the One Back Test and Two Back Test (working memory). Total administration time was approximately 20-25 minutes. Participants were familiarised with the full test battery during the familiarisation session 3-5 days prior to the first experimental condition.

Blood sampling

Venous blood samples were collected using an indwelling cannula inserted in an antecubital vein. Serum was collected for the analysis of BDNF which is the secondary outcome. Samples coagulated for 1 hour at room temperature (22–24 °C), prior to centrifuging at 2000 rpm (931 x g) for 15 min at 4 °C. Supernatants were removed and frozen immediately at −20 °C and subsequently moved to a −80 °C freezer at the end of the condition. Concentration of BDNF was determined from thawed serum using enzyme-linked immunosorbent assay kits (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instruction. All samples were assayed by the same lab technician who was blinded to the conditions. Intra- and inter-assay coefficients of variation were 3.2% and 11.3% respectively. Haematocrit and haemoglobin were determined from whole blood using a Beckman Coulter LH 785 according to standard methods, to calculate percent change in change plasma volume pre to post 8-hours.

Statistical analysis

Power calculations were made in relation to cognitive performance. We estimated based on recent evidence,[24] an effect size of ~0.40 (Cohen's *d* for repeated measures) for exposure of light-intensity walking breaks on executive function task performance. Sample size calculations estimated a final sample of 48 participants would be required.[25] The order of

conditions was block randomised and stratified by sex by an independent third party using a computer generated random sequence and stored in sealed envelopes as previously outlined.[25] Researchers were unblinded to the order of conditions when familiarisation was complete and participants were unblinded to the condition after the one hour steady state period for experimental visits one and two. Raw cognitive scores were standardised to the mean and standard deviation of baseline values to create a standardised z-score. A higher standardised z-score denotes a better performance and a lower z-score denotes a worse performance. A time-by-condition interaction term was included in regression models to examine the effect of conditions on the 8-hour time course. Marginal means from these models were used to plot the time course for each outcome. Change across the 8-hour condition was quantified by calculating net area under the curve (AUC). Specifically, this net change over the day is the area above baseline minus the area below baseline calculated using the trapezoidal method. Therefore, a negative net AUC value represents a net decrease across the day relative to baseline, and vice versa for a positive net AUC value. Following recommendations on data analysis of cross-over trials,[29] generalized linear mixed models with random intercepts were used to test the effect of the condition on each outcome. Data analysis were performed by technicians blinded to the study conditions. All models were adjusted for age, sex, waist circumference, treatment order, testing site and baseline values. Cognitive performance was adjusted for years of education as a categorical variable and BDNF was adjusted for change in plasma volume, calculated from haematocrit and haemoglobin using the Dill and Costill method.[30] Models with a time-by-condition interaction term comparing individual time points between conditions were adjusted for multiple comparisons using a Šidák correction. A probability level of 0.05 was adopted. The hypothesis that an acute bout of exercise would improve cognitive performance, relative to prolonged sitting, was tested by combining EX+SIT and EX+BR as one treatment to compare

to SIT. All combinations of individual conditions were compared to test the hypothesis that the magnitude of improvement in cognition following moderate-intensity exercise would be greater when combined with intermittent light-intensity breaks from sitting, relative to uninterrupted sitting. Pearson's correlations were used to test the association between cognitive performance and BDNF. Statistical analyses were performed using Stata 15 Windows (StataCorp LP).

RESULTS

Initially, 69 participants were randomised; however 67 completed at least one condition and 65 completed all conditions, due to dropout (Figure 2). Intention-to-treat analysis was performed on the full data set of 67 participants. Participants were older (67±7 years) adults who were overweight to obese (31.1±4.1 kg/m^2), with a majority being hypertensive (67%). Participant characteristics are included in Table 1.

Table 1. Participant characteristics

Demographic	Baseline
N	67
Sex (female/male)	35 / 32
Age (years)	67±7
Body mass index (kg/m^2)[a]	31.2±4.1
Waist circumference (cm)[a]	105.3±11.9
Hypertension, n (%)[a]	45 (67%)
Office systolic blood pressure (mmHg)[a]	130±14
Office diastolic blood pressure (mmHg)[a]	77±12
Mini Mental State Exam[a]	29±1
Years of education	14±3
Fasting glucose (mmol/L)[c]	5.3±0.7
Fasting insulin (pmol/L)[b]	50±24
Fasting total cholesterol (mmol/L)[b]	5.4±1.1
Fasting triglycerides (mmol/L)[b]	1.4±0.8
Fasting HDL cholesterol (mmol/L)[b]	1.4±0.3
Fasting LDL cholesterol (mmol/L)[b]	3.4±0.8

Data are mean±SD; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; [a]measured during familiarization visit, hypertension defined as ≥130 mmHg systolic or ≥80 mmHg diastolic according to recent guidelines;[31] [b]measured during first experimental visit.

Exercise responses

The initial 30-minute exercise bout induced similar HR and RPE responses (mean±SD) under each condition (EX+SIT: 109±12bpm, 71±8%HRmax, 11±2 RPE; EX+BR: 109±12bpm, 71±8%HRmax, 11±2 RPE). Average HR and RPE across all 12 walking breaks was 94±2bpm, 61±1%HRmax and 9±0.4 RPE.

Cognitive performance outcomes

When both exercise conditions (EX+SIT and EX+BR combined) were compared to SIT, no significant differences were observed in the net AUC for tests of attention, psychomotor function and visual learning. Working memory net AUC (z-score·hr marginal mean +SEM) in EX+SIT and EX+BR combined (+16±24) trended towards significance relative to SIT (-22±27, p=0.06). Executive function net AUC (z-score·hr marginal mean +SEM) trended towards significance in EX+SIT and EX+BR combined (-35±27) relative to SIT (-82±31, p=0.08). When conditions were compared individually, no between-condition differences in net AUC were observed for tests of attention, psychomotor function or visual learning (Figure 3D, 3E, 3F). However, working memory net AUC was improved in EX+BR relative to SIT (Figure 4C; p=0.04 SIT vs. EX+BR). Conversely, executive function net AUC was improved in EX+SIT, relative to SIT (Figure 4D; p=0.02 SIT vs. EX+SIT). In addition to the analysis of the standardised z-scores, similar results were observed in supplementary analyses of the unstandardised data (Figure S1 and S2).

Brain derived neurotrophic growth factor (BDNF)

Serum BDNF, analysed as eight-hour net AUC ($ng \cdot hr \cdot mL^{-1}$ marginal mean +SEM) without correction for change in plasma volume, was significantly higher in both EX (+142±295) and EX+BR (+185±295), relative to SIT (-329±294, $p<0.01$ SIT vs. EX+SIT or EX+BR). Correcting these values for change in plasma volume attenuated differences between

conditions. However, the corrected net AUC was still higher in EX+SIT and EX+BR relative to SIT (Figure 5B). No significant difference was observed between EX+SIT and EX+BR. There were no significant correlations between BDNF and cognitive performance outcomes.

DISCUSSION

We examined the effects of a morning bout of moderate-intensity exercise, with or without subsequent light-intensity walking breaks from sitting on cognitive performance in older adults who were overweight-to-obese. Our principal findings are that both activity conditions conferred some cognitive benefit across an 8-hour period, relative to uninterrupted sitting. While no impacts were apparent in terms of attention, psychomotor function or visual learning, we observed that a bout of moderate-intensity exercise improved executive function scores over a subsequent period of prolonged sitting. However, when exercise was combined with subsequent breaks from sitting, improvements in executive function were attenuated whilst working memory scores improved. Taken together, these findings suggest that different patterns of physical activity may improve distinct aspects of cognition.

Prolonged sitting, exercise and cognition

Improvements in both executive function and working memory have previously been observed following acute and chronic exercise.[4,32] Conversely, it has been demonstrated that executive function and working memory scores are impaired by prolonged sitting, and improved by intermittent breaks in prolonged sitting.[24] However, no previous study has examined the combined effects of exercise, prolonged sitting and breaks in sitting on cognitive performance in a controlled experimental setting. For working memory, we found scores improved most when exercise was combined with subsequent breaks in sitting. This represents an opportunity to optimise the cognitive benefits of a bout of exercise performed at

a level recommended in public health guidelines. Our finding suggests that future studies may gain insight by studying the combined effects of an exercise bout with prolonged sitting/breaks in sitting, behaviours which have traditionally been studied in isolation, but which coexist in the real world.

The finding that post-exercise improvements in executive function were attenuated by the addition of intermittent light-intensity breaks in sitting was unexpected and rejects our hypothesis regarding breaks in sitting. While this finding was unexpected, it may not be extraordinary in the context of previous evidence which has documented an inverted U pattern of response in cognitive performance with increasing exercise intensity.[4] However, the distinction is that increasing volume and/or frequency in the current study, more specifically than intensity, resulted in a pattern of improvement during exercise plus sitting, but impairment during exercise plus breaks. It may be that interrupting participants every 30-minutes to walk on a treadmill represented a distraction during the executive function task, inducing a "cognitive overload" effect. We must consider the possibility that this effect could be related to the fact that the test of executive function appeared first in the test battery. During EX+BR, cognitive assessment began approximately two minutes after the last walking break finished. Had executive function been the last test in the battery, the finding may have been different. In addition to corroborating our findings, future studies should specifically manipulate the frequency/volume of walking breaks and the order/variety of cognitive assessments, in order to build a more robust evidence base on the combined effects of exercise and sedentary behaviour that may inform optimal strategies for cognitive performance. In addition, it is worth noting that executive function is generally considered to have sub-components of which working memory is one. In addition to testing spatial working memory, the Groton Maze Learning Test for executive function assess learning efficiency and

error monitoring.[33] It is possible that the intervention affected these sub-components of executive function differently and future studies may gain further insight by testing these sub-components separately.

Potential mechanisms

Central to mediating the benefits of exercise on synaptic plasticity, learning and memory is brain derived neurotrophic growth factor (BDNF).[34,35] While the brain is a major source of BDNF production following exercise,[36] other circulating factors secreted from exercising muscle such as irisin [37] and cathepsin B,[38] or liver derived β-hydroxybutyrate [39] may contribute to BDNF expression in the brain. Therefore, BDNF seems to play a central role in a coordinated response to exercise from multiple organs. In the current study, increased concentrations of BDNF in both the exercise and exercise plus breaks conditions, relative to the sitting condition were observed. However, BDNF changes were not significantly correlated with cognitive outcomes. Disparity exists among studies investigating whether the effects of acute exercise on cognition are mediated in part by BDNF. In animal models, acutely increasing [38] or decreasing [38] BDNF improves or impairs cognition respectively. However, in humans, memory-related cognitive tasks have been correlated with changes in BDNF but non-memory related tasks are less likely to exhibit such a relationship.[40] Moreover, peripheral concentrations of BDNF likely do not reflect changes that occur centrally. We chose to assess BDNF in serum, rather than plasma, since the former provides an index of both free and platelet-stored BDNF.[41,42] Given the short half-life of free BDNF, secreted BDNF may be stored by the platelet before it is measured in the plasma. We also measured haematocrit and haemoglobin pre to post the 8-hour condition to calculate and adjust for change in plasma volume, which is important in the context of exercise.[43] However, a more accurate measurement of change across the day could be obtained by more

frequent measures of haematocrit and haemoglobin. The increases in BDNF over 8 hours demonstrate an effective 'whole of day' strategy that could be repeated over weeks or months with implications for learning and memory. It is also emphasised that such a strategy has other plausible beneficial effects throughout the body, such as in heart and vasculature,[44] and even for respiration.[45] This may indirectly benefit brain health by supporting increased capacity to exercise.

Implications and future directions

Our findings have several potential implications. First, it seems likely that prolonged uninterrupted sitting should be avoided in order to maintain optimal cognition across the day in older adults. In addition, our findings may have implications for the design of longer term exercise interventions seeking to improve aspects of cognitive performance. Such interventions sometimes demonstrate improved cognition,[46–49] but not always.[50–52] If it were possible to optimise cognitive performance over a whole day period using different exercise strategies, this may translate to improved design of exercise interventions. We demonstrated that for working memory, the benefit of a single bout of exercise was enhanced by subsequent breaks from prolonged sitting, albeit to the detriment of executive function. Future studies should focus on whether modifying the volume, frequency or intensity of active breaks can identify how best to maximise cognitive benefit.

Strengths and limitations

Strengths of our study design include the investigation of exercise and sitting patterns that reflect different behaviours that occur in society. While many people accumulate high amounts of sitting, some proportion of these individuals also engage in a daily exercise routine to a level recommended in public health guidelines. In addition, some guidelines also

recommend reducing and breaking up sitting.[53,54] The current study aimed to test different combinations of these behaviours, as there have been calls for more evidence in this area.[55,56] Another strength lies in the selection of multiple cognitive tests reflecting different aspects of cognition using a validated instrument. There are also limitations. We did not include a condition involving breaks in sitting only. As previous evidence strongly supports the cognitive benefits of an acute 30-minute bout of MVPA, our goal was to ascertain whether the addition of intermittent active breaks, to a single bout of exercise, further enhanced cognitive outcomes. Practice effects are a potential limitation of cognitive assessment. However, participants were familiarised with cognitive assessment on a separate occasion three to five days prior to the first experimental condition. In addition, the order of conditions was random spreading any remaining practice effect across all conditions. Finally, our study did not reveal a definitive mechanistic explanation for the observed changes in cognition. Future studies using techniques such as fMRI or EEG may offer more insight in this regard.

Summary

For older adults, engaging in a morning bout of moderate-intensity exercise increases brain derived neurotrophic growth factor and improves cognitive performance over 8 hours, relative to prolonged sitting. However, the specific aspect of cognition that improves following exercise may be influenced by whether or not breaks in sitting are also performed. This suggests that various patterns of physical activity may be used to optimise the daily maintenance of brain health.

What are the findings?

- A morning bout of moderate-intensity exercise improved executive function over an 8-hour period in older adults, relative to prolonged sitting.
- When exercise was combined with light-intensity breaks in sitting, working memory but not executive function was improved, relative to prolonged sitting.
- Exercise with or without subsequent breaks in sitting increased serum brain derived neurotrophic factor over 8-hours, relative to prolonged sitting.

How might it impact on clinical practice in the future?

- Uninterrupted sitting should be avoided, and moderate-intensity exercise should be encouraged for the daily maintenance of brain health.
- Different patterns of physical activity may be utilised to enhance distinct aspects of cognition.

Contributors

MJW, DJG, KAE, EC, LN, RL, PW, CJB, NTL, BAK, GL, NO and DWD contributed to the design of the study. MJW coordinated the trial and data collection and is the study guarantor. IH and JL assisted with recruitment and data collection. NE assisted with biochemical analysis. MJW and EC wrote the statistical analysis plan. MJW, DJG and DWD wrote the paper. KAE, EC, IH, LN, RL, PW, CJB, JL, NE, NTL, BAK, GL and NO reviewed and edited the paper. All authors approved the final version of the manuscript.

Competing Interests

None declared

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Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Ethical approval was obtained from The Alfred Hospital Ethics Committee (181-14) and The University of Western Australia Human Research Ethics Committee (RA/4/1/6990).

REFERENCES

1 Winblad B, Cedazo-Minguez A, Graff C, *et al.* The Lancet Neurology Commission Defeating Alzheimer's disease and other dementias: a priority for European science and society. *www.thelancet.com/neurology Lancet Neurol* 2016;**15**:455–532. doi:10.1016/S1474-4422(16)00062-4

2 Ng TP, Feng L, Nyunt MSZ, *et al.* Metabolic Syndrome and the Risk of Mild Cognitive Impairment and Progression to Dementia: Follow-up of the Singapore Longitudinal Ageing Study Cohort. *JAMA Neurol* 2016;**73**:456–63. doi:10.1001/jamaneurol.2015.4899

3 Baumgart M, Snyder HM, Carrillo MC, *et al.* Summary of the evidence on modifiable risk factors for cognitive decline and dementia: A population-based perspective. *Alzheimers Dement* 2015;**11**:718–26. doi:10.1016/j.jalz.2015.05.016

4 Chang YK, Labban JD, Gapin JI, *et al.* The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Res* 2012;**1453**:87–101. doi:10.1016/j.brainres.2012.02.068

5 McMorris T, Hale BJ. Differential effects of differing intensities of acute exercise on speed and accuracy of cognition: A meta-analytical investigation. *Brain Cogn* 2012;**80**:338–51. doi:10.1016/j.bandc.2012.09.001

6 Jensen CS, Hasselbalch SG, Waldemar G, *et al.* Biochemical Markers of Physical Exercise on Mild Cognitive Impairment and Dementia: Systematic Review and Perspectives. *Front Neurol* 2015;**6**:187. doi:10.3389/fneur.2015.00187

7 Jefferis BJ, Parsons TJ, Sartini C, *et al.* Objectively measured physical activity, sedentary behaviour and all-cause mortality in older men: does volume of activity matter more than pattern of accumulation? *Br J Sports Med* Published Online First: 19 January 2018.http://bjsm.bmj.com/content/early/2018/01/19/bjsports-2017-098733.abstract

8 Jefferis BJ, Sartini C, Lee I-M, *et al.* Adherence to physical activity guidelines in older adults, using objectively measured physical activity in a population-based study. *BMC Public Health* 2014;**14**:382. doi:10.1186/1471-2458-14-382

9 Gorman E, Hanson HM, Yang PH, *et al.* Accelerometry analysis of physical activity and sedentary behavior in older adults: a systematic review and data analysis. *Eur Rev Aging Phys Act* 2014;**11**:35–49. doi:10.1007/s11556-013-0132-x

10 Tremblay MS, Aubert S, Barnes JD, *et al.* Sedentary Behavior Research Network (SBRN) – Terminology Consensus Project process and outcome. *Int J Behav Nutr*

Phys Act 2017;**14**:75. doi:10.1186/s12966-017-0525-8

11 Matthews CE, Keadle SK, Troiano RP, *et al.* Accelerometer-measured dose-response for physical activity, sedentary time, and mortality in US adults. *Am J Clin Nutr* 2016;**104**:1424–32. doi:10.3945/ajcn.116.135129

12 Diaz KM, Howard VJ, Hutto B, *et al.* Patterns of Sedentary Behavior and Mortality in U . S . Middle-Aged and Older Adults. *Ann Intern Med* 2017;**167**:465–75. doi:10.7326/M17-0212

13 Falck RS, Davis JC, Liu-Ambrose T. What is the association between sedentary behaviour and cognitive function? A systematic review. *Br J Sports Med* 2017;**51**:800–11. doi:10.1136/bjsports-2015-095551

14 Steinberg SI, Sammel MD, Harel BT, *et al.* Exercise, sedentary pastimes, and cognitive performance in healthy older adults. *Am J Alzheimers Dis Other Demen* 2015;**30**:290–8. doi:10.1177/1533317514545615

15 Benatti FB, Ried-Larsen M. The Effects of Breaking Up Prolonged Sitting Time: A review of Experimental Studies. *Med Sci Sport Exerc* 2015;**47**:2053–61. doi:10.1249/MSS.0000000000000654

16 Grace MS, Dempsey PC, Sethi P, *et al.* Breaking up prolonged sitting alters the postprandial plasma lipidomic profile of adults with Type 2 Diabetes. *J Clin Endocrinol Metab* 2017;**102**:1991–9. doi:10.1210/jc.2016-3926

17 Bhammar DM, Sawyer BJ, Tucker WJ, *et al.* Breaks in sitting time: Effects on continuously monitored glucose and blood pressure. *Med Sci Sports Exerc* 2017;**49**:2119–30. doi:10.1249/MSS.0000000000001315

18 Dempsey PC, Sacre JW, Larsen RN, *et al.* Interrupting prolonged sitting with brief bouts of light walking or simple resistance activities reduces resting blood pressure and plasma noradrenaline in type 2 diabetes. *J Hypertens* 2016;**34**:2376–82. doi:10.1097/HJH.0000000000001101

19 Restaino RM, Holwerda SW, Credeur DP, *et al.* Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. *Exp Physiol* 2015;**100**:829–38. doi:10.1113/EP085238

20 Thosar SS, Bielko SL, Mather KJ, *et al.* Effect of prolonged sitting and breaks in sitting time on endothelial function. *Med Sci Sports Exerc* 2015;**47**:843–9. doi:10.1249/MSS.0000000000000479

21 Howard BJ, Fraser SF, Sethi P, *et al.* Impact on hemostatic parameters of interrupting sitting with intermittent activity. *Med Sci Sports Exerc* 2013;**45**:1285–91.

doi:10.1249/MSS.0b013e318285f57e

22 Wennberg P, Boraxbekk C-J, Wheeler M, *et al.* Acute effects of breaking up prolonged sitting on fatigue and cognition: a pilot study. *BMJ Open* 2016;**6**:e009630. doi:10.1136/bmjopen-2015-009630

23 Carter SE, Draijer R, Holder SM, *et al.* Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. *J Appl Physiol* 2018;**125**:790–8. doi:10.1152/japplphysiol.00310.2018

24 Mullane SL, Buman MP, Zeigler ZS, *et al.* Acute effects on cognitive performance following bouts of standing and light-intensity physical activity in a simulated workplace environment. *J Sci Med Sport* 2017;**20**:489–93. doi:10.1016/j.jsams.2016.09.015

25 Dunstan DW, Wheeler MJ, Ellis KA, *et al.* Interacting effects of exercise with breaks in sitting time on cognitive and metabolic function in older adults: Rationale and design of a randomised crossover trial. *Ment Health Phys Act* 2018;**15**:11–6. doi:10.1016/j.mhpa.2018.05.003

26 Collie A, Maruff P, Darby DG, *et al.* The effects of practice on the cognitive test performance of neurologically normal individuals assessed at brief test-retest intervals. *J Int Neuropsychol Soc* 2003;**9**:419–28. doi:10.1017/S1355617703930074

27 Falleti MG, Maruff P, Collie A, *et al.* Practice effects associated with the repeated assessment of cognitive function using the CogState battery at 10-minute, one week and one month test-retest intervals. *J Clin Exp Neuropsychol* 2006;**28**:1095–112. doi:10.1080/13803390500205718

28 Fredrickson J, Maruff P, Woodward M, *et al.* Evaluation of the usability of a brief computerized cognitive screening test in older people for epidemiological studies. *Neuroepidemiology* 2010;**34**:65–75. doi:10.1159/000264823

29 Kenward MG, Roger JH. The use of baseline covariates in crossover studies. *Biostatistics* 2010;**11**:1–17. doi:10.1093/biostatistics/kxp046

30 Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;**37**:247–8. doi:10.1152/jappl.1974.37.2.247

31 Whelton PK, Carey RM, Aronow WS, *et al.* 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of

Cardiology/American Heart Association Task F. *Hypertens* 2018;**71**:1269–324. doi:10.1161/HYP.0000000000000066

32 Smith PJ, Blumenthal JA, Hoffman BM, *et al.* Aerobic Exercise and Neurocognitive Performance: a Meta-Analytic Review of Randomized Controlled Trials. *Psychosom Med* 2010;**72**:239–52. doi:10.1097/PSY.0b013e3181d14633

33 Pietrzak RH, Maruff P, Mayes LC, *et al.* An examination of the construct validity and factor structure of the Groton Maze Learning Test, a new measure of spatial working memory, learning efficiency, and error monitoring. *Arch Clin Neuropsychol* 2008;**23**:433–45. doi:10.1016/j.acn.2008.03.002

34 Park H, Poo MM. Neurotrophin regulation of neural circuit development and function. *Nat Rev Neurosci* 2013;**14**:7–23. doi:10.1038/nrn3379

35 Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci* 2004;**20**:2580–90. doi:10.1111/j.1460-9568.2004.03720.x

36 Rasmussen P, Brassard P, Adser H, *et al.* Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* 2009;**94**:1062–9. doi:10.1113/expphysiol.2009.048512

37 Wrann CD, White JP, Salogiannnis J, *et al.* Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. *Cell Metab* 2013;**18**:649–59. doi:10.1016/j.cmet.2013.09.008

38 Moon HY, Becke A, Berron D, *et al.* Running-Induced Systemic Cathepsin B Secretion Is Associated with Memory Function. *Cell Metab* 2016;**24**:332–40. doi:10.1016/j.cmet.2016.05.025

39 Sleiman SF, Henry J, Al-Haddad R, *et al.* Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body beta-hydroxybutyrate. *Elife* 2016;**5**: e15092. doi:10.7554/eLife.15092

40 Piepmeier AT, Etnier JL. Brain-derived neurotrophic factor (BDNF) as a potential mechanism of the effects of acute exercise on cognitive performance. *J Sport Heal Sci* 2015;**4**:14–23. doi:10.1016/j.jshs.2014.11.001

41 Polacchini A, Metelli G, Francavilla R, *et al.* A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. *Sci Rep* 2015;**5**:17989. doi:10.1038/srep17989

42 Fujimura H, Altar CA, Chen R, *et al.* Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost* 2002;**87**:728–

34.

43 Pareja-Galeano H, Alis R, Sanchis-Gomar F, *et al.* Methodological considerations to determine the effect of exercise on brain-derived neurotrophic factor levels. *Clin Biochem* 2015;**48**:162–6. doi:10.1016/j.clinbiochem.2014.11.013

44 Donovan MJ, Lin MI, Wiegn P, *et al.* Brain derived neurotrophic factor is an endothelial cell survival factor required for intramyocardial vessel stabilization. *Development* 2000;**127**:4531–40.

45 Ogier M, Kron M, Katz DM. Neurotrophic factors in development and regulation of respiratory control. *Compr Physiol* 2013;**3**:1125–34. doi:10.1002/cphy.c120029

46 Jonasson LS, Nyberg L, Kramer AF, *et al.* Aerobic Exercise Intervention, Cognitive Performance, and Brain Structure: Results from the Physical Influences on Brain in Aging (PHIBRA) Study. *Front Aging Neurosci* 2016;**8**:336. doi:10.3389/fnagi.2016.00336

47 Zheng G, Xia R, Zhou W, *et al.* Aerobic exercise ameliorates cognitive function in older adults with mild cognitive impairment: a systematic review and meta-analysis of randomised controlled trials. *Br J Sport Med* Published Online First: 19 April 2016. doi:10.1136/bjsports-2015-095699

48 van Uffelen JGZ, Chin A Paw MJM, Hopman-Rock M, *et al.* The effects of exercise on cognition in older adults with and without cognitive decline: a systematic review. *Clin J Sport Med Off J Can Acad Sport Med* 2008;**18**:486–500. doi:10.1097/JSM.0b013e3181845f0b

49 Lautenschlager NT, Cox KL, Flicker L, *et al.* Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA* 2008;**300**:1027–37. doi:10.1001/jama.300.9.1027

50 Sink KM, Espeland MA, Castro CM, *et al.* Effect of a 24-Month Physical Activity Intervention vs Health Education on Cognitive Outcomes in Sedentary Older Adults: The LIFE Randomized Trial. *JAMA* 2015;**314**:781–90. doi:10.1001/jama.2015.9617

51 Snowden M, Steinman L, Mochan K, *et al.* Effect of exercise on cognitive performance in community-dwelling older adults: review of intervention trials and recommendations for public health practice and research. *J Am Geriatr Soc* 2011;**59**:704–16. doi:10.1111/j.1532-5415.2011.03323.x

52 Young J, Angevaren M, Rusted J, *et al.* Aerobic exercise to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev* 2015;:CD005381. doi:10.1002/14651858.CD005381.pub4

53 Australian Government Department of Health. Australia's Physical Activity and Sedentary Behaviour Guidelines For Adults (18-64 years). 2014.
54 Department of Health, Physical Activity Health Improvement and Protection. Start active, Stay active: a report on physical activity for health from the four home countries' chief medical officers. London: England: 2011.
55 Physical Activity Guidelines Advisory Committee. 2018 Physical activity guidelines advisory committee scientific report. Washington, DC:U.S. Department of Health and Human Services: 2018. doi:10.1111/j.1753-4887.2008.00136.x
56 Stamatakis E, Ekelund U, Ding D, *et al.* Is the time right for quantitative public health guidelines on sitting? A narrative review of sedentary behaviour research paradigms and findings. *Br J Sports Med* Published Online First: 10 June 2018. doi: 10.1136/bjsports-2018-099131

Figure Legends

Figure 1. Experimental design. Participants completed three conditions in a random order separated by a minimum of six days. Conditions were: Sitting (SIT): uninterrupted sitting (8hr, control); Exercise + Sitting (EX+SIT): sitting (1hr), moderate-intensity walking (30min, denoted by walking figure) followed by uninterrupted sitting (6.5hr); Exercise + Breaks (EX+BR): sitting (1hr), moderate-intensity walking (30min) followed by sitting (6.5hr) interrupted every 30 minutes with 3 minutes of light-intensity walking. Walking breaks are denoted by vertical lines in the EX+BR condition.

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) flow diagram. BMI, body mass index; ECG, electrocardiogram.

Figure 3. Cognitive test scores for attention, psychomotor function and visual learning. Panels A-C represent attention, psychomotor function and visual learning z-scores, respectively, displayed as a change from baseline across 8 hours. Panels D-F represent the 8-hour net area under the curve (AUC) z-scores for attention, psychomotor function and visual learning z-scores, respectively. In all panels, a positive value on the y-axis denotes an improved score relative to baseline and vice versa for negative values. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Dotted lines represent the timing of the standardised meals. Data are marginal means and 95% CI, adjusted for age, sex, waist circumference, years of education, baseline, treatment order, and testing site. Panels A-C are additionally adjusted for multiple comparisons.

Figure 4. Cognitive test scores for working memory and executive function. Panels A and B represent the working memory composite and executive function z-scores, respectively, displayed as a change from baseline across 8 hours. Panels C and D represent the 8-hour net area under the curve (AUC) z-scores for the working memory composite and executive function tests, respectively. In all panels, a positive value on the y-axis denotes an improved score relative to baseline and vice versa for negative values. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Dotted lines represent the timing of the standardised meals. Data are marginal means and 95% CI, adjusted for age, sex, waist circumference, years of education, baseline, treatment order, and testing site. Panels A and B are additionally adjusted for multiple comparisons. *$p<0.05$ vs. sitting (SIT), **$p<0.01$ vs. SIT.

Figure 5. Serum brain derived neurotrophic growth factor (BDNF). Data are marginal means ± SEM. Panel A represents the concentration of BDNF across 8 hours. Panel B represents the concentration of BDNF as the 8-hour net AUC, relative to the baseline measure in that condition. The shaded area represents the moderate-intensity exercise bout performed in exercise+sitting (EX+SIT) and exercise+breaks (EX+BR). Dotted lines represent the timing of the standardised meals. Data are marginal means and 95% CI, adjusted for age, sex, waist circumference, change in plasma volume, baseline, treatment order and testing site. Panel A is additionally adjusted for multiple comparisons. ***$p<0.001$ vs. sitting (SIT).