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

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Abbreviations: AD; Alzheimer's disease. BDNF; brain derived neurotrophic factor. ECG; electrocardiogram. FMD; flow mediated dilation. MoCA; Montreal cognitive assessment. MMSE; mini mental state exam. MVPA; moderate to vigorous intensity physical activity. T2D; type two diabetes. UWA; The University of Western Australia. VAS; visual analogue scale.

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Abstract

Background:

A single bout of moderate-to-vigorous intensity exercise improves both metabolic and cognitive function. In addition to exercise, emerging evidence suggests that reducing sitting time may be another strategy for improving metabolic function. However, the combined effects of acute exercise with reductions in sitting time on cognitive and metabolic function are largely unknown.

Methods/design:

This is a dual-site randomised crossover trial involving three acute experimental conditions separated by a minimum 6-day washout period. This trial includes inactive older adults (55-80 years) who are overweight to obese (body mass index 25-45kg/m²). Participants are recruited to complete the following eight hour laboratory-based conditions in a random order; 1) Sitting: uninterrupted sitting (8hrs, control); 2) Exercise: sitting (1hr), moderate intensity walking (30mins) followed by uninterrupted sitting (6.5hrs); 3) Exercise + Breaks: sitting (1hr), moderate intensity walking (30mins) followed by sitting (6.5hrs) interrupted every 30 minutes with 3 minutes of light intensity walking. The primary outcome will be cognitive function, assessed using a battery of memory tests (Cogstate). Secondary outcome measures will include postprandial glucose, insulin, triglycerides, brain derived neurotrophic factor (BDNF), catecholamines, brain blood flow, brachial artery flow mediated dilation (FMD) and blood pressure.

Discussion:

This evidence will inform practical preventive strategies aimed at optimising daily cognitive, vascular and metabolic function among the large number of older adults who are behaviourally exposed to prolonged uninterrupted sitting.

1. Introduction

As a consequence of population ageing, the total number of people living with dementia globally is estimated to increase from 35.6 million in 2010, to 115.4 million by 2050 (Prince et al., 2013). In the absence of effective pharmacotherapy, lifestyle modification remains the most effective strategy for reducing the risk of Alzheimer's disease (AD) and other causes of dementia (Winblad et al., 2016). For example, regular moderate-to-vigorous physical activity (MVPA) reduces many of the metabolic risk factors for cognitive decline such as obesity, hypertension and altered glucose metabolism (Baumgart et al., 2015). Indeed, several prospective studies support the idea that regular MVPA is associated with a reduced risk of cognitive decline, AD and other dementias (Buchman et al., 2012; Rovio et al., 2005; Xu et al., 2017). However, the evidence is mixed and not all prospective studies agree (Sabia et al., 2017).

Intervention studies aimed at improving cognition by increasing physical activity levels over a period of weeks or months also show mixed results. Some studies demonstrate improvements in cognitive function (van Uffelen, Chin A Paw, Hopman-Rock, & van Mechelen, 2008; Zheng, Xia, Zhou, Tao, & Chen, 2016), but some more recent studies show no improvement in cognitive function following exercise intervention (Sink et al., 2015; Snowden et al., 2011; Young, Angevaren, Rusted, & Tabet, 2015). These null findings contrast with experimental evidence from laboratory studies demonstrating that an acute bout of exercise can improve cognitive function (with the largest effect size seen for executive function tasks) (Chang, Labban, Gapin, & Etnier, 2012). Acute exercise is also a potent stimulus for the induction of growth factors such as brain derived neurotrophic factor (BDNF) (Piepmeier & Etnier, 2015), neurogenesis and angiogenesis which are beneficial to brain health and cognition (Cotman, Berchtold, & Christie, 2007; Ratey & Loehr, 2011; Tarumi & Zhang, 2015). Taken together, it appears difficult to translate the cognitive benefits

of acute exercise seen in laboratory settings into exercise interventions which seek to maintain improvements over a longer period of time.

Such discrepancies may be due to between-study differences in methodology, including varying degrees of effort to account for potential confounding factors. For example, an intervention which increases MVPA may result in a compensatory increase in sedentary behaviour which may be a confounding variable. Indeed, many exercise intervention studies focus on achieving the recommend level of MVPA; 150 minutes per week, accumulated in bouts of >10 minutes (Garber et al., 2011). However, outside of this time spent exercising, prolonged sitting may have a negative impact on health. Observational evidence indicates that increased sedentary time is associated with a higher risk of multiple chronic diseases such as type 2 diabetes [T2D], cancer and cardiovascular disease, after adjusting for MVPA (Biswas et al., 2015). Conversely, breaking up sitting with intermittent activity improves markers of disease risk, most notably, postprandial carbohydrate and lipid metabolism (Benatti & Ried-Larsen, 2015; Grace et al., 2017), blood pressure (Bhammar, Sawyer, Tucker, & Gaesser, 2017; Dempsey, Sacre, et al., 2016) and vascular function (Restaino, Holwerda, Credeur, Fadel, & Padilla, 2015; Thosar, Bielko, Mather, Johnston, & Wallace, 2015). There is rationale to suspect that prolonged sitting may have a negative impact on cognitive function, due to its effects on risk factors for cognitive decline (Wheeler et al., 2017).

Indeed, some observational evidence indicates that higher levels of sitting time are associated with lower cognitive function (Falck, Davis, & Liu-Ambrose, 2016). In an experimental setting, it has been demonstrated that reducing and breaking up sitting with intermittent light intensity activity may improve cognitive function over a 6 hour period (Mullane, Buman, Zeigler, Crespo, & Gaesser, 2016). However, not all studies support this idea (Wennberg et

al., 2016). Differences between studies may be due to the total volume of accumulated activity.

While acute exercise and breaking up sitting may both exert a positive influence on cognition, the combined effects of both activities on cognitive function have not been studied in a controlled, experimental setting.

1.1 Aims and Hypotheses

This study will aim to compare the acute effects of prolonged uninterrupted sitting to a single bout of moderate-intensity exercise, with or without subsequent light-intensity breaks in sitting time, on (i) cognitive function, (ii) metabolic function and (iii) cerebrovascular function in older, overweight adults during the course of an eight hour experimental condition.

Hypothesis 1: A continuous (30 min) bout of moderate-intensity exercise will improve cognitive function compared with uninterrupted sitting.

Hypothesis 2: The magnitude of the improvement in cognitive function resulting from a moderate-intensity exercise bout will be greater when combined with a series of brief intermittent light-intensity breaks from prolonged sitting, relative to uninterrupted sitting.

2. Methods

2.1 Participants

Participants were recruited from the local community at two sites: 1) Physical Activity Laboratory, Baker Heart and Diabetes, Melbourne, Australia; and 2) Human Cardiovascular

Exercise Research Laboratory, School of Human Sciences (Exercise and Sport Science), The University of Western Australia (UWA), Perth, Australia. Recruitment was complete by the time of manuscript submission. This study has been approved by the Alfred Human Research Ethics Committee and Human Research Ethics Committee of The University of Western Australia. All participants provided written, informed consent prior to testing.

Inclusion criteria included: Age ≥ 55 to ≤ 80 years; BMI ≥ 25 kg/m² to < 45 kg/m²; English-speaking. Exclusion criteria include: Pregnancy; self-reported sitting < 5 hours per day; self-reported engagement in moderate-intensity exercise ≥ 150 min/week for > 3 months; probable dementia (Telephone Interview of Cognitive Status score of < 19); cognitive impairment (Mini Mental State Exam (MMSE) < 24 or Montreal Cognitive Assessment (MoCA) ≥ 26 when MMSE is between 24-28); depressive symptoms of clinical relevance (Geriatric Depression Score > 6 or Hospital Anxiety and Depression Scale score > 8); diagnosed diabetes; use of glucose/lipid lowering medication; antidepressant medications; beta blockers; anti-anxiety medication; peri-menopausal or menopausal women (must be post-menopausal); excessive alcohol consumption (> 8 points on the Alcohol Use Disorders Identification Test); abnormal ECG (determined by study doctor); high resting blood pressure (measured systolic > 160 mmHg or diastolic > 100 mmHg); major illness/physical problems (acute or chronic) that may limit ability to perform moderate intensity exercise.

2.2 Screening

Participants were initially telephone screened for eligibility during which time the following was administered: Telephone Interview of Cognitive Status (Welsh, Breitner, & Magruder-Habib, 1993); and the Geriatric Depression Score assessment (Lyne et al., 1997). During the phone screening the study was verbally explained to the participant. After passing the telephone screening, participants were invited to attend a fasted blood test (after an overnight

fast ≥ 10 hours) at a local pathology centre to screen for diabetes and determine general health status. Following the blood test participants were booked in for a familiarisation visit.

2.3 Familiarisation

Participants were instructed to avoid caffeine and alcohol and MVPA for 48 hours prior to familiarisation. During familiarisation the following information was obtained: Written informed consent; completion of the MMSE (Folstein, Folstein, & McHugh, 1975), where participant's score was between 24 -28 on the MMSE the MoCA was also be administered (Nasreddine et al., 2005); Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983); Physical Activity Readiness Questionnaire + (Bredin, Gledhill, Jamnik, & Warburton, 2013); Dutch Eating Behavior Questionnaire (van Strien, Frijters, Bergers, & Defares, 1986); resting blood pressure; resting 12-lead ECG and anthropometric measurements. Participants were familiarised with cognitive testing on a commercially available computerised test battery (Cogstate Ltd, Melbourne, Australia). Following familiarisation of cognitive testing, participants were given the opportunity to walk on a motorised treadmill to allow for the determination of the incline that equates to a relative moderate-intensity (65-75% of age predicted maximal heart rate). To determine this incline, participants walked for a total of 30 minutes at a speed of 3.2 km/h^{-1} . Ratings of perceived exertion and heart rate (Polar Electro, Kempele, Finland) were tracked every 3 minutes and the incline was adjusted by between $\pm 0.5 - 2\%$ until the participant reached the required heart rate zone. The final incline was then applied during the experimental conditions with a speed of 3.2 km/h^{-1} .

2.4 Randomisation and blinding

The order of experimental conditions was randomised by a third party using a computer generated sequence of numbers and sealed envelopes. Block randomisation was performed with balanced blocks, stratified by sex. Study personnel were blinded to the order of conditions until the night before the first experimental visit. Participants were blinded to the order of conditions until the morning of the second experimental visit. Blood sample analysis and data analysis will be performed by technicians blinded to the study conditions.

2.5 Standardisation of dietary intake and physical activity

Participants were instructed to avoid caffeine, alcohol and moderate to vigorous intensity physical activity prior to each experimental condition. To assess physical activity levels, participants were fitted with two objective physical activity monitors – an accelerometer (Actigraph model GT3X+) worn on the hip and an inclinometer (activPAL3) worn on the thigh, in combination with an activity diary. Activity monitors were worn during the time between visits, starting from the familiarisation session. Fully charged devices replaced battery depleted devices during each experimental visit. Participants were instructed to keep a weighed food diary for the 48 hour period prior to each experimental condition (scales provided). Food records will be assessed using Australian-specific dietary analysis software (Foodworks: Xyris Software, Highgate Hill, AUS). Food intake was controlled from the evening before each experimental condition with a meal pack provided for consumption at home between 7pm -9pm in place of any other food, with no food allowed after that time. The evening meal packs were individualised using the Schofield equation (Schofield, 1985) to meet 33% of estimated daily energy requirements and using dietary analysis software, were designed to have an energy macronutrient profile of 55-58% carbohydrate, 29-31% fat

and 12-15% protein, as has been used previously in similar research (Dempsey, Larsen, et al., 2016). During the experimental conditions, breakfast and lunch meals were provided which are individualised in the same manner as the evening meal, and participants consumed the same breakfast and lunch during each experimental condition.

2.6 Experimental day protocol

Participants reported to the laboratory at ~0700 following an overnight fast (>10 hours) (Figure 2). Blood pressure and brachial artery flow mediated dilation (FMD) were measured prior to a “steady-state” sitting period. Baseline measures of cognitive function (and cerebrovascular function at the UWA site only) were taken during the steady state sitting period. A fasting blood sample was obtained prior to the breakfast meal. Following steady state sitting, conditions become unique as per their experimental protocol and blood draws, blood pressure, subjective appetite, cognitive and cerebrovascular function tests (at the UWA site only) occurred at multiple time points across the day. At the completion of the experimental condition brachial artery FMD was measured again.

Insert figure 1 about here

3. Outcomes

3.1 Cognitive function

Cognitive function is the primary outcome, measured using a computerized test battery (Cogstate - www.cogstate.com.au) that has been specifically developed for brief repeated

testing of cognitive performance in clinical and research trials, with good acceptability, efficiency and stability, and minimisation of practice effects (Collie, Maruff, Darby, & McStephen, 2003; Falletti, Maruff, Collie, & Darby, 2006; Fredrickson et al., 2010). Previous work has demonstrated that executive function tasks are the most sensitive to improvement following acute exercise (Chang et al., 2012). The test battery covers multiple cognitive domains using the following tasks: (1) The Groton Maze Learning Test to measure executive function; (2) Detection Test to measure psychomotor function and speed of processing; (3) Identification Test to measure attention and (4) One Card Learning Test to measure visual learning and (5) One Back Test and Two Back Test to measure working memory. The total administration time is approximately 25 minutes.

3.2 Blood sampling

Venous blood samples were collected using an indwelling cannula inserted in an antecubital vein. Coded samples were sent to an independent National Association of Testing Authorities /The Royal College of Pathologists of Australasia -accredited laboratory on the day of testing for the determination of glucose, triglycerides and full blood evaluation. Blood was collected into fluoride/oxalate tubes for analysis of plasma glucose using the hexokinase method.

Blood was collected into lithium heparin tubes for analysis of triglycerides using a COBAS Integra 400+ analyser (Roche Diagnostics, Indianapolis, IN). Plasma and serum samples were also collected at each time point for storage at -80°C and later analysis. Plasma samples were collected and centrifuged immediately prior to -80°C storage and serum samples were allowed to clot for 1 hour at room temperature (22-24°C) prior to centrifuging. Plasma and serum samples were spun at 2000rpm for 15 minutes at 4°C at room temperature (22-24°C) prior to subsequent storage at -80°C .

3.3 Brachial artery function:

This test was performed at the beginning and end of each eight hour condition, according to accepted guidelines (Thijssen et al., 2011). Participants remained supine for 15 minutes in a dimly lit, temperature controlled room (22-24°C) prior to initial recording. Participants were measured again at the conclusion of the eight hour testing condition. Brachial artery FMD was measured with a high-resolution ultrasound machine (Terason t3200™, Teratech, Burlington, MA, USA) in conjunction with a 10 MHz multifrequency linear array probe and insonation angle of 60°. A rapid inflatable cuff (SCI12D™, D.E. Hokanson Inc., Bellevue, Washington) was placed around the forearm, distal to the cubital fossa. Once an optimal image of the artery was obtained, a one minute recording of continuous resting vessel diameter and blood velocity was measured (live duplex mode). The cuff was then inflated for five minutes (220 mmHg). After five minutes of inflation, the cuff was released to induce reactive hyperemia. A further three minutes of continuous duplex ultrasound recording was undertaken to observe the post deflation diameter profile and peak response.

3.4 Cerebrovascular function

Cerebral blood flow was measured with transcranial Doppler only in participants recruited to the UWA site. Bilateral measures of middle cerebral artery flow velocities were determined with a 2 MHz probe transfixed to the posterior aspect of the temporal window of the skull using the Mark 600 headframe (Spencer technologies, Seattle, USA). The location of the middle cerebral artery within the brain was achieved by locating the trifurcation of the circle of Willis (~45-65 mm) in the anterior circulation of the brain, as previously outlined (Willie et al., 2011).

3.5 Blood pressure

Resting blood pressure and heart rate were measured in a seated position on the contralateral arm to the cannula; taken as the average of three serial measurements using an automated oscillatory method (HEM-907, Omron, Kyoto, Japan). During the exercise plus breaks condition, blood pressure and heart rate were measured prior to the three minute walking break.

3.6 Subjective measures

Subjective appetite was assessed as there is a body of literature suggesting that walking can acutely reduce sugary food cravings (Ledochowski, Ruedl, Taylor, & Kopp, 2015; Oh & Taylor, 2013) and ad libitum snacking in a simulated workplace environment (Oh & Taylor, 2012). This evidence represents an indirect mechanism by which walking may ultimately affect glucose levels. Appetite variables were assessed using visual analogue scales, where a mark is placed on a 100mm lined between two opposite states (i.e “not hungry at all” to “as hungry as I have ever felt”). Subjective states assessed include, hunger, fullness, desire to eat, prospective food consumption and food preference, as previously described (Flint, Raben, Blundell, & Astrup, 2000). Non-appetite sensations of calmness and drowsiness were also assessed by a visual analogue scale (VAS) as they have previously been shown to correlate with food intake (Parker et al., 2004). Subjective fatigue was assessed using an 18 item VAS, previously validated against the Stanford Sleepiness Scale and the Profile of Mood States (Lee, Hicks, & Nino-Murcia, 1991), and demonstrated to be sensitive to change from intermittent light intensity walking (Wennberg et al., 2016).

4. Statistical methods

4.1 Sample size calculation

Power calculations have been made in relation to the primary outcome measures of cognitive function and reflect the cross-over design of the study. Based on recent evidence (Mullane et al., 2016), we estimate the effect sizes (Cohen's d for repeated measures; ES) of additional exposure to light-intensity breaks (as compared to sole exposure to a moderate-intensity exercise bout) would be ~0.40 for executive function tasks. From a clinical perspective, this moderate effect size is similar in magnitude to the difference in cognitive decline over 36 months between older adults with high brain levels of amyloid beta versus low levels of amyloid beta (Lim et al., 2015). Sample size calculations indicate that 52 participants would be needed to detect an ES of 0.40 based on one baseline and one post assessment per experimental condition. However, this trial will include three post assessments per experimental condition, which gives 3 x participant data points per condition. Assuming a within-participant, within-condition correlation between assessments of 0.6, the effective sample size per condition is $[3 \times \text{participants}] / [1 + (3-1) \times 0.6] = [3 \times \text{participants}] / 2.2$. To achieve an effective sample size of 52, we need $52 \times (2.2/3) \sim 40$ participants. Thus, the sample size needed to detect an ES of 0.40 with a power of 0.80 and a two-tailed probability of 0.05 is 40 (with three post-assessment measures per person and experimental condition). In a recent trial adopting a similar design, we achieved a 15% attrition rate. However, as a safeguard, assuming a conservative attrition rate of ~20%, the required sample size is 48 participants.

4.2 Statistical Analyses

Following recent recommendations on data analysis of cross-over trials (Kenward & Roger, 2010), generalized linear mixed models with random intercepts will be used to evaluate the differential effects of the experimental conditions on the selected outcomes. All models will include a binary variable indicating the experimental condition (intervention vs. control), adjusted for relevant confounders. Mixed models are appropriate for correlated data (repeated measures) with various distributional assumptions and can easily accommodate missing data (Rabe-Hesketh, Yang, & Pickles, 2001). A probability level of 0.05 will be adopted. Statistical analyses will be performed using Stata 15 for Windows (StataCorp LP).

5. Discussion

Maintaining optimal cognitive, vascular and metabolic function are essential for health and quality of life, especially for older adults. Regular exercise is currently the most effective known strategy to prevent cognitive decline, but whether such benefits could be enhanced by avoiding prolonged periods of uninterrupted sitting during the 'non-exercise' periods of the day is currently unknown. This controlled acute experimental trial will provide evidence in the context of an eight hour experimental model, in a population at heightened risk of cognitive decline – overweight older adults. The study will contribute new knowledge pertaining to the effects of a morning bout of exercise (at an intensity and duration consistent with current public-health guidelines) on cognitive function across the day, and further, will examine the additive effects of subsequent breaks from prolonged sitting. With the prevalence of dementia and associated metabolic and vascular pathologies on the rise across ageing populations, there is the need for the identification of practical preventive strategies

that can optimise daily cognitive, vascular and metabolic function among the large number of older adults who are at risk.

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Figure 1.

Figure Legend

Figure 1. Experimental design. Participants will complete three conditions in a random order, separated by a minimum of 6 days. Conditions are as follows: 1) Sitting: uninterrupted sitting (8 hours, control); 2) Exercise: sitting (1 hour), moderate intensity walking (30 minutes, denoted by walking figure) followed by uninterrupted sitting (6.5 hours); 3) Exercise + Breaks: sitting (1 hour), moderate intensity walking (30 minutes) followed by sitting (6.5 hours) interrupted every 30 minutes with 3 minutes of light intensity walking. Walking breaks are denoted by vertical lines in the exercise + breaks, a total of 12 breaks will be accumulated with the first break beginning 30 minutes after exercise. During each condition, participants will consume a standardised breakfast and lunch meal and study outcomes will be measured at multiple time points across the day. FMD; flow mediated dilation.