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Effects of Breaking Up Prolonged Sitting on Fatigue and Cognition: A Randomised Trial

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Abstract (word count: 297)

Objectives: To compare the acute effects of uninterrupted sitting with sitting interrupted by brief bouts of light-intensity walking on self-reported fatigue, cognition, neuroendocrine biomarkers and cardio-metabolic risk markers in overweight/obese adults. We hypothesized that the pattern of intermittent short bouts of physical activity may confer greater benefit on fatigue and cognitive performance than uninterrupted sitting.

Design: Randomised two-condition crossover trial.

Setting: Laboratory study conducted in Melbourne, Australia between May and August 2013.

Participants: Nineteen overweight/obese adults (45-75 years) recruited from the general community.

Interventions: After an initial 2h period seated, participants consumed a meal-replacement beverage and completed each condition over the next 5h: uninterrupted sitting (sedentary condition) or sitting with 3min bouts of light-intensity walking every 30 min (active condition). Fatigue and cognitive assessments, and

Primary outcome measures: Self-reported fatigue, executive function and episodic memory conducted at 0h, 4h and 7h.

Secondary outcome measures: Neuroendocrine biomarkers and cardio-metabolic risk markers (blood collections were conducted at 0h, 4h and 7h, blood pressure and heart rate were measured hourly and interstitial glucose was measured using a continuous glucose monitoring system).

Results: Fatigue levels were lower and heart rate was higher at 4h and 7h in the active condition, relative to the sedentary condition (p=0.024 and p=0.038, respectively). There were no significant differences between conditions by time for other variables. In the sedentary

condition, changes in fatigue scores over time correlated with a decrease in heart rate and plasma dihydroxyphenylalanine (DOPA) and an increase in plasma dihydroxyphenylglycol (DHPG).

Conclusion: Interrupting prolonged sitting with light-intensity walking breaks may be an effective fatigue counter-measure across the working day. Fatigue levels corresponded with heart rate and neuroendocrine biomarker changes in uninterrupted sitting. Further research is needed to identify potential implications, particularly for the occupational health context.

Trial registration: Australian New Zealand Clinical Trials Registry, ACTRN12613000137796.

STRENGTH AND LIMITATIONS OF THIS STUDY

- Breaking up prolonged sitting time with light-intensity physical activity is associated with beneficial effects on glucose metabolism; breaking up sitting may also be relevant for work-related fatigue and cognitive performance.

- This is the first experimental study to compare the acute effects of uninterrupted sitting on subjective fatigue and cognitive functioning with sitting interrupted every 30 min with light-intensity walking in a 7 hour 'whole working day' model.

- The sample size may have limited the ability of the study to detect an effect from breaking up sitting in several of the outcome measures.

- We studied acute effects and the longer-term relevance of breaking up prolonged sitting should be further examined, particularly for office workers and others with highly sedentary occupations.

INTRODUCTION

There is accumulating evidence on the negative health consequences of prolonged sitting. Previous experimental studies have demonstrated that uninterrupted sitting is deleteriously associated with metabolic parameters [1 2], blood pressure [3] and markers of haemostasis [4] when compared to sitting that is interrupted with short bouts of physical activity. A recent experimental trial utilising a 7 hour 'whole working day' model found that in overweight and obese adults, regularly interrupting sitting time with short bouts of either light- or moderate-intensity walking lowered postprandial glucose and insulin concentrations when compared with prolonged sitting [1]. Although glucose ingestion has been suggested to acutely improve memory and cognitive function [5], meals that trigger postprandial hyperglycaemia have been associated with a decline in cognitive function relative to meals that cause a more gradual rise in blood glucose concentrations [6]. The emerging evidence relating changes in postprandial glucose to changes in cognitive function provides a rationale to investigate whether breaking up prolonged sitting with light-intensity physical activity may also be relevant for fatigue and cognitive performance.

Aside from the metabolic benefits, physical activity may mediate several putative pathways involved in fatigue and cognition. These include increased cerebral blood flow [7] and expression of neurotrophins such as brain derived neurotrophic factor (BDNF) [8], which have been linked to both acute neuronal activation and long-term functional and structural changes in the brain [9]. During physical activity, particularly of higher intensities, there is a

release of adrenaline and noradrenaline from the adrenal medulla. Also, peripheral catecholamine release has a direct effect on synthesis and release of noradrenaline in the brain via activation of adrenoreceptors on vagal afferent nerves [10] that may increase alertness and facilitate cognition.

The possible influence of prolonged sitting on cognition is of great potential importance for work productivity and cognitive health especially among office workers, who are particularly vulnerable to prolonged uninterrupted sedentary behaviour [11]. In previous research, office workers have reported reduced fatigue across the day with the use of sit-to-stand desks when compared to seated work [12 13], but also self-perceived hampered concentration and focus when sitting and standing work were combined [12]. Given this preliminary evidence, and from a physiological standpoint, we hypothesize that the pattern of intermittent short bouts of physical activity leading to reduced prolonged sedentary states may confer greater benefit on fatigue and cognitive performance than uninterrupted sitting. We examined, in a 7 hour 'whole working day' model, the acute effects of uninterrupted sitting on subjective fatigue and cognitive functioning (executive functions and episodic memory), compared with sitting interrupted every 30 min with light-intensity walking.

METHODS

Study Design

The study was a laboratory-based randomised crossover trial with two experimental conditions separated by a 6 day wash-out period. The study was registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (ACTRN12613000137796) and was approved by the Alfred Hospital Ethics Committee. All participants provided signed informed consent.

Participants

Participants were recruited from the general community between May and August 2013 (Supplemental Figure). Eligible participants were aged 45-75 years with a BMI ≥25 kg/m^2 but <40 kg/m^2, who reported sitting >5h a day and were not engaged in regular moderate-to-vigorous intensity physical activity (MVPA; ≥150 min/week for >3 months). Exclusion criteria included those who were: non-English speaking, pregnant, currently smoking, diagnosed with diabetes, reported to have cognitive or physical problems that may limit the ability to perform the activity bouts or complete the computer-based cognitive test program, currently using any of the following medications: glucose-lowering, lipid-lowering, antidepressant or oral cortisone medication.

Power calculations for this study were based on metabolic parameters (differences in glucose AUC) due to the exploratory nature of the study and a lack of previous published data on the effects of sedentary behaviour on cognitive outcomes. Assuming a conservative correlation of 0.50 between repeated measures of the parameters, it was estimated that 15 participants would be required to detect a clinically-meaningful change for glucose AUC between the control (prolonged sedentary bout) and the experimental condition (intermittent activity bouts) with 80% power at a 0.05 probability level (2-tailed test). To compensate for participant withdrawal, 19 were recruited.

Randomisation

Participants were randomised to one of two possible trial-condition orders using fixed block sizes for men and women. The computer-generated randomisation list and randomisation envelopes were prepared and kept by a third party. Research staff opened randomisation envelopes, once informed consent was obtained.

Pre-experimental procedure

Screening and familiarization

Following telephone screening, participants attended an assessment and familiarization visit at the research laboratory. During this visit, they underwent training on the cognitive test battery and were given the opportunity to ask questions and were allowed unlimited time to complete practice tests to ensure sufficient understanding of each task. They were then familiarized with walking on the motorized treadmill on a level incline.

Diet and physical activity

To control and account for any diet-induced variability in study outcomes, participants were provided with standardized meal packs for consumption on the evening prior to each experimental condition. The meal packs (consisting of a drink, snack and a commercially available microwave meal) were individualized to meet 33.3% of daily estimated energy requirements using the Schofield equation [14] with 1.5 physical activity factor and a target macronutrient profile of 12-15% energy from protein, 55-58% energy from carbohydrate and 29-31% energy from fat. To minimize potential carryover effects of physical activity, participants were instructed to avoid any moderate and/or vigorous physical activity for at least 48 h prior to each experimental condition. MVPA was assessed with an accelerometer, Actigraph GT3X+ (ActiGraph LLC, Pensacola, FL, USA) and sitting time with an inclinometer, ActivPAL3 (Pal Technologies Ltd., Glasgow, UK), in the 48 h prior to the first experimental condition and for the washout period. The accelerometer was worn on the hip during waking hours. The inclinometer was attached with an adhesive tape to the front of the thigh, mid-way between the hip and the knee, during both waking and non-waking hours. The participants wore both activity monitors commencing from the familiarization visit until the end of the second experimental day. Wear time and activity type, duration, and intensity undertaken during any non-wear periods were recorded in activity diaries.

Experimental procedure

The study protocol is shown in Figure 1. After having fasted overnight, participants arrived at the laboratory between 0700 and 0800 h. In both conditions participants remained seated for the initial two hours to ensure that a sedentary status was achieved prior to the consumption of a mixed meal. The mixed meal was provided as a 200ml drink containing 50 g fat (Calogen; Nutricia, Australia) and 75 g carbohydrate (100% maltodextrin powder; Natural Health, Australia). This meal was chosen to be consistent with previous work that revealed differences in postprandial glucose and insulin levels with prolonged sitting when compared with to an equivalent duration of sitting that is interrupted with short bouts of light-intensity walking [1]. A small snack (Oatmeal biscuits; Paradise, Australia) was also provided to participants at 5 h to circumvent any unwanted effects from postprandial hypoglycaemia.

INSERT FIGURE 1 ABOUT HERE

After the mixed meal, participants followed their respective trial condition protocols for the following five hours under the direct supervision of research staff. In the sedentary condition, participants remained seated during the experimental period, only rising from the chair to visit the toilet (on the same floor, approximately 15 meters from the chair). No participant visited the toilet on more than two occasions during the sedentary condition. In the active condition, participants rose from the seated position every 30 min throughout the experimental period and completed a 3-min bout of light-intensity walking on a motorized treadmill (Nautilus T916) at a level incline (0 gradient) at 3.2 km/h. They then returned to the seated position. This procedure was undertaken on 10 occasions, providing a total of 30 min of light-intensity

activity. The mean rating of perceived exertion (RPE), assessed by the Borg RPE scale [15], for the walking bouts was 9.1 ± 2.0 (9 corresponds with "Very light" on the Borg RPE scale).

At 0h, 4h and 7h participants completed the Visual Analogue Scale for fatigue (VAS-F) [16] directly followed by the cognitive test battery (described under "Cognitive assessment" below) (total time: 26±1 min). The cognitive tests were conducted using E-prime 2.0 (Psychology Software Tools Inc.) on a Toshiba Satellite Pro C650 laptop computer with a 1366x768, 15.6 inch screen in a seated position. Throughout both conditions, participants read books, magazines or newspapers, watched television or DVDs or worked on a laptop computer. However, participants were instructed to limit the amount of interactive multimedia or problem-solving tasks to avoid impact on the cognitive testing. Blood collections, fatigue and cognitive tests (in that order) were undertaken at 0h, 4h and 7h.

Fatigue assessment

Visual analogue scale for fatigue

A visual analogue scale to evaluate fatigue severity (VAS-F) was used during the experimental days. The VAS-F questionnaire consisted of 18 items related to the subjective experience of fatigue and energy and has shown high reliability (Cronbach's α 0.91-0.96), sensitivity for changes in fatigue over a day, and compares favourably with the Stanford Sleepiness Scale and the Profile of Mood States in healthy participants [16]. Each item asks participants to mark with a single vertical dash, representing how they currently feel, on a continuous horizontal line that extends between two extremes, for example from "not at all tired" to "extremely tired".

Cognitive assessment

A battery of cognitive tests covering functions including episodic memory, inhibition, and updating were used. The focus on central executive tasks was chosen based on previously described larger effect size for higher-order cognitive tasks compared to less complex tasks [17 18]. To test episodic memory function, a face-name association test was used [19]. Participants were instructed to remember a fictional name associated with an unknown face. During retrieval, they were presented with the previously viewed faces together with three letters. The task was to indicate which of the three letters corresponded to the first letter of the name that was associated with the face. To test the executive function of inhibition, two tests were used, Eriksen flanker task [20] and a modified Stroop colour-word task [21]. In the Eriksen flanker task the participants indicated the direction of the middle arrow in either congruent (>>>>>) or incongruent (>><>>) stimuli by pressing a button as fast and as accurate as possible. In the Stroop test, participants were asked to indicate the colour a colour-word was written in, e.g. “blue” written in the colour blue, responding as fast and as accurately as possible.

The executive function updating was also tested using two tests: n-back [22]; and letter memory [23]. In n-back the participants were asked to indicate whether each number (1-9) in a list matched a number occurring one, two or three numbers back. Accuracy and response time were used as dependent measures. In letter memory they viewed lists consisting of serially presented letters (A-D, 2s per letter). They did not know the length of each list. When the list ended they were asked to recall the last four presented letters in the correct order as fast and as accurately as possible. A cognitive composite score (Z-score transformed) was derived from the five cognitive subtests.

Continuous glucose monitoring

Glucose concentrations were recorded using an IPro2 (Medtronic) Continuous Glucose Monitoring System (CGMS) during both experimental conditions. The CGMS sensor was inserted under the skin in the lower back, lateral to the medioclavicular line between the iliac crest and the lowest rib and measured interstitial glucose concentrations every 5 min. Capillary finger-pricks (Optium Xceed) at 0h, 1h, and 4h were used for calibration. The total area under the glycaemic response curve (total AUC) and the net incremental AUC (net iAUC), the area under the curve above the baseline (fasting) value, was derived from the CGMS measurements.

Plasma analysis

Fasting and postprandial blood samples were collected from a venous cannula inserted into the lower arm. Blood was transferred in EDTA tubes for BDNF, insulin, IL-6, cortisol and in GSH/EGTA tubes for analysis of catechols after at least 10 min of seated rest following the cannulation. Samples were immediately put on ice, then centrifuged (1800 rpm for 20 min at 4°C) within 30 min of collection, and plasma was stored at -80°C for later analysis. To avoid intra-assay variability, analyses of biochemical markers from the EDTA plasma were conducted in a single batch after the study. Catechols, however, were analyzed within two months in three batches to minimize the risk of degradation from long-term storage. Plasma catechols were determined by high-performance liquid chromatography with coulometric detection, following extraction from plasma using alumina adsorption [24].

Blood pressure and heart rate measurements

Blood pressure and heart rate were measured hourly using an automated oscillometric blood pressure monitor (Digital Automatic Blood Pressure Monitor HEM-907, Omron, Kyoto, Japan) as the average of three single measurements. The measurements were taken at 5 min

before each walking bout in a seated upright position on the arm contralateral to the arm with the intravenous cannula.

Statistical analysis

Generalized linear mixed models (GLMMs) with a single random intercept (participant) were used to examine the differential effects of the experimental conditions in variables measured continuously (interstitial glucose), hourly and at 0h, 4h and 7 h. All analyses were adjusted for time and order effects and interstitial glucose changes were also adjusted for capillary fasting glucose concentration and BMI. Temporal changes between conditions were also assessed by including a condition-time interaction term. Pearson's pairwise correlation tests were used to assess the relationship of fatigue to plasma markers. A probability level of 0.05 was adopted. All statistical analyses were performed using Stata 12 for Windows (StataCorp LP). Data are reported as mean (± SD) in the text and tables and as estimated marginal means ± SEM in the figures unless otherwise indicated.

RESULTS

All 19 participants (mean age 59.7 ± 8.1 years; 10 men/9 women) completed both trial conditions and were included in the analyses. Participant characteristics are shown in Table 1.

INSERT TABLE 1 ABOUT HERE

According to the accelerometer data, participants engaged in a modest amount of daily MVPA during the washout period. Physical activity levels did not significantly differ in the two days leading up to each experimental condition (sedentary, 32.6 ± 27.6 min/day, vs. active 32.5 ± 30.8 min/day; p=0.99).

Within- and between-condition responses for the fatigue score, cognitive scores and biomarkers at 0h, 4h and 7h are presented in Table 2 and 3 respectively. An effect of time was seen for fatigue (at 4h, p= 0.008; at 7h, p=0.010), cognitive composite score (at 7h, p= 0.033) and heart rate (at 4h, p<0.001; at 7h, p<0.001) in both conditions combined. A difference between conditions over time was seen for the fatigue score (see Figure 2) and heart rate. A trend (p=0.077) for improvement in the episodic memory test was observed in the active condition compared to the sedentary condition. No statistically significant differences were seen for any of the other measured variables, including the individual executive function tests (results are only shown for the composite scores), interstitial glucose total AUC (p=0.48) and net iAUC (p=0.14). The changes in fatigue score over time correlated with a decrease in heart rate (0h to 4h: r_s=-0.60, p=0.007) and plasma level of DOPA (0h to 4h: r_s=-0.59, p=0.009) and an increase in plasma level of DHPG (0h to 4h: r_s=0.73, p<0.001; 0h to 7h: r_s=0.47, p=0.040) in the sedentary condition (but not in the active condition).

DISCUSSION

Increases in fatigue levels observed during uninterrupted sitting were attenuated significantly by intermittent light-intensity walking. A non-significant trend for an improvement in cognitive performance with light-intensity walking breaks was observed. The difference in fatigue between the two conditions was observed after 4h and, interestingly, persisted after 7h (even though stabilization in fatigue score was seen between 4h and 7h in the sedentary condition). The increase in fatigue during uninterrupted sitting correlated with a decrease in heart rate and changes in plasma levels of catechols (acute increase of DHPG and a decrease of DOPA).

Previous research on the relationship between sedentary behaviour and fatigue in adults is scarce. Ellingson and colleagues (23) reported that women who were categorized as being insufficiently active (<150 min/wk), but who also spent less time sedentary (≤1 hour per day, measured by accelerometer), had significantly lower levels of fatigue compared with their more sedentary peers. Our findings are consistent with those of Thorp et al. [12], who observed significant attenuations in fatigue levels in office workers transitioning from a seated to a standing work posture every 30 min across the workday, relative to seated work. These findings add to the existing literature, providing initial experimental evidence that the relationship of prolonged uninterrupted sitting and fatigue may be causal and by examining the effect of short intermittent light-walking breaks on increased fatigue.

Fatigue is a multifaceted phenomenon. *Acute fatigue* can be viewed as a normal and protective mechanism to physical and/or mental exertion, which usually decreases as the exertion recedes. In contrast, *persistent fatigue* is associated with several medical and psychiatric illnesses as well as impaired cognitive performance [25] and is one of the most common complaints in community and primary care settings [26]. The underlying neurobiological mechanisms that may be responsible for the increased fatigue from prolonged sitting and attenuated fatigue from intermittent walking breaks observed in this study are unclear. However, studies in individuals with chronic fatigue have found several alterations and/or dysfunctions in nervous and endocrine systems [27], such as impaired autonomic nervous functions [28]. Interestingly, studies have also found autonomic nervous alterations (detected via electrocardiography) associated with daily levels of fatigue in healthy individuals after a 30 min fatigue-inducing mental task session [29].

The increased fatigue observed during uninterrupted sitting corresponded with a lower heart rate, decreased plasma level of DOPA (a precursor of the catecholamines dopamine, adrenaline and noradrenaline) and increased plasma level of DHPG (a deaminated metabolite of noradrenaline). The changes in heart rate and plasma levels of these compounds may reflect alterations in the sympathetic nervous system [30], although more precise measures of sympathetic nervous activity should be used in order to clarify this issue. Thus, a potential contributing mechanism through which intermittent walking breaks attenuated increased fatigue may relate to the maintenance of a balanced regulation of the autonomic nervous system activity. In addition, walking breaks may also counteract fatigue by the direct effects on the central nervous system, in which the increased heart rate may serve to increase the delivery of glucose to active domains in the brain [31]. Functional neuroimaging studies have suggested that the basal ganglia (midbrain) and regions interacting with the basal ganglia play key roles in fatigue in both fatigued and healthy individuals [32]. The use of these techniques in future studies has the potential to provide significant advances in understanding whether similar brain mechanisms may mediate also the effect of intermittent walking breaks observed in this study.

In contrast to previous research that have used higher break frequencies [1], the 3 min walking breaks performed twice an hour in this study did not induce a significant reduction in postprandial glucose levels, indicating that a greater/more regular stimulus may be required to reduce postprandial glucose in the studied population. However, we cannot exclude that the difference in glucose response compared to the previous trial may to some extent relate to differences between interstitial glucose and plasma glucose (which was measured in the previous trial). Comparisons between the two methods have revealed a time-lag in interstitial glucose of 10-20 min [33]. This time-lag may increase when there is a rapid rise in the plasma

glucose [33] and decrease during physical activity due to activity-induced augmented blood flow [34] and may explain why a more rapid increase in postprandial interstitial glucose was observed in the active condition compared to the sedentary condition in the current trial.

We examined the effects of light-intensity walking breaks on acute fatigue, but the results may also have implications for persistent fatigue considering the increasing time spent sedentary in many countries [35], and the high prevalence of persistent fatigue [36]. In the prolonged uninterrupted sitting condition, the natural behavioural response to acute fatigue — rest — did not seem to reduce the fatigue. Consequently, it could be speculated that uninterrupted sitting may potentially lead to a vicious cycle of fatigue and further sedentary behaviour. Indeed, emerging research has found an association between time spent sedentary and poor sleep efficiency [37], which in turn may lead to increased fatigue during waking hours [38]. Intermittent light-intensity walking breaks may be a feasible way to reduce fatigue especially for individuals with low uptake of MVPA, but the role for walking breaks in the prevention of persistent fatigue needs to be examined in long-term interventions.

This is the first experimental study to examine the impact of walking breaks on cognition under conditions that might correspond to a typical sedentary office or domestic day. The effect on fatigue was not translated into a statistically significant effect on cognitive performance, contrary to what could be expected from previous research on fatigue and cognition [39]. There are several possible explanations for the lack of effect on cognition. First, the cognitive effect from repeated 3 min bouts of light-intensity physical activity may be too weak to be detected with our sample size. The optimal intensity of the physical activity for cognitive effects is still under debate. For example, the inverted U-hypothesis suggests that moderate intensity physical activity yields the largest effect while ‘drive’ theories predict that

vigorous intensity will be most beneficial [17 40]. A meta-analysis indicated that when performed directly after the physical activity, lighter (very light, light, and moderate) activity is more beneficial, but vigorous intensity is necessary for effects to be maximized if there is a delay between the physical activity and the cognitive test [17]. Second, it is likely that reduced fatigue over time also may lead to improved cognitive functioning; however, this needs to be evaluated in longitudinal studies. Third, the cognitive score improved over the day in both conditions, indicating a practice effect, a common problem in cognitive studies with repeated assessment which may have distorted the validity of performance outcomes. Potentially, the cognitive effect from walking breaks may also be moderated by age, fitness level, educational level and the activity performed between tests (doing strict office work or leisure activities such as reading, watching television).

There are several limitations to the study. First, as mentioned, the sample size may have been too small to detect an effect in cognitive performance (as well as several of the potential mediators). Second, we studied acute effects and thus, any long-term effects of walking breaks cannot be evaluated based on the results in the study. Finally, we did not investigate whether the effects on fatigue and cognition may be moderated by participant characteristics. We suggest that future studies should: 1) include a larger sample size, 2) conduct repeated cognitive training sessions before the experiment to minimize the practice effect, 3) include a higher frequency of regular blood collection time-points to enable more robust analyses of changes over time and, 4) apply functional neuroimaging techniques to increase the knowledge on neurophysiologic mechanisms for the relationships between prolonged sitting, light-walking breaks, fatigue and cognition.

In conclusion, intermittent light-walking breaks resulted in an attenuation of fatigue levels during uninterrupted sitting, however, the difference in fatigue did not translate into significantly improved cognitive performance. Our findings provide further support to the suggestion that the relationship between sedentary behaviour and fatigue may be causal and that light-intensity walking breaks may counteract increased fatigue. Although the current study only examined short-term effects, there may be longer-term relevance and implications for sedentary behaviour, particularly among office workers and others with highly sedentary occupations. Thus, the role that reduced sedentary behaviour and walking breaks may play in the prevention and/or treatment of fatigue warrants further investigation. Considering the current high prevalence of persistent fatigue in the general population, reductions in sedentary time may have considerable relevance from a public health perspective.

FOOTNOTES

Contributorship

PW, DWD, C-JB, RL and GL contributed to the conception and design of the study. PW coordinated the trial and data collection, analysed the data, wrote the paper draft and is the study guarantor. MW assisted in collecting the data. PS and JO wrote the statistical analysis plan and conducted the statistical analyses. C-JB, MW, BH, PCD, GL, NE, RL, PS, JO, JH-B, KAE, NO and DWD analysed the data and reviewed and edited the paper draft. All authors approved the final version of the manuscript**.**

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Competing interests

None declared.

Ethics approval

The Alfred Hospital Ethics Committee

Data sharing

No additional data available.

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Table 1. Characteristics of the 19 participants in the study (10 men and 9 women).

BMI (kg/m^2)	31.5 (4.7)
Waist circumference (cm)	105.2 (12.4)
Completed university degree*	78.9
Taking antihypertensive medication*	47.4
Habitual moderate-to-vigorous physical activity (minutes/day)**	32.7 (28.7)
Habitual sitting time (h/day)**	10.0 (1.8)
Sleep time (h/night)*	7.4 (0.8)

Data are % or mean (SD).
*Assessed using self-reports.
**Assessed using activity monitors during the wash-out period between the two experimental days.

Table 2. Within-condition responses for the fatigue and cognitive scores at 0h, 4h and 7h.

			TIME (h)			
SCORE	**CONDITION**	**N**	**0**	**4**	**7**	***P****
Fatigue score	*Active*	19	37.9 (18.19)	34.3 (17.28)	36.6 (19.09)	
	Sedentary	19	37.4 (16.0)	47.1 (18.84)	46.8 (18.42)	
						0.024
Cognitive composite score	*Active*	19	0.018 (0.52)	0.080 (0.45)	0.273 (0.28)	
	Sedentary	19	-0.016 (0.50)	0.058 (0.33)	0.156 (0.41)	0.669
Cognitive subtests:						
Executive functions composite score	*Active*	19	-0.002 (0.49)	0.127 (0.43)	0.171 (0.30)	
	Sedentary	19	0.003 (0.45)	0.079 (0.36)	0.154 (0.39)	0.886
Episodic memory score	*Active*	19	68.9 (10.70)	64.5 (13.84)	78.5 (11.6)	
	Sedentary	19	65.4 (14.77)	66.2 (11.61)	69.3 (11.8)	0.077

*P-values for difference in temporal changes by condition.

Table 3. Within-condition responses for biomarkers at 0h, 4h and 7h.

		TIME (h)						
MARKER	**CONDITION**	**0**	**N**	**4**	**N**	**7**	**N**	***P****
Heart rate (bpm)	*Active*	64.7 (8.1)	19	71.8 (10.0)	19	71.6 (11.2)	19	
	Sedentary	63.5 (8.6)	19	66.2 (9.4)	19	66.1 (9.7)	19	
								0.038
Systolic blood	*Active*	119.5 (10.3)	19	117.5 (11.9)	19	117.5 (11.1)	19	
pressure (mmHg)	*Sedentary*	119.9 (12.7)	19	117.5 (12.7)	19	119.7 (12.9)	19	
								0.801
Diastolic blood	*Active*	76.1 (7.9)	19	74.2 (9.3)	19	75.3 (7.2)	19	
pressure (mmHg)	*Sedentary*	77.9 (8.6)	19	72.1 (9.6)	19	74.8 (8.8)	19	
								0.223
Insulin (uU/ml)	*Active*	21.7 (9.2)	18	105 (48.54)	19	17.8 (6.57)	18	
	Sedentary	20.9 (7.84)	19	96.4 (51.76)	19	19.2 (6.52)	19	
								0.679
IL-6 (pg/ml)	*Active*	0.95 (0.93)	18	1.32 (0.85)	19	1.92 (1.42)	18	
	Sedentary	0.94 (0.7)	19	1.21 (0.51)	19	1.34 (0.79)	19	
								0.123
Cortisol (pg/ml)	*Active*	3452 (3038)	18	1512 (1226)	19	1364 (737)	18	
	Sedentary	3618 (5472)	19	1651 (1085)	19	2653 (3617)	19	
								0.611
BDNF (pg/ml)	*Active*	14905 (8324)	15	15201 (8628)	16	12605 (7603)	14	
	Sedentary	14226 (6852)	16	13098 (6854)	15	14180 (7172)	15	
								0.184
Catechols Noradrenaline (pg/ml)	*Active*	427 (220)	18	475 (238)	18	440 (195)	19	
	Sedentary	375 (192)	18	466 (281)	17	473 (250)	18	
								0.177
DHPG (pg/ml)	*Active*	1244 (292)	18	1259 (307)	18	1276 (298)	19	
	Sedentary	1243 (245)	18	1338 (381)	17	1349 (329)	18	
								0.447
Adrenaline (pg/ml)	*Active*	36.6 (48.35)	17	109.1 (226.7)	17	171.2 (274.89)	18	
	Sedentary	44.4 (54.31)	18	118.7 (355.09)	17	69.8 (99.55)	18	
								0.198
DOPA (pg/ml)	*Active*	1884 (497)	18	1616 (455)	18	1862 (557)	19	
	Sedentary	1919 (505)	18	1739 (476)	17	2051 (588)	18	
								0.676
Dopamine (pg/ml)	*Active*	23.7 (26.31)	18	63 (158.12)	18	42.4 (75.24)	19	
	Sedentary	49.6 (134.01)	18	39.6 (81.37)	17	61.2 (190.59)	18	
								0.234
DHPG/Noradrenaline	*Active*	4.0 (1.78)	18	3.4 (1.28)	17	3.3 (1.34)	18	
	Sedentary	3.4 (1.46)	18	3.0 (1.01)	18	3.2 (1.15)	19	
								0.434

*P-values for difference in temporal changes by condition. IL-6, interleukin 6; BDNF, brain derived neurotrophic factor; DHPG, dihydroxyphenylglycol; DOPA, dihydroxyphenylalanine.

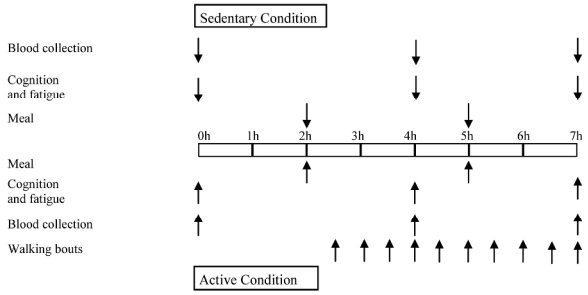


Figure 1: Study protocol
297x420mm (300 x 300 DPI)

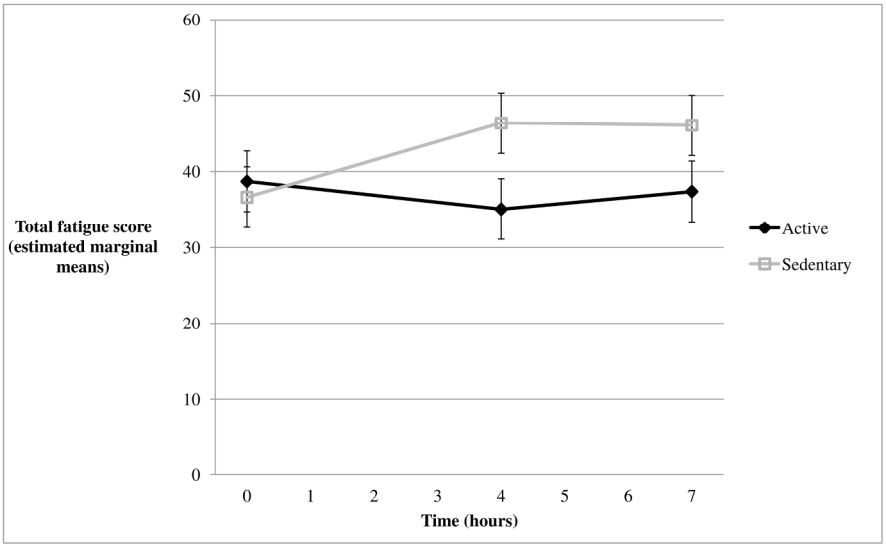
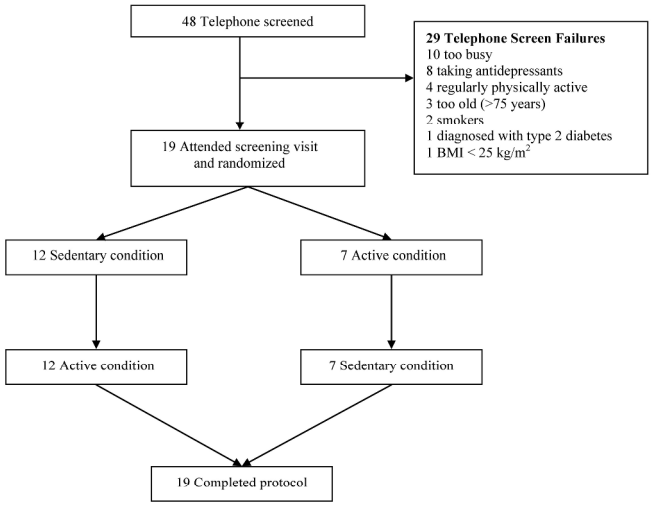


Figure 2: Total fatigue score (estimated marginal means) at 0h, 4h and 7h for the sedentary and the active conditions
209x148mm (300 x 300 DPI)



Supplemental Figure: Trial CONSORT diagram
297x420mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	5-17
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4-5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	6, 7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6, 7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	9-11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	None
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Not appl.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	Not appl.

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	Not appl.
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Not appl.
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	28
	13b	For each group, losses and exclusions after randomisation, together with reasons	28
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	24-26
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	24-26
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Not appl.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Not appl.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	None
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	17
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	15-16
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	5

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Acute Effects of Breaking Up Prolonged Sitting on Fatigue and Cognition: A Pilot Study

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Acute Effects of Breaking Up Prolonged Sitting on Fatigue and Cognition: A Pilot Study

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Running head: Sedentary behaviour, fatigue and cognition

Abstract (word count: 300)

Objectives: To compare the acute effects of uninterrupted sitting with sitting interrupted by brief bouts of light-intensity walking on self-reported fatigue, cognition, neuroendocrine biomarkers and cardio-metabolic risk markers in overweight/obese adults.

Design: Randomised two-condition crossover trial.

Setting: Laboratory study conducted in Melbourne, Australia.

Participants: Nineteen overweight/obese adults (45-75 years).

Interventions: After an initial 2h period seated, participants consumed a meal-replacement beverage and completed (on two days separated by a 6 day wash-out period) each condition over the next 5h: uninterrupted sitting (sedentary condition) or sitting with 3min bouts of light-intensity walking every 30 min (active condition).

Primary outcome measures: Self-reported fatigue, executive function and episodic memory at 0h, 4h and 7h.

Secondary outcome measures: Neuroendocrine biomarkers and cardio-metabolic risk markers (blood collections at 0h, 4h and 7h, blood pressure and heart rate measured hourly and interstitial glucose measured using a continuous glucose monitoring system).

Results: During the active condition fatigue levels were lower at 4h (-13.32 [95% CI -23.48 to -3.16]) and at 7h (-10.73 [95% CI -20.89 to -0.58]) compared to the sedentary condition. Heart rate was higher at 4h (4.47 [95% CI 8.37 to 0.58]) and at 7h (4.32 [95% CI 8.21 to 0.42]) during the active condition compared to the sedentary condition. There were no significant differences between conditions by time for other variables. In the sedentary condition, changes in fatigue scores over time correlated with a decrease in heart rate and

plasma dihydroxyphenylalanine (DOPA) and an increase in plasma dihydroxyphenylglycol (DHPG).

Conclusion: Interrupting prolonged sitting with light-intensity walking breaks may be an effective fatigue counter-measure acutely. Fatigue levels corresponded with heart rate and neuroendocrine biomarker changes in uninterrupted sitting in this pilot study. Further research is needed to identify potential implications, particularly for the occupational health context.

Trial registration: Australian New Zealand Clinical Trials Registry, ACTRN12613000137796.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first experimental study to compare the acute effects of uninterrupted sitting on subjective fatigue and cognitive functioning with sitting interrupted every 30 min with light-intensity walking in a 7 hour 'whole working day' model.
- A randomised crossover study design was used.
- Given this is a pilot study, the sample size may have limited the ability to detect an effect from breaking up sitting in several of the outcome measures.
- The longer-term relevance of acute effects of breaking up prolonged sitting are uncertain.

INTRODUCTION

There is accumulating evidence on the negative health consequences of prolonged sitting. Previous experimental studies have demonstrated that uninterrupted sitting is deleteriously associated with metabolic parameters [1 2], blood pressure [3] and markers of haemostasis [4] when compared to sitting that is interrupted with short bouts of physical activity. A recent experimental trial utilising a 7 hour 'whole working day' model found that in overweight and obese adults, regularly interrupting sitting time with short bouts of either light- or moderate-intensity walking lowered postprandial glucose and insulin concentrations when compared with prolonged sitting [1]. Although glucose ingestion has been suggested to acutely improve memory and cognitive function [5], meals that trigger postprandial hyperglycaemia have been associated with a decline in cognitive function relative to meals that cause a more gradual rise in blood glucose concentrations [6]. The emerging evidence relating changes in postprandial glucose to changes in cognitive function provides a rationale to investigate whether breaking up prolonged sitting with light-intensity physical activity may also be relevant for cognitive performance.

Aside from the metabolic benefits, physical activity may mediate several putative pathways involved in general mental fatigue and cognition. These include increased cerebral blood flow [7] and expression of neurotrophins such as brain derived neurotrophic factor (BDNF) [8], which have been linked to both acute neuronal activation and long-term functional and structural changes in the brain [9]. During physical activity, particularly of higher intensities, there is a release of adrenaline and noradrenaline from the adrenal medulla. Also, peripheral catecholamine release has a direct effect on synthesis and release of noradrenaline in the brain via activation of adrenoreceptors on vagal afferent nerves [10] that may increase alertness and facilitate cognition. Some sedentary behaviours, such as TV viewing, have been linked to

adverse long-term effects such as detrimental cognitive development in early childhood [11] and poorer cognitive function in older adults [12], but the mediating pathways are not well understood.

The possible influence of prolonged sitting on cognition is of great potential importance for work productivity and cognitive health especially among office workers, who are particularly vulnerable to prolonged uninterrupted sedentary behaviour [13]. In previous research, office workers have reported reduced fatigue across the day with the use of sit-to-stand desks when compared to seated work [14 15], but also self-perceived hampered concentration and focus when sitting and standing work were combined [14]. Given this preliminary evidence, and from a physiological standpoint, we hypothesize that the pattern of intermittent short bouts of physical activity leading to reduced prolonged sedentary states may confer greater benefit on fatigue and cognitive performance than uninterrupted sitting. We examined, in a 7 hour 'whole working day' model, the acute effects of uninterrupted sitting on subjective fatigue and cognitive functioning (executive functions and episodic memory), compared with sitting interrupted every 30 min with light-intensity walking. The study had an explorative approach and a small sample size and was conducted as an evaluation of technical procedures and logistic feasibility of a full-scale study (clinical trial registration ACTRN12614000737639).

METHODS

Study design

The study was a laboratory-based randomised crossover trial with two experimental conditions separated by a 6 day wash-out period. The study was registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (ACTRN12613000137796) and was

approved by the Alfred Hospital Ethics Committee. All participants provided signed informed consent.

Participants

Participants were recruited from the general community between May and August 2013 through the use of study fliers and posters or were contacted based on their previously registered expression of interest in future studies (Supplemental Figure). Eligible participants were aged 45-75 years with a BMI ≥25 kg/m^2 but <40 kg/m^2, who reported sitting >5h a day and were not engaged in regular moderate-to-vigorous intensity physical activity (MVPA; ≥150 min/week for >3 months). Exclusion criteria included those who were: non-English speaking, pregnant, currently smoking, diagnosed with diabetes, reported to have cognitive or physical problems that may limit the ability to perform the activity bouts or complete the computer-based cognitive test program, currently using any of the following medications: glucose-lowering, lipid-lowering, antidepressant or oral cortisone medication. Consistent with a previous trial with a similar experimental design [1], 19 participants were recruited.

Randomisation

Participants were randomised to one of two possible trial-condition orders using fixed block sizes for men and women. The computer-generated randomisation list and randomisation envelopes were prepared and kept by a third party. Research staff opened randomisation envelopes, once informed consent was obtained.

Pre-experimental procedure

Screening and familiarization

Following telephone screening, participants attended an assessment and familiarization visit at the research laboratory. During this visit, they underwent training on the cognitive test battery

and were given the opportunity to ask questions and were allowed unlimited time to complete practice tests to ensure sufficient understanding of each task. They were then familiarized with walking on the motorized treadmill on a level incline.

Diet and physical activity

To control and account for any diet-induced variability in study outcomes, participants were provided with standardized meal packs for consumption on the evening prior to each experimental condition. The meal packs (consisting of a drink, snack and a commercially available microwave meal) were individualized to meet 33.3% of daily estimated energy requirements using the Schofield equation [16] with 1.5 physical activity factor and a target macronutrient profile of 12-15% energy from protein, 55-58% energy from carbohydrate and 29-31% energy from fat. To minimize potential carryover effects of physical activity, participants were instructed to avoid any moderate and/or vigorous physical activity for at least 48 h prior to each experimental condition. Sitting time and MVPA were assessed with an accelerometer, Actigraph GT3X+ (ActiGraph LLC, Pensacola, FL, USA) which was worn on the hip during waking hours. The participants wore the accelerometer commencing from the familiarization visit until the end of the second experimental day. Wear time and activity type, duration, and intensity undertaken during any non-wear periods were recorded in activity diaries. Accelerometer data were downloaded using ActiLife 6.5.4 software and summarized using SAS 9.4 for each of the habitual days prior to the condition. Non-wear periods were deleted for analyses; these were periods with at least 60 minutes of zero counts per minute (cpm), allowing for up to two consecutive, one minute interruptions (count values between 1–49 cpm) per non-wear period [17]. Activity counts were categorised as sedentary (<100 cpm; predominantly sitting) [18], light-intensity activity (100-1951 cpm; typically gentle walking) [19], or MVPA (≥1952 cpm; typically at least brisk walking) [19]. Average of sitting time and MVPA

were calculated for each individual across valid days (days where the monitor was worn for at least 600 mins).

Experimental procedure

The study protocol is shown in Figure 1. After having fasted overnight, participants arrived at the laboratory between 0700 and 0800 h. In both conditions participants remained seated for the initial two hours to ensure that a sedentary status was achieved prior to the consumption of a mixed meal. The mixed meal was provided as a 200ml drink containing 50 g fat (Calogen; Nutricia, Australia) and 75 g carbohydrate (100% maltodextrin powder; Natural Health, Australia). This meal was chosen to be consistent with previous work that revealed differences in postprandial glucose and insulin levels with prolonged sitting when compared with to an equivalent duration of sitting that is interrupted with short bouts of light-intensity walking [1]. A small snack (Oatmeal biscuits; Paradise, Australia) was also provided to participants at 5 h to circumvent any unwanted effects from postprandial hypoglycaemia.

INSERT FIGURE 1 ABOUT HERE

After the mixed meal, participants followed their respective trial condition protocols for the following five hours under the direct supervision of research staff. In the sedentary condition, participants remained seated during the experimental period, only rising from the chair to visit the toilet (on the same floor, approximately 15 meters from the chair). During the sedentary condition 14 participants visited the toilet (8 participants on one occasion, 3 participants on two occasions, 2 participants on three occasions and 1 participant on four occasions) compared to the active condition where 8 participants visited the toilet only once.

In the active condition, participants rose from the seated position every 30 min throughout the experimental period and completed a 3-min bout of light-intensity walking on a motorized treadmill (Nautilus T916) at a level incline (0 gradient) at 3.2 km/h. They then returned to the seated position. This procedure was undertaken on 10 occasions, providing a total of 30 min of light-intensity activity. Previous research has shown that a 2-min bout of light-intensity walking every 20 min lowered postprandial glucose and insulin concentrations [1], but the intervals were expanded in the current study to enable a complete session of fatigue and cognitive assessments during a single bout of sitting.

At 0h, 4h and 7h participants completed the Visual Analogue Scale for fatigue (VAS-F) [20] directly followed by the cognitive test battery (described under "Cognitive assessment" below) (total time: 26±1 min). The cognitive tests were conducted using E-prime 2.0 (Psychology Software Tools Inc.) on a Toshiba Satellite Pro C650 laptop computer with a 1366x768, 15.6 inch screen in a seated position. Throughout both conditions, participants read books, magazines or newspapers, watched television or DVDs or worked on a laptop computer. However, participants were instructed to limit the amount of interactive multimedia or problem-solving tasks to avoid impact on the cognitive testing. Blood collections, fatigue and cognitive tests (in that order) were undertaken at 0h, 4h and 7h.

Fatigue assessment

Visual analogue scale for fatigue

A visual analogue scale to evaluate fatigue severity (VAS-F) was used during the experimental days. The VAS-F questionnaire consisted of 18 items related to the subjective experience of fatigue and energy and has shown high reliability (Cronbach's α 0.91-0.96), sensitivity for changes in fatigue over a day, and compares favourably with the Stanford Sleepiness Scale and the Profile of Mood States in healthy participants [20]. Each item asks participants to mark with a single vertical dash, representing how they currently feel, on a continuous horizontal line that extends between two extremes, for example from "not at all tired" to "extremely tired".

Cognitive assessment

A battery of cognitive tests covering functions including episodic memory, inhibition, and updating were used. The focus on central executive tasks was chosen based on previously described larger effect size for higher-order cognitive tasks compared to less complex tasks [21] [22]. To test episodic memory function, a face-name association test was used [23]. Participants were instructed to remember a fictional name associated with an unknown face. During retrieval, they were presented with the previously viewed faces together with three letters. The task was to indicate which of the three letters corresponded to the first letter of the name that was associated with the face. To test the executive function of inhibition, two tests were used, Eriksen flanker task [24] and a modified Stroop colour-word task [25]. In the Eriksen flanker task the participants indicated the direction of the middle arrow in either congruent (>>>>>) or incongruent (>><>>) stimuli by pressing a button as fast and as accurate as possible. In the Stroop test, participants were asked to indicate the colour a colour-word was written in, e.g. "blue" written in the colour blue, responding as fast and as accurately as possible.

The executive function updating was also tested using two tests: n-back [26]; and letter memory [27]. In n-back the participants were asked to indicate whether each number (1-9) in a list

matched a number occurring one, two or three numbers back. Accuracy and response time were used as dependent measures. In letter memory they viewed lists consisting of serially presented letters (A-D, 2s per letter). They did not know the length of each list. When the list ended they were asked to recall the last four presented letters in the correct order as fast and as accurately as possible. A cognitive composite score (Z-score transformed) was derived from the five cognitive subtests.

Continuous glucose monitoring

Glucose concentrations were recorded using an IPro2 (Medtronic) Continuous Glucose Monitoring System (CGMS) [28 29] during both experimental conditions. The CGMS sensor was inserted under the skin in the lower back, lateral to the medioclavicular line between the iliac crest and the lowest rib and measured interstitial glucose concentrations every 5 min. Capillary finger-pricks (Optium Xceed) at 0h, 1h, and 4h were used for calibration in accordance with the manufacturer's instructions. The total area under the glycaemic response curve (total AUC) and the net incremental AUC (net iAUC), includes all incremental area below the curve, including the area below the fasting concentration, was derived from the CGMS measurements.

Plasma analysis

Fasting and postprandial blood samples were collected from a venous cannula inserted into the lower arm. Blood was transferred in EDTA tubes for BDNF, insulin, IL-6, cortisol and in GSH/EGTA tubes for analysis of catechols after at least 10 min of seated rest following the cannulation. Samples were immediately put on ice, then centrifuged (1800 rpm for 20 min at 4°C) within 30 min of collection, and plasma was stored at -80°C for later analysis. To avoid intra-assay variability, analyses of biochemical markers from the EDTA plasma were conducted in a single batch after the study. Catechols, however, were analyzed within two

months in three batches to minimize the risk of degradation from long-term storage. Plasma catechols were determined by high-performance liquid chromatography with coulometric detection, following extraction from plasma using alumina adsorption [30].

Blood pressure and heart rate measurements

Blood pressure and heart rate were measured hourly using an automated oscillometric blood pressure monitor (Digital Automatic Blood Pressure Monitor HEM-907, Omron, Kyoto, Japan) as the average of three single measurements. The measurements were taken at 5 min before each walking bout in a seated upright position on the arm contralateral to the arm with the intravenous cannula.

Statistical analysis

Generalized linear mixed models (GLMMs) with a single random intercept (participant) were used to examine the differential effects of the experimental conditions in variables measured continuously (interstitial glucose), hourly and at 0h, 4h and 7 h. All analyses were adjusted for time and order effects and interstitial glucose changes were also adjusted for capillary fasting glucose concentration and BMI. Temporal changes between conditions were also assessed by including a condition-time interaction term. Pearson's pairwise correlation tests were used to assess the relationship of fatigue to plasma markers. A probability level of 0.05 was adopted. All statistical analyses were performed using Stata 12 for Windows (StataCorp LP). Data are reported as mean (± SD) in the text and tables and as estimated marginal means ± SEM in the figures unless otherwise indicated.

RESULTS

All 19 participants (mean age 59.7 ± 8.1 years; 10 men/9 women) completed both trial conditions and were included in the analyses. Participant characteristics are shown in Table 1.

INSERT TABLE 1 ABOUT HERE

According to the accelerometer data, participants engaged in a modest amount of daily MVPA during the washout period. Levels of MVPA in the 48 hours prior to each experimental condition did not differ significantly (36.29 ± 29.20 min/day before the sedentary condition vs. 30.18 ± 42.16 min/day before the active condition; p=0.607).

The mean rating of perceived exertion (RPE), assessed by the Borg RPE scale [31], for the walking bouts was 9.1 ± 2.0 (9 corresponds with "Very light" on the Borg RPE scale). Within- and between-condition responses for the fatigue score, cognitive scores and biomarkers at 0h, 4h and 7h are presented in Table 2 and 3 respectively. An effect of time was seen for fatigue (at 4h, p= 0.008; at 7h, p=0.010), cognitive composite score (at 7h, p= 0.033) and heart rate (at 4h, p<0.001; at 7h, p<0.001) in both conditions combined. During the active condition fatigue levels were lower at 4h (-13.32 [95% CI -23.48 to -3.16]) and at 7h (-10.73 [95% CI -20.89 to -0.58]) compared to the sedentary condition (see Figure 2). Heart rate was higher at 4h (4.47 [95% CI 8.37 to 0.58]) and at 7h (4.32 [95% CI 8.21 to 0.42]) during the active condition compared to the sedentary condition. A trend (p=0.077) for improvement in the episodic memory test was observed in the active condition compared to the sedentary condition. No statistically significant differences were seen for any of the other measured variables, including the individual executive function tests (results are only shown for the composite scores), interstitial glucose total AUC (p=0.48) and net iAUC (p=0.14). The changes in fatigue score over time correlated with a decrease in heart rate (0h to 4h: r_s=-0.60, p=0.007) and plasma level of DOPA (0h to 4h: r_s=-0.59, p=0.009) and an increase in plasma

level of DHPG (0h to 4h: r_s=0.73, p<0.001; 0h to 7h: r_s=0.47, p=0.040) in the sedentary condition (but not in the active condition).

DISCUSSION

Increases in fatigue levels observed during uninterrupted sitting were attenuated significantly by intermittent light-intensity walking. A non-significant trend for an improvement in cognitive performance with light-intensity walking breaks was observed. The difference in fatigue between the two conditions was observed after 4h and, interestingly, persisted after 7h (even though stabilization in fatigue score was seen between 4h and 7h in the sedentary condition). The increase in fatigue during uninterrupted sitting correlated with a decrease in heart rate and changes in plasma levels of catechols (acute increase of DHPG and a decrease of DOPA). As a comparison, the magnitude of change in fatigue score from 0h to 4h during uninterrupted sitting correspond to approximately 40 % of the difference in fatigue score recorded before and after one night's sleep in a study of 75 healthy individuals, aged 18-55 years [20].

Previous research on the relationship between sedentary behaviour and fatigue in adults is scarce. Ellingson and colleagues (23) reported that women who were categorized as being insufficiently active (<150 min/wk), but who also spent less time sedentary (≤1 hour per day, measured by accelerometer), had significantly lower levels of fatigue compared with their more sedentary peers. Our findings are consistent with those of Thorp et al. [14], who observed significant attenuations in fatigue levels in office workers transitioning from a seated to a standing work posture every 30 min across the workday, relative to seated work. These findings add to the existing literature, providing initial experimental evidence that the

relationship of prolonged uninterrupted sitting and fatigue may be causal and by examining the effect of short intermittent light-walking breaks on increased fatigue.

Fatigue is a multifaceted phenomenon. *Acute fatigue* can be viewed as a normal and protective mechanism to physical and/or mental exertion, which usually decreases as the exertion recedes. In contrast, *persistent fatigue* is associated with several medical and psychiatric illnesses as well as impaired cognitive performance [32] and is one of the most common complaints in community and primary care settings [33]. The underlying neurobiological mechanisms that may be responsible for the increased fatigue from prolonged sitting and attenuated fatigue from intermittent walking breaks observed in this study are unclear. However, studies in individuals with chronic fatigue have found several alterations and/or dysfunctions in nervous and endocrine systems [34], such as impaired autonomic nervous functions [35]. Interestingly, studies have also found autonomic nervous alterations (detected via electrocardiography) associated with daily levels of fatigue in healthy individuals after a 30 min fatigue-inducing mental task session [36].

The increased fatigue observed during uninterrupted sitting corresponded with a lower heart rate, decreased plasma level of DOPA (a precursor of the catecholamines dopamine, adrenaline and noradrenaline) and increased plasma level of DHPG (a deaminated metabolite of noradrenaline). The changes in heart rate and plasma levels of these compounds may reflect alterations in the sympathetic nervous system [37], although more precise measures of sympathetic nervous activity should be used in order to clarify this issue. Thus, a potential contributing mechanism through which intermittent walking breaks attenuated increased fatigue may relate to the maintenance of a balanced regulation of the autonomic nervous system activity. In addition, walking breaks may also counteract fatigue by the direct effects

on the central nervous system, in which the increased heart rate may serve to increase the delivery of glucose to active domains in the brain [38]. Functional neuroimaging studies have suggested that the basal ganglia (midbrain) and regions interacting with the basal ganglia play key roles in fatigue in both fatigued and healthy individuals [39]. The use of these techniques in future studies has the potential to provide significant advances in understanding whether similar brain mechanisms may mediate also the effect of intermittent walking breaks observed in this study.

In contrast to previous research that have used higher break frequencies [1], the 3 min walking breaks performed twice an hour in this study did not induce a significant reduction in postprandial glucose levels, indicating that a greater/more regular stimulus may be required to reduce postprandial glucose in the studied population. However, we cannot exclude that the difference in glucose response compared to the previous trial may to some extent relate to differences between interstitial glucose and plasma glucose (which was measured in the previous trial). Comparisons between the two methods have revealed a time-lag in interstitial glucose of 10-20 min [40]. This time-lag may increase when there is a rapid rise in the plasma glucose [40] and decrease during physical activity due to activity-induced augmented blood flow [41] and may explain why a more rapid increase in postprandial interstitial glucose was observed in the active condition compared to the sedentary condition in the current trial.

In many countries, increasing time is spent sedentary [42] and there is high prevalence of persistent fatigue [43]. We examined the effects of light-intensity walking breaks on acute fatigue and whether these results may have implications for persistent fatigue is uncertain. In the prolonged uninterrupted sitting condition, the natural behavioural response to acute fatigue — rest — did not seem to reduce the fatigue. Consequently, it could be speculated that

uninterrupted sitting may potentially lead to a vicious cycle of fatigue and further sedentary behaviour. Indeed, emerging research has found an association between time spent sedentary and poor sleep efficiency [44], which in turn may lead to increased fatigue during waking hours [45]. Intermittent light-intensity walking breaks may be a feasible way to reduce fatigue especially for individuals with low uptake of MVPA, but the role for walking breaks in the prevention of persistent fatigue needs to be examined in long-term interventions.

This is the first experimental study to examine the impact of walking breaks on cognition under conditions that might correspond to a typical sedentary office or domestic day. The effect on fatigue was not translated into a statistically significant effect on cognitive performance, contrary to what could be expected from previous research on fatigue and cognition [46]. There are several possible explanations for the lack of effect on cognition. First, the cognitive effect from repeated 3 min bouts of light-intensity physical activity may be too weak to be detected with our sample size. The optimal intensity of the physical activity for cognitive effects is still under debate. For example, the inverted U-hypothesis suggests that moderate intensity physical activity yields the largest effect while 'drive' theories predict that vigorous intensity will be most beneficial [21 47]. A meta-analysis indicated that when performed directly after the physical activity, lighter (very light, light, and moderate) activity is more beneficial, but vigorous intensity is necessary for effects to be maximized if there is a delay between the physical activity and the cognitive test [21]. Second, it is likely that reduced fatigue over time also may lead to improved cognitive functioning; however, this needs to be evaluated in longitudinal studies. Third, the cognitive score improved over the day in both conditions, indicating a practice effect, a common problem in cognitive studies with repeated assessment which may have distorted the validity of performance outcomes. Potentially, the cognitive effect from walking breaks may also be moderated by age, fitness level, educational

level and the activity performed between tests (doing strict office work or leisure activities such as reading, watching television).

There are several limitations to the study. First, given this is a pilot study, it was likely underpowered to detect an effect in cognitive performance (as well as several of the potential mediators). Second, we studied acute effects and thus, any long-term effects of walking breaks cannot be evaluated based on the results in the study. Third, our objective assessments indicated that the participants on average were engaged in MVPA > 30 min/day which may have dampened the effect of prolonged sitting during the trial. Finally, we did not investigate whether the effects on fatigue and cognition may be moderated by participant characteristics. We suggest that future studies should: 1) include a larger sample size, 2) conduct repeated cognitive training sessions before the experiment to minimize the practice effect, 3) include a higher frequency of regular blood collection time-points to enable more robust analyses of changes over time and, 4) apply functional neuroimaging techniques to increase the knowledge on neurophysiologic mechanisms for the relationships between prolonged sitting, light-walking breaks, fatigue and cognition.

In conclusion, intermittent light-walking breaks resulted in an attenuation of fatigue levels during uninterrupted sitting, however, the difference in fatigue did not translate into significantly improved cognitive performance. Our findings provide further support to the suggestion that the relationship between sedentary behaviour and fatigue may be causal and that light-intensity walking breaks may counteract increased fatigue. Although the current study only examined short-term effects, there may be longer-term relevance and implications for sedentary behaviour, particularly among office workers and others with highly sedentary occupations. Thus, the role that reduced sedentary behaviour and walking breaks may play in

the prevention and/or treatment of fatigue warrants further investigation in a full-scale study. Considering the current high prevalence of persistent fatigue in the general population, reductions in sedentary time may have considerable relevance from a public health perspective.

FOOTNOTES

Contributorship

PW, DWD, C-JB, RL and GL contributed to the conception and design of the study. PW coordinated the trial and data collection, analysed the data, wrote the paper draft and is the study guarantor. MW assisted in collecting the data. PS and JO wrote the statistical analysis plan and conducted the statistical analyses. C-JB, MW, BH, PCD, GL, NE, RL, PS, JO, JH-B, KAE, NO and DWD analysed the data and reviewed and edited the paper draft. All authors approved the final version of the manuscript**.**

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Competing interests

None declared.

Ethics approval

The Alfred Hospital Ethics Committee

Data sharing

No additional data available.

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Table 1. Characteristics of the 19 participants in the study (10 men and 9 women).

BMI (kg/m^2)	31.5 (4.7)
Waist circumference (cm)	105.2 (12.4)
Completed university degree*	78.9
Taking antihypertensive medication*	47.4
Habitual moderate-to-vigorous physical activity (minutes/day)**	35.80 (30.86)
Habitual sitting time (h/day)**	9.82 (2.19)
Sleep time (h/night)*	7.4 (0.8)

Data are % or mean (SD).
*Assessed using self-reports.
**Assessed using activity monitors during the wash-out period between the two experimental days.

Table 2. Within-condition responses for the fatigue and cognitive scores at 0h, 4h and 7h.

			TIME (h)			
SCORE	**CONDITION**	**N**	**0**	**4**	**7**	***P****
Fatigue score	*Active*	19	37.9 (18.19)	34.3 (17.28)	36.6 (19.09)	
	Sedentary	19	37.4 (16.0)	47.1 (18.84)	46.8 (18.42)	
						0.024
Cognitive composite score	*Active*	19	0.018 (0.52)	0.080 (0.45)	0.273 (0.28)	
	Sedentary	19	-0.016 (0.50)	0.058 (0.33)	0.156 (0.41)	0.669
Cognitive subtests:						
Executive functions composite score	*Active*	19	-0.002 (0.49)	0.127 (0.43)	0.171 (0.30)	
	Sedentary	19	0.003 (0.45)	0.079 (0.36)	0.154 (0.39)	0.886
Episodic memory score	*Active*	19	68.9 (10.70)	64.5 (13.84)	78.5 (11.6)	
	Sedentary	19	65.4 (14.77)	66.2 (11.61)	69.3 (11.8)	0.077

*P-values for difference in temporal changes by condition.

Table 3. Within-condition responses for biomarkers at 0h, 4h and 7h.

		TIME (h)						
MARKER	**CONDITION**	**0**	**N**	**4**	**N**	**7**	**N**	***P****
Heart rate (bpm)	*Active*	64.7 (8.1)	19	71.8 (10.0)	19	71.6 (11.2)	19	
	Sedentary	63.5 (8.6)	19	66.2 (9.4)	19	66.1 (9.7)	19	
								0.038
Systolic blood	*Active*	119.5 (10.3)	19	117.5 (11.9)	19	117.5 (11.1)	19	
pressure (mmHg)	*Sedentary*	119.9 (12.7)	19	117.5 (12.7)	19	119.7 (12.9)	19	
								0.801
Diastolic blood	*Active*	76.1 (7.9)	19	74.2 (9.3)	19	75.3 (7.2)	19	
pressure (mmHg)	*Sedentary*	77.9 (8.6)	19	72.1 (9.6)	19	74.8 (8.8)	19	
								0.223
Insulin (uU/ml)	*Active*	21.7 (9.2)	18	105 (48.54)	19	17.8 (6.57)	18	
	Sedentary	20.9 (7.84)	19	96.4 (51.76)	19	19.2 (6.52)	19	
								0.679
IL-6 (pg/ml)	*Active*	0.95 (0.93)	18	1.32 (0.85)	19	1.92 (1.42)	18	
	Sedentary	0.94 (0.7)	19	1.21 (0.51)	19	1.34 (0.79)	19	
								0.123
Cortisol (pg/ml)	*Active*	3452 (3038)	18	1512 (1226)	19	1364 (737)	18	
	Sedentary	3618 (5472)	19	1651 (1085)	19	2653 (3617)	19	
								0.611
BDNF (pg/ml)	*Active*	14905 (8324)	15	15201 (8628)	16	12605 (7603)	14	
	Sedentary	14226 (6852)	16	13098 (6854)	15	14180 (7172)	15	
								0.184
Catechols Noradrenaline (pg/ml)	*Active*	427 (220)	18	475 (238)	18	440 (195)	19	
	Sedentary	375 (192)	18	466 (281)	17	473 (250)	18	
								0.177
DHPG (pg/ml)	*Active*	1244 (292)	18	1259 (307)	18	1276 (298)	19	
	Sedentary	1243 (245)	18	1338 (381)	17	1349 (329)	18	
								0.447
Adrenaline (pg/ml)	*Active*	36.6 (48.35)	17	109.1 (226.7)	17	171.2 (274.89)	18	
	Sedentary	44.4 (54.31)	18	118.7 (355.09)	17	69.8 (99.55)	18	
								0.198
DOPA (pg/ml)	*Active*	1884 (497)	18	1616 (455)	18	1862 (557)	19	
	Sedentary	1919 (505)	18	1739 (476)	17	2051 (588)	18	
								0.676
Dopamine (pg/ml)	*Active*	23.7 (26.31)	18	63 (158.12)	18	42.4 (75.24)	19	
	Sedentary	49.6 (134.01)	18	39.6 (81.37)	17	61.2 (190.59)	18	
								0.234
DHPG/Noradrenaline	*Active*	4.0 (1.78)	18	3.4 (1.28)	17	3.3 (1.34)	18	
	Sedentary	3.4 (1.46)	18	3.0 (1.01)	18	3.2 (1.15)	19	
								0.434

*P-values for difference in temporal changes by condition. IL-6, interleukin 6; BDNF, brain derived neurotrophic factor; DHPG, dihydroxyphenylglycol; DOPA, dihydroxyphenylalanine.

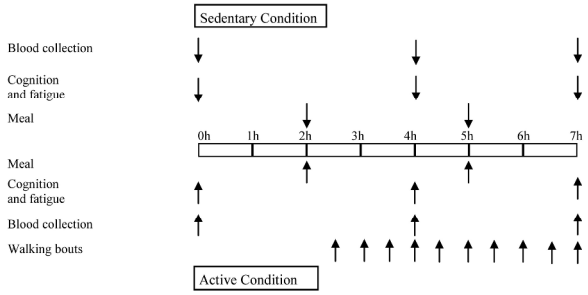


Figure 1: Study protocol
297x420mm (300 x 300 DPI)

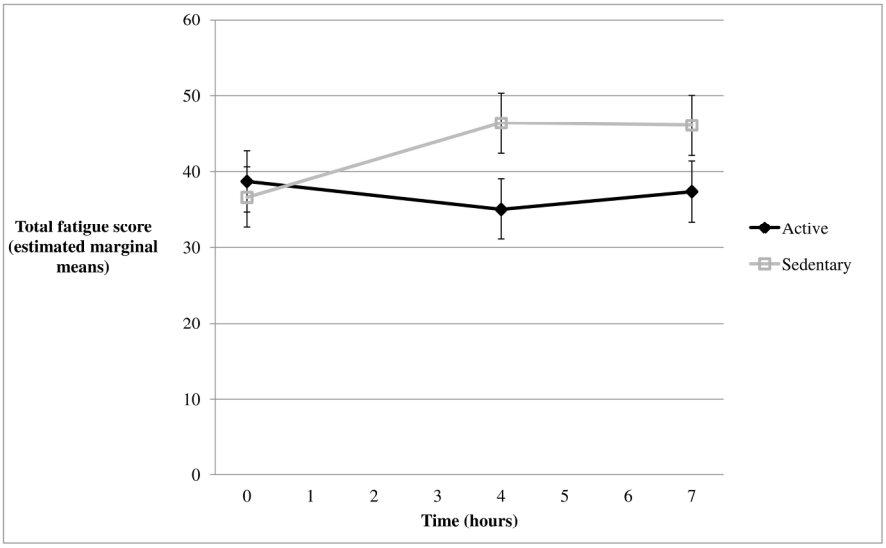
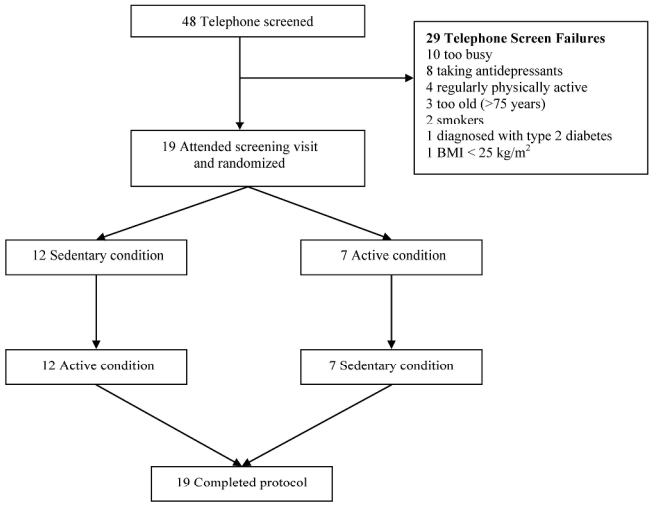


Figure 2: Total fatigue score (estimated marginal means) at 0h, 4h and 7h for the sedentary and the active conditions
209x148mm (300 x 300 DPI)



Supplemental Figure: Trial CONSORT diagram
297x420mm (300 x 300 DPI)