Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting

Running heading: Prolonged sitting and cerebral blood flow

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ABSTRACT

Decreased cerebrovascular blood flow and function are associated with lower cognitive functioning and increased risk of neurodegenerative diseases. Prolonged sitting impairs peripheral blood flow and function, but its effects on the cerebrovasculature are unknown. This study explored the effect of uninterrupted sitting and breaking up sitting time on cerebrovascular blood flow and function of healthy desk workers. Fifteen participants (10 male, 35.8±10.2 years, BMI: 25.5±3.2 $kg \cdot m^{-2}$) completed, on separate days, three 4-hr conditions in a randomised order: a) uninterrupted sitting (SIT), b) sitting with 2-min light intensity walking breaks every 30-min (2WALK) or c) sitting with 8-min light intensity walking breaks every 2-hrs (8WALK). At baseline and 4-hrs, middle cerebral artery blood flow velocity (MCAv), carbon dioxide reactivity (CVR) of the MCA and carotid artery were measured using transcranial Doppler (TCD) and duplex ultrasound respectively. Cerebral autoregulation (CA) was assessed with TCD using a squat-stand protocol and analysed to generate values of gain and phase in the very low, low, and high frequencies. There was a significant decline in SIT MCAv (-3.2±1.2 $cm.s^{-1}$) compared to 2WALK (0.6±1.5 $cm.s^{-1}$, p=0.02), but not between SIT and 8WALK (-1.2±1.0 $cm.s^{-1}$, p=0.14). For CA, the change in 2WALK very low frequency phase (4.47±4.07 degrees) was significantly greater than SIT (-3.38±2.82 degrees, p=0.02). There was no significant change in MCA or carotid artery CVR (p>0.05). Results indicate that prolonged, uninterrupted sitting in healthy desk workers reduces cerebral blood flow, however this is offset when frequent, short-duration walking breaks are incorporated.

Keywords sedentary behaviour, middle cerebral artery, cerebrovascular carbon dioxide reactivity, cerebral autoregulation, transfer function analysis

NEW & NOTEWORTHY

Prolonged, uninterrupted sitting in healthy desk workers reduces cerebral blood flow. However, this reduction in cerebral blood flow is offset when frequent, short-duration walking breaks are incorporated into this sitting period. For those who engage in long periods of sedentary behaviour, chronically breaking up these sitting periods with frequent active break strategies may have important implications for cerebrovascular health, however further research should explore this hypothesis.

INTRODUCTION

Sedentary behaviour (SB), defined as any waking behaviour in a sitting, reclining or lying posture (51), is an independent risk factor for multiple preventable diseases including cardiovascular disease and stroke (8, 11, 24, 57) and both cardiovascular and all-cause mortality (8, 57). Greater SB is also linked to impaired brain structure and function, which may contribute to cognitive decline and the development of neurodegenerative diseases such as dementia (53). Indeed, increased SB is associated with lower cognitive function (17). Understanding how SB affects the brain is therefore of great importance to delineate the association between cognition and SB.

The delivery and regulation of cerebral blood flow (CBF) is vital for normal brain function and survival (54). Cerebrovascular function describes the mechanisms regulating CBF to maintain constant cerebral perfusion (56), preventing the risk of ischemic brain injury and damage (52, 53, 56). Acute reductions in CBF are linked to impaired cognitive functioning (6, 23), whilst in the longer term impaired cerebrovascular function is implicit in neurodegenerative diseases including dementia, Alzheimer's disease and stroke (19, 22, 58). SB impairs peripheral blood flow, vascular function (36, 48) and glycemic control (15, 31). Whether a similar reduction occurs in cerebrovascular blood flow and function is unknown.

Alternatively, breaking up sitting with short bouts of low-intensity physical activity (PA) can prevent these detriments to vascular health and metabolic control (15, 31, 48). Furthermore, the frequency of these PA breaks appears to be an important modulator of these responses, as regularly breaking prolonged sitting with short PA bouts is more effective than a single PA bout at lowering postprandial glucose and insulin concentrations (31). Cerebrovascular function increases during exercise or following chronic exercise training (26, 28, 33),

additionally short duration low-intensity walking bouts can also elevate CBF (20, 27). Accordingly, regularly breaking up sitting with PA breaks may have beneficial effects on CBF and cerebrovascular function; however this is unknown.

This study explored the acute CBF and cerebrovascular function responses to prolonged, uninterrupted sitting, and assessed the cerebrovascular effects of breaking up prolonged sitting with short bouts of light intensity PA. We hypothesised that prolonged sitting would reduce CBF and impair cerebrovascular function, but this would be attenuated with light intensity PA breaks and that, in line with previous work, a more frequent PA break strategy would be more effective at preventing any impairment in cerebrovascular function.

MATERIAL AND METHODS

Participants

Fifteen (10 male) healthy desk workers employed in office and administrative jobs volunteered and written informed consent was obtained. Participants were recruited via advertising emails and posters which were distributed to University mailing lists, and by using newspaper advertisements. Participants were screened for exclusion criteria including: taking medication, smoker, BMI >35 or <18 $kg \cdot m^{-2}$, use of hormone-based contraception and diagnosis of cerebrovascular, cardiovascular or metabolic disease. Study procedures were approved by Liverpool John Moores University Ethics Committee and adhered to the Declaration of Helsinki.

Study design

Participants attended the temperature controlled (20-22 °C) laboratory at the same time of day (7.00-9.00 am) on three separate occasions. Testing procedures were the same across each test day (Figure 1). After arrival and 20-min supine rest, middle cerebral artery blood flow velocity (MCAv) and cerebrovascular carbon dioxide reactivity (CVR) were assessed. Participants were then seated and underwent measures of seated MCAv and cerebral autoregulation (CA). Following baseline measurements participants completed, in a randomised order: a) 4-hr uninterrupted sitting (SIT), b) 4-hr sitting+2-min light-intensity treadmill walking breaks every 30-min (2WALK) or, c) 4-hr sitting+8-min light intensity treadmill walking breaks every 120-min (8WALK). The measurement of seated MCAv was repeated immediately after each 4-hr intervention. MCAv was assessed while seated to assess the posture of interest, sitting, and to prevent the effects of moving to a supine posture altering hemodynamics. Participants then returned to a supine posture and supine MCAv and CVR

were assessed, followed by CA. Heart rate (HR) and MCAv were recorded immediately prior to and during each walking break.

Study procedures

Prior to each visit participants were instructed to avoid strenuous exercise for 24-hr, to complete an overnight fast and abstinence from caffeine and alcohol. Women were assessed in the follicular phase of the menstrual cycle (days 1-7). Participants completed the International Physical Activity Questionnaire (Long form, IPAQ) (9) to determine habitual PA (14) and SB (39). Given the duration of testing, participants were given low calorie, low fat, standardised snacks at specified time points (Figure 1). Following baseline tests, participants were given a breakfast cereal bar (Belvita Milk and Cereal Breakfast Biscuits, 220kcal, 33.6g carbohydrate, 7.2g fat, 3.6g protein) and a banana after 2-hr (~100kcal, ~27.0g carbohydrate, ~0.3g fat, ~1.0g protein). Water was available to drink *ad libitum*.

Interventions

Uninterrupted sitting (SIT). Participants remained seated at a desk for 4-hr and were permitted to perform low cognitively demanding desk-based activities such as reading, watching TV, surfing the internet or completing simple work tasks on a computer. Participants were prevented from standing or walking, with the exception of visiting the toilet (walking distance of ~7.5 m; on average participants visited the toilet once during each intervention), and from making vigorous movements. Participants were supervised at all times to ensure these conditions were adhered to.

2-min walking breaks (2WALK). Sitting was interrupted every 30-min with a 2-min light intensity treadmill walking break. Consequently, eight breaks were completed, totalling 16-min of activity. This break strategy was selected based on recommendations from the The

Sedentary Behaviour and Obesity Expert Working Group (7) which advises taking a break from sitting every 30-min. Walking was performed on a treadmill with no gradient (Run XT, Technogym, Italy) at a self-selected, habitual walking speed to represent an ecologically valid PA break that could be performed in a working environment. Walking speed was determined during a familiarisation session prior to the first experimental trial began and this speed was kept consistent for all walking breaks. Walking intensity was assessed during each PA bout using the rating of perceived exertion (RPE) and HR.

8-min walking breaks (8WALK). Sitting was interrupted every 120-min with an 8-min light intensity walk, using the same walking speed as previously described. Consequently, two breaks were completed, totalling 16-min of activity. Therefore, the total duration of PA performed in both walking break conditions was identical. This less frequent break strategy was based on recommendations that interventions to break up sitting must be feasible (5), which a high frequency breaks strategy may not be when translated into practise.

Measurements

All physiological data measurements were continuously acquired at 50 Hz using an analog-to-digital convertor (PowerLab ML880, ADInstruments, Colorado Springs, Colorado, USA) and displayed in real time on a computer with commercially available software (LabChart Version 7.0, ADInstruments).

Middle cerebral artery blood flow velocity (MCAv). MCAv was used as a surrogate measure for CBF as the MCA accounts for 70-80% of the brain's total perfusion (46). Continuous bilateral transcranial Doppler ultrasound (TCD) (ST3, Spencer Technologies, Redmond, WA, USA) was used to measure the left and right MCAv. A 2-MHz Doppler probe was positioned over the temporal window, located above the zygomatic arch and was secured using an

adjustable headband (Marc 600 Headframe, Spencer Technologies). Each MCA was identified based on the signal depth, peak and mean blood flow velocity as previously described (54). Once optimal signals had been obtained, the transducers were secured into position and the signal parameters were recorded to ensure within-subject consistency between tests. Additionally, photographs were taken of the probe positions as a reference for the acoustic window for subsequent visits. The sonographer had a between-day coefficient of variation of 7.8% for the MCAv.

Mean MCAv was calculated from the envelope of the velocity tracing using a weighted mean (1/3 maximum + 2/3 minimum) to account for the relative time spent in systolic and diastolic pressures (46). Supine and seated MCAv were acquired for 1-min. During the 1-min prior to each walking break (pre-walk) and throughout each subsequent walk, MCAv was continuously measured. Cerebrovascular conductance (CVC) was calculated by dividing MCAv by mean arterial pressure (MAP).

Cerebrovascular carbon dioxide reactivity (CVR). Maintenance of adequate CBF is influenced by the brain's ability to alter blood flow in response to changes in partial pressure of arterial carbon dioxide, termed CVR (56). Participants were instrumented with a face mask with a two-way non-rebreathing valve (MLA1028, ADInstruments, Colorado Springs, Colorado, USA). A Douglas bag filled with a 5% carbon dioxide (CO_2) mixture and fitted with a three-way valve, enabled the breathing circuit to be alternated between ambient air and the contents of the Douglas bag. Breath-by-breath CO_2 was sampled using a calibrated gas analyser (Ml206, ADInstruments) and the pressure of end-tidal carbon dioxide ($PETCO_2$) was calculated in LabChart with correction for the daily barometric pressure. After a 1-min baseline, participants were coached through a voluntary hyperventilation for 3-min or until $PETCO_2$ was reduced to 20 mmHg (whichever was achieved first). Immediately afterwards

188 the valve on the Douglas bag was switched so participants inhaled the 5% CO2 mixture.
189 Simultaneously, participants were instructed to return their respiratory rate to normal whilst
190 breathing the 5% CO_2 mixture for 3-min. Baseline $PETCO_2$ and MCAv were calculated as the
191 mean of the 1-min prior to hyperventilation, while MCAv and $PETCO_2$ data during 5% CO_2
192 breathing was collected as 10-sec averages for the entire 3-min period. Absolute and relative
193 MCAv were then plotted against $PETCO_2$ for each 10-sec of 5% CO_2 breathing and CVR was
194 subsequently quantified by linear regression (R^2 value). Relative MCAv was calculated as the
195 difference between baseline and 5% CO_2 MCAv divided by baseline MCAv (([5% CO_2
196 MCAv-baseline MCAv]/ baseline MCAv) x 100%).
197 Simultaneously, during the baseline and CO_2 breathing measurements, arterial diameter and
198 blood flow of the left common carotid artery (CCA) were acquired using a 10-MHz multi-
199 frequency linear array probe, attached to high resolution ultrasound machine (T3000; Terason,
200 Burlington, MA, USA). Using ultrasound to assess the dilation of larger extracranial neck
201 vessels during CO_2 alterations provides another means to monitor reactivity and vessel
202 dilation not assessable using TCD (3, 55). The extracranial arteries supplying the brain are
203 also sensitive to changes in CO_2 levels and therefore contribute to cerebrovascular CO_2
204 regulation. Images were acquired in accordance with methodological guidelines (47) and data
205 analysed as previously reported (21). To reduce any influence of turbulent flow on vascular
206 responsiveness, the CCA was imaged at least two centimetres below the point of bifurcation.
207 Data were used to determine the response of the CCA to elevations in $PETCO_2$ by averaging
208 30-sec of baseline diameter and blood flow data and comparing that to the diameter and blood
209 flow during the last 30-sec of 5% CO_2 breathing. All ultrasound measurements were
210 completed by the same sonographer, who has a between-day intraobserver coefficient of
211 variation of 3.5% for the CCA, in line with methodological guidelines (47).
212

Cerebral autoregulation (CA). A second key factor determining adequate CBF is effective CA, which maintains CBF over a range of perfusion pressures (56). Participants completed a squat-stand test, involving repeated cycles of 5-sec of standing and 5-sec of squatting (0.1 Hz) for 5-min to induce oscillations in blood pressure (BP) (12). MCAv and BP were continuously assessed. Data was analysed using transfer function analysis (TFA). TFA views CA as a linear control system where sinusoids at the input are transformed into sinusoids at output of the same frequency, however with a different amplitude (termed gain) and shifted in time (termed phase) (13). In the case of CA, BP is the input and MCAv the output, with CA as the regulator between the two (4). To ensure the statistical reliability of gain and phase values a coherence function is used (13). Coherence tests the linearity of the relationship between input and output and can be used to indicate whether data is reliable (4, 13). Data was processed and analysed in accordance with standardised TFA guidelines to produce values of gain, phase and coherence for three frequency domains: very low frequency (VLF: 0.02-0.07 Hz), low frequency (LF: 0.07-0.2 Hz) and high frequency (HF: 0.2-0.5 Hz) (13). TFA is a frequency-dependent phenomenon and these domains are within the frequency range CA is thought to operate. CA is viewed as a high-pass filter as the regulation of CBF is effective in the low frequency range of BP oscillations, but not in the high frequency range due to the time delay in initiating cerebrovascular adaptations to the changes in perfusion pressure (4). CA therefore allows rapid BP changes to be transmitted to CBF, whereas slow BP changes are filtered (4). As a consequence, the three frequency ranges have different responses and are likely controlled by different mechanisms (60).

Gain is a measure of how changes in BP are transmitted into MCAv (12). A low gain indicates efficient CA, with increases in gain consequently corresponding to reduced efficiency as for a given change in BP there are greater changes in MCAv (4). Phase describes the temporal relationship between changes in BP and MCAv (12). Waveforms that are in sync

are referred to as 'in phase', while if these waveforms are displaced from each other it describes a phase shift. Phase shift is considered a surrogate measure for the time delay of the autoregulatory response, with an increase in phase indicating a more efficient CA (4). Coherence describes the linearity of the relationship between the changes in MCAv and BP, with a coherence value approaching one indicating a linear relationship (4, 12). Coherence values were used to accept the validity of gain and phase estimates, with cut-off values for inclusion set at 0.4 in accordance with published guidelines (13). Analyses yielding coherence values lower than this cut-off value were excluded. As recommended, gain was normalised to control for possible baseline differences in BP and MCAv between conditions, therefore normalised gain was used during the interpretation of data (4, 13).

Hemodynamics. Participants were fitted with a photoplethysmographic cuff on the index or middle finger of the right hand (Finometer model 1, Finapres Medical Systems BV, Amsterdam, The Netherlands) and a 3-lead electrocardiogram to continuously assess MAP and HR throughout measurements.

Statistical analyses

Data was analysed using statistical software (SPSS Version 22.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p \leq 0.05$. Results are presented as means±standard error (SE). For each condition, the change in all outcomes parameters was calculated (4-hr–baseline, Δ). To assess differences between conditions, parameters were analysed using one-factor general linear mixed model with baseline values as a covariate. Differences in MCAv and HR between pre-walk and during each walk were analysed using paired samples t-tests. Post-hoc analyses were performed using the least significant difference (LSD) method.

RESULTS

Descriptive statistics are shown in Table 1.

Intervention effects

Cardiorespiratory and haemodynamic measures.

There were no significant main effects for the change in supine (p=0.78) or seated (p=0.33) MAP or the change in supine (p=0.90) or seated (p=0.82) HR (Table 2). Additionally, no differences in the change in supine (p=0.30) or seated (p=0.61) $PETCO_2$ were observed (Table 2).

Cerebral blood flow.

Values for MCAv are presented in Table 2. A significant main effect was observed for the change in supine MCAv (p=0.048), with post hoc analysis revealing a greater change in MCAv during SIT compared to 2WALK (p=0.02; Figure 2a), but not between SIT and 8WALK (p=0.14). Supine CVC however showed no significant main effect (p=0.09; Figure 2c). Seated MCAv showed a significant main effect (p=0.01), with significantly reduced MCAv observed in both SIT (p=0.01) and 8WALK (p=0.047) compared to 2WALK (Figure 2b). Seated CVC also differed significantly between conditions (p=0.01), with post hoc analysis revealing the change in 2WALK was significantly different compared to SIT (p=0.03; Figure 2d).

Cerebrovascular carbon dioxide reactivity.

Values of linear regression for MCA CVR are presented in Table 3. No significant main effect (p=0.30) was observed for the change in CVR. There was also no significant main effect (p=0.88) for the change in CCA diameter between baseline or during 5% CO_2 breathing

for each condition (SIT Baseline: -0.00±0.01 mm, 4hrs: -0.01±0.01 mm; 2WALK Baseline: 0.01±0.01 mm, 4hrs: -0.00±0.02 mm; 8WALK Baseline: -0.01±0.01 mm, 4hrs: -0.02±0.01 mm). Similarly, there was no significant main effect (p=0.28) for the change in CCA blood flow between baseline or during 5% CO_2 breathing for each condition (SIT Baseline: 1.22±0.95 $ml.s^{-1}$, 4hrs: -0.39±0.48 $ml.s^{-1}$; 2WALK Baseline: 1.24±0.48 $ml.s^{-1}$, 4hrs: -1.25±1.26 $ml.s^{-1}$; 8WALK Baseline: -0.51±0.82 $ml.s^{-1}$, 4hrs: -0.10±1.14 $ml.s^{-1}$).

Cerebral Autoregulation.

Mean values for coherence for each of the frequency domains were: VLF 0.5; LF 0.6; HF 0.4. Table 4 presents values for phase, gain and normalised gain for each domain. A significant main effect was observed in the VLF for the change in phase (p=0.047) and gain (p=0.001). For phase, post hoc analyses showed the change in SIT was significantly lower than the change in 2WALK (p=0.02). For gain, the change in 8WALK was significantly less compared to the change in 2WALK (p=0.01). In the LF the main effect for normalised gain approached significance (p=0.05). No significant main effect was observed in the HF for any parameters (p>0.05).

Physiological responses during walking breaks

Mean treadmill speed for each condition and every walking break was 3.6 km/h at an RPE of 8.6.

2WALK.

Walking breaks increased MCAv in seven out of the eight breaks. The increased MCAv was only significant at 60-min, with MCAv during walking 1.91 $cm.s^{-1}$ higher than prior to the walking bout (Pre Walk: 55.7±2.4 $cm.s^{-1}$; Walking: 57.8±2.3 $cm.s^{-1}$, p=0.02). HR also

312 significantly increased during each walking break, with an average increase of 33 bpm (Pre
313 Walk: 61±2 bpm; Walking: 94±2 bpm, $p<0.001$).
314
315 *8WALK.*
316 Both walking breaks significantly increased MCAv. At 120-min MCAv increased by 1.96
317 $cm.s^{-1}$ ($p=0.02$) while at 240-min a larger increase of 2.23 $cm.s^{-1}$ was observed ($p=0.004$).
318 Each break also significantly increased HR, with an average increase of 37 bpm (Pre Walk:
319 69±3 bpm; Walking: 96±6 bpm, $p<0.001$).

DISCUSSION

This study demonstrates that in healthy desk workers, prolonged, uninterrupted sitting causes a decrease in MCAv. Importantly, short duration, regular walking breaks (2WALK), rather than less frequent, longer duration walking breaks (8WALK), prevented the impairment of MCAv associated with uninterrupted sitting. Similarly, the frequent walking break strategy improved CA; an important factor in cerebrovascular function. In contrast, neither prolonged sitting nor walking breaks influenced CVR. Our results indicate that prolonged uninterrupted sitting impairs CBF, whilst taking regular PA breaks has positive effects on both CBF and CA. The promotion of active break strategies for those who engage in long periods of sitting may therefore have important clinical implications.

Uninterrupted sitting induced a decline in MCAv of 1.4-3.2 $cm.s^{-1}$. Translating this observation to the age-related decline in MCAv of 0.76 $cm.s^{-1}$ per year (1), this suggests the reductions observed following a one-off bout of uninterrupted sitting may equate to 2-4 years of age-related decline, albeit likely transient. Nonetheless, repeated exposure to this type of SB may have important implications for long-term cerebrovascular health. Indeed, chronically sedentary males (not regularly physically active) exhibit a 9.1 $cm.s^{-1}$ lower mean MCAv compared to their endurance trained counterparts (1). Interestingly, this observation aligns with our finding, in that breaking up sitting with frequent, short duration walking breaks (2WALK) prevented the sitting-induced decline in MCAv. This benefit was not observed in the less frequent, longer duration walking break condition (8WALK) despite larger increases in MCAv during the walking breaks. Taken together this implies the frequency of the breaks may be more important than the magnitude of the increase in MCAv during the breaks. This finding supports previous work showing, when directly compared to a single activity bout, regular activity breaks during sitting enhances postprandial glycaemia and insulinemia (31).

The importance of the frequency rather than the duration of PA is therefore replicated in our results.

Frequent walking breaks to interrupt sitting also enhanced markers of cerebrovascular function. Our results suggest the 2WALK condition significantly improved CA, as the change in VLF phase was greater compared to uninterrupted sitting, implying enhanced buffering capacity of CA with frequent activity breaks. This adds further support to the hypothesis that the frequency of breaking up sitting is more important than the break duration. The acute effects of PA breaks on CA has not been previously assessed, however some research has explored the effects of exercise. Static handgrip exercise for two minutes did not affect CA (30); whilst exhaustive cycling impairs CA (29). These findings indicate that different modalities, intensities and durations of exercise have varied effects on CA. Whilst the light walking breaks in our study are not directly comparable to exercise, our findings show that CA can be modified by low intensity PA and that this response is influenced by the frequency this activity.

CVR did not differ across the three conditions. Previous work has shown acute improvements in CVR following both moderate and strenuous intensity cycling for 50-min (34). In contrast, in our study the walking break interventions had no effect on CVR. A potential explanation for our observation is that we used light intensity, short duration PA interventions rather than exercise *per se*, the stimulus may therefore not have been large enough to alter CVR. Despite the decrease in MCAv following uninterrupted sitting, this did not manifest into a dysfunction in CVR, as has been observed for peripheral vascular function (48). This suggests the cerebrovasculature may have a greater functional capacity to resist the deleterious vascular effects of sitting and that more pronounced changes in CBF are required to mediate changes

in response to SB. Indeed, this may be expected based on the greater importance of the brain as an organ compared to the periphery (32).

There was no difference in the change in MAP between sitting and 2WALK, thus in line with MCAv, cerebrovascular conductance (CVC) was significantly higher following 2WALK compared to prolonged sitting, demonstrating changes in BP do not impact out findings. Instead, the neural stimulation of the cerebrovasculature may explain our cerebrovascular blood flow and function findings. The cerebral vasculature is extensively innervated by sympathetic fibres (28) and the progressive sympathoexcitation with ageing is suggested to contribute to age-associated decreases in CBF (1). Prolonged sitting elevates muscle sympathetic nerve activity (35), which may induce systemic vasoconstrictor effects, in turn inducing cerebral vasoconstriction and lower blood flow. The preservation of blood flow and function with frequent walking breaks may relate to cholinergic activity as cerebral blood vessels are also innervated by cholinergic fibres (56). In animals, cholinergic fibres are stimulated during walking, contributing to increased CBF (45, 50). Evidence in humans also supports that cholinergic vasodilation contributes to increased CBF during exercise, as acetylcholine blockade abolishes the exercise-induced increase in MCAv (44). It is therefore possible that in this study the more frequent walks led to a more sustained cholinergic activation, maintaining cerebral vasodilation and subsequently MCAv.

An alternative explanation for the decline in MCAv after uninterrupted sitting may relate directly to the function of cerebrovascular endothelial cells, which contribute to the regulation of CBF (49). Elevated levels of tissue plasminogen activator and Von Willebrand factor, markers of endothelial dysfunction, are associated with reduced CBF in older adults (42). Acute uninterrupted sitting induces peripheral endothelial dysfunction (36, 37, 48) therefore a

similar process may be present in cerebral arteries. Changes in cerebral glycaemic regulation may also contribute to sitting-induced reductions in MCAv, as the brain is highly sensitive to perturbations in circulating glucose levels (53). Prolonged sitting increases postprandial glycemia (15, 31), which can cause microvascular damage, impair endothelial function and reduce CBF (53). In this study, prolonged sitting may have elevated circulating glucose levels, subsequently reducing MCAv; whilst the frequent walking breaks may have prevented this hyperglycaemia, in turn maintaining MCAv. Future studies are warranted to understand the underlying mechanisms of decreased CBF during prolonged sitting and how physical activity breaks prevent these effects.

Workplace Application. As 65-75% of office workers' hours are spent sitting, the workplace has been identified as a key setting to reduce SB. However, as outlined by Buckley et al. (10), many health promotion and PA interventions aim to reduce SB by targeting moderate to vigorous PA, which is unlikely to be achievable within the constraints of a workplace. The frequent, light intensity walking break strategy used in our study is in line with recent workplace guidelines advising increasing light activity during working hours and regularly breaking up seated work (10). Importantly, accumulating evidence suggests that light intensity PA is beneficially associated with biomarkers of cardiometabolic health and may reduce mortality risk(18). Collectively this indicates that sedentary individuals should be encouraged to engage in PA of low intensities to confer improvements to health; such as by using the strategy employed in this study by interrupting prolonged sitting with light intensity walking breaks.

Limitations. Our study assessed the responses to a short sitting period, however of greater ecological interest would be examining the chronic responses to sitting. Whilst within an

experimental visit we controlled the activities that participants completed during sitting so that they were of a low-cognitive demand, these activities were not matched between visits. It is therefore possible that the activities they performed while seated differed between visits which may have influenced cerebrovascular responses. The use of TCD to assess MCAv and cerebrovascular function is associated with known limitations, including the inability to measure actual blood flow (54), the assumptions that measures from the MCA are representative of other cerebral vessels (2), and that MCA diameter is unaltered during varying levels of CO_2 (46). By recording the signal parameters and photographically recording the TCD probe placement, it was ensured as closely as possible the probe was in the same location and at the same angle for each visit; small variations may have occurred, however our coefficient of variation was 7.8% indicating good reproducibility. The analysis of CA using TFA is a developing method and lacks references values (13). Therefore whilst current assessment and analysis guidelines were adhered to (13), future research is required to fully understand the clinical value of our results.

Conclusion and implications

For the first time this study demonstrates that in healthy desk workers prolonged, uninterrupted sitting impairs CBF, whilst these reductions are offset when frequent, short duration walking breaks are incorporated. These observations may have clinical importance for both cognition and disease risk. Acutely cognitive performance declines following transient carotid artery occlusion that decreases CBF (23), but increases following pharmacologically elevated CBF (16). Given that UK office workers report sitting at work for 6.3-hr (25), reductions in CBF may have important ramifications for workers' productivity. More importantly, chronic reductions in CBF is a risk factor for cognitive impairment (40), is associated with cerebrovascular diseases such as Alzheimer's disease and dementia (41, 43,

445 58, 59) and correlates with cognitive dysfunction in Alzheimer's disease (38). Consequently,
446 in the long term the repeated exposure to sitting-induced decreases in CBF could cause
447 chronic downregulation of CBF and therefore have large implications in the development of
448 such diseases; which has previously been suggested (53). The high prevalence of SB in these
449 cerebrovascular disease populations further highlights the relevance of our findings. The
450 maintenance of CBF using frequent walking breaks to interrupt sitting therefore represents a
451 protective mechanism against disease risk. Indeed, in a nondemented cohort, greater CBF was
452 associated with a decreased chance of dementia development and less cognitive decline over a
453 6.5 year follow-up (40). Future work is needed to better understand the potential relation
454 between SB and development of cerebrovascular diseases.

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DISCLOSURES

SEC received PhD scholarship funding from a Biotechnology and Biological Sciences Research Council (BBSRC) grant. RD and LB are employed by Unilever, which has commercial interests in Food, Home and Personal Care products. All other authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

SEC, NDH, DHJT, RD and LB contributed to the conception and design of the study. SEC and SMH completed data collection. SEC analysed all data. SEC and NDH interpreted the data and drafted the initial manuscript. All authors contributed to the critical revision of the manuscript, approve the final submission and take responsibility for the integrity of the data and the accuracy of the data analysis.

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FIGURE CAPTIONS

Figure 1: Experimental design for the three test conditions, completed in a randomised order, on three separate days. a) 4-hr uninterrupted sitting, b) Sitting with 2-min treadmill walking breaks every 30-min, c) Sitting with 8-min treadmill walking breaks every 120-min. MCAv- middle cerebral artery blood flow velocity; CVR- cerebrovascular carbon dioxide reactivity; CA- cerebral autoregulation.

Figure 2: Change in middle cerebral artery blood flow velocity (MCAv) and cerebrovascular conductance (CVC) in the supine (a, c) and seated (b, d) positions measured at baseline and after four hours of each experimental condition with control for baseline blood flow and conductance. SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks. Error bars= ±SE. * Significant difference between conditions ($p<0.05$).

TABLES

Table 1: *Descriptive characteristics, self-reported physical activity scores and total sitting time of participants (n=15).*

	Mean±SD
Age (years)	35.8±10.2
Body Mass (kg)	74.5±11.9
Height (cm)	170.8±8.9
Body Mass Index ($kg.m^{-2}$)	25.5±3.2
Physical Activity Score (MET-minutes/week)	4524.3±2098.7
Sitting Time Per Week Day (Hours)	8.2±2.2
Sitting Time Per Weekend Day (Hours)	6.0±1.9
Sitting Time Per Week (Hours)	53.2±12.4

Table 2: *For each intervention, middle cerebral artery blood flow and cardiorespiratory measures at baseline, four hours and the overall change (Δ) with statistically adjusted baseline covariate control (Mean±SE).*

	SIT			2WALK			8WALK		
	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#
Supine position									
MCAv ($cm.s^{-1}$)	58.8±2.0	55.5±2.1	-3.2±1.2*	58.6±2.6	59.2±2.7	0.6±1.5	58.4±2.7	57.3±2.2	-1.2±1.0
CVC ($cm.s^{-1}.mmHg^{-1}$)	0.72±0.03	0.67±0.03	-0.06±0.02	0.73±0.03	0.71±0.03	-0.02±0.02	0.73±0.04	0.70±0.04	-0.03±0.02
MAP (mmHg)	83±2.8	84±2.5	2.3±1.8	80±1.9	84±2.3	2.6±1.8	81±2.3	83±2.9	1.8±2.3
HR (bpm)	59±3.4	56±2.4	-2.2±1.7	58±2.6	55±3.4	-3.1±3.0	56±2.3	55±2.1	-2.2±2.1
$PETCO_2$ (mmHg)	41.6±1.3	40.7±1.6	-0.9±0.8	42.6±1.5	41.3±1.7	-1.2±1.2	41.0±1.5	41.5±1.3	0.4±0.9
Seated position									
MCAv ($cm.s^{-1}$)	55.4±2.4	53.8±1.6	-1.4±1.8*	56.4±2.0	56.3±2.4	1.1±2.4	53.7±2.5	54.3±2.6	-0.8±2.7*
CVC ($cm.s^{-1}.mmHg^{-1}$)	0.62±0.03	0.59±0.03	-0.04±0.02*	0.65±0.03	0.64±0.04	0.01±0.03	0.61±0.03	0.62±0.04	-0.01±0.03
MAP (mmHg)	90±2.4	92±2.8	2.8±2.0	88±2.8	89±2.7	0.9±1.7	89±2.7	90±2.6	0.7±1.8
HR (bpm)	57±2.8	58±2.5	0.6±2.1	57±2.8	58±3.5	1.0±2.8	56±2.4	56±2.6	-0.4±2.6
$PETCO_2$ (mmHg)	37.6±1.3	37.8±1.4	-0.1±1.1	38.4±1.8	37.4±1.3	-0.8±0.7	38.2±1.6	37.1±1.4	-1.0±1.0

SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks; MCAv- middle cerebral artery blood flow velocity; CVC- cerebral vascular conductance; MAP- mean arterial pressure; HR- heart rate; $PETCO_2$- pressure of end-tidal carbon dioxide.
Delta change values expressed with statistically adjusted baseline covariate control.
* Significantly different to 2WALK ($p<0.05$).

Table 3: *R^2 values of linear regression of cerebrovascular carbon dioxide reactivity (CVR) for each intervention at baseline, four hours and the overall change (Δ) with statistically adjusted baseline covariate control (Mean±SE).*

	SIT			2WALK			8WALK		
	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$
CVR	0.83±0.03	0.83±0.03	0.00	0.80±0.04	0.79±0.04	-0.02	0.81±0.03	0.84±0.03	-0.03

Relatively high R^2 values confirm the linearity of the response.
SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks.
Delta change values expressed with statistically adjusted baseline covariate control.

Table 4: *For each intervention, cerebral autoregulation (CA) estimates of phase, gain and normalised gain ($Gain_n$) at baseline, four hours and the overall change (Δ) with statistically adjusted baseline covariate control (Mean±SE).*

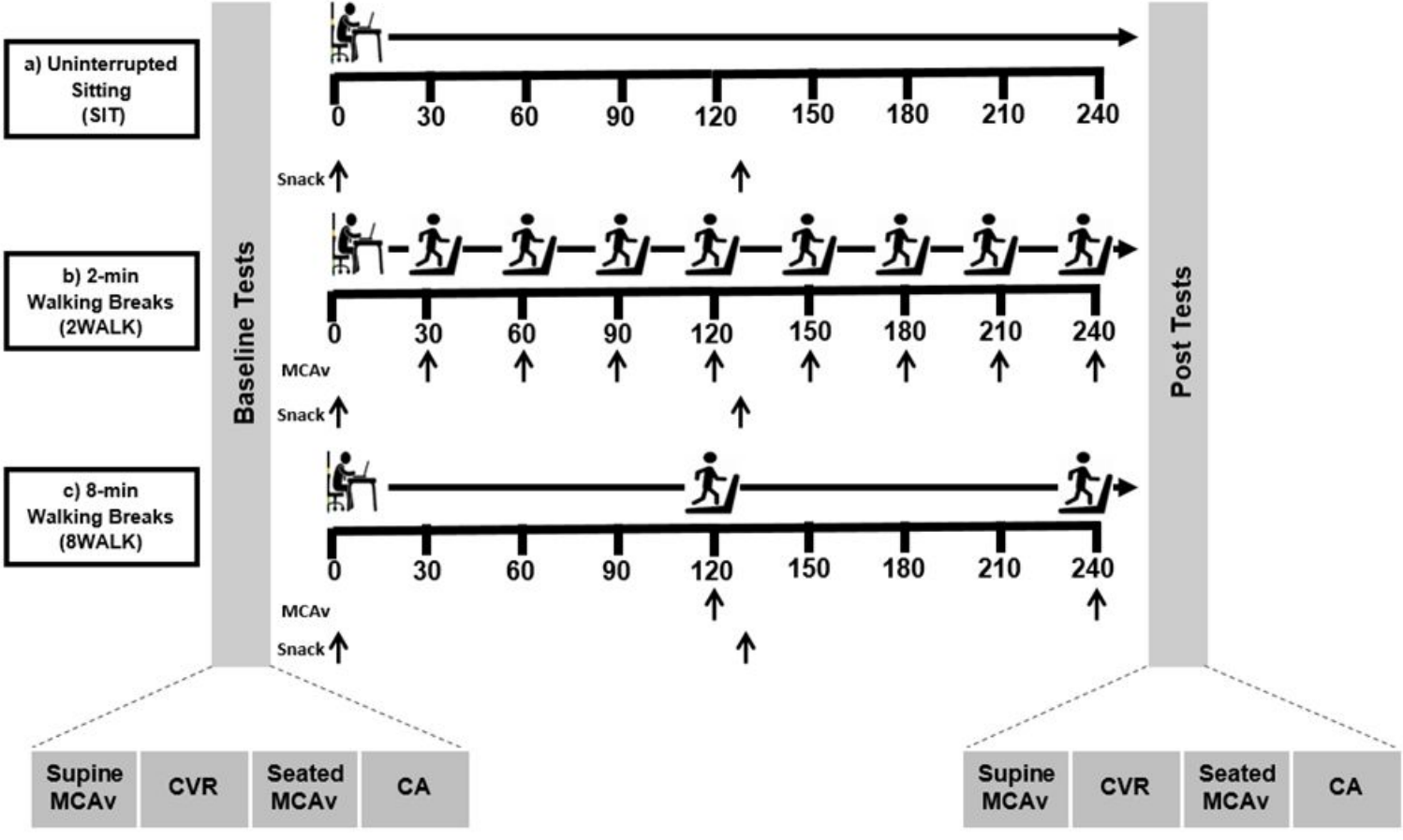
	SIT			2WALK			8WALK		
	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$
VLF Phase (degrees)	39.16±4.64	35.83±5.70	-3.38±2.82	41.93±6.19	46.91±7.49	4.47±4.07*	48.40±5.03	42.82±5.21	-2.03±8.20
VLF Gain ($cm.s^{-1}.mmHg^{-1}$)	0.52±0.04	0.49±0.02	-0.04±0.03	0.54±0.05	0.47±0.04	-0.10±0.05	0.47±0.03	0.49±0.03	-0.02±0.04$
VLF $Gain_n$ ($\%.mmHg^{-1}$)	0.91±0.09	0.88±0.05	-0.02±0.07	1.04±0.10	0.86±0.09	-0.23±0.08	0.86±0.07	0.91±0.05	-0.04±0.06
LF Phase (degrees)	24.34±2.49	24.94±3.46	-1.18±2.74	23.52±3.28	22.78±4.49	-2.67±3.75	25.26±2.54	28.66±4.76	1.37±3.27
LF Gain ($cm.s^{-1}.mmHg^{-1}$)	0.69±0.04	0.66±0.03	-0.05±0.03	0.78±0.06	0.76±0.07	0.04±0.05	0.71±0.06	0.86±0.10	0.17±0.11
LF $Gain_n$ ($\%.mmHg^{-1}$)	1.21±0.09	1.20±0.07	-0.12±0.10	1.43±0.10	1.36±0.13	0.04±0.10	1.27±0.09	1.52±0.22	0.30±0.19
HF Phase (degrees)	12.58±5.07	8.22±6.15	-2.39±6.80	5.95±3.73	9.52±6.69	6.58±6.14	8.04±3.42	10.15±5.04	-0.69±5.79
HF Gain ($cm.s^{-1}.mmHg^{-1}$)	0.70±0.04	0.69±0.03	0.01±0.04	0.78±0.06	0.72±0.06	0.02±0.04	0.68±0.08	0.86±0.10	0.13±0.06
HF $Gain_n$ ($\%.mmHg^{-1}$)	1.20±0.06	1.24±0.06	0.05±0.07	1.44±0.11	1.29±0.10	-0.03±0.07	1.22±0.12	1.53±0.18	0.27±0.16

SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks; VLF- very low frequency; LF- low frequency; HF- high frequency

Delta change values expressed with statistically adjusted baseline covariate control.

* Significantly different to SIT ($p<0.05$).

$ Significantly different to 2WALK ($p<0.05$).


a) Uninterrupted Sitting (SIT)
b) 2-min Walking Breaks (2WALK)
c) 8-min Walking Breaks (8WALK)
Baseline Tests
Post Tests
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Snack
MCAv
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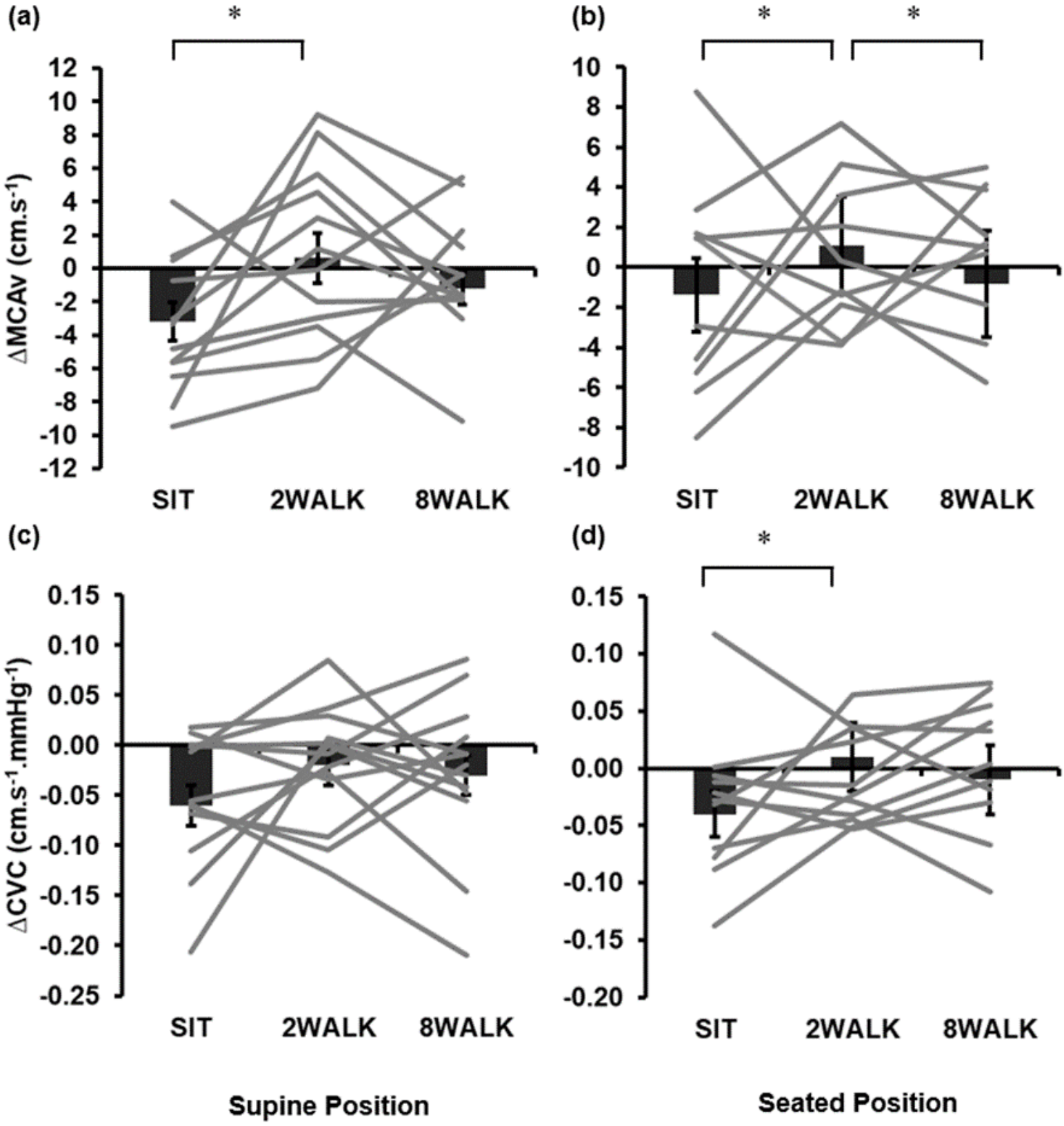
CVR
Seated MCAv
CA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ΔMCAv (cm.s-1)
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Supine Position
Seated Position