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RESEARCH ARTICLE

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

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Carter SE, Draijer R, Holder SM, Brown L, Thijssen DH, Hopkins ND. Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged sitting. *J Appl Physiol* 125: 790–798, 2018. First published June 7, 2018; doi:10.1152/japplphysiol.00310.2018.—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 yr, body mass index: 25.5 ± 3.2 kg/m^2) completed, on separate days, three 4-h conditions in a randomized order: *1*) uninterrupted sitting (SIT), *2*) sitting with 2-min light-intensity walking breaks every 30 min (2WALK), or *3*) sitting with 8-min light-intensity walking breaks every 2 h (8WALK). At baseline and 4 h, middle cerebral artery blood flow velocity (MCAv) and CO_2 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 analyzed 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) compared with 2WALK (0.6 ± 1.5 cm/s, $P = 0.02$) but not between SIT and 8WALK (−1.2 ± 1.0 cm/s, $P = 0.14$). 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.

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 behavior, 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.

cerebral autoregulation; cerebrovascular carbon dioxide reactivity; middle cerebral artery; sedentary behavior; transfer function analysis

INTRODUCTION

Sedentary behavior (SB), defined as any waking behavior 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 (5, 23), whereas 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, since 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 hypothesized that prolonged sitting would reduce CBF and impair cerebrovascular function, but this would be attenuated with light-

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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.

MATERIALS 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 that were distributed to university mailing lists, and by using newspaper advertisements. Participants were screened for exclusion criteria, including: taking medication, smoker, body mass index >35 or <18 kg/m^2, use of hormone-based contraception, and diagnosis of cerebrovascular, cardiovascular, or metabolic disease. Study procedures were approved by the 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 A.M.) on three separate occasions. Testing procedures were the same across each test day (Fig. 1). After arrival and 20 min of supine rest, middle cerebral artery blood flow velocity (MCAv) and cerebrovascular CO_2 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 randomized order: *1*) 4 h uninterrupted sitting (SIT), *2*) 4 h sitting + 2-min light-intensity treadmill walking breaks every 30 min (2WALK) or, *3*) 4 h sitting + 8-min light-intensity treadmill walking breaks every 120 min (8WALK). The measurement of seated MCAv was repeated immediately after each 4-h 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 before and during each walking break.

Study Procedures

Before each visit participants were instructed to avoid strenuous exercise for 24 h, to complete an overnight fast, and abstain 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) (9) to determine habitual PA (14) and SB (39). Given the duration of testing, participants were given low-calorie low-fat standardized snacks at specified time points (Fig. 1). Following baseline tests, participants were given a breakfast cereal bar (Belvita Milk and Cereal Breakfast Biscuits, 220 kcal, 33.6 g carbohydrate, 7.2 g fat, 3.6 g protein) and a banana after 2 h (~100 kcal, ~27.0 g carbohydrate, ~0.3 g fat, ~1.0 g protein). Water was available to drink ad libitum.

Interventions

SIT. Participants remained seated at a desk for 4 h 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 one time during each intervention), and from making vigorous movements.

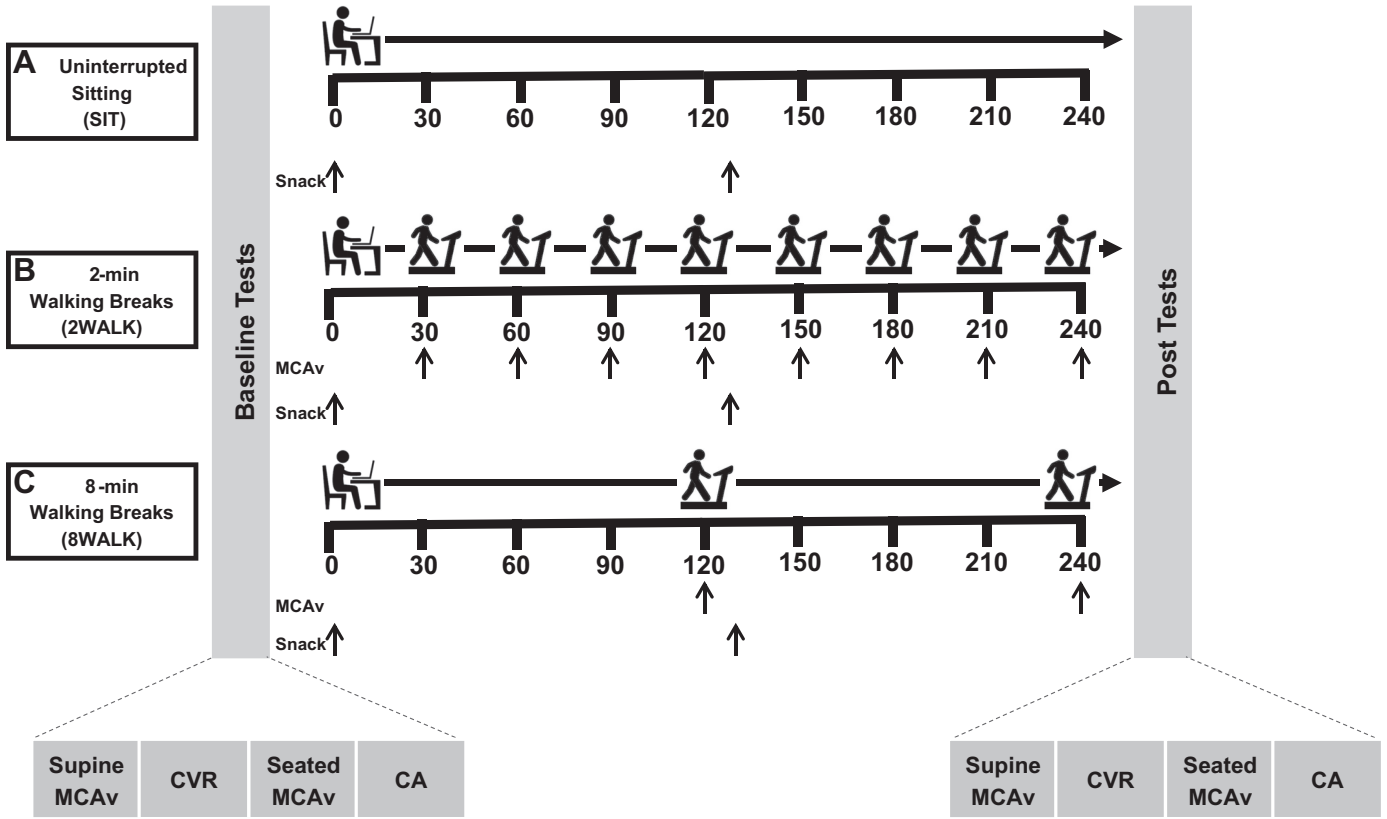

Fig. 1. Experimental design for the following three test conditions, completed in a randomized order, on three separate days: (*A*) 4 h uninterrupted sitting, (*B*) sitting with 2-min treadmill walking breaks every 30 min, and (*C*) sitting with 8-min treadmill walking breaks every 120 min. MCAv, middle cerebral artery blood flow velocity; CVR, cerebrovascular CO_2 reactivity; CA, cerebral autoregulation.

Participants were supervised at all times to ensure these conditions were adhered to.

2WALK. Sitting was interrupted every 30 min with a 2-min light-intensity treadmill walking break. Consequently, eight breaks were completed, totaling 16 min of activity. This break strategy was selected based on recommendations from The Sedentary Behavior 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) 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 familiarization session before 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.

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, totaling 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 (4), which a high-frequency breaks strategy may not be when translated into practice.

Measurements

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

Middle cerebral artery blood flow velocity. MCAv was used as a surrogate measure for CBF, since the MCA accounts for 70–80% of the brain's total perfusion (46). Continuous bilateral transcranial Doppler ultrasound (TCD) (ST3; Spencer Technologies, Redmond, WA) 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 in 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 before each walking break (prewalk) and throughout each subsequent walk, MCAv was continuously measured. Cerebrovascular conductance (CVC) was calculated by dividing MCAv by mean arterial pressure (MAP).

Cerebrovascular CO_2 reactivity. Maintenance of adequate CBF is influenced by the brain's ability to alter blood flow in response to changes in partial pressure of arterial CO_2, termed CVR (56). Participants were instrumented with a face mask with a two-way nonrebreathing valve (MLA1028; ADInstruments). A Douglas bag filled with a 5% 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 analyzer (Ml206; ADInstruments), and the pressure of end-tidal CO_2 ($P_{ET_{CO_2}}$) 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 $P_{ET_{CO_2}}$ was reduced to 20 mmHg (whichever was achieved first). Immediately afterward the valve on the Douglas bag was switched so participants inhaled the 5% CO_2 mixture. Simultaneously, participants were instructed to return their respiratory rate to normal while breathing the 5% CO_2 mixture for 3 min. Baseline $P_{ET_{CO_2}}$ and MCAv was calculated as the mean of the 1 min before hyperventilation, whereas MCAv and $P_{ET_{CO_2}}$ data during 5% CO_2 breathing were collected as 10-s averages for the entire 3-min period. Absolute and relative MCAv were then plotted against $P_{ET_{CO_2}}$ for each 10 s of 5% CO_2 breathing, and CVR was subsequently quantified by linear regression (R^2 value). Relative MCAv was calculated as the difference between baseline and 5% CO_2 MCAv divided by baseline MCAv [([5% CO_2 MCAv − baseline MCAv]/baseline MCAv) × 100%].

Simultaneously, during the baseline and CO_2 breathing measurements, arterial diameter and blood flow of the left common carotid artery (CCA) were acquired using a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA). Use of ultrasound to assess the dilation of larger extracranial neck vessels during CO_2 alterations provides another means to monitor reactivity and vessel dilation not assessable using TCD (3, 55). The extracranial arteries supplying the brain are also sensitive to changes in CO_2 levels and therefore contribute to cerebrovascular CO_2 regulation. Images were acquired in accordance with methodological guidelines (47), and data were analyzed as previously reported (21). To reduce any influence of turbulent flow on vascular responsiveness, the CCA was imaged at least 2 cm below the point of bifurcation. Data were used to determine the response of the CCA to elevations in $P_{ET_{CO_2}}$ by averaging 30 s of baseline diameter and blood flow data and comparing that with the diameter and blood flow during the last 30 s of 5% CO_2 breathing. All ultrasound measurements were completed by the same sonographer who has a between-day intraobserver coefficient of variation of 3.5% for the CCA, in line with methodological guidelines (47).

Cerebral autoregulation. 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 s of standing and 5 s of squatting (0.1 Hz) for 5 min to induce oscillations in blood pressure (BP) (12). MCAv and BP were continuously assessed. Data were analyzed 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 (52a). 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 are reliable (13, 52a). Data were processed and analyzed in accordance with standardized TFA guidelines to produce values of gain, phase, and coherence for the following 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, since the regulation of CBF is effective in the low-frequency range of BP oscillations but not in the high frequency range because of the time delay in initiating cerebrovascular adaptations to the changes in perfusion pressure (52a). CA therefore allows rapid BP changes to be transmitted to CBF, whereas slow BP changes are filtered (52a). 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, since for a given change in BP there are greater changes in MCAv (52a). Phase describes the temporal relationship between changes in BP and MCAv

Table 1. *Descriptive characteristics, self-reported physical activity scores, and total sitting time of participants*

	Mean ± SD
Age, yr	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 min/wk	4,524.3 ± 2,098.7
Sitting time/weekday, h	8.2 ± 2.2
Sitting time/weekend day, h	6.0 ± 1.9
Sitting time/wk, h	53.2 ± 12.4

MET, metabolic equivalent; $n = 15$ participants.

(12). Waveforms that are in sync are referred to as "in phase," whereas 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 (52a). Coherence describes the linearity of the relationship between the changes in MCAv and BP, with a coherence value approaching one indicating a linear relationship (12, 52a). 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 normalized to control for possible baseline differences in BP and MCAv between conditions; therefore, normalized gain was used during the interpretation of data (13, 52a).

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

Statistical Analyses

Data were analyzed using statistical software (SPSS version 22.0; IBM, Somers, NY), with significance accepted as $P \leq 0.05$. Results are presented as means ± SE. For each condition, the change in all outcome parameters was calculated (4 h minus baseline). To assess differences between conditions, parameters were analyzed using one-factor general linear mixed model with baseline values as a covariate. Differences in MCAv and HR between prewalk and during each walk were analyzed using paired-samples *t*-tests. Post hoc analyses were performed using the least-significant difference method.

RESULTS

Descriptive statistics are shown in Table 1.

Intervention Effects

Cardiorespiratory and hemodynamic 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 ($P = 0.61$) $P_{ET_{CO_2}}$ 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 with 2WALK ($P = 0.02$; Fig. 2*A*), but not between SIT and 8WALK ($P = 0.14$). Supine CVC, however, showed no significant main effect ($P = 0.09$; Fig. 2*C*). 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 with 2WALK (Fig. 2*B*). Seated CVC also differed significantly between conditions ($P = 0.01$), with post hoc analysis revealing the change in 2WALK was significantly different compared with SIT ($P = 0.03$; Fig. 2*D*).

Cerebrovascular CO_2 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, 4 h: −0.01 ± 0.01 mm; 2WALK baseline: 0.01 ± 0.01 mm, 4 h: −0.00 ± 0.02 mm; 8WALK baseline: −0.01 ± 0.01 mm, 4 h: −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, 4 h: −0.39 ± 0.48 ml/s; 2WALK baseline: 1.24 ± 0.48 ml/s, 4 h: −1.25 ± 1.26 ml/s; 8WALK baseline: −0.51 ± 0.82 ml/s, 4 h: −0.10 ± 1.14 ml/s).

Cerebral autoregulation. Mean values for coherence for each of the frequency domains were as follows: VLF 0.5; LF 0.6; HF 0.4. Table 4 presents values for phase, gain, and

Table 2. *For each intervention, middle cerebral artery blood flow and cardiorespiratory measures at baseline, 4 h, and the overall change with statistically adjusted baseline covariate control*

	SIT			2WALK			8WALK		
	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#
Supine position									
MCAv, cm/s	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, beats/min	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
PET_{CO_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	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, beats/min	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
PET_{CO_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

Values are means ± SE. SIT, uninterrupted sitting; 2WALK, 2-min walking breaks; 8WALK, 8-min walking breaks; Δ, change; MCAv, middle cerebral artery blood flow velocity; CVC, cerebral vascular conductance; MAP, mean arterial pressure; HR, heart rate; $P_{ET_{CO_2}}$, pressure of end-tidal CO_2. #Delta change values expressed with statistically adjusted baseline covariate control. *Significantly different from 2WALK ($P < 0.05$).

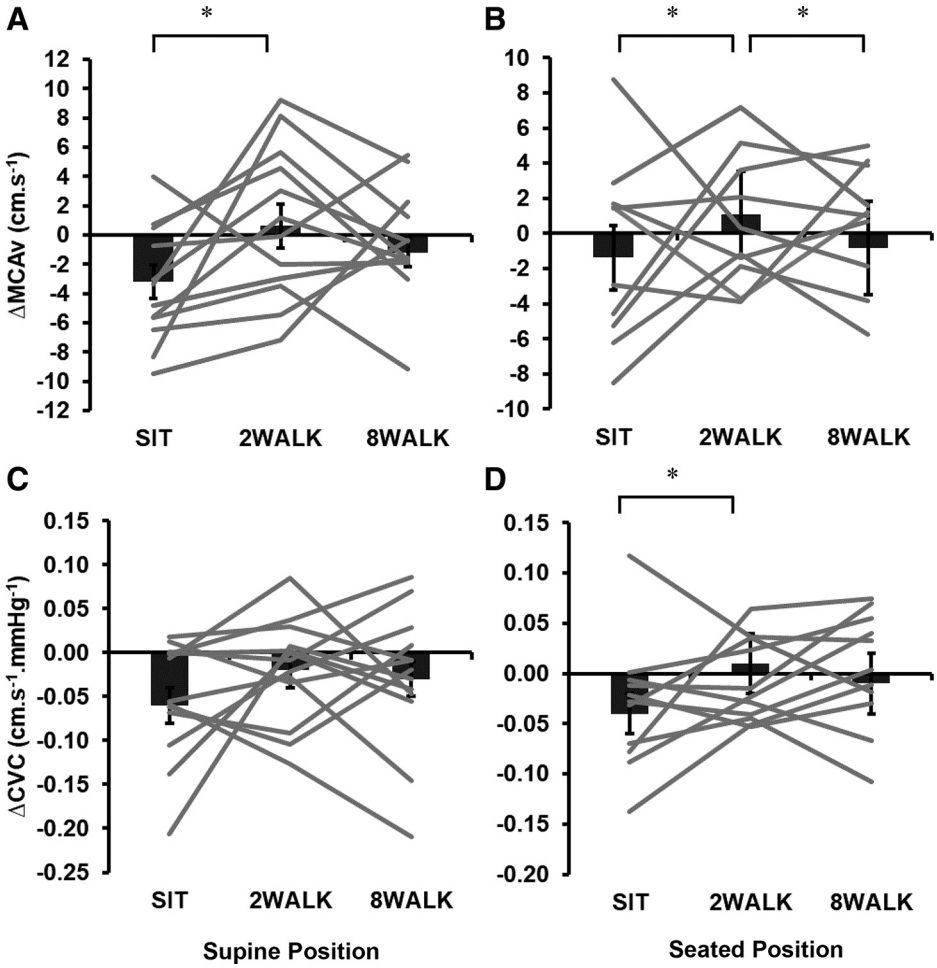

Fig. 2. Change in middle cerebral artery blood flow velocity (MCAv) and cerebrovascular conductance (CVC) in the supine (*A* and *C*) and seated (*B* and *D*) positions measured at baseline and after 4 h of each experimental condition with control for baseline blood flow and conductance. SIT, uninterrupted sitting; 2WALK, 2-min walking breaks; 8WALK, 8-min walking breaks. Error bars = ±SE. *Significant difference between conditions ($P < 0.05$).

normalized 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 with the change in 2WALK ($P = 0.01$). In the LF the main effect for normalized 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 higher than before the walking bout (prewalk: 55.7 ± 2.4 cm/s; walking: 57.8 ± 2.3 cm/s, $P = 0.02$). HR also significantly increased during each walking break, with an average increase of 33 beats/min (prewalk: 61 ± 2 beats/min; walking: 94 ± 2 beats/min, $P < 0.001$).

8WALK. Both walking breaks significantly increased MCAv. At 120 min MCAv increased by 1.96 cm/s ($P = 0.02$), whereas at 240 min a larger increase of 2.23 cm/s was observed ($P = 0.004$). Each break also significantly increased HR, with an average increase of 37 beats/min (prewalk: 69 ± 3 beats/min; walking: 96 ± 6 beats/min, $P < 0.001$).

Table 3. *R^2 values of linear regression of cerebrovascular CO_2 reactivity for each intervention at baseline, 4 h, and the overall change with statistically adjusted baseline covariate control*

	Sit			2WALK			8WALK		
	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#
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

Values are means ± SE. Relatively high R^2 values confirm the linearity of the response. SIT, uninterrupted sitting; 2WALK, 2-min walking breaks; 8WALK, 8-min walking breaks; Δ, change; CVR, cerebrovascular CO_2 reactivity. #Delta change values expressed with statistically adjusted baseline covariate control.

Table 4. *For each intervention, cerebral autoregulation estimates of phase, gain, and normalized gain at baseline, 4 h, and the overall change with statistically adjusted baseline covariate control*

	Sit			2WALK			8WALK		
	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#	Baseline	4 Hours	Δ#
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
Gain, $cm \cdot s^{-1} \cdot mmHg^{-1}$	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†
$Gain_n$, %/mmHg	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
Gain, $cm \cdot s^{-1} \cdot mmHg^{-1}$	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
$Gain_n$, %/mmHg	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
Gain, $cm \cdot s^{-1} \cdot mmHg^{-1}$	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
$Gain_n$, %/mmHg	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

Values are means ± SE. SIT, uninterrupted sitting; 2WALK, 2-min walking breaks; 8WALK, 8-min walking breaks; Δ, change; VLF, very low frequency; $Gain_n$, normalized gain; LF, low frequency; HF, high frequency. #Delta change values expressed with statistically adjusted baseline covariate control. *Significantly different from SIT ($P < 0.05$). †Significantly different from 2WALK ($P < 0.05$).

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 while 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. Translating this observation to the age-related decline in MCAv of 0.76 $cm \cdot s^{-1} \cdot yr^{-1}$ (1), this suggests the reductions observed following a one-off bout of uninterrupted sitting may equate to 2–4 yr 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 lower mean MCAv compared with 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 with a single activity bout, regular activity breaks during sitting enhance postprandial glycemia 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, since the change in VLF phase was greater compared with 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 have not been previously assessed; however, some research has explored the effects of exercise. Static handgrip exercise for 2 min did not affect CA (30) while exhaustive cycling impairs CA (29). These findings indicate that different modalities, intensities, and durations of exercise have varied effects on CA. Although the light-intensity 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 of 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, so 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 with the periphery (32).

There was no difference in the change in MAP between sitting and 2WALK; thus, in line with MCAv, CVC was significantly higher following 2WALK compared with prolonged sitting, demonstrating changes in BP do not impact our 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 fibers (28), and the progressive sympathoexcitation with aging is suggested to contribute to age-associated decreases in CBF (1). Prolonged sitting elevates muscle sym-

pathetic 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, since cerebral blood vessels are also innervated by cholinergic fibers (56). In animals, cholinergic fibers are stimulated during walking, contributing to increased CBF (45, 50). Evidence in humans also supports that cholinergic vasodilation contributes to increased CBF during exercise, since 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 glycemic regulation may also contribute to sitting-induced reductions in MCAv, since 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 while 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 PA breaks prevent these effects.

Workplace application. Because 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. Although 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 that the probe was in the same location and at the same angle for each visit; small variations may have occurred, but 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, while 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, whereas 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 United Kingdom office workers report sitting at work for 6.3 h (25), reductions in CBF may have important ramifications for workers' productivity. More importantly, chronic reductions in CBF are risk factors for cognitive impairment (40), are associated with cerebrovascular diseases such as Alzheimer's disease and dementia (41, 43, 58, 59), and correlate with cognitive dysfunction in Alzheimer's disease (38). Consequently, in the long term, the repeated exposure to sitting-induced decreases in CBF could cause chronic downregulation of CBF and therefore have large implications in the development of such diseases, which has previously been suggested (53). The high prevalence of SB in these cerebrovascular disease populations further highlights the relevance of our findings. The maintenance of CBF using frequent walking breaks to interrupt sitting therefore represents a protective mechanism against disease risk. Indeed, in a nondemented cohort, greater CBF was associated with a decreased chance of dementia development and less cognitive decline over a 6.5-yr follow-up (40). Future work is needed to better understand the potential relation between SB and development of cerebrovascular diseases.

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AUTHOR CONTRIBUTIONS

S.E.C., R.D., L.B., D.H.T., and N.D.H. conceived and designed research; S.E.C. and S.M.H. performed experiments; S.E.C. analyzed data; S.E.C. and

N.D.H. interpreted results of experiments; S.E.C. prepared figures; S.E.C. and N.D.H. drafted manuscript; S.E.C., R.D., S.M.H., L.B., D.H.T., and N.D.H. edited and revised manuscript; S.E.C., R.D., S.M.H., L.B., D.H.T., and N.D.H. approved final version of manuscript.

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