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ORIGINAL ARTICLE

Effects of alternating standing and sitting compared to prolonged sitting on cerebrovascular hemodynamics

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Abstract

Purpose Previous research suggests that prolonged sitting may acutely reduce cerebral blood flow velocity (CBFv). The purpose of this study was to evaluate the effects of alternating standing and sitting vs prolonged sitting on CBFv.

Methods This randomized crossover study enrolled working adults ($N=25$) with pre-to-stage 1 hypertension not using antihypertensive medications, and a body mass index (BMI) from 25 to < 40 kg/m^2. Subjects participated in two simulated workday conditions: (1) sitting continuously (SIT), and (2) alternating standing and sitting every 30 min (SS). Beat-to-beat systolic, mean and diastolic CBFv were recorded bilaterally for 1 min via insonation of the middle cerebral artery using transcranial Doppler ultrasonography before (morning), between (midday) and following (afternoon) two 3-h 40 min work periods.

Results Mean ± SD age was 42 ± 12 years, blood pressure (BP) was 132 ± 9/83 ± 8 mmHg, and BMI was 32 ± 5 kg/m^2. Cerebrovascular hemodynamics did not differ across condition ($P>0.05$). There were, however, significant nonlinear effects of time (decrease from morning to midday; increase from midday to afternoon) on systolic CBFv ($P=0.014$), mean CBFv ($P=0.001$), diastolic CBFv ($P=0.002$), and pulsatility index ($P=0.038$). When overall time effects were evaluated during each time interval, mean and diastolic CBFv significantly decreased morning to midday and all CBFv increased from midday to afternoon. When separated by condition, significant time effects were observed for all CBFv during SIT ($P<0.02$) but not SS ($P>0.05$).

Conclusions In individuals with elevated BP and BMI, CBFv significantly decreased by midday and increased by afternoon, especially during a workday of prolonged sitting. Future studies should evaluate the combination of frequent walks and a sit-stand desk to break up prolonged sitting.

Keywords Sedentary behaviour · Sit-stand desk · Cerebrovascular hemodynamics · Transcranial Doppler

Abbreviations

BP	Blood pressure
CBFv	Cerebral blood flow velocity
cfPWV	Carotid-femoral pulse wave velocity
crPWV	Carotid-radial pulse wave velocity
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
PP	Pulse pressure
PWV	Pulse wave velocity
TCD	Transcranial Doppler ultrasonography

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Introduction

Sedentary behaviour is defined as any waking behaviour while in a seated, reclining, or lying posture at ≤ 1.5 METs [1]. Prolonged sitting time is a known risk factor for cardiometabolic disease. It is independently associated with cardiovascular events such as stroke [2] and mortality [3]. Sitting time is also associated with retinal microvascular dysfunction [4], which is thought to reflect subclinical dysfunction in cerebral microcirculation [5]. In support of

these associations, several experimental studies have demonstrated that prolonged sitting results in unfavourable vascular effects. In these studies, 3–5 h of prolonged sitting resulted in increases in diastolic blood pressure (DBP) [6, 7] and mean arterial pressure (MAP) [6], and impairments in vascular function [8]. Furthermore, it has been demonstrated that 3 h of prolonged sitting impairs endothelial function, while breaking up sitting offsets this impairment [9].Whether these potentially deleterious vascular changes resulting from prolonged sitting also occur in the cerebrovasculature is not well established.

Normal cerebrovascular hemodynamics are crucial for healthy brain function as neurons are dependent on cerebral blood flow to provide oxygen and nutrients to support neuronal metabolism [10]. For example, inadequate cerebral blood flow disrupts neuronal function over time via impairments of protein synthesis, synaptic activity and glutamate excitotoxicity, leading to cognitive decline [11, 12]. Moreover, blood flow with large fluctuations between systole and diastole can be harmful to cerebrovascular health. This pulsatile blood flow can damage small vessels and result in reduced cerebral blood flow and increased cerebrovascular resistance [13], consequently increasing the risk of stroke, cognitive decline, dementia, and Alzheimer's disease [14–16]. Thus, reduced blood flow and increased pulsatile flow are detrimental to normal brain function and can lead to disease.

Preliminary evidence suggests that prolonged sitting may lead to suboptimal cerebrovascular hemodynamics. A crossover study used transcranial Doppler ultrasonography (TCD) to examine the effect of an energy drink vs water on cerebrovascular hemodynamics over a 2-h sitting period [17]. Consuming the energy drink resulted in a reduction of cerebral blood flow velocity (CBFv) and an increase in cerebrovascular resistance. Of note, however, is that there were also significant reductions in CBFv and increases in cerebrovascular resistance across the control session, suggesting that prolonged sitting alone may have unfavourable effects on cerebrovascular hemodynamics. Furthermore, a crossover study evaluated the effects of prolonged sitting and breaking up sitting via walking breaks on CBFv [18]. This study demonstrated that a 4-h sitting period resulted in a significant reduction in CBFv yet the addition of short-duration walking breaks (2-min of light intensity every 30 min) offset this decrease.

Given that 2-min light-intensity walking breaks every 30 min may not be feasible in all work environments (e.g., inability to leave workspace to perform walking breaks due to employer policies or the inability to interrupt work), sit-stand desks may be an alternative way to break up prolonged sitting. Sit-stand desks have been shown to significantly reduce sitting time and improve health outcomes such as blood pressure, postprandial glucose responses, musculoskeletal discomfort and pain [19–22]. It is unknown, however, whether interrupting prolonged sitting via standing has beneficial effects on cerebrovascular hemodynamics. Thus, the purpose of the current study was to evaluate the effects of prolonged sitting compared to alternating standing and sitting on cerebrovascular hemodynamics measured using TCD. We hypothesized that prolonged sitting would have undesirable effects on cerebrovascular hemodynamics and that alternating standing and sitting would attenuate these effects. An exploratory aim was to evaluate whether blood pressure (BP) and pulse wave velocity (PWV) were related to cerebrovascular hemodynamics and whether these explained observed condition or time effects [7].

Methods

Participants

Twenty-five working adults were recruited. Written informed consent was obtained for all subjects prior to any data collection. Approval for this study was granted by the Institutional Review Board at the University of Pittsburgh.

Inclusion criteria were comprised of ability to perform job-related deskwork remotely with a computer and internet access, a body mass index (BMI) of 25-39.9 kg/m^2, 20–65 years old, pre-to-stage I hypertension with systolic blood pressure (SBP) of 120–159 mmHg or DBP of 80–89 mmHg [23] and ability to comply with study protocols.

Exclusion criteria included use of antihypertensive or any other medication that would affect cardiovascular responses, cardiovascular event in the last 6 months, atrial fibrillation, SBP $\geq$ 160 mmHg or DBP of $\geq$ 100 mmHg, enrollment in weight loss program, current treatment for heart disease, cancer, end stage renal disease or other serious condition, inability to stand for 60 min, regular smoker, $\geq$ 90 min of moderate to vigorous intensity activity per week in the last 3 months, and pregnant in the past 6 months or breast feeding in the last 3 months.

Experimental procedure

Methods are described in detail elsewhere [7]. The outcomes reported herein were secondary outcomes collected during the protocol. Briefly, participants performed personal desk work on two, randomly ordered simulated workdays. On 1 day, they sat continuously (SIT) and on the other they alternated between standing and sitting every 30 min during the work periods via use of a height-adjustable sit-stand desk (SS) (Float or QuickStand, Humanscale, New York, New York, USA) [24]. Alternating between standing for 30-min and sitting for 30-min was selected based on recent

workplace expert guidelines for reducing sitting at work [24]. Vascular testing (BP, TCD and PWV) was performed at three time points each day following a 10-min seated rest: before (morning), between (midday), and following (afternoon) two 3-h and 40-min work periods (Fig. 1). We selected two 3-h and 40-min work period (with lunch in between) to simulate a typical workday: hourly workers typically work 7.5 h per day with a break for lunch. Testing period start times typically occurred between 7:30–8:00 am for morning, 11:30–12:00 pm for midday and 4:30–5:00 pm for afternoon. Following the morning testing period, participants consumed a standardized breakfast (30% of daily caloric need; 55% carbohydrate, 35% fat, and 10% protein), then completed the first work period. Next, participant underwent the midday testing period, after which they consumed a standardized lunch (30% daily caloric need; 55% carbohydrate, 35% fat, 10% protein). Following lunch, participants completed the second work period, which was followed by the afternoon testing period.

Number of bathroom breaks were also recorded and evaluated for influence on outcomes.

Prior to the experimental days, participants were instructed to abstain from food for 12 h and alcohol, nicotine, moderate to vigorous exercise and caffeine for 24 h. Verbal confirmation of abstention was obtained upon arrival. Sitting hours per day were assessed via the sedentary behaviour question on the Global Physical Activity Questionnaire [25].

Blood pressure

The vascular testing battery began with a 10-min rest in a seated posture followed by duplicate brachial BP and heart rate measured using an automated oscillometric device (HEM-075, Omron Healthcare, Inc., Lake Forest, IL). Readings were taken with a 1-min rest between measures and were averaged. Pulse pressure (PP) was calculated as SBP − DBP and MAP pressure was calculated as DBP + 1/3 (SBP − DBP).

Transcranial Doppler ultrasonography

TCD examinations were obtained immediately following BP measurements in a seated posture. Bilateral middle cerebral artery (MCA) CBFv was continuously measured for 1 min [18] using a 2-MHz TCD probe (Terumo; Spencer Technologies) by a single trained technician with intra-rater correlation coefficient of 85–97% across TCD measures. All TCD data was saved for later analysis. Participants were instructed to breathe normally and remain still. Insonation of the MCA was performed using a transtemporal window at depths ranging from 40 to 65 mm [26–28]. Care was taken, using depth and directionality of the signal, to ensure that the MCA was the intracranial vessel being insonated.

The depth, location of probe, and anatomic landmarks were recorded and marked to ensure the TCD recording probe position remained the same in each subject and the same approach was used for each condition as well as between conditions. Quality of TCD waveforms was assessed based on how well the envelope captured the waveforms and the strength of the signal.

Systolic, mean, and diastolic blood flow velocities and pulsatility index (a measure of pulsatile flow automatically calculated as the difference between systolic and diastolic flow velocities divided by mean flow velocity) were averaged over each minute recording. Right and left MCA values were then averaged to provide single MCA cerebrovascular hemodynamic values for each time point. One participant was excluded due to the inability to reliably insonate the bilateral MCA at the same depth.

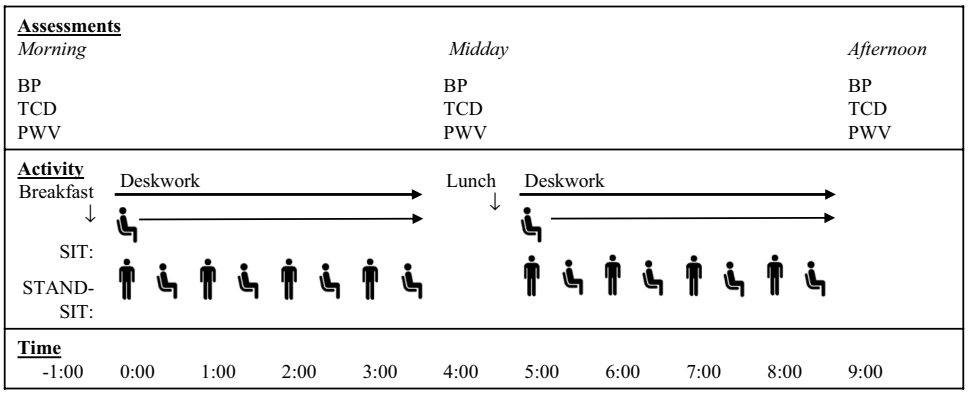

Fig. 1 Experimental protocol. Participants arrived at the laboratory between 7:00 and 7:30 am and completed two protocols in randomized order (continuously seated deskwork, alternating standing and sitting postures every 30 min during deskwork). Vascular testing (blood pressure, transcranial Doppler and pulse wave velocity) was performed in the morning, midday, and afternoon. Standardized meals were consumed after vascular testing in the morning and midday

Pulse wave velocity

Immediately following TCD, participants transitioned to a supine posture and rested for 10 min. After resting, carotid-femoral (cfPWV), carotid-radial (crPWV), and carotid-ankle (cdPWV) pulse wave velocities were measured using the Complior Analyse (Alam Medical, Vincennes, France) on the right side of the body by a trained technician. Using a tape measure, aortic distance was estimated by subtracting the distance from the carotid artery site to the sternal notch from the distance of the sternal notch to the femoral artery site [29–31]. Carotid-radial distance was estimated by measuring from the carotid artery site to the radial artery site while the subject's arm rested beside them. Carotid-ankle distance was estimated by measuring from the carotid artery site to the posterior tibialis site. Piezoelectric sensors were held in place on the skin (carotid, radial, posterior tibialis) or light clothing (femoral) over the arterial sites to obtain ten valid waveforms with low error. Low error was defined as a coefficient of variation across the ten waves of $\leq 5\%$. Error of $\leq 10\%$ was accepted for difficult scans. This process was repeated until three runs of ten waveforms were captured. Average crPWV, cfPWV and cdPWV (m s^{-1}) were calculated as distance divided by the average time differential between the foot of the waveform at the carotid and the radial, femoral and posterior tibialis sites within each run, and then averaged across the three runs [30–32]. Inter-rater reliability of technicians for cfPWV in our laboratory ranged from 85 to 96%.

Measures of sleepiness, mental effort and fatigue

Self-reported sleepiness, mental effort, and fatigue were considered as covariates that might influence cerebrovascular hemodynamics across the workday.

The Karolinska Sleepiness Scale [33] was administered at baseline (morning) and then immediately prior to midday and afternoon vascular testing. This scale is a self-report of level of drowsiness and has a 9-point Likert scale from 1 "extremely alert" to 9 "very sleepy, great effort to keep awake, fighting sleep".

Self-reported measures of mental effort and mental fatigue were also obtained. The mental fatigue scale was part of a modified [34] Physical Discomfort and Fatigue Questionnaire [35]. This self-report 100-point scale from "no fatigue" to "extreme fatigue" was administered at baseline (morning) and then prior to midday and afternoon vascular testing.

The mental effort scale [36] was administered prior to midday and afternoon vascular testing and was meant to reflect mental effort for each 3-h and 40-min work period. This scale is a self-report of perceived mental load and has a 9-point likert scale from 1 "very, very low mental effort" to 9 "very, very high mental effort".

Statistical analysis

A sample size of $n=25$ was selected for the parent study to achieve 80% power to detect a condition main effect size of 0.25 for hourly BP measurements, assuming $\alpha=0.05$ and a within-subject $r=0.7$. Post hoc calculations for the current study, assuming similar $\alpha=0.05$ and a within-subject $r=0.7$, and given the two follow-up measurements at midday and afternoon suggest we were powered to detect effect sizes of approximately 0.4.

Linear mixed models were used to evaluate effects of time of day and condition on cerebrovascular hemodynamics with adjustment for condition order, age, sex and heart rate [26]. Though we were not powered to detect an interaction effect, the absence of any interaction effects was confirmed prior to further analyses (all $P>0.05$). Subsequent models included only main effects of time and condition. Due to nonlinear effects over time, changes from morning to midday and midday to afternoon were modelled as separate coefficients in the same model. Statistical significance of the time effects was evaluated using the likelihood ratio test comparing models with both time coefficients to models with neither. Post hoc linear mixed models evaluated overall time effects at each time point (morning to midday; midday to afternoon) and the overall time effect by the likelihood ratio test within each condition. Sleepiness, mental fatigue and effort, and bathroom breaks were compared across condition and time and linear mixed models were used to evaluate whether BP, PWV, sleepiness, mental fatigue and effort, and bathroom breaks were significantly associated with cerebrovascular parameters. $P<0.05$ was used to accept significance. Analyses were performed using Stata version 14 (StataCorp, LLC, College Station, TX).

Results

Participant characteristics

Participants had a mean age of 42 ± 12 years, BMI of 31.9 ± 5.0 kg/m^2, and mean BP was $132\pm9/83\pm8$ mmHg. In addition, they were predominantly white (84%), had a post-graduate degree (60%) and were mostly male (65%). As previously reported [19], participant demographics are displayed in Table 1.

Effects on cerebrovascular hemodynamics

Effects of condition and time

There were no overall main effects of condition found on systolic CBFv ($\beta=0.98$ cm/s, $P=0.18$), mean CBFv

Table 1 Participant characteristics

	Mean (SD) or *n*, %
Age, years	42 (12)
Gender	
Male	16, 64%
Female	9, 36%
Race	
Non-Hispanic white	20, 80%
Non-Hispanic black	2, 8%
Hispanic white	1, 4%
Asian	2, 8%
Education	
High school graduate	5, 20%
College graduate	5, 20%
Post graduate degree	15, 60%
Occupational status	
Full-time	17, 68%
Part-time	5, 20%
Student	3, 12%
Body mass index, kg/m^2	31.9 (5.0)
Sitting time, h/day	9.8 (3.7)
Resting MCA CBFv	
Systolic CBFv, cm/s	73.2 (11.0)
Mean CBFv, cm/s	50.3 (7.4)
Diastolic CBFv, cm/s	36.1 (5.4)
Pulsatility index	0.74 (0.12)

($\beta = 0.62$ cm/s, $P = 0.22$), diastolic CBFv ($\beta = 0.71$ cm/s, $P = 0.07$) or pulsatility index ($\beta = -0.008$, $P = 0.231$).

There were, however, significant nonlinear main effects of time on systolic CBFv ($P = 0.014$), mean CBFv ($P = 0.001$), diastolic CBFv ($P = 0.002$), and pulsatility index ($P = 0.038$). Post hoc testing of each time point (overall) revealed CBFvs decreased from morning to midday for mean CBFv and diastolic CBFv and increased from midday to afternoon for all CBFv (Table 2). Pulsatility index significantly increased from morning to midday ($P = 0.019$), without significantly changing from midday to afternoon. Post hoc testing of nonlinear time effects within each condition (by the likelihood ratio test) revealed that time effects were present across SIT for systolic CBFv ($P = 0.017$), mean CBFv ($P = 0.003$), and diastolic CBFv ($P = 0.005$); time effects were not significant in the SS condition (all $P > 0.05$) (Table 2). Pulsatility time effects were not statistically significant within either condition. Statistical significance and magnitude of within-condition changes for each time interval are reported in Table 2 and Fig. 2.

Table 2 β coefficients (differences) between time points within condition

	Morning to midday	Midday to afternoon	*P* value for time effect
Systolic CBFv (cm/s)			
Overall	− 1.71 ($P = 0.071$)	2.55 ($P = 0.004$)**	0.014*
SIT	− 2.39 ($P = 0.069$)	3.55 ($P = 0.010$)*	0.017*
SS	− 1.33 ($P = 0.235$)	1.53 ($P = 0.074$)	0.353
Mean CBFv (cm/s)			
Overall	− 2.08 (P = 0.002)**	2.12 ($P < 0.001$)**	0.001**
SIT	− 2.76 ($P = 0.005$)**	2.85 ($P = 0.002$)**	0.003**
SS	− 1.56 ($P = 0.012$)*	1.40 ($P = 0.030$)*	0.097
Diastolic CBFv (cm/s)			
Overall	− 1.42 ($P = 0.004$)**	1.48 ($P = 0.001$)**	0.002**
SIT	− 1.59 ($P = 0.014$)*	2.14 ($P = 0.002$)**	0.005**
SS	− 1.13 ($P = 0.037$)*	0.840 ($P = 0.092$)	0.201
Pulsatility index			
Overall	0.022 ($P = 0.019$)*	− 0.007 ($P = 0.359$)	0.038*
SIT	0.014 ($P = 0.275$)	− 0.015 ($P = 0.188$)	0.333
SS	0.017 ($P = 0.266$)	− 0.0001 ($P = 0.987$)	0.187

Differences in CBFv values between time points across both conditions combined or within condition

*$P < 0.05$

**$P < 0.01$

Relationship of vascular measures to cerebrovascular hemodynamics

In models adjusting for condition order, age, sex, measures of BP and PWV were not significantly associated with systolic or mean CBFv ($P > 0.05$). DBP was directly associated with diastolic CBFv ($\beta = 0.108$ cm/s, $P = 0.042$). Both DBP ($\beta = -0.003$ cm/s, $P = 0.003$) and PP ($\beta = 0.0016$ cm/s, $P = 0.032$) were inversely and directly associated with pulsatility, respectively (Table 3).

Other potential influences on cerebral hemodynamics

In this subsample, sleepiness did not significantly differ by condition [SIT: 3.53 (1.79), SS: 3.17 (0.18), $P = 0.08$]. However, there was a significant effect by time [morning: 3.46 (1.95), midday: 3.02 (1.32), afternoon: 3.58 (1.69), $P = 0.047$].

Mental fatigue did not differ by condition [SIT: 14.1 (16.5), SS: 11.3 (12.6), $P = 0.09$] though there was a significant effect by time [morning: 10.8 (12.7), midday: 9.9 (10.7), afternoon: 17.3 (18.6), $P = 0.0005$]. Mental effort did not differ by condition [SIT: 5.7 (1.2), SS: 5.5 (1.4), $P = 0.20$] or by time [morning: 5.6 (1.4), afternoon: 5.7 (1.3), $P = 0.55$].

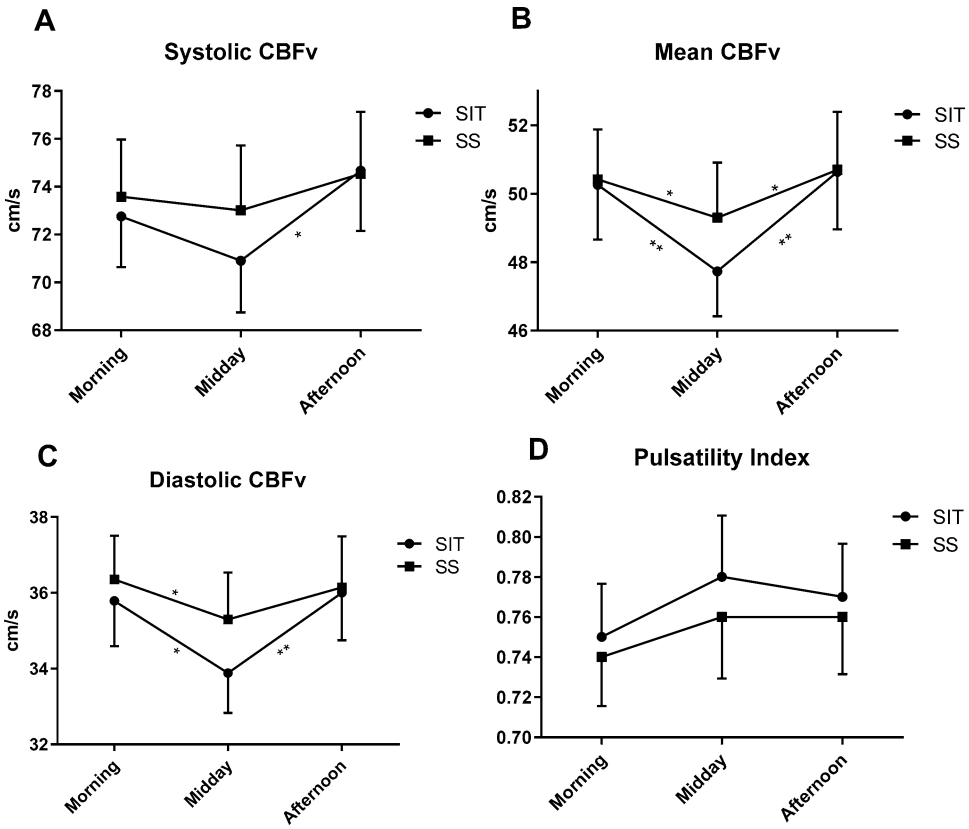

Fig. 2 Effects on cerebrovascular hemodynamics by condition. **a** Systolic CBFv. *P* for time = 0.014, *P* for condition = 0.178. **b** Mean CBFv. *P* for time = 0.001, *P* for condition = 0.219. **c** Diastolic CBFv. *P* for time = 0.002, *P* for condition = 0.065. **d** Pulsatility index. *P* for time = 0.038, *P* for condition = 0.231. **P* < 0.05, ***P* < 0.01 comparing midday to morning and afternoon. *P* < 0.05 comparing morning to afternoon. Error bars represent SEM

Table 3 β coefficients for vascular factors potentially explaining variability in CBFv

	Systolic CBFv	Mean CBFv	Diastolic CBFv	Pulsatility index
Systolic blood pressure	0.051 (*P* = 0.443)	0.042 (*P* = 0.357)	0.022 (*P* = 0.531)	− 0.00001 (*P* = 0.988)
Diastolic blood pressure	0.027 (*P* = 0.797)	0.091 (*P* = 0.199)	0.108 (*P* = 0.042)*	− 0.003 (*P* = 0.003)**
Mean arterial blood pressure	0.054 (*P* = 0.589)	0.087 (*P* = 0.200)	0.083 (*P* = 0.106)	− 0.0018 (*P* = 0.068)
Pulse pressure	0.056 (*P* = 0.470)	0.005 (*P* = 0.919)	− 0.032 (*P* = 0.431)	0.0016 (*P* = 0.032)*
Carotid-femoral PWV	0.456 (*P* = 0.467)	0.097 (*P* = 0.821)	− 0.014 (*P* = 0.965)	0.0075 (*P* = 0.218)
Carotid-radial PWV	− 0.377 (*P* = 0.401)	− 0.222 (*P* = 0.473)	− 0.138 (*P* = 0.552)	0.00076 (*P* = 0.862)
Carotid-ankle PWV	− 0.113 (*P* = 0.855)	− 0.095 (*P* = 0.815)	− 0.091 (*P* = 0.775)	0.0019 (*P* = 0.749)

**P* < 0.05, ***P* < 0.01

Bathroom breaks were not significantly different by condition [SIT: 3.71 (1.37), SS: 3.45 (1.67), *P* = 0.38] However, there was a significant effect by time [morning: 3.21 (1.72), afternoon: 3.96 (1.68), *P* = 0.01].

Due to the fact that some of these potential influences on CBFv differed by time, we investigated whether these factors were also associated with cerebrovascular hemodynamics. We found that sleepiness, mental effort, mental fatigue and bathroom breaks were not significantly associated with cerebrovascular hemodynamics parameters (Table 4).

Discussion

The aim of the current study was to evaluate the effects of alternating standing and sitting vs prolonged sitting on cerebrovascular hemodynamics. We found an effect of time of day on cerebrovascular hemodynamics such that CBFv parameters worsened by midday and returned to morning values by the afternoon across both simulated workdays. Though an overall condition effect was not found, post hoc testing of time effects revealed that the midday 'dip'

Table 4 β coefficients for other factors potentially explaining variability in CBFv

	Systolic CBFv	Mean CBFv	Diastolic CBFv	Pulsatility index
Sleepiness	0.474 ($P=0.135$)	0.337 ($P=0.123$)	0.313 ($P=0.059$)	− 0.003 ($P=0.409$)
Mental effort	− 0.521 ($P=0.365$)	− 0.235 ($P=0.537$)	− 0.160 ($P=0.591$)	− 0.002 ($P=0.672$)
Mental fatigue	− 0.008 ($P=0.840$)	− 0.002 ($P=0.949$)	0.002 ($P=0.918$)	9.13e−06 ($P=0.981$)
Bathroom breaks	0.798 ($P=0.265$)	0.638 ($P=0.175$)	0.546 ($P=0.138$)	− 0.004 ($P=0.577$)

*$P<0.05$, **$P<0.01$

in cerebrovascular hemodynamics was statistically significant during a workday of prolonged sitting but not when alternating postures.

The effect of time of day on cerebrovascular hemodynamics is consistent with previous literature suggesting that CBFv changes according to a circadian rhythm [37]. Conroy and colleagues demonstrated that the lowest CBFv occurs at 12:02 pm. This is corroborated by evidence of stroke having a peak incidence between the hours of 10:00 am and 12:00 pm, including subarachnoid haemorrhage, intracerebral haemorrhage and thromboembolic cerebral infarction [38]. This "time of day" effect is not explained by the potential influences for which we controlled. Though sleepiness followed the same pattern as CBFv measures (midday 'dip'), it was not associated with cerebrovascular hemodynamics. Similarly, neither mental fatigue nor mental effort were associated with cerebrovascular hemodynamics. Furthermore, though more bathroom breaks occurred during the afternoon across conditions, they were not associated with cerebrovascular hemodynamics.

Though we did not observe a significant overall condition effect, the midday 'dip' in CBFv (time effect) was only statistically significant during SIT. The lack of a statistically significant midday dip during the SS condition may have resulted from a modest offsetting of the circadian rhythm of CBFv that results in this midday 'dip'. Further, this slight offsetting may have not been a large enough stimulus to increase already normal values during the morning and afternoon. This is potentially important because sit-stand desks are easy to use without interrupting work. Interventions geared towards interrupting prolonged sitting time, such as regular use of sit-stand desks, may be a simple strategy by which to alter the circadian rhythm of CBFv by offsetting the midday 'dip'.

One other study has evaluated the effects of prolonged sitting and breaking up sitting on cerebrovascular hemodynamics [18]. The authors compared three conditions: prolonged sitting for 4 h, sitting interrupted by 2-min light intensity walking breaks every 30 min, and sitting interrupted by 8-min light intensity walking breaks every 2 h. They found that CBFv significantly decreased following prolonged sitting but not following the 2-min walks, suggesting that short frequent walks attenuated the CBFv reductions. These results are complimentary to the results of the present study that the midday 'dip' appeared to be larger in SIT vs SS. The difference in modality and intensity of standing vs walking could explain why there were not significant main effects of condition with standing in the present study, but there were with 2-min walks in the Carter (2018) study. Of note, however, is that Carter's volume-controlled but less frequent walking breaks (8 min every 2 h) did not offset the CBFv reductions during sitting, suggesting that the frequency of interruption to prolonged sitting is a key factor. Future studies should explore whether combining walking breaks and the use of a sit-stand desk to break up prolonged sitting has a synergistic effect on cerebrovascular hemodynamics.

Strengths of this study include its randomized crossover design with repeated measures across two simulated workdays and standardization of protocols. A sole trained sonographer performed all TCD recordings. Furthermore, the dose of standing used is consistent with current recommendations for decreasing sitting time at work [24]. A limitation in our methods was the unavailability of equipment to measure partial pressure of carbon dioxide ($PaCO_2$) and beat-to-beat BP during TCD measurement. Additional measurement of these cofactors could have influenced results and should be included in future studies. A second limitation is our sonographer was not blinded to condition and used of a hand-held probe rather than the best-practice head-frame mounted assessment method, though our single technician had excellent reliability and used a standardized, published approach [39]. Participants performed personal tasks that were not standardized within or between subjects, though mental effort and fatigue did not differ by condition. As this was a laboratory study, movement was limited by the experimental arrangements, and thus generalizability is limited. Generalizability is further limited as our study sample was comprised of individuals with overweight/obesity and pre-to-stage 1 hypertension. Additionally, CBFv is only a surrogate for CBF when the vessel area remains constant and is not quantitative for flow [28]. CBFv was only measured at the MCA and thus our results cannot be extrapolated to other cerebrovascular territories. Last, since the study was powered to detect differences in the main study outcome (BP), these secondary analyses may have been limited by insufficient power to detect differences in TCD outcomes across conditions.

Conclusions

In conclusion, CBFv differed across the workday such that a midday 'dip' occurred. Furthermore, this 'dip' was statistically significant during a work period of prolonged sitting and not when alternating standing and sitting. Future studies combining walking breaks with the use of a sit-stand desk to break up prolonged sitting and potentially yield greater improvement on cerebrovascular hemodynamics are warranted.

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Compliance with ethical standards

Conflict of interest Dr. Gibbs discloses funding from Humanscale. Drs. Perdomo, Kowalsky, Balzer and Mr. Taormina disclose no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all participants.

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