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

3

4 Running heading: Prolonged sitting and cerebral blood flow

5

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22 **ABSTRACT**

23 Decreased cerebrovascular blood flow and function are associated with lower cognitive
24 functioning and increased risk of neurodegenerative diseases. Prolonged sitting impairs
25 peripheral blood flow and function, but its effects on the cerebrovasculature are unknown.
26 This study explored the effect of uninterrupted sitting and breaking up sitting time on
27 cerebrovascular blood flow and function of healthy desk workers. Fifteen participants (10
28 male, 35.8 ± 10.2 years, BMI: 25.5 ± 3.2 $\text{kg} \cdot \text{m}^{-2}$) completed, on separate days, three 4-hr
29 conditions in a randomised order: a) uninterrupted sitting (SIT), b) sitting with 2-min light
30 intensity walking breaks every 30-min (2WALK) or c) sitting with 8-min light intensity
31 walking breaks every 2-hrs (8WALK). At baseline and 4-hrs, middle cerebral artery blood
32 flow velocity (MCAv), carbon dioxide reactivity (CVR) of the MCA and carotid artery were
33 measured using transcranial Doppler (TCD) and duplex ultrasound respectively. Cerebral
34 autoregulation (CA) was assessed with TCD using a squat-stand protocol and analysed to
35 generate values of gain and phase in the very low, low, and high frequencies. There was a
36 significant decline in SIT MCAv (-3.2 ± 1.2 $\text{cm} \cdot \text{s}^{-1}$) compared to 2WALK (0.6 ± 1.5 $\text{cm} \cdot \text{s}^{-1}$,
37 $p=0.02$), but not between SIT and 8WALK (-1.2 ± 1.0 $\text{cm} \cdot \text{s}^{-1}$, $p=0.14$). For CA, the change in
38 2WALK very low frequency phase (4.47 ± 4.07 degrees) was significantly greater than SIT ($-$
39 3.38 ± 2.82 degrees, $p=0.02$). There was no significant change in MCA or carotid artery CVR
40 ($p>0.05$). Results indicate that prolonged, uninterrupted sitting in healthy desk workers
41 reduces cerebral blood flow, however this is offset when frequent, short-duration walking
42 breaks are incorporated.

43

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

46

47 **NEW & NOTEWORTHY**

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

54 **INTRODUCTION**

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

63

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

72

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

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

82

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

89 MATERIAL AND METHODS

90 Participants

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

99

100 Study design

101 Participants attended the temperature controlled (20-22 °C) laboratory at the same time of day
102 (7.00-9.00 am) on three separate occasions. Testing procedures were the same across each test
103 day (Figure 1). After arrival and 20-min supine rest, middle cerebral artery blood flow
104 velocity (MCAv) and cerebrovascular carbon dioxide reactivity (CVR) were assessed.
105 Participants were then seated and underwent measures of seated MCAv and cerebral
106 autoregulation (CA). Following baseline measurements participants completed, in a
107 randomised order: a) 4-hr uninterrupted sitting (SIT), b) 4-hr sitting+2-min light-intensity
108 treadmill walking breaks every 30-min (2WALK) or, c) 4-hr sitting+8-min light intensity
109 treadmill walking breaks every 120-min (8WALK). The measurement of seated MCAv was
110 repeated immediately after each 4-hr intervention. MCAv was assessed while seated to assess
111 the posture of interest, sitting, and to prevent the effects of moving to a supine posture altering
112 hemodynamics. Participants then returned to a supine posture and supine MCAv and CVR

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

115

116 **Study procedures**

117 Prior to each visit participants were instructed to avoid strenuous exercise for 24-hr, to
118 complete an overnight fast and abstinence from caffeine and alcohol. Women were assessed
119 in the follicular phase of the menstrual cycle (days 1-7). Participants completed the
120 International Physical Activity Questionnaire (Long form, IPAQ) (9) to determine habitual
121 PA (14) and SB (39). Given the duration of testing, participants were given low calorie, low
122 fat, standardised snacks at specified time points (Figure 1). Following baseline tests,
123 participants were given a breakfast cereal bar (Belvita Milk and Cereal Breakfast Biscuits,
124 220kcal, 33.6g carbohydrate, 7.2g fat, 3.6g protein) and a banana after 2-hr (~100kcal, ~27.0g
125 carbohydrate, ~0.3g fat, ~1.0g protein). Water was available to drink *ad libitum*.

126

127 **Interventions**

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

135 *2-min walking breaks (2WALK)*. Sitting was interrupted every 30-min with a 2-min light
136 intensity treadmill walking break. Consequently, eight breaks were completed, totalling 16-
137 min of activity. This break strategy was selected based on recommendations from the The

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

145 *8-min walking breaks (8WALK)*. Sitting was interrupted every 120-min with an 8-min light
146 intensity walk, using the same walking speed as previously described. Consequently, two
147 breaks were completed, totalling 16-min of activity. Therefore, the total duration of PA
148 performed in both walking break conditions was identical. This less frequent break strategy
149 was based on recommendations that interventions to break up sitting must be feasible (5),
150 which a high frequency breaks strategy may not be when translated into practise.

151

152 **Measurements**

153 All physiological data measurements were continuously acquired at 50 Hz using an analog-to-
154 digital convertor (PowerLab ML880, ADInstruments, Colorado Springs, Colorado, USA) and
155 displayed in real time on a computer with commercially available software (LabChart Version
156 7.0, ADInstruments).

157

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

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

170 Mean MCAv was calculated from the envelope of the velocity tracing using a weighted mean
171 ($1/3$ maximum + $2/3$ minimum) to account for the relative time spent in systolic and diastolic
172 pressures (46). Supine and seated MCAv were acquired for 1-min. During the 1-min prior to
173 each walking break (pre-walk) and throughout each subsequent walk, MCAv was
174 continuously measured. Cerebrovascular conductance (CVC) was calculated by dividing
175 MCAv by mean arterial pressure (MAP).

176

177 *Cerebrovascular carbon dioxide reactivity (CVR)*. Maintenance of adequate CBF is
178 influenced by the brain's ability to alter blood flow in response to changes in partial pressure
179 of arterial carbon dioxide, termed CVR (56). Participants were instrumented with a face mask
180 with a two-way non-rebreathing valve (MLA1028, ADInstruments, Colorado Springs,
181 Colorado, USA). A Douglas bag filled with a 5% carbon dioxide (CO₂) mixture and fitted
182 with a three-way valve, enabled the breathing circuit to be alternated between ambient air and
183 the contents of the Douglas bag. Breath-by-breath CO₂ was sampled using a calibrated gas
184 analyser (MI206, ADInstruments) and the pressure of end-tidal carbon dioxide (PETCO₂) was
185 calculated in LabChart with correction for the daily barometric pressure. After a 1-min
186 baseline, participants were coached through a voluntary hyperventilation for 3-min or until
187 PETCO₂ 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% CO₂ mixture for 3-min. Baseline PETCO₂ and MCA_v were calculated as the
191 mean of the 1-min prior to hyperventilation, while MCA_v and PETCO₂ data during 5% CO₂
192 breathing was collected as 10-sec averages for the entire 3-min period. Absolute and relative
193 MCA_v were then plotted against PETCO₂ for each 10-sec of 5% CO₂ breathing and CVR was
194 subsequently quantified by linear regression (R² value). Relative MCA_v was calculated as the
195 difference between baseline and 5% CO₂ MCA_v divided by baseline MCA_v (([5% CO₂
196 MCA_v-baseline MCA_v]/ baseline MCA_v) x 100%).
197 Simultaneously, during the baseline and CO₂ 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 CO₂ 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 CO₂ levels and therefore contribute to cerebrovascular CO₂
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 PETCO₂ 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% CO₂ 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

213 *Cerebral autoregulation (CA)*. A second key factor determining adequate CBF is effective
214 CA, which maintains CBF over a range of perfusion pressures (56). Participants completed a
215 squat-stand test, involving repeated cycles of 5-sec of standing and 5-sec of squatting (0.1 Hz)
216 for 5-min to induce oscillations in blood pressure (BP) (12). MCAv and BP were
217 continuously assessed. Data was analysed using transfer function analysis (TFA). TFA views
218 CA as a linear control system where sinusoids at the input are transformed into sinusoids at
219 output of the same frequency, however with a different amplitude (termed gain) and shifted in
220 time (termed phase) (13). In the case of CA, BP is the input and MCAv the output, with CA
221 as the regulator between the two (4). To ensure the statistical reliability of gain and phase
222 values a coherence function is used (13). Coherence tests the linearity of the relationship
223 between input and output and can be used to indicate whether data is reliable (4, 13). Data
224 was processed and analysed in accordance with standardised TFA guidelines to produce
225 values of gain, phase and coherence for three frequency domains: very low frequency (VLF:
226 0.02-0.07 Hz), low frequency (LF: 0.07-0.2 Hz) and high frequency (HF: 0.2-0.5 Hz) (13).
227 TFA is a frequency-dependent phenomenon and these domains are within the frequency range
228 CA is thought to operate. CA is viewed as a high-pass filter as the regulation of CBF is
229 effective in the low frequency range of BP oscillations, but not in the high frequency range
230 due to the time delay in initiating cerebrovascular adaptations to the changes in perfusion
231 pressure (4). CA therefore allows rapid BP changes to be transmitted to CBF, whereas slow
232 BP changes are filtered (4). As a consequence, the three frequency ranges have different
233 responses and are likely controlled by different mechanisms (60).

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

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

248

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

253

254 **Statistical analyses**

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

262 **RESULTS**

263 Descriptive statistics are shown in Table 1.

264

265 **Intervention effects**

266 *Cardiorespiratory and haemodynamic measures.*

267 There were no significant main effects for the change in supine ($p=0.78$) or seated ($p=0.33$)

268 MAP or the change in supine ($p=0.90$) or seated ($p=0.82$) HR (Table 2). Additionally, no

269 differences in the change in supine ($p=0.30$) or seated ($p=0.61$) PETCO₂ were observed (Table
270 2).

271

272 *Cerebral blood flow.*

273 Values for MCAv are presented in Table 2. A significant main effect was observed for the

274 change in supine MCAv ($p=0.048$), with post hoc analysis revealing a greater change in

275 MCAv during SIT compared to 2WALK ($p=0.02$; Figure 2a), but not between SIT and

276 8WALK ($p=0.14$). Supine CVC however showed no significant main effect ($p=0.09$; Figure

277 2c). Seated MCAv showed a significant main effect ($p=0.01$), with significantly reduced

278 MCAv observed in both SIT ($p=0.01$) and 8WALK ($p=0.047$) compared to 2WALK (Figure

279 2b). Seated CVC also differed significantly between conditions ($p=0.01$), with post hoc

280 analysis revealing the change in 2WALK was significantly different compared to SIT

281 ($p=0.03$; Figure 2d).

282

283 *Cerebrovascular carbon dioxide reactivity.*

284 Values of linear regression for MCA CVR are presented in Table 3. No significant main

285 effect ($p=0.30$) was observed for the change in CVR. There was also no significant main

286 effect ($p=0.88$) for the change in CCA diameter between baseline or during 5% CO₂ breathing

287 for each condition (SIT Baseline: -0.00 ± 0.01 mm, 4hrs: -0.01 ± 0.01 mm; 2WALK Baseline:
288 0.01 ± 0.01 mm, 4hrs: -0.00 ± 0.02 mm; 8WALK Baseline: -0.01 ± 0.01 mm, 4hrs: -0.02 ± 0.01
289 mm). Similarly, there was no significant main effect ($p=0.28$) for the change in CCA blood
290 flow between baseline or during 5% CO₂ breathing for each condition (SIT Baseline:
291 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: -
292 1.25 ± 1.26 ml.s⁻¹; 8WALK Baseline: -0.51 ± 0.82 ml.s⁻¹, 4hrs: -0.10 ± 1.14 ml.s⁻¹).

293

294 *Cerebral Autoregulation.*

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

303

304 **Physiological responses during walking breaks**

305 Mean treadmill speed for each condition and every walking break was 3.6 km/h at an RPE of
306 8.6.

307

308 *2WALK.*

309 Walking breaks increased MCA_v in seven out of the eight breaks. The increased MCA_v was
310 only significant at 60-min, with MCA_v during walking 1.91 cm.s⁻¹ higher than prior to the
311 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, $p < 0.001$).

320 **DISCUSSION**

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

330

331 Uninterrupted sitting induced a decline in MCAv of 1.4-3.2 $\text{cm}\cdot\text{s}^{-1}$. Translating this
332 observation to the age-related decline in MCAv of 0.76 $\text{cm}\cdot\text{s}^{-1}$ per year (1), this suggests the
333 reductions observed following a one-off bout of uninterrupted sitting may equate to 2-4 years
334 of age-related decline, albeit likely transient. Nonetheless, repeated exposure to this type of
335 SB may have important implications for long-term cerebrovascular health. Indeed, chronically
336 sedentary males (not regularly physically active) exhibit a 9.1 $\text{cm}\cdot\text{s}^{-1}$ lower mean MCAv
337 compared to their endurance trained counterparts (1). Interestingly, this observation aligns
338 with our finding, in that breaking up sitting with frequent, short duration walking breaks
339 (2WALK) prevented the sitting-induced decline in MCAv. This benefit was not observed in
340 the less frequent, longer duration walking break condition (8WALK) despite larger increases
341 in MCAv during the walking breaks. Taken together this implies the frequency of the breaks
342 may be more important than the magnitude of the increase in MCAv during the breaks. This
343 finding supports previous work showing, when directly compared to a single activity bout,
344 regular activity breaks during sitting enhances postprandial glycaemia and insulinemia (31).

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

347

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

360

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

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

372

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

389

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

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

404

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

417

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

420 experimental visit we controlled the activities that participants completed during sitting so
421 that they were of a low-cognitive demand, these activities were not matched between visits. It
422 is therefore possible that the activities they performed while seated differed between visits
423 which may have influenced cerebrovascular responses. The use of TCD to assess MCAv and
424 cerebrovascular function is associated with known limitations, including the inability to
425 measure actual blood flow (54), the assumptions that measures from the MCA are
426 representative of other cerebral vessels (2), and that MCA diameter is unaltered during
427 varying levels of CO₂ (46). By recording the signal parameters and photographically
428 recording the TCD probe placement, it was ensured as closely as possible the probe was in the
429 same location and at the same angle for each visit; small variations may have occurred,
430 however our coefficient of variation was 7.8% indicating good reproducibility. The analysis
431 of CA using TFA is a developing method and lacks reference values (13). Therefore whilst
432 current assessment and analysis guidelines were adhered to (13), future research is required to
433 fully understand the clinical value of our results.

434

435 **Conclusion and implications**

436 For the first time this study demonstrates that in healthy desk workers prolonged,
437 uninterrupted sitting impairs CBF, whilst these reductions are offset when frequent, short
438 duration walking breaks are incorporated. These observations may have clinical importance
439 for both cognition and disease risk. Acutely cognitive performance declines following
440 transient carotid artery occlusion that decreases CBF (23), but increases following
441 pharmacologically elevated CBF (16). Given that UK office workers report sitting at work for
442 6.3-hr (25), reductions in CBF may have important ramifications for workers' productivity.
443 More importantly, chronic reductions in CBF is a risk factor for cognitive impairment (40), is
444 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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461

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467

468 **AUTHOR CONTRIBUTIONS**

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

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- 651

652 **FIGURE CAPTIONS**

653

654 **Figure 1:** Experimental design for the three test conditions, completed in a randomised order,
655 on three separate days. a) 4-hr uninterrupted sitting, b) Sitting with 2-min treadmill
656 walking breaks every 30-min, c) Sitting with 8-min treadmill walking breaks every
657 120-min. MCA_v- middle cerebral artery blood flow velocity; CVR- cerebrovascular
658 carbon dioxide reactivity; CA- cerebral autoregulation.

659

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

666

667

668 **TABLES**

669

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

672

	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

673

674

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

	SIT			2WALK			8WALK		
	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$	Baseline	4 Hours	$\Delta^{\#}$
Supine position									
<i>MCAv</i> ($cm.s^{-1}$)	58.8 \pm 2.0	55.5 \pm 2.1	-3.2 \pm 1.2*	58.6 \pm 2.6	59.2 \pm 2.7	0.6 \pm 1.5	58.4 \pm 2.7	57.3 \pm 2.2	-1.2 \pm 1.0
<i>CVC</i> ($cm.s^{-1}.mmHg^{-1}$)	0.72 \pm 0.03	0.67 \pm 0.03	-0.06 \pm 0.02	0.73 \pm 0.03	0.71 \pm 0.03	-0.02 \pm 0.02	0.73 \pm 0.04	0.70 \pm 0.04	-0.03 \pm 0.02
<i>MAP</i> (mmHg)	83 \pm 2.8	84 \pm 2.5	2.3 \pm 1.8	80 \pm 1.9	84 \pm 2.3	2.6 \pm 1.8	81 \pm 2.3	83 \pm 2.9	1.8 \pm 2.3
<i>HR</i> (bpm)	59 \pm 3.4	56 \pm 2.4	-2.2 \pm 1.7	58 \pm 2.6	55 \pm 3.4	-3.1 \pm 3.0	56 \pm 2.3	55 \pm 2.1	-2.2 \pm 2.1
<i>PETCO₂</i> (mmHg)	41.6 \pm 1.3	40.7 \pm 1.6	-0.9 \pm 0.8	42.6 \pm 1.5	41.3 \pm 1.7	-1.2 \pm 1.2	41.0 \pm 1.5	41.5 \pm 1.3	0.4 \pm 0.9
Seated position									
<i>MCAv</i> ($cm.s^{-1}$)	55.4 \pm 2.4	53.8 \pm 1.6	-1.4 \pm 1.8*	56.4 \pm 2.0	56.3 \pm 2.4	1.1 \pm 2.4	53.7 \pm 2.5	54.3 \pm 2.6	-0.8 \pm 2.7*
<i>CVC</i> ($cm.s^{-1}.mmHg^{-1}$)	0.62 \pm 0.03	0.59 \pm 0.03	-0.04 \pm 0.02*	0.65 \pm 0.03	0.64 \pm 0.04	0.01 \pm 0.03	0.61 \pm 0.03	0.62 \pm 0.04	-0.01 \pm 0.03
<i>MAP</i> (mmHg)	90 \pm 2.4	92 \pm 2.8	2.8 \pm 2.0	88 \pm 2.8	89 \pm 2.7	0.9 \pm 1.7	89 \pm 2.7	90 \pm 2.6	0.7 \pm 1.8
<i>HR</i> (bpm)	57 \pm 2.8	58 \pm 2.5	0.6 \pm 2.1	57 \pm 2.8	58 \pm 3.5	1.0 \pm 2.8	56 \pm 2.4	56 \pm 2.6	-0.4 \pm 2.6
<i>PETCO₂</i> (mmHg)	37.6 \pm 1.3	37.8 \pm 1.4	-0.1 \pm 1.1	38.4 \pm 1.8	37.4 \pm 1.3	-0.8 \pm 0.7	38.2 \pm 1.6	37.1 \pm 1.4	-1.0 \pm 1.0

677

678

679 SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks; MCAv- middle cerebral artery blood flow velocity;

680 CVC- cerebral vascular conductance; MAP- mean arterial pressure; HR- heart rate; PETCO₂- pressure of end-tidal carbon dioxide.

681 # Delta change values expressed with statistically adjusted baseline covariate control.

682 * Significantly different to 2WALK (p<0.05).

683

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

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

686

687 Relatively high R^2 values confirm the linearity of the response.

688 SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks.

689 # Delta change values expressed with statistically adjusted baseline covariate control.

690

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

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

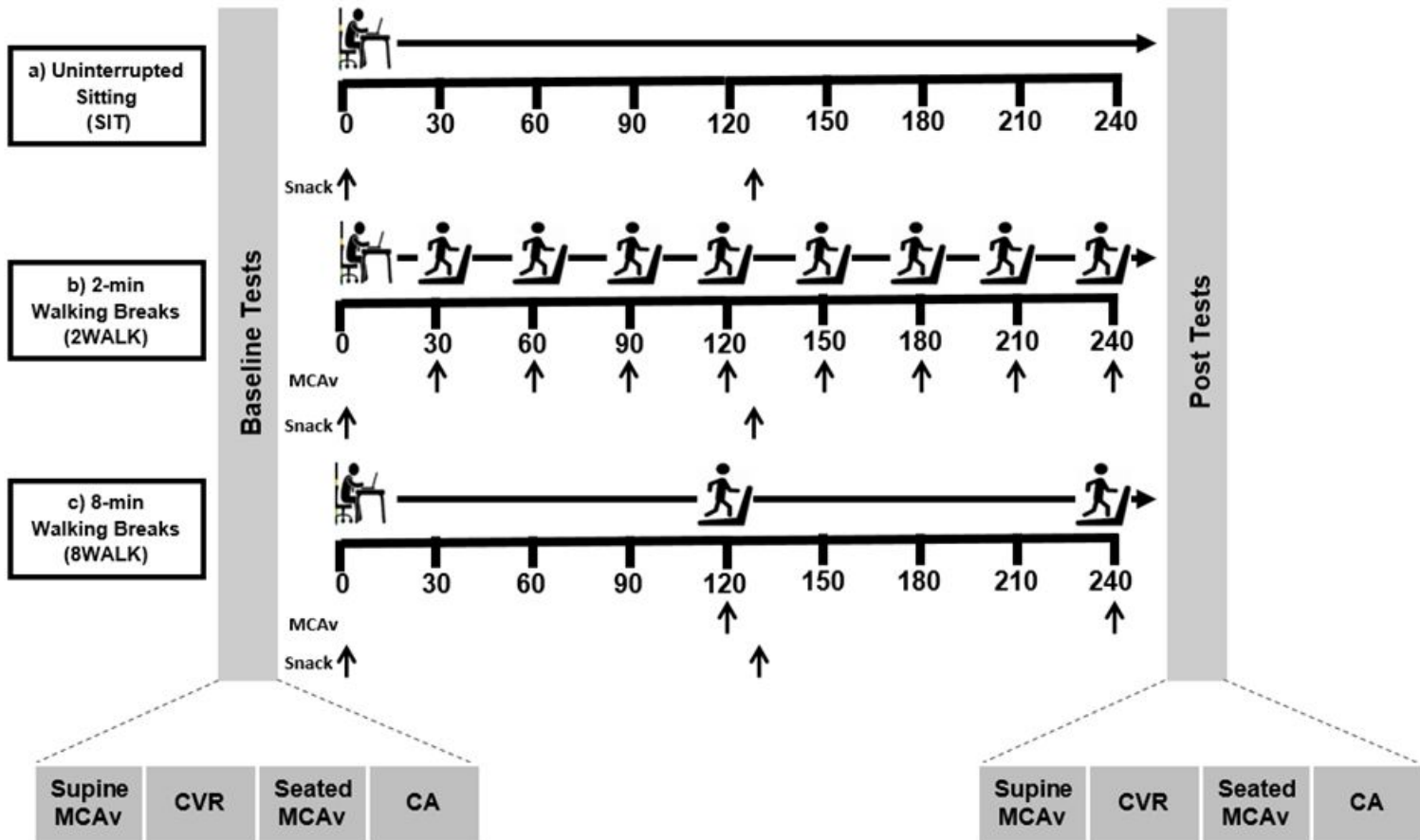
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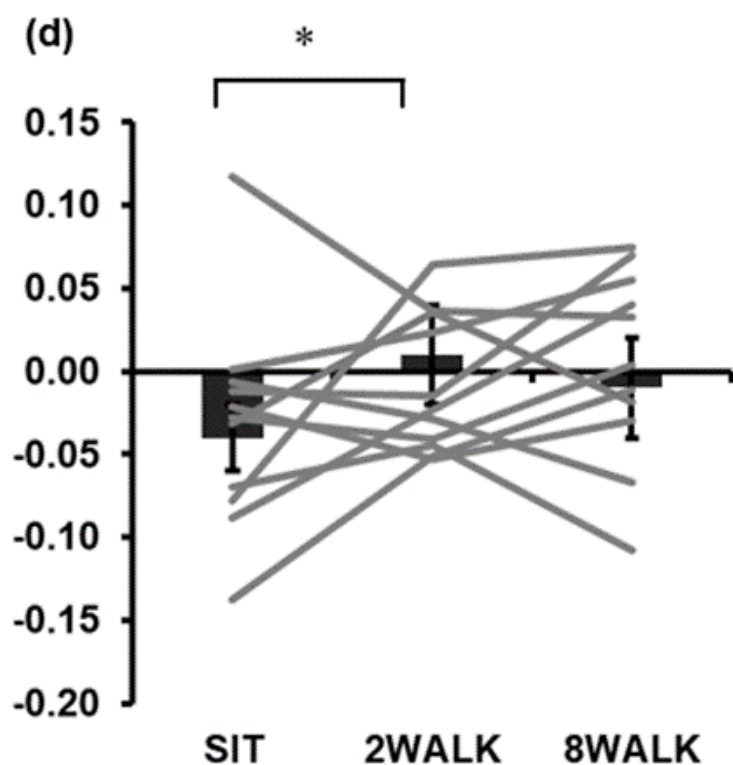
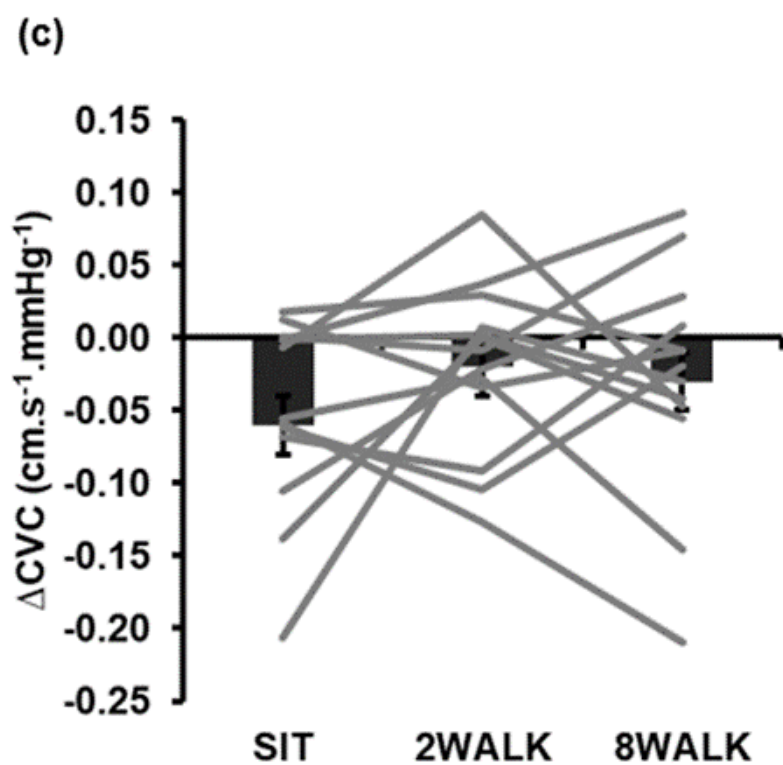
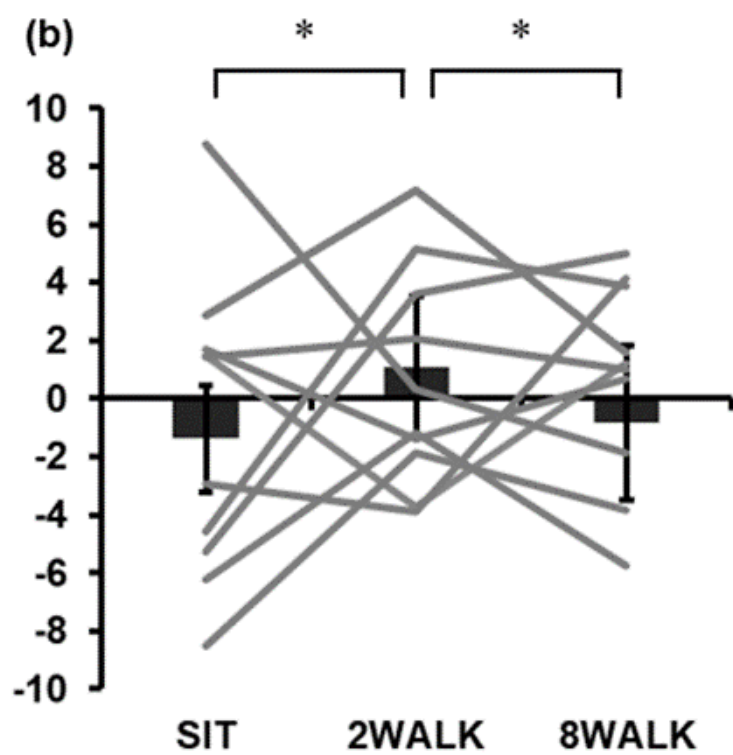
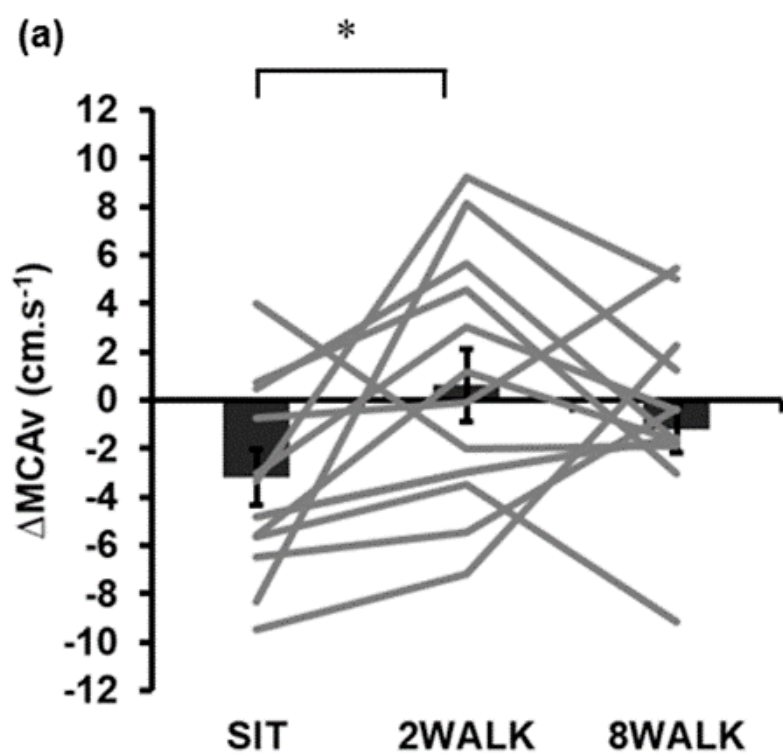
694 SIT- uninterrupted sitting; 2WALK- 2-min walking breaks; 8WALK- 8-min walking breaks; VLF- very low frequency; LF- low frequency; HF-
695 high frequency

696 # Delta change values expressed with statistically adjusted baseline covariate control.

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

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





Supine Position

Seated Position