

1 **Morning exercise mitigates the impact of prolonged sitting on cerebral blood flow in**  
2 **older adults**

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Running Head: Exercise improves the pattern of cerebral blood flow

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20 **ABSTRACT**

21 Preventing declines in cerebral blood flow is important for maintaining optimal brain health  
22 with aging. We compared the effects of a morning bout of moderate-intensity exercise, with  
23 and without subsequent light-intensity walking breaks from sitting, on cerebral blood velocity  
24 over 8-hours in older adults. In a randomized crossover trial, overweight/obese older adults  
25 ( $n=12$ , 2♀,  $70\pm 7$  years;  $30.4\pm 4.3$  kg/m<sup>2</sup>), completed 3 acute conditions (6-day washout); SIT:  
26 prolonged sitting (8hr, control); EX+SIT: sitting (1hr), moderate-intensity walking (30min),  
27 followed by uninterrupted sitting (6.5hr); EX+BR: sitting (1hr), moderate-intensity walking  
28 (30min), followed by sitting (6.5hr) interrupted with 3 minutes of light-intensity walking  
29 every 30 minutes. Bilateral middle cerebral artery velocities (MCA<sub>v</sub>) were determined using  
30 transcranial Doppler at 13 time points across the day. The temporal pattern and average  
31 MCA<sub>v</sub> over 8-hours was determined. The pattern of MCA<sub>v</sub> over 8-hours was a negative  
32 linear trend in SIT ( $p<0.001$ ), but a positive quadratic trend in EX+SIT ( $p<0.001$ ) and  
33 EX+BR ( $p<0.01$ ). Afternoon time points in SIT were lower than baseline within condition  
34 ( $p\leq 0.001$  for all). A morning dip in MCA<sub>v</sub> was observed in EX+SIT and EX+BR ( $p<0.05$   
35 relative to baseline), but afternoon time points were not significantly lower than baseline. The  
36 average MCA<sub>v</sub> over 8-hours was higher in EX+SIT than SIT ( $p=0.007$ ) or EX+BR  
37 ( $p=0.024$ ). Uninterrupted sitting should be avoided, and moderate-intensity exercise should  
38 be encouraged for the daily maintenance of cerebral blood flow in older adults. The clinical  
39 implications of maintaining adequate cerebral blood flow include the delivery of vital oxygen  
40 and nutrients to the brain.

41

42 **NEW & NOTEWORTHY**

43 This is the first study to measure the combined effects of an exercise bout with breaks in  
44 sitting on cerebral blood velocity in older adults. Using frequent recordings over an 8-hour  
45 period, we have performed a novel analysis of the pattern of cerebral blood velocity,  
46 adjusting for concurrent measures of mean arterial pressure and other potential confounders  
47 in a linear mixed effects regression.

48

49 **Keywords**

50 Acute exercise, sedentary behavior, transcranial Doppler, older adults, brain health

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## 63 INTRODUCTION

64 The prevalence of stroke and dementia is increasing due to population aging (14). Aging is  
65 also associated with an increased prevalence of cardiovascular risk factors for  
66 cerebrovascular disease such as physical inactivity, obesity, hypertension, hyperlipidaemia  
67 and dysglycemia (43). Therefore, strategies to maintain cerebrovascular health among older  
68 adults with cardiovascular risk factors are a public health priority. Evidence demonstrates that  
69 exercise in particular is associated with a reduced incidence of stroke (24, 39), and may also  
70 delay the progression of dementia (19, 25). However, the mechanisms underlying these  
71 benefits in humans remain unclear. Whilst exercise may exert some of its cardiovascular  
72 effects by modifying traditional risk factors (18, 22), there are also direct benefits of exercise  
73 on arterial function and health (17, 37). In addition, regular exercise can mitigate the decline  
74 in cerebral blood flow associated with ageing (1). Insight from animal studies demonstrates  
75 the importance of exercise-induced increases in cerebral blood flow for neurogenesis,  
76 cerebral angiogenesis and related growth factors (3, 28, 30, 35). In order to understand  
77 cerebrovascular regulation in response to exercise in humans, many studies focus on cerebral  
78 blood flow during or immediately after exercise (27). However, few experiments have  
79 characterized the cerebral blood flow response to different patterns of physical activity over  
80 the whole day, an imperative for the design of optimal exercise interventions.

81

82 Over a whole waking day, older adults spend about 5% of time engaged in exercise of  
83 moderate-to-vigorous intensity, but spend a majority of time in sedentary behavior which  
84 carries an increased risk for all-cause mortality (15, 20, 21). Recent evidence suggests that  
85 sedentary behaviors such as prolonged sitting may be negatively associated with aspects of  
86 brain health such as cognitive function and medial temporal lobe thickness (13, 33). In  
87 addition, laboratory studies which have investigated reducing and breaking up sitting with

88 intermittent physical activity have reported beneficial impacts on multiple systems relevant to  
89 brain health, including carbohydrate and lipid metabolism (4, 16), blood pressure (5, 10),  
90 sympathetic function (10) and vascular function (7, 32, 36). In response to accumulating  
91 evidence, some government guidelines now recommend reducing sitting in addition to  
92 engaging in moderate-to-vigorous intensity exercise (2, 11). In the US, the scientific report  
93 which informed the *2018 Physical Activity Guidelines for Americans* highlighted a need for  
94 future studies to investigate different patterns of physical activity and sedentary behavior on  
95 brain health outcomes (29). However, it is currently unknown whether engaging in moderate-  
96 to-vigorous intensity exercise would mitigate any potential decline in cerebral blood flow  
97 during a subsequent period of prolonged sitting. It is also unknown whether combining a bout  
98 of moderate-to-vigorous exercise with subsequent breaks in sitting would further enhance the  
99 cerebral blood flow response.

100

101 The aim of this study was to assess the impact of a moderate-intensity exercise bout, with or  
102 without subsequent breaks in sitting, on middle cerebral artery blood velocity (MCA<sub>v</sub>) in  
103 older adults. We hypothesized that an acute bout of exercise would enhance cerebrovascular  
104 responses over an eight hour period, relative to prolonged uninterrupted sitting. In addition,  
105 we hypothesized that cerebrovascular responses following acute exercise would be further  
106 enhanced by subsequent exposure to breaks in sitting.

107

## 108 **MATERIALS AND METHODS**

109 This experiment is a sub-study of a larger randomized crossover trial  
110 (ACTRN12614000737639) and the detailed methods have been published independently  
111 (12).

112

113 *Participants*

114 Men and postmenopausal women (n=12, 2♀, age ≥ 55 to ≤ 80 years; BMI ≥ 25 kg/m<sup>2</sup> to < 45  
115 kg/m<sup>2</sup>; English-speaking) were recruited from the local community via advertisement in  
116 Perth, Western Australia. Full participant characteristics are found in Table 1. This study was  
117 approved by the Human Research Ethics Committee of The University of Western Australia  
118 (RA/4/1/6990). Participants provided written informed consent prior to testing. All  
119 participants were screened for cardiovascular risk and previous cardiovascular events.  
120 Exclusion criteria included self-reported sitting < 5 hours per day, self-reported engagement  
121 in moderate-intensity exercise ≥ 150 min/week for > 3 months, probable dementia  
122 (Telephone Interview of Cognitive Status score of < 19), cognitive impairment (Mini Mental  
123 State Exam < 24), depressive symptoms of clinical relevance (Geriatric Depression Score >  
124 6 or Hospital Anxiety and Depression Scale score – depression score >8), diagnosed diabetes,  
125 use of glucose/lipid lowering medication, antidepressant medications, beta blockers, anti-  
126 anxiety medication, excessive alcohol consumption (> 8 points on the Alcohol Use Disorders  
127 Identification Test), abnormal ECG (determined by study doctor), high resting blood pressure  
128 (office systolic > 160 mm Hg or diastolic >100 mm Hg), or major illness/physical problems  
129 (acute or chronic) that would limit ability to perform moderate-intensity exercise.

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131

132 *Study Design*

133 Participants were randomized to participate in three laboratory sessions, separated by a  
134 minimum of six days (Figure 1). The order of conditions was block randomized and stratified  
135 by sex by an independent third party using a computer-generated random sequence and stored  
136 in sealed envelopes as previously outlined (12). Researchers were blinded to the order of  
137 conditions until familiarisation was complete and participants were blinded to the conditions

138 until each day of testing. Experimental conditions included: *Sitting (SIT)*: uninterrupted  
139 sitting (8hr, control); *Exercise + Sitting (EX+SIT)*: sitting (1hr), moderate-intensity treadmill  
140 walking (30min) followed by uninterrupted sitting (6.5hr); *Exercise + Breaks (EX+BR)*:  
141 sitting (1hr), moderate-intensity treadmill walking (30min) followed by sitting (6.5hr)  
142 interrupted every 30 minutes with 3 minutes of light-intensity treadmill walking. A  
143 familiarisation session was completed three to five days prior to testing, in which participants  
144 were familiarized with all testing equipment and procedures, including treadmill walking.  
145 During the 48 hours prior to testing, participants were instructed to avoid caffeine, alcohol  
146 and moderate-to-vigorous physical activity. In addition, food was controlled from the night  
147 before testing where participants consumed a standardized dinner at home between 7pm and  
148 9pm in place of their regular dinner. This meal was tailored for each participant to meet 33%  
149 of estimated daily energy requirement with a macronutrient profile of 55–58% carbohydrate,  
150 29–31% fat and 12–15% protein as previously described (12).

151

152 Insert figure 1 about here

153

#### 154 *Exercise*

155 The moderate-intensity exercise bout was performed on a treadmill at the same  
156 predetermined speed and incline for both EX+SIT and EX+BR. The speed was set at  
157  $3.2\text{km}\cdot\text{h}^{-1}$  and the incline was tailored for each participant during the familiarization session  
158 to induce a heart rate indicative of moderate-intensity, defined as 65% to 75% of age  
159 predicted maximum heart rate. Each three-minute light-intensity walking break performed  
160 during EX+BR was completed on a treadmill with 0% incline at a speed of  $3.2\text{km}\cdot\text{h}^{-1}$ , which  
161 was a walking speed for all participants. Heart rate (Polar Electro, Kempele, Finland) and  
162 ratings of perceived exertion (RPE scale 6-20; light intensity 9-11 RPE; moderate-intensity

163 12-15 RPE) were collected at 5-minute intervals during the 30-minute bout of exercise and at  
164 the end of each three-minute walking break.

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166

### 167 *Experimental day protocol*

168 Participants reported to the laboratory at ~7am following an overnight fast (>10 hours).

169 Participants remained seated while equipment was set up and the bilateral middle cerebral  
170 arteries were located as detailed below, prior to the start of the experiment at ~8am (0 hour).

171 The experiment began with baseline recordings of  $MCA_v$ , blood pressure and heart rate  
172 which were obtained prior to the administration of a standardized breakfast meal. Breakfast  
173 and lunch were administered at 40 and 280 minutes into the experiment and were consumed  
174 over a 20 minute period. All meals were standardized according the same criteria as the  
175 evening meal and remained the same for a given participant during all conditions. After  
176 breakfast the protocol was followed according to randomization and participants were  
177 instructed to remain seated apart from leaving the chair to void or to perform predetermined  
178 treadmill walking in the EX+SIT and EX+BR conditions. Study outcomes were measured at  
179 multiple time points across the day (Figure 1). All measures of  $MCA_v$  were taken during  
180 steady state sitting periods, such that in the EX+BR condition measures were collected at  
181 least 25 minutes after the most recent activity break.

182

### 183 *Cerebrovascular function*

184 Cerebral blood flow was indexed using transcranial Doppler (TCD; Spencer Technologies,  
185 Seattle, WA). Bilateral measures of middle cerebral artery velocity ( $MCA_v$ ) were determined  
186 with a 2MHz probe transfixted to the posterior aspect of the temporal window of the skull  
187 using the Mark 600 headframe (Spencer technologies, Seattle, USA). The headframe was



188 secured in place to negate movement effects on the insonation site and participants remained  
189 instrumented for the entire experiment to avoid relocating the MCA. The location of the  
190 middle cerebral artery was determined by locating the trifurcation of the circle of Willis (~45-  
191 65 mm) in the anterior circulation of the brain, as previously outlined (42). The  $MCA_v$  was  
192 continuously sampled for 5 minutes at baseline and for 30 seconds during subsequent time  
193 points, at 1000Hz via an analogue-to-digital converter (Powerlab, 16/30 AD Instruments,  
194 Colorado Springs, CO, USA). Data were analyzed offline using a specialized analytical  
195 software package (LabChart 8, AD Instruments, Colorado Springs, CO, USA). The sum of  
196 bilateral velocities was calculated for statistical analyses. The sum of bilateral velocities  
197 represents a surrogate measure of the total amount of blood being delivered to the brain.  
198 Summing the bilateral velocities also accounts for expected anatomical differences between  
199 the left and right MCA, detection of which would be diminished by averaging the bilateral  
200 velocities.

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202

### 203 *Assessment of hemodynamic variables*

204 Resting blood pressure and heart rate were measured in a seated position. A  
205 photoplethysmographic method was used for serial BP assessment (Finometer Pro, Finapres  
206 Medical Systems, Amsterdam, the Netherlands) and this was calibrated against automated  
207 brachial oscillometry (HEM-907, Omron, Kyoto, Japan). In all conditions, blood pressure and  
208 heart rate were measured contemporaneously with  $MCA_v$ , and at a time consistent with the  
209 period immediately preceding the 3-minute walking break during the EX+BR condition.

210

211 *Statistical analysis*

212 Based on previous evidence we estimated the effect size (Cohen's  $d$  for repeated measures) of  
213 exposure to intermittent light-intensity walking breaks relative to uninterrupted sitting, to be  
214  $\sim 1.1$  for  $MCA_v$  (6). Assuming a within participant correlation of 0.6, the effective sample  
215 size to detect this difference with a power of 0.80 and a two tailed probability of 0.05, is 9  
216 participants. The order of conditions was block randomized and stratified by sex by an  
217 independent third party using a computer generated random sequence and stored in sealed  
218 envelopes as previously outlined (12). Analysis was performed by technicians blinded to the  
219 study conditions. Following recent recommendations on data analysis of cross-over trials  
220 (23), generalized linear mixed models with random intercepts were used to evaluate the  
221 differential effects of the experimental conditions on the selected outcomes. Mixed models  
222 are appropriate for correlated data (repeated measures) with various distributional  
223 assumptions and can easily accommodate missing data (31). A treatment-by-time interaction  
224 term was included in regression models to examine between condition differences in  
225 temporal patterns of  $MCA_v$  across the day. Marginal means (i.e. adjusted mean of the  
226 dependent variable when fixed effects are held at their mean), were calculated for individual  
227 time points and within condition comparisons, relative to baseline, were performed for the  
228 sum of bilateral  $MCA_v$ . Between condition comparisons of individual time points were  
229 performed on heart rate and mean arterial pressure variables. All models were adjusted for  
230 baseline, age, sex, waist circumference and treatment order. Models with  $MCA_v$  as the  
231 dependent variable were additionally adjusted for mean arterial pressure (MAP), which was  
232 recorded simultaneously with  $MCA_v$ . Due to the large number of comparisons in the within  
233 and between condition analysis of individual time points, adjustment for multiple  
234 comparisons using a Šidák correction was performed. A probability level of 0.05 was  
235 adopted. Statistical analyses were performed using Stata 15 for Windows (StataCorp LP).

236

## 237 **RESULTS**

### 238 *Exercise response*

239 The initial 30-minute exercise bout induced similar ( $p>0.05$ ) heart rate and RPE responses  
240 (mean $\pm$ SD) under each condition (EX+SIT: 104 $\pm$ 10 bpm, 69 $\pm$ 7% HR<sub>max</sub>, 11 $\pm$ 3 RPE;  
241 EX+BR: 108 $\pm$ 15 bpm, 72 $\pm$ 11 %HR<sub>max</sub>, 11 $\pm$ 3 RPE). Average HR and RPE across all 12  
242 walking breaks was 93 $\pm$ 14 bpm, 62 $\pm$ 10%HR<sub>max</sub> and 8 $\pm$ 2 RPE.

243

### 244 *Temporal variation: 8hr pattern of cerebral blood velocity*

245 Recording the MCA<sub>v</sub> across an eight-hour time period enabled the assessment of the pattern  
246 of cerebral blood velocity across the day. Observation of the response across time revealed a  
247 persistent decline in SIT (Figure 2A). In the EX+SIT and EX+BR conditions, the initial  
248 decline of MCA<sub>v</sub> was followed by an afternoon recovery (Figure 2B, C). In support of these  
249 observations, a significant main effect of time was found for the sum of bilateral velocities  
250 ( $p<0.001$ ). Post hoc analysis revealed a negative linear trend in SIT ( $p<0.001$ ) but a positive  
251 quadratic trend for both EX+SIT ( $p<0.001$ ) and EX+BR ( $p<0.01$ ). A positive quadratic trend  
252 identifies the response as a convex curvilinear pattern. A significant main effect of time was  
253 also observed for left MCA<sub>v</sub> ( $p<0.001$ ) and right MCA<sub>v</sub> ( $p=0.04$ ). Left MCA<sub>v</sub> followed a  
254 negative linear trend in SIT ( $p<0.001$ ) but a positive quadratic trend in EX+SIT ( $p<0.001$ )  
255 and EX+BR ( $p<0.001$ ). Right MCA<sub>v</sub> followed a negative linear trend for SIT ( $p<0.001$ ), a  
256 positive quadratic trend in EX+SIT ( $p=0.02$ ) and no significant trend for EX+BR ( $p>0.05$ ).  
257 Within condition analysis of the time course data in the SIT condition revealed a significant  
258 decline in the sum of bilateral MCA<sub>v</sub> during the morning period relative to baseline, which  
259 was sustained until the end of the condition (Figure 2A). However, an initial decline in

260 MCA<sub>v</sub> relative to baseline was followed by a recovery, which was sustained for the final 2.5  
261 hours of the EX+SIT condition, and final 4 hours of the EX+BR condition (Figure 2B, C).

262

### 263 *Average cerebral blood velocity across the day*

264 The sum of bilateral velocities (cm/s), averaged across the day (Figure 3A), was higher in the  
265 EX+SIT condition 87 [95% CI 79-96] relative to SIT 85 [76-93, p=0.005] or EX+BR 85 [77-  
266 93, p=0.02]. These between condition differences in MCA<sub>v</sub> (cm/s) were largely driven by the  
267 left MCA, which was higher in EX+SIT 44 [42-46] compared to SIT 43 [41-45, p=0.009] or  
268 EX+BR 42 [40-44, p<0.001] (Figure 3C). However, no significant differences were observed  
269 between conditions in the average right MCA<sub>v</sub> (cm/s); SIT 45 [40-51], EX+SIT 45 [40-50],  
270 and EX+BR 46 [41-51] (Figure 3B).

271

### 272 *Comparison between conditions in hemodynamic data*

273 Heart rate, when averaged across the day, displayed a pattern of increase with increasing  
274 activity; SIT 68 [64-71], EX+SIT 72 [69-75, p<0.001 vs. SIT], and EX+BR 73 [70-77,  
275 p<0.001 vs. SIT]. This was predominantly due to increased heart rate following the 30-  
276 minute bout of moderate-intensity exercise. In EX+SIT and EX+BR, heart rate remained  
277 elevated for approximately 2 hours following the moderate-intensity exercise bout, relative to  
278 SIT (Figure 4A). Despite a higher heart rate, mean arterial pressure was lower for  
279 approximately 2 hours following the moderate-intensity exercise bout in EX+SIT and  
280 EX+BR, relative to SIT, although no significant differences between conditions were  
281 observed during this time. There was a small increase in the mean arterial pressure (mm Hg)  
282 averaged across the day in EX+BR 102 [96-107], compared to SIT 98 [92-104, p=0.02], but  
283 no difference compared to EX+SIT 99 [93-105].

284

285 **DISCUSSION**

286 We observed that the pattern of MCA<sub>v</sub> during prolonged uninterrupted sitting was that of  
287 negative linear trend, with significant declines relative to baseline during the final 3.5 hours  
288 of the experiment. In contrast, the pattern of MCA<sub>v</sub> following a morning bout of exercise  
289 with or without breaks in sitting, was that of a convex curvilinear response characterized by  
290 an initial decline followed by a subsequent recovery. Interestingly, the recovery of MCA<sub>v</sub>  
291 after the initial decline began earlier in the EX+BR condition, compared to EX+SIT, which  
292 may represent a benefit of intermittent walking on the temporal pattern of MCA<sub>v</sub>. The  
293 clinical implications of such a pattern of MCA<sub>v</sub> may be in avoiding sharp declines in the  
294 delivery of oxygen and nutrients to the brain (34). A decline in the delivery of glucose to the  
295 brain for example, risks exposing the brain to hypoglycaemia which can increase the risk of  
296 developing dementia (41). Previously, we hypothesized that *fluctuations* in glucose  
297 availability, more specifically than *absolute* concentrations, pose a risk to brain health and  
298 breaks in sitting may help mitigate this risk by maintaining a more stable supply of glucose to  
299 the brain (40). While the current data suggest that MCA<sub>v</sub> was most stable in the EX+BR  
300 condition, we did not measure glucose availability to the brain. Future studies to determine  
301 the effect of breaks in sitting on central glucose concentrations and oxygen delivery would be  
302 highly informative. To our knowledge, this is the first study to examine the 8-hour pattern of  
303 MCA<sub>v</sub> in this way. This type of analysis involving frequent transcranial Doppler assessment  
304 offers unique insights into the temporal regulation of cerebral blood flow, and may have  
305 implications for understanding cerebrovascular health.

306

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Insert figure 2 about here

308

309 We also observed that a morning bout of exercise sustained a higher average  $MCA_v$  across a  
310 subsequent period of prolonged sitting. However, the finding that adding regular activity  
311 breaks to a morning bout of exercise abolished the increase in average  $MCA_v$  was somewhat  
312 unexpected. There are some possible explanations worth exploring. 1) Day to day differences  
313 in the probe angle and location used when establishing the  $MCA_v$  signal may have introduced  
314 measurement error into the between condition comparisons of average  $MCA_v$ . Our within  
315 condition analysis of the pattern of  $MCA_v$  helps mitigate this potential source of error as  
316 participants remained instrumented for the entire experiment to avoid relocating the MCA. 2)  
317 Subtle changes in MCA diameter, undetectable by TCD, may have altered  $MCA_v$  during  
318 intermittent walking. Using magnetic resonance imaging, both increases and decreases in  
319 MCA diameter have been observed following hypercapnia and rhythmic handgrip exercise  
320 respectively (8, 38). While it is unknown what effect, if any, intermittent walking would have  
321 on MCA diameter, an increased diameter would translate to a decrease in velocity and vice  
322 versa for a decreased diameter, assuming constant flow.

323

324

Insert figure 3 about here

325

326 The effects of intermittent walking on  $MCA_v$  have been documented in one previous study of  
327 lean healthy ‘desk workers’ (6). The authors demonstrated an increase in  $MCA_v$  pre to post a  
328 4 hour period involving breaks in sitting, compared to prolonged uninterrupted sitting (6).  
329 Although we observed an attenuation in average  $MCA_v$  following intermittent walking, this  
330 was after an antecedent bout of morning exercise in a population of older overweight and  
331 obese adults (mean age 70 years), compared to walking breaks alone in a younger healthy  
332 population (mean age 36 years) in the study by Carter *et al.* These differences between

333 studies likely represented a different stimulus to a range of mechanisms responsible for  
334 regulating cerebral blood flow.

335

336 Whilst our study was not designed to address the mechanisms responsible for effects on  
337  $MCA_v$ , several possibilities may exist. Brain blood flow is controlled by multiple redundant  
338 and integrative mechanisms and is highly protected by local and reflex pathways. Although  
339 differences existed between conditions in blood pressure responses (Figure 4),  $MCA_v$  data  
340 were statistically adjusted for contemporaneous blood pressure in regression analyses and it is  
341 therefore unlikely that our cerebrovascular findings are primarily related to underlying  
342 changes in driving pressures. This type of correction avoids the need to meet the stringent  
343 assumptions required for ratio normalisation, where one number is divided by another (9). A  
344 further mechanism that controls cerebral blood flow is the partial pressure of carbon dioxide  
345 in the circulating blood and it is possible that the exercise bouts induced differences in this  
346 parameter. However, the major differences we observed between conditions occurred more  
347 than 4 hours after the morning exercise bout and all of our  $MCA_v$  data were obtained under  
348 quiet resting conditions. Furthermore, an impact of active breaks on carbon dioxide at the  
349 time of measurement seems unlikely, since there was ~25 minutes between these brief  
350 periods of walking and the subsequent resting measure of  $MCA_v$ .

351

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Insert figure 4 about here

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354

355 A strength of this study is the well-controlled randomized crossover design which controls  
356 for person-specific factors and affords smaller sample sizes. Trial conditions were also  
357 standardized for potential confounders such as diet, physical activity, medications and

358 baseline values. There are also some limitations to our study. The experiment was designed  
359 as a superiority trial, and we did not include a fourth condition involving walking breaks  
360 alone. This was due to the general acceptance of the health benefits associated with  
361 continuous exercise bouts; we considered this the minimum standard for prescription. Our  
362 aim in the present study was to determine whether additive benefit was possible beyond that  
363 obtained from a morning bout of exercise. Our measure of cerebrovascular function, based on  
364 transcranial Doppler ultrasound, is widely used, provides sensitive time course information  
365 and has been shown to be a useful surrogate measure of cerebral blood flow between  
366 individuals (26). However, direct measures of intracranial diameters are not currently  
367 possible using ultrasound and velocity is therefore relied upon as a surrogate measure of  
368 flow. This is less of an issue for within subject experimental designs because blood flow  
369 changes are heavily dependent upon velocity change. However, we cannot rule out distinct  
370 effects on artery diameter responses that went undetected. Future experiments utilizing  
371 electroencephalography (EEG) or near-infrared spectroscopy (NIRS) may help to better  
372 understand complementary and temporal patterns of change in cerebrovascular function in  
373 the future. Further, positron emission tomography (PET) and magnetic resonance imaging  
374 (MRI) would provide information on spatial distribution of brain blood flow. In addition, it is  
375 unknown whether the changes observed simply represent a local effect on the brain vessels  
376 per se, or an impact on cerebral activation that subsequently affected brain blood vessels.  
377 Future studies, perhaps including fMRI may be utilized to test how brain networks are  
378 affected by the combination of exercise and breaks in sitting. This is relevant since metabolic  
379 activity in the brain is known to also affect regional cerebral blood flow. Finally, given  
380 expected regional differences in cerebral blood flow, our findings are not generalizable to the  
381 posterior circulation.

382



383 *Conclusion*

384 We have demonstrated in older overweight to obese adults, that the pattern of cerebral blood  
385 velocity over eight hours is improved following a morning bout of moderate-intensity  
386 exercise with or without subsequent breaks in sitting. In addition, a morning bout of exercise  
387 sustained a higher average  $MCA_v$  during a period of subsequent sitting. Interestingly, adding  
388 intermittent walking breaks to a morning bout of exercise abolished the increase in average  
389  $MCA_v$ , which was unexpected. Future studies should seek to replicate these findings with  
390 more direct measures of cerebral blood flow using PET or MRI. In addition, future studies  
391 using TCD should take advantage of the high temporal resolution this measure offers, and  
392 collect frequent recordings to analyse the temporal *pattern* of cerebral blood velocity.  
393 Collecting and analysing data in this way can also take advantage of current statistical  
394 techniques such as linear mixed effects modelling, which are particularly suited to repeated  
395 measures analysis and within subject study designs. In conclusion, our findings suggest that  
396 uninterrupted sitting should be avoided, and moderate-intensity exercise should be  
397 encouraged for the daily maintenance of cerebral blood flow.

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408 **DISCLOSURES**

409 No conflicts of interest, financial or otherwise, are declared by the authors.

410

411 **AUTHOR CONTRIBUTIONS**

412 DJG, MJW, LN, KAE, EC and DWD were involved in the study design. PNA established the  
413 transcranial Doppler protocol. BS, KS, AS, JL, IH were involved in recruitment and data  
414 collection. MJW, BS and EC were involved in data analysis. DJG, MJW and DWD prepared  
415 the manuscript. All authors edited the manuscript and approved the final version.

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- 567



568 **Figure legends**

569

570 Figure 1. Experimental design. Participants completed three conditions in a random order  
571 separated by a minimum of six days. Conditions are as follows Sitting (SIT): uninterrupted  
572 sitting (8hr, control); Exercise + Sitting (EX+SIT): sitting (1hr), moderate-intensity walking  
573 (30min, denoted by walking figure) followed by uninterrupted sitting (6.5hr); Exercise +  
574 Breaks (EX+BR): sitting (1hr), moderate-intensity walking (30min) followed by sitting  
575 (6.5hr) interrupted every 30 minutes with 3 minutes of light-intensity walking. Walking  
576 breaks are denoted by vertical lines in the EX+BR condition. During each condition,  
577 participants consumed a standardized breakfast and lunch meal and transcranial Doppler,  
578 mean arterial pressure and heart rate were recorded simultaneously across the day.

579

580 Figure 2. The sum of bilateral velocities across the day. Panels A, B and C represent the  
581 velocity trace displayed as a change from baseline during the SIT, EX+SIT and EX+BR  
582 conditions, respectively. Baseline values (cm/s) in each condition are; SIT 96 [90-101];  
583 EX+SIT 95 [89-101]; and EX+BR 93 [87-99]. Dotted lines represent the timing of the  
584 standardized meals and the shaded area denotes the timing of the moderate-intensity exercise  
585 bout. Data are marginal mean + 95% CI, adjusted for baseline, age, sex, waist circumference,  
586 treatment order and mean arterial pressure. \* $p < 0.05$  within condition relative to baseline.

587

588 Figure 3. Between condition comparison of cerebral blood velocity. Panels A, B and C  
589 represent the sum of bilateral velocities, left MCA<sub>v</sub> and right MCA<sub>v</sub> respectively, displayed  
590 as an average across the day. Data are marginal means + 95% CI, adjusted for baseline, age,  
591 sex, waist circumference, treatment order and mean arterial pressure. MCA<sub>v</sub>, middle cerebral  
592 artery velocity.

593

594 Figure 4. Between condition comparison of heart rate and mean arterial pressure. Panels A  
595 and C represent heart rate and mean arterial pressure respectively, displayed as a time course  
596 across the day. Panels B and D represent the heart rate and mean arterial pressure  
597 respectively, displayed as an average across the day. Dotted lines represent the timing of the  
598 standardized meals and the shaded area denotes the timing of the moderate-intensity exercise  
599 bout. Data are marginal means  $\pm$  95% CI, adjusted for baseline, age, sex, waist circumference  
600 and treatment order. \* $p < 0.05$  relative to SIT. MAP, mean arterial pressure.

601

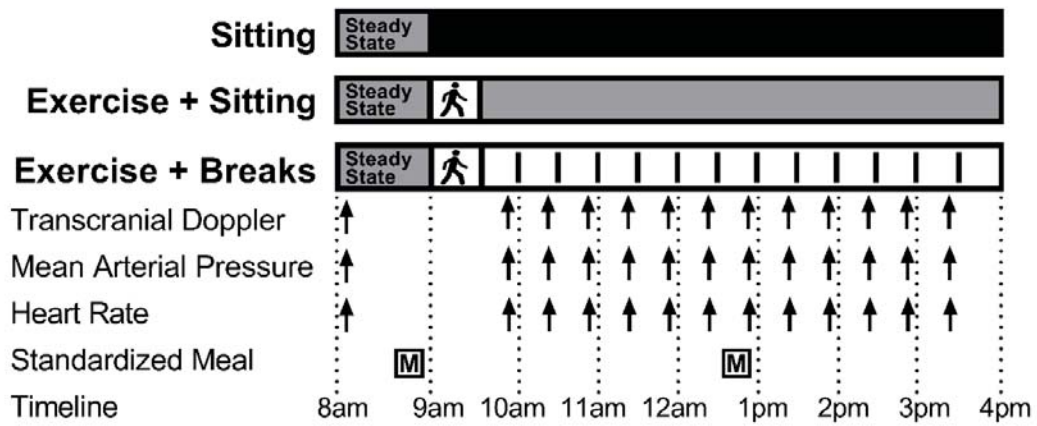


Figure 1.

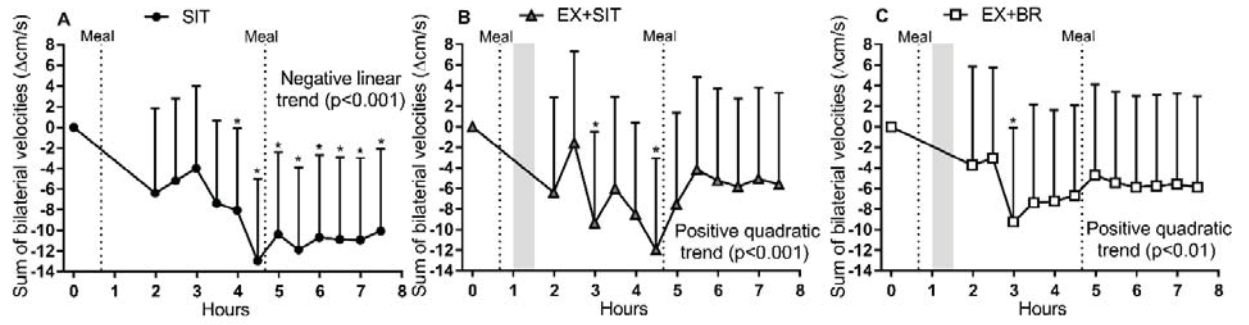


Figure 2.

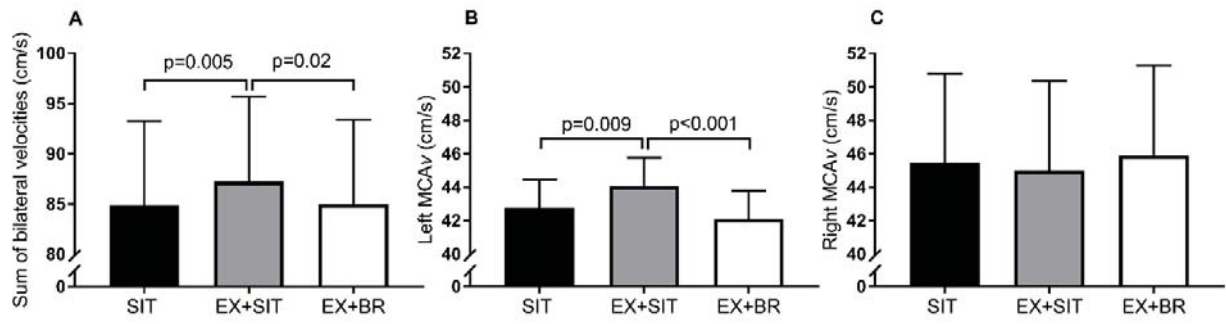


Figure 3.

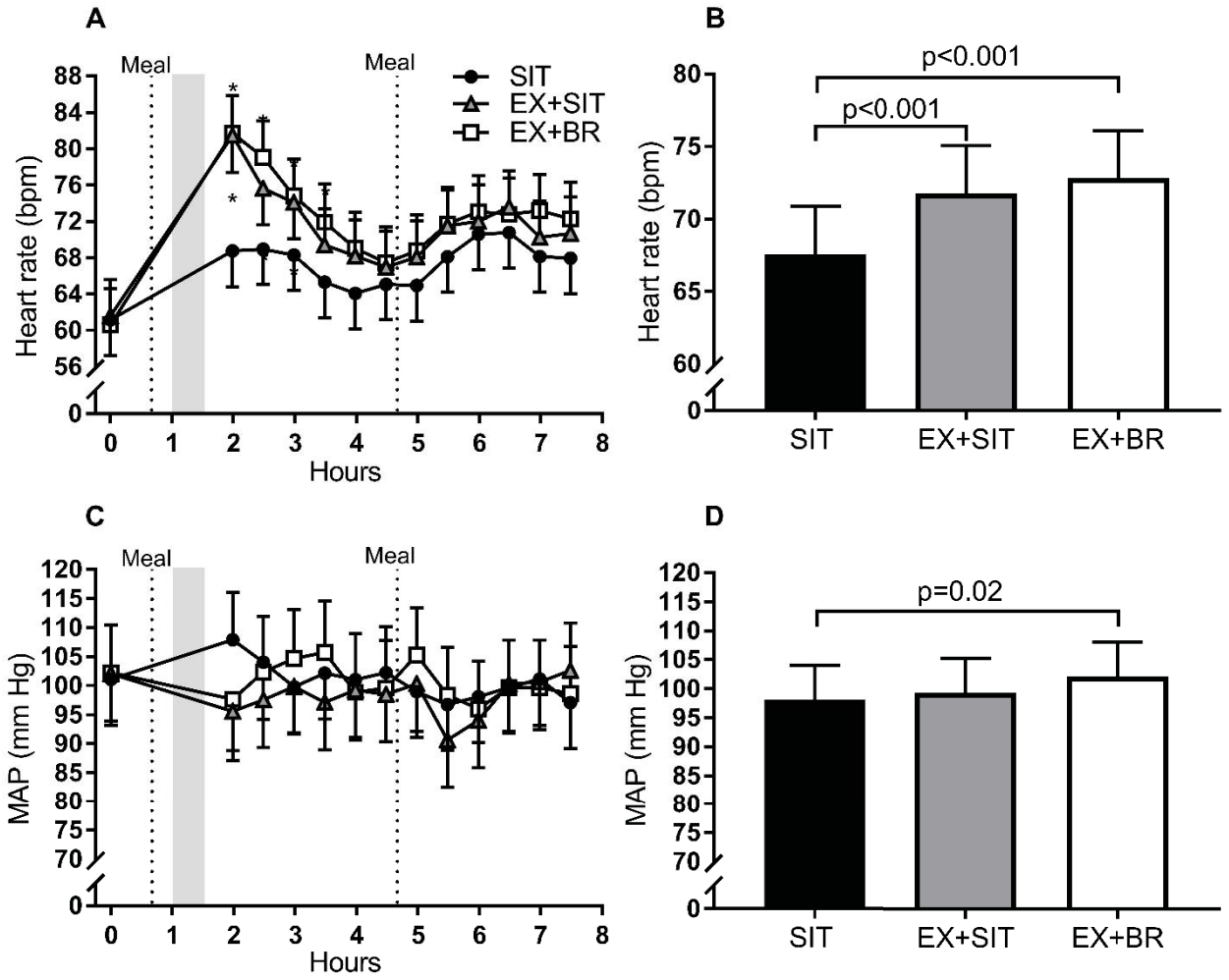


Figure 4.

## Tables

Table 1. Participant characteristics.

Demographic	Baseline
<i>N</i>	12
Sex (female/male)	2 / 10
Age (years)	70±7
Body mass index (kg/m <sup>2</sup> )	30.4±4.3
Waist circumference (cm)	103.4±11.0
Office SBP (mmHg)*	128±13
Office DBP (mmHg)*	76±13
Fasting glucose (mmol/L)†	5.0±0.5
Fasting insulin (pmol/L)†	30±24.7
Fasting cholesterol (mmol/L)†	5.1±0.9
Fasting triglycerides (mmol/L)†	1.1±0.4
Fasting HDL (mmol/L)†	1.3±0.3
Fasting LDL (mmol/L)†	3.3±0.6

Data are mean±SD; SBP, systolic blood pressure; DBP, diastolic blood pressure HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; \* measured during familiarisation visit, †measured during first experimental visit