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1 Effect of breaking up sedentary time with calisthenics on
2 endothelial function

3

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15

16 **Running Title:** Breaking up sitting with calisthenics: effect on endothelial function

17

18 **Key Words:** Sedentary behaviour; flow-mediated dilation; shear rate, calisthenics

19

20 Word Count: 3643

21 **Abstract**

22 **Background:** Periods of prolonged sitting impairs endothelial function in lower
23 limb conduit arteries, which is attenuated with physical activity breaks. The effect of
24 activity breaks on upper limb arteries has not been examined. This study assessed
25 changes in brachial artery endothelial function following either a prolonged sitting
26 period or breaking up this sedentary time by performing sets of calisthenics
27 exercises. **Methods:** Ten healthy participants (6 men) completed two conditions in a
28 counterbalanced order: a) 1-hr 26-min sitting, or b) breaking up this period every 20-
29 min by performing a set of five calisthenics exercises. Brachial artery endothelial
30 function was assessed via ultrasound using the flow-mediated dilation (FMD)
31 technique prior to and following each condition, while brachial shear rate (SR) was
32 acquired after each set of calisthenics. **Results:** There was no significant change in
33 FMD over time ($p=0.09$) or between conditions ($p=0.12$). Compared to sitting,
34 brachial SR increased following each set of calisthenics, with a significant difference
35 after the third break (Sit: $33.94 \pm 12.79 \text{ s}^{-1}$; Calisthenics: $57.16 \pm 30.48 \text{ s}^{-1}$, $p=0.02$).
36 **Conclusion:** Alterations in SR in the upper limbs suggest calisthenics may be an
37 effective intervention to break up sedentary time and attenuate the potentially
38 deleterious effects of prolonged sitting on cardiovascular health.

39

40 **Introduction**

41 Sitting is the most prevalent sedentary behaviour (Dunstan et al., 2012a; Owen et al.,
42 2010), particularly in workplaces (Parry et al., 2013), and is emerging as an
43 independent health risk factor (Dunstan et al., 2012a; Healy et al., 2008). Periods of
44 prolonged sitting impairs cardiometabolic health, however this is attenuated by
45 frequently breaking up this time (Dunstan et al., 2012b; Henson et al., 2015; Peddie
46 et al., 2013). Research is emerging to suggest that vascular health is also negatively
47 influenced by prolonged sedentary periods. Sitting for three (Thosar et al., 2014;
48 2015; McManus et al., 2015) and six (Restaino et al., 2015) hours caused superficial
49 femoral and popliteal artery endothelial function to decrease respectively. Whether
50 upper limb vascular function is also negatively influenced by sitting is less clear, as
51 over these time periods no decline in brachial artery endothelial function was
52 observed (Thosar et al., 2014; Restaino et al., 2015).

53

54 Reductions in shear stress appear to strongly mediate sitting-induced impairments in
55 vascular function. Shear stress is a key physiological mechanism in the regulation of
56 endothelial function (Carter et al., 2013; Padilla et al., 2011; Tinken et al., 2009),
57 chronic reductions of which augments the atherosclerotic process (Malek et al.,
58 1999). Prolonged sitting causes shear rate (SR) to decline in the superficial femoral
59 (Thosar et al., 2014; 2015), popliteal (Restaino et al., 2015, 2016) and brachial
60 (Thosar et al., 2014; Restaino et al., 2015) arteries. As brachial artery endothelial
61 function is maintained despite reductions in SR it suggests this vessel may be more
62 resistant to this negative effect of sitting (Restaino et al., 2015).

63

64 Using activity breaks to disrupt prolonged sitting periods appears to contribute to the
65 preservation of endothelial function by increasing shear stress. Frequently breaking
66 up prolonged sitting with walking bouts prevents the decline in femoral artery
67 endothelial function that is otherwise observed (Thosar et al., 2015). Physical
68 activity enhances endothelial function (Di Francescomarino et al., 2009) via
69 increased blood flow and shear stress (Tinken et al., 2009; 2010), but this
70 improvement is abolished if shear stress is attenuated using cuff inflation methods
71 (Birk et al., 2013; Tinken et al., 2009; 2010). Moreover, exercise training increases
72 endothelial function due to exposing the vasculature to repeated episodic elevations
73 in shear stress (Green et al., 2011; Tinken et al., 2009). Consequently, this pattern of
74 repeated increases in shear stress is replicated using activity breaks, which may
75 explain their protective role. However, in their study Thosar et al., (2015) assessed
76 SR 25 minutes after the activity breaks therefore any immediate changes were not
77 recorded, which may explain the lack of difference observed between the activity
78 and sitting conditions. Moreover, changes to upper limb endothelial function were
79 not considered.

80

81 Upper limb vascular function was assessed when a single ten minute walking bout
82 was completed following an extended sitting period (Restaino et al., 2015). This
83 intervention restored the decrease in popliteal but not brachial microvascular
84 function, possibly due to the lack of increased blood flow to the upper limbs
85 (Restaino et al., 2015). Consequently, a whole body exercise modality such as
86 calisthenics exercises (using body weight as a resistance) may be more effective in
87 enhancing vascular function as this activates both upper and lower limb musculature.
88 Additionally, calisthenics make an ideal workplace intervention as individuals are

89 not required to leave their working environment, purchase equipment or make
90 workplace adaptations (Carr et al., 2012; Carter et al., 2015).

91

92 The purpose of this study was to determine changes in brachial artery endothelial
93 function following either a prolonged sitting period or breaking up this sedentary
94 time performing sets of calisthenics exercises. We also aimed to assess the changes
95 in SR over this time period, to provide a greater mechanistic understanding of the
96 effects of sitting with or without activity breaks on upper limb vascular function.

97

98 **Methods**

99 **Study population**

100 Ten healthy participants (6 men) were screened prior to testing (PAR-Q) and
101 exclusion criteria included: smoker, current medication and presence of apparent
102 cardio-metabolic disease. The experimental procedures and potential risks were
103 explained to participants prior to testing and written informed consent obtained. The
104 University of Essex ethics committee approved the experimental protocol, which
105 conformed to the Declaration of Helsinki.

106

107 **Study design and procedures**

108 Participants attended the temperature controlled (20-22°C) laboratory at the same
109 time of day (between 9.00-9.30 am) on two separate occasions. Prior to testing
110 participants avoided strenuous exercise for 48-hr and any exercise for 12-hr prior,
111 and completed an overnight fast and abstinence from caffeine. Women were assessed
112 in days 1–7 of the menstrual cycle. Participants randomly completed either 1-hr 26-
113 min of: a) uninterrupted sitting (Sit) or b) disrupting this sitting every 20-min
114 performing a set of calisthenics (Calisthenics). This time period was chosen as
115 previous work has shown changes in SR occur within the first two hours of sitting
116 (Restaino et al., 2015; Thosar et al., 2015). Moreover, due to the novelty of the
117 calisthenics, it was important to assess the feasibility of participants completing this
118 intervention without too much participant burden.

119

120 After arrival, participants were fitted with a 3-lead electrocardiogram and separate
121 heart rate (HR) monitor, to assess HR during endothelial function measures and
122 during the physical activity breaks respectively. Participants rested in a supine

123 position for 20-min followed by baseline assessment of right brachial artery
124 endothelial function (Pre Condition) using the noninvasive flow mediated-dilation
125 (FMD) technique. Participants then moved to a seated position and baseline right
126 brachial artery mean SR was acquired, with SR used as an estimation of shear stress
127 (Johnson et al., 2011). Following this, participants completed one of the
128 experimental conditions. In the Calisthenics condition, mean brachial artery SR was
129 acquired following each activity break (Post Break). Time points were matched in
130 the Sit condition. After the condition baseline FMD and SR measures were repeated
131 (Post Condition) (Figure 1). The same researcher completed all measures.

132

133 Condition 1: Sit

134 Participants remained seated in a chair at a desk for 1-hr 26-min. During this time,
135 non-vigorous arm and leg movements were permitted, enabling participants to
136 complete desk-based activities such as reading and working on a computer (Figure
137 1a).

138

139 Condition 2: Calisthenics

140 The sedentary period was broken up every 20-min with a 2-min set of calisthenics.
141 This was repeated three times (Break 1, Break 2 and Break 3), followed by a final 20-
142 min sitting period, therefore totalling 1-hr 26-min (Figure 1b).

143

144 Five different exercises were performed: squats, arm circles, calf raises, knees to
145 elbows and forward lunges. Exercises alternatively activated upper and lower body
146 muscles groups to minimise fatigue. Participants performed 8 repetitions of each
147 exercise across a 3 second cycle (24 seconds per exercise) in time to an audible and

148 visual metronome. Prior to testing written and verbal instructions and demonstrations
149 for each exercise were given, and participants were provided the opportunity to
150 practise any unfamiliar exercises. Participants were instructed to keep exercise
151 technique consistent for each set of exercises.

152

153 To ensure consistent duration of break, a standardised transition time between sitting
154 to starting each activity bout and then returning to sitting was included of 25 and 15
155 seconds respectively.

156

157 **Vascular endothelial function testing**

158 Assessment of brachial artery FMD was performed according to published
159 guidelines (Harris et al., 2010; Stoner et al., 2012; Thijssen et al., 2011) using M-
160 mode imaging. Arterial blood flow and SRs were assessed using a linear array 10
161 Mhz Doppler ultrasound in triplex mode (Prosound Alpha 6, Hitachi Aloka Medical,
162 Tokyo, Japan), with an insonation angle of 60°. The ultrasound had built in software
163 enabling automated edge detection for diameter analysis in real time and fast Fourier
164 transform of raw audio data to determine mean blood velocity. Testing procedures
165 were completed as previously described (Carter et al., 2014).

166

167 For data analysis, basal arterial diameter was determined as the average of 30 heart
168 cycles prior to cuff inflation; while peak vessel dilation was calculated from the
169 highest average diameter of three consecutive heart cycles after cuff deflation. FMD
170 was calculated as: $\left(\frac{[\text{Peak post-occlusion arterial diameter} - \text{Baseline mean arterial}]{}}{\text{Baseline mean arterial diameter}}\right) \times 100 \%$. SR area under the curve
171

172 (AUC) was calculated from post cuff deflation until the point of peak vessel
173 diameter and formulated using the trapezoid rule (Harris et al., 2010).

174

175 **Shear rate measurements**

176 Brachial artery mean SR measurements were acquired at the same location as FMD
177 measures, as indicated by an indelible marking at the site. SRs were measured at Pre
178 Condition, Post Condition and Post each Break (at 22, 44 and 66-min during each
179 condition). Measures were collected at 1.5 minutes following each set of
180 calisthenics, providing time for probe positioning and imaging. Time points were
181 replicated in the Sit condition (Time 1, Time 2 and Time 3; Figure 1).

182

183 For each SR measurement 20 heart cycles of blood velocity and arterial diameter
184 data were collected. From this mean SR ($4 \times [\text{mean blood velocity}/\text{arterial diameter}$
185 $\text{at the measurement time}]$) could be calculated in accordance to published guidelines
186 (Harris et al., 2010).

187

188 **Heart rate and blood pressure**

189 HR was continually monitored (S610i, Polar Electro Oy, Kempele, Finland) with
190 measures taken at 5 second intervals. Blood pressure (BP) was measured at left
191 brachial artery (MX3 plus, Omron, USA) at the same time points as SR measures
192 and mean arterial pressure (MAP) subsequently calculated.

193

194 **Physical activity and sedentary behaviour assessment**

195 Participants completed the International Physical Activity Questionnaire (Long form,
196 IPAQ) (Booth, 2000), a validated and reliable measure of adult physical activity
197 (Craig et al., 2003) and sedentary behaviour (Rosenberg et al., 2008).

198

199 **Statistical analyses**

200 Data were normally distributed, assessed using Shapiro-Wilk test. Technical reasons
201 during data collection meant ten data sets for endothelial function and eight sets for
202 brachial SR were analysed.

203

204 Two-way repeated measures ANOVA were used to compare the effect of condition
205 on relative FMD, basal arterial diameters, mean SR and MAP. All post hoc analyses
206 were performed via paired samples t-test with Bonferroni adjustment. Data are
207 presented as means \pm standard deviation (SD) from which effects sizes were
208 calculated (partial eta squared η^2). These were interpreted as: $\eta^2=0.01$ considered
209 small, $\eta^2=0.06$ considered medium and $\eta^2=0.14$ considered large (Cohen, 1988).

210 Data was analysed using statistical software (SPSS Version 18.0, IBM Corporation,
211 Somers, NY, USA), with significance accepted as $p \leq 0.05$.

212 **Results**

213 **Descriptive statistics**

214 Descriptive statistics are shown in Table 1. Participants were classified as
215 highly active but spent over 6.5 hours per day seated (Table 1).

216

217 **Endothelial function**

218 For relative FMD, no significant interaction ($F(1,9)= 0.9, p=0.37, \eta^2=0.09$),
219 condition ($F(1,9)= 3.6, p=0.12, \eta^2=0.29$) or time ($F(1,9)= 2.9, p=0.09, \eta^2=0.25$)
220 effects were observed (Pre Sit: 4.58 ± 5.20 %; Post Sit: 5.33 ± 5.81 %; Pre
221 Calisthenics: 5.36 ± 4.07 %; Post Calisthenics: 8.32 ± 6.10 %; Figure 2). For SR AUC
222 there were no significant effects for interaction ($F(1,9)= 0.9, p=0.36, \eta^2=0.14$),
223 condition ($F(1,9)= 0.5, p=0.48, \eta^2=0.10$) or time ($F(1,9)= 0.8, p=0.40, \eta^2=0.12$) (Pre
224 Sit: 3261.6 ± 2887.8 s⁻¹; Post Sit: 3191.3 ± 2619.4 s⁻¹; Pre Calisthenics: 3903.9
225 ± 1948.5 s⁻¹; Post Calisthenics: 3249.8 ± 1893.3 s⁻¹). Normalising FMD for SR AUC
226 (dividing the percentage of FMD by SR AUC) resulted in no significant interaction
227 ($F(1,9)= 1.2, p=0.31, \eta^2=0.16$), condition ($F(1,9)= 0.7, p=0.44, \eta^2=0.11$) or time
228 ($F(1,9)= 2.9, p=0.12, \eta^2=0.33$) effects (Pre Sit: 0.001 ± 0.004 %/s⁻¹; Post Sit: 0.001
229 ± 0.002 %/s⁻¹; Pre Calisthenics: 0.001 ± 0.001 %/s⁻¹; Post Calisthenics: 0.003 ± 0.002
230 %/s⁻¹). There was no main effect for time ($F(1,9)= 2.5, p=0.15, \eta^2=0.21$) or condition
231 ($F(1,9)= 3.9, p=0.52, \eta^2=0.05$) for basal arterial diameters (Pre Sit: 3.72 ± 0.69 mm;
232 Post Sit: 3.55 ± 0.76 mm; Pre Calisthenics: 3.60 ± 0.66 mm; Post Calisthenics: 3.57
233 ± 0.79 mm).

234

235 **Shear rate**

236 A significant interaction effect was observed for brachial SR ($F(4,32)=2.0$ $p=0.02$,
237 $\eta^2=0.12$). Post Break SRs were increased in the Calisthenics condition compared to
238 the same time points in the Sit condition, however post-hoc analysis revealed this
239 difference was only statistically significant following Break 3 with Calisthenics
240 elevating SR by 23.22 s^{-1} more compared to Sit at Time 3 ($p=0.02$, $\eta^2=0.51$; Table
241 2). Post Condition, Sit SR remained depressed compared to Calisthenics ($p=0.07$,
242 $\eta^2=0.36$). Finally, Pre Condition SRs were not significantly different between
243 conditions ($p=0.88$, $\eta^2=0.01$).

244

245 **Cardiovascular response**

246 For MAP no significant interaction ($F(5,45)= 0.8$, $p=0.56$, $\eta^2=0.08$), time ($F(5,45)=$
247 1.2 , $p=0.33$, $\eta^2=0.12$) or condition ($F(1,9)= 0.8$, $p=0.41$, $\eta^2=0.08$) effects were
248 observed (Sit Pre: 91.1 ± 10.2 mmHg, Post: 88.1 ± 8.2 mmHg; Calisthenics Pre: 90.0
249 ± 9.9 mmHg, Post: 90.9 ± 7.4 mmHg). HR in the Calisthenics condition showed a
250 significant main effect for time ($F(11,88)= 23.3$, $p=0.001$, $\eta^2=1.0$). Post hoc analysis
251 revealed calisthenics significantly increased HR (Break 1: 90.05 ± 8.26 b.min⁻¹;
252 Break 2: 92.24 ± 6.16 b.min⁻¹; Break 3: 92.51 ± 7.24 b.min⁻¹; Figure 3) compared to
253 Pre Condition (68.90 ± 9.10 b.min⁻¹; $p=0.001$) and HR remained elevated above Pre
254 Condition at 30 seconds following each break ($p=0.001$). In the Sit condition, no
255 main effect for time was observed ($F(4,36)= 1.2$, $p=0.34$, $\eta^2=0.33$) with HR only
256 slightly reduced over time (Pre: 70.25 ± 8.92 b.min⁻¹; Post: 66.21 ± 8.72 b.min⁻¹).

257

258 **Discussion**

259 This study investigated changes in brachial artery endothelial function and SR in
260 response to a prolonged sitting period or breaking up this sedentary time. Sitting did
261 not attenuate brachial SR or FMD. Using calisthenics to disrupt this sitting time
262 caused significant transient increases in HR and brachial mean SR, however this did
263 not significantly increase brachial FMD. Results indicate that brachial artery
264 endothelial function is resistant to the negative effects of one hour of sitting. Data
265 also suggests that over a longer time period using calisthenics to break up sedentary
266 time may improve vascular function.

267

268 **Calisthenics activity breaks and endothelial function**

269 Using calisthenics to break up sedentary time did not significantly increase brachial
270 endothelial function. Despite what is known about activity-related improvements in
271 endothelial function (Di Francescomarino et al., 2009), this current result is in
272 support of existing research. Although in previous work, short walking bouts to
273 break up sitting attenuated the decline in FMD that otherwise occurred, FMD in the
274 activity condition was also not significantly increased compared to baseline (Thosar
275 et al., 2015). Whilst the FMD results from this current study were not significantly
276 different between conditions, unlike in Thosar et al. (2015) study, FMD was not
277 attenuated in the sitting condition. There are currently no guidelines as to clinically
278 meaningful changes in FMD and consequently, the 2.99 % greater increase in the
279 calisthenics condition compared to the sit condition may have important vascular
280 health implications; especially considering the relative acute nature of this study.

281

282 For the first time, changes in SR immediately after activity breaks to sitting have
283 been characterised. Brachial SR was elevated following each set of calisthenics and,
284 of particular interest, the increase in SR was greater after break three than break one,
285 suggesting a possible accumulative effect of the calisthenics interventions.
286 Moreover, SR measured 20 minutes after the final activity break had returned to a
287 value similar to baseline; highlighting the need to frequently disrupt sedentary
288 periods in order to maintain elevated SR.

289

290 Repeated episodic increases in SR are a suggested mechanism underlying exercise
291 related improvements in endothelial function (Padilla et al., 2008; Tinken et al.,
292 2010). In the current study, although over a short time period, each break replicated
293 this repetitive stimulus, suggesting that SR alterations could be a mechanism to
294 explain the slight increase brachial FMD observed. Indeed, this has previously been
295 proposed as an explanation for the maintenance of femoral artery endothelial
296 function when sedentary time was broken up with walking bouts (Thosar et al.,
297 2015). However, in the study by Thosar et al. (2015) SR measures were taken over
298 20 minutes after each walk, consequently assessing SR immediately post-activity
299 allows a clearer mechanistic explanation to describe the effects activity breaks
300 during sitting have on the vascular system. Future work should seek to characterise
301 the SR response following an activity break at more regular intervals to determine
302 the length of time over which elevations in SR persist.

303

304 Despite calisthenics not significantly changing brachial FMD, this does not imply it
305 is not an effective intervention choice to break up sedentary time. Results show that
306 mean brachial SR can be acutely increased using this intervention and, as the

307 calisthenics routine employed was a whole body exercise, such elevations may also
308 have been present in the lower limbs. Previous work (Thosar et al., 2015; Restaino et
309 al., 2015; 2016) has shown this vasculature is more susceptible to decreases in
310 endothelial function following prolonged sitting periods. Consequently, future
311 research should use calisthenics and investigate changes in lower limb vascular
312 function.

313

314 **Sitting did not attenuate endothelial function**

315 In agreement with existing literature (Thosar et al., 2014; Restaino et al., 2015)
316 continuous sitting did not decrease brachial FMD compared to baseline values.
317 Additionally, no significant reductions in brachial SR were observed. This may
318 provide an explanation for this outcome, as decreased SR is a proposed mechanism
319 for the reduction in lower limb endothelial function following sitting (Thosar et al.,
320 2014; 2015; Restaino et al., 2016). Importantly during these studies participants'
321 lower limbs remained motionless (Restaino et al., 2015; Thosar et al., 2015) whereas
322 in this current study participants were permitted to move their upper limbs to
323 complete desk-based tasks. This may have resulted in low level muscular
324 contractions, maintaining blood flow and SR. In support of this, upper limb
325 movement during bed rest enhanced brachial FMD (Hamburg et al., 2007), whilst
326 reducing daily step count attenuated femoral but not brachial FMD, related to a
327 larger reduction in lower limb perfusion compared to the upper limbs (Boyle et al.,
328 2013).

329

330 The absence of a decline in mean brachial SR after one hour and six minutes of
331 sitting is in line with existing research, as SR was not significantly reduced after the

332 first hour of a three hour sedentary period (Thosar et al., 2014). However, mean
333 brachial SR was significantly attenuated after two hours of sitting and remained
334 depressed for the further five hours of assessment (Restaino et al., 2015). This
335 indicates that the brachial artery is susceptible to SR reductions, however the results
336 from this current study and Thosar et al. (2014) suggest within an hour of sitting this
337 is not apparent. In the current study, the measure taken after one hour and six
338 minutes resulted in the largest reduction in SR which then remained slightly
339 depressed for the post-condition measure, indicating that reductions may have
340 continued if the time period was extended, in line with existing research (Restaino et
341 al., 2015). Taken together these results support the idea that the brachial artery is
342 more resistive to the negative effects of sitting (Restaino et al., 2015), as popliteal
343 and femoral mean SR are reduced within the first hour of sitting (Thosar et al., 2015;
344 Restaino et al., 2015). Moreover, even when reductions in brachial SR have been
345 observed the magnitude of decline was less than that in the lower limbs and only
346 microvascular function was attenuated (Restaino et al., 2015).

347

348 **Limitations and future research**

349 As aforementioned, it is likely that the continuous sitting condition duration was not
350 long enough for significant changes to endothelial function and SR to occur. As there
351 is no definition as to what is considered prolonged sitting (Dunstan et al., 2012a) the
352 current design was chosen to provide initial data on calisthenics and endothelial
353 function without excessive participant demands. Previous research considering
354 metabolic health and changes to endothelial function has utilised longer assessment
355 periods of up to nine hours (Dunstan et al., 2012b; Peddie et al., 2013; Thosar et al.,
356 2014; 2015; Restaino et al., 2015). Additionally, the population assessed were

357 young, healthy and had high levels of physical activity. Larger changes to
358 endothelial function may have occurred with an older or less active population.
359 Indeed, exercise status and age are known to influence endothelial responses (Black
360 et al., 2009). Furthermore, participants' transport to the laboratory was not
361 standardised, therefore pre-testing physical activity may have varied between
362 participants. However, based on the location of the university campus, it is assumed
363 participants would have used car or bus transportation; moreover participants were
364 asked to use the same form of transport for each test visit. Finally, due to the pilot
365 nature of this research, it is possible that the study was underpowered to detect
366 changes in FMD. Although previous work has shown significant differences with a
367 sample size of 12 (Thosar et al., 2015) a power calculation using our data suggests
368 our study may be underpowered and that 30 participants might be needed to detect a
369 significant change in relative FMD. As this study shows that calisthenics are feasible
370 for participants to complete future work should therefore use a larger sample size.

371

372 This study presents results following a single sitting period, however of greater
373 ecological interest is the influence of repeatedly performing either the calisthenics
374 intervention or prolonged sitting over several days or weeks. This would determine
375 if, as in exercise training studies, the repeated exposure of the vasculature to
376 increases in SR can lead to longer term improvements in endothelial function.
377 Furthermore, if repeated prolonged sitting periods does decrease endothelial function
378 it would further support the need for a reduction of this behaviour in the population.
379 In practical terms, longer term research within a workplace environment would
380 determine the feasibility of individuals carrying out multiple breaks to their daily
381 routine, alongside assessing behavioural change and adherence.

382

383 **Conclusions**

384 For the first time, this study demonstrates breaking up sedentary time with
385 calisthenics can lead to transient increases in HR and brachial artery mean SR. This
386 did not significantly increase brachial artery endothelial function, however a longer
387 assessment period more representative of a working day may be needed to result in a
388 significant improvement. Additionally results show that brachial artery endothelial
389 function is not negatively affected by an acute sitting period, when upper limb
390 movement typical of daily living is permitted. Overall, the SR results from this study
391 present calisthenics as a potential intervention to break up sedentary time and
392 enhance or maintain cardiovascular health. Longer term research, assessing upper
393 and lower limb endothelial function is required to assess whether repeatedly
394 exposing the vascular system to increases in SR can lead to larger improvements in
395 endothelial function.

396

397 **References**

- 398 Birk, G. K., Dawson, E. A., Cable, N. T., Green, D. J. and Thijssen, D. H. (2013).
399 Effect of unilateral forearm inactivity on endothelium-dependent vasodilator
400 function in humans. *European Journal of Applied Physiology*, 113: 933-940.
- 401 Black, M. A., Cable, N. T., Thijssen, D. H. and Green, D. J. (2009). Impact of age,
402 sex, and exercise on brachial artery flow-mediated dilatation. *American*
403 *Journal of Physiology. Heart and Circulatory Physiology*, 297: H1109-1116.
- 404 Booth, M. (2000). Assessment of physical activity: an international perspective.
405 *Research Quarterly Exercise Sport*, 71: S114-120.
- 406 Boyle, L. J., Credeur, D. P., Jenkins, N. T., Padilla, J., Leidy, H. J., Thyfault, J. P.
407 and Fadel, P. J. (2013). Impact of reduced daily physical activity on conduit
408 artery flow-mediated dilation and circulating endothelial microparticles.
409 *Journal of Applied Physiology*, 115: 1519-1529.
- 410 Carr, L. J., Walaska, K. A. and Marcus, B. H. (2012). Feasibility of a portable pedal
411 exercise machine for reducing sedentary time in the workplace. *British Journal*
412 *of Sport Medicine*, 46: 430-435.
- 413 Carter, H. H., Dawson, E. A., Birk, G. K., Spence, A. L., Naylor, L. H., Cable, N. T.,
414 Thijssen, D. H. and Green, D. J. (2013). Effect of SR manipulation on conduit
415 artery dilation in humans. *Hypertension*, 61: 143-150.
- 416 Carter, S. E., Faulkner, A. and Rakobowchuk, M. (2014). The role of prostaglandin
417 and antioxidant availability in recovery from forearm ischemia-reperfusion
418 injury in humans. *Journal of Hypertension*, 32: 339-351.

419 Carter, S. E., Jones, M. and Gladwell, V. F. (2015). Energy expenditure and heart
420 rate response to breaking up sedentary time with three different physical
421 activity interventions. *Nutrition Metabolism and Cardiovascular Diseases*, 25:
422 503-509.

423 Cohen, J., (1988). *Statistical Power Analysis for the Behavioral Sciences*, Second
424 Edition. Routledge.

425 Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L.,
426 Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F. and Oja, P.
427 (2003). International physical activity questionnaire: 12-country reliability and
428 validity. *Medicine and Science in Sports and Exercise*, 35: 1381-1395.

429 Di Francescomarino, S., Sciartilli, A., Di Valerio, V., Di Baldassarre, A. and Gallina,
430 S. (2009). The effect of physical exercise on endothelial function. *Sports*
431 *Medicine*, 39: 797-812.

432 Dunstan, D. W., Howard, B., Healy, G. N. and Owen, N. (2012a). Too much sitting-
433 A health hazard. *Diabetes Research and Clinical Practice*, 97: 368-376.

434 Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M.
435 T., Shaw, J. E., Bertovic, D. A., Zimmet, P. Z., Salmon, J. and Owen, N.
436 (2012b). Breaking up prolonged sitting reduces postprandial glucose and
437 insulin responses. *Diabetes Care*, 35: 976-983.

438 Green, D. J., Spence, A., Halliwill, J. R., Cable, N. T. and Thijssen, D. H. (2011).
439 Exercise and vascular adaptation in asymptomatic humans. *Experimental*
440 *Physiology*, 96: 57-70.

441 Hamburg, N. M., McMackin, C. J., Huang, A. L., Shenouda, S. M., Widlansky, M.
442 E., Schulz, E., Gokce, N., Ruderman, N. B., Keaney, J. F. and Vita, J. A.
443 (2007). Physical inactivity rapidly induces insulin resistance and microvascular
444 dysfunction in healthy volunteers. *Arteriosclerosis, Thrombosis, and Vascular*
445 *Biology*, 27: 2650-2656.

446 Harris, R. A., Nishiyama, S. K., Wray, D. W. and Richardson, R. S. (2010).
447 Ultrasound assessment of flow-mediated dilation. *Hypertension*, 55: 1075-
448 1085.

449 Healy, G. N., Dunstan, D. W., Salmon, J., Shaw, J. E., Zimmet, P. Z. and Owen, N.
450 (2008). Television time and continuous metabolic risk in physically active adults.
451 *Medicine and Science in Sports and Exercise*, 40: 639-645.

452 Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M. R., Stensel,
453 D. J., Tolfrey, K., Dunstan, D. W., Khunti, K. and Yates, T. (2015). Breaking
454 up prolonged sitting with standing or walking attenuates the postprandial
455 metabolic response in postmenopausal women: A randomized acute study.
456 *Diabetes Care*, 10.2337/dc15-1240.

457 Johnson, B. D., Mather, K. J. and Wallace, J. P. (2011). Mechanotransduction of
458 shear in the endothelium: Basic studies and clinical implications. *Vascular*
459 *Medicine*, 16: 365-377.

460 Malek, A. M., Alper, S. L. and Izumo, S. (1999). Hemodynamic shear stress and its
461 role in atherosclerosis. *Journal of the American Medical Association*, 282:
462 2035-2042.

463 McManus, A. M., Ainslie, P. N., Green, D. J., Simair, R. G., Smith, K. and Lewis,
464 N. (2015). Impact of prolonged sitting on vascular function in young girls.
465 *Experimental Physiology*, 100: 1379-1387.

466 Owen, N., Healy, G. N., Matthews, C. E. and Dunstan, D. W. (2010). Too much
467 sitting: The population-health science of sedentary behaviour. *Exercise and*
468 *Sport Science Reviews*, 28: 105-113.

469 Padilla, J., Harris, R. A., Rink, L. D. and Wallace, J. P. (2008). Characterization of
470 the brachial artery shear stress following walking exercise. *Vascular Medicine*,
471 13: 105-111.

472 Padilla, J., Simmons, G. H., Vianna, L. C., Davis, M. J., Laughlin, M. H. and Fadel,
473 P. J. (2011). Brachial artery vasodilatation during prolonged lower limb
474 exercise: Role of shear rate. *Experimental Physiology*, 96: 1019-1027.

475 Parry, S., Straker, L., Gilson, N. D. and Smith, A. J. (2013). Participatory workplace
476 interventions can reduce sedentary time for office workers- A randomised
477 controlled trial. *PLoS ONE*, 8: e78957.

478 Peddie, M. C., Bone, J. L., Rehrer, N. J., Skeaff, C. M., Gray, A. R. and Perry, T. L.
479 (2013). Breaking prolonged sitting reduces postprandial glycemia in healthy,
480 normal-weight adults: A randomized crossover. *American Journal of Clinical*
481 *Nutrition*, 98: 358-366.

482 Restaino, R. M., Holwerda, S. W., Credeur, D. P., Fadel, P. J. and Padilla, J. (2015).
483 Impact of prolonged sitting on lower and upper limb micro- and macrovascular
484 dilator function. *Experimental Physiology*, 100: 829-838.

- 485 Restaino, R. M., Walsh, L. K., Morishima, T., Vranish, J. R., Martinez-Lemus, L.
486 A., Fadel, P. J. and Padilla, J. (2016). Endothelial dysfunction following
487 prolonged sitting is mediated by a reduction in shear stress. *American Journal*
488 *of Physiology: Heart and Circulatory Physiology*, 310: H648-H653.
- 489 Rosenberg, D. E., Bull, F. C., Marshall, A. L., Sallis, J. F. and Bauman, A. E. (2008).
490 Assessment of sedentary behavior with the International Physical Activity
491 Questionnaire. *Journal of Physical Activity and Health*, 5: S30-S44.
- 492 Stoner, L. and Sabatier, M. J. (2012). Use of ultrasound for non-invasive assessment
493 of flow-mediated dilation. *Journal of Atherosclerosis and Thrombosis*, 19:
494 407-421.
- 495 Thijssen, D. H., Black, M. A., Pyke, K. E., Padilla, J., Atkinson, G., Harris, R. A.,
496 Parker, B., Widlansky, M. E., Tschakovsky, M. E. and Green, D. J. (2011).
497 Assessment of flow-mediated dilation in humans: a methodological and
498 physiological guideline. *American Journal of Physiology: Heart and*
499 *Circulatory Physiology*, 300: H2-H12.
- 500 Thosar, S. S., Bielko, S. L., Wiggins, C. C. and Wallace, J. P. (2014). Differences in
501 brachial and femoral artery responses to prolonged sitting. *Cardiovascular*
502 *Ultrasound*, 12: 50.
- 503 Thosar, S. S., Bielko, S. L., Mather, K. J., Johnston, J. D. and Wallace, J. P. (2015).
504 Effect of prolonged sitting and breaks in sitting time on endothelial function.
505 *Medicine and Science in Sports and Exercise*, 47: 843-849.
- 506 Tinken, T. M., Thijssen, D. H., Hopkins, N., Black, M. A., Dawson, E. A., Minson,
507 C. T., Newcomer, S. C., Laughlin, M. H., Cable, N. T. and Green, D. J. (2009).

508 Impact of shear rate modulation on vascular function in humans. *Hypertension*,
509 54: 278-285.

510 Tinken, T. M., Thijssen, D. H., Hopkins, N., Dawson, E. A., Cable, N. T. and Green,
511 D. J. (2010). Shear stress mediates endothelial adaptations to exercise training
512 in humans. *Hypertension*, 55: 312-318.

513

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517

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520 **Tables**

521 **Table 1:** Descriptive characteristics, self-reported physical activity scores and total
522 sitting time of participants (n= 10)

523

Variable	Mean	SD
Age (years)	27.3	8.1
Body Mass (kg)	82.6	19.7
Height (cm)	172.3	10.4
Physical Activity Score (MET-minutes/week)	3844.0	3271.8
Sitting Time Per Day (Hours)	6.6	2.3
Sitting Time Per Week (Hours)	46.2	16.3

524

525 **Table 2:** Brachial artery shear rate (SR) at baseline (Pre) and after (Post) two experimental conditions: a) uninterrupted sitting for 1-hr 26-min
 526 (Sit) or b) disrupting this sitting every 20-min performing a set of calisthenics (Calisthenics). In Calisthenics SR was measured after each break
 527 (Break 1, Break 2 and Break 3) and these time points were matched in Sit condition (Time 1, Time 2 and Time 3).

Measurement	Sit					Calisthenics				
	Pre	Time 1	Time 2	Time 3	Post	Pre	Break 1	Break 2	Break 3	Post
Brachial SR (s ⁻¹)	40.95 ±14.67	42.17 ±17.31	46.22 ±14.20	33.94 ±12.79	36.32 ±10.73	40.13 ±16.19	47.26 ±19.37	65.86 ±36.43	57.16 ±30.48*	46.41 ±20.22

528
 529 * Indicates Calisthenics was significantly greater than Sit at Break 3 (p<0.05).

530 **Figure Legends**

531 **Figure 1:** The experimental design and measurement time points for the two
532 conditions, completed in a randomised order on separate days. (a) Sitting for 1-hr 26-
533 min, or (b) breaking up this period every 20-min with a 2-min set of calisthenics
534 exercises. FMD- flow mediated dilation; SR- shear rate.

535

536 **Figure 2:** Brachial artery flow mediated dilation (FMD) before (Pre) and following
537 (Post) either 1-hr 26-min uninterrupted sitting (Sit) or, breaking up this period every
538 20-min with 2-min of performing calisthenics exercises (Calisthenics). (Error bars=
539 \pm SD)

540

541 **Figure 3:** Mean heart rate (HR) prior to each condition (Pre), during each
542 calisthenics activity break (Break 1, Break 2 and Break 3), and during the 5-min of
543 recovery following this intervention. * indicates a significantly greater HR than Pre
544 ($p=0.001$). (Error bars= \pm SD)