Effect of breaking up sedentary time with calisthenics on endothelial function

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2   endothelial function

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Abstract

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

Introduction

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

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

3

53   Using activity breaks to disrupt prolonged sitting periods appears to contribute to the

54   preservation of endothelial function by increasing shear stress. Frequently breaking

55   up prolonged sitting with walking bouts prevents the decline in femoral artery

56   endothelial function that is otherwise observed (Thosar et al., 2015). Physical

57   activity enhances endothelial function (Di Francescomarino et al., 2009) via

58   increased blood flow and shear stress (Tinken et al., 2009; 2010), but this

59   improvement is abolished if shear stress is attenuated using cuff inflation methods

60   (Birk et al., 2013; Tinken et al., 2009; 2010). Moreover, Eexercise training increases

61   endothelial function due to exposing the vasculature to repeated episodic elevations

62   in shear stress (Green et al., 2011; Tinken et al., 2009). Consequently, this pattern of

63   repeated increases in shear stress is replicated using activity breaks, which may

64   explain their protective role. However, in their study Thosar et al., (2015) assessed

65   SR 25 minutes after the activity breaks therefore any immediate changes were not

66   recorded, which may explain the lack of difference observed between the activity

67   and sitting conditions. Moreover, changes to upper limb endothelial function were

68   not considered.

69

70   Upper limb vascular function was assessed when a single ten minute walking bout

71   was completed following an extended sitting period (Restaino et al., 2015). This

72   intervention restored the decrease in popliteal but not brachial microvascular

73   function, possibly due to the lack of increased blood flow to the upper limbs

74   (Restaino et al., 2015). Consequently, a whole body exercise modality such as

75   calisthenics exercises (using body weight as a resistance) may be more effective in

76   enhancing vascular function as this activates both upper and lower limb musculature.

77   Additionally, calisthenics make an ideal workplace intervention as individuals are

4

78   not required to leave their working environment, purchase equipment or make

79   workplace adaptations (Carr et al., 2012; Carter et al., 2015).

80

81   The purpose of this study was to determine changes in brachial artery endothelial

82   function following either a prolonged sitting period or breaking up this sedentary

83   time performing sets of calisthenics exercises. We also aimed to assess the changes

84   in SR over this time period, to provide a greater mechanistic understanding of the

85   effects of sitting with or without activity breaks on upper limb vascular function.

86

5

Methods

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Study population

88

89 Ten healthy participants (6 men) were screened prior to testing (PAR-Q) and

90 exclusion criteria included: smoker, current medication and presence of apparent

91 cardio-metabolic disease. The experimental procedures and potential risks were

92 explained to participants prior to testing and written informed consent obtained. The

93 University of Essex ethics committee approved the experimental protocol, which

94 conformed to the Declaration of Helsinki.

95

Study design and procedures

96

97 Participants attended the temperature controlled (20-22°C) laboratory at the same

98 time of day (between 9.00-9.30 am) on two separate occasions. Prior to testing

99 participants avoided strenuous exercise for 48-hr and any exercise for 12-hr prior,

100 and completed an overnight fast and abstinence from caffeine. Women were assessed

101 in days 1–7 of the menstrual cycle. Participants randomly completed either 1-hr 26-

102 min of: a) uninterrupted sitting (Sit) or b) disrupting this sitting every 20-min

103 performing a set of calisthenics (Calisthenics). This time period was chosen as

104 previous work has shown changes in SR occur within the first two hours of sitting

105 (Restaino et al., 2015; Thosar et al., 2015). Moreover, due to the novelty of the

106 calisthenics, it was important to assess the feasibility of participants completing this

107 intervention without too much participant burden.

108

109 After arrival, participants were fitted with a 3-lead electrocardiogram and separate

110 heart rate (HR) monitor, to assess HR during endothelial function measures and

111 during the physical activity breaks respectively. Participants rested in a supine

6

112   position for 20-min followed by baseline assessment of right brachial artery

113   endothelial function (Pre Condition) using the noninvasive flow mediated-dilation

114   (FMD) technique. Participants then moved to a seated position and baseline right

115   brachial artery mean SR was acquired, with SR used as an estimation of shear stress

116   (Johnson et al., 2011). Following this, participants completed one of the

117   experimental conditions. In the Calisthenics condition, mean brachial artery SR was

118   acquired following each activity break (Post Break). Time points were matched in

119   the Sit condition. After the condition baseline FMD and SR measures were repeated

120   (Post Condition) (Figure 1). The same researcher completed all measures.

121

122   Condition 1: Sit

123   Participants remained seated in a chair at a desk for 1-hr 26-min. During this time,

124   non-vigorous arm and leg movements were permitted, enabling participants to

125   complete desk-based activities such as reading and working on a computer (Figure

126   1a).

127

128   Condition 2: Calisthenics

129   The sedentary period was broken up every 20-min with a 2-min set of calisthenics.

130   This was repeated three times (Break 1, Break 2 and Beak 3), followed by a final 20-

131   min sitting period, therefore totalling 1-hr 26-min (Figure 1b).

132

133   Five different exercises were performed: squats, arm circles, calf raises, knees to

134   elbows and forward lunges. Exercises alternatively activated upper and lower body

135   muscles groups to minimise fatigue. Participants performed 8 repetitions of each

136   exercise across a 3 second cycle (24 seconds per exercise) in time to an audible and

137 visual metronome. Prior to testing written and verbal instructions and demonstrations

138 for each exercise were given, and participants were provided the opportunity to

139 practise any unfamiliar exercises. Participants were instructed to keep exercise

140 technique consistent for each set of exercises.

141

142 To ensure consistent duration of break, a standardised transition time between sitting

143 to starting each activity bout and then returning to sitting was included of 25 and 15

144 seconds respectively.

145

146 **Vascular endothelial function testing**

147 Assessment of brachial artery FMD was performed according to published

148 guidelines (Harris et al., 2010; Stoner et al., 2012; Thijssen et al., 2011) using M-

149 mode imaging. Arterial blood flow and SRs were assessed using a linear array 10

150 Mhz Doppler ultrasound in triplex mode (Prosound Alpha 6, Hitachi Aloka Medical,

151 Tokyo, Japan), with an insonation angle of 60°. The ultrasound had built in software

152 enabling automated edge detection for diameter analysis in real time and fast Fourier

153 transform of raw audio data to determine mean blood velocity. Testing procedures

154 were completed as previously described (Carter et al., 2014).

155

156 For data analysis, basal arterial diameter was determined as the average of 30 heart

157 cycles prior to cuff inflation; while peak vessel dilation was calculated from the

158 highest average diameter of three consecutive heart cycles after cuff deflation. FMD

159 was calculated as: ((([Peak post-occlusion arterial diameter – Baseline mean arterial

160 diameter]/Baseline mean arterial diameter) x 100 %). SR area under the curve

8

161   (AUC) was calculated from post cuff deflation until the point of peak vessel

162   diameter and formulated using the trapezoid rule (Harris et al., 2010).

163

164   **Shear rate measurements**

165   Brachial artery mean SR measurements were acquired at the same location as FMD

166   measures, as indicated by an indelible marking at the site. SRs were measured at Pre

167   Condition, Post Condition and Post each Break (at 22, 44 and 66-min during each

168   condition). Measures were collected at 1.5 minutes following each set of

169   calisthenics, providing time for probe positioning and imaging. Time points were

170   replicated in the Sit condition (Time 1, Time 2 and Time 3; Figure 1).

171

172   For each SR measurement 20 heart cycles of blood velocity and arterial diameter

173   data were collected. From this mean SR (4 x [mean blood velocity/arterial diameter

174   at the measurement time]) could be calculated in accordance to published guidelines

175   (Harris et al., 2010).

176

177   **Heart rate and blood pressure**

178   HR was continually monitored (S610i, Polar Electro Oy, Kempele, Finland) with

179   measures taken at 5 second intervals. Blood pressure (BP) was measured at left

180   brachial artery (MX3 plus, Omron, USA) at the same time points as SR measures

181   and mean arterial pressure (MAP) subsequently calculated.

182

183   **Physical activity and sedentary behaviour assessment**

9

184 Participants completed the International Physical Activity Questionnaire (Long form,

185 IPAQ) (Booth, 2000), a validated and reliable measure of adult physical activity

186 (Craig et al., 2003) and sedentary behaviour (Rosenberg et al., 2008).

187

188 **Statistical analyses**

189 ~~All data are presented as mean ± standard deviation (SD).~~ Data were normally

190 distributed, assessed using Shapiro-Wilk test. Technical reasons during data

191 collection meant ten data sets for endothelial function and eight sets for brachial SR

192 were analysed.

193

194 Two-way repeated measures ANOVA were used to compare the effect of condition

195 on relative FMD, basal arterial diameters, mean SR and MAP. All post hoc analyses

196 were performed via paired samples t-test with Bonferroni adjustment. Data are

197 presented as means ± standard deviation (SD) from which effects sizes were

198 calculated (partial eta squared η^2). These were interpreted as: $\eta^2 = 0.01$ considered

199 small, $\eta^2 = 0.06$ considered medium and $\eta^2 = 0.14$ considered large (Cohen, 1988).

200 Data was analysed using statistical software (SPSS Version 18.0, IBM Corporation,

201 Somers,   NY,   USA),   with   significance   accepted   as $p \leq 0.05$.

202 **Results**

203 **Descriptive statistics**

204   Descriptive statistics are shown in Table 1. Participants were classified as

205 highly active but spent over 6.5 hours per day seated (Table 1).

206

207 **Endothelial function**

208   For relative FMD, no significant interaction $(F(1,9)= 0.9, p=0.37, \eta^2=0.09)$,

209 condition $(F(1,9)= 3.6, p=0.12, \eta^2=0.29)$ or time $(F(1,9)= 2.9, p=0.09, \eta^2=0.25)$

210 effects were observed (Pre Sit: 4.58 ±5.20 %; Post Sit: 5.33 ±5.81 %; Pre

211 Calisthenics: 5.36 ±4.07 %; Post Calisthenics: 8.32 ±6.10 %; Figure 2). For SR AUC

212 there were no significant effects for interaction $(F(1,9)= 0.9, p=0.36, \eta^2=0.14)$,

213 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

214 Sit: 3261.6 ±2887.8 s^{-1}; Post Sit: 3191.3 ±2619.4 s^{-1}; Pre Calisthenics: 3903.9

215 ±1948.5 s^{-1}; Post Calisthenics: 3249.8 ±1893.3 s^{-1}). Normalising FMD for SR AUC

216 (dividing the percentage of FMD by SR AUC) resulted in no significant interaction

217 $(F(1,9)= 1.2, p=0.31, \eta^2=0.16)$, condition $(F(1,9)= 0.7, p=0.44, \eta^2=0.11)$ or time

218 $(F(1,9)= 2.9, p=0.12, \eta^2=0.33)$ effects (Pre Sit: 0.001 ±0.004 %/s^{-1}; Post Sit: 0.001

219 ±0.002 %/s^{-1}; Pre Calisthenics: 0.001 ±0.001 %/s^{-1}; Post Calisthenics: 0.003 ±0.002

220 %/s^{-1}). There was no main effect for time $(F(1,9)= 2.5, p=0.15, \eta^2=0.21)$ or condition

221 $(F(1,9)= 3.9, p=0.52, \eta^2=0.05)$ for basal arterial diameters (Pre Sit: 3.72 ±0.69 mm;

222 Post Sit: 3.55 ±0.76 mm; Pre Calisthenics: 3.60 ±0.66 mm; Post Calisthenics: 3.57

223 ±0.79 mm).

224

225 **Shear rate**

11

226 A significant interaction effect was observed for brachial SR (F(4,32)=2.0 p=0.02,

227 η^2=0.12). Post Break SRs were increased in the Calisthenics condition compared to

228 the same time points in the Sit condition, however post-hoc analysis revealed this

229 difference was only statistically significant following Break 3 with Calisthenics

230 elevating SR by 23.22 s^{-1} more compared to Sit at Time 3 (p=0.02, η^2=0.51; Table

231 2). Post Condition, Sit SR remained depressed compared to Calisthenics (p=0.07,

232 η^2=0.36). Finally, Pre Condition SRs were not significantly different between

233 conditions (p=0.88, η^2=0.01).

234

235 **Cardiovascular response**

236 For MAP no significant interaction (F(5,45)= 0.8, p=0.56, η^2=0.08), time (F(5,45)=

237 1.2, p=0.33, η^2=0.12) or condition (F(1,9)= 0.8, p=0.41, η^2=0.08) effects were

238 observed (Sit Pre: 91.1 ±10.2 mmHg, Post: 88.1 ±8.2 mmHg; Calisthenics Pre: 90.0

239 ±9.9 mmHg, Post: 90.9 ±7.4 mmHg). HR in the Calisthenics condition showed a

240 significant main effect for time (F(11,88)= 23.3, p=0.001, η^2=1.0). Post hoc analysis

241 revealed calisthenics significantly increased HR (Break 1: 90.05 ±8.26 b.min^{-1};

242 Break 2: 92.24 ±6.16 b.min^{-1}; Break 3: 92.51 ±7.24 b.min^{-1}; Figure 3) compared to

243 ~~Baseline~~ Pre Condition (68.90 ±9.10 b.min^{-1}; p=0.001) and HR remained elevated

244 above ~~Baseline~~ Pre Condition at 30 seconds following each break (p=0.001). In the

245 Sit condition, no main effect for time was observed (F(4,36)= 1.2, p=0.34, η^2=0.33)

246 with HR only slightly reduced over time (Pre: 70.25 ±8.92 b.min^{-1}; Post: 66.21

247 ±8.72 b.min^{-1}).

248

12

Discussion

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

Calisthenics activity breaks and endothelial function

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

273

274   For the first time, changes in SR immediately after activity breaks to sitting have

275   been characterised. Brachial SR was elevated following each set of calisthenics and,

276   of particular interest, the increase in SR was greater after break three than break one,

277   suggesting a possible accumulative effect of the calisthenics interventions.

278   Moreover, SR measured 20 minutes after the final activity break had returned to a

279   value similar to baseline; highlighting the need to frequently disrupt sedentary

280   periods in order to maintain elevated SR.

281

282   Repeated episodic increases in SR are a suggested mechanism underlying exercise

283   related improvements in endothelial function (Padilla et al., 2008; Tinken et al.,

284   2010). In the current study, although over a short time period, each break replicated

285   this repetitive stimulus, suggesting that SR alterations could be a mechanism to

286   explain the slight increase brachial FMD observed. Indeed, this has previously been

287   proposed as an explanation for the maintenance of femoral artery endothelial

288   function when sedentary time was broken up with walking bouts (Thosar et al.,

289   2015). However, in the study by Thosar et al. (2015) SR measures were taken over

290   20 minutes after each walk, consequently assessing SR immediately post-activity

291   allows a clearer mechanistic explanation to describe the effects activity breaks

292   during sitting have on the vascular system. Future work should seek to characterise

293   the SR response following an activity break at more regular intervals to determine

294   the length of time over which elevations in SR persist.

295

296   Despite calisthenics not significantly changing brachial FMD, this does not imply it

297   is not an effective intervention choice to break up sedentary time. Results show that

14

298   mean brachial SR can be acutely increased using this intervention and, as the

299   calisthenics routine employed was a whole body exercise, such elevations may also

300   have been present in the lower limbs. Previous work (Thosar et al., 2015; Restaino et

301   al., 2015; 2016) has shown this vasculature is more susceptible to decreases in

302   endothelial function following prolonged sitting periods. Consequently, future

303   research should use calisthenics and investigate changes in lower limb vascular

304   function.

305

Sitting did not attenuate endothelial function

307   In agreement with existing literature (Thosar et al., 2014; Restaino et al., 2015)

308   continuous sitting did not decrease brachial FMD compared to baseline values.

309   Additionally, no significant reductions in brachial SR were observed. This may

310   provide an explanation for this outcome, as decreased SR is a proposed mechanism

311   for the reduction in lower limb endothelial function following sitting (Thosar et al.,

312   2014; 2015; Restaino et al., 2016). Importantly during these studies participants'

313   lower limbs remained motionless (Restaino et al., 2015; Thosar et al., 2015) whereas

314   in this current study participants were permitted to move their upper limbs to

315   complete desk-based tasks. This may have resulted in low level muscular

316   contractions, maintaining blood flow and SR. In support of this, upper limb

317   movement during bed rest enhanced brachial FMD (Hamburg et al., 2007), whilst

318   reducing daily step count attenuated femoral but not brachial FMD, related to a

319   larger reduction in lower limb perfusion compared to the upper limbs (Boyle et al.,

320   2013).

321

322   The absence of a decline in mean brachial SR after one hour and six minutes of

323   sitting is in line with existing research, as SR was not significantly reduced after the

324   first hour of a three hour sedentary period (Thosar et al., 2014). However, mean

325   brachial SR was significantly attenuated after two hours of sitting and remained

326   depressed for the further five hours of assessment (Restaino et al., 2015). This

327   indicates that the brachial artery is susceptible to SR reductions, however the results

328   from this current study and Thosar et al. (2014) suggest within an hour of sitting this

329   is not apparent. In the current study, the measure taken after one hour and six

330   minutes resulted in the largest reduction in SR which then remained slightly

331   depressed for the post-condition measure, indicating that reductions may have

332   continued if the time period was extended, in line with existing research (Restaino et

333   al., 2015). Taken together these results support the idea that the brachial artery is

334   more ~~resistance~~ resistive to the negative effects of sitting (Restaino et al., 2015), as

335   popliteal and femoral mean SR are reduced within the first hour of sitting (Thosar et

336   al., 2015; Restaino et al., 2015). Moreover, even when reductions in brachial SR

337   have been observed the magnitude of decline was less than that in the lower limbs

338   and only microvascular function was attenuated (Restaino et al., 2015).

339

340   **Limitations and future research**

341   As aforementioned, it is likely that the continuous sitting condition duration was not

342   long enough for significant changes to endothelial function and SR to occur. As there

343   is no definition as to what is considered prolonged sitting (Dunstan et al., 2012a) the

344   current design was chosen to provide initial data on calisthenics and endothelial

345   function without excessive participant demands. Previous research considering

346   metabolic health and changes to endothelial function has utilised longer assessment

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347   periods of up to nine hours (Dunstan et al., 2012b; Peddie et al., 2013; Thosar et al.,

348   2014; 2015; Restaino et al., 2015). Additionally, the population assessed were

349   young, healthy and had high levels of physical activity. Larger changes to

350   endothelial function may have occurred with an older or less active population.

351   Indeed, exercise status and age are known to influence endothelial responses (Black

352   et al., 2009). Furthermore, participants' transport to the laboratory was not

353   standardised, therefore pre-testing physical activity may have varied between

354   participants. However, based on the location of the university campus, it is assumed

355   participants would have used car or bus transportation; moreover participants were

356   asked to use the same form of transport for each test visit. Finally, dDue to the pilot

357   nature of this research, it is possible that the study was underpowered to detect

358   changes in FMD., Although as previous work has shown significant differences with

359   a sample size of 12 (Thosar et al., 2015) a power calculation using our data suggests

360   our study may be underpowered and that 30 participants might be needed to detect a

361   significant change in relative -FMD. This As this study shows that calisthenics are

362   feasible for participants to complete therefore future work should therefore use a

363   larger sample size.

364

365   This study presents results following a single sitting period, however of greater

366   ecological interest is the influence of repeatedly performing either the calisthenics

367   intervention or prolonged sitting over several days or weeks. This would determine

368   if, as in exercise training studies, the repeated exposure of the vasculature to

369   increases in SR can lead to longer term improvements in endothelial function.

370   Furthermore, if repeated prolonged sitting periods does decrease endothelial function

371   it would further support the need for a reduction of this behaviour in the population.

17

372 In practical terms, longer term research within a workplace environment would

373 determine the feasibility of individuals carrying out multiple breaks to their daily

374 routine, alongside assessing behavioural change and adherence.

375

376 **Conclusions**

377 For the first time, this study demonstrates breaking up sedentary time with

378 calisthenics can lead to transient increases in HR and brachial artery mean SR. This

379 did not significantly increase brachial artery endothelial function, however a longer

380 assessment period more representative of a working day may be needed to result in a

381 significant improvement. Additionally results show that brachial artery endothelial

382 function is not negatively affected by an acute siting period, when upper limb

383 movement typical of daily living is permitted. Overall, the SR results from this study

384 present calisthenics as a potential intervention to break up sedentary time and

385 enhance or maintain cardiovascular health. Longer term research, assessing upper

386 and lower limb endothelial function is required to assess whether repeatedly

387 exposing the vascular system to increases in SR can lead to larger improvements in

388 endothelial function.

389

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507 **Additional Information**

508 **Competing interests:**

509 None declared.

510

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512 None declared.

513 **Tables**

514 **Table 1:** Descriptive characteristics, self-reported physical activity scores and total

515 sitting time of participants (n= 10)

516

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

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26

518 **Table 2:** Brachial artery shear rate (SR) at baseline (Pre) and after (Post) two experimental conditions: a) uninterrupted sitting for 1-hr 26-min

519 (Sit) or b) disrupting this sitting every 20-min performing a set of calisthenics (Calisthenics). In Calisthenics SR was measured after each break

520 (Break 1, Break 2 and Break 3) and these time points were matched in Sit condition (Time 1, Time 2 and Time 3).

		Sit					Calisthenics			
Measurement	**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

521

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

27

Figure Legends

523

524 **Figure 1:** The experimental design and measurement time points for the two

525 conditions, completed in a randomised order on separate days. (a) Sitting for 1-hr 26-

526 min, or (b) breaking up this period every 20-min with a 2-min set of calisthenics

527 exercises. FMD- flow mediated dilation; SR- shear rate.

528

529 **Figure 2:** Brachial artery flow mediated dilation (FMD) before (Pre) and following

530 (Post) either 1-hr 26-min uninterrupted sitting (Sit) or, breaking up this period every

531 20-min with 2-min of performing calisthenics exercises (Calisthenics). (Error bars=

532 ±SD)

533

534 **Figure 3:** Mean heart rate (HR) prior to each condition (Pre), during each

535 calisthenics activity break (Break 1, Break 2 and Break 3), and during the 5-min of

536 recovery following this intervention. * indicates a significantly greater HR than Pre

537 (p=0.001). (Error bars= ±SD)

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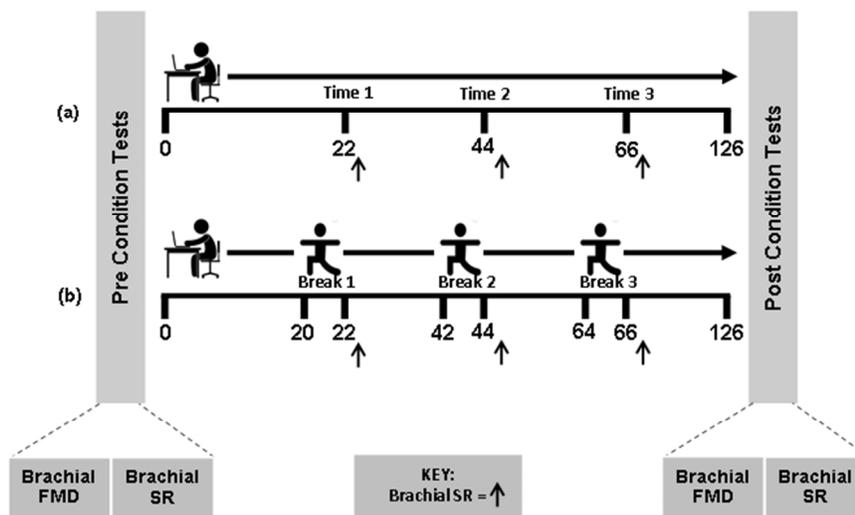
Figure 1: The experimental design and measurement time points for the two conditions, completed in a randomised order on separate days. (a) Sitting for 1-hr 26-min, or (b) breaking up this period every 20-min with a 2-min set of calisthenics exercises. FMD- flow mediated dilation; SR- shear rate.
162x84mm (120 x 120 DPI)
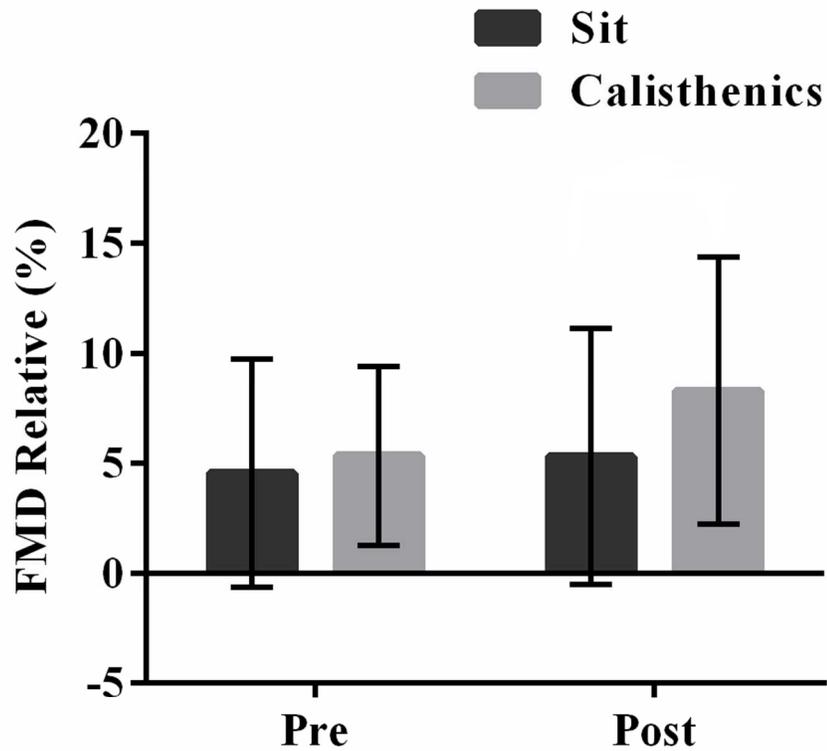
Figure 2: Brachial artery flow mediated dilation (FMD) before (Pre) and following (Post) either 1-hr 26-min uninterrupted sitting (Sit) or, breaking up this period every 20-min with 2-min of performing calisthenics exercises (Calisthenics). (Error bars= ±SD)
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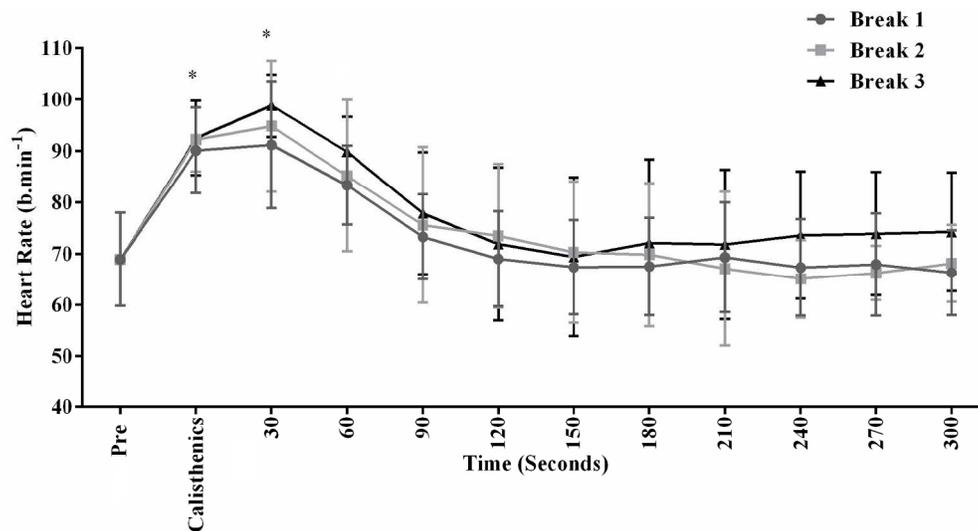
Figure 3: Mean heart rate (HR) prior to each condition (Pre), during each calisthenics activity break (Break 1, Break 2 and Break 3),and during the 5-min of recovery following this intervention * indicates a significantly greater HR than Pre (p=0.001). (Error bars= ±SD)
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