LJMU Research Online

Holder, SM, Dawson, EA, Brislane, Á, Hisdal, J, Green, DJ and Thijssen, DHJ

Fluctuation in Shear Rate, with Unaltered Mean Shear Rate, Improves Brachial Artery Flow-Mediated Dilation in Healthy, Young Men.

http://researchonline.ljmu.ac.uk/id/eprint/10657/

Article

For more information please contact researchonline@ljmu.ac.uk

FLUCTUATION IN SHEAR RATE, WITH UNALTERED MEAN SHEAR RATE, IMPROVES BRACHIAL ARTERY FLOW-MEDIATED DILATION IN HEALTHY, YOUNG MEN

Sophie M. Holder[1], Ellen A. Dawson[1], Áine Brislane[1,2], Jonny Hisdal[3], Daniel J. Green[4] & Dick H.J. Thijssen[1,5]

[1]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom.*

[2]*School of Sport and Exercise Science, York St John University, York, United Kingdom.*

[3]*Section of Vascular Investigations, Division of Cardiovascular and Pulmonary Diseases, Department of Vascular Surgery, Oslo University Hospital, Oslo, Norway.*

[4]*School of Human Sciences (Exercise and Sports Science), The University of Western Australia, Crawley, Western Australia, 6009.*

[5]*Radboud Institute for Health Sciences, Department of Physiology, Radboud University Medical Center, the Netherlands.*

Word count: 4718

Figures: 4

Tables: 2

Short title: Shear fluctuations improve brachial endothelial function

Corresponding author: Prof. Dick H.J. Thijssen, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Byrom Street, L3 3AF, Liverpool, United Kingdom, Tel: +441519046264, E-mail: d.thijssen@ljmu.ac.uk

Abstract

Aim: Increase in mean shear stress represents an important and potent hemodynamic stimulus to improve conduit artery endothelial function in humans. No previous study has examined whether fluctuations in shear rate patterns, without altering mean shear stress, impacts conduit artery endothelial function. This study examined the hypothesis that 30-minutes exposure to fluctuations in shear rate patterns, in the presence of unaltered mean shear rate, improves brachial artery flow-mediated dilation.

Methods: Fifteen healthy males (27.3±5.0 years) completed the study. Bilateral brachial artery flow-mediated dilation was assessed before and after unilateral exposure to 30-minutes of intermittent negative pressure (10seconds -40mmHg, 7seconds 0mmHg) to induce fluctuation in shear rate, whilst the contra-lateral arm was exposed to a resting period.

Results: Negative pressure significantly increased shear rate, followed by a decrease in shear rate upon pressure release (both $P<0.001$). Across the 30-minute intervention, mean shear rate was not different compared to baseline ($P=0.458$). A linear mixed model revealed a significant effect of time was observed for flow-mediated dilation ($P=0.029$), with exploratory post-hoc analysis showing an increase in the intervention arm (ΔFMD +2.0%, $P=0.008$), but not in the contra-lateral control arm (ΔFMD +0.5%, $P=0.664$). However, there was no effect for arm ($P=0.619$) or interaction effect ($P=0.096$).

Conclusion: In conclusion, we found that fluctuations in shear patterns, with unaltered mean shear, improves brachial artery flow-mediated dilation. These novel data suggest that fluctuations in shear pattern, even in the absence of altered mean shear, represents a stimulus to acute change in endothelial function in healthy individuals.

Key words: endothelial function, flow-mediated dilation, fluctuations, shear rate.

New & Noteworthy

Intermittent negative pressure applied to the forearm induced significant fluctuations in antegrade and retrograde shear rate, whilst mean shear was preserved relative to baseline. Our exploratory study revealed that brachial artery flow-mediated dilation was significantly improved following 30-minutes exposure to intermittent negative pressure. Fluctuations in blood flow or shear rate, with unaltered mean shear, may have important implications for vascular health, however further research is required to identify the underlying mechanisms and potential long-term health benefits.

Introduction

Hemodynamic stimuli play an important role in inducing functional and structural changes in the arterial wall via endothelial cell signal transduction (12). More specifically, increased mean shear stress represents a key stimulus for vascular adaptation, for example in response to exercise training (5, 12, 35). Manipulating shear rate through exercise or heating has provided *in vivo* evidence that elevation in mean shear rate mediates acute (13, 34) and chronic (19) improvement in endothelial function, measured by flow-mediated dilation (FMD). In addition to levels of mean shear stress, the pattern of shear stress is important, since increasing the antegrade shear component was associated with improved FMD, whilst increasing retrograde and oscillatory shear is associated with impaired FMD (22, 31).

Recently, Sundby and colleagues (27) showed that exposure to intermittent negative pressure (10-seconds negative pressure (-40 mmHg), 7-seconds atmospheric pressure) causes fluctuations in patterns of blood flow and shear rate. More specifically, increased antegrade and mean blood flow (velocity) was present at the onset of negative pressure, followed by marked reduction in antegrade and mean blood flow (and increase in retrograde blood flow) upon release of the negative pressure. Interestingly, frequent use of intermittent negative pressure in patients with lower limb ischaemia and ulcers is associated with improved wound healing (25, 26, 28). These clinical effects suggest that fluctuations in blood flow and shear stress patterns may impact vascular health in humans. Unfortunately, these studies did not **control for potential increases in mean shear levels**. Therefore, it remains unclear whether these observations are linked to repetitive exposure to fluctuations in shear, or whether observations were simply explained through increases in mean shear stress levels.

4

60

61 To the best of our knowledge, no previous study in animals or humans has directly

62 examined whether fluctuations in blood flow and shear stress patterns, in the presence

63 of unaltered mean blood flow and shear rate, impacts upon endothelial function.

64 Therefore, we assessed the effect of 30-minute exposure to intermittent negative

65 pressure, which mediates fluctuations in blood flow and shear rate patterns through

66 the brachial artery, on FMD (a measure of largely nitric oxide-mediated, endothelial

67 function (11)) in healthy young men. We hypothesised that fluctuations in blood flow

68 and shear stress patterns would induce improvement in brachial artery endothelial

69 function. Since fluctuations in mean shear stress are relevant to many activities of daily

70 living, we planned this study to provide insight into the potential clinical relevance of

71 fluctuations in shear stress as a hemodynamic stimulus for improvement in vascular

72 health *in vivo*.

73

74

75 **Materials and Methods**

76 *Participants*

77 Fifteen healthy males (age 27.3±5.0 years) were recruited for the study. All

78 participants were non-smokers, not taking medication and/or supplements known to

79 influence the cardiovascular system and free from cardiovascular/metabolic disease

80 risk factors. Based on a pre-screening health questionnaire, participants were

81 excluded if they had poor circulation (including diagnosis of peripheral vascular

82 disease or Reynaud's disease). Each participant provided written informed consent

83 before taking part in the experimental procedure. The research study was ethically

84 approved by the Liverpool John Moores School of Sport and Exercise Science

85 Research Ethics Committee and adhered to the Declaration of Helsinki.

86

87 *Experimental Design*

88 After 15 minutes of supine resting, we bilaterally examined brachial artery endothelial

89 function using the FMD test (29). This was followed by a 10-minute rest period to allow

90 blood flow and diameter to return to baseline levels. Subsequently, following a 1-

91 minute recording of baseline diameter and blood flow velocity, subjects underwent a

92 30-minute intervention involving intermittent negative pressure (i.e. left arm), whilst the

93 right arm served as a control arm. Within 2-minutes of this intervention, we repeated

94 bilateral brachial artery FMD testing.

95

96 *Preparations*

97 Prior to the laboratory visit, all participants were instructed to refrain from strenuous

98 exercise for at least 24 hours, alcohol for 12 hours, avoid all caffeinated products for

99 8 hours and food products high in polyphenols for 24 hours. Participants reported to

100 the quiet, temperature-controlled laboratory after fasting for at least 6 hours. After

101 reporting to our laboratory, stature and body mass were recorded to the nearest 0.1

102 unit using a stadiometer and digital scales respectively. Body mass index (BMI) was

103 calculated as body mass in kilograms divided by stature in metres squared (kg/m^2).

104

105 *Brachial artery flow-mediated dilation.* Brachial artery FMD was measured in

106 accordance with contemporary expert-consensus guidelines (29). Following 15

107 minutes of supine rest, left and right brachial artery diameter were assessed

108 simultaneously via high-resolution duplex ultrasound (Terason u-smart 3300, Teratech,

109   Burlington, MA) with a 10-12 MHz linear array probe. B-mode images were obtained

110   and optimised, and the probe was held in the same position for the duration of the test.

111   After 1 minute of baseline measurement, occlusion cuffs, connected to a rapid inflator

112   (Hokanson, Bellevue, WA), placed around both forearms, distal to the humeral

113   epicondyle, were inflated to a pressure of 220 mmHg for 5 minutes. Recording was

114   resumed 30-seconds prior to cuff deflation, and FMD was recorded for a further 3

115   minutes post cuff deflation. All measurements were taken by the same experienced

116   operators within participants. Bilateral FMD was repeated following the 30-minute

117   intervention period.

118

119   *Brachial artery diameter and shear rate.* High-resolution ultrasound (Terason u-smart

120   3300; Teratech, Burlington, MA) was used to examine brachial artery diameter and

121   shear rate as described above. Following the pre-intervention FMD, the participant's

122   skin was marked to ensure a consistent ultrasound probe position and therefore artery

123   segment during the visit. Furthermore, the ultrasound machine settings remained

124   constant (i.e. depth and Doppler cursor position) in order to assume the same probe

125   angle whilst imaging. Bilateral artery diameter and shear rate were recorded for 1-

126   minute baseline, and repeated at 5-minute intervals during the 30-minute intervention

127   period.

128

129   *Intervention.* During the laboratory visit, participants rested in the supine position with

130   both arms extended away from their body to approximately 80°, with their palms facing

131   upwards for optimal ultrasound imaging of the brachial artery. During the 10-minute

132   rest period following the pre-intervention FMD, the left arm was placed inside a rigid

133   plastic cylinder (8.5x40cm) connected to a pressure control box (FlowOx™, Otivio AS,

134 Oslo, Norway; Figure 1). The cylinder was sealed around the forearm with a

135 thermoplastic elastomer (TPS-SEBS). The arm was exposed to repeated bouts of

136 negative pressure (-40 mmHg; 10 seconds negative pressure, 7 seconds atmospheric

137 pressure) for 30 minutes (~105 full cycles of negative pressure).

138

139 *Blood pressure.* Blood pressure and heart rate were recorded continuously during the

140 protocol from the right (control) arm index/middle finger using a Portapres (Finapres

141 Medical Systems BV, Amsterdam, The Netherlands). This data were displayed,

142 recorded and exported using PowerLab software (ADInstruments, Australia). The

143 difference in blood pressure and heart rate was calculated from a 1-minute recording

144 before the intervention period started, and the last minute of the intervention.

145

146 *Data analysis.* All FMD data analysis was performed blinded by the same observer,

147 using a specialised custom-designed edge-detection and wall-tracking software, of

148 which the reproducibility and validity have been demonstrated elsewhere (39). This

149 software tracks the vessel walls and blood flow velocity trace in B-mode frames via

150 pixel density and frequency distribution algorithm. An optimal region of interest to be

151 analysed was selected by the sonographer, chosen on the basis of the quality of the

152 image, in regards to clear distinction between the artery walls and lumen. The FMD

153 was defined as the maximum percentage change in artery diameter from baseline to

154 peak during the 3 minutes post cuff release. The software automatically calculated the

155 relative diameter change, time to peak (following cuff release) and shear rate area-

156 under-the-curve (SRAUC). Despite the initial region of interest selection being

157 operator-determined, the remaining analysis was independent of operator bias.

158

159 Brachial artery diameter and shear rate were analysed using the custom-designed

160 software described above. The region of interest location (selected by the operator)

161 remained consistent for each 1-minute recording *within* participants. Using markers

162 placed by the operator, the software calculated the average artery diameter and shear

163 rate across the minute recordings. The fluctuations in shear stress were analysed as

164 an average during the application of negative pressure (10secs; On), atmospheric

165 pressure (7secs; Off), and the full cycle, then repeated for the 3 full cycles captured

166 during each 1-minute recording. These processes were repeated for each time point

167 during the intervention. Data from a representative individual are presented in Figure

168 2.

169

170 *Statistical analysis.* Statistical analysis was conducted using IBM SPSS version 25

171 (SPSS Inc., Chicago, IL). Allometric scaling was performed on FMD data to control for

172 differences in baseline diameter (3, 4). A linear mixed model with covariate control for

173 SRAUC and scaled baseline diameter determined the main effect for time (pre-post)

174 and arm. A general linear model assessed the changes in blood pressure and heart

175 rate across the intervention period. Paired T-tests determined the difference in

176 antegrade and retrograde shear during intermittent negative pressure compared to

177 baseline in both arms. Statistical significance was recognised when a *P* value <0.05

178 was observed. Data are presented as mean±standard deviation unless stated

179 otherwise.

180

181

182 **Results**

183 Subject characteristics are presented in Table 1.

184 *Brachial artery blood flow and shear rate.* There were no significant changes across

185 the 30-minute intervention in heart rate (52±7 bpm *versus* 54±8 bpm, P=0.47) or in

186 systolic (129±9 mmHg *versus* 135±12 mmHg, P=0.16), diastolic (55±8 mmHg *versus*

187 59±9 mmHg, P=0.36) or mean blood pressure (80±8 mmHg *versus* 84±9 mmHg,

188 P=0.23). Negative pressure was associated with a significant increase in mean shear

189 rate, whilst pressure release was followed by a significant decrease in mean shear

190 rate, to levels below baseline ("pressure on": Δ+34.2s^{-1}, "pressure off": Δ-26.5s^{-1}; both

191 *P*<0.001; Figure 3A). Consequently, mean shear rate across the intervention period

192 was not different from baseline ("pressure on/off cycle": Δ+3.8s^{-1}; *P*=0.458). In the

193 control arm, negative pressure did not change mean shear from baseline levels

194 ("pressure on": Δ+1.6 *P*=0.805, "pressure off": Δ+3.5s^{-1} *P*=0.613). Therefore, mean

195 shear rate remained unchanged throughout the intervention period compared to

196 baseline ("pressure on/off cycle": Δ+2.5s^{-1} *P*=0.702; Figure 3B).

197

198 When examining shear patterns, negative pressure increased antegrade shear rate

199 (*P*<0.001) and decreased retrograde shear rate (*P*=0.006, Figure 3). Upon pressure

200 release, compared to baseline levels, a decrease in antegrade shear rate and increase

201 in retrograde shear rate was found (*P*=0.003 and *P*<0.001, respectively). As a result,

202 mean antegrade and retrograde shear rate across the 30-minute intervention period

203 was not different from baseline (*P*=0.504 and 0.777, respectively). Antegrade and

204 retrograde shear rate remained unaltered from baseline in the control arm during

205 "pressure on" (antegrade: Δ+2.5s^{-1}, *P*=0.730; retrograde: Δ-1.9s^{-1}, *P*=0.190) and

206 "pressure off" (antegrade: Δ+1.9s^{-1}, *P*=0.779; retrograde: Δ-2.0s^{-1}, *P*=0.164).

207 Therefore, mean antegrade and retrograde shear rate was not different from baseline

208 across the intervention (antegrade: Δ+2.2s^{-1}, *P*=0.750; retrograde: Δ-1.9s^{-1}, *P*=0.173).

209 *Brachial artery FMD.* Linear mixed model analysis revealed a significant main effect

210 for time (P=0.029; F-ratio=5.146), whilst no effect was observed for arm (P=0.619; F-

211 ratio=0.251) or interaction effect (P=0.096; F-ratio=2.906). Post-hoc exploratory

212 analysis revealed a significant increase in FMD in the intervention arm (Δ+2.0%,

213 P=0.008), whilst no change was observed in the control arm (Δ+0.5%, P=0.664).

214 Individual FMD responses are presented in Figure 4 and all associated parameters

215 (mean and 95% confidence intervals) are presented in Table 2.

216

217

218 **Discussion**

219 We show that application of intermittent negative pressure to the forearm increases

220 antegrade blood flow and shear rate, whilst pressure release mediates increased

221 retrograde blood flow and shear rate measured at the brachial artery, relative to

222 baseline and the contralateral control arm. Despite these marked fluctuations in blood

223 flow and shear rate patterns throughout the 30-minute intervention, mean blood flow

224 and shear rate was not different from baseline. We therefore successfully preserved

225 average resting levels of flow and shear rate, despite inducing fluctuations of these

226 variables. Although exploratory in nature, we observed improved brachial artery FMD

227 as a result of these fluctuations in blood flow and shear rate, an effect that was not

228 apparent in the contralateral control limb. Taken together, these findings suggest that

229 fluctuations in shear rate, independent of mean blood flow and shear rate, may impact

230 acute vascular function in healthy young individuals. Whilst further research is required,

231 this contributes to improving our understanding of shear stress as an important

232 hemodynamic stimulus in the adaptation of vascular health in humans *in vivo.*

233

11

234 Our findings regarding the impact of cyclical negative pressure are in line with a

235 previous study in the lower limbs (27). Importantly, our study adds the novel

236 knowledge that these fluctuations were associated with improvements in endothelial

237 function, as measured with the brachial artery FMD. Blood pressure and heart rate

238 remained unaltered during the intervention period, effectively excluding the possibility

239 that systemic factors contributed to our observations. To further support this notion,

240 no changes in brachial artery blood flow or shear rate were found in the contralateral

241 arm. This strongly suggests that the mechanisms contributing to the increase in FMD

242 in the intervention arm relate to local effects (i.e. fluctuations in shear rate) rather than

243 systemic/circulating factors.

244

245 Our novel results may be somewhat surprising, in that the fluctuations in shear rate

246 were not accompanied by changes in mean shear rate, but still caused an increase in

247 FMD. In our previous work, supported by studies in animals (21, 38), we consistently

248 found that changes in mean shear rate are essential to change FMD (31, 34). More

249 specifically, selective increases in antegrade shear rate (and therefore mean shear

250 rate) were related to improved FMD (13, 34), whilst an isolated increase in retrograde

251 shear rate (i.e. lower mean shear rate) was associated with a dose-dependent

252 decrease in brachial and femoral artery FMD (22, 31). One potential explanation for

253 the increase in FMD is the relative larger importance of increases in antegrade shear

254 rate compared to changes in retrograde shear rate. To support this idea, moderate-

255 intensity cycling exercise acutely increases retrograde shear rate (10, 30), followed by

256 normalisation after ~15 minutes with a concomitant increase in antegrade shear rate

257 (23). Nonetheless, acute or chronic performance of cycling exercise (i.e. 30-/40-min

258 bouts) leads to improvement in brachial artery FMD (5, 12). This evolving hypothesis

259   that changes in antegrade shear rate may be relatively more important than changes

260   in retrograde shear rate warrants further investigation.

261

262   Another explanation for our findings relates to the importance of fluctuations in shear

263   rate patterns, rather than mean shear rate. In the microcirculation, previous work used

264   mathematical simulation to support the concept that fluctuations of capillary blood flow,

265   rather than steady-state conditions, improve oxygenation of tissue (36). Follow-up

266   work in humans examining skin perfusion and oxygenation demonstrated that periodic

267   fluctuations in vasomotion may be beneficial for local oxygenation (32). In conduit

268   arteries, some studies have found that enhanced external counterpulsation increased

269   shear rate fluctuations and FMD in the brachial artery (6, 15). However, these changes

270   were also accompanied by an overall increase in mean shear rate, making it

271   impossible to isolate the impact of fluctuations *per se* (i.e. in the absence of changes

272   in mean shear). Finally, indirect support for a potential clinically-relevant, beneficial

273   effect on vascular health for these fluctuations is provided by the observation of

274   improved wound healing upon repeated exposure to intermittent negative pressure

275   (26, 28). These observations may contribute to improved microcirculatory blood flow

276   and therefore the delivery of oxygen and nutrients to promote wound healing (25, 26).

277   Although speculative, our findings suggest that these benefits of intermittent negative

278   pressure stimulus on wound healing (26, 28) may be related to enhanced endothelial

279   function.

280

281   A final possible explanation for our findings relates to the impact of intermittent

282   negative pressure on changes in the pressure gradient across the artery wall (24) and,

283   therefore, transmural pressure (20). Although changes in transmural pressure may

284 affect vascular health (2, 12), it seems unlikely this can explain our findings. First,

285 negative pressure likely increases transmural pressure (due to the drop in external

286 pressure), which is typically associated with impaired vascular health (2). Secondly,

287 vascular function was examined in the brachial artery, i.e. not directly exposed to the

288 changes in (transmural) pressure, and we observed no significant systemic effects on

289 blood pressure of unilateral forearm suction.

290

291 The clinical relevance of our findings is that fluctuations in blood flow or shear rate *per*

292 *se* represent a hemodynamic stimulus capable of improving vascular health. Previous

293 studies manipulating shear rate have increased mean shear rate to improve FMD. In

294 contrast to these stimuli, we have not changed mean shear rate, but still found

295 improved FMD, most likely due to the fluctuations in shear and blood flow patterns.

296 Furthermore, these fluctuations in blood flow and shear rate may be more ecologically

297 valid compared to sustained increases in shear rate. More specifically, fluctuations in

298 blood flow and shear rate are more related to activities of daily living, such as those

299 associated with low-intensity physical activity and changes in posture. Therefore,

300 repetitive exposure to these stimuli may be efficient in improving vascular health.

301 Indeed, recent work has demonstrated that regular exposure to mild physical activity

302 stimuli, such as walking breaks (8, 33) or fidgeting (18), prevents decline in cerebro-

303 and cardiovascular health associated with prolonged sitting. Although speculative,

304 activity-induced fluctuations in blood flow may be the underlying mediator contributing

305 to the preserved vascular health.

306 *Limitations.* The present study possesses several strengths, including strict adherence

307 to contemporary expert-consensus guidelines for FMD (29) and blinded data analysis

308 using custom-designed edge-detection software to eliminate operator bias. There are

309   some limitations to the study. Firstly, we recruited healthy recreationally active males,

310   which makes it difficult to extrapolate our findings to other populations (e.g. females)

311   (7, 16, 37) or clinical groups. However, larger improvements in FMD may be observed

312   in those with *a priori* endothelial dysfunction (17). A second limitation is that we did not

313   perform additional measurements such as blood analysis for markers of endothelial

314   cell activity. *In vitro* studies in cultured endothelial cells and isolated arteries, reviewed

315   elsewhere (12), demonstrate the release of pro- and anti-atherogenic substances in

316   response to exposure to oscillatory (or low) and laminar (or high) shear stress

317   respectively. Insight into the impact of fluctuations in shear stress (with preserved

318   mean shear) would have contributed to further understanding the underlying

319   mechanisms of our findings. A final limitation relates to the relatively small sample size

320   of our study. Post-hoc statistical power analysis using G*Power software (9) revealed

321   a power of 0.77 to detect within-subject changes in FMD, but a power of 0.27 to find a

322   significant interaction effect. Therefore, our results should be interpreted with caution,

323   and further work is required to better understand the potency of fluctuations in shear

324   rate patterns on vascular function.

325

326

327   **Conclusion**

328   In conclusion, our findings suggest that 30-minutes exposure to fluctuations in shear

329   rate improves endothelial function, despite the absence of concomitant changes in

330   mean shear rate compared to resting baseline levels. Our work implies that

331   fluctuations in blood flow or shear rate may represent a hemodynamic stimulus to

332   potentially improve vascular health. Future research to examine the underlying

333   mechanisms and potential long-term effects would be of interest.

334

339 **Disclosures**

340 None.

References

341 **References**

342 1.   **Atkinson CL, Carter HH, Dawson EA, Naylor LH, Thijssen DH, and Green DJ**.
343 Impact of handgrip exercise intensity on brachial artery flow-mediated dilation. *Eur J Appl*
344 *Physiol* 115: 1705-1713, 2015.
345 2.   **Atkinson CL, Carter HH, Naylor LH, Dawson EA, Marusic P, Hering D,**
346 **Schlaich MP, Thijssen DH, and Green DJ**. Opposing effects of shear-mediated dilation and
347 myogenic constriction on artery diameter in response to handgrip exercise in humans.
348 *Journal of Applied Physiology* 119: 858-864, 2015.
349 3.   **Atkinson G, and Batterham AM**. Allometric scaling of diameter change in the
350 original flow-mediated dilation protocol. *Atherosclerosis* 226: 425-427, 2013.
351 4.   **Atkinson G, Batterham AM, Thijssen DH, and Green DJ**. A new approach to
352 improve the specificity of flow-mediated dilation for indicating endothelial function in
353 cardiovascular research. *Journal of Hypertension* 31: 287-291, 2013.
354 5.   **Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, Thijssen DH, and**
355 **Green DJ**. Brachial artery adaptation to lower limb exercise training: role of shear stress.
356 *Journal of Applied Physiology* 112: 1653-1658, 2012.
357 6.   **Braith RW, Conti CR, Nichols WW, Choi CY, Khuddus MA, Beck DT, and**
358 **Casey DP**. Enhanced external counterpulsation improves peripheral artery flow-mediated
359 dilation in patients with chronic angina: a randomized sham-controlled study. *Circulation*
360 122: 1612-1620, 2010.
361 7.   **Brandão AHF, Serra PJ, Zanolla K, Cabral ACV, and Geber S**. Variation of
362 endothelial function during the menstrual cycle evaluated by flow-mediated dilatation of
363 brachial artery. *JBRA Assisted Reproduction* 18: 148-150, 2014.
364 8.   **Carter SE, Draijer R, Holder SM, Brown L, Thijssen DHJ, and Hopkins ND**.
365 Regular walking breaks prevent the decline in cerebral blood flow associated with prolonged
366 sitting. *J Appl Physiol (1985)* 2018.
367 9.   **Faul F, Erdfelder E, Lang AG, and Buchner A**. G*Power 3: a flexible statistical
368 power analysis program for the social, behavioral, and biomedical sciences. *Behav Res*
369 *Methods* 39: 175-191, 2007.
370 10.   **Green D, Cheetham C, Reed C, Dembo L, and O'Driscoll G**. Assessment of
371 brachial artery blood flow across the cardiac cycle: retrograde flows during cycle ergometry.
372 *J Appl Physiol* 93: 361-368, 2002.
373 11.   **Green DJ, Dawson EA, Groenewoud HM, Jones H, and Thijssen DH**. Is flow-
374 mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension* 63: 376-382, 2014.
375 12.   **Green DJ, Hopman MT, Padilla J, Laughlin H, and Thijssen DH**. Vascular
376 adaptation to exercise in humans: role of hemodynamic stimuli. *Physiological Reviews* 97: 1-
377 33, 2017.
378 13.   **Greyling A, Schreuder TH, Landman T, Draijer R, Verheggen RJ, Hopman MT,**
379 **and Thijssen DH**. Elevation in blood flow and shear rate prevents hyperglycemia-induced
380 endothelial dysfunction in healthy subjects and those with type 2 diabetes. *J Appl Physiol*
381 *(1985)* 118: 579-585, 2015.
382 14.   **Greyling A, van Mil AC, Zock PL, Green DJ, Ghiadoni L, Thijssen DH, and**
383 **Dilation TIWGoFM**. Adherence to guidelines strongly improves reproducibility of brachial
384 artery flow-mediated dilation. *Atherosclerosis* 248: 196-202, 2016.
385 15.   **Gurovich AN, and Braith RW**. Enhanced external counterpulsation creates acute
386 blood flow patterns responsible for improved flow-mediated dilation in humans. *Hypertens*
387 *Res* 36: 297-305, 2013.

388 16. **Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y,**
389 **Taketani Y, Orimo H, and Ouchi Y**. Modulation of endothelium-dependent flow-mediated
390 dilatation of the brachial artery by sex and menstrual cycle. *Circulation* 92: 3431-3435, 1995.
391 17. **Maiorana A, O'Driscoll G, Taylor R, and Green D**. Exercise and the nitric oxide
392 vasodilator system. *Sports Med* 33: 1013-1035, 2003.
393 18. **Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, and Padilla J**.
394 Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. *American*
395 *Journal of Physiology Heart and Circulatory Physiology* 311: H177-182, 2016.
396 19. **Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH, and Green DJ**.
397 Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator
398 function in humans. *American Journal of Physiology Heart and Circulatory Physiology* 300:
399 H664-669, 2011.
400 20. **Pfitzner J**. Poiseuille and his law. *Anaesthesia* 31: 273-275, 1976.
401 21. **Pohl U, Holtz J, Busse R, and Bassenge E**. Crucial role of endothelium in the
402 vasodilator response to increased flow in vivo. *Hypertension* 8: 37-44, 1986.
403 22. **Schreuder TH, Green DJ, Hopman MT, and Thijssen DH**. Impact of retrograde
404 shear rate on brachial and superficial femoral artery flow-mediated dilation in older subjects.
405 *Atherosclerosis* 241: 199-204, 2015.
406 23. **Simmons GH, Padilla J, Young CN, Wong BJ, Lang JA, Davis MJ, Laughlin**
407 **MH, and Fadel PJ**. Increased brachial artery retrograde shear rate at exercise onset is
408 abolished during prolonged cycling: role of thermoregulatory vasodilation. *J Appl Physiol*
409 *(1985)* 110: 389-397, 2011.
410 24. **Smyth CN**. Effect of suction on blood-flow in ischaemic limbs. *Lancet* 2: 657-659,
411 1969.
412 25. **Sundby OH, Hoiseth LO, Irgens I, Mathiesen I, Lundgaard E, Haugland H,**
413 **Weedon-Fekjaer H, Sundhagen JO, Sanbaek G, and Hisdal J**. Intermittent negative
414 pressure applied to the lower limb increases foot macrocirculatory and microcirculatory
415 blood flow pulsatility in people with spinal cord injury. *Spinal Cord* 56: 382-391, 2018.
416 26. **Sundby OH, Hoiseth LO, Mathiesen I, Jorgensen JJ, Sundhagen JO, and Hisdal**
417 **J**. The effects of intermittent negative pressure on the lower extremities' peripheral
418 circulation and wound healing in four patients with lower limb ischemia and hard-to-heal leg
419 ulcers: a case report. *Physiological Reports* 4: 2016.
420 27. **Sundby OH, Hoiseth LO, Mathiesen I, Jorgensen JJ, Weedon-Fekjaer H, and**
421 **Hisdal J**. Application of intermittent negative pressure on the lower extremity and its effect
422 on macro- and microcirculation in the foot of healthy volunteers. *Physiological Reports* 4:
423 2016.
424 28. **Sundby OH, Irgens I, Hoiseth LO, Mathiesen I, Lundgaard E, Haugland H,**
425 **Weedon-Fekjaer H, Sundhagen JO, Sandbaek G, and Hisdal J**. Intermittent mild negative
426 pressure applied to the lower limb in patients with spinal cord injury and chronic lower limb
427 ulcers: a crossover pilot study. *Spinal Cord* 56: 372-381, 2018.
428 29. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B,**
429 **Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation
430 in humans: a methodological and physiological guideline. *American Journal of Physiology*
431 *Heart and Circulatory Physiology* 300: H2-12, 2011.
432 30. **Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, and Green DJ**.
433 Brachial artery blood flow responses to different modalities of lower limb exercise. *Med Sci*
434 *Sports Exerc* 41: 1072-1079, 2009.
435 31. **Thijssen DH, Dawson EA, Tinken TM, Cable NT, and Green DJ**. Retrograde flow
436 and shear rate acutely impair endothelial function in humans. *Hypertension* 53: 986-992,
437 2009.

438   32.   **Thorn CE, Kyte H, Slaff DW, and Shore AC**. An association between vasomotion
439   and oxygen extraction. *Am J Physiol Heart Circ Physiol* 301: H442-449, 2011.
440   33.   **Thosar SS, Bielko SL, Mather KJ, Johnston JD, and Wallace JP**. Effect of
441   prolonged sitting and breaks in sitting time on endothelial function. *Medicine and Science in*
442   *Sports and Exercise* 47: 843-849, 2015.
443   34.   **Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT,**
444   **Newcomer SC, Laughlin MH, Cable NT, and Green DJ**. Impact of shear rate modulation
445   on vascular function in humans. *Hypertension* 54: 278-285, 2009.
446   35.   **Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, and Green DJ**.
447   Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension*
448   55: 312-318, 2010.
449   36.   **Tsai AG, and Intaglietta M**. Evidence of flowmotion induced changes in local tissue
450   oxygenation. *Int J Microcirc Clin Exp* 12: 75-88, 1993.
451   37.   **Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K,**
452   **and Komesaroff PA**. Variations in endothelial function and arterial compliance during the
453   menstrual cycle. *Journal of Clinical Endocrinology & Metabolism* 86: 5389-5395, 2001.
454   38.   **Woodman CR, Price EM, and Laughlin MH**. Shear stress induces eNOS mRNA
455   expression and improves endothelium-dependent dilation in senescent soleus muscle feed
456   arteries. *J Appl Physiol (1985)* 98: 940-946, 2005.
457   39.   **Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR,**
458   **Puddey IB, Beilin LJ, Burke V, Mori TA, and Green D**. Improved analysis of brachial
459   artery ultrasound using a novel edge-detection software system. *Journal of Applied*
460   *Physiology* 91: 929-937, 2001.
461

462

463   **Table 1:** Subject characteristics of the participants (n=15).

Parameter	Mean±SD
Age (years)	27.3±5.0
Height (m)	1.75±0.06
Body mass (kg)	75.1±7.5
BMI (kg/m^2)	24.4±2.0
Systolic blood pressure (mmHg)	115±3
Diastolic blood pressure (mmHg)	62±7
Mean arterial pressure (mmHg)	80±5
Heart rate (bpm)	52±8

464
465   BMI – body mass index; bpm – beats per minute

Table 2: Brachial artery FMD for the intervention and control arm before and after 30-minute exposure to unilateral intermittent negative pressure in healthy young individuals (n=15). P-values refer to a linear mixed model to examine the main effect of 'time' (pre- *versus* post-intervention), 'arm' (intervention-arm *versus* contra-lateral control arm) and the interaction-effect between 'time'*'arm'. Data are presented as mean (95% confidence intervals).

	Intervention arm		Control arm		'time'	'arm'	'time*arm'
	Pre	Post	Pre	Post			
Baseline diameter (mm)	4.04 (3.82-4.26)	4.02 (3.79-4.24)	3.82 (3.60-4.05)	3.79 (3.57-4.01)	0.671	0.002	0.957
Peak diameter (mm)	4.26 (4.03-4.48)	4.31 (4.09-4.54)	4.07 (3.84-4.30)	4.05 (3.82-4.27)	0.797	0.001	0.603
FMD (%)	5.5 (3.9-7.0)	7.5 (5.9-9.0)	6.4 (4.9-8.0)	6.9 (5.4-8.5)	0.029	0.619	0.096
SRAUC ($s^{-1}x10^3$)	19.3 (15.0-23.5)	17.9 (13.6-22.1)	17.1 (12.8-21.3)	17.5 (13.2-21.7)	0.762	0.428	0.572
Time to peak (secs)	48 (40-56)	43 (35-51)	43 (35-51)	47 (39-55)	0.950	0.919	0.217

FMD – flow-mediated dilation; SRAUC – shear rate area-under-the-curve

FIGURE LEGENDS

Figure 1: Photo of the experimental set-up. The participant lay supine with both arms extended for optimal ultrasound scanning of the brachial artery. Ultrasound machines and probes remained consistent throughout the study (Terason u-smart 3300, Teratech, Burlington, MA) with 10-12 Hz probes. Furthermore, the settings on the ultrasound machine (i.e. depth, Doppler cursor position) were maintained for the duration of the laboratory visit. The participant's left arm was inside the rigid cylinder, connected to a pressure control box (not seen in the image) and exposed to 30 minutes of intermittent negative pressure, whilst the right arm served as a control.

Figure 2: Shear rate data of the brachial artery calculated as 1-s averages at rest, followed by 3 cycles of intermittent negative pressure (grey bars: negative pressure) in 15 healthy young men. Values are mean ± standard error. Note the clear fluctuations in brachial artery shear rate, with higher levels of mean and antegrade shear rate during (the first part of) negative pressure, followed by a rapid decline and normalisation of mean and antegrade shear rate upon release of the pressure. Mean shear rate is presented as the dashed line.

Figure 3: Presentation of average levels of antegrade (white bars), retrograde (black bars) and mean (grey bars) shear rate at baseline and during the intermittent negative pressure intervention in the intervention arm (A) and control arm (B). Data during the intermittent negative pressure were presented during negative pressure ('on'), during pressure release ('off') and as the average across the entire 30-minute intervention ('average'). Error bars represent SD. *Significantly different from baseline at $P < 0.05$.

Figure 4: Individual brachial artery FMD% responses to 30-minutes intermittent negative pressure in the intervention and control arms of healthy young individuals (n=15). Black dotted line represents mean change in FMD. Error bars represent SD. P-values refer to a linear mixed model.
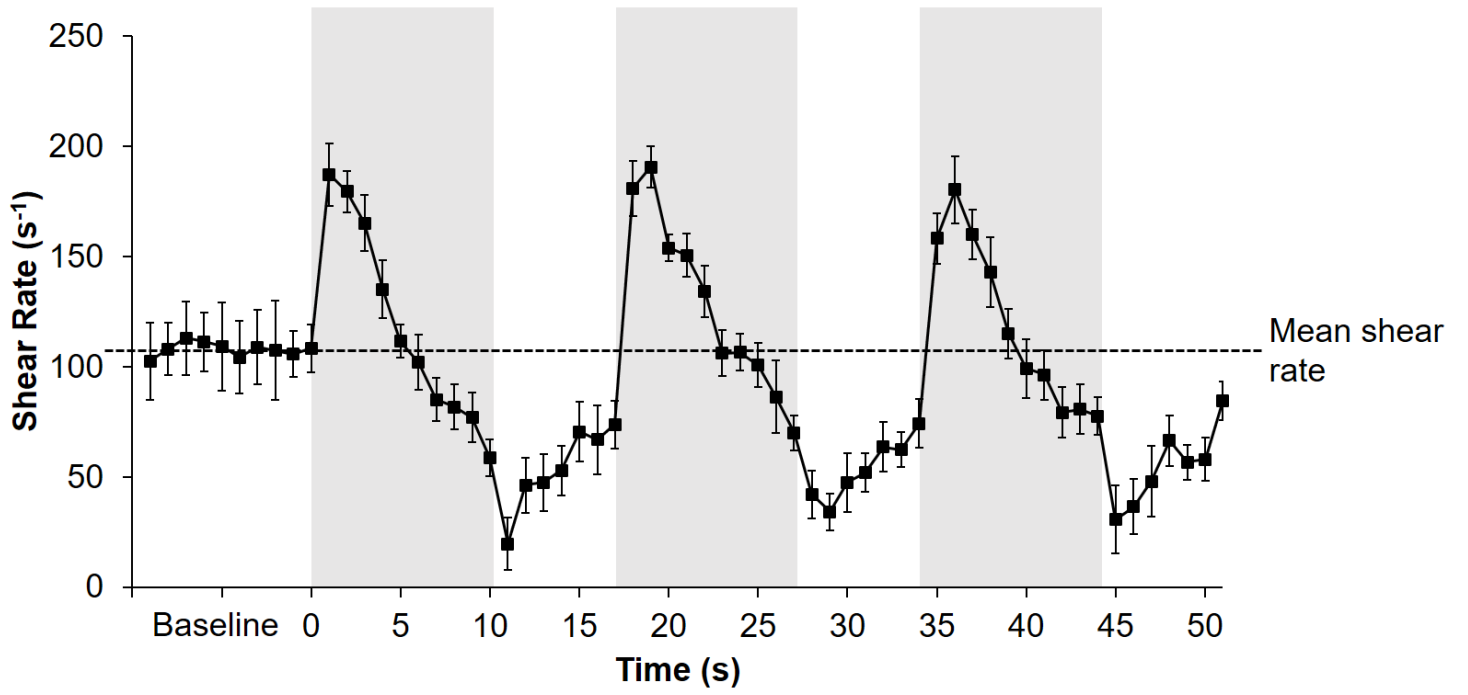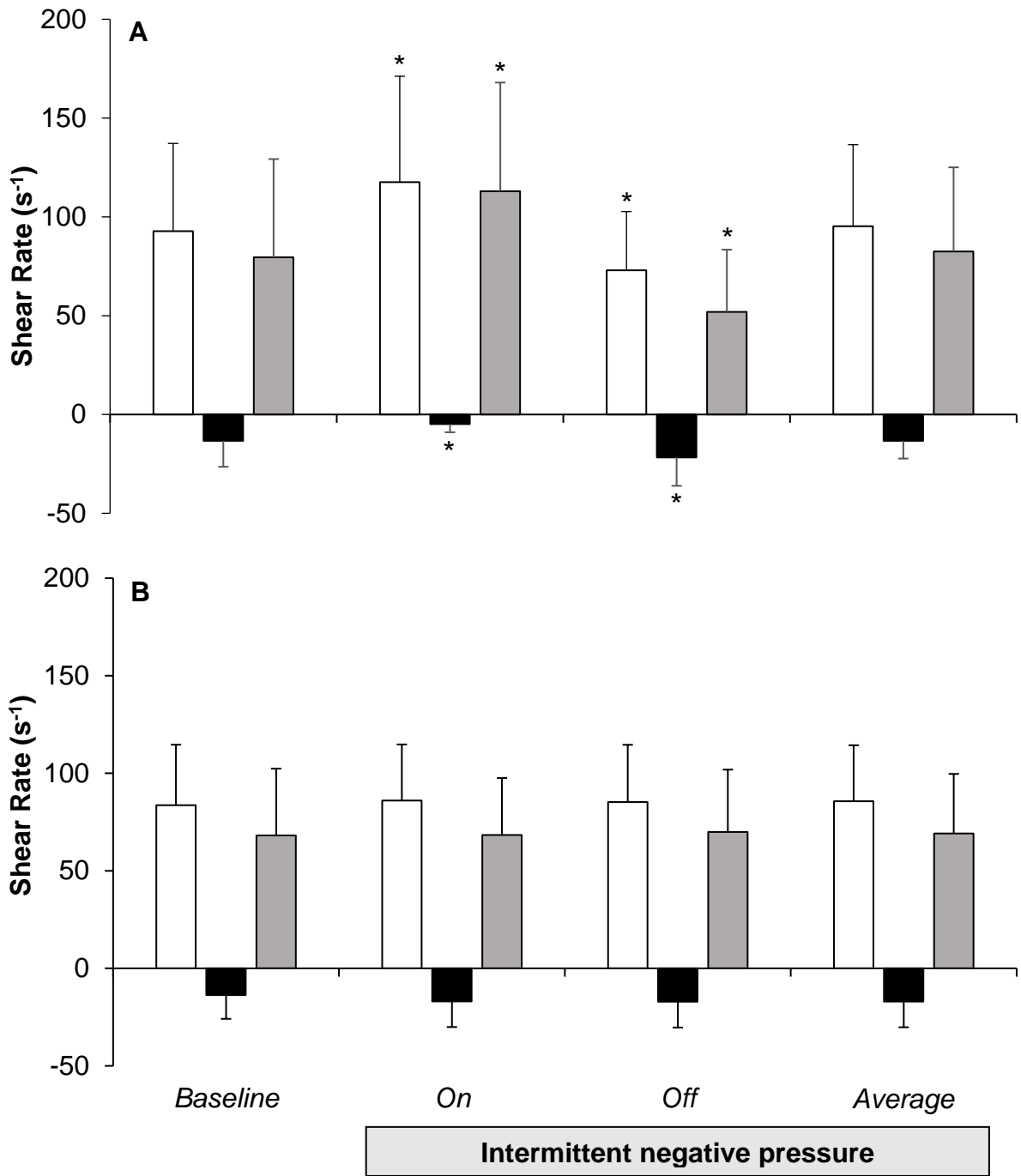
Figure 1

Figure 2

Figure 3

Figure 4.

Linear Mixed Model:
Time *P*=0.029
Arm *P*=0.619
Time*Arm *P*=0.096