



## 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

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Holder, SM, Dawson, EA, Brislane, Á, Hisdal, J, Green, DJ and Thijssen, DHJ (2019) Fluctuation in Shear Rate, with Unaltered Mean Shear Rate, Improves Brachial Artery Flow-Mediated Dilation in Healthy, Young Men. Journal of Applied Physiology. ISSN 8750-7587**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://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<sup>1</sup>, Ellen A. Dawson<sup>1</sup>, Áine Brislane<sup>1,2</sup>, Jonny Hisdal<sup>3</sup>, Daniel J. Green<sup>4</sup> & Dick H.J. Thijssen<sup>1,5</sup>

<sup>1</sup>*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom.*

<sup>2</sup>*School of Sport and Exercise Science, York St John University, York, United Kingdom.*

<sup>3</sup>*Section of Vascular Investigations, Division of Cardiovascular and Pulmonary Diseases, Department of Vascular Surgery, Oslo University Hospital, Oslo, Norway.*

<sup>4</sup>*School of Human Sciences (Exercise and Sports Science), The University of Western Australia, Crawley, Western Australia, 6009.*

<sup>5</sup>*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

1 **Abstract**

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

9 **Methods:** Fifteen healthy males ( $27.3 \pm 5.0$  years) completed the study. Bilateral  
10 brachial artery flow-mediated dilation was assessed before and after unilateral  
11 exposure to 30-minutes of intermittent negative pressure (10seconds -40mmHg,  
12 7seconds 0mmHg) to induce fluctuation in shear rate, whilst the contra-lateral arm was  
13 exposed to a resting period.

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

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

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

27 **New & Noteworthy**

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

35 **Introduction**

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

46

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

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  $27.3 \pm 5.0$  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 ( $\text{kg/m}^2$ ).

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 ( $52\pm 7$  bpm *versus*  $54\pm 8$  bpm,  $P=0.47$ ) or in  
186 systolic ( $129\pm 9$  mmHg *versus*  $135\pm 12$  mmHg,  $P=0.16$ ), diastolic ( $55\pm 8$  mmHg *versus*  
187  $59\pm 9$  mmHg,  $P=0.36$ ) or mean blood pressure ( $80\pm 8$  mmHg *versus*  $84\pm 9$  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”:  $\Delta+34.2\text{s}^{-1}$ , “pressure off”:  $\Delta-26.5\text{s}^{-1}$ ; 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”:  $\Delta+3.8\text{s}^{-1}$ ;  $P=0.458$ ). In the  
193 control arm, negative pressure did not change mean shear from baseline levels  
194 (“pressure on”:  $\Delta+1.6$   $P=0.805$ , “pressure off”:  $\Delta+3.5\text{s}^{-1}$   $P=0.613$ ). Therefore, mean  
195 shear rate remained unchanged throughout the intervention period compared to  
196 baseline (“pressure on/off cycle”:  $\Delta+2.5\text{s}^{-1}$   $P=0.702$ ; 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:  $\Delta+2.5\text{s}^{-1}$ ,  $P=0.730$ ; retrograde:  $\Delta-1.9\text{s}^{-1}$ ,  $P=0.190$ ) and  
206 “pressure off” (antegrade:  $\Delta+1.9\text{s}^{-1}$ ,  $P=0.779$ ; retrograde:  $\Delta-2.0\text{s}^{-1}$ ,  $P=0.164$ ).  
207 Therefore, mean antegrade and retrograde shear rate was not different from baseline  
208 across the intervention (antegrade:  $\Delta+2.2\text{s}^{-1}$ ,  $P=0.750$ ; retrograde:  $\Delta-1.9\text{s}^{-1}$ ,  $P=0.173$ ).

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 ( $\Delta+2.0\%$ ,  
213  $P=0.008$ ), whilst no change was observed in the control arm ( $\Delta+0.5\%$ ,  $P=0.664$ ).  
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

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.





335 **Acknowledgements.**

336 We acknowledge the assistance of Dr. Nicola D Hopkins and Dr. Guilherme Speretta.

337 D. J. Green is supported by National Health and Medical Research Council Principal

338 Research Fellowship Grant APP1080914.

339 **Disclosures**

340 None.

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. **Pfutzner 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).

<b>Parameter</b>	<b>Mean±SD</b>
Age (years)	27.3±5.0
Height (m)	1.75±0.06
Body mass (kg)	75.1±7.5
BMI (kg/m <sup>2</sup> )	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			
<i>Baseline diameter (mm)</i>	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
<i>Peak diameter (mm)</i>	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
<i>FMD (%)</i>	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
<i>SRAUC (s<sup>-1</sup>x10<sup>3</sup>)</i>	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
<i>Time to peak (secs)</i>	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  $\pm$  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

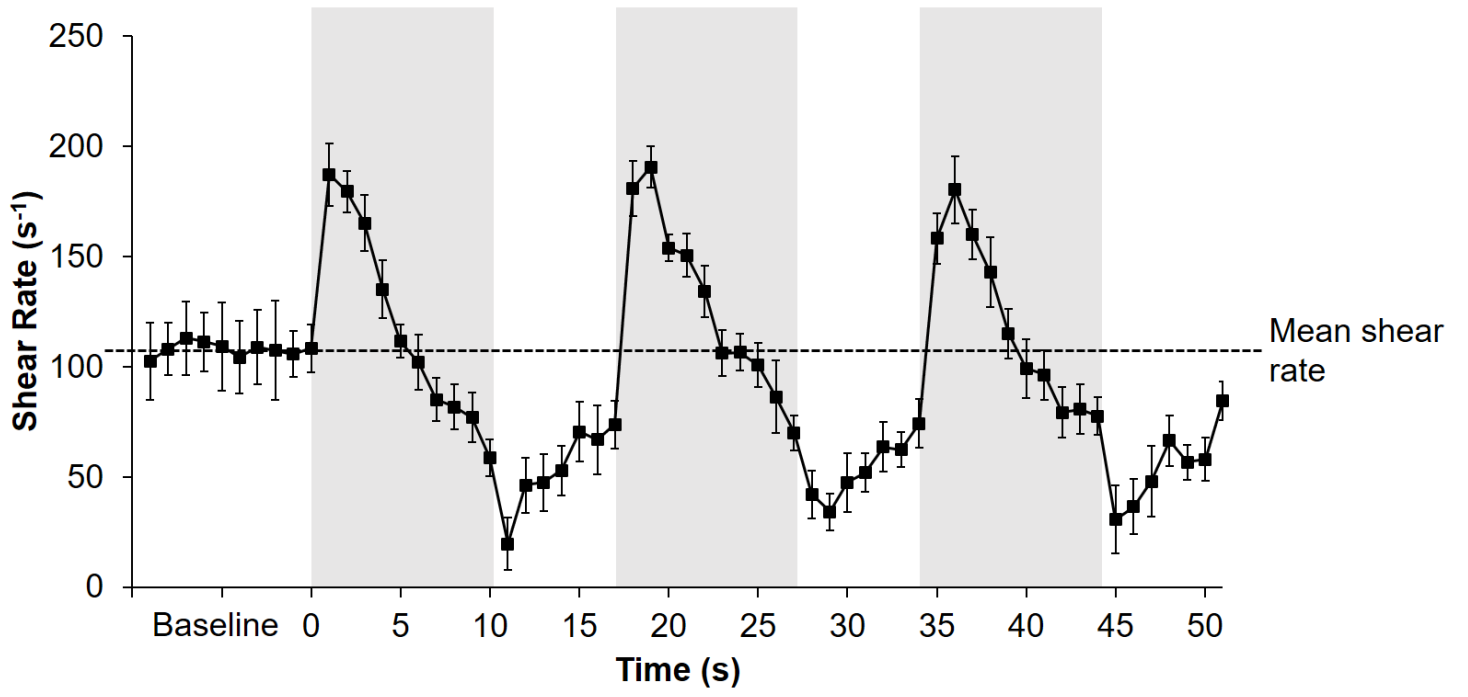


Figure 3

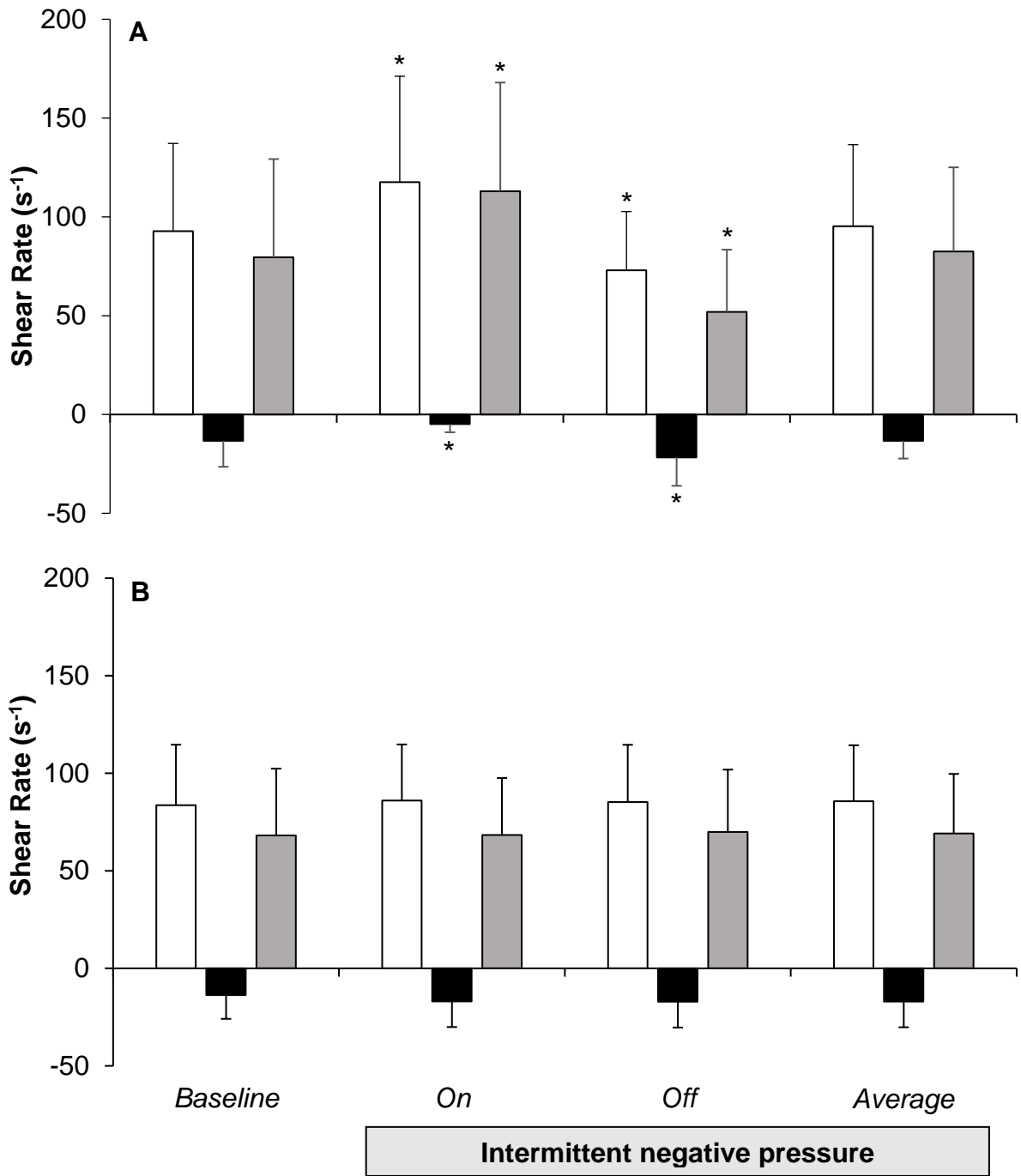


Figure 4.

