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

4
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34 **Abstract**

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

42 **Methods:** Fifteen healthy males (27.3±5.0 years) completed the study. Bilateral
43 brachial artery flow-mediated dilation was assessed before and after unilateral
44 exposure to 30-minutes of intermittent negative pressure (10seconds -40mmHg,
45 7seconds 0mmHg) to induce fluctuation in shear rate, whilst the contra-lateral arm
46 was exposed to a resting period.

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

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

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

61 **New & Noteworthy**

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

69 **Introduction**

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

80

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

93 or whether observations were simply explained through increases in mean shear
94 stress levels.

95 To the best of our knowledge, no previous study in animals or humans has directly
96 examined whether fluctuations in blood flow and shear stress patterns, in the
97 presence of unaltered mean blood flow and shear rate, impacts upon endothelial
98 function. Therefore, we assessed the effect of 30-minute exposure to intermittent
99 negative pressure, which mediates fluctuations in blood flow and shear rate patterns
100 through the brachial artery, on FMD (a measure of largely nitric oxide-mediated,
101 endothelial function (11)) in healthy young men. We hypothesised that fluctuations in
102 blood flow and shear stress patterns would induce improvement in brachial artery
103 endothelial function. Since fluctuations in mean shear stress are relevant to many
104 activities of daily living, we planned this study to provide insight into the potential
105 clinical relevance of fluctuations in shear stress as a hemodynamic stimulus for
106 improvement in vascular health *in vivo*.

107

108

109 **Materials and Methods**

110 *Participants*

111 Fifteen healthy males (age 27.3 ± 5.0 years) were recruited for the study. All
112 participants were non-smokers, not taking medication and/or supplements known to
113 influence the cardiovascular system and free from cardiovascular/metabolic disease
114 risk factors. Based on a pre-screening health questionnaire, participants were
115 excluded if they had poor circulation (including diagnosis of peripheral vascular
116 disease or Reynaud's disease). Each participant provided written informed consent
117 before taking part in the experimental procedure. The research study was ethically

118 approved by the Liverpool John Moores School of Sport and Exercise Science
119 Research Ethics Committee and adhered to the Declaration of Helsinki.

120 *Experimental Design*

121 After 15 minutes of supine resting, we bilaterally examined brachial artery endothelial
122 function using the FMD test (29). This was followed by a 10-minute rest period to
123 allow blood flow and diameter to return to baseline levels. Subsequently, following a
124 1-minute recording of baseline diameter and blood flow velocity, subjects underwent
125 a 30-minute intervention involving intermittent negative pressure (i.e. left arm), whilst
126 the right arm served as a control arm. Within 2-minutes of this intervention, we
127 repeated bilateral brachial artery FMD testing.

128

129 *Preparations*

130 Prior to the laboratory visit, all participants were instructed to refrain from strenuous
131 exercise for at least 24 hours, alcohol for 12 hours, avoid all caffeinated products for
132 8 hours and food products high in polyphenols for 24 hours. Participants reported to
133 the quiet, temperature-controlled laboratory after fasting for at least 6 hours. After
134 reporting to our laboratory, stature and body mass were recorded to the nearest 0.1
135 unit using a stadiometer and digital scales respectively. Body mass index (BMI) was
136 calculated as body mass in kilograms divided by stature in metres squared (kg/m^2).

137

138 *Brachial artery flow-mediated dilation.* Brachial artery FMD was measured in
139 accordance with contemporary expert-consensus guidelines (29). Following 15
140 minutes of supine rest, left and right brachial artery diameter were assessed
141 simultaneously via high-resolution duplex ultrasound (Terason u-smart 3300,
142 Teratech, Burlington, MA) with a 10-12 MHz linear array probe. B-mode images were

143 obtained and optimised, and the probe was held in the same position for the duration
144 of the test. After 1 minute of baseline measurement, occlusion cuffs, connected to a
145 rapid inflator (Hokanson, Bellevue, WA), placed around both forearms, distal to the
146 humeral epicondyle, were inflated to a pressure of 220 mmHg for 5 minutes.
147 Recording was resumed 30-seconds prior to cuff deflation, and FMD was recorded
148 for a further 3 minutes post cuff deflation. All measurements were taken by the same
149 experienced operators within participants. Bilateral FMD was repeated following the
150 30-minute intervention period.

151

152 *Brachial artery diameter and shear rate.* High-resolution ultrasound (Terason u-smart
153 3300; Teratech, Burlington, MA) was used to examine brachial artery diameter and
154 shear rate as described above. Following the pre-intervention FMD, the participant's
155 skin was marked to ensure a consistent ultrasound probe position and therefore
156 artery segment during the visit. Furthermore, the ultrasound machine settings
157 remained constant (i.e. depth and Doppler cursor position) in order to assume the
158 same probe angle whilst imaging. Bilateral artery diameter and shear rate were
159 recorded for 1-minute baseline, and repeated at 5-minute intervals during the 30-
160 minute intervention period.

161

162 *Intervention.* During the laboratory visit, participants rested in the supine position with
163 both arms extended away from their body to approximately 80°, with their palms
164 facing upwards for optimal ultrasound imaging of the brachial artery. During the 10-
165 minute rest period following the pre-intervention FMD, the left arm was placed inside
166 a rigid plastic cylinder (8.5x40cm) connected to a pressure control box (FlowOx™,
167 Otivio AS, Oslo, Norway; Figure 1). The cylinder was sealed around the forearm with

168 a thermoplastic elastomer (TPS-SEBS). The arm was exposed to repeated bouts of
169 negative pressure (-40 mmHg; 10 seconds negative pressure, 7 seconds
170 atmospheric pressure) for 30 minutes (~105 full cycles of negative pressure).

171

172 *Blood pressure.* Blood pressure and heart rate were recorded continuously during
173 the protocol from the right (control) arm index/middle finger using a Portapres
174 (Finapres Medical Systems BV, Amsterdam, The Netherlands). This data were
175 displayed, recorded and exported using PowerLab software (ADInstruments,
176 Australia). The difference in blood pressure and heart rate was calculated from a 1-
177 minute recording before the intervention period started, and the last minute of the
178 intervention.

179

180 *Data analysis.* All FMD data analysis was performed blinded by the same observer,
181 using a specialised custom-designed edge-detection and wall-tracking software, of
182 which the reproducibility and validity have been demonstrated elsewhere (39). This
183 software tracks the vessel walls and blood flow velocity trace in B-mode frames via
184 pixel density and frequency distribution algorithm. An optimal region of interest to be
185 analysed was selected by the sonographer, chosen on the basis of the quality of the
186 image, in regards to clear distinction between the artery walls and lumen. The FMD
187 was defined as the maximum percentage change in artery diameter from baseline to
188 peak during the 3 minutes post cuff release. The software automatically calculated
189 the relative diameter change, time to peak (following cuff release) and shear rate
190 area-under-the-curve (SRAUC). Despite the initial region of interest selection being
191 operator-determined, the remaining analysis was independent of operator bias.

192

193 Brachial artery diameter and shear rate were analysed using the custom-designed
194 software described above. The region of interest location (selected by the operator)
195 remained consistent for each 1-minute recording *within* participants. Using markers
196 placed by the operator, the software calculated the average artery diameter and
197 shear rate across the minute recordings. The fluctuations in shear stress were
198 analysed as an average during the application of negative pressure (10secs; On),
199 atmospheric pressure (7secs; Off), and the full cycle, then repeated for the 3 full
200 cycles captured during each 1-minute recording. These processes were repeated for
201 each time point during the intervention. Mean (\pm standard error) shear rate data at
202 baseline and during 3 cycles of intermittent negative pressure are presented in
203 Figure 2.

204

205 *Statistical analysis.* Statistical analysis was conducted using IBM SPSS version 25
206 (SPSS Inc., Chicago, IL). Allometric scaling was performed on FMD data to control
207 for differences in baseline diameter (3, 4). A linear mixed model with covariate
208 control for SRAUC and scaled baseline diameter determined the main effect for time
209 (pre-post) and arm. A general linear model assessed the changes in blood pressure
210 and heart rate across the intervention period. Paired T-tests determined the
211 difference in antegrade and retrograde shear during intermittent negative pressure
212 compared to baseline in both arms. Statistical significance was recognised when a *P*
213 value <0.05 was observed. Data are presented as mean \pm standard error unless
214 stated otherwise.

215

216

217 **Results**

218 Subject characteristics are presented in Table 1.

219

220 *Brachial artery blood flow and shear rate.* There were no significant changes across
221 the 30-minute intervention in heart rate (mean 52bpm \pm SD 7 bpm *versus* 54 \pm 8 bpm,
222 $P=0.47$) or in systolic (129 \pm 9 mmHg *versus* 135 \pm 12 mmHg, $P=0.16$), diastolic (55 \pm 8
223 mmHg *versus* 59 \pm 9 mmHg, $P=0.36$) or mean blood pressure (80 \pm 8 mmHg *versus*
224 84 \pm 9 mmHg, $P=0.23$). Negative pressure was associated with a significant increase
225 in mean shear rate, whilst pressure release was followed by a significant decrease in
226 mean shear rate, to levels below baseline (“pressure on”: $\Delta+34.2s^{-1}$, “pressure off”:
227 $\Delta-26.5s^{-1}$; both $P<0.001$; Figure 3A). Consequently, mean shear rate across the
228 intervention period was not different from baseline (“pressure on/off cycle”: $\Delta+3.8s^{-1}$;
229 $P=0.458$). In the control arm, negative pressure did not change mean shear from
230 baseline levels (“pressure on”: $\Delta+1.6$ $P=0.805$, “pressure off”: $\Delta+3.5s^{-1}$ $P=0.613$).
231 Therefore, mean shear rate remained unchanged throughout the intervention period
232 compared to baseline (“pressure on/off cycle”: $\Delta+2.5s^{-1}$ $P=0.702$; Figure 3B).

233

234 When examining shear patterns, negative pressure increased antegrade shear rate
235 ($P<0.001$) and decreased retrograde shear rate ($P=0.006$; Figure 3A). Upon
236 pressure release, compared to baseline levels, a decrease in antegrade shear rate
237 and increase in retrograde shear rate was found ($P=0.003$ and $P<0.001$,
238 respectively). As a result, mean antegrade and retrograde shear rate across the 30-
239 minute intervention period was not different from baseline ($P=0.504$ and 0.777 ,
240 respectively). Antegrade and retrograde shear rate remained unaltered from baseline
241 in the control arm during “pressure on” (antegrade: $\Delta+2.5s^{-1}$, $P=0.730$; retrograde: $\Delta-$

242 1.9s^{-1} , $P=0.190$) and “pressure off” (antegrade: $\Delta+1.9\text{s}^{-1}$, $P=0.779$; retrograde: $\Delta-$
243 2.0s^{-1} , $P=0.164$; Figure 3B). Therefore, mean antegrade and retrograde shear rate
244 was not different from baseline across the intervention (antegrade: $\Delta+2.2\text{s}^{-1}$,
245 $P=0.750$; retrograde: $\Delta-1.9\text{s}^{-1}$, $P=0.173$).

246

247 *Brachial artery FMD*. Linear mixed model analysis revealed a significant main effect
248 for time ($P=0.029$; F-ratio=5.146), whilst no effect was observed for arm ($P=0.619$; F-
249 ratio=0.251) or interaction effect ($P=0.096$; F-ratio=2.906). Post-hoc exploratory
250 analysis revealed a significant increase in FMD in the intervention arm ($\Delta+2.0\%$,
251 $P=0.008$), whilst no change was observed in the control arm ($\Delta+0.5\%$, $P=0.664$).
252 Individual FMD responses are presented in Figure 4 and all associated parameters
253 (mean and 95% confidence intervals) are presented in Table 2.

254

255

256 **Discussion**

257 We show that application of intermittent negative pressure to the forearm increases
258 antegrade blood flow and shear rate, whilst pressure release mediates increased
259 retrograde blood flow and shear rate measured at the brachial artery, relative to
260 baseline and the contralateral control arm. Despite these marked fluctuations in
261 blood flow and shear rate patterns throughout the 30-minute intervention, mean
262 blood flow and shear rate was not different from baseline. We therefore successfully
263 preserved average resting levels of flow and shear rate, despite inducing fluctuations
264 of these variables. Although exploratory in nature, we observed improved brachial
265 artery FMD as a result of these fluctuations in blood flow and shear rate, an effect
266 that was not apparent in the contralateral control limb. Taken together, these findings

267 suggest that fluctuations in shear rate, independent of mean blood flow and shear
268 rate, may impact acute vascular function in healthy young individuals. Whilst further
269 research is required, this contributes to improving our understanding of shear stress
270 as an important hemodynamic stimulus in the adaptation of vascular health in
271 humans *in vivo*.

272

273 Our findings regarding the impact of cyclical negative pressure are in line with a
274 previous study in the lower limbs (27). Importantly, our study adds the novel
275 knowledge that these fluctuations were associated with improvements in endothelial
276 function, as measured with the brachial artery FMD. Blood pressure and heart rate
277 remained unaltered during the intervention period, effectively excluding the
278 possibility that systemic factors contributed to our observations. To further support
279 this notion, no changes in brachial artery blood flow or shear rate were found in the
280 contralateral arm. This strongly suggests that the mechanisms contributing to the
281 increase in FMD in the intervention arm relate to local effects (i.e. fluctuations in
282 shear rate) rather than systemic/circulating factors.

283

284 Our novel results may be somewhat surprising, in that the fluctuations in shear rate
285 were not accompanied by changes in mean shear rate, but still caused an increase
286 in FMD. In our previous work, supported by studies in animals (21, 38), we
287 consistently found that changes in mean shear rate are essential to change FMD (31,
288 34). More specifically, selective increases in antegrade shear rate (and therefore
289 mean shear rate) were related to improved FMD (13, 34), whilst an isolated increase
290 in retrograde shear rate (i.e. lower mean shear rate) was associated with a dose-
291 dependent decrease in brachial and femoral artery FMD (22, 31). One potential

292 explanation for the increase in FMD is the relative larger importance of increases in
293 antegrade shear rate compared to changes in retrograde shear rate. To support this
294 idea, moderate-intensity cycling exercise acutely increases retrograde shear rate (10,
295 30), followed by normalisation after ~15 minutes with a concomitant increase in
296 antegrade shear rate (23). Nonetheless, acute or chronic performance of cycling
297 exercise (i.e. 30-/40-min bouts) leads to improvement in brachial artery FMD (5, 12).
298 This evolving hypothesis that changes in antegrade shear rate may be relatively
299 more important than changes in retrograde shear rate warrants further investigation.

300

301 Another explanation for our findings relates to the importance of fluctuations in shear
302 rate patterns, rather than mean shear rate. In the microcirculation, previous work
303 used mathematical simulation to support the concept that fluctuations of capillary
304 blood flow, rather than steady-state conditions, improve oxygenation of tissue (36).
305 Follow-up work in humans examining skin perfusion and oxygenation demonstrated
306 that periodic fluctuations in vasomotion may be beneficial for local oxygenation (32).
307 In conduit arteries, some studies have found that enhanced external
308 counterpulsation increased shear rate fluctuations and FMD in the brachial artery (6,
309 15). However, these changes were also accompanied by an overall increase in
310 mean shear rate, making it impossible to isolate the impact of fluctuations *per se* (i.e.
311 in the absence of changes in mean shear). Finally, indirect support for a potential
312 clinically-relevant, beneficial effect on vascular health for these fluctuations is
313 provided by the observation of improved wound healing upon repeated exposure to
314 intermittent negative pressure (26, 28). These observations may contribute to
315 improved microcirculatory blood flow and therefore the delivery of oxygen and
316 nutrients to promote wound healing (25, 26). Although speculative, our findings

317 suggest that these benefits of intermittent negative pressure stimulus on wound
318 healing (26, 28) may be related to enhanced endothelial function.

319

320 A final possible explanation for our findings relates to the impact of intermittent
321 negative pressure on changes in the pressure gradient across the artery wall (24)
322 and, therefore, transmural pressure (20). Although changes in transmural pressure
323 may affect vascular health (2, 12), it seems unlikely this can explain our findings.
324 First, negative pressure likely increases transmural pressure (due to the drop in
325 external pressure), which is typically associated with impaired vascular health (2).
326 Secondly, vascular function was examined in the brachial artery, i.e. not directly
327 exposed to the changes in (transmural) pressure, and we observed no significant
328 systemic effects on blood pressure of unilateral forearm suction.

329

330 The clinical relevance of our findings is that fluctuations in blood flow or shear rate
331 *per se* represent a hemodynamic stimulus capable of improving vascular health.
332 Previous studies manipulating shear rate have increased mean shear rate to
333 improve FMD. In contrast to these stimuli, we have not changed mean shear rate,
334 but still found improved FMD, most likely due to the fluctuations in shear and blood
335 flow patterns. Furthermore, these fluctuations in blood flow and shear rate may be
336 more ecologically valid compared to sustained increases in shear rate. More
337 specifically, fluctuations in blood flow and shear rate are more related to activities of
338 daily living, such as those associated with low-intensity physical activity and changes
339 in posture. Therefore, repetitive exposure to these stimuli may be efficient in
340 improving vascular health. Indeed, recent work has demonstrated that regular
341 exposure to mild physical activity stimuli, such as walking breaks (8, 33) or fidgeting

342 (18), prevents decline in cerebro- and cardiovascular health associated with
343 prolonged sitting. Although speculative, activity-induced fluctuations in blood flow
344 may be the underlying mediator contributing to the preserved vascular health.

345

346

347 *Limitations.* The present study possesses several strengths, including strict
348 adherence to contemporary expert-consensus guidelines for FMD (29) and blinded
349 data analysis using custom-designed edge-detection software to eliminate operator
350 bias. There are some limitations to the study. Firstly, we recruited healthy
351 recreationally active males, which makes it difficult to extrapolate our findings to
352 other populations (e.g. females) (7, 16, 37) or clinical groups. However, larger
353 improvements in FMD may be observed in those with *a priori* endothelial dysfunction
354 (17). A second limitation is that we did not perform additional measurements such as
355 blood analysis for markers of endothelial cell activity. *In vitro* studies in cultured
356 endothelial cells and isolated arteries, reviewed elsewhere (12), demonstrate the
357 release of pro- and anti-atherogenic substances in response to exposure to
358 oscillatory (or low) and laminar (or high) shear stress respectively. Insight into the
359 impact of fluctuations in shear stress (with preserved mean shear) would have
360 contributed to further understanding the underlying mechanisms of our findings. A
361 final limitation relates to the relatively small sample size of our study. Post-hoc
362 statistical power analysis using G*Power software (9) revealed a power of 0.77 to
363 detect within-subject changes in FMD, but a power of 0.27 to find a significant
364 interaction effect. Therefore, our results should be interpreted with caution, and
365 further work is required to better understand the potency of fluctuations in shear rate
366 patterns on vascular function.

367

368

369 **Conclusion**

370 In conclusion, our findings suggest that 30-minutes exposure to fluctuations in shear
371 rate improves endothelial function, despite the absence of concomitant changes in
372 mean shear rate compared to resting baseline levels. Our work implies that
373 fluctuations in blood flow or shear rate may represent a hemodynamic stimulus to
374 potentially improve vascular health. Future research to examine the underlying
375 mechanisms and potential long-term effects would be of interest.

376

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382 **Disclosures**

383 None.

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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

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BMI – body mass index; bpm – beats per minute

509 **Table 2:** Brachial artery FMD for the intervention and control arm before and after 30-minute exposure to unilateral intermittent
 510 negative pressure in healthy young individuals (n=15). P-values refer to a linear mixed model to examine the main effect of 'time'
 511 (pre- versus post-intervention), 'arm' (intervention-arm versus contra-lateral control arm) and the interaction-effect between
 512 '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	0.957 ⁵¹³
<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	0.603 ⁵¹⁴
<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	0.096 ⁵¹⁵
<i>SRAUC (s⁻¹x10³)</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	0.572 ⁵¹⁶
<i>Time to peak (secs)</i>	48 (40-56)	43 (35-51)	43 (35-51)	47 (39-55)	0.950	0.919	0.217 ⁵¹⁷

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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 in the intervention arm (A) and control arm (B), 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. In panel A, 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. Panel B demonstrates no change in shear rate in the control arm during the intermittent negative pressure intervention. 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) of 15 healthy young men. 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 standard error. Paired T-tests determined differences in shear rate compared to baseline. *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 standard error. A linear mixed model determined the main effect for time and arm.

Figure 1



Figure 2

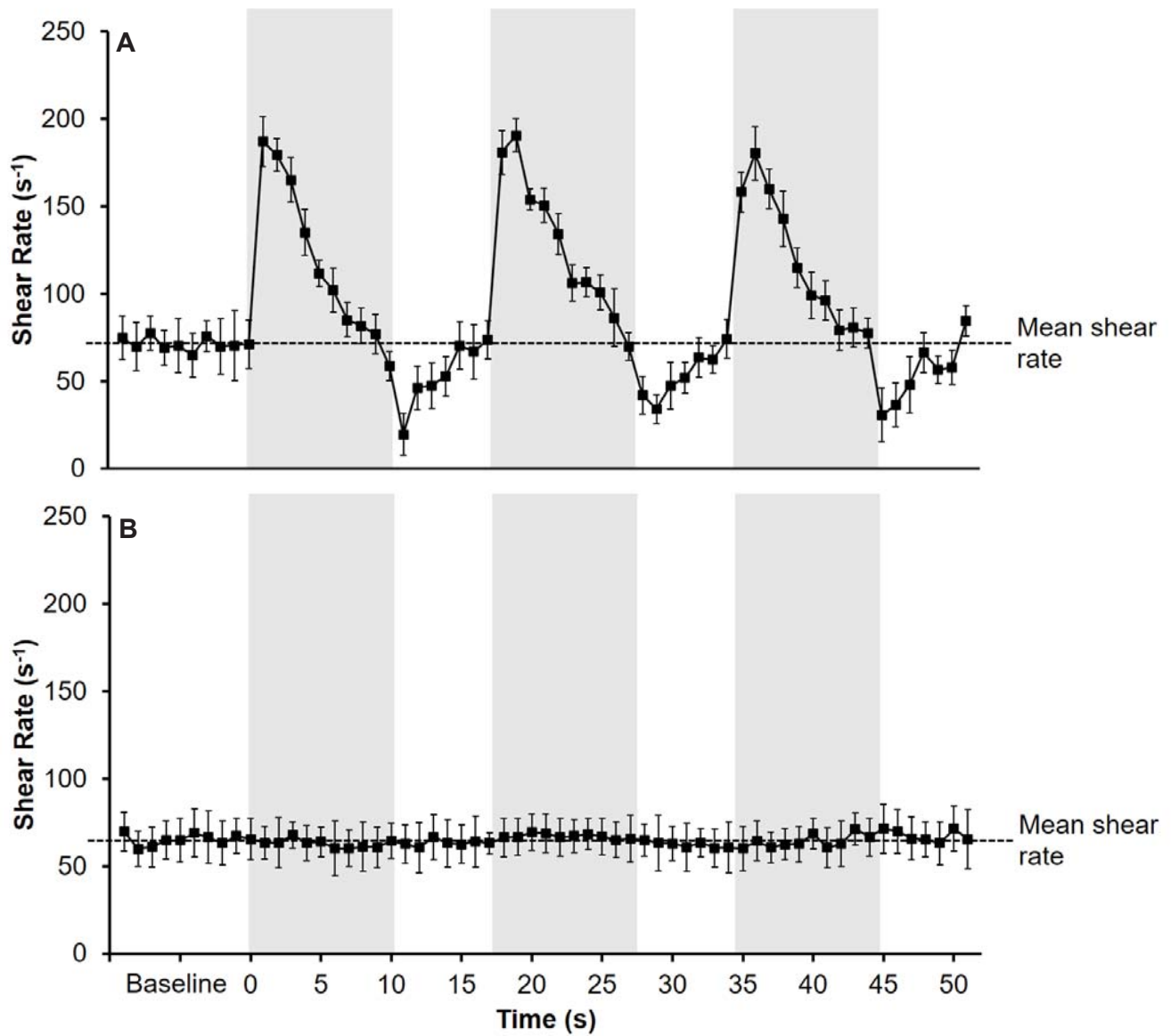


Figure 3

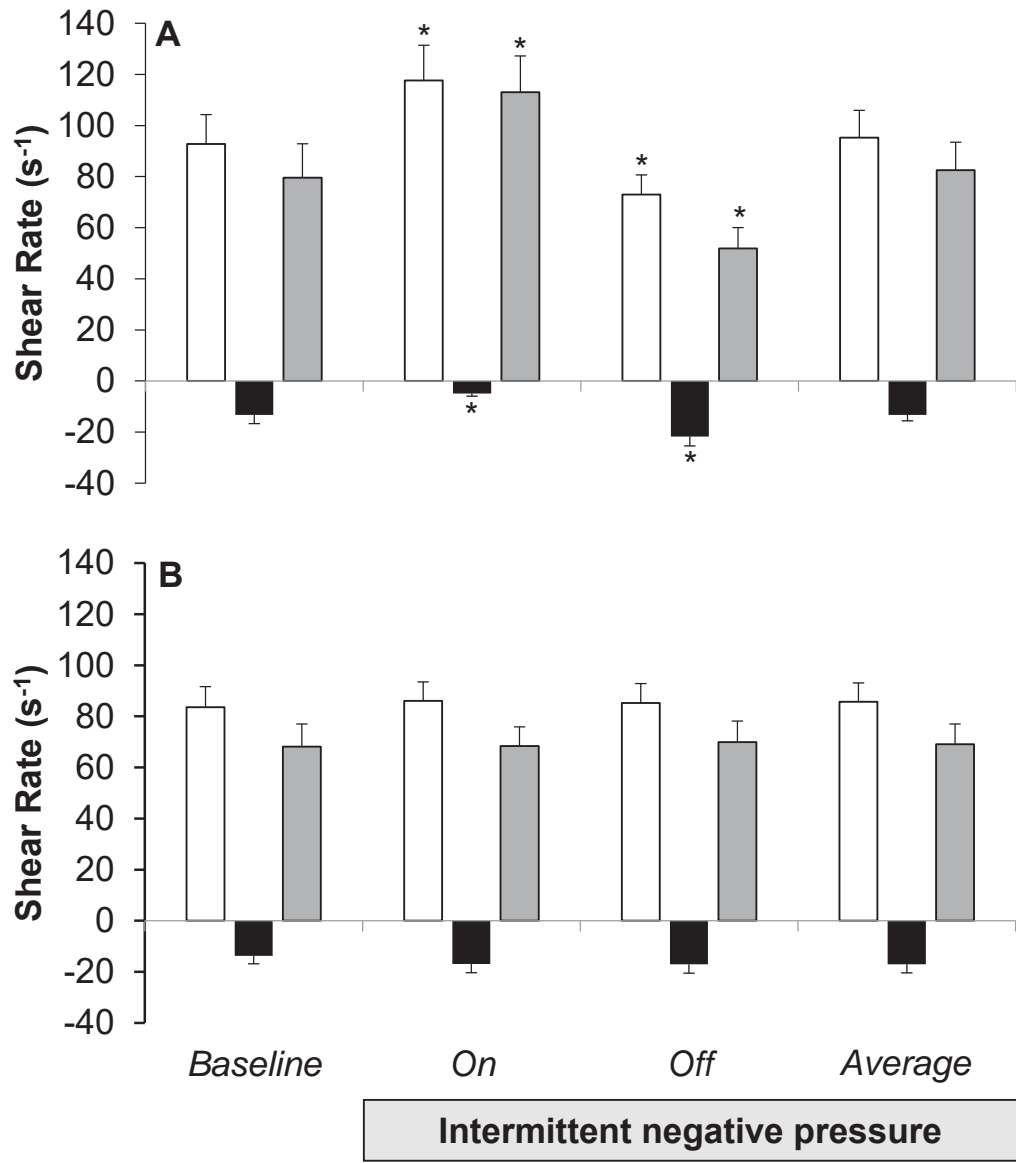


Figure 4.

