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

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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.

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.

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.

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

Materials and Methods

Participants

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

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

Experimental Design

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

Preparations

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

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

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

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

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

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

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

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

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 (± 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 ± standard error unless
214 stated otherwise.
215
216

Results

Subject characteristics are presented in Table 1.

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

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

1.9s^{-1}, *P*=0.190) and "pressure off" (antegrade: Δ+1.9s^{-1}, *P*=0.779; retrograde: Δ-2.0s^{-1}, *P*=0.164; Figure 3B). Therefore, mean antegrade and retrograde shear rate was not different from baseline across the intervention (antegrade: Δ+2.2s^{-1}, *P*=0.750; retrograde: Δ-1.9s^{-1}, *P*=0.173).

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

Discussion

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

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

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

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

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

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

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

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

Conclusion

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

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Disclosures

None.

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506 **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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508 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**				
	Pre	*Post*	*Pre*	*Post*	**'time'**	**'arm'**	**'time*arm'**
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.

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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 ± 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

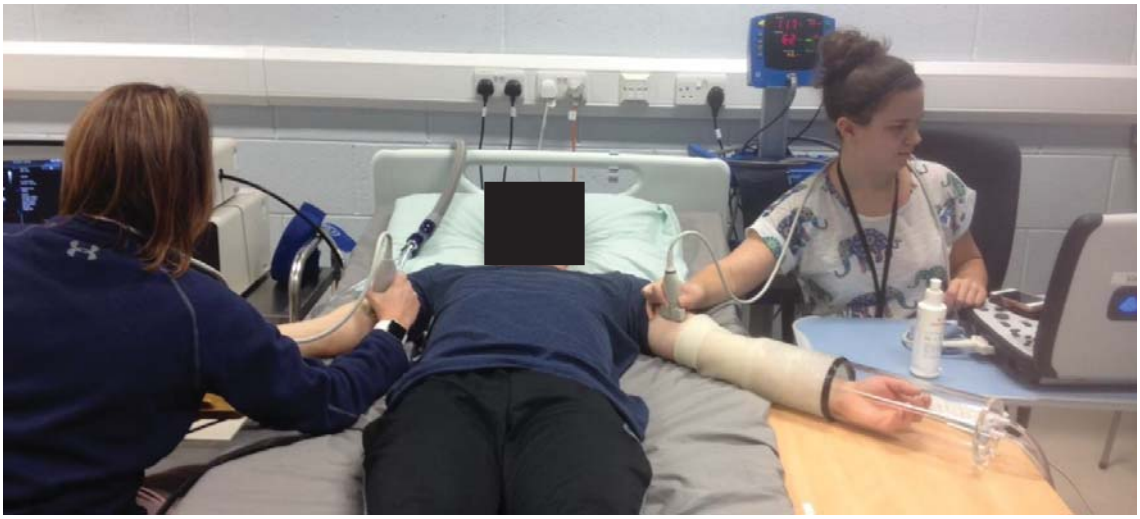

Figure 2

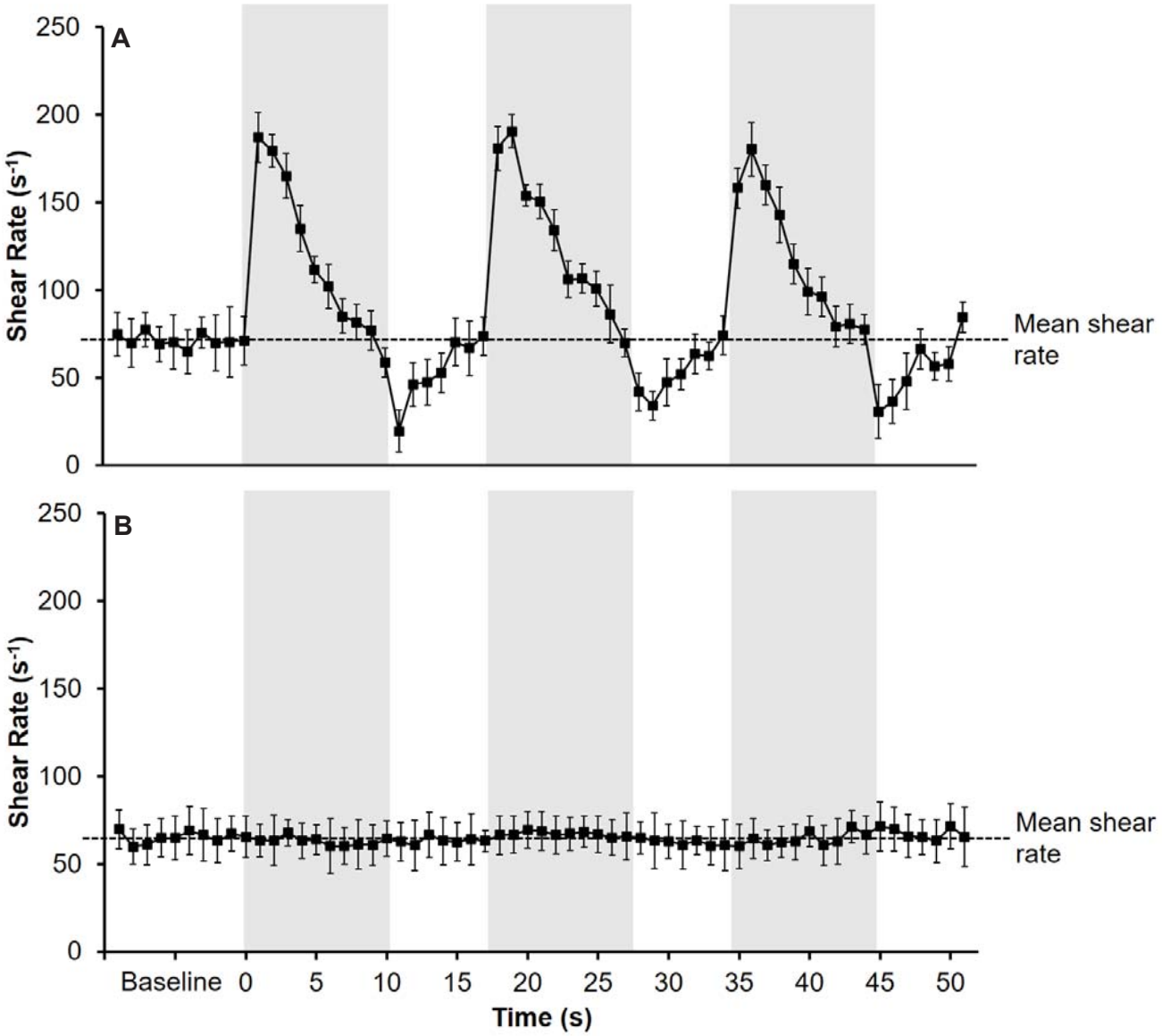

Figure 3

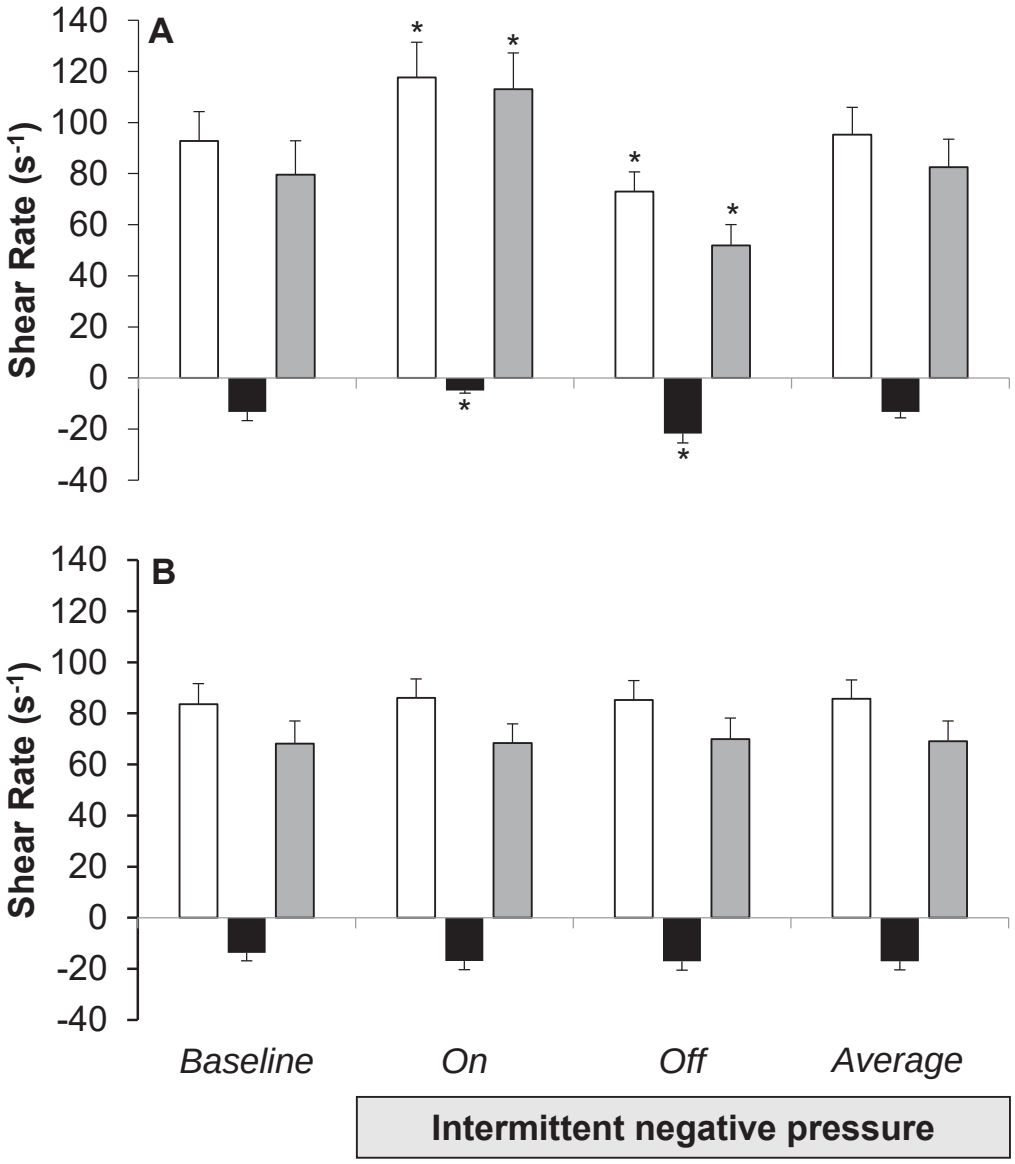

Figure 4.