

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

Sophie M. Holder¹, Ellen A. Dawson¹, Áine Brislane^{1,2}, Jonny Hisdal³, Daniel J. Green⁴ & Dick H.J. Thijssen^{1,5}

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

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

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

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

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

Word count: 4718

Figures: 4

Tables: 2

Short title: Shear fluctuations improve brachial endothelial function

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

Abstract

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

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

Results: Negative pressure significantly increased shear rate, followed by a decrease in shear rate upon pressure release (both $P < 0.001$). Across the 30-minute intervention, mean shear rate was not different compared to baseline ($P = 0.458$). A linear mixed model revealed a significant effect of time was observed for flow-mediated dilation ($P = 0.029$), with exploratory post-hoc analysis showing an increase in the intervention arm ($\Delta\text{FMD} +2.0\%$, $P = 0.008$), but not in the contra-lateral control arm ($\Delta\text{FMD} +0.5\%$, $P = 0.664$). 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.

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.

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.

60

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

73

74

75 **Materials and Methods**

76 *Participants*

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

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™, 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.

Brachial artery diameter and shear rate were analysed using the custom-designed software described above. The region of interest location (selected by the operator) remained consistent for each 1-minute recording *within* participants. Using markers placed by the operator, the software calculated the average artery diameter and shear rate across the minute recordings. The fluctuations in shear stress were analysed as an average during the application of negative pressure (10secs; On), atmospheric pressure (7secs; Off), and the full cycle, then repeated for the 3 full cycles captured during each 1-minute recording. These processes were repeated for each time point during the intervention. Data from a representative individual are presented in Figure 2.

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

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 (52 ± 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”: $\Delta+34.2\text{s}^{-1}$, “pressure off”: $\Delta-26.5\text{s}^{-1}$; both $P<0.001$; Figure 3A). Consequently, mean shear rate across the intervention period was not different from baseline (“pressure on/off cycle”: $\Delta+3.8\text{s}^{-1}$; $P=0.458$). In the control arm, negative pressure did not change mean shear from baseline levels (“pressure on”: $\Delta+1.6$ $P=0.805$, “pressure off”: $\Delta+3.5\text{s}^{-1}$ $P=0.613$). Therefore, mean shear rate remained unchanged throughout the intervention period compared to baseline (“pressure on/off cycle”: $\Delta+2.5\text{s}^{-1}$ $P=0.702$; Figure 3B).

When examining shear patterns, negative pressure increased antegrade shear rate ($P<0.001$) and decreased retrograde shear rate ($P=0.006$, Figure 3). 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: $\Delta+2.5\text{s}^{-1}$, $P=0.730$; retrograde: $\Delta-1.9\text{s}^{-1}$, $P=0.190$) and “pressure off” (antegrade: $\Delta+1.9\text{s}^{-1}$, $P=0.779$; retrograde: $\Delta-2.0\text{s}^{-1}$, $P=0.164$). Therefore, mean antegrade and retrograde shear rate was not different from baseline across the intervention (antegrade: $\Delta+2.2\text{s}^{-1}$, $P=0.750$; retrograde: $\Delta-1.9\text{s}^{-1}$, $P=0.173$).

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 ($\Delta+2.0\%$, $P=0.008$), whilst no change was observed in the control arm ($\Delta+0.5\%$, $P=0.664$). 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 suggest that these benefits of intermittent negative pressure stimulus on wound healing (26, 28) may be related to enhanced endothelial function.

A final possible explanation for our findings relates to the impact of intermittent negative pressure on changes in the pressure gradient across the artery wall (24) and, therefore, transmural pressure (20). Although changes in transmural pressure may

affect vascular health (2, 12), it seems unlikely this can explain our findings. First, negative pressure likely increases transmural pressure (due to the drop in external pressure), which is typically associated with impaired vascular health (2). Secondly, vascular function was examined in the brachial artery, i.e. not directly exposed to the changes in (transmural) pressure, and we observed no significant systemic effects on blood pressure of unilateral forearm suction.

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

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.

References

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

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

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

463

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

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

464

465

BMI – body mass index; bpm – beats per minute

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

	Intervention arm		Control arm		‘time’	‘arm’	‘time*arm’
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>			
<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⁻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
<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

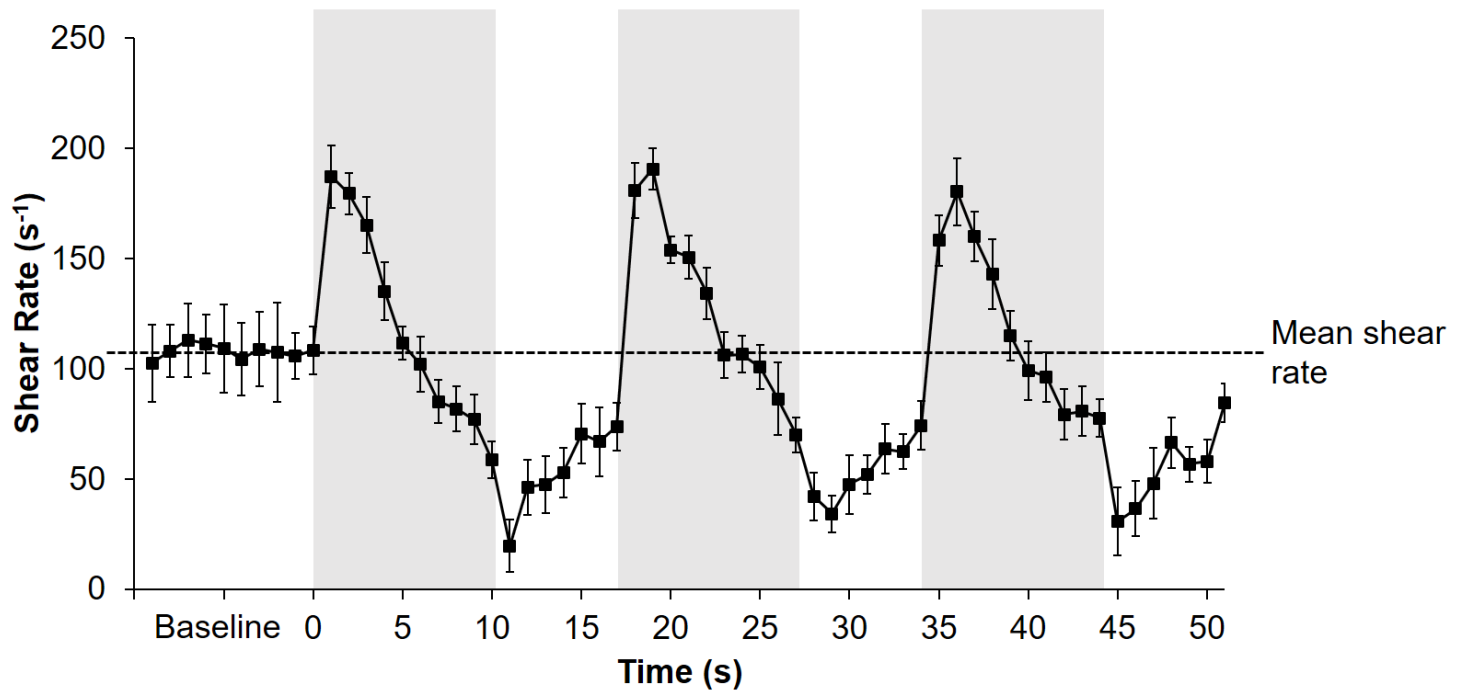


Figure 3

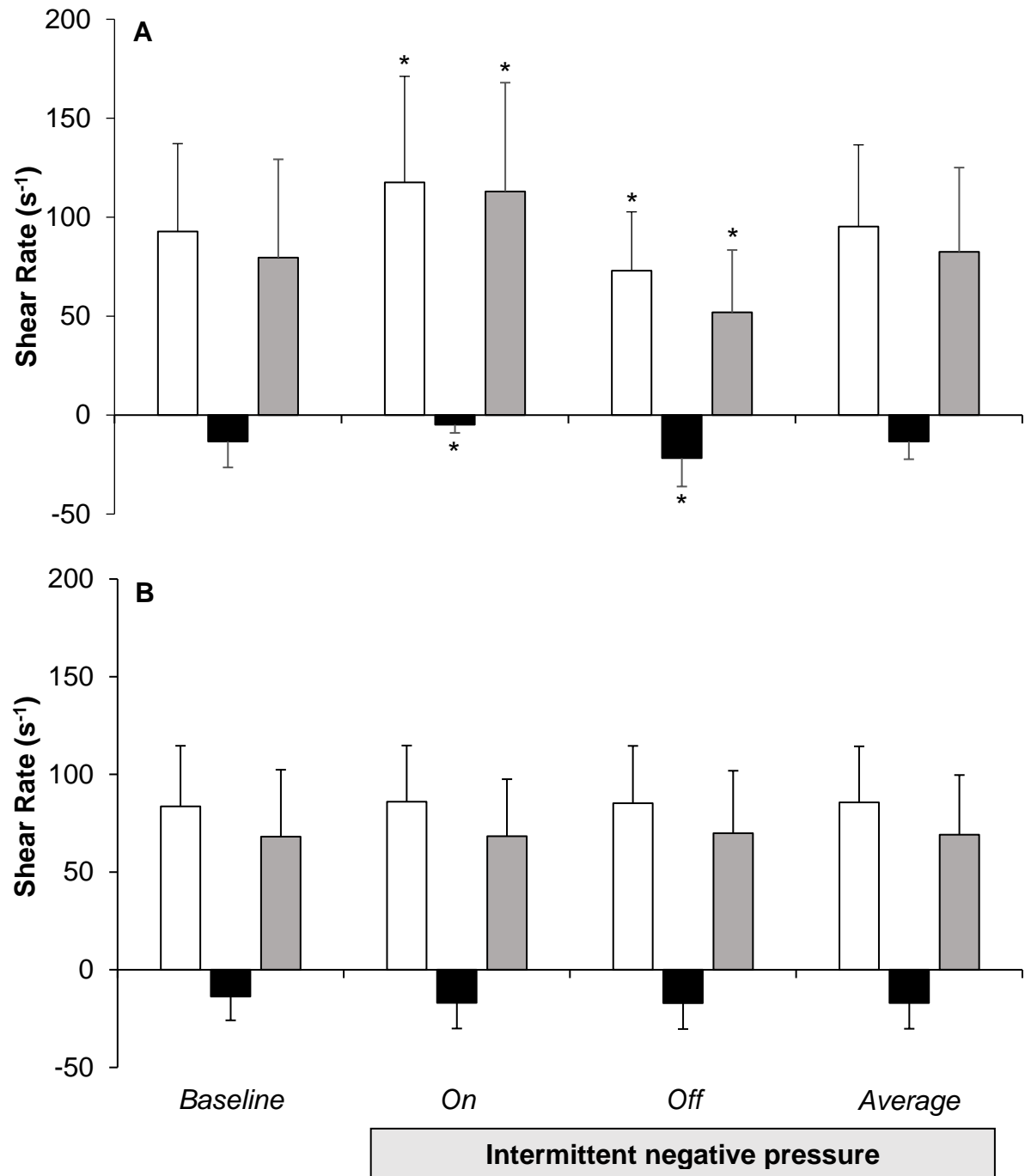


Figure 4.

