Impact of proximal and distal cuff inflation on brachial artery endothelial function in healthy individuals

Ellen A. Dawson,[1] Maxime Boidin,[1,2,3] Ruth Thompson,[1] Nigel T. Cable,[4] Dick H.J. Thijssen,[1,5*] Daniel J. Green[6*]

*Contributed equally as senior

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

[2]Cardiovascular Prevention and Rehabilitation (EPIC) Center, Montreal Heart Institute, Montreal, Canada

[3]School of Kinesiology and Exercise Science, Faculty of Medicine, Université de Montréal, Montreal, Canada

[4School] of Sport, Exercise and Rehabilitations Sciences, University of Birmingham, Birmingham, United Kingdom

[5]Research Institute for Health Sciences, Department of Physiology, Radboud university medical center, Nijmegen, the Netherlands

[6]School of Human Sciences (Exercise and Sport Science), The University of Western Australia, Perth, Western Australia

Corresponding author:
Dr Ellen A. Dawson
E.Dawson@ljmu.ac.uk

ORCID
https://orcid.org/0000-0003-3226-2921

Word account of the main text: 3007
Word account of the abstract: 213

ABSTRACT

Purpose. In this study, we examined whether the decrease in endothelial function associated with short-term exposure to elevated retrograde shear rate (SR), could be prevented when combined with a concurrent drop in transmural pressure in humans.

Methods. Twenty-five healthy individuals reported to our laboratory on 3 occasions to complete 30-min experimental conditions, preceded and followed by assessment of endothelial function using flow-mediated dilation (FMD). We used cuff inflation for 30-min to manipulate retrograde SR and transmural pressure in the brachial artery. Subjects underwent, in randomised order: 1. forearm cuff inflation to 60 mmHg (Distal cuff; causing increase in retrograde SR), 2. Upper arm cuff inflation to 60 mmHg (Proximal cuff; causing increase in retrograde SR + decrease in transmural pressure), and 3. No cuff inflation (Control).

Results. The Distal and Proximal cuff conditions both increased brachial artery retrograde SR ($p<0.001$) and oscillatory shear index ($p<0.001$). The Control intervention did not alter SR patterns or FMD ($p>0.05$). A significant interaction-effect was found for FMD ($p<0.05$), with the decrease during Distal cuff (from 6.9±2.3% to 6.1±2.5%), being reversed to an increase with Proximal cuff (from 6.3±2.0% to 6.9±2.0%). The proximal cuff related increase in FMD could not be explained by the decrease in antegrade or increase in retrograde shear.

Conclusion. This study suggests that a decrease in transmural pressure may ameliorate the decline in endothelial function that occurs following exposure to elevated retrograde shear in healthy individuals.

Key words: Atherosclerosis, vascular function, shear stress, hemodynamics

Cardiovascular diseases, in particular those associated with atherosclerosis, remain the world's leading causes of morbidity and mortality (Townsend et al. 2016). Impaired function of the endothelium is an early and integral atherosclerotic event (Behrendt and Ganz 2002; Widlansky et al. 2003). In fact, impaired endothelial function precedes thickening of the arterial wall and plaque formation, both characteristics of atherosclerosis (Glowinska-Olszewska et al. 2007; Halcox et al. 2009; Juonala et al. 2004; Kobayashi et al. 2004). Hemodynamic factors, including shear stress and (transmural) pressure, represent key stimuli for adaptation in endothelial function and vascular structure (Green et al. 2017; Laughlin et al. 2008), and understanding the interplay between such hemodynamic factors can improve our knowledge of their role in the development of atherosclerosis and cardiovascular disease.

In a series of previous studies, we altered shear stress patterns utilising cuff inflation, which induced an increase in retrograde shear stress and increase in oscillatory shear index. Exposure to these altered shear stress patterns caused a dose-dependent decrease in endothelial function (Schreuder et al. 2014; Thijssen et al. 2009), possibly through inhibition of NO bioavailability (Johnson et al. 2011; Laughlin et al. 2008; Newcomer et al. 2008; Thijssen et al. 2009; Widlansky et al. 2003). Interestingly, inflation of a cuff may cause similar hemodynamic effects as an arterial stenosis. Altered shear stress patterns have been observed both proximal and distal to a stenosis (Koskinas et al. 2009; Laughlin et al. 2008; Lee et al. 1978) and from cuff inflation (Betik et al. 2004; Schreuder et al. 2014; Thijssen et al. 2009; Tinken et al. 2009). In keeping with the stenosis analogy (Ku 1997; Lee et al. 1978), a drop in transmural pressure may be present distal, but not proximal, from a partially inflated cuff (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018). Accordingly, cuff manipulation may represent a non-invasive model to assess the impact of hemodynamics on endothelial function.

The aim of this study was to examine the impact of sub-systolic cuff inflation (to 60 mmHg) on shear rate patterns and endothelial function (examined using the flow-mediated dilation technique) in young healthy individuals. We hypothesised that the decrease in endothelial function as a result of exposure to elevated levels of retrograde SR might be offset when these SR patterns are combined with a drop in transmural pressure in healthy individuals.

Methods

Study design and participants' recruitment

Twenty-five men (27±4 yrs) were recruited at the Liverpool John Moores University from 2013 to 2015. The study procedures were approved by the ethics committee of Liverpool John Moores University and adhered to the declaration of Helsinki. All participants gave written consent before experimental testing. Participants diagnosed with cardiovascular diseases, who reported cardiovascular risk factors (e.g. hypertension or hypercholesterolemia) or were using any medication that could influence the cardiovascular system, were excluded.

Experimental design

In random order, all participants reported to our laboratory on 3 occasions to undergo testing, separated by at least 24 hours between visits. Endothelial function of the mid-brachial artery was assessed at each visit (using the flow-mediated dilation [FMD] technique), before and immediately after the 30-minute intervention. The 3 interventions consisted of: 1. a cuff inflation around the forearm to alter SR patterns, elevating the oscillatory shear index with minimal impact on transmural wall pressure (Distal cuff condition), 2. a cuff inflation around the upper arm to alter SR patterns, elevating the oscillatory shear index (Proximal cuff) and decreasing transmural wall pressure (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018), and 3. no cuff inflation (i.e. Control) (Figure 1). All measurements were performed at the same time of the day to help correct for circadian rhythm, under standardized conditions, in the same respective condition, and on the right arm (Jones et al. 2010).

Assessment of brachial artery function, and blood flow and shear rate pattern were conducted in accordance with recent guidelines (Thijssen et al. 2011; Thijssen et al. 2019). Participants were requested to fast for six hours, abstain from alcohol and caffeine for 18 hours and avoid physical activity for 24 hours prior to the measurements (Thijssen et al. 2019). Although fitness and physical activity levels were not formally assessed, different conditions under which subjects were studied, were conducted within, weeks and subjects did not change their usual levels of activity in that time period.

Participants rested in a supine posture for at least 15 minutes, to ensure steady state conditions and to facilitate baseline examination of heart rate, blood pressure and brachial artery function. Heart rate and blood pressure (i.e. systolic blood pressure [SBP], diastolic blood pressure [DBP] and mean arterial pressure [MAP]) were measured before each brachial artery function measurement, using an automated sphygmomanometer (Dinamap, GE Pro 300V2), placed around the left upper arm.

Brachial artery endothelial function. FMD measurement was performed in all participants to assess NO mediated endothelium-dependent vasodilation (Green et al. 2011). To measure brachial artery FMD, the right arm was extended and positioned at an angle of ~80° from the torso. Immediately distal to the olecranon process of the right arm, a rapid inflated and deflated pneumatic cuff of 5 cm (D.E. Hokanson, Bellevue, WA) was placed, to provide a stimulus for local ischemia in the forearm (Corretti et al. 2002; Thijssen et al. 2019). A 10-MHz multifrequency linear probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA) was used to image the brachial artery. The probe was positioned on the distal one-third of the upper arm during the measurements. Once an optimal image was found, the probe was held stable, whilst ultrasound parameters were set to optimize the longitudinal, B-mode images of lumen-arterial wall interface. Continuous Doppler velocity assessment was simultaneously obtained, and was collected using the same insonation angle (always <60°). After a 1-minute baseline, the cuff placed round the forearm was inflated at ~220 mmHg for 5 minutes, and then deflated for 3 minutes. Brachial artery diameter and blood flow were continuously recorded (Camtasia, TechSmith, MI, USA) during the first minute baseline, the last 30-second of cuff inflation, and the 3-minute of cuff deflation. FMD was calculated as the maximum percent increase in brachial arterial diameter after cuff deflation as compared with resting diameter. Measurements also included baseline (mm) and peak (mm) brachial diameters, adjusted FMD (%), shear rate area under the curve (SR_{auc}, s), and time to peak (TTP, s) (Thijssen et al. 2019).

Interventions. Immediately after the initial FMD measurement, participants remained supine for 15 minutes to restore diameter and blood flow to normal level. Subsequently, individuals underwent one of the 3 interventions. Each intervention lasted 30 minutes and consisted of the manipulation of the brachial artery by inflation of a blood pressure cuff to 60 mmHg, as reported by previous studies (Schreuder et al. 2014; Thijssen et al. 2009). Participants underwent one of the following interventions: 1) cuff placed on the forearm inflated to 60 mmHg (Distal cuff); 2) cuff placed on the upper arm inflated to 60 mmHg (Proximal cuff); and 3): cuff placed on the forearm + not inflated (Control). Since all measurements were performed in the mid-brachial artery, cuff position on the forearm meant that we insonated the artery proximal to the cuff location (Distal cuff), while cuff position of the upper arm means that we insonated distal to the cuff location (Proximal cuff). Brachial artery mean shear rate, pattern of shear rate (antegrade *versus* retrograde, and oscillatory shear index [OSI]) (Black et al. 2008; Newcomer et al. 2008; Wu et al. 2004), diameter, mean blood flow and pattern of blood flow (antegrade *versus* retrograde) were recorded for 1 minute at every 5-minute interval for each intervention. After 25 minutes of intervention heart rate and blood pressure were again measured.

Data analysis

Analysis of brachial artery diameter and shear rate during both FMD measurements and also during the interventions was performed using custom-designed-edge-detection and wall-tracking software, with an intra-observer coefficient of variation of 6.7% (Woodman et al. 2001). After calibration, regions of interest (ROI) were selected for analysis of diameter (from B-mode image) and blood flow (from blood flow velocity envelope) at 30 Hz (Black et al. 2008). All data were written to a file and used for further analysis in a custom designed analysis package. Correction for within subject changes for baseline diameter and SR_{auc} were made by using these parameters as covariates (Atkinson et al. 2013). After computing the logged values of baseline and peak diameter, the difference between the logged baseline and logged peak diameter were used in a general linear model as the outcome and logged baseline diameter was used as a covariate. The same procedure was used for SR_{auc} analysis.

Statistical analysis

Data are presented as mean ± standard deviation unless stated otherwise. The statistical analyses were performed with GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, California, USA). Differences were defined as statistically significant when $p<0.05$. After ensuring a normal distribution, a one-way analysis of variance (ANOVA) was used to compare baseline over the three conditions. A two-way repeated measures ANOVA (condition x time) was used to compare all time points (every 5 minutes for 30 minutes) between conditions for OSI. A similar two-way ANOVA with repeated measures for time was used to examine whether the impact of cuff placement ("time": pre *versus* post) on endothelial function (FMD), blood pressure and heart rate differs between conditions ("condition"). Tukey's post hoc analyses on the Δ were used to identify differences. Post-hoc paired *t*-test analyses (pre-post) were used for each variable. The analysis was repeated with the correction for within-subject changes for baseline diameter and SR_{auc}.

Results

Mean (±SD) weight and body mass index were 75.9±11.1 kg and 23.8±2.6 kg.m^{-2} respectively. No cardiovascular risk factors or disease were reported. We found no significant differences between the three conditions at baseline or during the interventions for systolic blood pressure, diastolic blood pressure, or mean arterial pressure. We found a significant time effect for heart rate after the intervention (Table 1), possibly influenced by a longer resting period.

Effect of cuff position on blood flow and shear pattern

Two-way ANOVAs revealed an interaction for all SR variables. One-way ANOVAs post hoc analyses on the Δ showed a significant decrease in mean SR during Proximal (p=0.003) and Distal (p=0.03) cuff compared to Control with no differences between both cuff interventions (p=0.13) (Figure 2). The same analysis showed a significant decrease in antegrade SR in the Proximal cuff compared to the Distal cuff (p=0.003) and a trend compared to Control (p=0.08). Retrograde SR were significantly increased in both cuff interventions compared to Control (p<0.001 for both), with a greater effect on retrograde SR for the Distal cuff condition (p=0.02 for Δ retrograde SR between both experimental conditions, Figure 2). We also observed an increase in OSI during Distal cuff and Proximal cuff compared to control, with no difference between cuff conditions (Figure 3). Blood flow demonstrated similar results to SR (Table 2).

Effect of cuff position on vessel diameter and FMD

There were no differences for baseline or peak diameter, SR_{auc}, and TTP between interventions at baseline (Table 3). A two-way ANOVA performed on pre and post data across the 3 conditions revealed an interaction for FMD (p=0.03). Post-hoc paired *t*-tests for time effect (pre-post) within conditions showed trends for both Distal (p=0.07) and Proximal cuff (p=0.06) (Figure 4). Post-hoc analysis on the ΔFMD showed a significantly different between the 2 experimental conditions (p=0.04, Table 3, Figure 4). Repeating the analysis when correcting for within-subject changes for baseline diameter and SR_{auc} confirmed our initial analysis.

Discussion

Our study compared the impact of different cuff placements on brachial artery endothelial function. Our data suggest that an increase in retrograde SR impairs FMD, and that the magnitude of this impairment can be mitigated by a contemporaneous decrease in transmural pressure (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018).

In the present study, we used cuff inflation to manipulate blood flow and SR patterns. Both cuff placements altered SR pattern by reducing the mean and retrograde blood flow and SR, whereas antegrade blood flow and transmural pressure were only affected by the proximal cuff placement. Elevated retrograde blood flow and SR have been shown to increase endothelin 1 expression (Ziegler et al. 1998), expression of adhesion molecules (Chappell et al. 1998; Himburg et al. 2007) and ROS-producing enzymes (De Keulenaer et al. 1998; Hwang et al. 2003), the release of superoxide anions (McNally et al. 2003), and decrease endothelial NO synthase expression (De Keulenaer et al. 1998; Hwang et al. 2003). Such changes result in impaired vasodilation and a pro-atherogenic phenotype in the vascular wall (Green et al. 2017; Laughlin et al. 2008). In a previous study we observed that forearm cuff inflation at 50 and 75 mmHg, during which antegrade SR remained relatively stable and retrograde SR increased (Thijssen et al. 2009), resulted in decreased FMD. In similar studies, our team have demonstrated that elevation in retrograde SR attenuates brachial and superficial femoral artery FMD (Schreuder et al. 2014; Thijssen et al. 2009; Tinken et al. 2009). In fact, a dose-dependent relationship is apparent between the drop in FMD and increase in retrograde SR ($r=0.51$; $p=0.006$) (Thijssen et al. 2009). These findings are consistent with our observations in the present study, in that the distal cuff condition involving a large increase in retrograde SR and was also associated with a decrease in FMD. While we cannot exclude possible impacts on coagulation and platelet function, our within-subject design, and previous studies, suggest that retrograde shear rate plays a key role in modulating FMD (Padilla et al. 2008).

We observed a significant interaction between cuff position on FMD responses (p=0.03). In contrast to our findings with forearm cuff placement, placement of a cuff around the upper arm resulted in an increase in FMD. Similar findings in 8 healthy males after a 5-minute suprasystolic occlusion were observed in another study (Betik et al. 2004). However, that study did not report blood flow and SR pattern. Both cuff conditions in our study showed an increase of retrograde SR. Such an increase in retrograde shear could explain the reduced FMD we observed in the Distal cuff condition. However, we also found an increase in retrograde shear in the Proximal cuff condition, which was associated with an *increase* in FMD. Such an increase in FMD cannot be explained on the basis of an increase in retrograde shear, as such change is typically linked to a decrease in FMD (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018; Stegehuis et al. 2018; Thijssen et al. 2009; van de Hoef et al. 2013). The increase in FMD is also unlikely to be related to the decrease in antegrade shear, given that previous *in vitro* (Lie et al. 1970) and *in vivo* (Doshi et al. 2001; Holder et al. 2019; Simmons et al. 2011; Tinken et al. 2009) studies have related *enhanced* anterograde flow with improvement in endothelial function.

An alternative explanation for the increase in FMD after proximal cuff placement relates to reduced transmural pressure. A previous study from our team assessed the impact of acute exposure to elevations in transmural pressure on brachial artery diameter (Atkinson et al. 2015). We observed a decrease in brachial artery function as a result of increased transmural pressure which was independent of SR. This accords with our current study where, despite decreases in antegrade and increases in retrograde flow and shear in the proximal cuff condition, FMD did not decrease. A drop in transmural pressure seems the most likely explanation for the enhanced FMD we observed after proximal cuff placement. Previous *in vitro* and animal studies have reported a decrease in transmural pressure as a result of stenosis or cuff inflation (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018). Altering transmural pressure modifies vascular smooth muscle function and tone in a manner that is not dependent of the endothelium-derived NO (Ekelund et al. 1992). Based on previous studies (Anderson et al. 1979; Bache and Schwartz 1982; de Leeuw et al. 2018; Stegehuis et al. 2018; van de Hoef et al. 2013) we therefore suggest that, whilst increases in retrograde SR impair FMD, the magnitude of this impairment can be mitigated by a contemporaneous decrease in transmural pressure.

In coronary artery disease patients, invasive techniques have demonstrated that pressure drop distal to a stenosis can improve the selection of patients who benefit from coronary revascularization, versus the use of the coronary angiogram alone (van Nunen et al. 2015). Such an approach may reduce exposure to mechanical revascularization and improve the benefit of coronary interventions. Our study used cuff inflation to emulate shear patterns that may also be observed in coronary arteries, providing insight into the impact of partial occlusion on artery function using a non-invasive technique.

There are several limitations and caveats associated with our study. It is possible that the increase FMD after the proximal cuff condition that we observed may have resulted from a direct effect of proximal cuff placement on distal arterial smooth muscle (Agewall et al. 1999). However, our 30 min cuff inflation was not constrictive or ischemic and did not induce muscle pain or any symptoms, suggesting that that direct downstream effects on the artery wall were unlikely. Stenosis has an impact on blood flow, shear rate, and at extreme levels, tissue oxygenation and perfusion. We did not assess the impact of 60 mmHg cuff inflation on artery wall oxygenation and perfusion in our study. Considering that oxygenation and perfusion in tissues were affected with a cuff above 80 mmHg (Abay and Kyriacou 2016), it could be interesting to repeat our experimental design using a higher cuff inflation. An omission of our study was that we did not directly measure transmural pressure in the brachial artery. However, several previous studies have demonstrated the presence of a drop in pressure distal from a stenosis, either present as a result of a pathophysiologic process or induced mechanically under experimental conditions (Bache and Schwartz 1982; Chatzizisis et al. 2007; Dirksen et al. 1998; Koskinas et al. 2009; Laughlin et al. 2008; Stegehuis et al. 2018). Although shear stress could be different depending on the placement of the probe in relation to the cuff, we standardised the location of the cuff in both experimental conditions. Finally, our study was undertaken in healthy young male volunteers and we did not have measures of fitness or physical activity levels. Future studies could investigate the impacts of cuff inflation in women, with a range of fitness levels and in clinical populations, such as those of advanced age, with obesity, and hypertension which are characterized by elevated peripheral vascular tone and elevated retrograde shear rate in conduit arteries.

In conclusion, the positioning of a cuff above or below the brachial artery alters blood flow, SR, transmural pressure and endothelial function. Impaired endothelial function observed after distal cuff inflation can be explained by the greater retrograde SR, in keeping with previous evidence. We conclude that the increase in FMD we observed after proximal cuff placement may be explained by a countervailing decrease in transmural pressure. Future studies might utilize our dual cuff location approach to determine whether the balance between shear and transmural pressure effects is protective, or detrimental, in distinct clinical populations.

Compliance with ethical standards

Conflict of interest

All authors declare no conflict of interests.

Funding

MB is financially supported by a grant from the Fonds de Recherche du Québec – Santé (FRQ-S) and the Cardiometabolic Healthy, Diabetes and Obesity Research Network (CMDO). DG is supported by a NHMRC Principal Research Fellowship (APP1080914).

References

Abay T, Kyriacou P (2016) Comparison of NIRS, laser Doppler flowmetry, photoplethysmography, and pulse oximetry during vascular occlusion challenges Physiological measurement 37:503

Agewall S, Whalley G, Doughty R, Sharpe N (1999) Handgrip exercise increases postocclusion hyperaemic brachial artery dilatation Heart 82:93-95

Anderson W, Johnston C, Korner P (1979) Acute renal haemodynamic and renin-angiotensin system responses to graded renal artery stenosis in the dog The Journal of physiology 287:231-245

Atkinson CL et al. (2015) 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

Atkinson G, Batterham AM, Thijssen DH, Green DJ (2013) A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research J Hypertens 31:287-291 doi:10.1097/HJH.0b013e32835b8164

Bache RJ, Schwartz JS (1982) Effect of perfusion pressure distal to a coronary stenosis on transmural myocardial blood flow Circulation 65:928-935

Behrendt D, Ganz P (2002) Endothelial function. From vascular biology to clinical applications Am J Cardiol 90:40L-48L

Betik AC, Luckham VB, Hughson RL (2004) Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions American Journal of Physiology-Heart and Circulatory Physiology 286:H442-H448

Black MA, Cable NT, Thijssen DH, Green DJ (2008) Importance of measuring the time course of flow-mediated dilatation in humans Hypertension 51:203-210 doi:10.1161/HYPERTENSIONAHA.107.101014

Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW (1998) Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium Circ Res 82:532-539

Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH (2007) Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior J Am Coll Cardiol 49:2379-2393 doi:10.1016/j.jacc.2007.02.059

Corretti MC et al. (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force J Am Coll Cardiol 39:257-265

De Keulenaer WG, Alexander RW, Ushio-Fukai M, Ishizaka N, Griendling KK (1998) Tumour necrosis factor α activates a p22phox-based NADH oxidase in vascular smooth muscle Biochemical Journal 329:653-657

de Leeuw PW, Postma CT, Spiering W, Kroon AA (2018) Atherosclerotic Renal Artery Stenosis: Should we Intervene Earlier? Current hypertension reports 20:35

Dirksen MT, van der Wal AC, van den Berg FM, van der Loos CM, Becker AE (1998) Distribution of inflammatory cells in atherosclerotic plaques relates to the direction of flow Circulation 98:2000-2003

Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, Goodfellow J (2001) Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide Clinical science 101:629-635

Ekelund U, Björnberg J, Grande PO, Albert U, Mellander S (1992) Myogenic vascular regulation in skeletal muscle in vivo is not dependent of endothelium-derived nitric oxide Acta physiologica scandinavica 144:199-207

Glowinska-Olszewska B, Tolwinska J, Urban M (2007) Relationship between endothelial dysfunction, carotid artery intima media thickness and circulating markers of vascular inflammation in obese hypertensive children and adolescents Journal of Pediatric Endocrinology and Metabolism 20:1125-1136

Green DJ, Hopman MT, Padilla J, Laughlin MH, Thijssen DH (2017) Vascular adaptation to exercise in humans: role of hemodynamic stimuli Physiological reviews 97:495-528

Green DJ, Jones H, Thijssen D, Cable N, Atkinson G (2011) Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension 57:363-369

Halcox JP et al. (2009) Endothelial function predicts progression of carotid intima-media thickness Circulation 119:1005-1012

Himburg HA, Dowd SE, Friedman MH (2007) Frequency-dependent response of the vascular endothelium to pulsatile shear stress American Journal of Physiology-Heart and Circulatory Physiology 293:H645-H653

Holder SM, Dawson EA, Brislane A, Hisdal J, Green DJ, Thijssen DH (2019) Fluctuation in shear rate, with unaltered mean shear rate, improves brachial artery flow-mediated dilation in healthy, young men Journal of Applied Physiology 126:1687-1693

Hwang J et al. (2003) Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation Circ Res 93:1225-1232 doi:10.1161/01.RES.0000104087.29395.66

Johnson BD, Mather KJ, Wallace JP (2011) Mechanotransduction of shear in the endothelium: basic studies and clinical implications Vasc Med 16:365-377 doi:10.1177/1358863X11422109

Jones H, Green DJ, George K, Atkinson G (2010) Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans Am J Physiol Regul Integr Comp Physiol 298:R427-432 doi:10.1152/ajpregu.00442.2009

Juonala M, Viikari JS, Laitinen T, Marniemi J, Helenius H, Rönnemaa T, Raitakari OT (2004) Interrelations between brachial endothelial function and carotid intima-media thickness in young adults: the cardiovascular risk in young Finns study Circulation 110:2918-2923

Kobayashi K, Akishita M, Yu W, Hashimoto M, Ohni M, Toba K (2004) Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity Atherosclerosis 173:13-18

Koskinas KC, Chatzizisis YS, Baker AB, Edelman ER, Stone PH, Feldman CL (2009) The role of low endothelial shear stress in the conversion of atherosclerotic lesions from stable to unstable plaque Curr Opin Cardiol 24:580-590 doi:10.1097/HCO.0b013e328331630b

Ku DN (1997) Blood flow in arteries Annual Review of Fluid Mechanics 29:399-434 doi:DOI 10.1146/annurev.fluid.29.1.399

Laughlin MH, Newcomer SC, Bender SB (2008) Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype J Appl Physiol (1985) 104:588-600 doi:10.1152/japplphysiol.01096.2007

Lee BY, Assadi C, Madden JL, Kavner D, Trainor FS, McCann WJ (1978) Hemodynamics of arterial stenosis World J Surg 2:621-627

Lie M, Sejersted OM, Kiil F (1970) Local regulation of vascular cross section during changes in femoral arterial blood flow in dogs Circulation Research 27:727-737

McNally JS et al. (2003) Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress Am J Physiol Heart Circ Physiol 285:H2290-2297 doi:10.1152/ajpheart.00515.2003

Newcomer SC, Sauder CL, Kuipers NT, Laughlin MH, Ray CA (2008) Effects of posture on shear rates in human brachial and superficial femoral arteries Am J Physiol Heart Circ Physiol 294:H1833-1839 doi:10.1152/ajpheart.01108.2007

Padilla J et al. (2008) Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus Cardiovasc Ultrasound 6:44 doi:10.1186/1476-7120-6-44

Schreuder TH, Green DJ, Hopman MT, Thijssen DH (2014) Acute impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in humans Physiol Rep 2:e00193 doi:10.1002/phy2.193

Simmons GH et al. (2011) Increased brachial artery retrograde shear rate at exercise onset is abolished during prolonged cycling: role of thermoregulatory vasodilation Journal of Applied Physiology 110:389-397

Stegehuis VE, Wijntjens GW, Murai T, Piek JJ, van de Hoef TP (2018) Assessing the haemodynamic impact of coronary artery stenoses: intracoronary flow versus pressure measurements European Cardiology Review 13:46

Thijssen DH et al. (2011) Assessment of flow-mediated dilation in humans: a methodological and physiological guideline Am J Physiol Heart Circ Physiol 300:H2-12 doi:10.1152/ajpheart.00471.2010

Thijssen DH et al. (2019) Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans European heart journal 40:2534-2547

Thijssen DH, Dawson EA, Tinken TM, Cable NT, Green DJ (2009) Retrograde flow and shear rate acutely impair endothelial function in humans Hypertension 53:986-992 doi:10.1161/HYPERTENSIONAHA.109.131508

Tinken TM et al. (2009) Impact of shear rate modulation on vascular function in humans Hypertension 54:278-285 doi:10.1161/HYPERTENSIONAHA.109.134361

Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M (2016) Cardiovascular disease in Europe: epidemiological update 2016 European heart journal 37:3232-3245

van de Hoef TP, Meuwissen M, Escaned J, Davies JE, Siebes M, Spaan JA, Piek JJ (2013) Fractional flow reserve as a surrogate for inducible myocardial ischaemia Nat Rev Cardiol 10:439-452 doi:10.1038/nrcardio.2013.86

van Nunen LX et al. (2015) Fractional flow reserve versus angiography for guidance of PCI in patients with multivessel coronary artery disease (FAME): 5-year follow-up of a randomised controlled trial Lancet 386:1853-1860 doi:10.1016/s0140-6736(15)00057-4

Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA (2003) The clinical implications of endothelial dysfunction J Am Coll Cardiol 42:1149-1160

Woodman RJ et al. (2001) Improved analysis of brachial artery ultrasound using a novel edge-detection software system J Appl Physiol (1985) 91:929-937

Wu SP, Ringgaard S, Oyre S, Hansen MS, Rasmus S, Pedersen EM (2004) Wall shear rates differ between the normal carotid, femoral, and brachial arteries: An in vivo MRI study Journal of Magnetic Resonance Imaging 19:188-193 doi:Doi 10.1002/Jmri.10441

Ziegler T, Bouzourene K, Harrison VJ, Brunner HR, Hayoz D (1998) Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells Arterioscler Thromb Vasc Biol 18:686-692

Tables and figures

Table 1. Blood pressure and heart rate responses to the interventions.

	Control (n=25)		Distal cuff (n=25)		Proximal cuff (n=25)		ANOVA P-value		
	Pre	Post	Pre	Post	Pre	Post	Group effect	Time effect	Interaction
SBP, mmHg	118±7	118±8	117±9	116±9	119±9	119±9	0.41	0.47	0.97
DBP, mmHg	66±5	68±5	66±6	65±5	66±5	67±6	0.65	0.78	0.08
MAP, mmHg	86±4	87±4	87±5	86±3	87±5	87±6	0.71	0.95	0.53
HR, bpm	56±13	54±10	56±11	53±9	56±12	54±10	0.95	<0.001	0.95

Variables are expressed as mean ± SD.

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate.

Table 2. Blood flow pattern at baseline and during each intervention

	Control (n=25)		Distal cuff (n=25)		Proximal cuff (n=25)		ANOVA p-value		
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention	Group effect	Time effect	Interaction
Blood flow pattern									
Mean blood flow, $mL.min^{-1}$	60.6±36.1	56.6±29.7	47.3±31.0	28.1±22.7	59.2±41.1	26.0±19.3**	<0.001	<0.001	<0.001
Antegrade blood flow, $mL.min^{-1}$	68.8±32.8	62.9±28.6	57.1±28.2	55.5±19.1	67.3±38.3	44.9±17*, ¶	0.03	<0.01	0.001
Retrograde blood flow, $mL.min^{-1}$	-8.3±11.3	-6.4±6.1	-9.8±9.6	-27.5±19.4§§	-8.2±9.2	-18.9±12.3**, ¶	<0.001	<0.001	<0.001
Blood flow velocity, $cm.s^{-1}$	8.8±5.5	8.1±4.6	6.9±4.3	4.2±3.3§	8.1±5.0	3.7±2.8**	<0.001	<0.001	0.001

Variables are expressed as mean ± SD. Intervention value were averaged over the six time points (i.e. at 5, 10, 15, 20, 25 and 30 minutes).

Tukey's multiple comparisons on the Δ: Proximal cuff vs. control, *p<0.05, **p<0.001; Distal cuff vs. control §p<0.05, §§p<0.001; Distal cuff vs. Proximal cuff ¶p<0.05, ¶¶p<0.001.

Table 3. Brachial artery function before and after the interventions

	Control (n=25)		Distal cuff (n=25)		Proximal cuff (n=25)		ANOVA p-value		
	Pre	Post	Pre	Post	Pre	Post	Group effect	Time effect	Interaction
Baseline diameter, mm	3.86±0.44	3.88±0.43	3.90±0.41	3.87±0.39	3.88±0.38	3.89±0.37	0.98	0.91	0.48
Peak diameter, mm	4.10±0.44	4.14±0.44	4.16±0.44	4.10±0.40	4.13±0.40	4.16±0.40	0.97	0.81	0.11
FMD, %	6.3±2.8	6.7±3.3	6.9±2.3	6.1±2.5	6.3±2.0	6.9±2.0[¶]	0.97	0.69	0.03
SR_{auc}, s^{-1} 10^3	23.0±10.9	19.3±9.6	18.8±8.9	17.8±6.5	19.8±8.3	18.1±7.6	0.42	0.005	0.28
Time to peak, s	52±20	48±20	45±17	44±13	48±15	40±9	0.19	0.03	0.56

Variables are expressed as mean ± SD.
FMD: Flow-mediated dilation; SR_{auc}: Shear rate area under the curve.
Distal cuff vs. Proximal cuff, [¶]$p<0.05$.

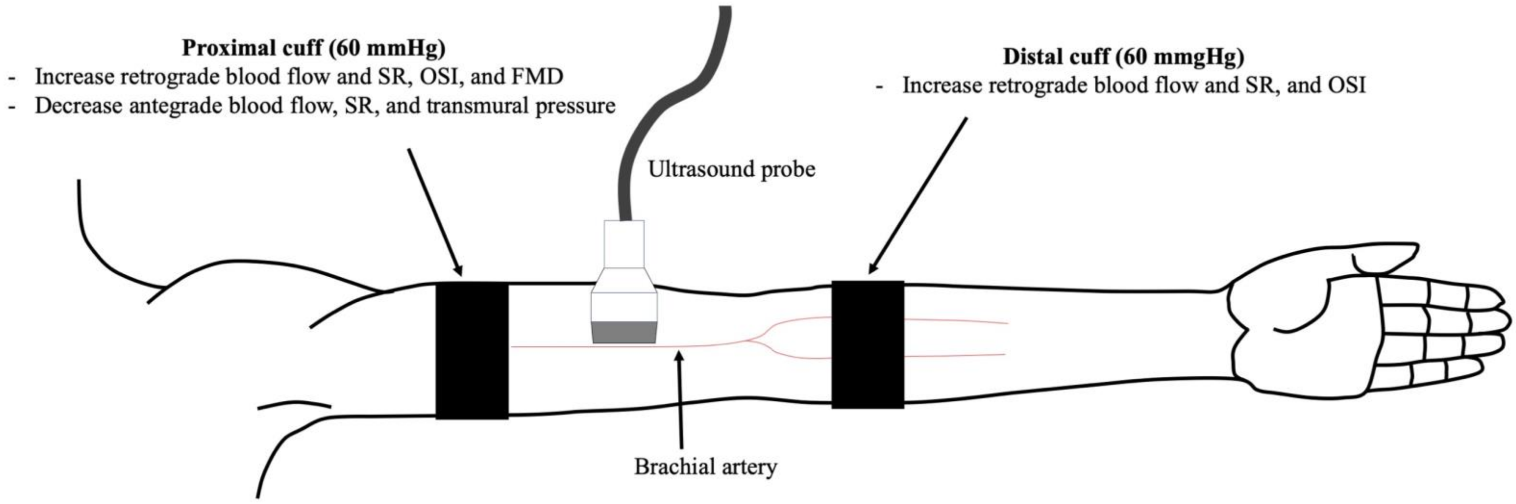

Figure 1: Schematic diagram of the both cuff interventions and placement of the ultrasound probe during the cuff intervention. The position for cuff used for the FMD assessment (cuff pressure >200 mmHg) was the same as that used for the Distal cuff condition (60 mmHg).
SR: Shear rate; OSI: Oscillatory shear index; FMD: Flow-mediated dilation.

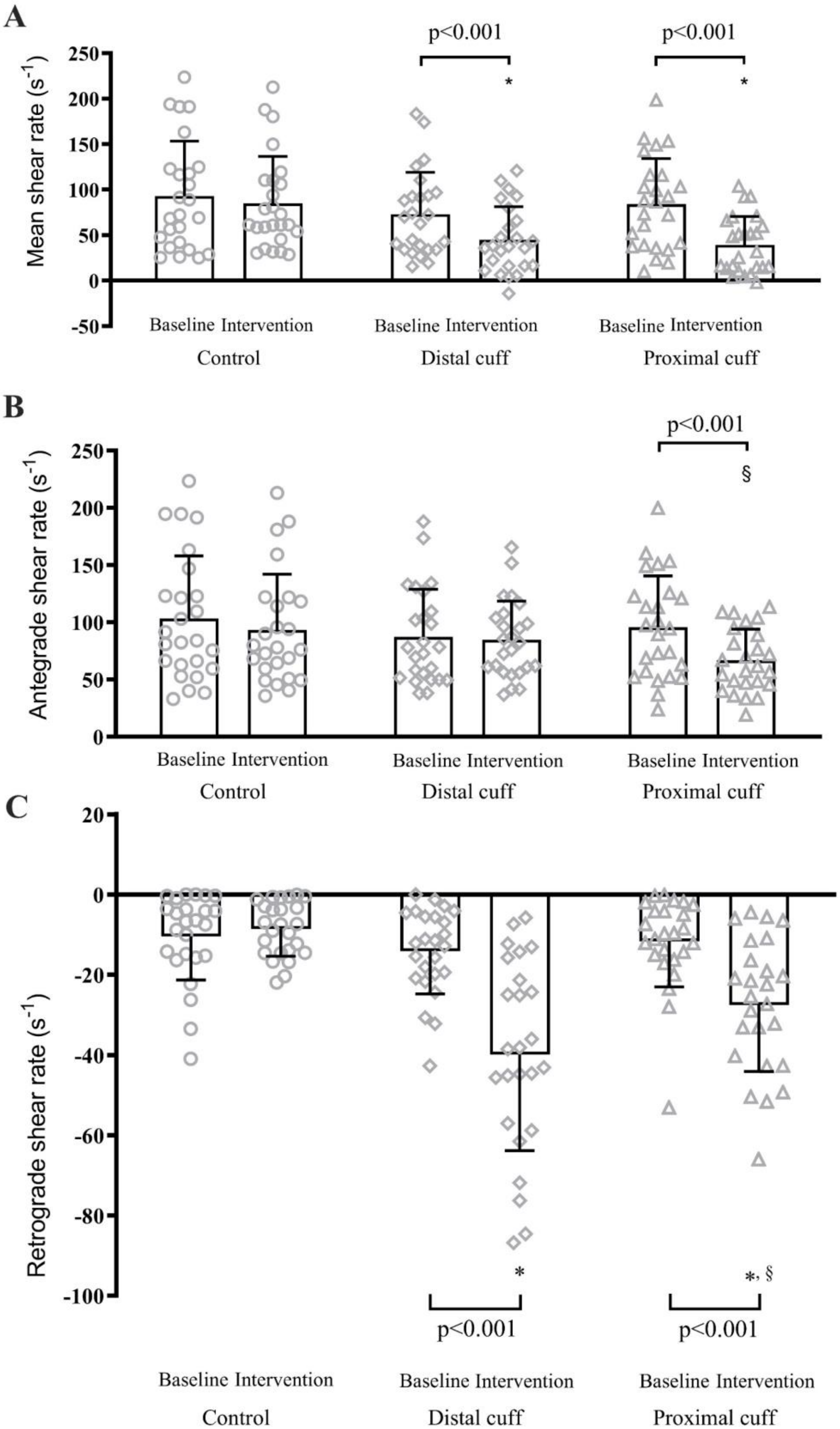

Figure 2: Group and individual mean shear rate (panel A) and shear rate pattern (antegrade shear rate (panel B), and retrograde shear rate (panel C)) at baseline and during intervention for each condition (Control, Distal cuff, and Proximal cuff) in healthy young men (n=25).
*: Compared to Control, p<0.05; §: Distal cuff vs. Proximal cuff, p<0.05, using a two-way ANOVA with repeated measures. P-values represent the paired *t*-test analysis (pre-post) in each condition. Error bars represent SD.

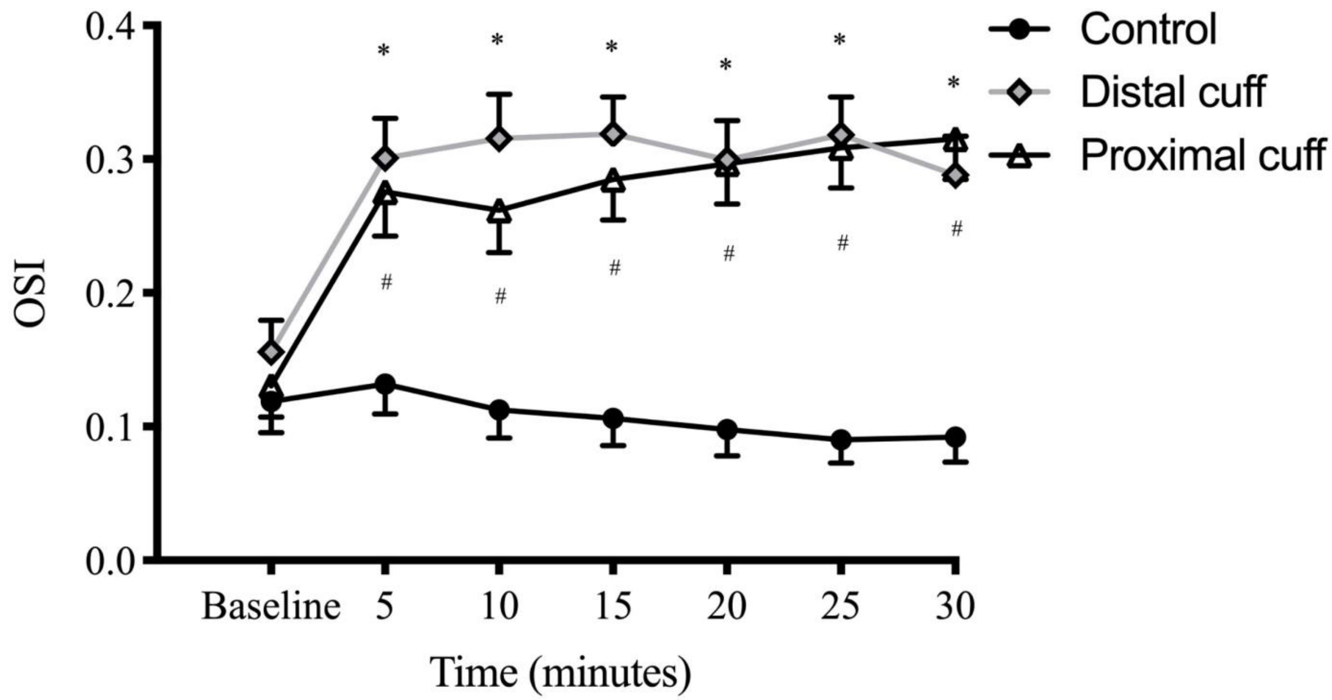

Figure 3: Oscillatory shear index (OSI) during the 30-minute intervention between the three conditions (Control condition ● ; Proximal cuff condition ■ ; Distal cuff condition △) in healthy young men (n=25). *: Distal cuff vs. Control, $p<0.001$; #: Proximal cuff vs. Control, $p<0.001$ at the same time point. Error bars represent SD.

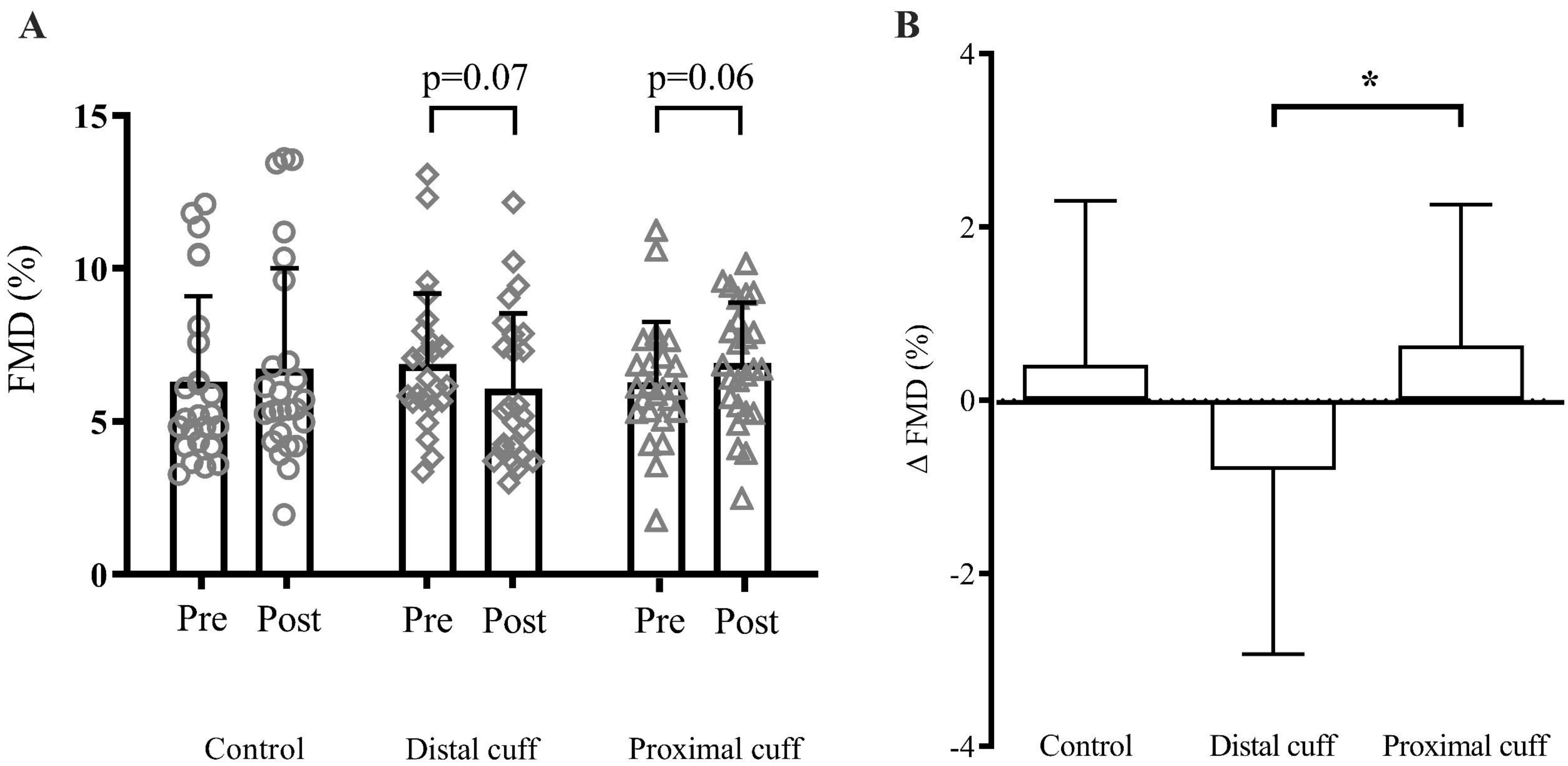

Figure 4: Group and individual flow-mediated dilation (FMD) responses (panel A) and changes in FMD (post-pre: Δ FMD, panel B,) to the Control, Distal cuff and Proximal cuff interventions in healthy young men (n=25). *p<0.05. Error bars represent SD.