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

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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 ($\text{SR}_{\text{auc}}$, s), and time to peak ($\text{TTP}$, 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 ∆ 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 ∆ 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 ∆ 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$_{\text{auc}}$, 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 $\Delta$FMD 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$_{\text{auc}}$
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.

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Nat Rev Cardiol 10:439-452 doi:10.1038/nrcardio.2013.86


**Tables and figures**

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=25)</th>
<th>Distal cuff (n=25)</th>
<th>Proximal cuff (n=25)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118±7</td>
<td>118±8</td>
<td>117±9</td>
<td>116±9</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>66±5</td>
<td>68±5</td>
<td>66±6</td>
<td>65±5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86±4</td>
<td>87±4</td>
<td>87±5</td>
<td>86±3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>56±13</td>
<td>54±10</td>
<td>56±11</td>
<td>53±9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Blood flow pattern</th>
<th>Control (n=25)</th>
<th>Distal cuff (n=25)</th>
<th>Proximal cuff (n=25)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Intervention</td>
<td>Baseline</td>
<td>Intervention</td>
</tr>
<tr>
<td>Mean blood flow, mL.min⁻¹</td>
<td>60.6±36.1</td>
<td>56.6±29.7</td>
<td>47.3±31.0</td>
<td>28.1±22.7</td>
</tr>
<tr>
<td>Antegrade blood flow, mL.min⁻¹</td>
<td>68.8±32.8</td>
<td>62.9±28.6</td>
<td>57.1±28.2</td>
<td>55.5±19.1</td>
</tr>
<tr>
<td>Retrograde blood flow, mL.min⁻¹</td>
<td>-8.3±11.3</td>
<td>-6.4±6.1</td>
<td>-9.8±9.6</td>
<td>-27.5±19.4§§</td>
</tr>
<tr>
<td>Blood flow velocity, cm.s⁻¹</td>
<td>8.8±5.5</td>
<td>8.1±4.6</td>
<td>6.9±4.3</td>
<td>4.2±3.3§§</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Control (n=25)</th>
<th>Distal cuff (n=25)</th>
<th>Proximal cuff (n=25)</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>3.86±0.44</td>
<td>3.88±0.43</td>
<td>3.90±0.41</td>
<td>3.87±0.39</td>
</tr>
<tr>
<td>Peak diameter, mm</td>
<td>4.10±0.44</td>
<td>4.14±0.44</td>
<td>4.16±0.44</td>
<td>4.10±0.40</td>
</tr>
<tr>
<td>FMD, %</td>
<td>6.3±2.8</td>
<td>6.7±3.3</td>
<td>6.9±2.3</td>
<td>6.1±2.5</td>
</tr>
<tr>
<td>SR&lt;sub&gt;auc&lt;/sub&gt;, s&lt;sup&gt;-1&lt;/sup&gt; 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>23.0±10.9</td>
<td>19.3±9.6</td>
<td>18.8±8.9</td>
<td>17.8±6.5</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>52±20</td>
<td>48±20</td>
<td>45±17</td>
<td>44±13</td>
</tr>
</tbody>
</table>

Variables are expressed as mean ± SD.
FMD: Flow-mediated dilation; SR<sub>auc</sub>: Shear rate area under the curve.
Distal cuff vs. Proximal cuff, °p<0.05.
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.