HIGHLIGHTS

- Endothelial dysfunction is common in patients with severe aortic stenosis.
- This study demonstrates early improvements in endothelial function post-TAVR.
- Importantly, improvements in endothelial function are sustained at late follow up.
- Improved endothelial function is likely related to the altered haemodynamics.
Patients with aortic stenosis exhibit early improved endothelial function following transcatheter aortic valve replacement: the eFAST study

Andrea Comella¹,4 **, Michael Michail¹,2,4 MBBS**, Jasmine Chan¹,4 MBBS(Hons), James D. Cameron¹,4 PhD, Robert Gooley PhD¹,4, Anthony Mathur²,3,4 PhD, Alun D. Hughes²,4 PhD, Adam J. Brown¹,4 PhD

¹Monash Cardiovascular Research Centre, MonashHeart, Monash University and Monash Health, Melbourne, Australia

²Institute of Cardiovascular Science, University College London, London, United Kingdom

³Centre for Cardiovascular Medicine and Device Innovation, Queen Mary University of London, London, United Kingdom

⁴This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

** Joint first authors.

Study was performed at Monash Health

Running Head: Endothelial function recovery post-TAVR

Correspondence:

Dr Adam J Brown

Monash Cardiovascular Research Centre and MonashHeart, Monash Health, Melbourne, Australia

Email: adam.brown@monash.edu

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ABSTRACT (250 words)

Background: Patients with severe aortic stenosis (AS) exhibit systemic endothelial dysfunction, which can be associated with myocardial ischaemia in absence of obstructive coronary disease. Transcatheter aortic valve replacement (TAVR) is used to treat severe AS in patients with high or prohibitive surgical risk. However, it remains unknown whether endothelial function recovers post-TAVR. We therefore sought to assess the early and late changes in flow-mediated dilation (FMD), a measure of endothelial function, following TAVR.

Methods: Patients undergoing TAVR for severe AS had ultrasound assessment of brachial endothelial-independent and -dependent FMD. Measurements were performed pre-TAVR, at early follow-up (<48 hours post-TAVR) and late follow-up (4-6 weeks post-TAVR).

Results: 27 patients (mean age 82.0±7.0; 33.3% female) were recruited; 37.0% had diabetes mellitus and 59.3% had hypertension. Brachial artery FMD increased from 4.2±1.6% (pre-TAVR) to 9.7±3.5% at early follow-up (p<0.0001). At late follow-up, improvement compared with early follow-up was sustained (8.7±1.9%, p=0.27). Resting brachial arterial flow velocities decreased significantly at late follow-up (11.24±5.16 vs. 7.73±2.79 cm/s, p=0.003). Concordantly, at late follow-up, there was decrease in resting wall shear stress (WSS; 14.8±7.8 vs. 10.6±4.8dyne/cm², p=0.01), peak WSS (73.1±34.1 vs. 58.8±27.8dyne/cm², p=0.03) and cumulative WSS (3543±1852 vs. 2504±1089dyne·s/cm², p=0.002). Additionally, a favourable inverse correlation between cumulative WSS and FMD was restored at late follow-up (r=-0.21 vs. r=0.49).

Conclusion: Endothelial function in patients with AS improves early post-TAVR and this improvement is sustained. This likely occurs as a result of improved arterial haemodynamics, leading to lower localised WSS and release of vasoactive mediators that may also alleviate myocardial ischaemia.
KEY WORDS

transcatheter aortic valve replacement; aortic stenosis; endothelial dysfunction; flow mediated dilatation
1. INTRODUCTION

1.1. Aortic stenosis (AS) is the most common valvular pathology requiring intervention in developed countries and the burden of the disease continues to grow with an ageing population\(^1\). The underlying disease mechanisms responsible for the development of AS shares some common aetiological mechanisms with coronary atherosclerosis\(^2\). This includes disturbance in endothelial cell function, which plays an important role in regulating vascular wall function and organ blood flow\(^3\) through vasoactive factors such as nitric oxide and prostaglandins. Endothelial dysregulation (ED) is characteristic of atherosclerotic disease\(^4\) and reduced flow mediated dilation in the brachial artery, an indicator of ED, has also been demonstrated in the early stages of AS\(^5\). Furthermore, the physiological changes in patients with severe AS have been demonstrated to lead to increased wall shear stress (WSS) which further dysregulate endothelial function\(^6\)\(^-\)\(^8\). ED contributes to the pathophysiological alterations in coronary blood flow in AS patients,\(^9\) including impaired coronary flow reserve (CFR)\(^10\)\(^,\)\(^11\). The mechanisms for impairment of CFR in AS are multifactorial but include submaximal ED-mediated microcirculatory vasodilatation in hyperaemia and increased resting secretion of NO causing higher baseline coronary flow velocities\(^9\). As one of the proposed mechanisms underpinning impaired CFR, ED is likely a contributing mechanism to the exertional symptoms seen in patients with severe AS. Transcatheter aortic valve replacement (TAVR) is a percutaneous alternative to surgical aortic valve replacement and is treatment of choice in those with high or prohibitive surgical risk.\(^12\) In patients undergoing coronary angioplasty, persistent ED is associated with adverse cardiovascular outcomes.\(^13\) It remains unknown whether ED recovers following TAVR and previous studies have yielded conflicting results\(^14\)\(^,\)\(^15\)\(^,\)\(^16\)\(^,\)\(^17\). Importantly, it is unknown whether the minimally invasive nature of this procedure is associated with early recovery, or whether changes are dependent on other
factors which may resolve at a later stage. Furthermore, a better understanding of ED recovery may provide insights into the underlying mechanisms in those with persistent symptoms following TAVR. We hypothesised that once the pathological AS haemodynamic environment is restored to premorbid physiological conditions post-TAVR, the improvement in aortic flow patterns will induce an improvement in the endothelial function. We also hypothesised that any improvement in endothelial function will not occur immediately following TAVR but may occur over several weeks.
2. METHODS

2.1. This was a prospective, single centre study carried out at Monash Medical Centre, Melbourne between February and August, 2019. The study protocol was approved by the institutional research ethics committee (Human Research Ethics Committees Australia reference: HREC/45959/MonH-2018-152246). All recruited patients provided written informed consent.

2.2. All patients with severe AS with an indication for TAVR as per international guidelines\(^1^8\) were screened. Exclusion criteria included (1) patients undergoing concomitant coronary revascularisation, (2) stage 5 chronic kidney disease, (3) systemic inflammatory conditions and (4) active malignancy.

2.3. Endothelial assessment was performed at: (1) baseline pre-TAVR; on the day of the procedure, (2) early follow-up within 48 hours post-TAVR, and (3) late follow-up, 4-6 weeks post-TAVR. At each assessment, the patients were graded according to the New York Heart Association (NHYA) class and Canadian Cardiovascular Society (CCS) score for angina pectoris.

2.4. Endothelial function was assessed non-invasively measuring (FMD) of the brachial artery.\(^1^9\) FMD was performed in accordance to standardised practice by the same experienced investigator\(^1^9\). Prior to assessment, patients fasted and abstained from smoking, coffee and exercise. All tests were conducted in a temperature-controlled environment (21°C). Measurements were taken using a high-resolution linear vascular probe (Mindray L14-6Ns, Mindray, China), placed 1 to 2 cm above the elbow. Duplex recordings were taken for 30 seconds before cuff inflation and then again for 2.5 minutes, beginning 30 seconds before cuff
deflation. Cuff was placed on the forearm, to ensure that vessel dilation was due solemnly to increase in WSS and not by build-up off metabolic waste products. In order to assess for non-endothelial-dependant vasodilatation, sublingual nitroglycerin was administered after 10 minutes of rest and then the ultrasound measurements were repeated.

2.5. Offline analysis was performed on recorded cine-loops to measure diastolic brachial artery diameters and the angle-corrected time mean-averaged velocities for WSS calculation. FMD was calculated as the percentage change in diameter:

\[
FMD(\%) = \left( \frac{\text{diameter}_{\text{hyperaemia}} - \text{diameter}_{\text{baseline}}}{\text{diameter}_{\text{baseline}}} \right) \times 100
\]

WSS was derived from velocity measurements and vessel diameter using the relationship:

\[
WSS = \frac{8 \times v}{d}
\]

where \( v \) is mean blood flow velocity and \( d \) is diameter.

Intra-observer reproducibility for FMD was assessed on 15 healthy volunteers. Measurements were taken on two separate occasions at least one week apart. Intraclass correlation coefficient demonstrated excellent reproducibility for FMD measurements (ICC = 0.96).

2.6. Our primary end-point was change in FMD at early post-TAVR follow-up. Our preliminary data demonstrated that 21 patients would be required to show a difference between FMD pre- and post-TVAR (\( \alpha 0.05, \beta 0.2 \)). We therefore recruited a total of 33 patients to account for an anticipated dropout rate of 5-10%. Secondary endpoints included change in brachial WSS and changes in FMD at late post-TAVR follow-up assessment. All results are reported as mean ± standard deviation (SD). Distribution of FMD measurements was assessed according to the
Shapiro-Wilk test. Comparison between FMD and WSS values pre-TAVR were compared to values post-TAVR in the same subjects using a paired t-test. Correlations between variables were assessed using Pearson’s correlation coefficient. Statistical analysis was performed using Python 3.8’s statistical packages (scipy.stats and statsmodels), with a p-value ≤0.05 being deemed significant.
3. RESULTS

3.1. Twenty-seven patients were included in the study, with all participants undergoing successful assessment of FMD pre-TAVR. Two patients (7.4%) were lost to follow-up at the late post-TAVR assessment (Figure S1). The mean time to early and late follow-up assessments were 1.4 days and 40.4 days, respectively. The baseline demographics and pharmacotherapy of this cohort are presented in Table 1. 10 patients (37.0%) had diabetes mellitus and 16 (59.3%) had hypertension.

All TAVR procedures were successful with a reduction of mean aortic gradient from 48.8±8.5 to 13.3±6.7 mmHg, (p<0.0001). Echocardiographic and symptom data pre- and post-TAVR are presented in Table S1. At baseline only two patients (7.4%) had CCS III angina, with the remainder having no symptoms of chest pain. Twenty-four patients (88.9%) exhibited symptoms of breathlessness prior to TAVR, with 45.8% of these categorised as NYHA class III. Following TAVR, only 1 patient (3.7%) had resolution of breathlessness at early follow-up. At late follow-up, all but two patients (92.0%) were in NYHA class I.

3.2. FMD improved from 4.2±1.6% pre-TAVR to 9.7±3.5% at early post-TAVR assessment (p<0.0001). The improvement was sustained at late follow-up assessment (FMD 8.7±1.9%, p=0.27 when compared to early follow-up; Table 2, Figure 1). All patients responded to GTN at baseline resulting in percentage dilation greater than that induced by the increase in shear (4.2±1.6 vs. 10.9±3.4%, p<0.0001). Time to peak diameter in hyperaemia did not change significantly between pre- and post-TAVR (Table 2; pre-TAVR 66.4 secs±24.0; early post-TAVR 61.9secs±25.1, p=0.64; late post-TAVR 55.6secs±25.3, p=0.10).
3.3. A subgroup analysis was performed on patients with type II diabetes mellitus given the known association with endothelial dysfunction. Diabetic patients were also found to have significant improvement in brachial FMD following TAVR, both at early and late follow-up assessments (4.46±2.14% vs. 8.87±4.45% early post-TAVR, p=0.006; vs. 8.89±1.34% late post-TAVR, p= 0.003). Furthermore, there was no convincing evidence that change in FMD differed between people with and without diabetes (ΔFMD at early follow-up for diabetics 4.4±3.9% vs. 7.0±3.1% for non-diabetics, p=0.07; ΔFMD at late follow-up for diabetics 4.1±2.6% vs. 4.6±2.7% for non-diabetics, p = 0.71; overall ΔFMD for diabetics 4.3±3.3% vs. 5.8±3.1% for non-diabetics, p = 0.12; Figure S2).

3.4. Compared to pre-TAVR, resting brachial arterial flow velocities were unchanged at early assessment but decreased significantly at late post-TAVR follow-up (11.24±5.16 vs. 7.73±2.79 cm/s, p=0.003). Concordantly, at late post-TAVR follow-up, there were decreases in resting WSS (14.8±7.8 vs. 10.6±4.8 dyne/cm², p=0.01; Figure S3A) and peak WSS (73.1±34.1 vs. 58.8±27.8 dyne/cm², p=0.03; Figure S3B). Whilst a trend was apparent, changes in resting and peak WSS did not reach significance at early post-TAVR assessment (all p>0.05).

3.5. Similarly, at late post-TAVR follow-up, there were decreases in the cumulative WSS (total area under the curve, AUC; 3543±1852 vs. 2503±1089 dyne·s/cm², p=0.002; Figure S3C) and AUC to peak diameter (2476±1315 vs. 1542±829 dyne·s/cm², p=0.001; Figure S3D). Additionally, there was poor correlation of cumulative WSS with FMD pre-TAVR and this was not restored at early post-TAVR follow-up; however, at late follow-up, the correlation was moderate (total AUC r=0.42, p=0.035; Figure 2).
3.6. Further evidence of haemodynamic changes post-TAVR can be observed in the brachial arterial flow pattern. The time to the peak of the resting Doppler waveform decreased at early post-TAVR follow-up, a finding sustained at late follow-up (both p≤0.05; Table 2). Similarly, there was a reduction in the time to the peak of the hyperaemic Doppler waveform at early and late follow-up. The time to peak of the hyperaemic Doppler waveform measured before TAVR correlated well with the dimensionless index (DI; r=-0.51, p= 0.008).
4. DISCUSSION

4.1. Our study demonstrates significant improvements in endothelial-dependent FMD following TAVR. This improvement occurs early post-TAVR and is sustained at late follow-up. Additionally, there are observed changes post-TAVR in the brachial arterial haemodynamics as evidenced by decreased blood flow velocities and WSS, although many of these changes were only evident at late post-TAVR follow-up.

4.2. Early improvements in endothelial function may be a consequence of the early haemodynamic changes associated with the relief of aortic valve obstruction. The time to peak flow velocity decreased at rest and in hyperaemia at early follow-up, likely a manifestation of the central haemodynamic changes. Pre-TAVR, this correlated well with DI, consistent with the prolonged ejection time and delayed peaked velocity associated with AS severity. In addition to the haemodynamic changes, the early improvements in FMD may also reflect the quicker global physiological recovery associated with a minimally invasive approach such as TAVR. This is in contrast to cardiac surgery, which itself can cause early impaired endothelial function, potentially due to factors such as surgical stress, trauma, anaesthesia, pain, hypoxaemia and hypovolaemia.

Importantly, patients with comorbidities that are known to impair endothelial function, such as hypertension, diabetes mellitus and smoking were not excluded from our study. This permitted a realistic reflection of the effect of TAVR on endothelial function in a wider patient demographic with severe AS referred for TAVR. Of interest, the subgroup analysis demonstrated that diabetic patients also benefited from improved endothelial function post-TAVR at early and late follow-up.
4.3. Following TAVR there is an observed decrease in brachial WSS at rest and at peak
hyperaemic flow, likely representing a transition towards normalisation of cardiovascular
physiology. Whilst early trends were observed, these changes were only convincingly seen at
late follow-up, suggesting a slower, adaptive process. Previous evidence has similarly
demonstrated that patients with severe AS have high aortic WSS\textsuperscript{8, 24} that subsequently
decreases following AVR\textsuperscript{25,26, 27}. The mechanisms underlying increases in FMD following
TAVR may be partially explained by changes in WSS. In severe AS, high WSS may stimulate
eNOS (endothelium Nitric Oxide Synthase) to produce NO\textsuperscript{28, 29} with consequent
vasodilatation, thus increasing resting blood flow as seen in peripheral vessels in this study,
but also typically seen in the coronary arteries of such patients.\textsuperscript{9} This partial hyperaemic state
allows less scope for further vasodilatation and upregulation of blood flow due to the partially
exhausted mechanisms and results in impaired FMD, thus suggesting that in AS the
endothelium is not dysfunctional but rather dysregulated as the high resting WSS depletes the
endothelial NO reserves. Once WSS decreases post-TAVR, resting NO release is reduced,
and the brachial artery regains greater scope for vasodilation during hyperaemia and thus
restoration of FMD. These explanations are hypothesis-generating and are not conclusive
given the presence of other conflicting data which suggests an increase in WSS following
relief of AS.\textsuperscript{14, 30, 31} Unusually, these were observed in the absence of changes in blood flow
velocities or vessel diameter.

4.4. Further evidence of delayed physiological normalisation is observed in the relationship
between cumulative WSS (AUC) and FMD. Whilst this normally correlates in healthy
subjects\textsuperscript{32}, this relationship is lost in our study cohort, prior to treatment. Following TAVR, a
linear relationship is restored, although this does not occur until late follow-up (when WSS had decreased). Delayed physiological normalisation is similarly observed in myocardial blood flow in patients with severe AS. Resting coronary flow velocities are elevated, with impaired scope for further upregulation (CFR). These abnormalities are not normalised immediately following TAVR. In particular, CFR does not improve immediately post-TAVR\textsuperscript{33, 34}, but rather over a longer time period\textsuperscript{34}; this delay may be attributed to factors such as ventricular remodelling and its influence on coronary perfusion\textsuperscript{35}. The delays associated with changes in brachial haemodynamics and the reestablishment of the correlation between cumulative WSS and FMD supports the notion whereby some aspects of endothelial function recovery may also be delayed. The slower normalisation of coronary physiology may therefore be dependent on late improvements in endothelial function in addition to ventricular remodelling.

4.5. This is a single-centre study with a limited population size. Nonetheless, the study was adequately powered to demonstrate restoration in endothelial function from the early follow-up. FMD is an operator-dependant technique that requires experienced operators trained in 2D and Doppler ultrasonography and is therefore subject to observer bias\textsuperscript{19}. Our site has extensive experience in FMD and all assessments in this study were performed by the same experienced investigator with a demonstrated high reproducibility for FMD measures within a validation cohort.
5. CONCLUSION

5.1. In conclusion, our data shows that endothelial function in patients with AS improves early following TAVR and that this improvement is sustained. These changes are likely in response to the improved arterial haemodynamics resulting from the relief of AS. There is evidence of ongoing late normalisation of arterial haemodynamics, including lower WSS. These late adaptations likely contribute to the delayed improvements seen in coronary physiology and in patient’s symptoms.
DISCLOSURES

RPG has received consultancy fees from Boston Scientific Inc.

Other authors have no conflict of interest to declare.
REFERENCES


<table>
<thead>
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<th>Patient characteristics</th>
<th>Mean ± SD / n (%)</th>
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<td><strong>Baseline characteristics</strong></td>
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<tr>
<td>Age, years</td>
<td>82.0±7.0</td>
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<tr>
<td>Female</td>
<td>9 (33.3)</td>
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<tr>
<td>BMI, kg/m²</td>
<td>24.8±5.5</td>
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<tr>
<td>Diabetes mellitus</td>
<td>10 (37.0)</td>
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<tr>
<td>Family history of CAD</td>
<td>9 (33.3)</td>
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<tr>
<td>Hypertension</td>
<td>16 (59.3)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>24 (88.9)</td>
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<td>Smokers</td>
<td>3 (11.1)</td>
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<td>Ex-smokers</td>
<td>11 (41)</td>
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<tr>
<td>Previous MI</td>
<td>9 (33.3)</td>
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<td>Peripheral artery disease</td>
<td>1 (3.7)</td>
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<tr>
<td>Previous stroke</td>
<td>5 (18.5)</td>
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<tr>
<td><strong>Medications</strong></td>
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<tr>
<td>Beta blockers</td>
<td>10 (37.0)</td>
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<tr>
<td>ACEi/ARB</td>
<td>17 (63.0)</td>
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<tr>
<td>Nitrates</td>
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<tr>
<td>Statin</td>
<td>16 (59.2)</td>
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<tr>
<td>Aspirin</td>
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<td>Warfarin</td>
<td>1 (3.7)</td>
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<td>NOAC</td>
<td>7 (25.9)</td>
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<tr>
<td>Calcium channels blockers</td>
<td>10 (37.0)</td>
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</tbody>
</table>

Values are presented as n (%) or mean ± SD. ACEi indicates angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blocker; BMI, body mass index; CAD, coronary artery disease; MI, myocardial infarction; NOAC, novel oral anticoagulants
### TABLE 2: CHANGES IN BRACHIAL FLOW MEDIATED DILATATION AND ARTERIAL HAEMODYNAMICS PRE- AND POST-TAVR

<table>
<thead>
<tr>
<th></th>
<th>Pre-TAVR</th>
<th>Early follow-up</th>
<th>p value</th>
<th>Late follow-up</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>FMD, %</td>
<td>4.2±1.6</td>
<td>9.7±3.5</td>
<td>&lt;0.0001</td>
<td>8.7±1.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time to peak diameter, s</td>
<td>66.4±24.0</td>
<td>61.9±25.11</td>
<td>0.64</td>
<td>55.6±25.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Resting velocities, cm/s</td>
<td>11.24±5.16</td>
<td>11.18±5.93</td>
<td>0.96</td>
<td>7.73±2.79</td>
<td>0.003</td>
</tr>
<tr>
<td>Resting WSS, dyne/cm²</td>
<td>14.8±7.8</td>
<td>15.3±9.3</td>
<td>0.74</td>
<td>10.6±4.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Peak WSS, dyne/cm²</td>
<td>73.1±34.1</td>
<td>64.1±22.7</td>
<td>0.08</td>
<td>58.8±27.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Cumulative WSS (AUC), dyne·s/cm²</td>
<td>3543±1852</td>
<td>3323±1405</td>
<td>0.42</td>
<td>2504±1089</td>
<td>0.002</td>
</tr>
<tr>
<td>Cumulative WSS to peak diameter, dyne·s/cm²</td>
<td>2476±1315</td>
<td>2312±917</td>
<td>0.49</td>
<td>1542±829</td>
<td>0.001</td>
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<tr>
<td>Time to peak of resting Doppler waveform, s</td>
<td>0.09±0.02</td>
<td>0.07±0.02</td>
<td>&lt;0.0001</td>
<td>0.06±0.01</td>
<td>&lt;0.0001</td>
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<tr>
<td>Time to peak of hyperaemic Doppler waveform, s</td>
<td>0.18±0.04</td>
<td>0.09±0.03</td>
<td>&lt;0.0001</td>
<td>0.09±0.03</td>
<td>&lt;0.0001</td>
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<td>Diabetic, FMD, %</td>
<td>4.5±2.14</td>
<td>8.9±4.4</td>
<td>0.006</td>
<td>8.9±1.3</td>
<td>0.003</td>
</tr>
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</table>

Values are presented as mean ± SD. AUC indicates area under the WSS curve; FMD, flow mediated dilation; WSS, wall sheath stress.