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Low Coronary Wall Shear Stress is Associated with Severe Endothelial Dysfunction in Patients with Non-Obstructive Coronary Artery Disease

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Abstract

Objectives: We investigated the relationship between low wall shear stress (WSS) and severe endothelial dysfunction (EDFx).

Background: Local hemodynamic forces, such as WSS play an important role in atherogenesis through their effect on endothelial cells. We hypothesized that low WSS independently predicts severe EDFx in patients with coronary artery disease (CAD).
Methods: Forty-four patients with CAD underwent coronary angiography, fractional flow reserve (FFR) and endothelial function testing. Segments with >10% vasoconstriction after acetylcholine (Ach) infusion were defined as having severe EDFx. WSS, calculated using 3D angiography, velocity measurements and computational fluid dynamics, was defined as low (< 1 Pa), intermediate (1–2.5 Pa) or high (> 2.5 Pa).

Results: Median age was 52 years, 73% were females. Mean FFR was 0.94 ± 0.06. In 4,510 coronary segments, median WSS was 3.67 Pa. 24% had severe EDFx. A higher proportion of segments with low WSS had severe EDFx (71%) compared to intermediate WSS (22%) or high WSS (23%) (p < 0.001). Segments with low WSS demonstrated greater vasoconstriction in response to Ach than intermediate or high WSS segments (−10.7% vs. −2.5% vs. +1.3%, respectively, p < 0.001). In a multivariable logistic regression analysis, female sex (OR: 2.44, p = 0.04), diabetes (OR: 5.01, p = 0.007) and low WSS (OR: 9.14, p < 0.001) were independent predictors of severe EDFx.

Conclusion: In patients with non-obstructive CAD, segments with low WSS demonstrated more vasoconstriction in response to ACh than intermediate or high WSS segments. Low WSS was independently associated with severe endothelial dysfunction.

CONDENSED ABSTRACT

44 patients with CAD underwent endothelial function testing. Segments with >10% vasoconstriction after acetylcholine (Ach) were defined as having severe EDFx. WSS, calculated using computational fluid dynamics, was defined as low (< 1 Pa), intermediate (1–2.5 Pa) or high (> 2.5 Pa). In 4,510 coronary segments, higher proportion of segments with low WSS had severe EDFx (71%) compared to intermediate WSS (22%) or high WSS (23%) (p < 0.001). Segments with low WSS demonstrated greater vasoconstriction in response to ACh than intermediate or high WSS segments (p < 0.001). Low WSS (OR: 9.14, p < 0.001) independently predicted severe EDFx.

Keywords

Coronary Wall Shear Stress; Endothelial Dysfunction; Computational Fluid Dynamics; Non-obstructive Coronary Artery Disease

INTRODUCTION

Atherosclerosis is a systemic disease with focal manifestations often initiating in the outer hips of bifurcations and inner curvatures of vessels. Local hemodynamic forces, such as wall shear stress (WSS), play an important role in atherogenesis, likely mediated through endothelial cells. WSS, the tangential frictional force exerted by blood flow on the arterial wall, has been associated with atherosclerotic plaque progression in human coronary arteries (1–7). Endothelial dysfunction (EDFx) occurs early in the development of atherosclerosis and is believed to be the final common pathway by which systemic risk factors impact the vasculature (8,9). Moreover, EDFx is characterized by a local imbalance between atheroprotective endothelium-dependent vasodilators such as nitric oxide and pro-inflammatory, proliferative, and pro-coagulopathic endothelium-derived vasoconstrictive factors that favor atherogenesis (10). Interestingly, regional coronary arterial endothelial responses to
acetylcholine (ACH) are heterogeneous in the same patient despite exposure of the entire vessel to the same systemic risk factors (9,11,12). This focal variability in EDFx may be related to differences in WSS. Arterial endothelial cell alignment is known to follow WSS direction and low WSS has been theorized to cause EDFx by altering the activity of several inflammatory pathways (13). To date the role of WSS in promoting EDFx in-vivo has not been fully characterized. We hypothesized that in patients with non-obstructive coronary artery disease (CAD), coronary segments with low WSS demonstrate greater endothelial dysfunction compared to segments with intermediate or high WSS. Accordingly, we investigated the relationship between WSS and endothelial dysfunction in patients with non-obstructive CAD using a 3-dimensional angiographic reconstruction and patient specific coronary velocity measurements to derive CFD computations of WSS.

**METHODS**

**Subjects and Study Design**

We selected all patients who were enrolled in our prospective Emory intravascular registry between August 2012 and October 2014. The Emory intravascular registry consists of patients who had an abnormal non-invasive stress test or presented with refractory anginal syndromes, underwent cardiac catheterization, were found to have non-obstructive epicardial coronary atherosclerotic lesions (fractional flow reserve >0.80), and underwent further coronary reactivity testing with intracoronary ACh infusion. Exclusion criteria included acute myocardial infarction with cardiogenic shock or hemodynamic instability, ejection fraction <30%, coronary artery bypass surgery, severe valvular heart disease, lesions requiring percutaneous or surgical revascularization, presence of visual coronary collaterals, inability to provide informed consent, or significant kidney (creatinine > 1.5mg/dl), liver disease (AST/ALT/ALP higher than 3 times the normal limit). We also excluded patients with Hgb < 7 gm/dl, platelet count <100 K/mcL or INR > 1.5. For the present analysis, in addition to the clinical exclusion criteria, patients who did not have sufficiently high-quality baseline and post Ach angiograms for accurate 3D reconstruction for purposes of wall shear stress calculations and endothelial function assessment were also excluded. All patients provided written informed consent and the Emory University Institutional Review Board approved the study.

**Coronary Angiography, Coronary Velocity Measurement with Endothelial Function Assessment**

As described previously (1,2,5,14), all vasoactive medications were held for at least 24–48 hours prior to cardiac catheterization. Systolic blood pressure, diastolic blood pressure, and heart rate were measured prior to the start of the procedure. Patients underwent angiography in a biplane cardiac catheterization system (Philips Medical Systems, Andover, MA or Toshiba America Medical Systems, Tustin, CA). Pressure and velocity measurements were obtained using a 0.014-inch pressure and Doppler flow velocity monitoring guidewire (ComboWire®, Volcano Corporation, Rancho Cordova, CA). The ComboWire® was advanced to the guide catheter tip where the aortic pressure and guidewire pressures were equalized. The guidewire was advanced into the proximal, non-tortuous portion of the left anterior descending coronary artery or left circumflex artery (based on operator discretion).
at least 5mm from major angiographic side branches (>2mm diameter) and the inlet velocity was recorded. The ComboWire was then advanced into the mid or distal artery and the outlet velocity and pressure was recorded. Patients were first evaluated with $10^{-8}$M ACh for safety prior to proceeding with $10^{-6}$M Ach (18.2 mcgc/ml at the rate of 90/ml/hr infused intracoronary through a 3F microcatheter over 3 minutes). Bi-plane coronary angiography was performed after each infusion to assess vascular response. Patients were not moved or re-positioned in between serial coronary angiograms. The same radiographic system, magnification and views were used for the serial angiograms. Intravenous nitroglycerin (200 μg) was then given to reverse residual vasoconstrictive effects and angiography repeated. Subsequently, 140μg/kg/min adenosine was infused through a ≥ 20 gauge intravenous catheter for 2 minutes to induce maximal coronary hyperemia. Continuous recordings of aortic pressure, distal pressure, and velocity were obtained during baseline and drug infusion periods. During maximal hyperemia, fractional flow reserve (FFR) was defined as the ratio of mean distal to aortic pressure and coronary flow velocity reserve (CFR) was defined as the ratio of hyperemic to basal average peak velocity. Velocity measurements demonstrated good reproducibility with a concordance correlation coefficient (CCC) of 0.979 (95% CI 0.966–0.988) (1).

**Endothelial Function, Computational Fluid Dynamics and Wall Shear Stress Methodology and Calculations**

Three-dimensional geometric reconstructions of each patient’s target vessel were performed offline on pre- and post-ACh cineangiograms using QAngio XA 3D RE (Medis Medical Imaging Systems, Leiden, the Netherlands). All visible branching vessels were included in the 3D reconstruction. The pre- and post-ACh reconstructions were co-registered using branching points as fiduciary anatomical landmarks. The resulting pre- and post-ACh 3D models were analyzed by means of fully automated software tools for the extraction of centerlines, computation of point-wise radius map, and the discretization of each vessel in longitudinal segments of 0.5 mm length (Figure 1A) (see supplemental methods) (19,20). Percent change in diameter (%ΔD) was calculated for each 0.5mm segment as 100% x $(D_{post} - D_{pre}) / D_{pre}$. Based on prior studies, each coronary segment demonstrating %ΔD > −10% was defined as having severe EDFx (21–25). Similarly, segments with %ΔD between −10% to +10% in response to ACh were defined as having mild EDFx, while those with %ΔD ≥ +10% were defined as having normal endothelial function. After the reconstructed baseline 3D surface was meshed, computational fluid dynamics was carried out using the finite element library LifeV (EPFL, Switzerland; Politecnico di Milano, Italy; INRIA, France; and Emory University, USA – www.lifev.org). Post-processing was carried out by the open source software Paraview (www.paraview.org). The fluid (blood) was assumed to be a homogeneous pulsatile isothermal incompressible Newtonian fluid (viscosity constant with respect to shear rate),(26) allowing for the use of the incompressible Navier-Stokes equations that can be numerically solved by applying the finite element method (27). Three cardiac cycles were simulated to reach the desired pulsatile regime with satisfactory accuracy in the velocity field. Boundary conditions of the target vessel and its branches were specified as a series of velocity and pressure profiles measured by the Combowire® in conjunction with an application of Murray’s law (28,29) that weighs the flux of each arterial branch, including the distal portion of the target vessel, with respect to its neighbor. This has
been demonstrated to be a reasonable mathematical assumption for mildly stenotic coronaries, as in the present study (30). Spatial velocity profiles were assumed to be flat in the 80% inner part of the proximal vessel, with a linear decrease in the profile in the outermost part of the proximal vessel (to satisfy the no-slip boundary condition on the wall), and parabolic in the arterial branch outlets. After computing the pulsatile flow field in the region of interest, the WSS map was determined on the vessel surface as a function of time in the cardiac cycle,(31,32) then averaged over time and circumference at each 0.5mm segment for quantitative analysis (Figure 1B). Based on previous experimental and human data, low WSS was categorized as < 1Pa (1,2,5,33–35). Intermediate WSS was categorized as ≥1 Pa but < 2.5 Pa, while high WSS was defined as ≥2.5 Pa.

**Statistical Analysis:**

Continuous variables are summarized as mean ± standard deviation (SD) or median and interquartile range (IQR), as appropriate, and categorical variables as counts and proportions. The association between WSS and severe EDFx was investigated using logistic regression models. To account for the correlations within patient due to repeated measurements (segments), a robust “sandwich” variance estimator was used to estimate the variance-covariance matrix of the regression parameter coefficients by generalized estimating equations (GEE). An independence working correlation structure was chosen based on the QIC (quasi-likelihood under the independence model criterions) statistic. The covariates included age, sex, race (black vs. non-black), diabetes mellitus (HbA1c > 7%), hypertension (systolic blood pressure >140 or Diastolic blood pressure BP >90 mmHg), hyperlipidemia (LDL>100 mg/dL) and active smoking (yes vs. no). Subsequently, a linear regression model was used to investigate the relationship between WSS and change in diameter (%ΔD), again with GEE to account for within-subject correlations and adjusted for the aforementioned covariates. In these models, WSS was treated as a binary variable (<1 Pa vs. ≥1 Pa) and then a continuous variable (measured in Pascals). In addition, a generalized additive mixed model (GAMM) was used to explore the relationship between WSS and severe EDFx (with logit link). The model was fit by a smooth term of WSS using a cubic spline smoothing with a basis dimension of 3.A P-value <0.05 was considered statistically significant. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC), R version 3.4.1, and SPSS 24.0 software (SPSS Inc., Chicago Illinois).

**RESULTS**

We evaluated 44 coronary arteries (93 % LAD) in 44 patients. Baseline demographics and clinical characteristics of the study cohort are shown in Table 1. Median age of the study population was 52 (44.0, 66) years and 73% of patients were female. Median angiographic diameter stenosis was 18.3 (13.6, 26.5) %. Median FFR was 0.95 (0.91, 0.99) while median CFR was 2.2 (1.8, 2.5). Overall, 4,510 vessel segments (each 0.5 mm in length, a total of 2,255 mm) were analyzed. Median segmental baseline diameter was 2.3 (1.9, 2.7) mm. Median post-ACh diameter was 2.3 (1.9, 2.7) mm, while median %ΔD was 0 (−9.0, 10.0).

Among 4,510 analyzed vessel segments, median WSS was 3.67 (2.39, 5.53) Pa. Low, intermediate, and high WSS were found in 110 (2.4%), 1,117 (24.8%), and 3,283 (72.8%)
segments, respectively. Normal endothelial function was observed in 1,171 (26%) segments, mild EDFx in 2,273 (50.4%) segments, and severe EDFx in 1,066 (23.6%) segments.

**Segment Level Relationship between Endothelial Function and Wall Shear Stress**

After accounting for within subject correlation, segments with low WSS demonstrated greater vasoconstriction in response to ACh than segments with intermediate WSS or high WSS (−10.7% [IQR −12.1, −8.8] vs. −2.5% [IQR −8.3, +5.5] vs. +1.3% [IQR −8.6, +11.3], respectively, p < 0.001) (Figure 2). Furthermore, low WSS was associated with a mean −0.11 %ΔD in response to ACh (p < 0.001) after adjusting for various demographic and cardiovascular risk factors (Table 2).

A higher proportion of segments with low WSS had severe EDFx (71%) compared to intermediate WSS (22%) or high WSS (23%) (p < 0.001) (Figure 3). Interestingly, no segment with normal endothelial function had low WSS. In a univariable logistic regression analysis, low WSS was associated with severe EDFx [Odds Ratio (OR): 8.42, p < 0.001]. In a multivariable logistic regression model, female sex (OR: 2.44, p = 0.04), diabetes (OR: 5.01, p = 0.007) and low WSS (OR: 9.14, p < 0.001) were independent predictors of severe EDFx (Table 3). Figure 4A shows a representative coronary vessel with numerous low WSS segments and figure 4B shows the corresponding distribution of endothelial function in the same vessel. Notice the high number of segments with severe or mild EDFx co-localized with low WSS areas. Figure 5 shows the estimated probability of severe EDFx as a function of WSS between 0 and 10 Pa. The estimated probability of severe EDFx increases from 0.5 (50%) to 0.95 (95%) as WSS decreases from 4 to 1 Pa.

**DISCUSSION**

This clinical study investigates the relationship between time-averaged WSS and coronary endothelial function derived from baseline and post-ACh three-dimensional coronary angiography in patients with non-obstructive CAD. The results demonstrate that in patients with non-obstructive CAD: (1) Coronary segments with low WSS show a higher degree of vasoconstriction and severe endothelial dysfunction compared to segments with intermediate or high WSS. (2) Low WSS is independently associated with severe endothelial dysfunction. (3) The estimated probability of severe endothelial dysfunction increases at lower WSS values and reaches its highest peak at <1 Pa.

**Mechanisms of Wall Shear Stress and Endothelial Dysfunction: From Bench to Bedside**

There is a growing body of molecular data linking low WSS to the development of endothelial dysfunction. Experimental ex vivo studies have demonstrated multiple mechanisms by which low WSS induces changes in endothelial cell morphology with loss of alignment and increased turnover (36–38). Sustained low WSS affects the structure and function of endothelial cells resulting in transformation to a proatherogenic phenotype with polygonal morphology (36,38,39). Moreover, low WSS down-regulates the expression of endothelial nitric oxide synthase (eNOS), (40–42) which generates nitric oxide and plays a crucial role in maintaining coronary arterial vasodilation. The loss of eNOS, and decreased NO production, leads to graded impairment of normal dilatory response of the coronary...
arteries to endothelium-dependent vasodilators and ultimately vasospasm. Furthermore, in animal models it has been shown that low WSS promotes atherosclerotic plaque progression by increasing endothelial LDL uptake and synthesis,\(^{(43)}\) up-regulating the expression of growth factors and endothelin, down-regulating the expression of growth inhibitors (e.g. TGF-\(\beta\)),\(^{(34,44)}\) and enhancing the pro-inflammatory gene expression through the mitogen-activated protein kinase and nuclear factor-kappa B pathway \((45,46)\).

Whether these cellular and experimental observations relating low WSS to numerous proatherosclerotic pathways are relevant in human coronary atherosclerosis was previously unknown. We demonstrate that coronary segments with low WSS demonstrate greater absolute vasoconstriction in response to Ach compared to segments with intermediate and high WSS. Our finding that, even after adjusting for demographic and clinical risk factors, low WSS is a strong predictor of severe endothelial dysfunction with an odds ratio of 9.14, higher than that of diabetes (5.01) and female sex (2.44) provides a strong underpinning of the association between low WSS with severe endothelial dysfunction in patients with non-obstructive CAD. Moreover, we show that the probability of severe EDFx increases with decrease in WSS and a value of \(< 1\) Pa is could be used to identify segments with severe EDFx.

**Co-localization of Endothelial Dysfunction and Low Wall Shear Stress**—Our study demonstrates that a significantly greater number of segments with severe endothelial dysfunction reside in low WSS areas. Furthermore, none of the segments with normal endothelial function co-localize with low WSS. While it is known that WSS patterns are largely driven by regional vascular geometry (low WSS in inner curvature of vessels, outer hips of bifurcations, and upstream and downstream from stenoses), the vascular response to Ach infusion is likely not primarily driven by geometry. Indeed, endothelial responses to Ach are heterogeneous in the same patient despite exposure of all vessel segments to the same systemic risk factors \((9,11,12)\). These data demonstrate the complex interplay between WSS and endothelial function in the presence of non-obstructive CAD. Prognostic data demonstrate that low WSS is associated with coronary plaque progression \((1,3,4)\) and that patients with endothelial dysfunction have adverse cardiovascular events. Future studies evaluating the relative predictive value of regional endothelial dysfunction and WSS with respect to plaque progression and clinical events are warranted.

**Clinical Implications**—This study demonstrates that an important regional precursor of coronary plaque progression namely, low wall shear stress, is independently associated with severe endothelial dysfunction, the final common pathway through which cardiovascular risk factors contribute to atherosclerosis. Interestingly, majority of coronary segments with low wall shear stress had severe endothelial dysfunction. While it is known the patients with severe coronary endothelial dysfunction have adverse cardiovascular outcomes and that coronary segments with low wall shear stress are associated with plaque progression, the long-term outcomes of coronary segments that display both endothelial dysfunction and low WSS are not known \((1,47)\). Future studies are warranted to investigate the prognostic value of such segments coronary. Another opportunity with such detailed physiologic phenotyping of patients is to refine our diagnosis of patients with ongoing chest pain, ischemia and non-
obstructive CAD. Indeed, almost two thirds of patients with non-obstructive CAD and persistent chest pain with or without a positive stress tests have evidence of coronary endothelial dysfunction (48). Whether patients with severe EDFx and evidence of segmental low WSS should undergo more intense risk factor modification, antiatherosclerotic or anti-inflammatory therapies remains to be investigated.

**Strengths and Limitations**

A strength of our analysis is the methodology of WSS calculation using three-dimensional reconstruction, patient specific pulsatile flow, and boundary conditions imposed on the CFD simulations. The 3D QCA methodology employed has been shown to be more accurate than 2D QCA (conventional single-vessel QCA) measurements (15–18). While accurate coregistration of 3D WSS values and 2D QCA-derived diameter measurements for endothelial function assessment is usually challenging, in the present study both these variables were derived from the same 3D reconstructed geometry, allowing for seamless integration and analysis of the data. Furthermore, once the 3D models were reconstructed, all subsequent operations on surfaces and meshes (centerlines computation, longitudinal discretization, etc.) were fully automated, strengthening the reproducibility of the proposed investigation.

A number of limitations require consideration when interpreting the results of the present study. First, the described relationship between WSS and endothelial dysfunction does not imply cause and effect but rather an association. However, the vascular biological underpinnings of both disturbed WSS and endothelial dysfunction are well established and elucidating the mechanistic links between these two variables was not the goal of this investigation. Second, correlated error is introduced by the clustering of numerous arterial segments within patients; however, appropriate statistical methods were used to adjust for correlated error. Third, although both WSS and endothelial function data were derived from the same 3D reconstructed geometries, inherent inaccuracies due to vessel foreshortening can occur during post-processing of the cineangiograms. We have attempted to minimize these errors through stringent quality control, including comparison of vessel lengths at each clearly demarcated branch point, reconstruction using different views and angles, and standard operating protocols. Furthermore, as per our pre-specified protocol, majority of the vessels included in the study were LAD, future studies could investigate the relationship between low WSS and severe EDFx in the RCA. In addition, prospective multi-centric studies investigating the impact of low WSS and EDFx on clinical outcomes can add provide further insights to the results of our study. Lastly, although we observed that diabetes associated with severe EDFx even after accounting for data clustering, with only one patient with diabetes in our dataset, we had limited ability to test this association.

**CONCLUSIONS**

Among patients with non-obstructive CAD, segments with low WSS demonstrated more vasoconstriction in response to ACh than intermediate or high WSS segments. Low WSS was independently associated with severe endothelial dysfunction. A value of < 1 Pa had the highest estimated probability of severe endothelial dysfunction.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENT:

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ABBREVIATIONS AND ACRONYMS

- Ach indicates acetylcholine
- ALT indicates alanine aminotransferase
- ALP indicates alkaline phosphatase
- AST indicates aspartate aminotransferase
- CAD indicates coronary artery disease
- CFD indicates computational fluid dynamics
- CFR indicates coronary flow velocity reserve
- DM indicates diabetes mellitus
- EDFx indicates endothelial dysfunction
- GEE indicates generalized estimating equations
- HTN indicates Hypertension
- HLD indicates Hyperlipidemia
- IQR indicates interquartile range OR indicates odds ratio
- eNOS indicates endothelial nitric oxide synthase
- WSS indicates wall shear stress
- %ΔD indicates percent change in vessel diameter in response to acetylcholine

REFERENCES


PERSPECTIVES

WHAT IS KNOWN?
Wall Shear Stress has been shown to play a role in atherogenesis, possibly through the endothelial cells. Endothelial dysfunction occurs early in the development of atherosclerosis.

WHAT IS NEW?
Results of our study show that low wall shear stress plays an important role in the development of severe endothelial dysfunction.

WHAT IS NEXT?
Further, coronary segments with low WSS demonstrated a net vasoconstrictive response to acetylcholine. Since endothelial dysfunction is associated with atherosclerosis, further large prospective studies elucidating the association between WSS and endothelial dysfunction, and importantly developing WSS as a clinical tool to risk stratify patients with early atherosclerosis, are required.
Figure 1. 3D geometric reconstruction showing (A) 0.5mm segmentation and (B) calculated segment-level WSS.
Biplane angiographic projections were used to create a 3-Dimensional model of the target coronary vessel, which was subsequently divided into 0.5mm longitudinal segments (seen as circumferential bands) and meshed using automated software (A). After performing CFD with patient-specific boundary conditions, WSS was averaged across each 0.5mm segment; the resulting values are displayed as 0.5mm color-coded bands (B) representing the calculated average WSS per segment. Abbreviations: CFD indicates Computational Fluid Dynamics; WSS indicates Wall Shear Stress.
Figure 2. Box plots of percent change in diameter in response to ACh in segments with low, intermediate, and high segmental WSS.

The line subdividing the box represents median. Error bars are drawn to span all data points within 1.5 IQR of the nearer quartile. Any data points beyond 1.5 IQR are shown individually. Both high (n=3283) and intermediate WSS segments (n=1117) demonstrated higher %ΔD when compared to low WSS segments (n=110) after adjusting for within subject correlations using GEE (p<0.001).

Abbreviations: Ach indicates Acetylcholine; %ΔD indicates percent change in diameter (%ΔD) in response to Ach; GEE indicates generalized estimating equations; IQR indicates Interquartile Range; n indicates number of segments; WSS indicates Wall Shear Stress.
Figure 3. Distribution of segments with endothelial dysfunction.
Bar graphs represent the percentage distribution of segments with severe EDFx, mild EDFx, and normal endothelial function in segments with low, intermediate, and high WSS.
Abbreviations: EDFx indicates Endothelial Dysfunction; WSS indicates Wall Shear Stress.
Figure 4. Co-localization of segments with low wall shear stress and endothelial dysfunction (A, B).

A coronary vessel displaying a significant number of segments with low WSS (marked blue) is shown (A). The same vessel, assessed post-Ach infusion, contains a high number of segments with severe EDFx (marked red) or mild EDFx (marked yellow) in the same regions as baseline low WSS. Notice that no segment with normal endothelial function (marked white) colocalizes with low WSS (B). Abbreviations: ACh indicates Acetylcholine; EDFx indicates Endothelial Dysfunction; WSS indicates Wall Shear Stress.
Figure 5. Estimated relationship between wall shear stress and endothelial dysfunction using a generalized additive mixed model.

The X axis indicates WSS in Pascal, along with the number of observations shown; the Y axis indicates the estimated probability of severe EDFx. Shaded area represents 95% confidence interval. Abbreviations: EDFx indicates Endothelial Dysfunction; WSS indicates Wall Shear Stress.
Table 1.

Demographic and Clinical Characteristics of Study Patients (N=44).

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<thead>
<tr>
<th></th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Age (years) *</td>
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<tr>
<td>Females</td>
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<tr>
<td>African-American</td>
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* Median and interquartile range are presented.
Table 2.

Associations between Diameter Change in Response to Acetylcholine (%ΔD) and Low Wall Shear Stress using Linear Regression with Generalized Estimating Equations to Account for Within-Subject Correlations.

<table>
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<th>Variables</th>
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<th>Multivariable Model</th>
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<td></td>
<td>Estimated difference in %ΔD (95% CI)</td>
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<tr>
<td>Diabetes Mellitus</td>
<td>-0.145 (-0.178 – 0.112)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Active Smoker</td>
<td>-0.041 (-0.110 – 0.028)</td>
<td>0.24</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>-0.006 (-0.074 – 0.062)</td>
<td>0.87</td>
</tr>
<tr>
<td>Low Wall Shear Stress</td>
<td>-0.110 (-0.142 – 0.077)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 3.

Association between Severe Endothelial Dysfunction and Low Wall Shear Stress using Logistic Regression Analysis with Generalized Estimating Equations to Account for Within-Subject Correlations.

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio Estimate (95% CI)</th>
<th>p-value</th>
<th>Odds Ratio Estimate (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (0.98 – 1.05)</td>
<td>0.30</td>
<td>0.99 (0.96 – 1.03)</td>
<td>0.80</td>
</tr>
<tr>
<td>Females</td>
<td>2.39 (1.00 – 5.71)</td>
<td>0.51</td>
<td>2.44 (1.04 – 5.72)</td>
<td>0.04</td>
</tr>
<tr>
<td>African American</td>
<td>0.84 (0.35 – 2.02)</td>
<td>0.70</td>
<td>0.80 (0.38 – 2.12)</td>
<td>0.80</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.19 (0.48 – 2.97)</td>
<td>0.71</td>
<td>1.33 (0.45 – 3.94)</td>
<td>0.60</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>4.44 (2.84 – 6.93)</td>
<td>&lt; 0.001</td>
<td>5.01 (1.57 – 16.01)</td>
<td>0.007</td>
</tr>
<tr>
<td>Active Smoker</td>
<td>1.27 (0.46 – 3.49)</td>
<td>0.64</td>
<td>1.41 (0.37 – 5.44)</td>
<td>0.62</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.23 (0.51 – 2.96)</td>
<td>0.64</td>
<td>1.40 (0.58 – 3.38)</td>
<td>0.46</td>
</tr>
<tr>
<td>Wall Shear Stress (&lt;1 Pa vs. ≥1 Pa)</td>
<td>8.42 (4.07 – 17.42)</td>
<td>&lt; 0.001</td>
<td>9.14 (2.60 – 32.11)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>