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Local Low Shear Stress and Endothelial Dysfunction in Patients With Nonobstructive Coronary Atherosclerosis

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ABSTRACT

BACKGROUND Local hemodynamic factors are important determinants of atherosclerotic plaque development and progression.

OBJECTIVES The goal of this study was to determine the association between low endothelial shear stress (ESS) and microvascular and epicardial endothelial dysfunction in patients with early atherosclerosis.

METHODS Sixty-five patients (mean age 52 ± 11 years) with nonobstructive coronary atherosclerosis (luminal diameter stenosis <30%) were included. Microvascular and epicardial coronary endothelial function was assessed by using intracoronary acetylcholine infusion. Vascular profiling, using 2-plane coronary angiography and intravascular ultrasound, was used to reconstruct the three-dimensional anatomy of the left anterior descending artery. Each reconstructed artery was divided into sequential 3-mm segments and analyzed for local ESS with computational fluid dynamics; that is, lower ESS levels at both a 3-mm regional level (average ESS and low ESS) and at a vessel level (lowest ESS per artery) and for plaque characteristics (plaque area, plaque thickness, and plaque burden).

RESULTS Coronary segments in arteries with abnormal microvascular function exhibited lower ESS compared with segments in arteries with normal microvascular function (average ESS: 1.67 ± 1.04 Pa vs. 2.03 ± 1.72 Pa [$p = 0.050$]; lowest ESS: 0.54 ± 0.25 Pa vs. 0.72 ± 0.32 Pa [$p = 0.014$]). Coronary segments in arteries with abnormal epicardial endothelial function also exhibited significantly lower ESS compared with segments in arteries with normal epicardial function (average ESS: 1.49 ± 0.89 Pa vs. 1.93 ± 1.50 Pa [$p < 0.0001$]; low ESS: 1.26 ± 0.81 Pa vs. 1.56 ± 1.30 Pa [$p = 0.001$]; lowest ESS: 0.51 ± 0.27 Pa vs. 0.65 ± 0.29 Pa [$p = 0.080$]). Patients with abnormal microvascular endothelial function exhibited a progressive decrease in average and low ESS, starting from patients with normal epicardial endothelial function to those with both microvascular and epicardial endothelial dysfunction ($p < 0.0001$ and $p = 0.004$, respectively).

CONCLUSIONS These data indicate an association between dysfunction of the microvascular and epicardial endothelium and local ESS at the early stages of coronary atherosclerosis in humans. (J Am Coll Cardiol 2018;71:2092–102)

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Endothelial dysfunction is the initiating feature and cornerstone of developing atherosclerosis and is characterized by decreased bioavailability of nitric oxide (NO) and promotion of vascular dysfunction and atherosclerosis progression (1). Although the entire vasculature is exposed to the atherogenic effects of traditional risk factors (e.g., diabetes, hypertension, smoking, hyperlipidemia), other local anatomic, hemodynamic, and biochemical factors play a significant role in focal atherosclerotic plaque progression and in the clinical manifestations of coronary artery disease (CAD) (1). However, these factors have not been fully elucidated.

Interestingly, 20% to 50% of patients with chest pain undergoing coronary angiography have nonobstructive coronary atherosclerosis with coronary artery luminal stenosis <30% (2–4). Functional changes, driven mainly by endothelial dysfunction, cannot be detected visually by coronary angiography or with intravascular imaging modalities; these changes may be present in the coronary microvasculature (small resistance vessels), in the epicardial coronary arteries, or in both (2).

Low endothelial shear stress (ESS), the tangential stress produced by the friction of the flowing blood on the endothelial surface, is a focal pro-inflammatory stimulus that is associated with the development and progression of coronary atherosclerosis (5–7). Atherosclerotic lesions characterized by large plaque burden (PB), small minimum lumen area (MLA), and thin-cap fibroatheroma morphology are prone to cause major adverse cardiovascular events, particularly in the presence of pro-inflammatory low ESS (8,9).

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The relationship between ESS and coronary microvascular and epicardial endothelial function in patients with nonobstructive coronary atherosclerosis has not been studied to date. The objective of the present study was to determine whether low ESS was associated with significant impairment in microvascular and epicardial endothelial function in patients with mild coronary atherosclerosis. In addition, we examined the association between microvascular and epicardial endothelial dysfunction and high-risk plaque characteristics, such as PB, MLA, plaque thickness (PT), and plaque area (PA).

PATIENTS AND METHODS

STUDY POPULATION. Patients randomly selected from the prospective Mayo Clinic Database, which utilized a rigorous, routine invasive protocol (Online Figure 1), were retrospectively studied. All patients were referred for elective coronary angiography for assessment of chest pain. Exclusion criteria included the following: >30% diameter stenosis of any coronary artery, left ventricular ejection fraction ≤50%, or left ventricular hypertrophy on echocardiography; acute coronary syndrome; uncontrolled hypertension; angioplasty or bypass surgery; valvular heart disease; significant endocrine or renal disorder; pregnancy; comorbidities such as malignancies and immunological diseases; and subjects receiving systemic glucocorticoids or immunosuppressant agents, as previously described (10,11).

Diabetes was considered present if a patient was prescribed insulin or oral agents or had a fasting glucose level ≥126 mg/dl. Hypertension was defined as blood pressure ≥140/90 mm Hg or the current use of antihypertensive treatment. Hyperlipidemia was defined as a total cholesterol level ≥200 mg/dl, the current use of lipid-lowering treatment, or both. Nonsmokers were defined as patients who had never smoked cigarettes regularly; former smokers as those who had quit smoking at least 5 years before the procedure; and smokers as those who smoked regularly.

The detailed protocol described here was approved by the Mayo Clinic Institutional Review Board.

CORONARY ANGIOGRAPHY. Patients presented to the cardiac catheterization laboratory in the fasting state, and all cardiovascular medications had been discontinued for at least 48 h. Routine diagnostic coronary angiography was performed by using standard clinical protocols, as previously described (10,11). Angiograms were reviewed before the infusion of any pharmacological agent. In cases in which the severity of the stenosis was uncertain, online quantitative coronary angiography was used.

ENDOTHELIAL FUNCTION ASSESSMENT. All patients underwent evaluation of endothelial-dependent and endothelial-independent coronary vascular function as previously described (Online Appendix, Online Figure 1) (10,11). A 0.014-inch Doppler guidewire (FloWire, Volcano Corporation, Rancho Cordova, California) within a 2.2-F coronary infusion catheter (Transit Microcatheter, DePuy Synthes Companies, Warsaw, Indiana) was advanced and positioned into the proximal portion of the left anterior descending artery (LAD). This vessel was chosen for accessibility and because it supplies the largest territory of myocardium. Subsequently, endothelium-dependent coronary vascular function was assessed by a selective infusion of increasing concentrations of

ABBREVIATIONS AND ACRONYMS

CBF = coronary blood flow

ESS = endothelial shear stress

hs-CRP = high-sensitivity C-reactive protein

IVUS = intravascular ultrasound

LAD = left anterior descending artery

MLA = minimum lumen area

NO = nitric oxide

PA = plaque area

PB = plaque burden

PT = plaque thickness

intracoronary acetylcholine for 3 min at each (10^{-6} M, 10^{-5} M, and 10^{-4} M to achieve estimated coronary bed concentrations of 10^{-8} M, 10^{-7} M, and 10^{-6} M, respectively) into the LAD. Hemodynamic data (heart rate and mean blood pressure), Doppler parameter (average peak velocity), and coronary angiography were obtained after each infusion level. The infusion was terminated when the highest molar concentration of acetylcholine (1,024 mol/l) was reached.

Coronary artery diameter was measured at baseline and after the infusion of acetylcholine by an independent investigator blinded to Doppler velocity data using a previously described computer-based image analysis system (12,13). Endothelial-dependent coronary blood flow (CBF) was then calculated by using the following formula, as previously described: CBF = π(average peak velocity)(coronary artery diameter/2)^2 (14). The interobserver and intraobserver reproducibility of the CBF calculation was 5%. The maximal percent increase in CBF in response to acetylcholine compared with the CBF at baseline was then calculated. For quality control, all measurements were performed in the segment 5 mm distal to the tip of the Doppler guidewire; after each infusion, the diameter was measured in the same segment of the vessel. CBF was calculated for each patient at baseline and after each infusion of acetylcholine. The maximal effect of acetylcholine was expressed as percent change in coronary artery diameter using quantitative coronary angiography (QCA, Medis Corporation, Leiden, the Netherlands) (representing epicardial endothelial function) and percent change in the CBF (representing microvascular endothelial function) relative to baseline.

DEFINITION OF TERMS. Impaired microvascular endothelial-dependent function was defined as a maximal percent increase in CBF ≤50% in response to any dose of acetylcholine (10,11). Epicardial endothelial dysfunction was defined as a decrease in the coronary artery diameter ≥20% in response to acetylcholine.

ESTIMATION OF ESS AND PLAQUE/ARTERIAL WALL CHARACTERISTICS. Local intracoronary ESS was assessed by using validated vascular profiling techniques with coronary angiography and intravascular ultrasound (IVUS) to reconstruct the coronary artery lumen and perform computational fluid dynamics calculations, as previously described (Online Appendix, Online Figure 2) (5,8). The methods of the IVUS examination have been described previously (10,11). The IVUS examination was performed after intracoronary administration of 100 to 200 mg of nitroglycerin. A 20-MHz, 2.9-F monorail, electronic Eagle Eye Gold IVUS catheter (Volcano Corporation) was advanced into the proximal LAD, and automatic pullback at 0.5 mm/s was performed. Custom software was used for 3-dimensional reconstruction of coronary arteries by merging the coronary angiographic centerline with the IVUS centerline, and local blood flow patterns were calculated by using routine computational fluid dynamics methods (15). The reproducibility of this approach has been previously described (16). Each reconstructed coronary artery was divided along its entire length into serial 3-mm segments for assessment of vascular characteristics.

Local ESS values for each 3-mm segment were quantified over a moving 90° arc for each 3-mm segment lumen circumference (average ESS, low ESS, and maximum ESS). The objective was to differentiate segments with eccentric ESS distribution with extremes of low and high ESS around the lumen circumference but resulting in an average similar to segments with uniform ESS distribution. The lowest local ESS along the length of each artery was also identified (lowest ESS per artery). PA, external elastic membrane area, lumen area, and the derived variables of PB, PT, and MLA were calculated. PA, PT, PB, and MLA were estimated in a 90° arc around the circumference of each segment of the artery. Maximum PB, minimum MLA, and maximum PT were estimated per artery.

BIOCHEMICAL ANALYSES. Venous blood samples, obtained from all participants in the study, were centrifuged at 3,000 rotations/min, and serum/plasma samples were collected and stored at −80°C until assayed. Serum levels of high-sensitivity C-reactive protein (hs-CRP) were measured as an inflammatory biomarker.

STATISTICAL METHODS. Continuous variables are reported as means ± SDs. Variables with skewed distribution (hs-CRP) were log-transformed to improve normality and are presented as median with interquartile range. For continuous variables, the Student's *t*-test was used to test for differences between 2 groups (normal vs. abnormal) for either microvascular or epicardial endothelial function. In situations in which there were multiple observations per patient, mixed models were used to correct for potential correlated error. To examine the association between the presence of abnormal microvascular or epicardial endothelial function and selected covariates, logistic regression analysis was performed. Variables were selected based on findings from univariate analysis; we also considered variables that were highly correlated with each other and removed

1 of the 2 variables. The correlation between lowest ESS and change in CBF was assessed with the Pearson r correlation coefficient. We also combined categories of microvascular and epicardial endothelial function to construct a 3-part variable defined as normal-normal, normal-abnormal, or abnormal-abnormal. We then compared ESS and plaque characteristics with 3 pairwise comparisons for each measure.

The p values were adjusted by using the Scheffé method for measures in which there was 1 observation per patient and the least significant difference method in which there were multiple observations per patient. Normal distribution of continuous variables was tested with inspection of p-p plots. All reported p values are based on 2-sided tests. The level of statistical significance was set at 0.05. All data were analyzed by using SPSS version 18.0 (IBM SPSS Statistics, IBM Corporation, Armonk, New York).

RESULTS

CATEGORIZATION OF CASES ACCORDING TO ENDOTHELIAL FUNCTION ASSESSMENT. Imaging acquisition was sufficient for ESS vascular profiling analyses in 65 of 114 patients. Of these 65 patients, 17 patients had diffuse epicardial endothelial dysfunction, and 48 patients had normal epicardial endothelial function (Table 1). Thirty-nine patients had abnormal microvascular endothelial function, and 26 patients had normal microvascular endothelial function. All patients with abnormal epicardial endothelial function also had abnormal microvascular endothelial function (n = 17) (Online Figure 3). Patients with normal epicardial endothelial function exhibited either abnormal (n = 22) or normal (n = 26) microvascular endothelial function. All patients with normal microvascular endothelial function also had normal epicardial endothelial function (n = 26). The demographic characteristics of the study patients (Table 2) were comparable to those in the patients excluded from the study due to insufficient image quality (Online Appendix Table).

NORMAL VERSUS ABNORMAL MICROVASCULAR ENDOTHELIAL FUNCTION. The characteristics of coronary endothelial function, ESS, and plaques in patients with normal versus those with abnormal microvascular endothelial function are displayed in Table 3.

Patients with abnormal microvascular endothelial function had significantly smaller changes in coronary artery diameter in response to acetylcholine compared with patients with normal microvascular endothelial function ($p < 0.012$). Coronary epicardial segments in arteries with abnormal downstream microvascular endothelial function (n = 555 segments) exhibited lower ESS (average ESS, low ESS, maximum ESS, and lowest ESS per artery) compared with segments in arteries with normal microvascular endothelial function (n = 391 segments).

Plaque characteristics were significantly different in coronary segments with abnormal microvascular endothelial function compared with segments with normal microvascular endothelial function: increased PA ($p < 0.001$), PB ($p < 0.001$), and PT ($p < 0.001$); maximum PB per artery ($p = 0.02$); and maximum PT per artery ($p = 0.02$). There were no statistically significant differences in MLA between the 2 groups.

Concerning hs-CRP values, there was no statistically significant difference between patients with normal and abnormal microvascular endothelial function (1.44 [0.60 to 2.78] mg/l vs. 1.40 [0.49 to 5.15] mg/l; $p = 0.64$). Similarly, there was no significant correlation between CRP values and ESS

TABLE 1 Categorization in Groups/Subgroups According to Microvascular and Epicardial Endothelial Function Measurements (N = 65)

	Epicardial Function	
Microvascular Function	**Normal (n = 48)**	**Abnormal (n = 17)**
Normal (n = 26)	26	0
Abnormal (n = 39)	22	17

TABLE 2 Demographic Characteristics of Study Patients

General characteristics	
Age, yrs	52 ± 11
Male	7 (11)
Body mass index, kg/m^2	31 ± 6
Cardiovascular risk factors	
Hypertension	34 (52)
Diabetes	5 (8)
Hyperlipidemia	37 (57)
Cigarette smokers (never/former/current)	39 (64)/20 (33)/2 (3)
Biochemical measurements	
Fasting blood glucose, mg/dl	100 ± 20
Total cholesterol, mg/dl	185 ± 40
High-density lipoprotein cholesterol, mg/dl	60 ± 19
Low-density lipoprotein cholesterol, mg/dl	99 ± 33
Triglyceride, mg/dl	128 ± 72
Medication	
ACE inhibitors	9 (14)
Beta-blockers	26 (40)
Calcium-channel blockers	20 (31)
Statins	24 (37)
Aspirin	38 (58)

Values are mean ± SD or n (%). Clinical data are missing in 4 of 65 patients included in the study.

ACE = angiotensin-converting enzyme.

TABLE 3 Coronary Artery Characteristics in Patients Stratified According to Microvascular Endothelial Function

	Normal	Abnormal	p Value
No. of vessels/segments	26/391	39/555	
Lumen area, mm^2	9.77 ± 3.56	10.12 ± 4.01	0.17*
Flow (estimated in VP), ml/s	1.38 ± 0.65	1.25 ± 0.60	0.41
Estimation of endothelial function			
Change in coronary artery diameter, %	−3.02 ± 7.45	−14.73 ± 26.36	0.012
Estimation of ESS			
Average ESS, Pa	2.03 ± 1.72	1.67 ± 1.04	0.050*
Low ESS, Pa	1.64 ± 1.49	1.36 ± 0.92	0.12*
Maximum ESS, Pa	2.46 ± 2.03	2.01 ± 1.30	0.008*
Lowest ESS per artery, Pa	0.72 ± 0.32	0.54 ± 0.25	0.014
Plaque characteristics			
Plaque area, mm^2	2.72 ± 1.74	3.78 ± 2 .34	<0.0001*
Plaque burden, %	21.33 ± 9.72	26.46 ± 12.59	<0.0001*
MLA, mm^2	9.40 ± 3.46	9.57 ± 3.91	0.45*
Plaque thickness, mm	0.28 ± 0.18	0.39 ± 0.24	<0.0001*
Maximum plaque burden per artery, %	29.88 ± 12.58	37.99 ± 14.70	0.024
Minimum MLA per artery, mm^2	6.43 ± 2.29	7.26 ± 3.48	0.29
Maximum plaque thickness per artery, mm	0.46 ± 0.26	0.64 ± 0.32	0.012

Values are mean ± SD, unless otherwise indicated. *The p values are corrected for clustering of segments within patients.

ESS = endothelial shear stress; average ESS = average ESS in 90° arcs around the circumference of each 3-mm arterial segment; low ESS = lowest local ESS in 90° arcs around the circumference of each segment; maximum ESS = highest local ESS in 90° arcs around the circumference of each segment; lowest ESS per artery = the lowest ESS value in each artery; plaque area = plaque area in 90° arcs around the circumference of each segment; plaque burden = plaque burden in 90° arcs around the circumference of each segment; MLA = minimum lumen area in 90° arcs around the circumference of each segment; plaque thickness = plaque thickness in 90° arcs around the circumference of each segment; maximum plaque burden per artery = the maximum plaque burden in 1 segment per artery; Pa = pascal; VP = vascular profiling procedure.

measurements (lowest ESS per artery, maximum ESS per artery, and average ESS per artery, p = NS for all).

Binary logistic regression analysis for the probability of abnormal microvascular endothelial function indicated that lowest ESS was the only significant factor associated with the presence of abnormal microvascular endothelial function (Table 4). A decrease in lowest ESS by 1 Pa increased the odds of abnormal microvascular endothelial function by 11 times (p = 0.048). Other variables (MLA, maximum PB, and change in coronary artery diameter) were not significantly associated with microvascular endothelial dysfunction.

TABLE 4 Adjusted Odds Ratios of the Presence of Abnormal Microvascular Endothelial Function According to Multivariate Logistic Regression Analysis (n = 60)

	Odds Ratio (95% Confidence Interval)	p Values
Lowest ESS, Pa	0.084 (0.007-0.976)	0.048
MLA, mm^2	1.18 (0.92-1.52)	0.19
Maximum PB, %	1.04 (0.99-1.09)	0.09
Change in coronary artery diameter, %	0.97 (0.94-1.01)	0.13

Lowest ESS = the lowest ESS value in each artery; MLA = minimum lumen area in each artery; maximum PB = maximum plaque burden in each artery; change in coronary artery diameter = the change in coronary artery diameter after acetylcholine infusion in each artery. Other abbreviation as in Table 3.

The lowest ESS value in each artery was significantly correlated with the change in CBF after acetylcholine infusion (r = 0.33, p = 0.009, r^2 = 0.1) (Figure 1).

NORMAL VERSUS ABNORMAL EPICARDIAL ENDOTHELIAL FUNCTION. Coronary endothelial function measurements, ESS values, and plaque measurements between patients with normal and abnormal epicardial endothelial function are presented in Table 5.

Patients with abnormal epicardial endothelial function (n = 17) had a significantly lower increase in CBF in response to acetylcholine compared with patients with normal epicardial endothelial function (n = 48) (p < 0.001). Coronary segments in arteries with abnormal epicardial endothelial function (250 segments) exhibited lower local ESS (average ESS, low ESS, maximum ESS, and lowest ESS per artery) compared with segments in arteries with normal epicardial endothelial function (696 segments).

There were no significant differences in PA, PB, MLA, PT, maximum PB, minimum MLA, and maximum PT between the 2 groups. There was no statistically significant difference in hs-CRP levels between patients with normal and abnormal epicardial endothelial function (1.61 [0.59 to 4.78] mg/l vs. 1.10 [0.36 to 3.25] mg/l; p = 0.81).

Binary logistic regression analysis for the probability of abnormal epicardial endothelial function (Table 6) indicated that microvascular endothelial dysfunction (i.e., a blunted change in CBF) was the only significant factor associated with the presence of abnormal epicardial endothelial function. A decrease in CBF by 10% in response to acetylcholine was associated with a 58% increased likelihood of abnormal epicardial endothelial function being present (p < 0.004). Other anatomic and hemodynamic variables (lowest ESS, minimum MLA, and maximum PB) were not significantly associated with epicardial endothelial dysfunction.

SUBGROUP ANALYSES COMBINING MICROVASCULAR AND EPICARDIAL ENDOTHELIAL FUNCTION. Coronary segments in patients with both microvascular and epicardial endothelial dysfunction (250 segments) exhibited lower ESS compared with segments in patients with abnormal microvascular and normal epicardial endothelial function (306 segments) (average ESS: 1.49 ± 0.88 Pa vs. 1.79 ± 1.12 Pa [p < 0.0001]; low ESS: 1.26 ± 0.81 Pa vs. 1.44 ± 0.98 Pa [p = 0.006]; lowest ESS per artery: 0.50 ± 0.27 Pa vs.

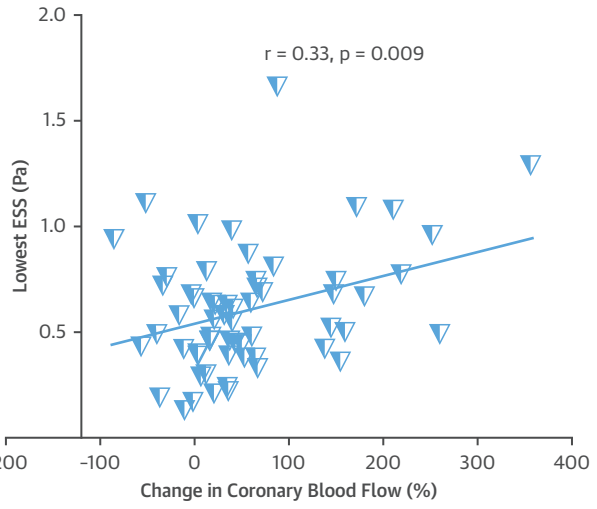
0.56 ± 0.23 Pa [p = 0.81]) (Figure 2). Similarly, coronary segments in patients with both microvascular and epicardial endothelial dysfunction (250 segments) exhibited lower ESS compared with segments in patients with microvascular and epicardial endothelial function that were both normal (389 segments) (average ESS: 1.49 ± 0.88 Pa vs. 2.02 ± 1.720 Pa [p < 0.0001]; low ESS: 1.26 ± 0.81 Pa vs. 1.64 ± 1.48 Pa [p = 0.004]; lowest ESS per artery: 0.50 ± 0.27 Pa vs. 0.71 ± 0.31 Pa [p = 0.06]).

Coronary segments in patients with abnormal microvascular endothelial function but normal epicardial endothelial function exhibited a trend of increased maximum PB and maximum PT compared with segments with microvascular and epicardial endothelial function that were both normal. There were no statistically significant differences in minimum MLA between the 3 subgroups (Figure 3).

DISCUSSION

The present study found a significant association of microvascular and epicardial endothelial dysfunction with ESS patterns in patients with nonobstructive CAD. We observed that coronary arteries exposed to abnormal microvascular endothelial function exhibited significantly lower ESS compared with arteries with normal microvascular endothelial function, and that epicardial ESS was lower in coronary segments with abnormal compared with normal epicardial endothelial function. Importantly, patients with abnormal microvascular endothelial function exhibited a decrease in local ESS, and there was a progressive decrease in ESS from those with concomitant normal epicardial endothelial function to those with microvascular and epicardial endothelial function that were both abnormal. The lowest ESS value in each artery also significantly correlated with the degree of microvascular endothelial dysfunction, which in turn was associated with the presence of abnormal epicardial endothelial function. Patients with abnormal microvascular endothelial function exhibited worse plaque characteristics of maximum PB and maximum PT compared with patients with normal microvascular function, regardless of epicardial endothelial function. The maximum ESS in each arterial segment was also lower in patients with microvascular, as well as epicardial, endothelial dysfunction, compared with patients with normal endothelial function. However, the absolute values of maximum ESS observed in these patients with nonobstructive CAD remained in the physiological, vasculoprotective range under all conditions and likely did not contribute to vascular dysfunction.

FIGURE 1 Correlation Between Lowest ESS and Change in Coronary Blood Flow in Each Artery



The lowest endothelial shear stress (ESS) value in each artery is indicated.

The current hypothesis-generating study suggests a possible association between endothelial function and low ESS in the coronary circulation in humans, and it may contribute to our understanding of the mechanisms responsible for the initiation and progression of coronary atherosclerosis.

TABLE 5 Coronary Artery Characteristics in Patients Stratified According to Epicardial Endothelial Function

	Normal	Abnormal	p value
No. of vessels/segments	48/696	17/250	
Lumen area, mm^2	9.97 ± 3.68	10.01 ± 4.23	0.58*
Flow (estimated in VP), ml/s	1.39 ± 0.64	1.05 ± 0.45	0.043
Estimation of endothelial function			
Change in coronary blood flow, %	80.98 ± 84.99	−10.06 ± 37.56	<0.0001
Estimation of ESS			
Average ESS, Pa	1.93 ± 1.50	1.49 ± 0.89	<0.0001*
Low ESS, Pa	1.56 ± 1.30	1.26 ± 0.81	0.001*
Maximum ESS, Pa	2.36 ± 1.81	1.75 ± 1.01	<0.0001*
Lowest ESS per artery, Pa	0.65 ± 0.29	0.51 ± 0.27	0.080
Plaque characteristics			
Plaque area, mm^2	3.27 ± 2.02	3.50 ± 2.57	0.29*
Plaque burden, %	24.42 ± 12.03	24.13 ± 10.96	0.78*
MLA, mm^2	9.49 ± 3.61	9.52 ± 4.03	0.58*
Plaque thickness, mm	0.34 ± 0.21	0.35 ± 0.26	0.85*
Maximum plaque burden per artery, %	34.36 ± 13.99	35.83 ± 15.74	0.72
Minimum MLA per artery, mm^2	6.78 ± 2.56	7.34 ± 4.25	0.52
Maximum plaque thickness per artery, mm	0.56 ± 0.27	0.60 ± 0.41	0.59

Values are mean ± SD, unless otherwise indicated. Variables are as defined in Table 3. *p value corrected for clustering of segments within patients.

TABLE 6 Adjusted Odds Ratios of the Presence of Abnormal Epicardial Endothelial Function According to Multivariate Logistic Regression Analysis (n = 60)

	Odds Ratio (95% Confidence Interval)	p Values
Lowest ESS, Pa	7.11 (0.20–247.90)	0.28
MLA, mm^2	1.20 (0.80–1.30)	0.89
Maximum PB, %	1.01 (0.96–1.07)	0.64
Change in CBF, %	1.05 (1.02–1.08)	0.002

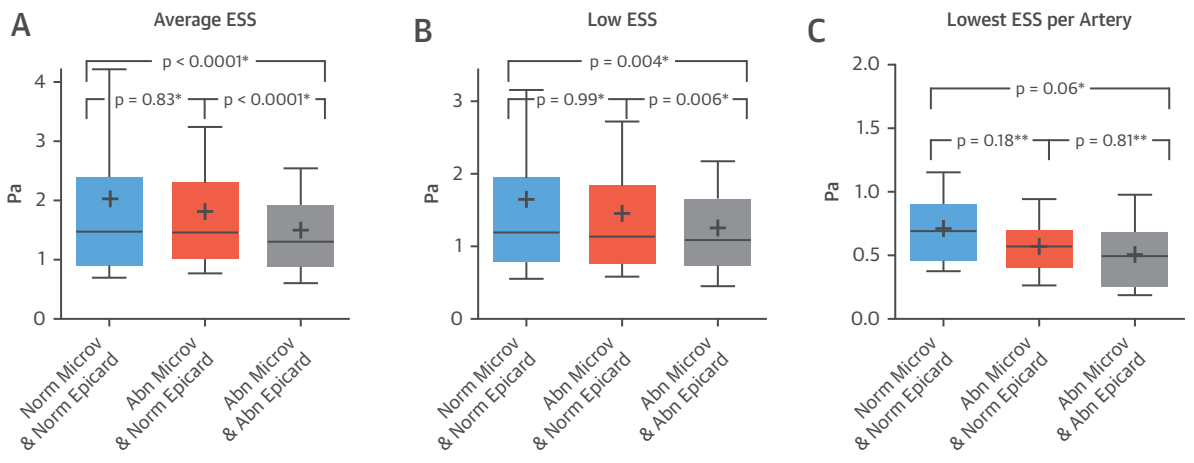
Change in CBF = the change in coronary blood flow after acetylcholine infusion in each artery; other abbreviations as in Tables 3 and 4.

Despite a comparable exposure of the coronary vasculature to systemic cardiovascular risk factors, localization of epicardial endothelial dysfunction in select segments along the coronary arteries provides evidence that other local factors contribute to focal epicardial function deterioration. Low ESS is a local pro-inflammatory stimulus and a main driver for atherosclerosis progression that is correlated with plaque progression, plaque vulnerability, and adverse cardiac prognosis (5-8,17,18). The results of the present study support the concept that, in addition to systemic risk factors, local factors such as low ESS significantly contribute to the promotion of early focal epicardial endothelial dysfunction and, potentially, plaque progression.

The present findings suggest a novel insight concerning the natural history of coronary atherosclerosis as a continuous process that progresses from microvascular to epicardial endothelial dysfunction manifestations over time, with likely mechanistic contributions by local ESS patterns, especially low ESS, at multiple time points. At the earliest stages of the disease, established systemic risk factors may promote microvascular endothelial dysfunction and initiate coronary atherosclerosis. Microvascular endothelial dysfunction may subsequently trigger a fall in upstream local ESS, further provoking and exacerbating inflammatory processes in the upstream coronary endothelium. Over time, the low ESS secondary to microvascular endothelial dysfunction may drive focal epicardial endothelial dysfunction as well as atherosclerosis progression, resulting in more advanced phenotypic manifestations of CAD (**Central Illustration,** Online Appendix, Online Figure 4).

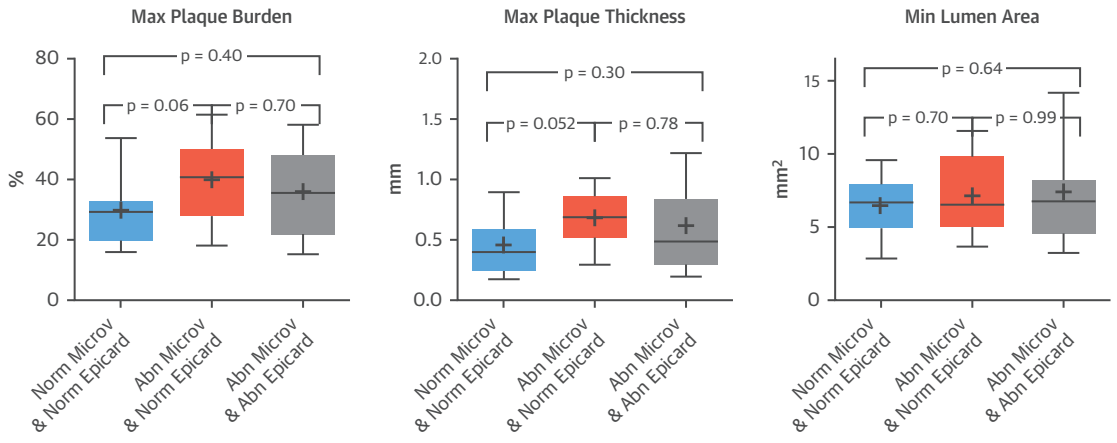
MICROVASCULAR ENDOTHELIAL DYSFUNCTION AND ESS. Established risk factors for coronary atherosclerosis are associated with microvascular endothelial dysfunction (19,20). Microvascular endothelial dysfunction is one of the first recognizable signs of coronary atherosclerosis and precedes clinical manifestations of coronary disease (13). In subjects without coronary atherosclerosis or risk factors of atherosclerosis, the coronary microcirculation is capable of raising its basal CBF in response to demand at least 3-fold (1). The main factor regulating blood flow is the coronary endothelium, which produces vasodilator substances such as NO (1).

FIGURE 2 ESS Measurements: Analysis Based on Both Microvascular and Epicardial Endothelial Function



Coronary segments in patients with both microvascular and epicardial endothelial dysfunction (250 segments) compared with segments in patients with abnormal microvascular and normal epicardial endothelial function. **(A)** Average ESS = average endothelial shear stress (ESS) in 90° arcs around the circumference of each segment of the artery; **(B)** low ESS = lowest local ESS in 90° arcs around the circumference of each segment of the artery; **(C)** lowest ESS per artery = the lowest ESS value in each artery. Abn = abnormal; norm = normal; Pa = pascal. p values in **A and B** are corrected for clustering of segments within patients. *p values adjusted for multiple comparisons by least significant difference method; **p values adjusted for multiple comparisons by using the Scheffé method.

FIGURE 3 Plaque Characteristics in 1 Segment Per Artery: Analysis Based on Both Microvascular and Epicardial Endothelial Function



The p values were adjusted for multiple comparisons by using the Scheffé method. Max = maximum; Min = minimum; other abbreviation as in Figure 2.

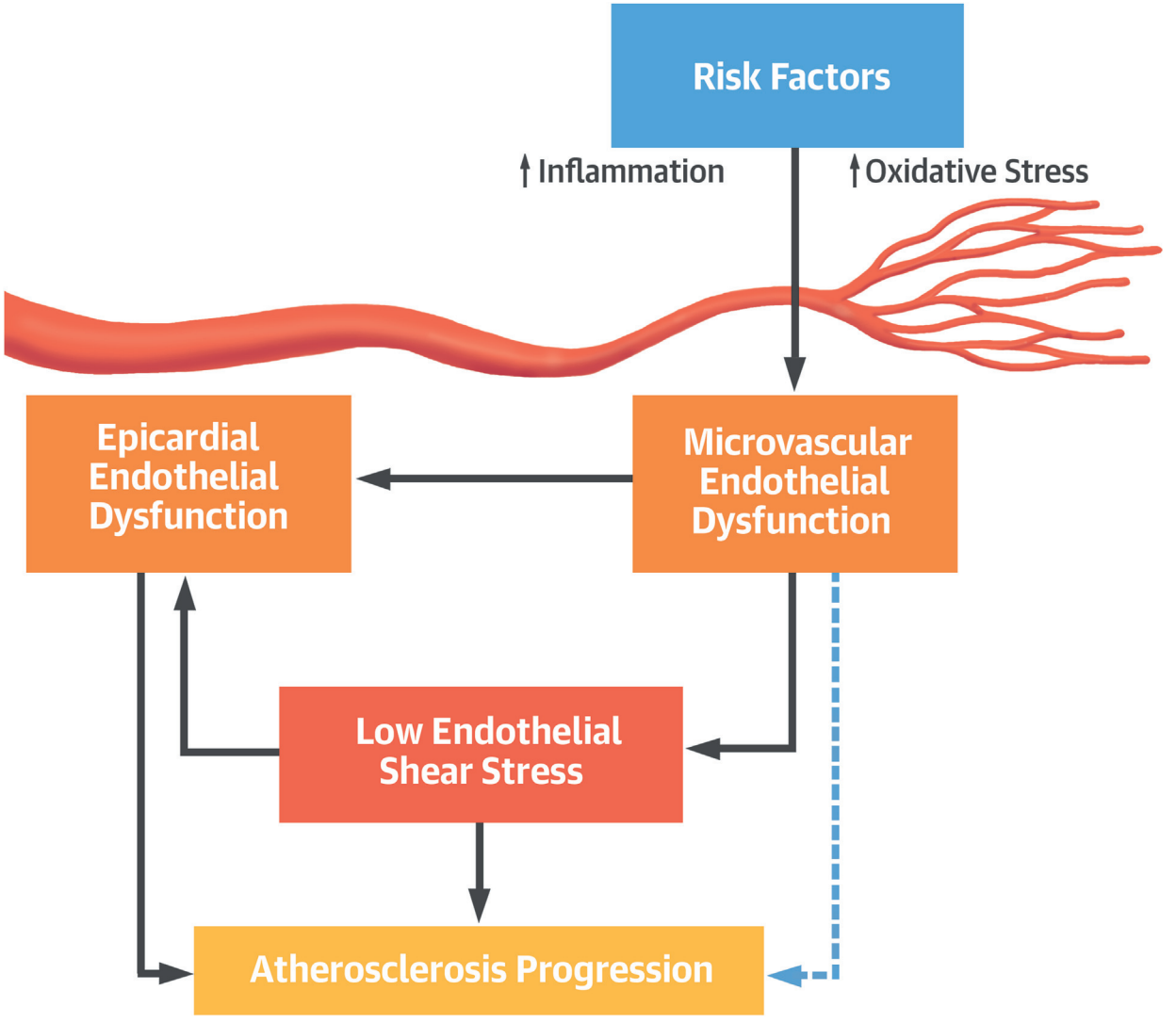
Atherosclerosis progression is strongly associated with inflammation. The increase in systemic and vascular inflammation, mainly caused by systemic risk factors, inhibits NO synthase activity and impairs endothelial NO availability (21). As a result, systemic and coronary inflammation is correlated with coronary microvascular dysfunction (22). Local factors, such as low ESS, exacerbate focal inflammation and may further exaggerate dysfunction of the endothelium (23). The increase in inflammatory status, directed in large part by local low ESS, along with the increase in oxidative stress, promote an ongoing process of endothelial injury (23). Moreover, low ESS when combined with elevated cholesterol levels increases the expression of adhesion molecules and lipoprotein receptors, suggesting an interrelating pathobiologic role of systemic and local factors in atherosclerosis (23). The local nature of the arterial inflammatory process is underscored by the lack of association between systemic hs-CRP levels and either microvascular or epicardial endothelial dysfunction.

Endothelial dysfunction in the microvasculature may thus serve not only as a marker but also as an active contributor to progression of atherosclerosis by promoting inflammation, coagulation, vasoconstriction, and inadequate vascular repair (1,22). Microvascular endothelial dysfunction is characterized by increased resistance at the level of microcirculation, further resulting in a reduction in resting CBF and particularly its response to increased demand (24,25), which may significantly affect ESS upstream throughout the coronary vasculature. Our observation that most ESS parameters were diminished in otherwise normal coronary arteries facing downstream microvascular endothelial dysfunction underscores the link between low ESS and microvascular dysfunction. Furthermore, subsequent development of epicardial endothelial function magnified the phenotype and further diminished ESS.

MICROVASCULAR ENDOTHELIAL DYSFUNCTION AND ESS DRIVE EPICARDIAL ENDOTHELIAL DYSFUNCTION. We observed that although patients with microvascular endothelial dysfunction do not necessarily manifest epicardial endothelial dysfunction, all patients with epicardial endothelial dysfunction also had microvascular endothelial dysfunction, suggesting that epicardial endothelial function may be affected by microvascular endothelial dysfunction. Microvascular endothelial dysfunction was associated with a significant increase in the likelihood of the presence of epicardial endothelial dysfunction. The low epicardial ESS associated with microvascular endothelial dysfunction may act as the stimulus triggering local inflammatory responses and vascular injury culminating in epicardial endothelial dysfunction.

ENDOTHELIAL DYSFUNCTION, ESS, AND PLAQUE CHARACTERISTICS. Clinical imaging studies have shown that epicardial endothelial dysfunction is associated with coronary plaque progression and vulnerability (10,12,22,26–28). In patients with minimal coronary atherosclerosis, coronary segments with epicardial endothelial dysfunction exhibit smaller lumen area, larger PB, larger necrotic core plaques, and larger lipid content (10,26); coronary

CENTRAL ILLUSTRATION **Role of Local Low ESS in Coronary Microvascular and Epicardial Endothelial Dysfunction**



Siasos, G. et al. J Am Coll Cardiol. 2018;71(19):2092–102.

The present study introduces innovative hypotheses linking the natural history of coronary atherosclerosis as a continuous process that progresses from microvascular to epicardial endothelial dysfunction manifestations over time, with likely mechanistic contributions by local endothelial shear stress (ESS) patterns, especially low ESS, at multiple time points.

segments with epicardial endothelial dysfunction also showed accelerated plaque progression (measured by percent atheroma volume with IVUS) after a 6-month follow-up (12).

In the present study, we found for the first time that microvascular endothelial dysfunction was associated with low ESS and more adverse epicardial plaque characteristics of PA, maximum PB, and maximum PT compared with patients with normal microvascular function. The presence of epicardial low ESS from the downstream microvascular dysfunction may be the stimulus for more advanced epicardial atherosclerotic manifestations. Longitudinal studies in patients with microvascular endothelial dysfunction will be necessary to address this hypothesis.

STUDY LIMITATIONS. Although our results indicate a strong association between coronary endothelial function (microvascular and epicardial) and ESS,

these observations remain hypothesis-generating. Although we cannot conclude that there is causality, the proposed concept is novel and may contribute to our understanding of the mechanisms responsible for the initiation and progression of coronary atherosclerosis. Moreover, the study was limited by the small sample size of patients. The process of "vascular profiling" to calculate local ESS patterns requires near-orthogonal projections of the coronary angiogram, as well as a long IVUS pullback along the course of the artery. Most cases with insufficient image quality excluded from this study had foreshortened angiogram projections, IVUS artifacts, poor pullback, or manual pullbacks that are unanalyzable in our interface. However, we successfully identified significant associations between ESS and coronary endothelial dysfunction. Moreover, although a 20 MHz IVUS transducer is not ideal, it is at the edge of acceptability for ESS measurements.

The present study also provides data regarding the plaque characteristics (i.e., PA, PB, PT, MLA). Data regarding the composition of the plaque (virtual histology data) would be of complementary value to anatomic measurements, but these data were not acquired during the intracoronary imaging procedure and are therefore not available in our study population. Although coronary artery dysfunction in patients with early atherosclerosis is driven mainly by endothelial alterations, additional factors, including ventricular hypertrophy, thrombus, and smooth muscle cell dysfunction, can also affect the CBF (29), and we cannot rule out such contributions. Furthermore, baseline characteristics of the population show that only 11% were male, 8% had diabetes mellitus, and 3% were current smokers, which is not representative of most patients with CAD. However, the prevalence of these characteristics is similar to those in a much larger patient cohort with a similar clinical phenotype (13), with the exception of cigarette smoking. Larger cohort studies will be necessary to determine the true prevalence of these important risk factors in microvascular disease. Lastly, our study was not designed to detect an association of ESS with advanced coronary atherosclerotic lesions, given our focus on patients with nonobstructive coronary arterial disease.

CONCLUSIONS

Microvascular and epicardial endothelial dysfunction are associated with a decrease in ESS. These observations provide a potential contribution of local hemodynamic factors in the initiation and progression of CAD and underscore the need to therapeutically target the microcirculation early during atherogenesis to protect the entire coronary vascular tree.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Low local ESS is associated with progression of coronary atherosclerosis and predicts an unfavorable prognosis beyond anatomic plaque characteristics through mechanisms that involve both microvascular and epicardial endothelial dysfunction.

TRANSLATIONAL OUTLOOK: Further studies are needed to determine whether therapies (including lifestyle interventions) that raise ESS improve coronary endothelial function and clinical outcomes in patients without flow-limiting coronary stenosis.

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KEY WORDS atherosclerosis, coronary artery disease, endothelial function, endothelial shear stress, endothelium, microvascular disease

APPENDIX For supplemental methods, a table, and figures, please see the online version of this paper.