The dynamic interplay between coronary endothelial dysfunction and epicardial plaque burden is thought to play a pivotal role in the pathogenesis of acute coronary syndromes. The degree of endothelial dysfunction has been found to be greater in the setting of acute coronary syndromes, compared to a stable clinical presentation. As a result, our traditional understanding is that enhanced vessel wall reactivity in unstable patients is activated, at least in part, by systemic inflammatory mechanisms, in concert with local plaque-related features.

Clinical Perspective on p 682

Background—Epicardial plaque burden and endothelial function are recognized predictors of coronary events. We aimed to investigate mechanistic relationships between atheroma volume and endothelial function in patients with non–ST-segment–elevation myocardial infarction (NSTEMI) using intravascular ultrasound.

Methods and Results—in coronary vessels of patients with near-normal or minimal angiographic disease (n=23) and NSTEMI (n=24), intravascular ultrasound-derived measures (percent atheroma volume), arterial remodeling index, and segmental lumen volumes were performed in contiguous 5-mm epicardial segments. Repeat intravascular ultrasound imaging was performed after consecutive 5-minute intracoronary infusions (vehicle solution, 0.30 μg/min and 0.60 μg/min intracoronary salbutamol) to measure changes in segmental lumen volume (endothelium-dependent function). Male sex, diabetes mellitus, smoking, higher triglycerides, and lower high-density lipoprotein cholesterol were more prevalent in the NSTEMI group. Patients with NSTEMI demonstrated greater segmental percent atheroma volume (40.4±12 versus 27.5±14%, P<0.001), remodeling index (1.2 [1.0–1.5] versus 1.0 [0.9–1.0], P<0.001), and displayed less endothelium-dependent vasomotion (% change segmental lumen volume: 2.1±0.89 versus 5.1±0.89%, P=0.02) compared to patients with minimal angiographic disease. No significant difference in endothelial function between both groups was observed when controlling for plaque burden. Multivariate analysis for change in segmental lumen volume identified percent atheroma volume (β=−0.18, P=0.0004), high-sensitivity C-reactive protein >2 mg/L (β=−3.1, P=0.03), diabetes mellitus (β=−6.9, P<0.0001), low-density lipoprotein cholesterol levels (β=−0.04, P=0.01), and smoking (β=−3.2, P=0.01) as independent associates.

Conclusions—Although coronary endothelial vasoreactivity is blunted in the setting of NSTEMI, this is a reflection of the greater volume of atherosclerosis and cardiovascular risk factors. Thus, the relationship between coronary endothelium-dependent vasomotor reactivity and atheroma volume remains constant irrespective of the nature of the clinical presentation. (Circ Cardiovasc Imaging. 2013;6:674-682.)

Key Words: adrenergic receptor ▪ endothelial function ▪ intravascular ultrasonography ▪ NSTEMI ▪ plaque burden ▪ salbutamol
Several investigators have reported the association of plaque burden with disease progression and incident coronary events. Similarity, the prognostic significance of coronary arterial vasoreactivity has also been well described, whereby endothelial dysfunction, shown to occur in a segmental fashion, independently associated with future cardiovascular events. A better understanding of the relationship between plaque burden and corresponding segmental vasomotor reactivity may be important in elucidating mechanisms underlying plaque progression and instability.

In a recent intravascular ultrasound (IVUS) study that validated intracoronary (IC) salbutamol (selective β2-adrenoreceptor agonist) as a novel endothelium-dependent vasomotor stimulus, an exploratory subanalysis revealed a significant inverse relationship between segmental plaque burden and corresponding endothelium-dependent lumen vasoreactivity. As such, the chief aim of this study was to use IVUS to further explore this relationship in the setting of non-ST-segment–elevation myocardial infarction (NSTEMI) and to contrast these findings in stable patients with near-normal or no significant angiographic coronary disease. Our primary hypothesis was that for each unit measure of IVUS-derived coronary atheroma volume, patients with NSTEMI would display a difference in endothelial function compared with stable subjects with near-normal coronary arteries.

Methods

Study Subjects

We enrolled 47 patients (aged ≥18 years) referred for a clinically indicated coronary angiogram. Our near-normal cohort was defined by those patients with normal or minimally detectable (<30% visual angiographic stenosis) coronary disease throughout the entire epicardial tree and who were troponin T negative. This group comprised consecutive patients undergoing coronary angiography for investigation of chest pain/chest pain equivalent syndromes and were thought to represent a population being as close to normal as deemed ethically possible undergoing an invasive study. The chosen vessel for IV provocation and imaging in this group of patients was invariably the longest vessel containing numerous side branches (for ease of anatomic matching) and minimal tortuosity, for more stable IVUS imaging. In parallel, a separate group of 23 consecutive patients administered with chest discomfort in concert with a significant acute coronary syndrome (NSTEMI) and were considered for study enrollment. In these patients, the invasive research protocol was performed in a noncritically diseased (<30% angiographic stenosis), nonculprit (study) vessel, prior to percutaneous coronary intervention within the culprit vessel, adopting the same angiographic inclusion/exclusion criteria for selection of the study vessel as the stable, near-normal cohort. Following informed consent, vasoactive medications were held for at least 24 hours prior to the invasive study. In the NSTEMI group, we only included patients who were pain free following hospitalization and initial medical stabilization and had no form of nitrate therapy in the 12 hours prior to their angiogram. All procedures were performed in the morning prior to an overnight fast. Exclusion criteria included significant valvular heart disease, left ventricular dysfunction (known ejection fraction ≤40%), prior percutaneous or surgical revascularization, acute coronary syndrome within the preceding 4 weeks (in the near-normal cohort), known coronary spasm, severe obstructive lung disease, creatinine clearance ≤60 mL/min, β-blocker use in the preceding 24 hours, or the use of short- or long-acting β2-agonists within the previous 12 hours. This study was approved by the Royal Adelaide Hospital Human Research Ethics Committee.

Cardiac Catheterization and Intravascular Imaging Protocols

Coronary angiography was performed via a standard 6F technique. Intravenous heparin (70 IU/kg) was administered for the research protocol. A 0.014-inch coronary guide wire was placed into the study vessel within its midsegment away from major side branches. This wire was also used to deliver a 2.5F 40-MHz Atlantis Pro IVUS catheter (Boston Scientific, Natick, MA) into the study artery. This was undertaken without pretreatment with IC nitroglycerin. If percutaneous coronary intervention was to be performed to a culprit lesion, this was done immediately after the invasive research protocol. All IC infusions were administered through an infusion pump at 2 mL/min via the coronary guiding catheter for a period of 5 minutes. After 3 minutes of IC infusion (of either vehicle solution or salbutamol), the IVUS catheter was moved from within the guiding catheter into the distal conduit vessel and images were acquired during automated catheter withdrawal at 0.5 mm/s. Our previous validation study showed that repeated, consecutive, IC vehicle infusions for 5 minutes during IC instrumentation with IVUS has no significant impact on changes in lumen measurements over time. The IVUS images were recorded on a digital video disc for off-line analysis.

Coronary Infusion and Endothelial Function Testing Protocols

The infusion protocols that were performed for the validation of IC salbutamol as an endothelium-dependent coronary vasomotor stimulus have been previously described in detail. IC salbutamol was chosen instead of IC acetylcholine. In patients with NSTEMI undergoing repeated IVUS imaging without pretreatment with IC nitroglycerin, there was concern regarding the possibility of inducing clinically significant coronary spasm with an acetylcholine-based protocol. Moreover, a series of in vivo human observations have implicated coronary β2-adrenoreceptor stimulation to cause nitric oxide (NO)-mediated peripheral and coronary arterial vasomotor responses. However, underlying mechanisms for β2-adrenergic endothelial NO release appear distinct to those following acetylcholine stimulation. It is likely that β2-adrenergic signaling may involve similar actions on adenylyl cyclase to endothelial prostaglandins, direct stimulation of endothelial NO synthase, and/or potassium channel activation to induce NO synthase. For the purpose of further exploring the relationship between epicardial plaque burden and segmental β2-adrenergic mediated endothelium-dependent vasoreactivity, our endothelial function testing protocol involved (following baseline IC 5% dextrose [vehicle] infusion) sequential 5-minute infusions of IC salbutamol at the doses of 0.30 µg/min and 0.60 µg/min, respectively (Figure 1D).

Data Acquisition and Analysis

Analysis of IVUS data were performed by the Atherosclerosis Imaging Core Laboratory, Cleveland Clinic, according to prior experience and published guidelines. Technicians were blinded to clinical details and imaging sequence. Proximal and distal fiduciary markers (anatomic side branches) were chosen to define the overall region of vessel to be analyzed, as well as for segment matching. Cross-sectional images were selected every 30 frames (0.5 mm apart). Frames that precluded complete lumen or vessel wall planimetry were excluded from analysis, as were segments that involved branch points. Each IVUS pullback was divided into 5-mm segments comprising 11 frames taken at 10 evenly spaced cross-sectional (0.5 mm) intervals (Figure 1A through 1C). Given the known segmental heterogeneity of coronary vasomotor reactivity, each segment was thus analyzed separately as an individual entity, with appropriately used statistical methods. Matching frames of anatomic side branches from the baseline and postsalbutamol stimulation IVUS runs were coregistered to ensure accurate segment matching between runs. Leading edges of the lumen and external elastic membrane (EEM) were manually planimetered. Percent atheroma volume (PAV) was calculated to determine segmental plaque burden.
Observer Variability

For coronary lumen measurements, the intraobserver coefficient of variation was 1.1%, and interobserver coefficient of variation was 2.6%. For plaque measurements, the intraobserver coefficient of variation was 1.8%, and the interobserver coefficient of variation was 3.8%.

Statistical Analysis

Data are expressed as mean±SD or median and 25th and 75th percentiles, as appropriate, for continuous data. Categorical data are presented as percent of nonmissing data. Comparisons between the near-normal and NSTEMI groups were made using Student t test for continuous data (or Wilcoxon rank-sum for nonnormally distributed data) and χ² tests for categorical data. Absolute and percent changes in SLV were calculated for each segment and summarized across PAV tertiles. Least-square means were calculated within each tertile for change in SLV parameters using mixed models (via a variance component structure), accounting for multiple segment measurements within a patient and controlling for baseline SLV. A separate subgroup analysis was performed comparing absolute and percent change in SLV in patients with high-sensitivity C-reactive protein (hsCRP) <2 and ≥2 mg/L across PAV tertiles. Tests for trend across PAV tertiles were calculated for the near-normal/NSTEMI groups, as well as baseline hsCRP levels. Multivariable mixed models were created to identify independent predictors of change in SLV. All variables that were univariately associated with change in lumen volume (P value <0.20) were considered for multivariable adjustment. Variables were then kept in the final model if they reached statistical significance (P value <0.05). Further adjustment was made for near-normal/NSTEMI categorization of patients and baseline lumen volume. Correlations between segmental PAV and endothelial function were computed using the Spearman rank calculation. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). P values <0.05 were considered statistically significant.

Results

Clinical and IVUS Data

Table 1 summarizes clinical, biochemical, angiographic, and IVUS characteristics of the 2 patients groups. The NSTEMI cohort comprised significantly more males, patients with diabetes mellitus, and a trend toward more hypertensive individuals. This group also demonstrated lower serum high-density lipoprotein cholesterol, higher triglyceride levels, and a trend toward increased hsCRP levels. All patients with an NSTEMI underwent successful percutaneous coronary intervention and stent insertion to the culprit lesion after undertaking the experimental protocol within the nonculprit vessel. Despite similar angiographic inclusion/exclusion criteria between
Table 1. Clinical, Biochemical, Angiographic, and Ultrasonic Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Near-Normal Cohort</th>
<th>NSTEMI Cohort</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 24 Patients</td>
<td>n = 23 Patients</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 193 Segments</td>
<td>n = 193 Segments</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58±12</td>
<td>59±12</td>
<td>0.93</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (42)</td>
<td>19 (83)</td>
<td>0.004</td>
</tr>
<tr>
<td>Smoker*, n (%)</td>
<td>10 (42)</td>
<td>15 (65)</td>
<td>0.11</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>3 (13)</td>
<td>6 (26)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>10 (42)</td>
<td>16 (70)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>177±46</td>
<td>161±42</td>
<td>0.22</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>113±40</td>
<td>104±35</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>49±13</td>
<td>35±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>66 (44–102)</td>
<td>97 (89–133)</td>
<td>0.009</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>2.4 (1.4–4.6)</td>
<td>4.0 (2.2–12)</td>
<td>0.07</td>
</tr>
<tr>
<td>Statin use</td>
<td>9 (38)</td>
<td>13 (59)</td>
<td>0.14</td>
</tr>
<tr>
<td>Troponin T, ng/mL</td>
<td>0</td>
<td>1.7±3.6</td>
<td>N/A</td>
</tr>
<tr>
<td>Nonculprit study artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD, n (%)</td>
<td>21 (88)</td>
<td>11 (48)</td>
<td>0.004</td>
</tr>
<tr>
<td>LCx, n (%)</td>
<td>2 (8)</td>
<td>9 (39)</td>
<td>0.01</td>
</tr>
<tr>
<td>RCA, n (%)</td>
<td>1 (4)</td>
<td>3 (13)</td>
<td>0.28</td>
</tr>
<tr>
<td>Segments per patient</td>
<td>8 (6–8)</td>
<td>8 (7–10)</td>
<td>0.66</td>
</tr>
<tr>
<td>PAV</td>
<td>27.5±14</td>
<td>40.4±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SLV, mm³</td>
<td>93±37</td>
<td>78±40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EEM, mm³</td>
<td>131±49</td>
<td>134±47</td>
<td>0.59</td>
</tr>
<tr>
<td>RI</td>
<td>1.0 (0.9–1.0)</td>
<td>1.2 (1.0–1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>El</td>
<td>9.0 (6.4–14)</td>
<td>4.3 (3.0–6.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

EEM indicates external elastic membrane; EI, eccentricity index; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LAD, left anterior descending artery; LCx, left circumflex artery; LDL-C, low-density lipoprotein cholesterol; N/A, not applicable (as by definition, all stable patients were tested negative for cardiac Troponin T levels); NSTEMI, non-ST-segment–elevation myocardial infarction; PA V, percent atheroma volume; RCA, right coronary artery; RI, remodeling index; SLV, segmental lumen volume; and TAV, total atheroma volume.

Data expressed as mean±SD or median and interquartile range when appropriate.

*Definition of smoking was taken as current or within 4 wk.

Lumen Response: Near Normal Versus NSTEMI Controlled for IVUS-derived Plaque Burden

Figure 2 illustrates segmental endothelium-dependent vasomotor reactivity to IC salbutamol, according to clinical presentation, in a dose-response manner. Following baseline IC vehicle infusion, patients in the near-normal group displayed greater increases in SLV to both the 0.30 and 0.60 μg/min doses of IC salbutamol (% change SLV: 5.1±0.89 and 6.0±1.53%, P<0.001 from baseline, respectively; absolute change SLV: 3.7±4.0.73 and 4.1±1.3 mm³, P<0.001 and P=0.002 from baseline, respectively) compared to the NSTEMI group at each of the 0.30 and 0.60 μg/min doses, respectively (% change SLV of 2.05±0.89 and 3.21±1.12, P=0.02 and 0.005 from baseline, respectively; absolute change SLV: 1.2±0.73 and 1.9±0.97 mm³, P=0.11 and 0.05 from baseline, respectively). The degree of vasomotor response observed following the 0.30 μg/min dose of IC salbutamol was significantly less in the NSTEMI group compared with the near-normal group (differences in % change SLV: P=0.02; differences in absolute change SLV: P=0.01). There was a significant, inverse correlation between segmental PA V and endothelial function (Spearman correlation coefficients and nonparametric P value): absolute change in SLV versus PA V −0.26 (−0.36, −0.16), P<0.001; % change in SLV versus PA V −0.21 (−0.31, −0.11), P<0.001.

Multivariable Predictors of Segmental Endothelium-dependent Coronary Vasomotor Responses

Table 2 describes the multivariable analysis for predictors of change in SLV. The considered univariate factors associated with more expansive remodeling of the vessel wall.

Dose–Response Analysis: Near Normal Versus NSTEMI not Stratified for IVUS-derived Plaque Burden

Two patients in the near-normal group experienced transient coronary spasm during instrumentation, prior to IC infusions, who responded promptly to administration of IC nitroglycerin. These subjects did not proceed to the study protocol and hence data were not acquired. Successive doses of IC salbutamol exerted no significant change from baseline on blood pressure or heart rate.

Figure 2 illustrates segmental endothelium-dependent vasomotor reactivity to IC salbutamol, according to clinical presentation, in a dose-response manner. Following baseline IC vehicle infusion, patients in the near-normal group displayed greater increases in SLV to both the 0.30 and 0.60 μg/min doses of IC salbutamol (% change SLV: 5.1±0.89 and 6.0±1.53%, P<0.001 from baseline, respectively; absolute change SLV: 3.7±4.0.73 and 4.1±1.3 mm³, P<0.001 and P=0.002 from baseline, respectively) compared to the NSTEMI group at each of the 0.30 and 0.60 μg/min doses, respectively (% change SLV of 2.05±0.89 and 3.21±1.12, P=0.02 and 0.005 from baseline, respectively; absolute change SLV: 1.2±0.73 and 1.9±0.97 mm³, P=0.11 and 0.05 from baseline, respectively). The degree of vasomotor response observed following the 0.30 μg/min dose of IC salbutamol was significantly less in the NSTEMI group compared with the near-normal group (differences in % change SLV: P=0.02; differences in absolute change SLV: P=0.01). There was a significant, inverse correlation between segmental PA V and endothelial function (Spearman correlation coefficients and nonparametric P value): absolute change in SLV versus PA V −0.26 (−0.36, −0.16), P<0.001; % change in SLV versus PA V −0.21 (−0.31, −0.11), P<0.001.

Our results show that within each tertile of plaque burden, there were no significant differences in endothelium-dependent luminal responses between the near-normal and NSTEMI groups. However, a consistent finding was the inverse relationship between the burden of segmental epicardial atherosclerosis and corresponding lumen response. Irrespective of the nature of coronary syndrome, segments containing the lowest tertile of plaque exhibited the greatest degree of vasodilatation (measured as absolute or percent change from baseline). On the contrary, segments containing the highest tertile of plaque burden exhibited the least degree of epicardial vasomotion and occasionally vasoconstriction.

Multivariable Predictors of Segmental Endothelium-dependent Coronary Vasomotor Responses

Table 2 describes the multivariable analysis for predictors of change in SLV. The considered univariate factors associated with more expansive remodeling of the vessel wall.
with a change in SLV (P value <0.20) that were entered into the multivariable model included the nature of clinical presentation (near normal versus NSTEMI), age, hsCRP levels ≥2 mg/L, sex, total cholesterol levels, low-density lipoprotein cholesterol levels, high-density lipoprotein cholesterol levels, triglyceride levels, hypertension, diabetes mellitus, smoking status, and PAV. An hsCRP level of ≥2 mg/L was used to dichotomize patients with higher levels of systemic subclinical inflammation. The multivariable analysis revealed that higher segmental plaque burden was independently associated with lower degrees of endothelial function (P=0.0004). Similar significant, independent relationships were found in active smokers (P=0.01), patients with diabetes mellitus (P<0.0001), higher low-density lipoprotein cholesterol levels (P=0.01), patients with an hsCRP level ≥2 mg/L (0.03), increasing age (P<0.0001), and greater baseline SLV (P<0.0001) per se. After controlling for these factors, clinical status (near normal versus NSTEMI) was not significantly associated with a change in SLV (P=0.91).

**Associations Between Inflammation, Atheroma Burden, and Vascular Reactivity**

A further subgroup analysis was undertaken to explore the association between inflammation, coronary atheroma burden, and endothelium-dependent coronary vasomotion. Patients were dichotomized into 2 groups: hsCRP <2 mg/L and hsCRP ≥2 mg/L, with median CRP levels in each group being 1.1 (0.5–1.5) and 4.4 (2.9–11), respectively. Those with elevated hsCRP levels (≥2 mg/L) demonstrated greater PAV (35.3±15 versus 30.2±14%, P=0.002), larger EEM volume (136.0±48 versus 123.7±48 mm³, P=0.026), but similar baseline lumen volume volumes (134±47 versus 131±49 mm³, P=0.59), compared to those with less systemic inflammation (hsCRP <2 mg/L). Segmental remodeling indices were similar between both groups (1.1 [1.0–1.3] versus 1.0 [0.9–1.3], P=0.60). Without stratification for the degree of plaque burden, coronary segments in patients with higher levels of systemic inflammation displayed less endothelium-dependent coronary vasomotion than segments from individuals with less systemic inflammation (% change SLV: 2.51±0.73 versus 6.52±1.2%, P=0.005; absolute change SLV: 1.67±0.59 versus 4.63±0.98 mm³, P=0.01). In the lowest PAV tertile, the degree of systemic inflammation failed to impart any effect on endothelium-dependent luminal response (Figure 4). However, in the presence of greater amounts of plaque, systemic inflammation seemed to influence lumen response. In patients with hsCRP levels ≥2 mg/L, blunted and even constrictive lumen responses were observed (for both relative and absolute changes in SLV, P value for trend across all PAV tertiles <0.001). Yet, preservation of lumen responses was observed in those individuals with hsCRP levels ≥2 mg/L (for both relative and absolute changes in SLV, P values for trend across...
PAV tertiles were 0.36 and 0.27, respectively) (Figure 4). A trend was observed regarding the interaction between coronary atheroma volume (tertiles of PAV) and hsCRP (<2 versus ≥2 mg/L) on endothelium-dependent coronary vasoreactivity (change in SLV) (P value for interaction=0.09).

**Discussion**

We show for the first time that the volume of coronary atheroma significantly associates with endothelium-dependent lumen vasoreactivity, regardless of the nature of clinical syndrome. With the higher image resolution provided by IVUS, we found that the magnitude of endothelium-dependent lumen responses did not significantly differ according to clinical presentation, when the degree of atheroma volume was controlled equally across clinical groups. Endothelium-dependent lumen vasoreactivity related inversely with coronary atheroma volume, with similar associations found with a number of other systemic cardiovascular risk factors, but not with the nature of clinical presentation. Of particular note was the observation of an inverse relationship between endothelium-dependent lumen vasoreactivity and subclinical systemic inflammation, characterized by hsCRP levels ≥2 mg/L. Subgroup analysis of the association between the degree of systemic inflammation, plaque burden, and lumen vasoreactivity revealed a possible interaction between hsCRP levels ≥2 mg/L and greater atheroma volumes, in mediating endothelium-dependent lumen vasoreactivity. These observations provide a mechanistic understanding of why patients with acute coronary syndromes frequently display greater degrees of conduit segment endothelial dysfunction. These data also provide intriguing insight into the dynamic interplay between coronary atheroma volume, systemic inflammation, and subsequent vasomotor reactivity, and how the interaction between these components may mediate plaque instability, and subsequent clinical risk.

Following the notion that patients with unstable coronary disease display enhanced coronary vasoreactivity, we anticipated that the magnitude of lumen vasoreactivity to an endothelium-dependent stimulus, per unit of coronary atheroma volume, would be greater in the setting of an NSTEMI than compared to a stable, near-normal patient cohort. This suspicion stemmed from prior observations of greater lumen vasoreactivity in unstable patients with evidence of systemic inflammation. However, our study findings are at partial odds with these prior observations and challenge prior mechanistic appraisals of why epicardial lumen vasoreactivity has been shown to be impaired in the setting of an acute coronary syndrome. Given that prior evaluations of lumen vasoreactivity used coronary angiography, it is likely that the extent of atheroma volume in these vessels was greatly underestimated. Furthermore, angiography did not permit previous investigators to conduct a detailed appraisal of the differences in magnitudes of lumen responses, stratified for the degree of atheroma volume, in the way IVUS has enabled us to do in this study. Despite adoption of the same angiographic inclusion/exclusion criteria for identifying the study vessel, our findings of more severe diffuse disease demonstrated in patients with NSTEMI, compared to patients with stable coronary syndromes, are in agreement with prior observations. Our data

**Table 2. Multivariable Analysis for Change in Segmental Lumen Volume**

<table>
<thead>
<tr>
<th>Variable</th>
<th>β Coefficient (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near-normal presentation (vs NSTEMI)</td>
<td>−0.15 (−2.9 to 2.6)</td>
<td>0.91</td>
</tr>
<tr>
<td>Baseline SLV, mm³</td>
<td>−0.12 (−1.15 to −0.08)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age, y</td>
<td>−0.24 (−0.34 to −0.13)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hsCRP ≥2 mg/L (vs hsCRP &lt;2 mg/L)</td>
<td>−3.1 (−5.9 to −0.29)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>−6.9 (−10.0 to −3.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.0 (−1.5 to 3.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>−0.05 (−0.08 to −0.01)</td>
<td>0.01</td>
</tr>
<tr>
<td>Smoker</td>
<td>−3.2 (−5.8 to −0.64)</td>
<td>0.01</td>
</tr>
<tr>
<td>PAV</td>
<td>−0.18 (−0.27 to −0.08)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NSTEMI, non–ST-segment–elevation myocardial infarction; PAV, percent atheroma volume; and SLV, segmental lumen volume.
with statins might have not only improved NO bioavailability, but also subsequently improved endothelial function; however, lipid lowering per se reduces the inflammatory content of atheroma, with a resultant plaque stabilizing effect. Although some consider the prognostic role of hsCRP levels to be equivalent to serum cholesterol levels, further studies are required to assess whether hsCRP levels, used in concert with measured coronary atheroma volume, further improves coronary risk prediction.

Our observations of a mechanistic link between coronary structure and corresponding vessel function may have implications for promoting the direct evaluation of the coronary vasculature as a means to further stratify coronary risk. Moreover, this approach also avoids uncertainties of extrapolating measurements from remote vascular beds to events known to arise from within the coronary tree. Complementary to the findings from IVUS studies, which have shown consistent associations between the baseline volumetric extent of coronary plaque and incident clinical events, semiquantitative measures of coronary plaque burden with noninvasive imaging similarly associated with cardiovascular mortality. Moreover, these observations augmented the prediction of mortality beyond traditional methods of quantifying cardiovascular risk. In addition, the recent demonstration of a noninvasive means to evaluate vasomotor responses to isometric handgrip further outlined the potential clinical use of noninvasive coronary imaging for clinical risk stratification. However, as it stands, there is currently no hard evidence to support the use of noninvasive coronary imaging as a means to better predict coronary risk. Ultimately, supportive data from large-scale clinical trials will be needed to justify the use of direct atherosclerosis imaging as a means to further stratify individuals in routine clinical practice. Nevertheless, our observations of a direct relationship between coronary atheroma volume and segmental endothelium-dependent vasoreactivity, independent to other systemic risk factors and the nature of clinical presentation, might suggest that simply measuring plaque burden may be sufficient as a novel risk-stratification tool. Although it remains to be seen whether the incorporation of vasomotor reactivity testing during direct coronary imaging will provide further incremental patient and lesion-specific prognostic capabilities.

Several limitations of this study should be noted. Because of the invasive nature of our experimental protocol and possibility of coronary spasm/constriction during simultaneous IC salbutamol infusion and IVUS interrogation, we evaluated nonculprit vessels with mild angiographic disease. Hence, these findings cannot be extrapolated to culprit segments containing critical stenoses, where platelet-rich thrombus may also impact on focal vascular reactivity. However, a large natural history study showed that IVUS-derived plaque burden in nonculprit vessels was an independent predictor of half of the future coronary events observed in the study, underscoring the systemic nature of the disease process and importance of nonculprit vessel pathology following an NSTEMI. Nearly half of the near-normal population comprised females, some of whom are known to present with cardiac syndrome X, with such patients possibly having an atypical relationship between plaque burden and vasomotor reactivity. However, on multivariable analysis, female sex was not found to independently...

### Table 1

<table>
<thead>
<tr>
<th>PAV Tertile (%)</th>
<th>T1: (6.01-26.59)</th>
<th>T2: (26.6-40.6)</th>
<th>T3: (40.75-74.65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>128</td>
<td>129</td>
<td>129</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salbutamol 0.30 μg/min</th>
<th>% Δ in SLV</th>
<th>Absolute change in SLV (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salbutamol 0.30 μg/min</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Salbutamol 0.30 μg/min</td>
<td>T3</td>
<td>T3</td>
</tr>
</tbody>
</table>

**Figure 4.** Lumen responses stratified according to high-sensitivity C-reactive protein (hsCRP) levels and plaque burden. Segmental epicardial endothelium-dependent vasoreactivity (% change and absolute change in segmental lumen volume [SLV]) in all coronary segments stratified according to common tertiles of measured plaque burden (percent atheroma volume [PAV]) across patients with hsCRP levels <2 mg/L vs ≥2 mg/L, following the 0.30 μg/min salbutamol dose.
associate with endothelial function. The reference group from which we drew comparisons were not entirely normal, in the sense that overt coronary atherosclerosis was still detectable on IVUS, despite appearing negligible on coronary angiography. However, this represented a population being as close to normal as deemed ethically possible undergoing an invasive coronary evaluation. Direct vasodilator responses to IC nitroglycerin injection were not evaluated. This would have allowed us to test if smooth muscle cell dysfunction, rather than impairment in NO-dependent function, contributed to blunted vessel wall reactivity in a number of segments. Salbutamol, however, has minimal direct smooth muscle cell dilating properties, as shown in our previous validation study.11 Plaque composition was not assessed in this study. Although current techniques of assessing plaque composition via interrogation of the radiofrequency backscatter signal currently possess certain limitations,41,42 its evaluation might have yielded added mechanistic insight into the possibility of atheroma phenotype mediating segmental lumen vasoreactivity.

Conclusions
Irrespective of the nature of clinical presentation, the magnitude of segmental lumen vasoreactivity was not significantly different, when controlled for by the degree of coronary atheroma volume. Rather, coronary lumen vasoreactivity appeared primarily dependent on the volume of atheroma, established cardiovascular risk factors, and the presence of subclinical inflammation. Systemic inflammation seems to play a role in mediating coronary endothelium-dependent lumen reactivity, in the presence of a greater, but not lesser, burden of coronary atheroma. This suggests a possible interaction between inflammation and plaque in determining coronary vasomotor reactivity. These findings outline the functional significance of coronary atheroma volume in vivo, thereby providing further mechanistic validation of its future potential clinical role as an imaging biomarker of coronary risk.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Similar to coronary endothelial dysfunction, emerging evidence points to coronary atheroma volume as an imaging biomarker of coronary risk, yet its dynamic interplay with underlying corresponding endothelial-dependent vasoreactivity is poorly defined in humans in vivo. Using a novel approach of intracoronary provocation during simultaneous intravascular ultrasound interrogation of a nonculprit epicardial coronary artery in vivo, we demonstrated that coronary segments of patients with non–ST-segment–elevation myocardial infarction displayed less endothelium-dependent vasomotion compared with segments in patients with stable, near-normal coronary arteries. This was a reflection of a greater risk-factor profile and underlying plaque burden in patients with non–ST-segment–elevation myocardial infarction, despite use of the same angiographic inclusion and exclusion criteria across both patient groups. Irrespective of the nature of clinical presentation, the magnitude of segmental lumens vasoreactivity, when controlled for by the degree of segmental coronary atheroma volume, did not significantly differ. Rather, multivariable analysis revealed coronary lumen vasoreactivity to be primarily dependent on the volume of atheroma, established cardiovascular risk factors, and the presence of subclinical inflammation. Subclinical systemic inflammation (high-sensitivity C-reactive protein >2 mg/L) also seemed to mediate endothelium-dependent lumen reactivity, in the presence of a greater, but not lesser, burden of coronary atheroma, suggesting a possible interaction between inflammation and plaque in determining coronary vasomotor reactivity. Collectively, these findings outline the potential role of the interplay between epicardial plaque burden, endothelial function, and systemic inflammation in mediating adverse coronary outcomes.
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