Natural History of Experimental Coronary Atherosclerosis and Vascular Remodeling in Relation to Endothelial Shear Stress

A Serial, In Vivo Intravascular Ultrasound Study

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Background—The natural history of heterogeneous atherosclerotic plaques and the role of local hemodynamic factors throughout their development are unknown. We performed a serial study to assess the role of endothelial shear stress (ESS) and vascular remodeling in the natural history of coronary atherosclerosis.

Methods and Results—Intravascular ultrasound–based 3-dimensional reconstruction of all major coronary arteries (n=15) was performed serially in vivo in 5 swine 4, 11, 16, 23, and 36 weeks after induction of diabetes mellitus and hyperlipidemia. The reconstructed arteries were divided into 3-mm-long segments (n=304). ESS was calculated in all segments at all time points through the use of computational fluid dynamics. Vascular remodeling was assessed at each time point in all segments containing significant plaque, defined as maximal intima-media thickness ≥0.5 mm, at week 36 (n=220). Plaque started to develop at week 11 and progressively advanced toward heterogeneous, multifocal lesions at all subsequent time points. Low ESS promoted the initiation and subsequent progression of plaques. The local remodeling response changed substantially over time and determined future plaque evolution. Excessive expansive remodeling developed in regions of very low ESS, further exacerbated the low ESS, and was associated with the most marked plaque progression. The combined assessment of ESS, remodeling, and plaque severity enabled the early identification of plaques that evolved to high-risk lesions at week 36.

Conclusions—The synergistic effect of local ESS and the remodeling response to plaque formation determine the natural history of individual lesions. Combined in vivo assessment of ESS and remodeling may predict the focal formation of high-risk coronary plaque. (Circulation. 2010;121:2092-2101.)

Key Words: atherosclerosis ■ endothelial shear stress ■ natural history ■ remodeling

Atherosclerosis is a systemic disease with multifocal heterogeneous manifestations.1 Because multiple plaques at different stages of progression and variable morphology typically coexist within the same patient or even artery,2 characterizing and risk-stratifying each individual plaque in vivo would be invaluable. Increasing attention has focused on the in vivo identification of high-risk plaques most likely to rupture and cause an acute coronary event. Although previous evidence exists on the heterogeneous nature of atherosclerotic disease, there have been no previous in vivo studies to characterize the natural history of atherosclerosis and to determine the factors responsible for the heterogeneity of plaque development. As a result, no widely accepted method to prospectively identify high-risk plaque in vivo is available at present.

Clinical Perspective on p 2101

The focal distribution and heterogeneity of plaques, despite the exposure of the entire vasculature to the same systemic risk factors, have been attributed to the effect of local hemodynamic forces. Endothelial shear stress (ESS) critically determines the regional localization of atherosclerosis and the evolution of individual lesions.1,3,4 However, previous studies were often limited by the use of only 2 time points to assess...
natural history\textsuperscript{5–7} or by the lack of animal models of human-like coronary atherosclerosis.\textsuperscript{8} Currently, there are no data prospectively assessing the role of ESS in the continuum of atherosclerosis manifestations or the progression of heterogeneous advanced plaques over multiple time points. The vascular remodeling response to the development of plaque is an essential component of atherosclerotic disease and strongly influences the composition of the individual plaque and its ultimate clinical presentation.\textsuperscript{9,10} Little is currently known about the natural course of the remodeling response of the wall to local plaque, the dynamic interplay between remodeling and the changing local hemodynamic environment, and the impact of the nature of remodeling on future plaque growth.

The objective of the present study was dual. First, we sought to perform a serial intravascular ultrasound (IVUS) study of the natural history of coronary atherosclerosis and to determine the role of ESS throughout the natural course of developing lesions using a diabetic porcine model that can develop human-like advanced plaques.\textsuperscript{3,11} We further investigated the evolution of the vascular remodeling response to plaque and the association of remodeling with local ESS and subsequent plaque progression. Second, we hypothesized that in vivo assessment of local ESS, plaque, and remodeling characteristics may predict the heterogeneity of plaque development and the formation of high-risk plaque at an early stage of its development. Early identification of coronary lesions likely to evolve to a rupture-prone phenotype may enable the appropriate application of a focused systemic treatment or highly selective local prophylactic intervention to avert an adverse clinical outcome.

\section*{Methods}

A detailed description of the methods is presented in the online-only Data Supplement. Briefly, 5 male Yorkshire swine 12 to 14 weeks of age were rendered diabetic by injection of streptozotocin (50 mg/kg in 0.1 mol/L Na-citrate daily for 3 days) and were fed a high-fat diet (containing 1.5% cholesterol and 15% lard supplemented with sucrose,\textsuperscript{11} Intracoronary vascular profiling with IVUS and angiography, as described previously,\textsuperscript{12} was serially performed in vivo on each of the animals’ major epicardial coronary arteries at weeks 4, 11, 16, 23, and 36 after induction of diabetes mellitus and initiation of high-fat diet (Figure 1 in the online-only Data Supplement). The experimental protocol was approved by the Harvard Medical School Institutional Animal Care and Use Committee. All measurements were made blinded to animal age.

\subsection*{Vascular Profiling for the Assessment of Local ESS}

Vascular profiling used a methodology previously described and validated in vivo.\textsuperscript{3,4,12,13} Each 3-dimensional reconstructed artery was divided at week 36 into 3-mm-long segments along its entire length. To locate the segments in IVUS investigations at multiple time points, 2 to 3 readily visible side branches were identified and used as reference markers for accurate comparison of the same segments over time. ESS at the lumen surface of the 3-dimensional reconstructed artery was calculated at all time points as the product of blood viscosity and the gradient of blood velocity at the wall. Mean ESS was calculated in each segment at all time points.

\subsection*{Assessment of Plaque Severity and Plaque Progression by IVUS}

Atherosclerotic plaque severity over time was estimated in each segment of the 3-dimensional reconstructed arteries by maximum intima-media thickness (maxIMT) assessed by IVUS. To account for possible circumferential and longitudinal variations in plaque thickness, the 30° arch with the greatest average IMT was identified around the circumference of each 3-dimensional reconstructed segment and represented the maxIMT of the segment at each time point. Plaque severity was categorized as not significant (grade 0; \textless 0.5 mm), minor (grade 1; 0.5 to 0.7 mm), intermediate (grade 2; 0.71 to 1.0 mm), and severe (grade 3; \textgreater 1.0 mm) (Figure 1A). The progression of plaque severity between consecutive time points was assessed by the relative change in maxIMT (%ΔmaxIMT) according to the following equation:

\[
%\Delta \text{maxIMT} = \frac{\text{maxIMT (follow-up) - baseline}}{\text{maxIMT (baseline)}} \times 100
\]

Plaque progression between consecutive time points was assessed by change in plaque (intima-media) volume (ΔPV). We calculated the PV of each 3 mm-long 3-dimensional reconstructed segment as the sum of PVs contained between all consecutive end-diastolic IVUS frames that were included in each 3-mm-long segment. The PV contained between each 2 consecutive end-diastolic IVUS frames was in turn computed as the product of plaque area multiplied by the distance between each 2 consecutive frames.

Plaque burden (percent) was calculated for each segment at all time points according to the following equation:

\[
\text{%plaque burden} = \frac{\text{plaque volume/EEM volume}}{\times 100}
\]

where EEM is external elastic membrane.

\subsection*{Assessment of Vascular Remodeling}

The nature of the remodeling response to plaque growth was assessed over time in each arterial segment that contained significant plaque at the final week 36, defined as maxIMT \textgeq 0.5 mm by IVUS. Remodeling was assessed in these segments at each time point by comparing the local remodeling behavior of each individual segment with the global remodeling response of the entire artery as previously described.\textsuperscript{3,14} We identified 3 patterns of vascular remodeling: excessive expansive remodeling, compensatory expansive remodeling, and constrictive remodeling.

\subsection*{Identification of High-Risk Plaque by IVUS}

High-risk plaques were characterized by IVUS at week 36. We considered plaques with maximal severity and excessive expansive remodeling at week 36 to represent the lesions with the highest risk of rupture, consistent with previous human IVUS\textsuperscript{15} and histopathology studies,\textsuperscript{2,9,16} as well as our previous IVUS and histopathology results using the same diabetic, hyperlipidemic swine model.\textsuperscript{3} Accordingly, segments with the combination of grade 3 plaque (maxIMT \textgeq 1.0 mm) and excessive expansive remodeling at week 36 made up the subpopulation of high-risk plaques.

\subsection*{Determination of a Risk Score for the Prediction of High-Risk Plaque}

A composite risk score was used at weeks 11, 16, and 23 to predict the formation of high-risk plaques at week 36. Risk score points were assigned to local ESS, plaque severity, and the vascular remodeling pattern using an arbitrary grading system summarized in the Table. The grading system was developed using categorical versions of all included variables. Plaque severity was categorized into 4 grades based on maxIMT by IVUS as described above. ESS was categorized into 3 grades: \textless 1.0, 1.0 to 1.5, and \textgreater 1.5 Pa. The vascular remodeling pattern was categorized as excessive expansive, compensatory, and constrictive. The predictive risk score was assessed for each segment at weeks 11, 16, and 23 as the sum of all points at each time point.

\subsection*{Statistical Analyses}

Statistical analyses were performed with SPSS version 17.0 (SPSS Inc, Chicago, Ill) or STATA version 10.1 (Stata Corp, College Station, Tex). Continuous variables are summarized as mean±SEM;
categorical variables, as actual numbers and percentages. Correlation between 2 continuous variables was measured with the Pearson $r$ correlation coefficient. For analyses with a continuous dependent and a categorical independent variable, ANOVA was used. Because observations were not statistically independent, the animal was specified as a random effect to account for the clustering of arteries within animals. The method of Scheffe was used to adjust for multiple comparisons of data. Linear regression was used when there were both continuous independent and dependent variables. The Huber-White sandwich estimator was used to correct for the clustering of arteries within animals. Logistic regression was used to examine the association between the risk score at week 11, 16, or 23, and plaque status at week 36, which was defined as high-risk versus all other lesions. First, separate bivariate analyses examining the effect of each score were conducted; second, a multivariate model with the best set of predictors was developed. Given the hierarchical data structure, 95% confidence intervals were adjusted with the Huber-White sandwich estimator. Findings were considered statistically significant at the 0.05 level.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

### Table. Grading System for the Assessment of the Predictive Risk Score

<table>
<thead>
<tr>
<th>Plaque Severity</th>
<th>ESS</th>
<th>Vascular Remodeling</th>
</tr>
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<tbody>
<tr>
<td>Category</td>
<td>Points</td>
<td>Category</td>
</tr>
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<td>Grade 0</td>
<td>0</td>
<td>&gt;1.5 Pa</td>
</tr>
<tr>
<td>Grade 1</td>
<td>1</td>
<td>1.0–1.5 Pa</td>
</tr>
<tr>
<td>Grade 2</td>
<td>2</td>
<td>&lt;1.0 Pa</td>
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<tr>
<td>Grade 3</td>
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### Results

Fifteen coronary arteries from 5 pigs were serially profiled (left anterior descending artery, $n=5$; left circumflex artery, $n=5$; right coronary artery, $n=5$). The mean time-averaged total cholesterol and blood glucose during follow-up were $728\pm161$ and $265\pm53$ mg/dL, respectively.

#### Assessment of Plaque Severity and Plaque Progression Over Time

Plaque started to develop at week 11 and progressively evolved to more advanced grades, leading to marked heterogeneity at all subsequent time points. The frequency of grade 1 plaques peaked at week 16 and subsequently decreased, with a parallel increase in the frequency of grade 2 and 3 plaques, suggesting the transition of minor to more advanced lesions (Figure 1B). We investigated the association of individual plaque severity at follow-up with the corresponding plaque severity at earlier time points. There was a strong correlation of maxIMT of individual segments at week 36 with maxIMT at week 23 ($r=0.72, P<0.001$) and week 16 ($r=0.57, P<0.001$) and a moderate correlation with maxIMT at week 11 ($r=0.39, P<0.001$). We focused on segments with each grade of plaque severity at week 36 and assessed their evolution throughout their natural history. We observed nonuniform patterns of progression rate such that segments with severe (grade 3) plaque at week 36 had on average higher maxIMT at weeks 16 and 23 and higher plaque progression ($\Delta PV$) during all consecutive time intervals compared with segments that eventually developed less severe or no plaque at week 36 (Figure 1C and 1D). These
results indicate that arterial segments culminating in the most severe plaque persistently exhibited more marked progression throughout their evolution. Of note, total cholesterol did not significantly differ in segments that culminated in each grade of plaque at week 36 (P=0.19).

Proatherogenic Effect of Low ESS Over Time
To assess the proatherogenic effect of ESS throughout the evolution of minor lesions to heterogeneous, advanced plaques, all segments (n=304) were categorized on the basis of low ESS (<1.2 Pa) or moderate/higher ESS (≥1.2 Pa) at each time point as previously reported.4 Segments exposed to low ESS at weeks 11, 16, and 23 exhibited greater subsequent progression of plaque severity (%ΔmaxIMT) compared with segments with moderate/higher ESS (Figure 2A). To investigate the proatherogenic effect of low ESS in relation to the presence of significant plaque, we focused on week 23 on the basis that almost half of all segments (n=147 of 304, 48.4%) already contained significant plaque (maxIMT ≥0.5 mm) at that time point. Higher progression of plaque severity in response to low ESS compared with moderate/higher ESS occurred in segments free of significant plaque at week 23 (P=0.09) and in segments with significant plaque by IVUS at that time point (P=0.054; Figure 2B).

Assessment of Vascular Remodeling Over Time
Vascular remodeling response to plaque was determined at all time points in all segments that contained significant plaque at week 36 (maxIMT ≥0.5 mm; n=220, 72.4% of all segments). There was marked heterogeneity of remodeling patterns; at each time point, the majority of segments exhibited compensatory expansive remodeling, ranging between 70% and 80% over time, whereas the rest were almost equally divided between an excessive expansive (10% to 17%) and constrictive (10% to 15%) phenotype (Figure 3A). The remodeling response of each individual segment also exhibited remarkable heterogeneity throughout its evolution such that segments did not consistently manifest the same remodeling pattern over time but often evolved through different patterns (Figure 3B). Although in general the proportion of segments with each remodeling type remained relatively stable over time, the specific segments within each category changed very substantially. The majority (73% to 86%) of segments initially displaying compensatory remodeling remained with that remodeling pattern over time. A large proportion (26% to 56%) of initially excessively expansive remodeled segments remained with the same remodeling pattern, although a substantial proportion (40% to 74%) evolved toward a more compensatory pattern. The majority (75% to 85%) of constrictively remodeled segments at each time point developed compensatory remodeling over time, and only a small minority (14% to 22%) remained constrictive or developed constriction by 36 weeks. Individual remodeling trajectories of segments that culminated in compensatory, excessive expansive, or constrictive remodeling are detailed in Figure 3C, 3D, and 3E, respectively.

We assessed the progression of plaque burden in segments that developed each remodeling pattern at week 36. Lesions culminating in constrictive remodeling had on average a plaque burden of 62±6.1% at week 36, whereas the plaque burden at week 36 for segments with compensatory or excessive expansive remodeling at week 36 was 48.3±5.2% and 41.8±4.4%, respectively (Figure II in the online-only Data Supplement). These results indicate the highly dynamic nature of the remodeling response to individual lesions and the relation between the gradually increasing plaque burden and the ultimate pattern of the remodeling response of the wall to advanced plaque.

Association of Vascular Remodeling With Local ESS
We evaluated the ESS environment that preceded each remodeling phenotype and the effect of remodeling on local ESS for all intervals between each 2 consecutive time points. At each time point, excessive expansive remodeling developed in regions of lower ESS at the immediately preceding time point compared with segments that developed compensatory or constrictive remodeling (Figure 4A).

ESS generally decreased in segments with excessive expansive remodeling compared with each preceding time point. In segments in which plaque progression resulted in compensatory or constrictive remodeling, however, ESS generally tended to increase in relation to the preceding ESS (Figure 4B).
We further assessed the impact of each remodeling pattern on the rate of subsequent plaque progression. Plaques with excessive expansive remodeling at any time point throughout their evolution tended to have more marked subsequent progression, as assessed by $\Delta PV$ (Figure 5). Plaque progression tended to be more moderate in segments with compensatory remodeling and even more attenuated in constrictive lesions, particularly at later time points.

Identification and Prediction of High-Risk Plaque Based on Low ESS, Plaque Severity, and Excessive Expansive Remodeling

We focused on the subpopulation of severe plaques with maxIMT $\geq 1.0$ mm at week 36 ($n=113$ of 304, 37.2%). Shear stress in severe plaques exhibited significant heterogeneity at week 36, ranging from very low to physiological to high (Figure 6A). Only a minority of these severe plaques (12 of 113, 10.6% of severe plaques, 3.9% of all segments) displayed excessive expansive remodeling; these plaques likely represent the highest-risk lesions in that the pathophysiological stimulus for ongoing severe inflammation and internal elastic laminae degradation remained prominent. These excessively remodeled, high-risk severe lesions had on average lower ESS (Figure 6B) and larger plaque volume compared with severe plaques with compensatory or constrictive remodeling ($40.1\pm 3.5$ versus $29.9\pm 2.9$ versus $24.2\pm 2.3$ mm$^3$; $P=0.002$ and $P<0.001$, respectively) at week 36.

Furthermore, these high-risk lesions at week 36 had been more frequently exposed to low ESS (Figure 7A) and exhibited greater plaque severity (Figure 7B and 7C) at all preceding time points compared with all other segments throughout their evolution. These high-risk plaques also had a higher frequency of excessive expansive remodeling over time compared with all other segments that developed significant plaque (maxIMT $\geq 0.5$ mm) by week 36 (Figure 7D).

**Prediction of High-Risk Plaque Based on the Combined Assessment of Preceding ESS, Plaque Severity, and Vascular Remodeling**

We investigated the prognostic value of the combined assessment of ESS, plaque severity, and remodeling pattern at preceding points for the early identification of high-risk plaque at the final follow-up. Segments that culminated in high-risk plaque at week 36, as defined above ($n=12$), had higher values of the risk score at preceding weeks 11, 16, and 23 compared with all other segments that developed significant plaque (maxIMT $\geq 0.5$ mm) by week 36 (Figure 8). On multivariable analysis, the risk score at week 16 (odds


Discussion

The present study systematically explored in vivo the natural history of coronary atherosclerosis and arterial remodeling, as assessed by vascular profiling, in an animal model that develops human-like focal advanced lesions. Our major finding is that a complex interplay among plaque progression, wall remodeling response, and corresponding changes in local ESS synergistically determined the future vascular behavior of developing lesions. Low ESS was associated with the early initiation and ongoing progression of focal plaque. Vascular remodeling was a highly dynamic, temporally changing response to local plaque formation. Regions of very low ESS resulted in plaques with excessive expansive remodeling, which further exacerbated the low-ESS environment and augmented subsequent plaque progression. Conversely, lesions with compensatory or constrictive remodeling were characterized by amelioration of the adverse low-ESS stimulus and a trend toward less marked further growth. A small subpopulation of coronary segments was exposed to a progressively worsening stimulus of low ESS throughout their evolution and ultimately evolved to presumed highest-risk plaques. These high-risk lesions could be identified in vivo at earlier stages of their natural history by the combined assessment of local ESS, plaque thickness, and vascular remodeling.

Different Patterns of Plaque Progression

We observed that plaques developed focally, independently of each other, and displayed remarkable heterogeneity at all time points. Specific coronary regions remained spared of atherosclerosis, whereas other regions progressively developed variable disease manifestations despite the exposure to comparable systemic atherogenic risk factors. Segments that evolved toward the most severe plaques were persistently characterized by more advanced severity and more accelerated plaque progression throughout their natural history compared with segments culminating in less severe or no plaque. Assessment of plaque distribution and severity within the coronary vasculature at any time point may thus enable the prediction of regions in which worsening subsequent plaque progression is more likely to occur.

Natural History of Remodeling

Our results show remarkable heterogeneity of the remodeling response at each time point, ranging from constrictive to...
compensatory expansive to excessive expansive. Notably, individual plaques also evolved through a variety of remodeling patterns throughout their natural history, thus exhibiting a heterogeneous course, analogous to the dynamic nature of plaque progression itself. Although serial human IVUS studies have previously reported that the remodeling pattern of a lesion tends to remain relatively constant over time,5 these studies used only 2 time points of investigation, whereas our study used 5 time points. Compensatory expansive remodeling was the predominant vascular response at all time points, and the majority of segments with compensatory remodeling remained in the same pattern at any subsequent stage. However, remarkable changes in remodeling patterns were observed during individual plaque progression, with the most common transitions occurring between compensatory and either excessive expansive or constrictive remodeling. Overall, our findings indicate that the extent of vessel expansion or the development of constriction and thereby the status of relative plaque stability or instability9,10,15,16 may change in response to plaque growth. Although remodeling is influenced by genetics,19 the arterial wall dynamically responds to the progressing plaque and the accordingly changing local hemodynamic environment.

### ESS and Vascular Remodeling Determine the Natural History of Individual Plaques

We serially investigated in vivo, for the first time, coronary arteries initially without plaque that progressively became severely atherosclerotic. We found that in the setting of systemic atherogenic risk factors, low ESS, as determined by native arterial geometry, induces the initiation of atherosclerosis in originally plaque-free arterial regions; furthermore, low ESS occurring in the setting of plaque-induced changes in the local hemodynamic environment also exacerbates additional growth in regions that already contain significant plaque. Low ESS elicits a molecular and cellular proatherogenic phenotype in intact endothelial cells and continues to exert a proatherogenic effect at more advanced stages of atherosclerosis.1 The proatherogenic role of low ESS has been evaluated extensively in vitro and ex vivo20,21 and validated in vivo in either plaque-free animal3,8 or minimally diseased human arteries.4,12,22 In keeping with the notion that the proatherogenic endothelial phenotype is reversible on restoration of atheroprotective flow conditions,23 our results indicate that local low ESS is critical in persistently promoting coronary plaque growth in the continuum of atherogenesis and atherosclerotic progression.

The nature of remodeling is a recognized determinant of plaque stability. Expansive remodeling is related to plaque vulnerability9,16 and unstable clinical presentation10,15 whereas constrictive remodeling is considered to represent fibrocalcific, stable plaques.6,9,16 A novel finding of the present study is that the nature of remodeling is also related to subsequent plaque progression. Excessive expansive remodeling is associated with the most marked subsequent plaque progression, whereas constrictive remodeling attenuates further growth at all time points. To the best of our

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**Figure 5.** A, Plaque progression assessed by ΔPV between consecutive time points in relation to the baseline remodeling pattern. B, Plaque progression (ΔPV) for segments with each baseline remodeling pattern plotted against baseline ESS for time intervals of weeks 11 to 16, 16 to 23, and 23 to 36. Horizontal and vertical lines represent the SEM of ESS and ΔPV, respectively. Excess indicates excessive expansive; Comp, compensatory; and Constrict, constrictive.

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**Figure 6.** A, Frequency distribution of ESS in segments with severe plaque at week 36 defined as maxIMT >1.0 mm by IVUS. B, ESS at week 36 in segments with severe plaque and excessive expansive (Excess), compensatory (Comp), or constrictive (Constrict) remodeling at week 36.
knowledge, only 1 serial IVUS study has directly investigated the effect of remodeling on plaque progression. It found that baseline remodeling does not predict the rate of subsequent plaque growth. The seeming discrepancy with our results could be attributed to the use of only 2 time points of investigation in the previous study, differences in the definition and frequency of each remodeling pattern, and the use of statins, which are recognized regulators of plaque development and remodeling.

The arterial wall remodeling response to plaque development is clearly a critical determinant of subsequent local ESS and consequently the magnitude of the ongoing atherogenic stimulus and the natural history of the individual lesion. One can conceptually speculate that in a susceptible arterial region and with the synergistic effect of systemic risk factors, naturally occurring low ESS leads to local plaque formation essentially as an adaptive endothelial response to the detrimental local ESS conditions in an effort to increase local ESS and thereby restore a more vasculoprotective environment. If the plaque encroaches on the lumen and thus increases local ESS, compensatory expansive remodeling response of the arterial wall may again normalize the low-ESS stimulus and attenuate further plaque progression. If plaque forms in an extremely low-ESS environment, the wall response may not be compensatory expansive remodeling but excessive expansive remodeling instead as a result of more intense local inflammation and disruption of the internal elastic laminae. This focal area of intense inflammation in turn further exacerbates the proatherogenic low-ESS stimulus and establishes a vicious cycle promoting accelerated plaque growth and ultimate creation of high-risk plaque.

Constrictive vascular remodeling develops in regions of higher ESS and further increases the local ESS, thereby ameliorating the proatherogenic stimulus and attenuating the rate of additional plaque growth. The development of constrictive remodeling may not be causally related to the higher levels of ESS per se but rather to the absence of a proinflammatory low-ESS environment that would promote local inflammation, extracellular matrix degradation, and vessel expansion. In this local hemodynamic setting, constrictive remodeling may either occur persistently throughout the natural course of a developing lesion or represent a late-stage phenomenon (Figure 3E). In either case, and relevant to the human autopsy findings first described by Glagov et al, our serial IVUS results demonstrate in a prospective manner that constrictive remodeling eventually developed in advanced lesions with an average plaque burden exceeding the 50% value, which in our experimental model of atherosclerosis...
may represent the threshold of the capacity of the wall to further expand and accommodate the growing plaque (Figure II in the online-only Data Supplement).

In Vivo Assessment of Low ESS and Vascular Remodeling for the Identification and Prediction of High-Risk Plaques

Although low ESS is a critical pathophysiological factor leading to localized plaque initiation and progression to severe, highly inflamed lesions, the local ESS environment within which each severe plaque is located is remarkably heterogeneous. The vast majority of severe plaques reside in a physiological ESS environment, likely related to the previous remodeling response of the artery to normalize ESS. Only a minority of severe plaques are in either a low- or a high-ESS milieu. High-risk severe plaques with excessive expansive remodeling had been exposed to a persistently low local ESS throughout their natural history and continued to exhibit the lowest ESS values at an advanced stage of their evolution. The magnitude of low ESS is dose dependently related to the intensity of local inflammation and the severity of high-risk plaque characteristics; thus, very low ESS in advanced lesions may represent an ongoing stimulus for more marked vessel expansion, worsening local inflammation, and increased proclivity to rupture.

Our results indicate that the value of vascular profiling as a prognostic tool to risk-stratify early individual plaques lies in the persistence of an adverse local hemodynamic milieu in a small subpopulation of developing lesions. The integration of local ESS, plaque severity, and the nature of vascular remodeling into 1 predictive risk score enabled the early identification of regions that subsequently evolved toward remodeling had been exposed to a persistently low local ESS and continued to exhibit the lowest ESS values at an advanced stage of their evolution. The magnitude of low ESS is dose dependently related to the intensity of local inflammation and the severity of high-risk plaque characteristics; thus, very low ESS in advanced lesions may represent an ongoing stimulus for more marked vessel expansion, worsening local inflammation, and increased proclivity to rupture.

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Study Limitations

The small population size is acknowledged as a limitation. The power of the study increased, however, by profiling the entire length of 15 arteries divided into 304 segments at 5 consecutive time points.

The direct extrapolation of our results to humans may be limited because of the severe diabetic, hyperlipidemic conditions in our model. The combined risk score was developed through the use of an arbitrary grading system and was not applied in an independent validation sample. The risk score aims to underscore the synergistic effect of local ESS, plaque severity, and the remodeling response of the wall as a critical determinant of the natural history of individual lesions in our experimental model. The applicability of this score has not been tested in humans and remains to be determined in adequately powered clinical trials, which are currently underway.

ESS was averaged within a 3-mm-long arterial segment. However, by dividing the arteries into relatively short segments that exhibit homogeneity of ESS along their length, we eliminated the possible error of averaging.

The assumption of steady-state coronary blood flow ignored phasic phenomena, but as we previously demonstrated, using the average flow in steady-flow calculations yields essentially the same values of ESS as calculating the average ESS from the phasic solution. The errors produced by the assumptions of newtonian viscosity and rigid arterial walls were insignificant in the flow ranges observed in our study.

Although ESS is a continuous variable, analyses were performed by dichotomizing ESS as a categorical variable. Changes in ESS over time in relation to the remodeling response could be related in part to regression to the mean.

Conclusions

The present study provides insight into the natural history of coronary atherosclerosis, focusing on the mechanistic role of ESS. Atherosclerosis-prone segments exposed to low ESS are the regions in which plaque forms and progressively evolves. The remodeling response to local plaque formation may change over time, is determined by the preceding ESS milieu, and in turn dictates the subsequent progression rate of the individual lesion. Plaques with excessive expansive remodeling develop in regions of very low ESS, further augment the low-ESS stimulus, and are characterized by more marked additional growth. In vivo combined assessment of local ESS, plaque severity, and vascular remodeling may enable the early identification of advanced plaques with the most intense ongoing proinflammatory stimulus and thereby the highest likelihood of rupture.

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Disclosures

None.

References

Knowledge of the natural history of atherosclerosis and of the determinants of heterogeneous atherosclerotic manifestations is a precondition for the early identification of coronary regions most likely to evolve to culprit lesions of acute coronary events. This experimental study indicates that the in vivo combined assessment of local low endothelial shear stress, excessive expansive remodeling, and advanced plaque severity at earlier stages of the disease course may enable the prediction of lesions most likely to possess or to acquire a high-risk phenotype. Assessment of local hemodynamic and arterial wall characteristics might thereby be used as a clinical tool for individual plaque risk stratification and prognostication. Percutaneous coronary interventions are currently performed either in severely obstructive plaques causing myocardial ischemia or in culprit lesions of acute coronary syndromes, the majority of which are minimally obstructive. Early in vivo identification of high-risk lesions before they actually become prone to rupture may guide the appropriate application of a focused systemic treatment or highly selective local prophylactic interventions to avert the thrombotic complications of coronary plaque rupture. A large-scale, human natural history study currently underway (the PREDICTION Trial) may confirm the applicability of evaluating shear stress and arterial wall morphology to predict the development of lesions that cause new coronary events.
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SUPPLEMENTAL MATERIAL

Supplemental Methods

Animals

The experimental protocol was approved by the Harvard’s Institutional Animal Care and Use Committee and conforms to the Guide for the Care and Use of Laboratory Animals. Five male Yorkshire swine, age 12-14 week, initially weighing 18.9±1.0 kg, were sedated with Telazol (10 mg/kg) and injected via ear vein with filter-sterilized b-cell cytotoxin streptozotocin (50 mg/kg in 0.1 mol/l Na-citrate, pH 4.5) daily for 3 days to induce diabetes. Animals were subsequently given 25 g glucose twice daily at feeding for 2 days to offset insulin release from b-cells. Animals were maintained without exogenous insulin throughout the study protocol. Starting at the time of diabetes induction, the animals were fed a diet containing 1.5% cholesterol and 15% lard supplemented with sucrose, as previously described, in quantities titrated to maintain serum total cholesterol (TC) and blood glucose (BG) levels between 500-800 mg/dl and 150-350 mg/dl, respectively, while allowing steady weight gain. If the serum cholesterol level exceeded 800 mg/dl the diet was adjusted by adding normal pig chow to the 1.5% cholesterol diet.

Animal weight was monitored every month, as well on the day of each catheterization procedure. TC and BG were monitored daily for 2 weeks, then weekly thereafter, following overnight (≥12 hours) fasting. Standard enzymatic assay kits were used for TC and BG measurement (Sigma, St. Louis, MO and Thermo Scientific, Waltham, MA, respectively). BG in particular was checked daily in cases in which the glucose level was >350 mg/dl. Blood (500 µl)
for all measurements was obtained without sedation by pricking an ear vein, cleaned with Nolvosan, with a lancet and collecting drops in a hematocrit tube.

**Study protocol**

All pigs underwent serial coronary angiography and IVUS, as described below, in all the major epicardial coronary arteries (left anterior descending, left circumflex, and right coronary artery) at five consecutive time points *in vivo*: 4, 11, 16, 23 and 36 weeks after the induction of diabetes and initiation of a high-fat diet (Figure 1S).

**Serial cardiac catheterizations**

Animals were fasted overnight before each catheterization procedure. Pre-procedural evaluation included physical examination and blood glucose measurement. Animals were at a surgical plane of anesthesia prior to each catheterization procedure, and remained under anesthesia for the entire procedure, as well as throughout euthanasia following the final procedure at week 36. The anesthesia protocol consisted of the combination of tiletamine with zolazepam (4.4 mg/kg, Telazol, Wyeth), Xylazine (2.2 mg/kg, Rompun, Bayer), and Atropine (0.05 mg/kg). After endotracheal intubation with 7-7.5mm sized tubes, the animals were ventilated with isoflurane (0.1% to 5.0%) and oxygen for maintenance of anesthesia throughout the procedure. Monitoring during each procedure included continuous electrocardiogram, arterial blood pressure and $\text{O}_2$ saturation ($\text{SaO}_2$) monitoring.

Access site for vascular access was the femoral groin area. The skin at the access site was aseptically prepared with povidone iodine (Betadine) solution. All incisions were made using a muscle-sparing technique and were kept as small as possible (~2.5 cm in length) in order to minimize injury, facilitate fast healing and minimize post-operative pain. After the incision and the arteriotomy, an appropriate sized introducer sheath was placed in the artery. The sheath was
removed immediately after completion of the procedure. All incisions were closed with a 3-layer closure of vicryl. Animals were monitored for post-operative pain and given analgesics (Buprenorphine, Buprenex, Reckitt & Colman, 0.05 mg/kg IM / BID for 24 hours, appropriately adjusted thereafter).

**Vascular profiling for ESS calculation**

Intracoronary vascular profiling methodology has been previously described and validated in-vivo. In brief, the 3D anatomy of the coronary artery was reconstructed from IVUS images and biplane coronary angiography. IVUS (ClearView, Boston Scientific, Natick, MA) was performed with automated pullback at 0.5 mm/sec. The arterial lumen and external elastic membrane (EEM) were segmented from digitized end-diastolic IVUS images. The physical 3D path of the IVUS transducer during pullback was reconstructed using the corresponding biplane angiographic projections, and the segmented IVUS images were located along this path and oriented appropriately. Lumen and EEM boundary points were connected by spline curves to rebuild the lumen and EEM geometry in 3D space, respectively. A structured grid was employed to represent the lumen volume. Coronary blood flow for the reconstructed arterial segment was calculated directly from the time required for opacified blood to fill a known volume of coronary artery during a contrast injection. Blood was considered as Newtonian fluid and its viscosity was estimated using the hematocrit and serum TC. Detailed intravascular flow characteristics were obtained by computational fluid dynamics solving the transport equations governing the conservation of mass and momentum (PHOENICS, Cham Ltd, London, UK). The governing equations of blood flow were determined assuming that the arterial wall is stiff, blood is incompressible, and coronary blood flow is steady with uniform inlet flow velocity. ESS at the
lumen surface of the geometrically correct 3D reconstructed artery was calculated at all time points as the product of viscosity and the gradient of blood velocity at the wall.4

Each 3D reconstructed artery was divided at week 36 into 3mm-long segments along its entire length. To locate the segments in IVUS investigations at multiple time points, baseline and follow-up reconstructed arteries were matched using IVUS-derived anatomical landmarks (i.e. side branches, veins, calcified areas) for accurate comparison of the same segments over time.3,4,8 Mean ESS was calculated in each 3D segment at all time points.

Assessment of vascular remodeling

The nature of the remodeling response to plaque growth was assessed over time in each arterial segment that contained significant plaque at final week 36, defined as maxIMT≥0.5mm by IVUS. Remodeling was assessed in these segments at each time point by comparing the local remodeling behavior of each individual segment with the global remodeling response of the entire artery, as previously described.2,9 Briefly, the EEM areas of all the IVUS cross-sections along each reconstructed artery were measured at each time point and plotted against the corresponding intima-media areas. The global reference of the entire reconstructed artery at each time point was determined by the linear regression line and its 90% prediction band in the EEM area vs. intima-media area plot. The EEM and intima-media area of each individual segment were then identified within the corresponding plot, and three local remodeling patterns were defined: (a) excessive expansive remodeling if the EEM area of the segment was above the upper limit of the 90% prediction band of the entire artery remodeling behavior, (b) compensatory expansive remodeling if the EEM area of the segment was within the 90% prediction band, and (c) constrictive remodeling if the EEM area of the segment was below the lower limit of the 90% prediction band.
Supplemental Results

To assess the lifetime exposure of pigs to hyperlipidemia and hyperglycemia, the time-average of TC and BG was calculated for all measurements once the pigs were rendered diabetic and started on the high-fat diet. The time-averaged TC and BG during the 36-week study duration were 728±161 mg/dl and 265±53 mg/dl, respectively.

The mean body weight at study entry was 18.9±1.0 kg (range: 17.9-20.2kg). At the end of the study the body weight increased to 49.9±2.9 kg (range: 46.1-53.3 kg), representing a 164±16.9% increase (Table 1S). Animal body weight and the rate of body weight gain did not exhibit significant differences among the animals throughout the study protocol. The most marked body weight gain, quantified as absolute increase of body weight (21.0±2.6 kg), percent rate of weight gain (73.0±9.9%), and weight gain rate (1.6±0.2 kg/week), was observed during the last of the four intervals of the study period, i.e. between weeks 23 and 36.
Study Limitations

We acknowledge some assumptions we made concerning blood flow. Instead of using intracoronary Doppler flow-wire, the coronary flow was measured by applying a previously published methodology based on the fundamental definition of flow rate, i.e. the time required for opacified blood to fill a known volume of coronary artery. We determined the true volume of the arterial segment under study and measured the time required to fill that volume by tracking the wave front of contrast medium through it. Utilizing our technique for flow measurement we were able to minimize the use of additional catheters, thereby minimizing the risk of complications, such as vascular injury that could affect the progression of native atherosclerotic disease. The assumption of steady-state coronary blood flow ignored phasic phenomena, but, as we previously demonstrated, using the average flow in steady flow calculations yields essentially the same values of ESS as calculating the average ESS from the phasic solution. The errors produced by the assumptions of Newtonian viscosity and rigid arterial walls were insignificant in the flow ranges observed in the current study. At the Reynolds numbers observed in this study, the distortions introduced by the assumption of uniform inlet velocity were also insignificant for inlet diameters above 1 mm\(^3\).

Our model did not enable us to study pathophysiologic mechanisms implicated in abrupt plaque progression, including healing of subclinical plaque rupture. However, we have previously shown no histological evidence of plaque rupture using the same experimental model in a similar time frame of investigation. We may therefore conclude that plaque progression assessed by IVUS represents increase of plaque volume itself.
Supplemental Tables

**Table 1S.** Progression of individual animal body weight over time. No significant differences were observed among animals at any time point.

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<thead>
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<th>Animal</th>
<th>Total Body Weight (kg)</th>
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<td>18.3</td>
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Supplemental Figure Legends

**Figure 1S.** Schematic presentation of the study protocol.

**Figure 2S.** Evolution of plaque burden in segments that culminated in compensatory (Comp.), excessive expansive (Excess), and constrictive remodeling (Constrict.) at week 36. Plaques that culminated in constrictive remodeling had on average plaque burden >50% at week 36, as opposed to plaques that culminated in compensatory, or excessive expansive remodeling. Values are presented as mean ± SEM.
Supplemental Figures

Figure 1S.

<table>
<thead>
<tr>
<th>Week 0</th>
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<th>Week 16</th>
<th>Week 23</th>
<th>Week 36</th>
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<td>5 male Yorkshire pigs</td>
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↑ Induction of diabetes / initiation of high-fat diet

↑ In vivo vascular profiling (IVUS / Coronary angiography)
Figure 2S.
Supplemental References


