

Prediction of the Localization of High-Risk Coronary Atherosclerotic Plaques on the Basis of Low Endothelial Shear Stress

An Intravascular Ultrasound and Histopathology Natural History Study

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Background—Low endothelial shear stress (ESS) promotes the development of atherosclerosis; however, its role in the progression of atherosclerotic plaques and evolution to inflamed high-risk plaques has not been studied. Our hypothesis was that the lowest values of ESS are responsible for the development of high-risk coronary atherosclerotic plaques associated with excessive expansive remodeling.

Methods and Results—Twenty-four swine, treated with streptozotocin to induce diabetes and fed a high-fat diet, were allocated into early (n=12) and late (n=12) atherosclerosis groups. Intima-media thickness was assessed by intravascular ultrasound in the coronary arteries at weeks 4 and 8 in the early group and weeks 23 and 30 in the late group. Plaques started to develop after week 8, leading to marked heterogeneity in plaque severity at week 30. ESS was calculated in plaque-free subsegments of interest (n=142) in the late group at week 23. Coronary arteries (n=31) of this group were harvested at week 30, and the subsegments of interest were identified and analyzed histopathologically. Low ESS was an independent predictor of the development of high-risk plaques, characterized by intense lipid accumulation, inflammation, thin fibrous cap, severe internal elastic lamina degradation, and excessive expansive remodeling. The severity of high-risk plaque characteristics at week 30 was significantly correlated with the magnitude of low ESS at week 23.

Conclusions—The magnitude of low ESS determines the complexity and heterogeneity of atherosclerotic lesions and predicts the development of high-risk plaque. (*Circulation*. 2008;117:993-1002.)

Key Words: coronary atherosclerosis ■ plaque ■ inflammation ■ remodeling ■ shear stress

Although the entire vasculature is exposed to the atherogenic effect of systemic risk factors, atherosclerotic lesions form at specific regions of the arterial tree where there is disturbed flow.¹ Local hemodynamic factors, in particular low endothelial shear stress (ESS), play a major role in the regional localization of atherosclerosis.¹⁻⁷ Low ESS modulates endothelial gene expression through complex mechanotransduction processes, inducing an atherogenic endothelial phenotype that promotes lipid accumulation and oxidation, inflammatory cell infiltration, smooth muscle cell proliferation, and extracellular matrix produc-

tion, ultimately leading to the formation of early atherosclerotic plaques.¹ Each of these early plaques subsequently exhibits an individual natural history: A portion of them may evolve to high-risk plaques responsible for acute coronary syndromes, some may remain quiescent, and others may become stenotic, leading to stable angina.¹ Although the detailed pathophysiological triggers responsible for the individual natural history trajectory of each atherosclerotic plaque are unknown, the local, dynamic interplay between low ESS and atherosclerosis progression is likely to be critical. The first aim of this study was to test

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the hypothesis that arterial subsegments with the lowest values of ESS are those regions where high-risk plaques with large necrotic lipid core, intensive inflammation, and thin fibrous cap will develop.

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Although several arterial regions along the coronary arteries are characterized by low ESS, the lesions that develop in these areas are quite heterogeneous.¹ Prior *in vitro* and *in vivo* human studies suggested that the proatherogenic endothelial response and the magnitude of atherosclerosis progression are associated with the lowest levels of ESS.^{3,4} However, direct data to confirm the “dose-response” relationship between the magnitude of low ESS and the severity of atherosclerosis do not exist. Our second aim was to use histopathology to evaluate whether the severity of high-risk characteristics is associated with the magnitude of low ESS.

Many histopathology and intravascular ultrasound (IVUS) studies have confirmed that expansive remodeling is associated with high-risk plaques and acute coronary syndromes, whereas constrictive remodeling is associated with fibrous plaques and stable angina.⁸ Compensatory expansive remodeling is well understood to preserve the lumen in response to plaque growth,⁹ but the pathobiological mechanisms responsible for excessive expansive remodeling, an exaggerated form of compensatory expansive remodeling in which both the vessel and lumen size increase,¹ have not been investigated. The dynamic interplay between the local hemodynamic milieu and the biology of the arterial wall is likely to be important.¹ The third aim of the present study was to evaluate the hypothesis that arterial subsegments with the lowest values of ESS are those regions where excessive expansive remodeling occurs, thereby perpetuating or even exacerbating the local low-ESS environment.

Methods

A detailed description of the methods is presented in the online-only Data Supplement. Briefly, 24 male Yorkshire swine were rendered diabetic and fed a high-fat diet supplemented with sucrose in quantities titrated to maintain serum total cholesterol and blood glucose levels from 500 to 700 mg/dL and 150 to 350 mg/dL, respectively.¹⁰ The pigs were allocated into early (n=12) and late (n=12) atherosclerosis groups. Intracoronary vascular profiling (ie, IVUS and angiography)⁴ was performed for assessment of local ESS along the lumen surface of 3-dimensional-reconstructed major coronary arteries at weeks 4 and 8 for the early group and weeks 23 and 30 for the late group (Figure 1). Progression of plaque burden and severity over time were estimated in the reconstructed arteries by IVUS.

To investigate the effect of ESS on lesion differentiation to high-risk plaques, we focused on the late group. In these arteries, ESS was calculated on the reconstructed lumen surface at baseline (week 23), and 3-mm-long subsegments of interest of different ESS magnitude were selected (Figure 2B). To be considered in the analysis, each subsegment of interest was required to be free of apparent atherosclerotic plaque at baseline, defined by IVUS as maximum intima-media thickness (IMT) ≤ 0.5 mm (Figure 2C). To account for the longitudinal histopathological heterogeneity of atherosclerotic plaques, (1) we analyzed only subsegments with uniform IMT (by IVUS) along their length at follow-up, and (2) we selected subsegments with the most severe appearance in IVUS when atherosclerotic plaques >3 mm long were present at follow-up (Figure 2C). We also required each subsegment of interest to have a surface area of similar ESS $\geq 30\%$ of the total surface area of the

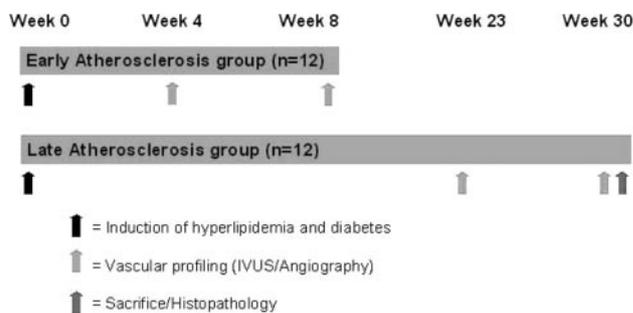


Figure 1. Schematic presentation of the study protocol.

subsegment.¹¹ To reduce the impact of local flow disturbances on our results, subsegments free of side branches and located at least 1 mm away from adjacent side branches were selected (Figure 2B).

Coronary arteries were harvested at follow-up (week 30), and the subsegments of interest were located. To locate the subsegments of interest, 2 to 3 readily visible side branches were identified on the baseline and follow-up IVUS pullbacks, as well as on the ESS and IMT 2-dimensional maps (Figure 2). The same side branches were also identified on the harvested arteries with the use of magnifying lenses ($\times 2$). With the use of these branches as landmarks, the exact location of each subsegment of interest was then identified on the preserved coronary arteries. Subsegments of interest were frozen, cut at the middle, and cryosectioned. Verhoeff’s elastin, oil red O, and picrosirius red staining and CD45 immunostaining were performed in each cryosection for the assessment of intima-to-media ratio and maximum intimal thickness, lipid accumulation, collagen content, and inflammatory cell infiltration, respectively. To assess the impact of ESS on the heterogeneity of atherosclerotic lesions, the lesions were histopathologically classified into (1) minimal lesions, (2) intermediate lesions, and (3) fibroatheromas.^{12,13} Plaque vulnerability, indicated by minimum fibrous cap thickness (μm), was assessed and correlated with local baseline ESS. The integrity of the internal elastic lamina (IEL) was also assessed and correlated with local baseline ESS.

The nature of the arterial remodeling response to plaque growth (ie, change in plaque area by IVUS ≥ 0.2 mm²) in each subsegment of interest was estimated by comparing the local remodeling behavior of each subsegment with the global remodeling response of the entire reconstructed artery, as described previously.¹⁴ The remodeling behavior of each subsegment was correlated with the histopathology, as well as the baseline and follow-up ESS.

Statistical Analyses

All analyses were performed with SPSS 15.0 (SPSS Inc, Chicago, Ill) and Stata 10.0 (StataCorp LP, College Station, Tex). Continuous variables with normal distribution are summarized as mean \pm SEM, nonnormally distributed variables as median and 25th and 75th percentiles, and categorical variables as actual numbers and percentages. To correct for systematic error introduced by the clustering of arterial subsegments within animals, several statistical methods were used. First, to investigate the association of continuous variables (eg, histopathological characteristics) with categorical variables (eg, baseline ESS group, lesion category, remodeling pattern, IEL grade), mixed-effects ANOVA with the animal, artery, and subsegment of interest as random effects was used. Second, for analyses in which the dependent variable (eg, IMT, ESS) was measured at baseline and follow-up, repeated-measures ANOVA was employed, and the animal, artery, and subsegment of interest were specified as random effects. Third, for analyses with continuous independent (eg, baseline ESS) and dependent variables (eg, minimum fibrous cap thickness), linear regression was employed. Finally, in cases in which the dependent variable was categorical (eg, IEL grade, lesion category, remodeling pattern), ordered logistic regression was implemented. In linear regression and logistic regression analyses, the standard errors of the regression coefficient were adjusted for clustering of arterial subsegments within animals with the Huber

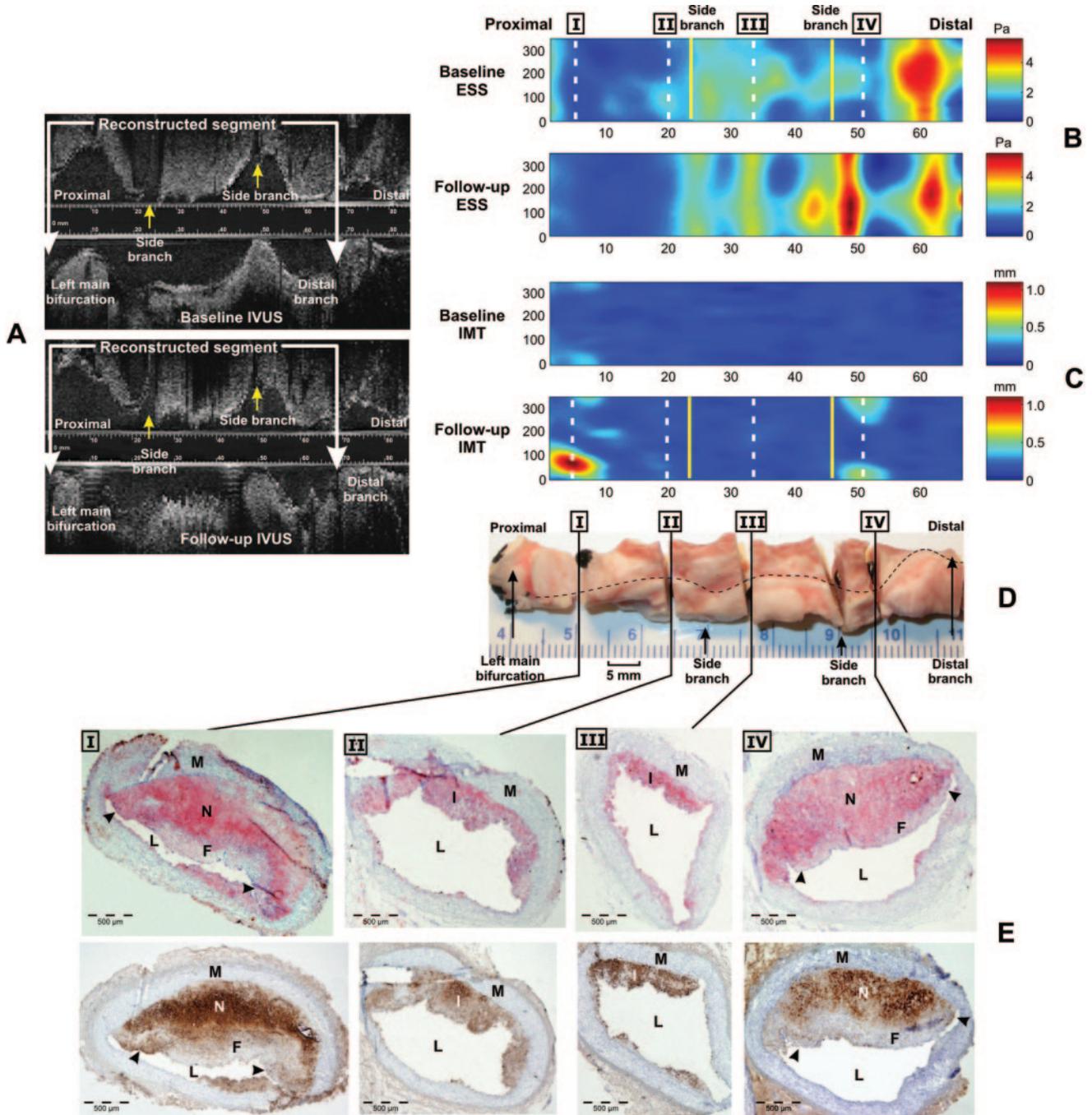


Figure 2. Representative case of a 3-dimensional-reconstructed and profiled left circumflex artery at baseline (week 23) and follow-up (week 30), matched with histopathology at follow-up. A, Longitudinal aspects of baseline and follow-up IVUS pullbacks appropriately matched with the use of major side branches as landmarks (yellow arrows). B and C, Two-dimensional maps showing the ESS (B) and IMT (C) distribution along the artery length at baseline and follow-up; in each map, the vertical axis denotes the artery circumference ($^{\circ}$) and the horizontal axis the artery length (mm). D, The reconstructed left circumflex artery was harvested and sectioned at follow-up (artery depicted with dashed black line). Two major side branches were located on the ESS and IMT maps at baseline and follow-up (solid yellow lines) and also identified on the harvested artery (black arrows). Four arterial subsegments 3 mm long without evidence of plaque on IMT maps were identified at the baseline corresponding to areas of low, moderate, and higher ESS (sections I to IV, depicted with dashed white lines on the ESS and IMT maps). The same sections were identified on the harvested artery with the side branches used as landmarks. Oil red O and CD45 staining were performed in sections derived from the middle of each subsegment (E). Sections I and IV revealed eccentric fibroatheromas with thin inflamed fibrous cap (black arrowheads) and large lipid core corresponding to subsegments with very low ESS at baseline (B; L indicates lumen; I, intima; M, media; F, fibrous cap; and N, necrotic core). Of note, ESS remained low at follow-up in these subsegments (B). Sections II and III revealed small intermediate lesions that developed in subsegments with higher baseline ESS (B). The actual lumen dimensions cannot be assessed in the photomicrographs because of the shrinkage associated with tissue preparation at -80°C .

White Sandwich Estimator. In all analyses, probability values were adjusted for multiple comparisons of data with the use of either the Scheffé or modified Bonferroni method. Findings were considered statistically significant at the 0.05 level.

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

Results

One pig in the early group and 1 pig in the late group died before the follow-up vascular profiling. In addition, 1 pig in the early group died before the follow-up vascular profiling of its left anterior descending artery, and 2 pigs in the late group died before the follow-up vascular profiling of their left anterior descending artery and right coronary artery, respectively. Therefore, 32 coronary arteries from 11 pigs were profiled in the early group (left anterior descending artery, n=10; left circumflex artery, n=10; obtuse marginal artery, n=1; and right coronary artery, n=11), and 31 coronary arteries from 11 pigs were profiled in the late group (left anterior descending artery, n=10; left circumflex artery, n=10; obtuse marginal artery, n=1; and right coronary artery, n=10).

The mean weight of pigs in the early and late groups on the day of euthanasia was 33±2 and 66±4 kg, respectively. The mean total cholesterol during the follow-up period was 454±42 for the early group and 611±28 mg/dL for the late group, whereas the mean blood glucose was 277±33 and 230±26 mg/dL, respectively.

Changes in Plaque Progression and Severity Over Time

Atherosclerotic burden, expressed by the intima-media volume normalized over the artery length, significantly increased after week 8 (Figure 3B). The maximum IMT by IVUS was classified into 4 grades (ie, grade 0, ≤0.50 mm; grade 1, 0.51 to 0.70 mm; grade 2, 0.71 to 1.00 mm; grade 3, >1.00 mm; Figure 3A), and the percent length of the artery occupied by each grade was measured. Plaques started to gradually progress to more advanced grades after week 8, leading to marked heterogeneity in plaque severity at week 30 (Figure 3C).

Histomorphometric and Histomorphological Differences Among Fibroatheromas and Intermediate and Minimal Lesions

To assess the role of low ESS in plaque development and composition, 142 subsegments free of apparent atherosclerosis by IVUS were selected at week 23, and these subsegments were sectioned and stained at week 30. Figure 2 depicts a representative case of a 3-dimensional-reconstructed left circumflex artery with baseline and follow-up IVUS pull-back, as well as ESS and IMT distribution along the artery length matched with histopathology at follow-up. Baseline ESS of the subsegments studied was 1.0 (0.7 to 1.4) Pa (limits, 0.0 to 3.2 Pa), whereas the intima-to-media ratio of atherosclerotic lesions that developed at follow-up was 0.50 (0.20 to 1.00).

There was marked heterogeneity of atherosclerotic lesions by histology at follow-up. On the basis of histomorphometric

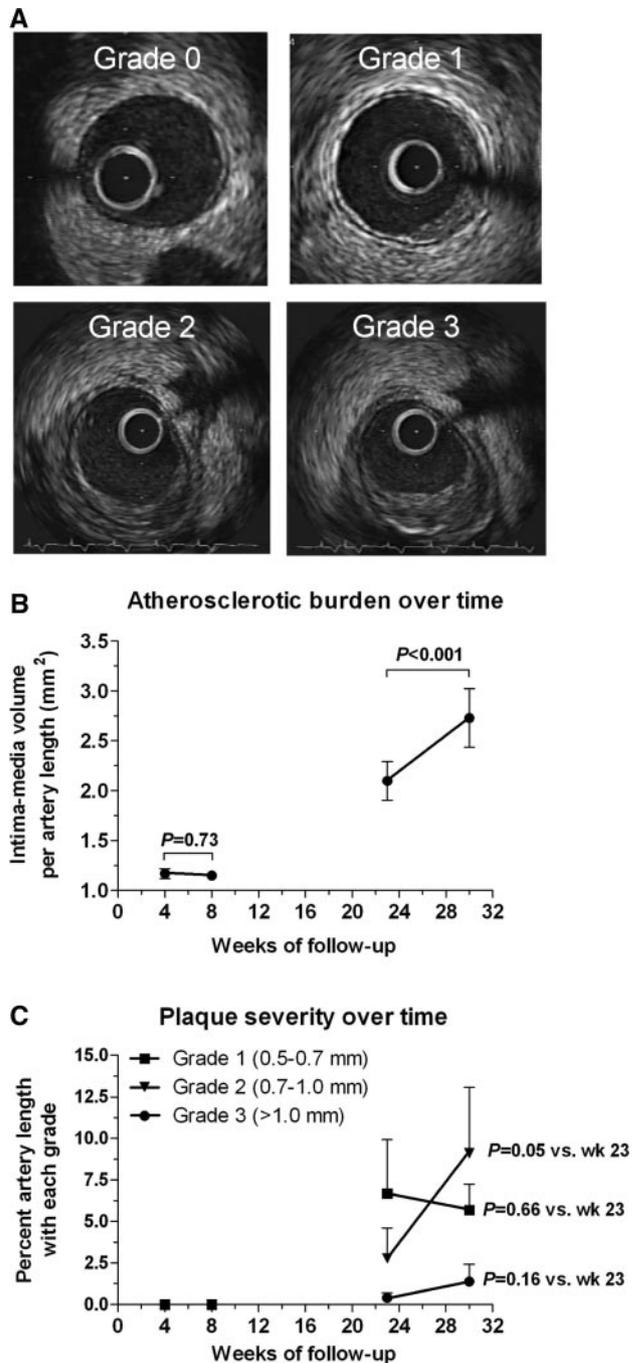


Figure 3. A, Atherosclerotic plaque grades by IVUS. B, Progression of atherosclerotic burden over time. C, Progression of each plaque grade over time. Data were derived from the early atherosclerosis group (32 arteries from 11 pigs; weeks 4 and 8) and the late atherosclerosis group (31 arteries from 11 pigs; weeks 23 and 30).

and histomorphological characteristics, lesions were classified into 3 categories representing distinct stages of the natural history of atherosclerosis: minimal lesions (n=26; 18.3%), intermediate lesions (n=56; 39.4%), and fibroatheromas (n=60; 42.3%) (Figure 4A to 4F). Fibroatheromas were significantly larger lesions compared with intermediate and minimal lesions, with increased lipids, inflammation, and

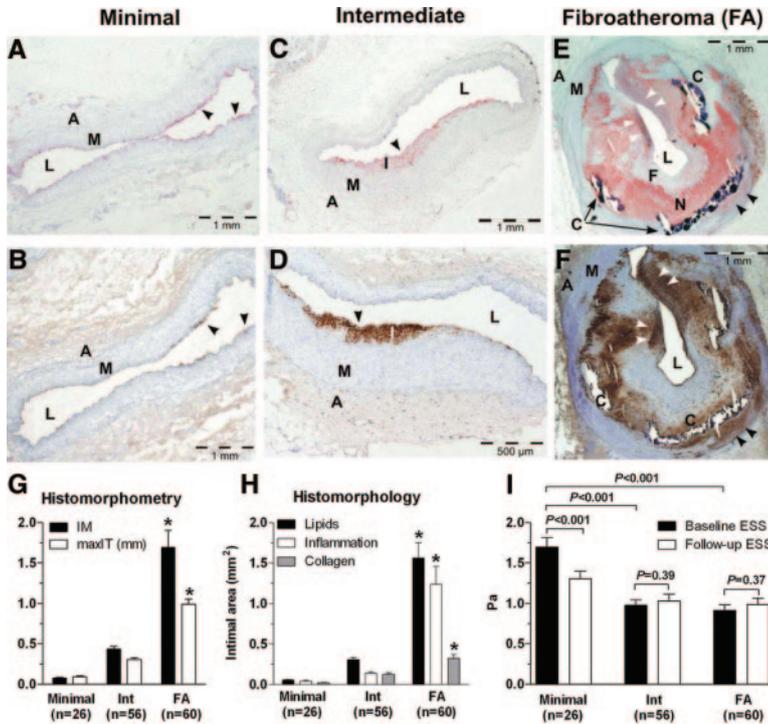


Figure 4. Representative digital photomicrographs of coronary atherosclerotic lesions that developed at week 30 (L indicates lumen; I, intima; M, media; and A, adventitia). Oil red O (A) and CD45 (B) staining of a minimal lesion (arrowheads). Oil red O (C) and CD45 (D) staining of an intermediate lesion (Int; arrowheads), ie, a small mass of lipid-laden inflammatory cells without evidence of fibrous cap is shown. E, Oil red O staining of a fibroatheroma (FA) reveals a humanlike high-risk atherosclerotic plaque with a thin fibrous cap (F; white arrowheads) overlying a large necrotic lipid core (N), extensive calcification (C) at the base of the plaque, and regional IEL degradation and media thinning beneath the plaque (black arrowheads). F, CD45 staining of the same plaque shows extensive inflammatory cell infiltration at the shoulders of the fibrous cap (white arrowheads), at the base of the plaque surrounding the calcified areas, and within the underlying media (black arrowheads). Differences in histomorphometry (G), histomorphology (H), and baseline and follow-up ESS (I) among minimal lesions, intermediate lesions, and fibroatheromas are shown. * $P < 0.001$ for fibroatheromas vs minimal and intermediate lesions.

collagen (Figure 4G and 4H). The absolute values of plaque constituents were markedly different across the plaque categories, whereas their relative content (ie, percentage of intima) was similar (Table I in the online-only Data Supplement). All the fibroatheromas had thin fibrous caps, with minimum fibrous cap thickness 9.0 (7.0 to 15.0) μm (limits, 4.0 to 50.0 μm).

Effect of Low ESS on Development of High-Risk Plaques

Both fibroatheromas and intermediate lesions developed in subsegments with significantly lower baseline ESS compared with minimal lesions, and the ESS remained low at these lesions at follow-up (Figures 2B and 4I). To adjust for the effect of total cholesterol and blood glucose, we applied ordered logistic regression using lesion category as the dependent variable, and we found that the lowest baseline ESS ($P=0.011$) and the increased total cholesterol ($P=0.005$) were predictors of the development of fibroatheromas versus intermediate or minimal lesions.

Association of Magnitude of Low ESS With Severity of High-Risk Characteristics

To determine the effect of magnitude of ESS on the severity of high-risk characteristics, baseline ESS was classified into 4 groups (ie, ≤ 0.50 , 0.51 to 1.00, 1.01 to 1.50, > 1.50 Pa) and correlated with histopathology at follow-up (Figure 5). Subsegments with very low ESS (≤ 0.50 Pa) developed significantly larger plaques with more lipids and inflammation compared with subsegments with moderate (1.01 to 1.50 Pa) or higher ESS (> 1.50 Pa), suggesting a dose-response effect of low ESS.

Association of Low ESS With Fibrous Cap Thinning

To investigate the association of baseline ESS with fibrous cap thickness of lesions at follow-up, minimum fibrous cap thickness was measured in all the lesions with evidence of fibrous cap, ie, fibroatheromas ($n=60$), and correlated with baseline ESS. All the fibrous caps were thin (minimum fibrous cap thickness $< 50 \mu\text{m}$) and highly inflamed, especially at the shoulders. There was a significant association between minimum fibrous cap thickness and the lowest ESS values (Figure 6).

Association of Low ESS With IEL Degradation

To assess the association of baseline ESS with IEL degradation in lesions that developed at follow-up, IEL integrity was classified into 4 categories (grade 0, intact,

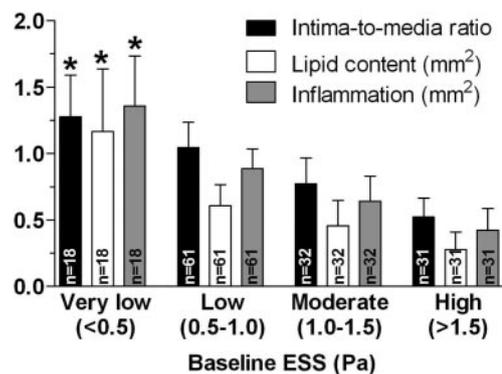


Figure 5. Association of ESS magnitude at baseline with the severity of high-risk plaque characteristics at follow-up. * $P < 0.05$ for each characteristic in very low ESS vs respective characteristic in moderate or high ESS; $P=0.13$ for inflammation in very low ESS vs low ESS.

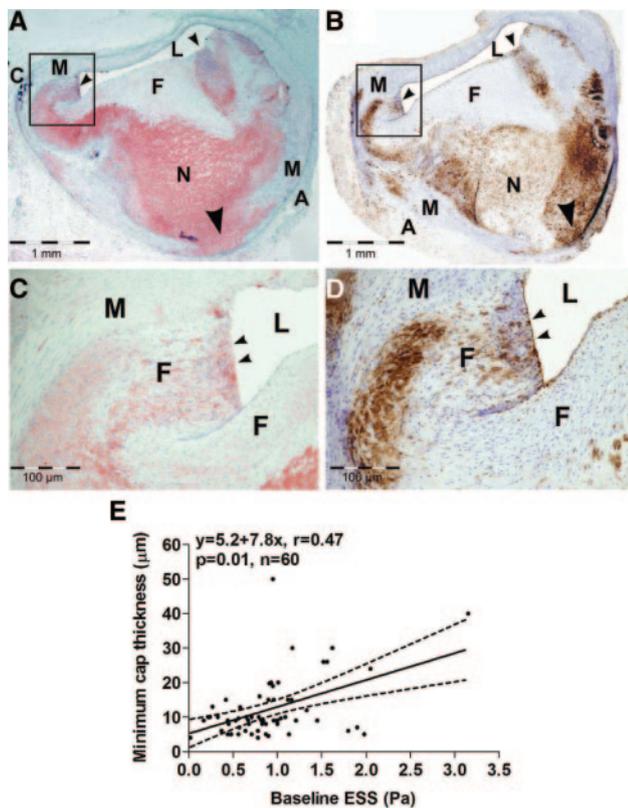


Figure 6. Digital photomicrographs of oil red O-stained (A and C) and CD45-stained (B and D) fibroatheroma with an inflamed thin fibrous cap at its shoulders (small black arrowheads). L indicates lumen; M, media; A, adventitia; F, fibrous cap; N, necrotic core; and C, calcification. C and D are magnifications of the black box in A and B, respectively. The necrotic core is extended into the media through the disrupted IEL (A and B; large black arrowheads). E, Association of minimum cap thickness with the magnitude of baseline ESS; dashed lines represent 95% CI for the regression line.

well-organized IEL; grade 1, IEL with a few breaks; grade 2, IEL with many breaks but intact media; grade 3, IEL with severe fragmentation associated with degradation of the underlying media; Figure 7).¹⁵ Severe IEL disintegration (grades 2 and 3) occurred in subsegments with the lowest baseline ESS values (<1 Pa), as did severe inflammation (Figure 7A), which was localized at the base of the plaques close to the IEL (Figure 4E and 4F). In contrast, no or minimal IEL degradation (grades 0 and 1) and limited inflammation were observed in subsegments with the highest ESS levels (>1.5 Pa).

Figure 7B displays the baseline ESS values and IEL grades associated with each plaque category. The great majority of fibroatheromas (n=54 of 60; 90%) and intermediate lesions (n=41 of 56; 73.2%) were associated with more intense degradation of IEL beneath the plaque (grade 2 or 3; $P<0.001$) and low ESS ($P<0.05$), whereas the great majority of minimal lesions (n=23 of 26; 88.5%) were associated with no or minimal IEL breaks (grade 0 or 1; $P<0.001$) and higher ESS ($P<0.05$). Of note, no grade 0 (intact IEL) occurred in fibroatheromas, whereas no grade 3 (severe IEL fragmentation) occurred in minimal lesions.

These data indicate that the severity of IEL degradation beneath the atherosclerotic intima is related to the magnitude of low baseline ESS and suggest that the magnitude of inflammation is the causative link.

Association of Low ESS With Excessive Expansive Remodeling

Plaque growth (ie, change in plaque area by IVUS ≥ 0.2 mm²) was observed in 102 of 142 subsegments (72%) of interest. Three local remodeling responses were identified in these subsegments, ie, excessive expansive (n=26; 22.5%), compensatory expansive (n=53; 52%), and inadequate remodeling (n=23; 25.5%), and were correlated with the histopathology, as well as the baseline and follow-up ESS.

Compared with subsegments with compensatory expansive remodeling, subsegments with excessive expansive remodeling contained larger plaques with more lipid deposition, inflammation, and collagen (Figure 8A); baseline ESS was not different between subsegments with excessive expansive and compensatory expansive remodeling (Figure 8B). Excessively remodeled subsegments were associated with lower baseline ESS and more inflammation than subsegments with inadequate remodeling, indicating that the lowest values of baseline ESS may promote wall expansion beneath the plaque through upregulation of local inflammation (Figure 8B).

Intriguingly, we observed that follow-up ESS tended to decrease by 14% ($P=0.10$) in subsegments with excessive remodeling despite the growth of local plaque (Figure 8C). This decrease in ESS at follow-up in excessively remodeled subsegments was in contrast to no significant change in follow-up ESS in subsegments with compensatory expansive remodeling (-7.6% ; $P=0.24$) and to an increase in the follow-up ESS of 23.5% in subsegments with inadequate remodeling (associated with a statistical trend, $P=0.11$).

Discussion

In the present study, we explored in detail the effect of local baseline ESS and systemic risk factors (hyperlipidemia, hyperglycemia) on the natural history of local coronary atherosclerotic plaque, as assessed by IVUS and histopathology. We observed after 30 weeks a broad spectrum of atherosclerotic lesions, from minimal lesion, to intermediate lesion, to inflamed thin cap fibroatheroma. All these lesions were highly focal and developed independently of each other, driven by both low ESS and systemic factors. We showed for the first time that low ESS is a critically important predictor of coronary plaque location, development, and progression to high-risk plaque and that there is a strong association between the magnitude of low ESS and the complexity of high-risk characteristics, ie, lipid accumulation, inflammation, and fibrous cap thinning. We also found that low ESS leads to severe IEL degradation beneath the plaque and eventually excessive expansive remodeling, thereby perpetuating the low-ESS environment and local plaque inflammation.

We focused on subsegments free of apparent atherosclerosis by IVUS at baseline because this allowed us to study the role of ESS in the initiation and progression of coronary atherosclerosis through multiple natural history stages culminating in severe high-risk plaque. We observed enormous

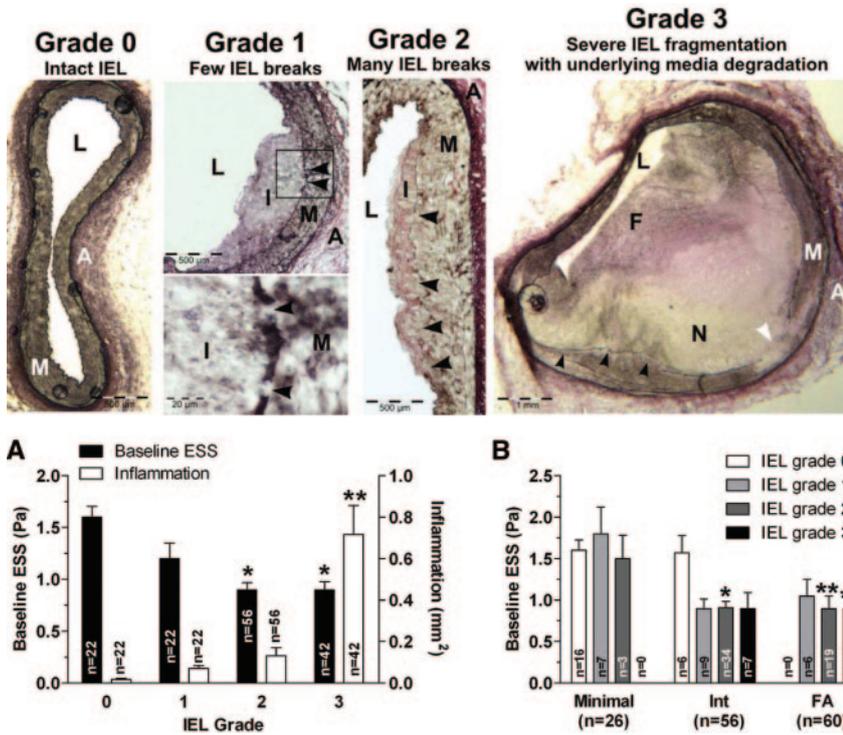


Figure 7. Digital photomicrographs of Verhoeff's elastin-stained sections showing the IEL grades. Black arrowheads denote the IEL breaks, and the white arrowhead in IEL grade 3 denotes the media degradation. L indicates lumen; I, intima; M, media; A, adventitia; F, fibrous cap; and N, necrotic core. A, Association of IEL grades with baseline ESS (left axis) and severity of inflammation (right axis). * $P < 0.001$ for baseline ESS in grades 2 and 3 vs grade 0; ** $P < 0.001$ for inflammation in grade 3 vs grades 0, 1, and 2. B, Association of baseline ESS with lesion categories and IEL grades. * $P < 0.05$ for baseline ESS in intermediate lesions with IEL grade 2 vs ESS in minimal lesions with IEL grade 0; ** $P < 0.05$ for baseline ESS in fibroatheromas (FA) with IEL grades 2 and 3 vs ESS in minimal lesions with IEL grade 0.

differences in the absolute value, but not the relative content, of plaque constituents within progressively more complex plaque, indicating that the fundamental pathobiological significance of an atherosclerotic plaque is dependent on the severity and the distribution of its constituents (Table I in the online-only Data Supplement).

Role of Low ESS as a Predictor of Development of High-Risk Plaque

Although the causative role of low ESS in atherogenesis has been studied previously in endothelial cell cultures,^{1,3} as well as in vivo in animals and humans,^{1,2,4} the role of low ESS in the progression of early coronary atherosclerotic lesions and differentiation into high-risk plaque has not been explored. Recent studies in mouse carotid arteries investigated the effect of ESS patterns on the nature of plaque development and progression, showing that low ESS is associated with lesions with high-risk characteristics.⁵ However, the direct applicability of these studies to coronary arteries may be limited because of ESS differences in arterial beds. Our experimental model demonstrated a marked histopathological heterogeneity of coronary atherosclerotic lesions at week 30 covering a broad spectrum of the natural history of atherosclerosis.¹² Fibroatheromas and intermediate lesions, which are considered precursors of fibroatheromas,^{12,13} developed in regions with significantly lower ESS at week 23 compared with minimal lesions. Although fibroatheromas and intermediate lesions were localized in subsegments of similar baseline ESS magnitude, the presence of more intensive systemic risk factors (eg, hyperlipidemia) promoted the evolution of early lesions to fibroatheromas versus intermediate lesions, suggesting the synergistic role of local hemodynamic and systemic factors in the modulation of the natural history of atherosclerosis.

Role of Magnitude of Low ESS in Severity of High-Risk Characteristics

Another novel aspect of our study is that we demonstrated that the severity of high-risk plaque characteristics is closely related to the magnitude of ESS at baseline, such that as ESS becomes lower, lipid deposition, inflammatory cell infiltration, and fibrous cap degradation are enhanced, leading to the formation of plaques with larger lipid core, severe inflammation, and thin fibrous cap. This dose-response effect of ESS may provide an explanation for the heterogeneity of atherosclerotic lesions in atherosclerosis-prone arterial regions with low ESS exposed to the same systemic atherogenic profile in the same individual.^{1,3}

Interaction of Low ESS With Excessive Expansive Remodeling Promotes Development of High-Risk Plaque

Traditionally, arterial remodeling has been assessed by the ratio of lumen and external elastic membrane area at the diseased region over the lumen and external elastic membrane area of an adjacent, presumed healthy, reference site at a single time point.¹⁶ However, this approach has several major limitations (eg, occurrence of atherosclerosis and remodeling in the reference site, effect of arterial tapering, and subjectivity in identifying a single presumed "normal" reference site).¹⁶ Recently, a serial approach was also introduced in which the lumen and external elastic membrane area in a given arterial region are compared between 2 time points.¹⁶ Although this approach is pathobiologically valid, it was not applicable in our experimental model because of the confounding effect of normal arterial growth on arterial remodeling. To overcome the limitations of the aforementioned remodeling approaches, we used a revised approach, which is basically a modification of the standard methodol-

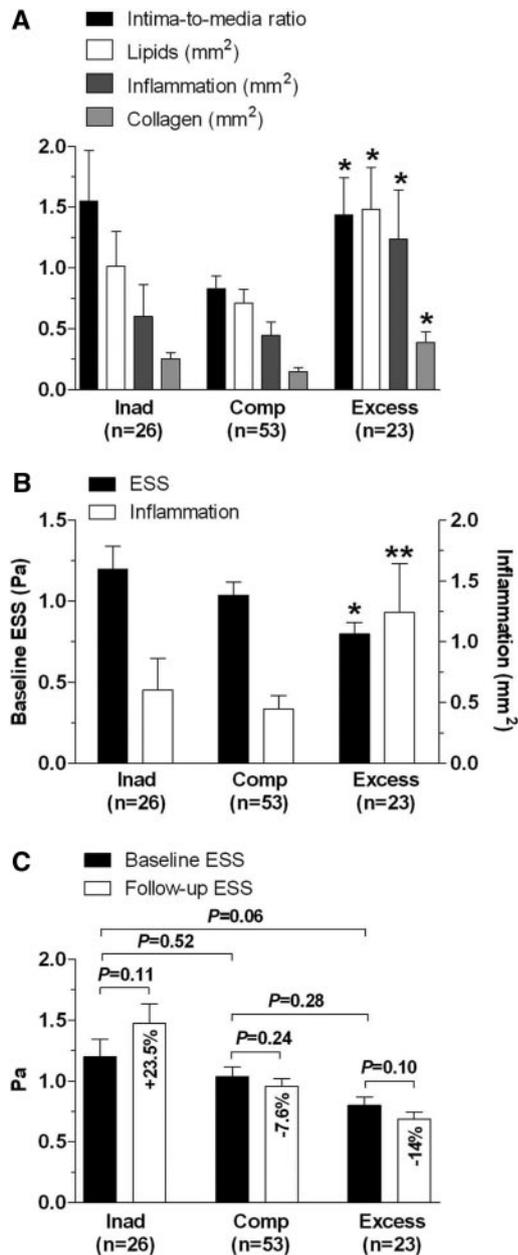


Figure 8. A, Histomorphometric and histomorphological characteristics of arterial subsegments with excessive expansive (Excess), compensatory expansive (Comp), and inadequate remodeling (Inad). * $P < 0.01$ for each histopathological characteristic in excessive vs respective characteristic in compensatory expansive remodeling; $P = 0.03$ for inflammation in excessive vs inflammation in inadequate remodeling. B, Baseline ESS (left axis) and severity of inflammation (right axis) in each remodeling pattern. * $P = 0.28$ for baseline ESS in excessive vs compensatory remodeling and $P = 0.06$ for baseline ESS in excessive vs inadequate remodeling; ** $P < 0.01$ for inflammation in excessive vs compensatory remodeling and $P = 0.03$ for inflammation in excessive vs inadequate remodeling. C, ESS level at baseline and follow-up in each remodeling pattern.

ogy for remodeling assessment described by Glagov et al.⁹ Instead of using a single arterial site, we assessed the global remodeling of the entire artery as reference,¹⁴ and we compared that reference to the local remodeling behavior of each individual subsegment of interest. Utilization of a global arterial reference minimized any errors that might be associ-

ated with incorrect assumptions related to remodeling in a single reference site or arterial growth. We observed that the critical discriminating feature characterizing excessive expansive remodeling versus compensatory or inadequate remodeling is the presence of very low ESS at baseline and the subsequently increased accumulation of inflammatory cells, especially close to the IEL.

Although low ESS in normal arteries elicits an adaptive response of the arterial wall leading to constrictive remodeling and restoration of ESS to physiological baseline levels,¹⁷ in atherosclerotic arteries the response to low ESS is very complex. Human and animal studies have shown that atherosclerotic regions with low ESS exhibit expansive remodeling.^{1,4–6,11,18} Wentzel et al¹⁸ showed that as the atherosclerotic plaques grow and encroach into the lumen, the local ESS increases over the whole arterial cross section, including the nondiseased wall, which thereby promotes nitric oxide-dependent compensatory expansive remodeling. However, the factors that determine whether the expansive remodeling response to atherosclerosis becomes either compensatory or excessive, with consequent implications fostering high-risk plaques, have not been studied previously. Our observations indicate that the excessive expansive remodeling occurs in regions with very low ESS due to structural changes of the wall underneath the plaque. The differences between baseline and follow-up ESS in the different remodeling environments demonstrate important directional differences in ESS, although the small sample size limits the statistical power of these differences. Low ESS is the initial stimulus for lipid accumulation and intense plaque inflammation, inducing disintegration of the underlying IEL. Severe IEL disintegration provides the gateway for the inflammatory cells to enter the media, where they promote the enzymatic degradation of the collagen and elastin fibers, ultimately inducing excessive wall and lumen expansion. Simultaneously, disintegration of the IEL facilitates the migration of smooth muscle cells from the media to the intima, where they elaborate extracellular matrix, thereby promoting plaque growth.^{15,19} Excessive expansive remodeling likely perpetuates or even exacerbates the local low-ESS environment, which further enhances lipid accumulation, inflammation, and intensive matrix degradation in the atherosclerotic vascular wall and the shoulders of the fibrous cap, thereby promoting additional wall expansion and fibrous cap thinning. This self-perpetuating vicious cycle among local low ESS, inflammation, and excessive expansive remodeling may transform an early atherosclerotic lesion to a high-risk plaque, especially in the presence of intense systemic and genetic risk factors.¹

Study Limitations

The major limitation in our study pertains to the experimental model we utilized. The diabetic, hyperlipidemic animals were exposed to severe atherogenic conditions, which allowed us to investigate the development of high-risk plaque; however, such conditions rarely exist in humans, thereby preventing the direct extrapolation of these results to humans. Furthermore, our experimental model did not develop late atherosclerotic lesions (ie, ruptured plaques or fibrous plaques) at week 30.¹² Although the pigs were exposed to highly atherosclerotic

conditions for almost 11 months, this time frame was not enough for the development of such plaques.

A single, representative cross section was taken from the middle of each 3-mm subsegment of interest, and it is possible that the cross section utilized was not adequately representative. Although plaque composition changes longitudinally from the upstream portion to the downstream portion of the plaque, plaque composition remains essentially homogeneous within a 3-mm region around the area of maximal plaque formation.²⁰ Furthermore, we selected 3-mm areas for sectioning that were clearly homogeneous by IVUS.

Although the vascular remodeling behavior we observed supported the concept that areas of low ESS lead to excessive expansive remodeling, animal growth during the period of our investigation may have in itself contributed to the vascular remodeling patterns we observed. However, our methodological approach to assess the remodeling behavior of each subsegment of interest within the context of the changes of the entire artery enabled us to assess the specific effect of baseline ESS on remodeling behavior, eliminating the confounding role of vascular growth.

There was a selection bias because subsegments of interest were not selected randomly. This bias was limited by intentionally selecting subsegments of different baseline ESS magnitude so that the entire spectrum of ESS values was represented. We also studied a limited number of animals and a limited number of time points. However, the power of the study increased by investigating multiple subsegments in each coronary artery (average of 5 subsegments per artery).

Several assumptions we made concerning computational fluid dynamics are described in the online-only Data Supplement.

Conclusion

The findings of this study extend the current understanding of low ESS as an atherogenic factor to a predictor of the localization and development of a high-risk plaque. In coronary arterial regions with low ESS, lipids accumulate and inflammatory cells infiltrate the intima, leading to the development and progression of early atherosclerotic lesions. The magnitude of local low ESS, in combination with the local remodeling response and the severity of systemic risk factors, determines the subsequent natural history of these early lesions. In the setting of very low levels of ESS and substantial systemic risk factors, early lesions evolve into high-risk plaques with excessive expansive remodeling, which perpetuates and may even exacerbate the adverse low-ESS environment.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The present study describes experiments in diabetic hyperlipidemic swine capable of developing humanlike high-risk plaques (ie, thin cap fibroatheromas). Local endothelial shear stress (ESS) was calculated in vivo with the use of vascular profiling techniques (intravascular ultrasound and coronary angiography) in plaque-free subsegments of interest at baseline (week 23), and these subsegments were analyzed histopathologically at follow-up (week 30), demonstrating that (1) arterial subsegments with the lowest values of ESS are those regions where high-risk plaques with large lipid core, intensive inflammation, and thin fibrous cap will develop; (2) the severity of high-risk plaque characteristics (ie, lipid accumulation, inflammatory cell infiltration, and fibrous cap thinning) is correlated with the magnitude of low ESS; and (3) very low ESS induces an intense inflammatory response that leads to severe internal elastic lamina degradation and subsequent excessive expansive remodeling (ie, excessive lumen and wall expansion). These wall changes further reduce local ESS, establishing a cascade of inflammation and excessive expansive remodeling, which can transform an early atherosclerotic lesion into a high-risk plaque. These findings indicate that application of vascular profiling methods for the in vivo understanding of local ESS and vascular remodeling response, which are responsible for individual plaque behavior and natural history, may allow for detailed risk stratification and identification of a high-risk plaque in its early stages of development. Early in vivo identification of a high-risk plaque may provide a rationale for highly selective, prophylactic local coronary interventions (eg, implantation of stents), supplemented by an intensive systemic pharmacological approach, to avert a future acute coronary event.