Published in final edited form as:

Heart. 2009 September ; 95(18): 1525–1530. doi:10.1136/hrt.2009.166017.

Segmental coronary endothelial dysfunction in patients with minimal atherosclerosis is associated with necrotic core plaques

S Lavi^{1,2}, J-H Bae^{1,3}, C S Rihal¹, A Prasad¹, G W Barsness¹, R J Lennon⁴, D R Holmes Jr¹, and A Lerman¹

¹Division of Cardiovascular Diseases and the Center for Coronary Physiology and Imaging, Mayo Clinic, Rochester, Minnesota, USA

²Division of Cardiology, London Health Sciences Centre, London, Ontario, Canada

³Cardiology Division, Konyang University Hospital, Daejeon, South Korea

⁴Division of Biostatistics, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Background/objective—Endothelial dysfunction and atherosclerosis are systemic disorders, but are often characterised by segmental involvement and complications. A potential mechanism for local involvement early in the disease process may be related to plaque composition. This study was designed to test the hypothesis that in patients with minimal coronary atherosclerosis, coronary artery segments with abnormal endothelial function have specific plaque characteristics.

Methods—Intravascular ultrasound (IVUS) images were obtained from 30 patients who underwent coronary endothelial function assessment. Spectral analysis of the IVUS radiofrequency data was used for assessment of plaque composition. IVUS findings of the coronary sections were compared according to the corresponding endothelial response to acetylcholine.

Results—Sections with a decrease epicardial coronary arterial diameter in response to acetylcholine had smaller baseline lumen (7.5 (2.4) mm² vs 8.8 (3.3) mm², $p = 0.006$) but larger plaque burden (37.1% (9.4%) vs 31% (7%), $p = 0.003$) than sections with normal endothelial response. Sections with endothelial dysfunction had larger necrotic core plaques: 0.13 (0.03–0.33) mm² vs 0.0 (0.0–0.07), $p < 0.001$ and more dense calcium: 0.03 (IQR 0.0–0.13) mm² vs 0.0 (0.0–0.10) mm², $p < 0.01$, than those with normal endothelial response. Only necrotic core area was associated with endothelial dysfunction ($p < 0.001$) after adjusting for other measures.

Correspondence to: Dr Amir Lerman, Division of Cardiovascular Diseases, Mayo Clinic, Mary Brigh 4-523, First Street SW, Rochester, MN 55905, USA; Lerman.Amir@mayo.edu.
SL and J-HB contributed equally to this work.

Competing interests: None.

Provenance and peer review: Not commissioned; externally peer reviewed.

Conclusions—This study suggests that local coronary endothelial dysfunction in patients with minimal coronary atherosclerosis is associated with plaque characteristics that are typical of vulnerable plaques.

Although atherosclerosis is a diffuse and systemic disease, there is also a segmental process that may involve selected segments along the coronary vascular tree.¹ The segmental process is manifested both in the progression of atherosclerosis as well as in complications of atherosclerosis such as acute coronary syndrome. The exact mechanism by which specific sites are affected differently is unknown, but a potential mediator may be endothelial function.

Coronary endothelial dysfunction is characterised by segmental vasoconstrictive response to the endothelium-dependent vasodilator acetylcholine.² It represents an early stage of atherosclerosis and is associated with cardiovascular events.³ Although coronary events are associated with specific plaque composition,⁴ it is unknown whether a direct relation exists between plaque features and endothelial dysfunction.

Spectral analysis of the intravascular ultrasound (IVUS) radiofrequency data allows detailed assessment of plaque composition and may potentially shed light on this association.⁵ Recent studies have demonstrated that this technique may serve as a useful imaging tool for tissue characteristics in patients with coronary artery disease^{6–8} and is capable of classifying different components of the plaque: fibrous, fibrofatty, calcified and necrotic core. The virtual histology (VH) IVUS technique was first validated ex vivo⁵ and has been shown to accurately differentiate plaque constituents. This was followed by in vivo validation studies that showed high accuracy both in patients with stable angina pectoris and in patients with acute coronary syndrome in up to 97%.^{9,10} More recently this method was validated by comparing VH-IVUS of carotid plaque to histopathology following endarterectomy.¹¹

The current study was designed to test the hypothesis that in patients without obstructive coronary artery disease, sections with endothelial dysfunction are associated with specific tissue characteristics.

METHODS

Study subjects

The study included consecutive patients who were referred for elective coronary angiography with assessment of coronary endothelial function by an independent cardiologist for the evaluation of chest pain. All patients had at least one coronary section with endothelial dysfunction as well as sections with a relatively normal response. Exclusion criteria included presence of angiographic stenosis of more than 30%, vessel tortuosity, history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, acute coronary syndrome, uncontrolled hypertension, valvular heart disease, significant endocrine, hepatic, renal or inflammatory disease. Long-acting nitrates or calcium channel blockers were withheld for 36–48 hours before the study to allow assessment of baseline coronary physiology. Patients were provided with short-acting nitrates for emergency use. Withholding of the medications was not associated with

substantial exacerbation of symptoms. Patients' demographics and laboratory data, including fasting lipid profile and serum glucose, were obtained. The Mayo Foundation institutional review board approved the study, and informed consent was obtained from all patients.

Coronary angiography

Coronary angiography was performed according to standard techniques using the femoral artery approach. Coronary artery diameter (CAD) was measured offline by a single independent investigator using a quantitative coronary angiography (QCA) program (Medis Corporation).¹²

Coronary endothelial function test

The left main coronary artery was engaged with a 6F or 7F guiding catheter. Next, a Doppler guide wire (0.014-inch diameter, FloWire, Volcano Therapeutics, Rancho Cordova, CA, USA) was advanced within a 2.2F coronary infusion catheter (Ultrafuse, SciMed Life System, Minneapolis, MN, USA) and positioned in the middle portion of the left anterior descending coronary artery (LAD). Assessment of the endothelium-dependent coronary endothelial function was performed by selective infusion of acetylcholine into the LAD. Acetylcholine at increasing doses (10^{-6} mol/l, 10^{-5} mol/l and 10^{-4} mol/l) was infused at 1 ml/min, each dose for 2 minutes.³¹³ Haemodynamic data, Doppler measurements and coronary angiography were obtained after each infusion. Average peak velocity (APV) was determined from instantaneous velocity signals from the Doppler wire by an online fast Fourier transform. Coronary blood flow was calculated from the Doppler-derived time velocity integral and vessel diameter as $\pi \times (\text{CAD})^2 \times (\text{APV}/2)$. After assessment of the endothelium-dependent function, nitroglycerin (200 µg, Abbott Laboratories) was injected as an intracoronary bolus. Since all our patients had coronary endothelial dysfunction, we chose sections with more endothelial dysfunction as those with visible vasoconstriction. This corresponded to a decrease of more than 10% in CAD in response to 10^{-4} mol/l acetylcholine.³¹⁴ Data analysis from our laboratory demonstrated a variability of 8% (3%) in repeated measurements of coronary endothelial function.¹⁵

Intravascular ultrasound virtual histology examination

The methods of the IVUS examination have been described previously.¹⁶ The VH-IVUS examination was performed with a dedicated VH-IVUS console (Volcano Therapeutics) after intracoronary administration of 100–200 µg nitroglycerin. A 20-MHz, 2.9F monorail, electronic Eagle Eye Gold IVUS catheter (Volcano Therapeutics) was advanced into the distal LAD, and automatic pullback at 0.5 mm/s was performed. The VH-IVUS image was recorded on a DVD-Rom for later offline analysis.

Spectral analysis of IVUS radiofrequency data

Each patient who was included in the study had one or more sections with vasoconstriction and sections with normal response. At first, we identified the coronary artery sections with vasoconstriction in the IVUS image using distance from anatomical landmarks that are seen on angiography and IVUS such as side branches. Then, for each section with vasoconstriction, an adjacent section without vasoconstriction was selected. The IVUS

images from each section were saved and afterwards the grey scale and tissue characteristics were analysed by investigators who were blind to the vasoconstriction results. Only sections that were large enough for IVUS imaging were studied. Three coronary artery IVUS cross-sections in each segment were chosen for analysis at a minimum three-frames interval before obtaining the VH analysis. Three coronary artery IVUS images in an adjacent normal section were also chosen.

Analyses were performed with customised software (IVUSLab; Volcano Therapeutics) by two independent examiners who were unaware of the clinical characteristics of the patients. The lumen and the media-adventitia interface were determined manually according to the ACC consensus document.¹⁷ Afterwards, the software automatically calculates areas and displays the results. For each frame, VH findings were expressed in colours, as previously described (green for fibrous, green-yellow for fibrofatty, white for dense calcified and red for necrotic core area). In addition, the area (mm²) and percentage of each tissue component of plaque was expressed. The accuracy of this method compared to histology has been validated.⁵⁹¹¹

Statistical analysis

The distribution normality of the variables was tested using the Shapiro-Wilk statistic. Continuous variables are summarised as mean (SD), or median and interquartile range, and dichotomous variables as numbers and percentages. Data were averaged separately for each patient from all the sections with and without endothelial dysfunction, such that each patient contributed two measurements for statistical analysis: one with endothelial dysfunction and one without. IVUS results were normally distributed. Averaged results were compared with a paired t test. For continuous variables that were not normally distributed we used the signed rank test. Conditional logistic regression was used to model partial associations with endothelial dysfunction as the outcome. Variables included the grey scale and VH absolute measures (eight variables total). Partial associations were tested for each variable by removing it from the full model. Statistical significance was accepted when the p value was less than 0.05. We used SAS version 9.1.3 for all analyses.

RESULTS

Patient population

Thirty patients were included in the study. The clinical characteristics of the patients are shown in table 1.

Each of the patients had at least one coronary section with visible vasoconstriction in response to acetylcholine. For each section with vasoconstriction, one adjacent section with a relatively angiographic normal response was analysed. Examples of IVUS findings are shown in figure 1. The absolute change in diameter in the sections with and without visible vasoconstriction in response to acetylcholine was -0.39 (0.46) mm and -0.16 (0.37) mm, $p = 0.01$. The segmental response to acetylcholine and nitroglycerin compared to baseline is presented in figure 2. The response to nitroglycerin was similar in the normal and abnormal

sections. At baseline, coronary blood flow in the LAD artery was 74 (51) ml/min and increased to 96 (77) ml/min with acetylcholine.

Comparison of VH-IVUS findings according to the presence of endothelial dysfunction

IVUS and VH findings are shown in table 2.

By grey scale IVUS, coronary artery sections with endothelial dysfunction had a significantly smaller lumen area, but more plaque burden compared to those without endothelial dysfunction.

VH-IVUS analysis showed that coronary artery sections with endothelial dysfunction had significantly larger areas of necrotic core and dense calcified plaque compared to those without endothelial dysfunction. Since the degree of plaque burden was different in the two groups, we compared the plaque constituents after adjusting to plaque burden. Plaque constituents from relatively normal sections within interquartile range of plaque burden (26–37%, $n = 15$) were compared to those of 14 sections with vasoconstriction in response to acetylcholine that had a similar plaque burden. The percentage of necrotic core and dense calcified areas of the plaque were higher in coronary artery sections with vasoconstriction (fig 3).

VH-IVUS predictors of endothelial dysfunction

A conditional logistic regression model for endothelial dysfunction was constructed using the eight variables in table 2. The likelihood function for the model diverged to infinity, indicating “perfect” prediction with the eight variables. However, the lack of convergence results in unestimable partial effects (that is, risk ratios) for each of the eight variables. Thus, each variable was tested with a likelihood ratio test by removing it from the eight-variable model and comparing the likelihood of the seven-variable model to the full model. Only necrotic core area was a predictor of endothelial dysfunction when adjusting for the other VH-IVUS measures. The likelihood ratio test statistic for necrotic core area was 15.5, indicating that the model is significantly worse ($p < 0.001$) with its exclusion. For all other variables, the likelihood ratio test statistic resulting from their exclusion was 0, indicating that they had no additional predictive information over the other seven variables. An increase of 0.01 mm^2 in necrotic core area is associated with an unadjusted relative risk ratio of 1.34 (95% CI 1.10 to 2.03) in endothelial dysfunction.

DISCUSSION

The current study demonstrates that similar to the segmental nature of obstructive coronary artery disease, coronary endothelial dysfunction has a segmental pattern along the coronary artery. Sections with normal and abnormal endothelial function coexist, but there is a significant difference in the tissue characterisation between these sections. Our study shows for the first time that in patients with early coronary atherosclerosis, coronary segments with abnormal endothelial function are associated with specific tissue characteristics that may imply vulnerability. The current study further supports the role of endothelial dysfunction in the mechanism of coronary atherosclerosis in humans.

Atherosclerosis and its early stage, endothelial dysfunction, are systemic processes, with segmental involvement.¹¹¹ Major complications of atherosclerosis such as acute coronary syndrome and sudden cardiac death are often not associated with obstructive coronary disease but usually occur at sites of localised plaque rupture or erosion.¹⁸ Therefore, early identification of the disease process is desirable. Although coronary endothelial function assessment is unlikely to be used routinely for identification of vulnerable plaques, our results show that coronary artery segments with vasoconstriction in response to administration of the endothelial-dependent vasodilator acetylcholine have specific features of vulnerable plaque. Our results are supported by recent studies that describe the relation between coronary spasm and acute coronary syndrome. Coronary spasm was provoked with acetylcholine in 70% of patients with acute myocardial infarction in the infarct-related artery and in 50% of the non-culprit arteries. Provoked coronary spasm was a predictor of subsequent events.¹⁹ In almost half of the patients with acute coronary syndrome without obstructive coronary disease, coronary spasm was induced with acetylcholine.²⁰ Taken together, the results of these studies suggest that coronary spasm and endothelial dysfunction are associated with early non-obstructive vulnerable plaques.

In the current study we found an association between necrotic core areas and segmental vasoconstriction in response to acetylcholine. The necrotic core areas contain sites of active inflammation and oxidative stress.¹⁸ Both oxidative stress and inflammation may be a link between plaque composition and endothelial dysfunction. We have shown that local oxidative stress and local production of inflammatory mediators such as Lp-PLA₂, are associated with coronary endothelial dysfunction.²¹²² In a recent study in pigs, coronary segments with inflammatory response and oxidative stress had impaired endothelial function.²³ Some degree of vasoconstriction was apparent even in the relatively normal sections, which underscores the systemic nature of endothelial dysfunction.

Endothelial dysfunction is a potential initiating event in vascular injury and local inflammation. The cause of endothelial dysfunction in localised segments along the coronary artery despite exposure of all areas of the artery to the same risk factors may be speculative. In apolipoprotein E-deficient mice, endothelial dysfunction was associated locally with plaque formation and correlated to lesion size.²⁴

A possible mechanism may be a local vascular injury, which is followed by oxidative stress, inflammation and abnormal repair of the endothelium.²⁵ Normally there is a constant process of injury to the endothelium by mechanical and chemical factors that is accompanied by repair mechanisms.²⁶ Systemic environment or vascular conditions may impair these mechanisms and shift the process towards endothelial dysfunction. Other potential mechanisms for segmental endothelial dysfunction are abnormal flow pattern at bending points and near bifurcations²⁷ and the increased endothelial cell apoptosis in these areas.²⁸

A few studies reported an association between endothelial dysfunction and ultrasound findings in the coronary or the peripheral circulation. Carotid intimal-medial thickening was associated with peripheral endothelial dysfunction,²⁹ and patients who underwent heart transplant and had evidence of coronary endothelial dysfunction, subsequently had coronary

intimal thickening by IVUS.³⁰ In contrast to heart transplant patients, the disease in the early stage of atherosclerosis is more segmental and this may explain in part why a direct relation between endothelial dysfunction and early atherosclerosis as assessed by IVUS was not found previously.³³¹ The lack of direct relation between endothelial dysfunction and IVUS findings may be also related to the limited ability to define plaque composition by grey scale IVUS.³² In patients with minimal atherosclerosis, the sensitivity of IVUS systems is crucial to detect small differences between groups. With improvement in IVUS technology, current systems have better resolution. Newer technology such as virtual histology provides a better assessment of the plaque composition and therefore is more likely to detect differences.

The more detailed assessment of plaque composition by spectral analysis of IVUS radiofrequency data in the current study, combined with analysis according to sections, enabled us to show such a relation for the first time. The validity of IVUS-based spectral analysis has been assessed in ex vivo and in vivo studies, in animals and humans, in coronary and non-coronary vasculature, and has been shown to correlate well with the tissue components of the plaque.⁵⁹¹¹ Identification of plaque components by virtual histology has potential clinical implications. We now show that this technique may also identify areas of endothelial dysfunction.

Study limitations and clinical perspective

There are no established VH criteria for vulnerable plaque, and VH-IVUS-derived thin cap fibroatheroma is not proved as a surrogate for future complications. The VH-IVUS findings in our patients with mild atherosclerosis did not fulfil the definition of thin cap fibroatheroma. The clinical characteristics of our study population are not typical for patients with coronary artery disease, but are usual for patients with chest pain without significant coronary disease.¹³ Therefore, our findings may not be generalised to patients with more advanced atherosclerosis. Only the LAD was assessed and therefore the results may not apply to other coronary arteries.

While we cannot deduce causality, the current study provides a unique opportunity to investigate an important step in the progression of coronary atherosclerosis in humans. Larger studies like the PROSPECT trial evaluate the prognostic implications of plaque constitute; similarly, it will be needed to evaluate whether coronary artery segments with endothelial dysfunction progress into culprit lesions and acute coronary syndromes.

Conclusions

The present study demonstrates for the first time that in humans with early coronary atherosclerosis, coronary segments with a higher degree of endothelial dysfunction are associated with more necrotic plaque. The clinical and prognostic implications of our findings remain to be determined.

Acknowledgments

Funding: The study was supported by grants from the NIH: NIH K24 HL-69840, NIH R01 HL-63911, HL-77131 and from Mayo and University of Minnesota: MAYO-UOFM #4 PROJ1-2.

References

1. Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation*. 2005; 111:363–8. [PubMed: 15668353]
2. El-Tamimi H, Mansour M, Wargovich T, et al. Constrictor and dilator responses to intracoronary acetylcholine in adjacent segments of the same coronary artery in patients with coronary artery disease. Endothelial function revisited. *Circulation*. 1994; 89:45–51. [PubMed: 8281679]
3. Suwaidi JA, Hamasaki S, Higano ST, et al. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*. 2000; 101:948–54. [PubMed: 10704159]
4. Fujii K, Kobayashi Y, Mintz GS, et al. Intravascular ultrasound assessment of ulcerated ruptured plaques: a comparison of culprit and nonculprit lesions of patients with acute coronary syndromes and lesions in patients without acute coronary syndromes. *Circulation*. 2003; 108:2473–8. [PubMed: 14610010]
5. Nair A, Kuban BD, Tuzcu EM, et al. Coronary plaque classification with intravascular ultrasound radiofrequency data analysis. *Circulation*. 2002; 106:2200–6. [PubMed: 12390948]
6. Rodriguez-Granillo GA, Garcia-Garcia HM, Valgimigli M, et al. Global characterization of coronary plaque rupture phenotype using three-vessel intravascular ultrasound radiofrequency data analysis. *Eur Heart J*. 2006; 27:1921–7. [PubMed: 16840496]
7. Rodriguez-Granillo GA, Garcia-Garcia HM, McFadden EP, et al. In vivo intravascular ultrasound-derived thin-cap fibroatheroma detection using ultrasound radiofrequency data analysis. *J Am Coll Cardiol*. 2005; 46:2038–42. [PubMed: 16325038]
8. Fujii K, Carlier SG, Mintz GS, et al. Association of plaque characterization by intravascular ultrasound virtual histology and arterial remodeling. *Am J Cardiol*. 2005; 96:1476–83. [PubMed: 16310425]
9. Nasu K, Tsuchikane E, Katoh O, et al. Accuracy of in vivo coronary plaque morphology assessment: a validation study of in vivo virtual histology compared with in vitro histopathology. *J Am Coll Cardiol*. 2006; 47:2405–12. [PubMed: 16781367]
10. Nair A, Margolis M, Kuban BD, et al. Automated coronary plaque characterisation with intravascular ultrasound backscatter: ex vivo validation. *Eurointervention*. 2007; 3:113–20. [PubMed: 19737694]
11. Diethrich EB, Paulina Margolis M, Reid DB, et al. Virtual histology intravascular ultrasound assessment of carotid artery disease: the Carotid Artery Plaque Virtual Histology Evaluation (CAPITAL) study. *J Endovasc Ther*. 2007; 14:676–86. [PubMed: 17924734]
12. Al Suwaidi J, Higano ST, Holmes DR Jr, et al. Measuring maximal percent area stenosis poststent placement with intracoronary Doppler and the continuity equation and correlation with intracoronary ultrasound and angiography. *Am J Cardiol*. 1999; 84:650–4. [PubMed: 10498133]
13. Lavi S, Prasad A, Yang EH, et al. Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease. *Circulation*. 2007; 115:2621–7. [PubMed: 17485580]
14. Hasdai D, Gibbons RJ, Holmes DR Jr, et al. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. *Circulation*. 1997; 96:3390–5. [PubMed: 9396432]
15. Yang EH, McConnell JP, Lennon RJ, et al. Lipoprotein-associated phospholipase A2 is an independent marker for coronary endothelial dysfunction in humans. *Arterioscler Thromb Vasc Biol*. 2006; 26:106–11. [PubMed: 16239595]
16. Tobis JM, Mallery J, Mahon D, et al. Intravascular ultrasound imaging of human coronary arteries in vivo. Analysis of tissue characterizations with comparison to in vitro histological specimens. *Circulation*. 1991; 83:913–26. [PubMed: 1999040]
17. Mintz GS, Nissen SE, Anderson WD, et al. American College of Cardiology clinical expert consensus document on standards for acquisition, measurement and reporting of intravascular ultrasound studies (IVUS): a report of the American College of Cardiology task force on clinical expert consensus documents developed in collaboration with the European Society of Cardiology endorsed by the Society of Cardiac Angiography and Interventions. *J Am Coll Cardiol*. 2001; 37:1478–92. [PubMed: 11300468]

18. Naghavi M, Libby P, Falk E, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation*. 2003; 108:1664–72. [PubMed: 14530185]
19. Wakabayashi K, Suzuki H, Honda Y, et al. Provoked coronary spasm predicts adverse outcome in patients with acute myocardial infarction: a novel predictor of prognosis after acute myocardial infarction. *J Am Coll Cardiol*. 2008; 52:518–22. [PubMed: 18687243]
20. Ong P, Athanasiadis A, Hill S, et al. Coronary artery spasm as a frequent cause of acute coronary syndrome: the CASPAR (Coronary Artery Spasm in Patients With Acute Coronary Syndrome) Study. *J Am Coll Cardiol*. 2008; 52:523–7. [PubMed: 18687244]
21. Lavi S, McConnell JP, Rihal CS, et al. Local production of lipoprotein-associated phospholipase A2 and Lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation*. 2007; 115:2715–21. [PubMed: 17502572]
22. Lavi S, Yang EH, Prasad A, et al. The interaction between coronary endothelial dysfunction, local oxidative stress, and endogenous nitric oxide in humans. *Hypertension*. 2008; 51:127–33. [PubMed: 18086957]
23. Pendyala LK, Li J, Shinke T, et al. Endothelium-dependent vasomotor dysfunction in pig coronary arteries with paclitaxel-eluting stents is associated with inflammation and oxidative stress. *J Am Coll Cardiol Interv*. 2009; 2:253–62.
24. Crauwels HM, Van Hove CE, Holvoet P, et al. Plaque-associated endothelial dysfunction in apolipoprotein E-deficient mice on a regular diet. Effect of human apolipoprotein AI. *Cardiovasc Res*. 2003; 59:189–99. [PubMed: 12829190]
25. Lesnik P, Chapman MJ. A new dimension in the vasculoprotective function of HDL: progenitor-mediated endothelium repair. *Arterioscler Thromb Vasc Biol*. 2006; 26:965–7. [PubMed: 16627821]
26. Lerman A. Restenosis: another “dysfunction” of the endothelium. *Circulation*. 2005; 111:8–10. [PubMed: 15630036]
27. Cooke JP. Flow, NO, and atherogenesis. *PNAS*. 2003; 100:768–70. [PubMed: 12552094]
28. Tricot O, Mallat Z, Heymes C, et al. Relation between endothelial cell apoptosis and blood flow direction in human atherosclerotic plaques. *Circulation*. 2000; 101:2450–3. [PubMed: 10831515]
29. Ghiadoni L, Taddei S, Virdis A, et al. Endothelial function and common carotid artery wall thickening in patients with essential hypertension. *Hypertension*. 1998; 32:25–32. [PubMed: 9674633]
30. Davis SF, Yeung AC, Meredith IT, et al. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. *Circulation*. 1996; 93:457–62. [PubMed: 8565162]
31. Nishimura RA, Lerman A, Chesebro JH, et al. Epicardial vasomotor responses to acetylcholine are not predicted by coronary atherosclerosis as assessed by intracoronary ultrasound. *J Am Coll Cardiol*. 1995; 26:41–9. [PubMed: 7797774]
32. MacNeill BD, Lowe HC, Takano M, et al. Intravascular modalities for detection of vulnerable plaque: current status. *Arterioscler Thromb Vasc Biol*. 2003; 23:1333–42. [PubMed: 12805071]

Figure 1.

Examples of intravascular ultrasound (IVUS) findings. Grey scale IVUS and tissue characterisation of plaques by use of radiofrequency intravascular ultrasound analysis are shown for five of the patients. For each patient, two images are shown on the left from a segment in the left anterior descending (LAD) coronary artery with endothelial dysfunction. Two images on the right are from a segment with normal endothelial function. Virtual histology colour code: Grey, arterial wall; dark green, fibrous; light green, fibrofatty; red, necrotic core; white, dense calcified area.

Figure 2.

Coronary artery diameter response to acetylcholine and nitroglycerin. Segmental response to intracoronary acetylcholine (Ach) and nitroglycerin (NTG) as determined by percentage change in coronary artery diameter.

Figure 3.

Plaque composition and endothelial function. Plaque composition according to spectral analysis of intravascular ultrasound radiofrequency data (mean (SEM)).

Table 1

Demographics of study patients (n = 30)

Age (years)	48 (10)
Gender, male	9 (30)
Hypertension	13 (43)
Diabetes	2 (7)
Hyperlipidaemia	19 (63)
Smoking	1 (3)
Family history of CAD	21 (70)
Body mass index (kg/m ²)	30 (5)
Fasting blood glucose (mg/dl)	104 (20)
Total cholesterol (mg/dl)	187 (45)
Triglyceride (mg/dl)	109 (53)
High-density lipoprotein cholesterol (mg/dl)	59 (15)
Low-density lipoprotein cholesterol (mg/dl)	105 (37)
Ejection fraction (%)	64 (6)
Medication	
ACE inhibitors	5 (17)
B-blockers	12 (40)
Calcium channel blockers	12 (40)
Statins	17 (57)

Values are numbers (%) or mean (SD).

ACE, angiotensin-converting enzyme; CAD, coronary artery disease.

Table 2

Intravascular ultrasound (IVUS) and virtual histology (VH) findings according to segmental response to acetylcholine

	Normal response	Endothelial dysfunction	Difference	p Value [*]
Analysis of grey scale IVUS				
Vessel area (mm ²)	12.6 (4)	11.8 (3.4)	0.8 (2.8)	0.11
Lumen area (mm ²)	8.8 (3.3)	7.5 (2.4)	1.3 (2.4)	0.006
Plaque area (mm ²)	3.9 (1.1)	4.3 (1.7)	−0.4 (1.3)	0.09
Plaque burden (%)	31.7 (6.9)	37.1 (9.4)	5.4 (9.0)	0.003
Area of the each plaque constituent				p Value^{**}
Fibrous area (mm ²)				0.18
Median (Q1–Q3)	0.44 (0.13–0.80)	0.30 (0.10–1.20)	0.12 (−0.23–0.77)	
Min, max	0.0, 2.37	0.0, 4.43	−1.50, 2.07	
Fibrofatty area (mm ²)				0.23
Median (Q1–Q3)	0.04 (0–0.17)	0.08 (0.0–0.13)	0.0 (−0.03–0.10)	
Min, max	0.0, 0.57	0.0, 0.67	−0.20, 0.30	
Dense calcium area (mm ²)				<0.001
Median (Q1–Q3)	0.0 (0.0–0.0)	0.03 (0.0–0.13)	0.0 (0.0–0.13)	
Min, Max	0.0, 0.10	0.0, 0.53	−0.05, 0.53	
Necrotic core area (mm ²)				<0.001
Median (Q1–Q3)	0.0 (0.0–0.07)	0.13 (0.03–0.33)	0.10 (0.01–0.29)	
Min, max	0.0, 0.30	0.0, 1.33	−0.05, 1.10	

* Paired t test. Mean (SD).

** Signed rank test.