

Coronary Artery Disease: Pathogenesis and Acute Coronary Syndromes

STEPHEN G. WORTHLEY, M.D.^{1,2}, JULIO I. OSENDE, M.D.^{1,2}, GÉRARD HELFT, M.D.^{1,2}, JUAN J. BADIMON, PH.D.^{1,2},
AND VALENTIN FUSTER, M.D., PH.D.²

Abstract

Atherosclerotic diseases and their thrombotic complications remain the leading causes of mortality and morbidity in Western society. In the United States, cardiovascular disease is responsible for one in every 2.4 (41.4%) deaths and is the leading single cause of mortality. Furthermore, the presence of atherosclerotic disease (defined as thickening of the arterial wall through the accumulation of lipids, macrophages, T-lymphocytes, smooth muscle cells, extracellular matrix, calcium and necrotic debris) is more prevalent, but by itself rarely fatal. The crucial, final common process for the conversion of a nonocclusive, often clinically silent atherosclerotic lesion to a potentially fatal condition is often plaque disruption. The mortality associated with atherosclerotic disease relates to the acute coronary syndromes, including acute myocardial infarction, unstable angina pectoris and sudden cardiac death. Substantial clinical, experimental and postmortem evidence demonstrates the central role that a superimposed acute thrombosis on a disrupted atherosclerotic plaque plays in the onset of acute coronary syndromes. Therefore, therapeutic approaches to date have focused on reducing such thrombotic complications of atherosclerotic plaques (i.e., antiplatelet, anticoagulant and thrombolytic therapies) to reduce the resulting morbidity and mortality.

In this review, we will focus on the current theories of atherogenesis and how they impact on our understanding of acute coronary syndromes.

Key Words: Atherogenesis, atherosclerosis, platelets, platelet aggregation, thrombogenicity, thrombosis, acute coronary syndrome, plaque formation, plaque disruption, hemodynamics, Q-wave myocardial infarction, rheology, collateral circulation, risk factors.

Atherogenesis: The Early Stages

Progression and Classification of Atherosclerotic Lesions

PATHOLOGICAL STUDIES have provided insights into the early changes within the artery wall that may be associated with atherosclerosis. The earliest findings noted were of eccentric intimal thickening in arterial regions opposite the flow divider of arterial bifurcations. The earliest signs of lipid retention were isolated foam

cells (macrophages) found within the intima of 45% of infants (1). By puberty, such foam cell accumulations were accompanied by lipid droplets both extracellularly and within smooth muscle cells. These early lesions are the so-called fatty streaks, noted in 65% of children between 12 and 14 years of age. By the third decade of life, these lesions had developed a cap of smooth muscle cells and collagen, thus forming a fibroatheroma. The formation of these fibrous caps is generally slow. However, they may thicken rapidly with the deposition of platelets and fibrin on their surfaces because of thrombus-dependent fibrotic organization.

Based on the above pathological data, atherosclerotic plaque progression was subdivided into five phases and lesion types (Fig. 1) by the American Heart Association Committee on Vascular Lesions (1). Although not severely stenotic by coronary angiography, type IV and type Va lesions are particularly important because they are more susceptible or "vulnerable" to disruption and subsequent thrombosis. Type

¹Cardiovascular Biology Research Laboratory and ²Zena and Michael A. Weiner Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Adapted from a Grand Rounds presentation to the Division of Cardiology, Department of Medicine, Mount Sinai School of Medicine, New York, NY on March 29, 1999.

Address correspondence to Juan J. Badimon, Ph.D., Cardiovascular Biology Research Laboratory, Zena and Michael A. Weiner Cardiovascular Institute, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029-6574.

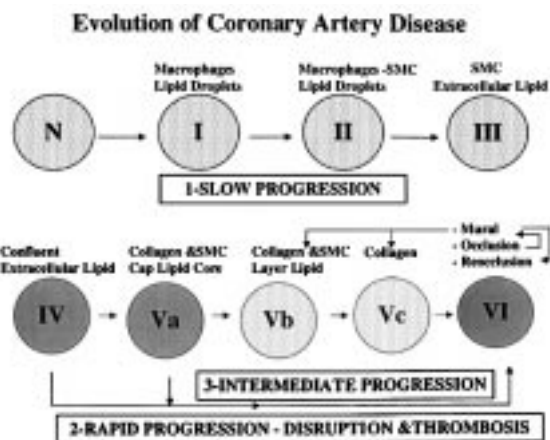


Fig. 1. Schematic representation of atherosclerotic lesion progression. The solid dark circles indicate those phases where plaque disruption and subsequent thrombosis play a critical role. (taken from ref. #105)

IV lesions have a high extracellular lipid content intermixed with fibrous tissue beneath a fibrous cap. In contrast, type Va lesions contain a larger lipid-rich core with a thin fibrous cap. Upon disruption, these minimally stenotic coronary lesions may lead to acute occlusive thrombosis associated with the acute coronary syndromes or a small nonocclusive thrombus. The latter event may lead to a severe stenotic lesion after fibrous organization within the underlying atherosclerotic plaque. This abrupt episodic progression of disrupted “vulnerable” plaques leads to the frequent complicated type VI lesion that accounts for about 75% of the patients with acute coronary syndromes.

Importance of Rheology, Endothelial Dysfunction and Inflammation in Early Atherosclerosis

The arterial vessel wall and the endothelial cells are subject to mechanical and hydrostatic forces exerted by blood within the vessel, circumferential stress from motion of the vessel during the cardiac cycle and shear stress resulting from blood flow within the vessel. The biological implication of these mechanical forces is clearly demonstrated by the preferential localization of atherosclerotic lesions at certain sites within the arterial tree despite the presence of the same systemic, genetic and environmental factors. It is the last of these forces, shear stress, which appears to have the greatest impact upon events occurring at the interface of the blood and vessel wall. Shear stress stimulates the release of vasoactive substances, and

changes such cellular processes as gene expression (i.e., via shear-stress-responsive elements), cell metabolism and cell morphology (2, 3). At areas of abrupt curvature in the vessel (such as at the carotid bulb), laminar blood flow is disrupted, which results in recirculation vortices and low mean shear stress and flow reversal. The correlation is strong between endothelial dysfunction and areas of low mean shear stress and oscillatory flow with flow reversal (4). The predilection of the endothelial cell surface at these sites for cellular adhesion molecules, increased uptake of lipoproteins, inflammatory cell transmigration, and the secretion of chemokines and cytokines leads to the proliferation of smooth muscle cells and macrophages within the vessel wall. Thus, high mean shear stress inhibits leukocyte binding and chemokine and cytokine expression, while low mean shear stress promotes inflammatory cell binding.

The endothelium plays a central role in arterial hemostasis through the regulation of plasma lipoprotein permeability and leukocyte adhesion, and the production of prothrombotic and antithrombotic factors, growth factors and vasoactive substances (Fig. 2). There is substantial data demonstrating that shear stress is an important stimulus for the secretion of prostacyclin (5, 6) and nitric oxide (NO) (7–9), both of which are potent inhibitors of platelet aggregation. Furthermore, shear stress has been shown



Fig. 2. Endothelium-derived substances. Note the list of pro- and anti-atherogenic factors that could be produced by the endothelium (see text). **PDGF.** -Platelet Derived Growth Factor; **bFGF.** -basic Fibroblast Growth Factor; **VCAM.** -Vascular Adhesion molecules; **ICAM.** - Intercellular Adhesion Molecules; **ELAM.** -Endothelial Leukocyte Adhesion Molecule; **TGF- β .** - Transforming Growth Factor Beta; **TFPI.** -Tissue Factor Pathway Inhibitor; **tPA.** -tissue-type Plasminogen Activator; **PAI-1.** -Plasminogen Activator Inhibitor-1; **vWF.** -von Willebrand factor.

to regulate the production of thrombomodulin (10), which (through interaction with proteins C and S) inactivates specific clotting factors, stimulates the expression of tissue plasminogen activator (10–12) and reduces the secretion of plasminogen activator inhibitor type 1 (12), thus promoting fibrinolysis as well. The genes for the production of tissue factor, one of the most potent stimuli for thrombin generation via the extrinsic pathway of the coagulation cascade, are upregulated in conditions of low mean shear stress (13), and promote the existence of a pro-thrombotic endothelial surface. Thus, in conditions of low mean shear stress, anticoagulant mechanisms are inhibited, in contrast to the activation of procoagulant pathways.

Recruitment of monocytes into the vessel wall is an early step in the formation of an atherosclerotic lesion. The fatty streak, the precursor for atherosclerotic lesions, contains macrophages and T lymphocytes exclusively (1), although the deposition of lipids precedes this inflammatory cellular influx in patients with hypercholesterolemia (14, 15). The subsequent migration of leukocytes across the endothelium depends on chemotactic factors such as monocyte chemoattractant protein 1 (MCP-1) and oxidized low density lipoproteins (LDL) (16) as well as platelet-endothelial cell adhesion molecules (17). Substances such as monocyte colony stimulating factor seem important for the survival and multiplication of macrophages within the growing atherosclerotic lesions (18, 19). T-cells are similarly dependent on interleukin-2 (20). Macrophages produce many growth factors (including platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF), in addition to the inflammatory agents described above (21). However, it is the production of matrix-degrading substances (matrix metalloproteinases (MMP) and heparanases) which are crucial to the perpetuation and growth of the early lesion (22). Gelatinase A (MMP-2) degrades the collagen found in the basement membranes, and appears to be critical in facilitating smooth muscle migration through the basement membrane (23). The major control of MMP activity, once activated from the inactive zymogen, lies with the production of tissue inhibitors of MMPs (TIMPs) (22), of which three have been identified to date. It appears that the ratio of MMPs to TIMPs is crucial in determining connective tissue and basement membrane breakdown. Lymphocytes, including CD4 and CD8 positive T-

cells, have been identified in significant numbers within atherosclerotic lesions, and invariably play a role in the inflammatory processes in plaque genesis and progression (24, 25). These T-cells are activated when they bind antigens processed and presented by both macrophages and smooth muscle cells. One such antigen may be oxidized LDL (26). Smooth muscle cell proliferation, an important feature of atherosclerotic lesions, is stimulated and regulated by endothelial factors, of which shear stress is one (27). Low mean shear stress (which is pro-atherogenic) has been shown to be associated with increased production of endothelial PDGF (27). High mean shear stress (which is anti-atherogenic or atheroprotective) has been associated with reduced production of endothelin-1 (28) and angiotensin II (29), both of which are smooth muscle mitogens, and increased production of NO (30, 31) and transforming growth factor β (TGF- β). These latter two substances are inhibitors of smooth muscle cell growth. Thus, substantial evidence exists for shear stress mediated modulation of vascular smooth muscle cell proliferation.

Platelets and Thrombosis in Early Atherosclerosis and Acute Coronary Syndromes

Platelet deposition and thrombosis atop an atherosclerotic lesion lead to one of two broad events (Fig. 3). First, nonocclusive luminal thrombosis leads to silent, rapid plaque growth.

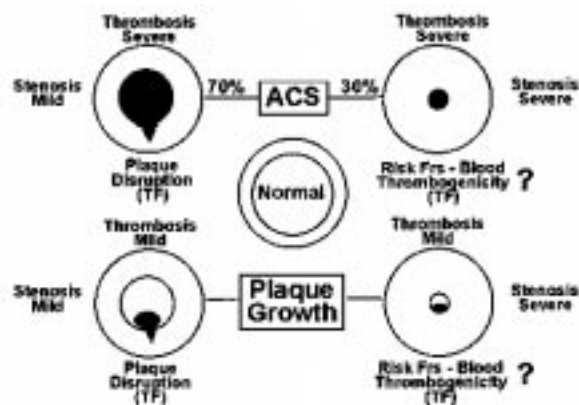


Fig. 3. Diagram showing the various outcomes that result from the thrombotic complications of atherosclerotic disease. Plaque disruption and subsequent thrombosis are associated with 70% of the acute coronary syndromes, while the remaining 30% seem to be caused by the existence of a severe stenosis triggering thrombosis. ACS. -Acute Coronary syndromes. The ? marks refer to the potential effect of a newly reported systemic pool of tissue factor (TF).

During serial angiographic studies, the presence of a mild or moderately stenotic plaque (<50%) was the most frequent cause of acute ischemic events (32–35). These studies also noted that even without clinical symptoms, some minor lesions had progressed rapidly in size over a short period. Postmortem studies of patients dying from ischemic events have revealed the cause of both the acute events and lesion progression to be intimately related to plaque-associated thrombosis (36–41). The atherosclerotic lesions that appeared susceptible or prone to such thrombotic phenomena were noted to have common histological characteristics. These so-called “vulnerable” plaques are more prone to plaque disruption and subsequent thrombus formation.

Second, occlusive (transiently or permanently) luminal thrombosis is associated with unstable angina pectoris, acute myocardial infarction or sudden cardiac death. Plaques containing a large atheromatous core are more prone to disruption, since 75% of such plaques are responsible for the atherothrombotic complications leading to the acute coronary syndromes (42–45). Most of the other cases are associated with plaque thrombosis atop a macrophage-rich intimal erosion in a more fibrotic plaque, often in association with a severe arterial stenosis (42–44).

Plaque Disruption

Plaque disruption is a central feature of atherothrombotic syndromes, and the risk that this will occur relates to the existence of several factors that can be divided into intrinsic (related to plaque composition) and extrinsic (mostly related to the geographical location of the lesion) (Table 1).

Intrinsic Factors

Atherosclerotic plaque disruption generally occurs at sites where the fibrous cap is thinnest and most heavily infiltrated with macrophage-derived foam cells (i.e., its weakest point). This usually is seen at the shoulder region of eccentric lesions (43) (the interface between the normal vessel wall and the atherosclerotic plaque). Factors associated with fibrous cap rupture include size of the atheromatous core (i.e., ratio of lipid to fibrotic components), thickness and composition of the fibrous cap (i.e., ratio of smooth muscle cells to macrophages), inflammation within the fibrous cap and cap fatigue.

While the composition of most atherosclerotic lesions is mainly fibrotic, a significant

TABLE 1

Determinants of Atherosclerotic Plaque Vulnerability

•	INTRINSIC FACTORS	
	COMPOSITION	→ Lipid vs Fibrotic content
		→ Cell Population (Macrophages vs SMC)
•	EXTRINSIC FACTORS	
	Circumferential Tensile Stress (moderate stenosis > tension than severe stenosis)	
	Compressive Stress (vasoconstriction)	
	Circumferential Bending	
	Longitudinal flexion stress	
	Shear stress (laminar vs oscillatory)	

atheromatous core does exist in most so-called culprit lesions for acute coronary syndromes (46). Several studies confirm the association between size of the atheromatous core and risk for subsequent plaque rupture. One study found that in aortic plaque an atheromatous core of > 40% of the plaque content was at a particularly high risk of disruption and subsequent thrombosis (47). Based on numerous animal studies, lipid-lowering approaches are believed to decrease the lipid content of the plaque (i.e., decrease the size of the lipid-rich core), resulting in a more fibrotic and stable plaque (48–50).

Fibrous caps vary widely in their thickness and composition. However, fibrous caps are thinnest at the shoulder regions of the vulnerable plaques (43). Collagen (in particular, type 1 collagen) is a critical determinant of fibrous cap strength. In disrupted aortic plaques, smooth muscle cells (the source of collagen in the cap) and the collagen content itself are decreased (47, 51). One mechanism postulated for the reduced number of smooth muscle cells in the fibrous cap is apoptosis (51). It is uncertain whether this is the only mechanism responsible.

Pathological studies (with immunohistochemistry) have revealed evidence of ongoing inflammation within the fibrous cap at sites of disruption, and other studies have shown macrophage infiltration at the disrupted shoulder regions of fibrous caps (42, 52, 53). An important mechanism appears to be the production of matrix-degrading enzymes, including MMPs, which played an important role in atherogenesis also. Activated macrophages within the fibrous cap produce a variety of MMPs, and *in-vitro* studies have confirmed the ability of these enzymes to degrade fibrous caps (54). Although many of these enzymes have been implicated, including the interstitial collagenases MMP-1 and -3 (55), only gelatinase B (MMP-9) has been associated with rupture-prone areas in

human coronary artery specimens obtained at atherectomy (56). T-cells are present in increased numbers at these rupture-prone sites also, and can stimulate macrophages to produce MMP-9 (57).

Extrinsic Factors

Atherosclerotic lesions within the coronary arterial system are subject to mechanical and hemodynamic forces that may trigger disruption of atherosclerotic plaques (58, 59). Cap tension refers to the circumferential wall tension exerted on the vessel due to the blood pressure. This force is governed by Laplace's law; the higher the blood pressure and the larger the luminal diameter, the more tension will develop in the wall (58). The soft atheromatous core is unable to bear these forces well, and the tension is redistributed to adjacent structures such as the fibrous cap (43). Thus, we can appreciate that mildly-to-moderately stenotic lesions will be subject to greater circumferential stresses, in accord with Laplace's law, than severely stenotic lesions. Other forces to which the coronary artery wall and atherosclerotic lesions are subject include longitudinal flexion and circumferential bending associated with the motion of the heart and the propagating pulse wave. It appears that part of the mechanism by which β -blockers exert their favorable effect on reducing reinfarction is by attenuating these forces. Finally, vasospasm and plaque hemorrhage could potentially enhance plaque rupture by compressing the atheromatous core, and cause the plaque to "blow out" into the vessel lumen (53, 60). However, there are little data to confirm the significance of this mechanism in plaque rupture.

Acute Thrombosis

Substrate and Tissue Factor Dependent Thrombosis

Studies of the relative thrombogenicity of the various components of atherosclerotic plaques have demonstrated that lipid-rich plaques are up to six times more thrombogenic than all other components (61). The exact mechanisms for the thrombogenicity of the lipid core are uncertain. However, it has been shown that lipid cores have a high tissue factor content (62), and this may account for some or most of the thrombogenicity (Fig. 4). This tissue factor may originate in macrophage-derived

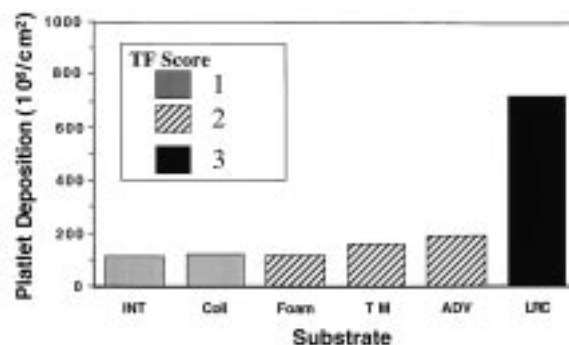


Fig. 4. Platelet deposition and Tissue Factor (TF) activity score in human tissues. Mean values for platelet deposition are illustrated on the ordinate. The intensity of tissue staining is expressed as the average of the scores determined by two independent observers. Note the positive correlation between platelet deposition and TF score on the exposed human substrates. **INT** - normal intima; **Coll** - collagen-rich matrix; **Foam** - foam cell-rich matrix; **TM** - normal tunica media; **ADV** - adventitia; **LRC** - lipid-rich core. Atherosclerotic lesions characterized by the presence of a lipid-rich core (LRC) are most thrombogenic and have the highest content of TF. (taken from ref. #62)

foam cells (63, 64). Studies with directional atherectomy specimens from patients with unstable coronary syndromes showed a higher population of macrophage-rich areas than specimens from stable angina patients. Moreover, there was a significant correlation between these macrophage-rich areas and positive tissue factor staining in atherectomy samples from those patients with unstable coronary syndromes (65–67). Based upon immunohistochemical evidence, it is likely that macrophages are responsible for the bulk of the tissue factor found in the core. This tissue factor may derive from cell debris or microparticles released during apoptosis (68). Plaque disruption may thus expose active tissue factor to circulating blood, leading to acute thrombosis. Furthermore, in models of thrombosis induced by a damaged arterial wall, tissue factor pathway inhibitor (TFPI) has a potent antithrombotic effect (Fig. 5). Despite this evidence, tissue factor in the lipid core may potentially be derived from other sources.

Preliminary evidence is emerging that identifies a plasma source of tissue factor. A modest increase in plasma tissue factor levels (measured by enzyme-linked immunosorbent assay, ELISA) was reported in a study of 31 patients experiencing acute and chronic phases of myocardial infarction versus those with stable angina or controls (69). Tissue factor activity was not measured. In a follow-up study (70), this research group also reported that plasma TF

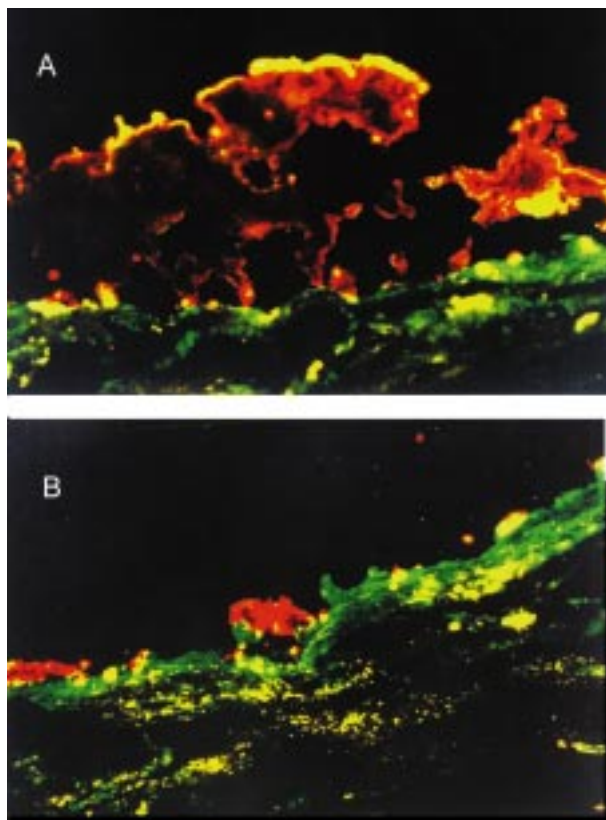


Fig. 5. Effect of the inhibition of TF activity by rTFPI on the thrombogenicity of human atherosclerotic lesions. **A.** Representative immunofluorescence images of control. **B.** TFPI-treated human lipid-rich atherosclerotic lesions. Fibrin(ogen) deposition is shown as green, platelet deposition as red, and their co-localization as orange. Note significant reduction in both platelet and fibrin(ogen) induced by specific inhibition of TF in atherosclerotic lesions. (taken from ref. #106)

and TFPI levels were increased in the acute and subacute phase of patients with unstable angina and correlated with increases in free TFPI and TF (Fig. 6). Most important, patients with unstable angina and heightened tissue factor were at increased risk for unfavorable outcomes. However, in this study too, tissue factor activity was not measured, nor was the source of the plasma tissue factor addressed.

The source of this circulating tissue factor remains to be determined. Although endothelial cells, macrophages, smooth muscle cells and myocardial cells may be the source of circulating tissue factor, the possibility that this circulating moiety derives, in part, from blood monocytes and/or neutrophils is being actively investigated. Preliminary data by immunoelectron microscopy indicates that tissue factor antigen is most abundant in membrane vesicles that cluster near the surface of platelets (71). More recent

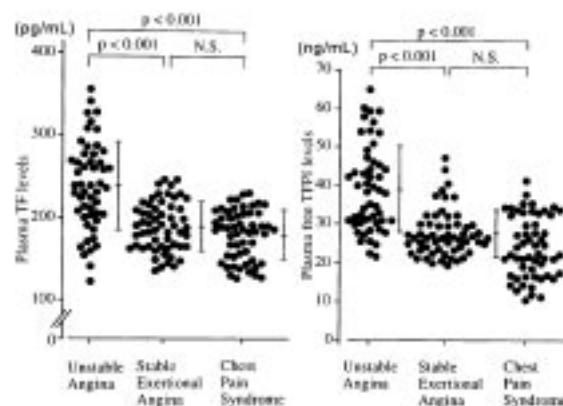


Fig. 6. Evidence of increased tissue factor (TF) and tissue factor pathway inhibitor (TFPI) antigen plasma levels in patients with acute coronary syndromes. Unstable angina patients show higher levels of both TF and TFPI than stable angina or chest pain syndrome patients. (taken from ref. #70)

research has demonstrated tissue factor in monocytes and neutrophils in peripheral blood.

Systemic Thrombogenicity

There is substantial experimental and clinical evidence that a primary hypercoagulable or thrombogenic state that promotes focal thrombus formation may exist in the circulation (Table 2). This is important when considering the risk of complicating thrombosis after plaque rupture and confirms that factors beyond the atherosclerotic plaque are also important in predicting thrombotic risk. Systemic factors, including alterations in lipid and hormonal metabolism, hemostasis, fibrinolysis, and platelet and leukocyte function, are associated with increased blood reactivity and thrombogenicity.

TABLE 2

Factors Modulating Platelet-Arterial Wall Interaction

LOCAL FLUID DYNAMICS

- Shear stress
- Tensile Stress

NATURE OF THE EXPOSED SUBSTRATE

- Degree of injury (mild vs severe arterial injury)
- Composition of atherosclerotic plaque
- Residual mural thrombus

SYSTEMIC THROMBOGENIC FACTORS

- Hypercholesterolemia
- Catecholamines (smoking, cocaine, etc.)
- Smoking
- Diabetes
- Homocysteine
- Lipoprotein (a)
- Infections (*Chlamydia pneumoniae*, *Helicobacter pylori*, CMV)
- Hypercoagulable state (Fibrinogen, vWF, TF, factor VII)
- Defective fibrinolytic state, etc.

CMV, -Cytomegalovirus; TF, -tissue factor; vWF, -von Willebrand Factor

Increased plasma levels of catecholamines may favor platelet reactivity. Platelet aggregation and the generation of thrombin by circulating catecholamines have been documented experimentally (72–74). It seems probable that this association helps to explain the link between emotional stress (75) and circadian variation (i.e., early morning clustering of events) (76–78) with myocardial infarction. There has been increasing evidence of enhanced platelet reactivity in cigarette smokers (Fig. 7) (79–81); this reactivity may or may not be related to catecholamine levels (82). The enhanced thrombogenicity of smoking is further confirmed by the finding that there is a sharp decline in acute vascular events most often associated with thrombosis when smoking ceases (83, 84).

Hypercholesterolemia has been linked with hypercoagulability (85–87) and enhanced platelet reactivity (88–90) in many studies. Young patients with a strong family history of coronary artery disease seem to have increased platelet reactivity. The hypercoagulable state, associated with hypercholesterolemia, can be reversed with the normalization of lipid levels with lipid-lowering therapy (87, 91).

Homocysteine has been shown to be associated with arterial thrombosis and atherosclerosis.

Homocysteine increases tissue factor activity of the endothelial cells, possibly with lipoprotein (a). Homocysteine also inhibits the expression of endothelial cell surface thrombomodulin (the substance central to the activation of protein C), and the binding activity of antithrombin III to the endothelial heparan sulfate. Thus, homocysteine reduces the natural anticoagulant properties of the normal endothelium (92–95).

It seems clear that defects within the fibrinolytic pathways lead to an increased thrombotic risk in patients with coronary artery disease (96–98). A correlation between high levels of plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (tPA) and crosslinked fibrin with the progression of atherosclerotic disease has been documented (99). Furthermore, it has been shown that in patients with angina pectoris, plasma levels of fibrinogen, von Willebrand factor (vWF) and tPA are independent predictors of subsequent myocardial infarction or sudden death (86). In patients with some types of dyslipidemia, high levels of PAI-1 correlate with the cholesterol levels. While this suggests a potential mechanism by which hypercholesterolemia is associated with increased thrombogenicity, the association between PAI-1 and coronary artery disease and acute myocardial infarction is unclear, with conflicting reports in the literature (86, 100).

Other hemostatic proteins have also been investigated with regard to their role as thrombotic risk factors. Several prospective studies have indicated that high plasma fibrinogen concentrations are independent risk factors for coronary artery disease and myocardial infarction (101). The mechanism by which fibrinogen contributes to atherogenesis is not well understood. Hypotheses include increased fibrin formation, increased viscosity, platelet aggregation and stimulation of smooth muscle cell proliferation. It is, however, also important to note that high plasma fibrinogen levels correlate with age, degree of obesity, hyperlipidemia, diabetes, smoking, and emotional stress. All of these conditions are associated with atherosclerosis.

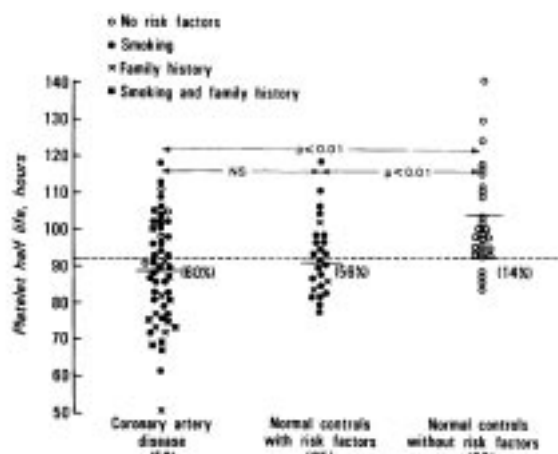


Fig. 7. Effect of smoking and other risk factors on platelet survival. Distribution of platelet survival half-life in three groups of subjects. The absence or presence of cigarette smoking or family history in each subject is indicated. The dashed line (at 92 hours) separates those subjects with a normal platelet half-life (≥ 92 hours) from those with a shorter platelet half-life. The solid short horizontal line shown for each group denotes the average platelet survival half-life for that group. The percentage of individuals in each group whose platelet half-life survival was less than normal is indicated. The number of individuals in each of the three groups was 50, 25 and 28 respectively. (taken from ref. #80)

Coronary Vasoconstriction

Coronary vasospasm may play an important role in the pathogenesis of acute coronary syndromes, as documented through electrocardiographic and angiographic studies (102). In the setting of a minor plaque disruption with a small thrombotic response, there can still be the

release of vasoactive substances by both the platelet and the arterial wall, leading to further compromise of coronary blood flow (103). Coronary artery vasospasm was found to be an important contributor to the phenomenon of intermittent coronary artery occlusion in patients with acute myocardial infarction (104).

Clinical Manifestations of Atherothrombosis

The clinical manifestations of atherosclerotic plaques depend on several factors, including the degree and abruptness of blood flow obstruction, duration of decreased myocardial perfusion, myocardial oxygen demand at the time of the blood flow obstruction, and extent of the thrombotic response to plaque disruption.

Plaque disruption (whether erosion or rupture) may be accompanied by hemorrhage into the plaque and with a variable amount of luminal thrombosis. If the thrombus is small, the plaque disruption probably proceeds unnoticed. If the thrombus is large enough to compromise blood flow through the coronary artery, the individual may experience an acute ischemic syndrome.

Disruption of an atherosclerotic plaque in the coronary arteries, whether ruptured or fissured, plays a fundamental role in the development of the acute coronary syndromes, including unstable angina pectoris, acute myocardial infarction or sudden cardiac death (20, 36, 105, 106). Coronary thrombosis almost exclusively occurs in the setting of underlying atherosclerosis, with disruption of the underlying plaque triggering thrombosis (107). Angioscopic studies have documented the presence of intraluminal thrombi both in unstable angina (108–112) and in acute myocardial infarction (111, 113). The incidence of thrombi in unstable angina varied significantly among different studies. These variations were related to the time between anginal symptoms and the angiographic study (110, 114–116). The shorter the interval between the two, generally the higher the likelihood of finding occlusive thrombi.

When injury to the vessel wall is minimal, the thrombogenic stimulus is relatively mild. Any resulting thrombotic occlusion is probably transient, as may occur in unstable angina (105, 106). On the other hand, deep vessel injury, as is seen with plaque rupture, results in the exposure of collagen, lipids and other intravascular components, which leads to a more persistent thrombotic occlusion and acute myocardial infarction (107).

Plaque fissuring or rupture with subsequent thrombosis accounts for many episodes of un-

stable angina or acute myocardial infarction. However, other mechanisms may be important in the etiology of acute coronary syndromes; one such mechanism involves alterations in the balance between myocardial oxygen supply and demand. In patients with stable coronary artery disease, symptoms often result from increases in myocardial oxygen demand. Unstable angina, non-Q-wave and Q-wave myocardial infarction represent a continuum of the same disease process and, in contrast to the setting of stable angina, are usually characterized by an abrupt reduction in coronary artery blood flow (107). In unstable angina, the thrombotic vessel occlusion tends to be transient and episodic, leading to anginal symptoms at rest. In addition to plaque disruption, other mechanisms may contribute to the reduction in coronary flow. As mentioned earlier, platelets that have attached to the disrupted plaque release vasoactive substances including thromboxane A₂ and serotonin, promoting the aggregation of further platelets to the area and inducing vasoconstriction (103). Alterations in perfusion probably account for 60–70% of cases of unstable angina. The remainder appears to be mainly due to transient increases in myocardial oxygen demand (117).

In non-Q-wave myocardial infarction, the angiographic morphology of the responsible lesion is similar to that seen in unstable angina, confirming that plaque disruption is common to both syndromes. However, about 25% of patients with non-Q-wave myocardial infarction have a totally occluded, infarct-related artery at early angiography, with the distal myocardium supplied by collateral vessels (107). The presence of ST-segment elevation in the electrocardiogram, the early peak in the plasma creatine kinase and the high angiographic patency rate of the infarct-related artery all suggest that complete coronary occlusion followed by early reperfusion (< two hours) due to resolution (partial or total) of the thrombus and/or of the vasospasm is important in the pathogenesis of most non-Q-wave myocardial infarctions. Thus, limiting the duration of myocardial ischemia by enhancing spontaneous thrombolysis, inhibiting or resolving vasospasm, and promoting a well-developed collateral circulation can prevent the formation of Q-wave myocardial infarction (107).

Deep arterial injury or ulceration results in the formation of a fixed and persistent thrombus leading to the abrupt cessation of myocardial perfusion, and necrosis associated with

Q-wave myocardial infarction (105, 106). The coronary artery lesion responsible for the infarction is frequently only mild to moderately stenotic, suggesting that plaque rupture with subsequent thrombosis is the primary source of the occlusion, rather than the severity of the underlying lesion (Fig. 8) (36). Although an individual severe stenosis has been shown to occlude more frequently than a milder stenosis, the latter accounts for more coronary artery occlusions, due to its much greater frequency (118). Moreover, the milder stenoses are much less likely to be associated with a collateral circulation that might protect against an acute clinical event (119) if an occlusion does occur. In approximately 25% of patients with Q-wave infarction, coronary thrombosis results from superficial intimal injury in association with a high-grade stenosis (105, 106).

The acute onset of malignant ventricular dysrhythmias (ventricular tachycardia and ventricular fibrillation) appears to account for the syndrome of sudden cardiac death in patients with extensive myocardial infarction or cardiomyopathy (120). A malignant ventricular dysrhythmia and sudden cardiac death may also occur with a rapidly progressive coronary artery lesion following plaque rupture and thrombosis in the face of little or no collateral

flow and acute myocardial hypoperfusion (120).

Vascular Biology of Risk Factors

Lipoproteins

Elevated low-density lipoprotein (LDL) is associated with endothelial injury and inflammatory responses in the vessel wall (121–123). These effects of LDL are especially potent when modified by oxidation or glycation, or associated with immune complexes (121, 124–126). Oxidized LDL is avidly taken up by tissue macrophages within the vessel wall, either via LDL receptors (which are subject to down-regulation) or scavenger receptors (which are not subject to feedback mechanisms), leading to the accumulation of cholesterol esters and eventually foam cell formation (127). These foam cells may undergo necrosis due to the direct cytotoxic effects of modified LDL or a process of programmed cell death called apoptosis, induced by certain inflammatory cytokines (20, 128). These two processes lead to the accumulation of extracellular lipid that may coalesce, forming a lipidic, necrotic core.

The density, not just the quantity, of the systemic LDL particles has been shown to correlate with risk of atherosclerotic disease (129). Small, dense LDL particles are more susceptible to peroxidation (130, 131) and thus promote atherogenesis by the mechanisms described above.

Antioxidants have been shown to reduce the size of atherosclerotic lesions (123, 132–135) and fatty streaks (135) in animal models. Antioxidants have also been shown to increase the resistance of human LDL to oxidation *ex vivo*, commensurate with the plasma vitamin E levels (136). Although the incidence of myocardial infarction has been reduced by vitamin E supplementation in some preliminary clinical trials, a clear mortality benefit from this therapy has not been shown (137–140). Other antioxidants (e.g., β -carotene) appear to have no independent cardiovascular benefits (136, 141, 142).

Triglyceride-rich lipoproteins appear to be important contributors to coronary atherosclerosis. Mechanisms include increased thrombogenicity; small, dense LDLs; postprandial lipemia with increased chylomicrons and very low density lipoproteins (VLDLs); decreased high-density lipoprotein (HDL) levels; and insulin resistance.

STENOSIS SEVERITY - OCCLUSION - MI

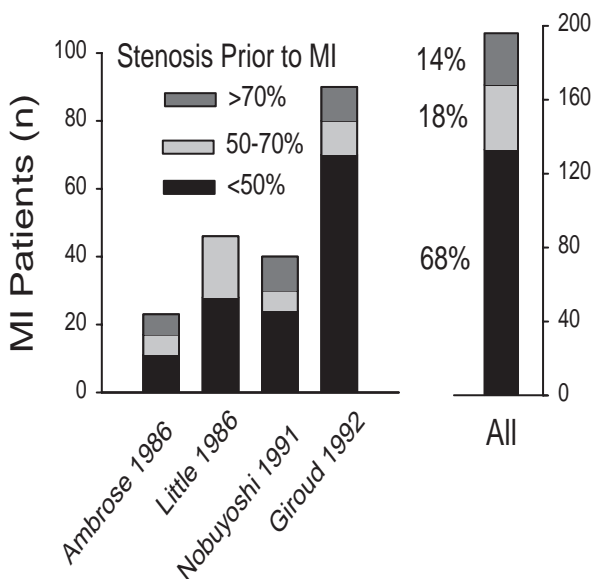


Fig. 8. Meta-analysis of studies showing the association between stenosis severity and associated risk of coronary occlusion and myocardial infarction (MI). (taken from ref. #36)

HDL fractions appear to counter the atherogenic effects of LDL, although it is unclear which mechanism(s) is responsible. There may be lipid dependent and independent effects (143). Clearly, the role of HDL in reverse cholesterol transport (the removal of cholesterol from peripheral, extrahepatic sites including the vessel wall and returning it to the liver for metabolism or excretion) can explain some of its atheroprotective effect (143). However, HDL (of which there are discrete subfractions with variable effects) has also been shown to have antioxidant (144) and anti-inflammatory effects (145, 146). In a recent, large-scale clinical trial, therapy specifically directed at elevating the HDL in patients with coronary artery disease and low HDL level was shown to reduce significantly the risk of a major cardiovascular event (147).

Lipoprotein (a) is an independent risk factor for coronary artery disease (148–151). Apolipoprotein (a), the major apoprotein found in lipoprotein (a), has close structural homology with plasminogen (152). There is evidence that high levels of lipoprotein (a) result in competitive inhibition of the fibrinolytic activity of plasminogen (153). However, this metabolic condition appears more important in atherogenesis than thrombogenesis (154).

Diabetes

Diabetes is associated with a severely dysfunctional endothelium. Impaired endothelium-dependent relaxation, the best characterized of these abnormalities, can be induced by short exposure to high glucose concentrations. Diabetes may impair endothelium-dependent relaxation by an increased generation of advanced glycosylation end products and increased oxygen free radicals in the arterial wall (155). High glucose levels have also been shown to impair endothelial regeneration (156, 157).

Platelet aggregation and coagulation are increased in diabetes mellitus. Platelets from diabetic patients have shown enhanced adhesiveness and hyperaggregability in response to a wide range of agonists (156, 157). Elevated thromboxane A₂ synthesis occurs in diabetic patients, facilitating platelet aggregation and thrombus formation (158). The primary reason for altered platelet behavior in diabetes is not well understood, but there is evidence that the derangement may start with the megakaryocyte. Other abnormalities in the coagulation system of diabetic patients include increased fibrino-

gen and vWF levels and decreased antithrombin III activity in response to hyperglycemia. In addition, a typical feature of insulin resistance and hyperinsulinemia is an increased PAI-1 activity, resulting in reduced plasma fibrinolytic activity (159).

Hypertension

One of the mechanisms by which hypertension promotes atherogenesis is through the induction of endothelial dysfunction. Furthermore, hypertension appears to attenuate responses of the vessel wall to endothelium-dependent vasodilators, to increase vascular permeability to macromolecules such as lipoproteins, and to increase endothelin production and inflammatory cell adherence. Hypertension is also associated with phenotypic changes in vascular smooth muscle cells, increasing their proliferative potential and response to growth factors.

Smoking

Smoking is clearly linked to many events known to induce atherosclerosis, including increased fibrinogen levels, enhanced platelet reactivity and increased whole blood viscosity associated with secondary polycythemia. Smoking is a potent stimulus for the induction of endothelial dysfunction. Smoking is known to induce changes in the lipid profile, including lower HDL and increased oxidation of LDL, the latter of which is presumed to be associated with exposure of LDL to free radicals present in cigarette smoke.

Obesity and Physical Inactivity

Obesity, in the absence of other risk factors for atherosclerosis, may be associated with only a small increased risk of coronary artery disease. However, it is clearly associated with the development of dyslipidemia, hypertension and diabetes. Physical activity positively alters the lipid profile, blood pressure, and glucose tolerance, and improves cardiovascular and pulmonary functional capacity. Physical fitness, which can be more objectively quantified than physical activity, independently reduces the risk of coronary heart disease.

Genetic Factors (Family History)

Single gene mutations that alter lipid metabolism and thus are associated with acceler-

ated atherogenesis have been identified. However, no other single gene mutations enhance the currently known atherogenic risk factors. Some patients with dyslipidemias, hypertension, diabetes mellitus and homocystinemia have complex polygenic disorders. Currently identifiable genetic abnormalities, however, account for only a small number of patients with premature coronary artery disease.

Miscellaneous

Many other markers for risk of acute coronary events have emerged, and research continues into the role they play in the pathogenesis of atherosclerosis and its complications (Fig. 9). When considered together, they can provide important information regarding risk stratification of patients (160, 161).

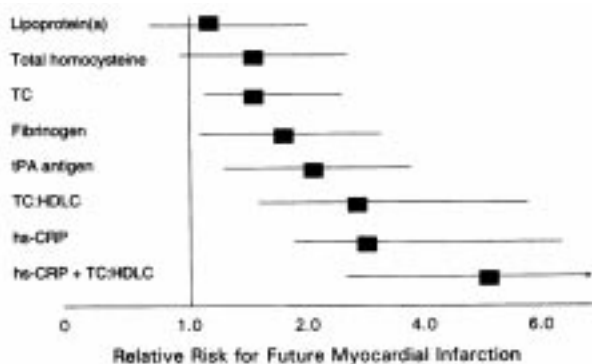


Fig. 9. Cardiovascular risk factor and relative risk for recurrent myocardial infarction among healthy middle-aged men. **TC.** - total cholesterol; **tPA.** - tissue plasminogen activator; **TC:HDL-C.** - ratio of total to HDL-cholesterol; **hs-CRP.** - high-sensitivity C-reactive protein. (taken from ref. #160 and 161).

Conclusions

Many recent advances in our understanding of the molecular biology of atherogenesis and the mechanisms involved in the acute coronary syndromes have provided insights into potential future therapies. Risk factor modification is the cornerstone of prevention and should be pursued aggressively. However, we may in the future be actively treating at-risk patients with therapies that act at a cellular or molecular level (such as inhibitors of specific macrophage functions or direct MMP inhibitors) to impede atherosclerosis, rather than just modifying risk factors.

References

1. Stary HC, Chandler AB, Glagov S, et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994; 89:2462–2478.
2. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995; 75:519–560.
3. Gimbrone MA, Jr. Vascular endothelium, hemodynamic forces, and atherogenesis. *Am J Pathol* 1999; 155:1–5.
4. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. *Arteriosclerosis* 1985; 5:293–302.
5. Ross R, Bowen-Pope DF, Raines EW. Platelet-derived growth factor and its role in health and disease. *Philos Trans R Soc Lond B Biol Sci* 1990; 327:155–169.
6. Grabowski EF, Jaffe EA, Weksler BB. Prostacyclin production by cultured endothelial cell monolayers exposed to step increases in shear stress. *J Lab Clin Med* 1985; 105:36–43.
7. Vanhoutte PM. Endothelium and control of vascular function. State of the art lecture. *Hypertension* 1989; 13:658–667.
8. Busse R, Pohl U, Luckhoff A. Mechanisms controlling the production of endothelial autacoids. *Z Kardiol* 1989; 78:64–69.
9. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol* 1986; 250:H1145–1149.
10. Malek AM, Jackman R, Rosenberg RD, Izumo S. Endothelial expression of thrombomodulin is reversibly regulated by fluid shear stress. *Circ Res* 1994; 74:852–860.
11. Takada Y, Shinkai F, Kondo S, et al. Fluid shear stress increases the expression of thrombomodulin by cultured human endothelial cells. *Biochem Biophys Res Commun* 1994; 205:1345–1352.
12. Kawai Y, Matsumoto Y, Ikeda Y, Watanabe K. Regulation of antithrombogenicity in endothelium by hemodynamic forces. *Rinsho Byori* 1997; 45:315–320.
13. Lin MC, Almus-Jacobs F, Chen HH, et al. Shear stress induction of the tissue factor gene. *J Clin Invest* 1997; 99:737–744.
14. Simionescu N, Vasile E, Lupu F, et al. Prelesional events in atherogenesis. Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. *Am J Pathol* 1986; 123:109–125.
15. Napoli C, D'Armiento FP, Mancini FP, et al. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 1997; 100:2680–2690.
16. Rajavashisth TB, Andalibi A, Territo MC, et al. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* 1990; 344:254–257.
17. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993; 178:449–460.
18. Qiao JH, Tripathi J, Mishra NK, et al. Role of macrophage colony-stimulating factor in atherosclerosis: Studies of osteopetrotic mice. *Am J Pathol* 1997; 150:1687–1699.
19. de Villiers WJ, Smith JD, Miyata M, et al. Macrophage phenotype in mice deficient in both macrophage-colony-stimulating factor (op) and apolipoprotein E. *Arterioscler Thromb Vasc Biol* 1998; 18:631–640.

20. Ross R. Atherosclerosis — an inflammatory disease. *N Engl J Med* 1999; 340:115–126.
21. Libby P, Ross R. Cytokines and growth regulatory molecules. Philadelphia: Lippincott-Raven; 1996.
22. Celentano DC, Frishman WH. Matrix metalloproteinases and coronary artery disease: A novel therapeutic target. *J Clin Pharmacol* 1997; 37:991–1000.
23. Pauly RR, Passaniti A, Bilato C, et al. Migration of cultured vascular smooth muscle cells through a basement membrane barrier requires type IV collagenase activity and is inhibited by cellular differentiation. *Circ Res* 1994; 75:41–54.
24. de Boer OJ, van der Wal AC, Becker AE. Atherosclerosis, inflammation, and infection. *J Pathol* 2000; 190(3):237–243.
25. Jonasson L, Holm J, Skalli O, et al. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. *Arteriosclerosis* 1986; 6:131–138.
26. Stemme S, Faber B, Holm J, et al. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci U S A* 1995; 92:3893–3897.
27. Kraiss LW, Raines EW, Wilcox JN, et al. Regional expression of the platelet-derived growth factor and its receptors in a primate graft model of vessel wall assembly. *J Clin Invest* 1993; 92:338–348.
28. Sharefkin JB, Diamond SL, Eskin SG, et al. Fluid flow decreases preproendothelin mRNA levels and suppresses endothelin-1 peptide release in cultured human endothelial cells. *J Vasc Surg* 1991; 14:1–9.
29. Rieder MJ, Carmona R, Krieger JE, et al. Suppression of angiotensin-converting enzyme expression and activity by shear stress. *Circ Res* 1997; 80:312–319.
30. Buga GM, Gold ME, Fukuto JM, Ignarro LJ. Shear stress-induced release of nitric oxide from endothelial cells grown on beads. *Hypertension* 1991; 17:187–193.
31. Ohno M, Cooke JP, Dzau VJ, Gibbons GH. Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. Modulation by potassium channel blockade. *J Clin Invest* 1995; 95:1363–1369.
32. Ambrose JA, Tannenbaum MA, Alexopoulos D, et al. Angiographic progression of coronary artery disease and the development of myocardial infarction. *J Am Coll Cardiol* 1988; 12:56–62.
33. Giroud D, Li JM, Urban P, et al. Relation of the site of acute myocardial infarction to the most severe coronary arterial stenosis at prior angiography. *Am J Cardiol* 1992; 69:729–732.
34. Little WC, Constantinescu M, Applegate RJ, et al. Can coronary angiography predict the site of a subsequent myocardial infarction in patients with mild-to-moderate coronary artery disease? *Circulation* 1988; 78:1157–1166.
35. Nobuyoshi M, Tanaka M, Nosaka H, et al. Progression of coronary atherosclerosis: Is coronary spasm related to progression? *J Am Coll Cardiol* 1991; 18:904–910.
36. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995; 92:657–671.
37. Davies MJ, Thomas AC. Plaque fissuring — the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. *Br Heart J* 1985; 53:363–373.
38. Mann JM, Davies MJ. Vulnerable plaque. Relation of characteristics to degree of stenosis in human coronary arteries. *Circulation* 1996; 94:928–931.
39. Falk E. Why do plaques rupture? [review] *Circulation* 1992; 86(6 Suppl):III30–III42.
40. Davies MJ. Stability and instability: Two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. *Circulation* 1996; 94:2013–2020.
41. Felton CV, Crook D, Davies MJ, Oliver MF. Relation of plaque lipid composition and morphology to the stability of human aortic plaques. *Arterioscler Thromb Vasc Biol* 1997; 17:1337–1345.
42. Falk E. Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis. Characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. *Br Heart J* 1983; 50:127–134.
43. Richardson PD, Davies MJ, Born GV. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet* 1989; 2:941–944.
44. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994; 89:36–44.
45. Frink RJ. Chronic ulcerated plaques: New insights into the pathogenesis of acute coronary disease. *J Invasive Cardiol* 1994; 6:173–185.
46. Falk E. Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. *Am J Cardiol* 1989; 63:114E–120E.
47. Davies MJ, Richardson PD, Woolf N, et al. Risk of thrombosis in human atherosclerotic plaques: Role of extracellular lipid, macrophage, and smooth muscle cell content. *Br Heart J* 1993; 69:377–381.
48. Wagner WD, St. Clair RW, Clarkson TB, Connor JR. A study of atherosclerosis regression in *Macaca mulatta*: III. Chemical changes in arteries from animals with atherosclerosis induced for 19 months and regressed for 48 months at plasma cholesterol concentrations of 300 or 200 mg/dL. *Am J Pathol* 1980; 100:633–650.
49. Loree HM, Tobias BJ, Gibson LJ, et al. Mechanical properties of model atherosclerotic lesion lipid pools. *Arterioscler Thromb* 1994; 14:230–234.
50. Small DM. George Lyman Duff memorial lecture. Progression and regression of atherosclerotic lesions. Insights from lipid physical biochemistry. *Arteriosclerosis* 1988; 8:103–129.
51. Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 1995; 146:3–15.
52. Constantinides P. Plaque fissures in human coronary thrombosis. *J Atheroscler Res* 1966; 6:1–17.
53. Friedman M. The coronary thrombus: Its origin and fate. *Hum Pathol* 1971; 2:81–128.
54. Shah PK, Falk E, Badimon JJ, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* 1995; 2:1565–1569.
55. Sukhova GK, Schonbeck U, Rabkin E, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation* 1999; 99:2503–2509.
56. Brown DL, Hibbs MS, Kearney M, et al. Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation* 1995; 91:2125–2131.
57. Malik N, Greenfield BW, Wahl AF, Kiener PA. Activation of human monocytes through CD40 induces matrix metalloproteinases. *J Immunol* 1996; 156:3952–3960.
58. Lee RT, Kamm R. Vascular mechanics for the cardiologist. *J Am Coll Cardiol* 1994; 23:1289–1295.
59. MacIsaac AI, Thomas JD, Topol EJ. Toward the quiescent coronary plaque. *J Am Coll Cardiol* 1993; 22:1228–1241.

60. Lin CS, Penha PD, Zak FG, Lin JC. Morphodynamic interpretation of acute coronary thrombosis, with special reference to volcano-like eruption of atheromatous plaque caused by coronary artery spasm. *Angiology* 1988; 39:535–547.
61. Fernandez-Ortiz A, Badimon JJ, Falk E, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: Implications for consequences of plaque rupture. *J Am Coll Cardiol* 1994; 23:1562–1569.
62. Toschi V, Gallo R, Lettino M, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation* 1997; 95:594–599.
63. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A* 1989; 86:2839–2843.
64. Thiruvikraman SV, Guha A, Roboz J, et al. In situ localization of tissue factor in human atherosclerotic plaques by binding of digoxigenin-labeled factors VIIa and X. *Lab Invest* 1996; 75:451–461.
65. Annex BH, Denning SM, Channon KM, et al. Differential expression of tissue factor protein in directional atherectomy specimens from patients with stable and unstable coronary syndromes. *Circulation* 1995; 91:619–622.
66. Moreno PR, Falk E, Palacios IF, et al. Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. *Circulation* 1994; 90:775–778.
67. Moreno PR, Bernardi VH, Lopez-Cuellar J, et al. Macrophages, smooth muscle cells, and tissue factor in unstable angina. Implications for cell-mediated thrombogenicity in acute coronary syndromes. *Circulation* 1996; 94:3090–3097.
68. Mallat Z, Hugel B, Ohan J, et al. Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques: A role for apoptosis in plaque thrombogenicity. *Circulation* 1999; 99:348–353.
69. Suefujii H, Ogawa H, Yasue H, et al. Increased plasma tissue factor levels in acute myocardial infarction. *Am Heart J* 1997; 134:253–259.
70. Soejima H, Ogawa H, Yasue H, et al. Plasma tissue factor pathway inhibitor and tissue factor antigen levels after administration of heparin in patients with angina pectoris. *Thromb Res* 1999; 93:17–25.
71. Giesen PL, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: Another view of thrombosis. *Proc Natl Acad Sci U S A* 1999; 96:2311–2315.
72. Badimon L, Martinez-Gonzalez J, Royo T, et al. A sudden increase in plasma epinephrine levels transiently enhances platelet deposition on severely damaged arterial wall. *Thrombosis and Haemostasis* 1999; 82:1736–1742.
73. Spalding A, Vaitkevicius H, Dill S, et al. Mechanism of epinephrine-induced platelet aggregation. *Hypertension* 1998; 31:603–607.
74. Goto S, Handa S, Takahashi E, et al. Synergistic effect of epinephrine and shearing on platelet activation. *Thromb Res* 1996; 84:351–359.
75. Krantz DS, Kop WJ, Santiago HT, Gottdiener JS. Mental stress as a trigger of myocardial ischemia and infarction. *Clin Clin* 1996; 14:271–287.
76. Muller JE, Stone PH, Turi ZG, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 1985; 313:1315–1322.
77. Johnstone MT, Mittleman M, Tofler G, Muller JE. The pathophysiology of the onset of morning cardiovascular events. *Am J Hypertens* 1996; 9:22S–28S.
78. Willich SN, Linderer T, Wegscheider K, et al. Increased morning incidence of myocardial infarction in the ISAM Study: Absence with prior beta-adrenergic blockade. ISAM Study Group. *Circulation* 1989; 80:853–855.
79. Winniford MD, Wheelan KR, Kremers MS, et al. Smoking-induced coronary vasoconstriction in patients with atherosclerotic coronary artery disease: Evidence for adrenergically mediated alterations in coronary artery tone. *Circulation* 1986; 73:662–667.
80. Fuster V, Chesebro JH, Frye RL, Elveback LR. Platelet survival and the development of coronary artery disease in the young adult: Effects of cigarette smoking, strong family history and medical therapy. *Circulation* 1981; 63:546–551.
81. Blann AD, Kirkpatrick U, Devine C, et al. The influence of acute smoking on leucocytes, platelets and the endothelium. *Atherosclerosis* 1998; 141:133–139.
82. Powell JT. Vascular damage from smoking: disease mechanisms at the arterial wall. *Vasc Med* 1998; 3:21–28.
83. Paul O. Background of the prevention of cardiovascular disease. II. Arteriosclerosis, hypertension, and selected risk factors. *Circulation* 1989; 80:206–214.
84. Buhler FR, Vesanen K, Watters JT, Bolli P. Impact of smoking on heart attacks, strokes, blood pressure control, drug dose, and quality of life aspects in the International Prospective Primary Prevention Study in Hypertension. *Am Heart J* 1988; 115:282–288.
85. Hunt BJ. The relation between abnormal hemostatic function and the progression of coronary disease. *Curr Opin Cardiol* 1990; 5:758–765.
86. Thompson SG, Kienast J, Pyke SD, et al. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *N Engl J Med* 1995; 332:635–641.
87. Rauch U, Osende JJ, Chesebro JH, et al. Statins and cardiovascular diseases: The multiple effects of lipid-lowering therapy by statins. *Atherosclerosis* 2000; 153:181–189.
88. Badimon JJ, Badimon L, Turitto VT, Fuster V. Platelet deposition at high shear rates is enhanced by high plasma cholesterol levels. In vivo study in the rabbit model. *Arterioscler Thromb* 1991; 11:395–402.
89. Carvalho AC, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. *N Engl J Med* 1974; 290:434–438.
90. Henry PD, Cabello OA, Chen CH. Hypercholesterolemia and endothelial dysfunction. *Curr Opin Lipidol* 1995; 6:190–195.
91. Lacoste L, Lam JY, Hung J, et al. Hyperlipidemia and coronary disease. Correction of the increased thrombogenic potential with cholesterol reduction. *Circulation* 1995; 92:3172–3177.
92. Boers GH. Hyperhomocysteinemia as a risk factor for arterial and venous disease. A review of evidence and relevance. *Thromb Haemost* 1997; 78:520–522.
93. Boers GH, Smals AG, Trijbels FJ, et al. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med* 1985; 313:709–715.
94. de Jong SC, van den Berg M, Rauwerda JA, Stehouwer CD. Hyperhomocysteinemia and atherothrombotic disease. *Semin Thromb Hemost* 1998; 24:381–385.
95. Prasad K. Homocysteine, a risk factor for cardiovascular disease. *Int J Angiology* 1999; 8:76–86.
96. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med* 2000; 342:1792–1801.

97. Olofsson BO, Dahlen G, Nilsson TK. Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary artery disease. *Eur Heart J* 1989; 10:77–82.
98. Geppert A, Graf S, Beckmann R, et al. Concentration of endogenous tPA antigen in coronary artery disease: relation to thrombotic events, aspirin treatment, hyperlipidemia, and multivessel disease. *Arterioscler Thromb Vasc Biol* 1998; 18:1634–1642.
99. Thompson SG, Kienast J, Pike SDM, et al. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with unstable angina. *N Engl J Med* 1995; 332:635–641.
100. Hamsten A, Wiman B, de Faire U, Blomback M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. *N Engl J Med* 1985; 313:1557–1563.
101. Meade TW. Fibrinogen and cardiovascular disease. *J Clin Pathol* 1997; 50:13–15.
102. Maseri A, L'Abbate A, Baroldi G, et al. Coronary vasospasm as a possible cause of myocardial infarction. A conclusion derived from the study of "preinfarction" angina. *N Engl J Med* 1978; 299:1271–1277.
103. Willerson JT, Golino P, Eidt J, et al. Specific platelet mediators and unstable coronary artery lesions. Experimental evidence and potential clinical implications. *Circulation* 1989; 80:198–205.
104. Gasser RN, Dienstl F, Puschendorf B, et al. New perspectives on the function of coronary artery spasm in acute myocardial infarction: the thromboischemic reentry mechanism. A review of 10 years research on the pathophysiology of AMI. *Angiology* 1986; 37:880–887.
105. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992; 326:242–250.
106. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (2). *N Engl J Med* 1992; 326:310–318.
107. Theroux P, Fuster V. Acute coronary syndromes: Unstable angina and non-Q-wave myocardial infarction. *Circulation* 1998; 97:1195–1206.
108. de Feyter PJ, Ozaki Y, Baptista J, et al. Ischemia-related lesion characteristics in patients with stable or unstable angina. A study with intracoronary angiography and ultrasound. *Circulation* 1995; 92:1408–1413.
109. Silva JA, Escobar A, Collins TJ, et al. Unstable angina. A comparison of angiographic findings between diabetic and nondiabetic patients. *Circulation* 1995; 92:1731–1736.
110. Sherman CT, Litvack F, Grundfest W, et al. Coronary angiography in patients with unstable angina pectoris. *N Engl J Med* 1986; 315:913–919.
111. Mizuno K, Satomura K, Miyamoto A, et al. Angiographic evaluation of coronary-artery thrombi in acute coronary syndromes. *N Engl J Med* 1992; 326:287–291.
112. Nesto RW, Waxman S, Mittleman MA, et al. Angiography of culprit coronary lesions in unstable angina pectoris and correlation of clinical presentation with plaque morphology. *Am J Cardiol* 1998; 81:225–228.
113. Van Belle E, Lablanche JM, Bauters C, et al. Coronary angiographic findings in the infarct-related vessel within 1 month of acute myocardial infarction: Natural history and the effect of thrombolysis. *Circulation* 1998; 97:26–33.
114. Uchida Y, Tomaru T, Nakamura F, et al. Percutaneous coronary angiography in patients with ischemic heart disease. *Am Heart J* 1987; 114:1216–1222.
115. Uchida Y, Nakamura F, Tomaru T, et al. Prediction of acute coronary syndromes by percutaneous coronary angiography in patients with stable angina. *Am Heart J* 1995; 130:195–203.
116. Rehr R, Disciascio G, Vetovec G, Cowley M. Angiographic morphology of coronary artery stenoses in prolonged rest angina: Evidence of intracoronary thrombosis. *J Am Coll Cardiol* 1989; 14:1429–1437.
117. Braunwald E, Jones RH, Mark DB, et al. Diagnosing and managing unstable angina. Agency for Health Care Policy and Research. *Circulation* 1994; 90:613–622.
118. Alderman EL, Corley SD, Fisher LD, et al. Five-year angiographic follow-up of factors associated with progression of coronary artery disease in the Coronary Artery Surgery Study (CASS). CASS Participating Investigators and Staff. *J Am Coll Cardiol* 1993; 22:1141–1154.
119. Danchin N. Is myocardial revascularisation for tight coronary stenoses always necessary? *Lancet* 1993; 342:224–225.
120. Mehta D, Curwin J, Gomes JA, Fuster V. Sudden death in coronary artery disease: Acute ischemia versus myocardial substrate. *Circulation* 1997; 96:3215–3223.
121. Griendling KK, Alexander RW. Oxidative stress and cardiovascular disease. *Circulation* 1997; 96:3264–3265.
122. Morel DW, Hessler JR, Chisolm GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. *J Lipid Res* 1983; 24:1070–1076.
123. Navab M, Berliner JA, Watson AD, et al. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol* 1996; 16:831–842.
124. Khoo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. *Arteriosclerosis* 1988; 8:348–358.
125. Khoo JC, Miller E, Pio F, et al. Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. *Arterioscler Thromb* 1992; 12:1258–1266.
126. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997; 272:20963–20966.
127. Brown MS, Goldstein JL. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem* 1983; 52:223–261.
128. Libby P, Geng YJ, Aikawa M, et al. Macrophages and atherosclerotic plaque stability. *Curr Opin Lipidol* 1996; 7:330–335.
129. Slyper AH. Low-density lipoprotein density and atherosclerosis. Unraveling the connection. *JAMA* 1994; 272:305–308.
130. Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 1992; 93:189–199.
131. de Graaf J, Hak-Lemmers HL, Hectors MP, et al. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscler Thromb* 1991; 11:298–306.
132. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci U S A* 1987; 84:7725–7729.

133. Kita T, Nagano Y, Yokode M, et al. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci U S A* 1987; 84:5928–5931.
134. Sasahara M, Raines EW, Chait A, et al. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. I. Is the extent of atherosclerosis related to resistance of LDL to oxidation? *J Clin Invest* 1994; 94:155–164.
135. Chang MY, Sasahara M, Chait A, et al. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. II. Cellular composition and proliferation. *Arterioscler Thromb Vasc Biol* 1995; 15:1631–1640.
136. Reaven PD, Khouw A, Beltz WF, et al. Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by beta-carotene. *Arterioscler Thromb* 1993; 13:590–600.
137. The Heart Outcomes Prevention Evaluation (HOPE) Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. *N Engl J Med* 2000; 342:154–160.
138. Rimm EB, Stampfer MJ, Ascherio A, et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; 328:1450–1456.
139. Stampfer MJ, Hennekens CH, Manson JE, et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; 328:1444–1449.
140. Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; 347:781–786.
141. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996; 334:1150–1155.
142. Hennekens CH, Buring JE, Manson JE, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996; 334:1145–1149.
143. Barter PJ, Rye KA. High density lipoproteins and coronary heart disease. *Atherosclerosis* 1996; 121:1–12.
144. Decossin C, Tailleux A, Fruchart JC, Fievet C. Prevention of *in vitro* low-density lipoprotein oxidation by an albumin-containing Lp A-I subfraction. *Biochim Biophys Acta* 1995; 1255:31–38.
145. Ulevitch RJ, Johnston AR, Weinstein DB. New function for high density lipoproteins. Isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma. *J Clin Invest* 1981; 67:827–837.
146. Ashby DT, Rye KA, Clay MA, et al. Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells. *Arterioscler Thromb Vasc Biol* 1998; 18:1450–1455.
147. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999; 341:410–418.
148. Dahlen GH, Guyton JR, Attar M, et al. Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. *Circulation* 1986; 74:758–765.
149. Djurovic S, Berg K. Epidemiology of Lp(a) lipoprotein: Its role in atherosclerotic/thrombotic disease. *Clin Genet* 1997; 52:281–292.
150. Seed M, Hoppichler F, Reaveley D, et al. Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. *N Engl J Med* 1990; 322:1494–1499.
151. Hopkins PN, Hunt SC, Schreiner PJ, et al. Lipoprotein(a) interactions with lipid and non-lipid risk factors in patients with early onset coronary artery disease: Results from the NHLBI Family Heart Study. *Atherosclerosis* 1998; 141:333–345.
152. McLean JW, Tomlinson JE, Kuang WJ, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987; 330:132–137.
153. Scanu AM. Atherothrombogenicity of lipoprotein(a): The debate. *Am J Cardiol* 1998; 82:26Q–33Q.
154. Allen S, Khan S, Tam S, et al. Expression of adhesion molecules by lp(a): A potential novel mechanism for its atherogenicity. *FASEB J* 1998; 12:1765–1776.
155. Chappey O, Dosquet C, Wautier MP, Wautier JL. Advanced glycation end products, oxidant stress and vascular lesions. *Eur J Clin Invest* 1997; 27:97–108.
156. Aronson D, Rayfield EJ, Chesebro JH. Mechanisms determining course and outcome of diabetic patients who have had acute myocardial infarction. *Ann Intern Med* 1997; 126:296–306.
157. Winocour PD. Platelet abnormalities in diabetes mellitus. *Diabetes* 1992; 41 Suppl 2:26–31.
158. Davi G, Catalano I, Averna M, et al. Thromboxane biosynthesis and platelet function in type II diabetes mellitus. *N Engl J Med* 1990; 322:1769–1774.
159. McGill JB, Schneider DJ, Arfken CL, et al. Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. *Diabetes* 1994; 43:104–109.
160. Badimon JJ, Lettino M, Toschi V, et al. Local inhibition of tissue factor reduces the thrombogenicity of disrupted human atherosclerotic plaques: Effects of tissue factor pathway inhibitor on plaque thrombogenicity under flow conditions. *Circulation* 1999; 99:1780–1787.
161. Ridker PM. Evaluating novel cardiovascular risk factors: Can we better predict heart attacks? *Ann Intern Med* 1999; 130:933–937.