The existence of atherosclerosis has been recognized for >500 years; as a pathological condition, it has been recognized for >150 years. Understanding of atherosclerotic vascular disease (AVD) has evolved most dramatically over the past 25 years with the growth of the field of vascular biology. Numerous studies have described this disease as a diffuse and progressive process with a variable distribution and clinical presentation that is dependent on the regional circulation involved. Factors that may influence these differences include the size and structure of the affected artery, local and regional flow, changes in microcirculatory alterations, and end-organ damage.

This report discusses the general concepts of atherosclerosis, pathophysiology, microcirculatory disturbances, regional responses to atherosclerosis and ischemia, and recommendations for future research, programs, and advocacy.

Pathophysiology of Atherosclerosis in Peripheral Arterial Disease
Atherosclerosis involves several highly interrelated processes, including lipid disturbances, platelet activation, thrombosis, endothelial dysfunction, inflammation, oxidative stress, vascular smooth cell activation, altered matrix metabolism, remodeling, and genetic factors. This sequence is shown schematically in Figure 1 and described in detail below.

Risk Factors
Risk factors play an important role in initiating and accelerating the complex process of atherosclerosis. Risk factors for atherosclerosis are also the primary method of risk assessment and the target for therapeutic intervention in the prevention of premature vascular disease. Interestingly, the impact of these risk factors on disease development and progression in the peripheral vasculature are not the same as those in the coronary vessels and may represent an avenue of investigation to explain variations in clinical presentation among diverse groups.

The risk factor most correlated with onset and progression of peripheral arterial disease (PAD) is cigarette smoking. Smokers have a 1.7- to 5.6-fold increase in development of disease compared with nonsmokers. Worse, smoking increases development of intermittent claudication, the symptomatic form of PAD, by as much as 8- to 10-fold. In this population, smoking cessation is associated with lower rates of amputation and longer survival and should be vigorously pursued despite poor rates of long-term cessation.

Diabetes mellitus confers a similar increase in risk, augmenting rates of intermittent claudication in men with glycosuria 3.5-fold compared with men who are not diabetic. Interestingly, in women, risk increased 860% to a level similar to that in men with glycosuria. Thus, diabetes may eliminate the protection against atherosclerosis that is a benefit of being female. Diabetes adversely modifies the clinical course of PAD and is the most common cause of amputation in the United States, accounting for 45% to 70% of all nontraumatic amputations.

The effects of dyslipidemia and hypertension are less impressive than diabetes and cigarette smoking. Relative risk for PAD is ~1.1 for each 10-mg/dL increase in total cholesterol, with similar increases for development of claudication. Abnormalities in components of the lipid profile, including elevated LDL, decreased HDL, and hypertriglyceridemia, are more common in patients with PAD than in control subjects but do not carry the same import as the first 2 risk factors. Probably the best evidence for the role...
of lipids is derived from the Program on Surgical Control of Hyperlipidemia (POSCH) trial, in which patients who were randomly assigned to ideal bypass to lower cholesterol levels were significantly less likely than control subjects to develop new intermittent claudication after 10 years.16

The effect of hypertension, which is large for risk of stroke, is more muted in the peripheral vasculature. Hypertension increased risk of PAD by 10% in 1 study, whereas it was demonstrated only in patients with severe but not moderate elevations in another.4,17 Decreases in prevalence of hypertension in the United States over a 9-year period were associated with decreased rates of lower-extremity arterial reconstruction and amputation.18

Platelet Activation and Thrombosis
Platelet activation and thrombosis have long been recognized as important components of atherosclerosis. Coronary thrombosis often occurs at sites of peripheral plaque rupture or erosion.19 Immediately after plaque rupture or erosion, subendothelial collagen, the lipid core, and procoagulants such as tissue factor and von Willebrand factor are exposed to circulating blood. Platelets rapidly adhere to the vessel wall through the platelet glycoproteins (GP) Ia/IIa and GP Ib/IX20 with subsequent aggregation to this initial monolayer through linkage with fibrinogen and the exposed GP Ib/IIa on activated platelets. Platelets are a rich source of NO, and deficiency of this nitric oxide produces a hypercoagulable state.21 Platelets activate the coagulopathy to a greater extent than is observed in healthy blood, consistent with increased thrombosis compared with healthy subjects. In healthy subjects, activation of eNOS causes vasodilation in both muscular conduit vessels and resistance arterioles. In contrast, in subjects with atherosclerosis, similar stimulation yields attenuated vasodilation in peripheral vessels and causes paradoxical vasoconstriction in coronary arteries, thus indicating a decrease in the bioavailability of NO.30,31 Interestingly, endothelial dysfunction can be demonstrated in patients with risk factors for atherosclerosis in the absence of atherosclerosis itself.32,33 These observations lend credence to the concept that endothelial dysfunction is integral to the development and progression of disease.

A decrease in the bioavailability of NO and an increase in production of vasoconstrictors such as angiotensin II would create an environment favorable for thrombosis and development of atherosclerosis.21 In addition to adversely affecting blood flow and nutrient delivery, another important aspect of endothelial dysfunction would likely be the increase in inflammation.2 Endothelial cells participate importantly in the

Endothelial Dysfunction
Initially regarded as an inert barrier between blood and blood vessel, the vascular endothelium is now recognized as an important center of vascular control. Indeed, since the semi-
recruitment, adhesion, and diapedesis of leukocytes into the vascular wall through production of chemokines and cytokines and intracellular transcription factors such as nuclear factor κB and activator protein-1. Indeed, these processes are central to formation of nascent atherosclerotic lesions. NO antagonizes each of these processes, whereas attenuation of its bioavailability creates an environment favorable for atherogenesis. Moreover, decreased NO increases the tendency for lesion progression by enhancing vascular smooth muscle proliferation and migration, augmenting platelet activation and thrombosis, possibly participating in intravascular neovascularization, and favoring adverse lipid modification. Once lesions have developed, endothelial dysfunction may exacerbate development of clinical events. Impaired endothelium may abnormally reduce vascular perfusion, produce factors that decrease plaque stability, and augment the thrombotic response to plaque rupture.

The clinical relevance of endothelial dysfunction has been borne out in recent studies; the presence of endothelial dysfunction predicts the presence of significant coronary artery disease and provides prognostic information about the likelihood of events in patients with coronary artery disease. Peripheral vascular endothelial dysfunction may also predict PAD, but evidence for this is limited.

**Inflammation**

A large body of experimental and clinical research studies shows clearly that inflammation plays a central role in atherosclerosis. Inflammation develops concurrently with accumulation of minimally oxidized LDL in the arterial wall. The endothelial cell expresses several adhesion molecules, including P- and E-selectins, intercellular adhesion molecule, and vascular cell adhesion molecule-1, which bind to circulating leukocytes. Transmigration of leukocytes into the arterial wall is mediated through chemoattractants such as monocyte chemotactic protein. This leads to accumulation of inflammatory macrophages and T-cells within the arterial wall. These activated leukocytes release proteolytic enzymes and a variety of peptide growth factors and cytokines that degrade matrix proteins and stimulate smooth muscle cells, endothelial cells, and macrophages. Foam cells aggregate as a result of macrophage accumulation of oxidized LDL. CD40 receptor and CD40 ligand are expressed on several inflammatory cells, including macrophages, B and T lymphocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts. It is thought that this system contributes to leukocyte adhesion, matrix degeneration, and cytokine-induced inflammation. Interruption of the CD40 signaling pathway reduces progression of atherosclerosis in experimental models.

The inflammatory process also can lead to plaque disruption and thrombosis. Vulnerable plaques are characterized by a large lipid core, a thin fibrous cap, and inflammatory cells at the thinnest portion of the cap surface. Several studies have shown that matrix metalloproteinases (MMPs) and other substances expressed by macrophages lead to degradation of the cap, resulting in an unstable plaque that is susceptible to rupture (Figure 2). Exposure of the underlying atheroma and tissue factor to circulating platelets and thrombin can then lead to formation of a thrombus. CD40 may also play a role in plaque rupture through effects on MMP production and thrombogenicity. Plaque rupture is believed to be a major process in the pathophysiology of acute coronary syndromes. Serum markers of inflammation such as high-sensitivity C-reactive protein are elevated in patients with acute coronary syndromes and PAD, and the level independently predicts subsequent events. Although there is evidence that this process occurs in PAD, its importance in clinical presentation or progression of disease is not known.

Multiple factors are responsible for initiation of this inflammatory response. Hemorheological factors also likely contribute to initiation of arterial disease. In addition, the role of oxidized lipoproteins within the arterial wall is well established. Other lipoproteins such HDL and VLDL may play an important role as well. Risk factors such as cigarette smoking, hypertension, diabetes, and obesity also contribute.

There has been considerable interest in infectious agents as stimuli for this inflammatory response.\textsuperscript{45} Pathological analysis has shown evidence of \textit{Chlamydia pneumoniae} within the plaque.\textsuperscript{54} Patients with atherosclerosis have increased titers of antibodies against chlamydia, \textit{Helicobacter pylori}, herpes simplex virus, and cytomegalovirus. Several ongoing clinical trials are evaluating the role of antibiotics in prevention of atherosclerosis.

**Oxidant Stress**

Excess generation of reactive oxygen species (ROS) represents an important pathological process in atherogenesis. Each component of the atherosclerotic blood vessel has been demonstrated to increase production of ROS, primarily superoxide anion ($\text{O}_2^-$).\textsuperscript{53} Important sources of ROS are vascular smooth muscle cells, endothelial cells, fibroblasts, and infiltrating leukocytes.\textsuperscript{55} Production of ROS affects gene transcription, damages DNA, and increases production of inflammatory transcription factors.\textsuperscript{56} The 2 best-characterized effects include oxidation of LDL and scavenging of endothelium-derived NO.

Although the mechanism of oxidative modification of LDL remains unknown, the importance of oxidation can be seen by the presence of oxidized LDL in atherosclerotic lesions. Experimentally, the amount of oxidized LDL, as measured by autoantibody titers, is reflective of the atherosclerotic burden.\textsuperscript{57} Oxidized LDL induces a series of atherogenic processes, including transcription of proatherogenic genes, production of matrix metalloproteinases and tissue factor, antagonism of endothelial cell production of NO, and promotion of vascular smooth muscle cell apoptosis.\textsuperscript{58} The augmented production of superoxide anion also rapidly reacts with NO to produce peroxynitrite, a potent oxidant.\textsuperscript{59} As described in the section on endothelial dysfunction, scavenging of NO increases inflammation, platelet activation, and vasoconstriction.

However, recent large trials of antioxidant vitamins, including the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI) Prevention Trial, the Heart Outcomes Prevention Evaluation Study (HOPE), and the Heart Protection Study (HPS), have not demonstrated any reduction in clinical events with antioxidant vitamin E therapy.\textsuperscript{50–53} The antioxidants used in these trials, however, have limitations that may have precluded an adequate test of the hypothesis.\textsuperscript{64} For example, the rate constant for reaction of vitamin E or C with superoxide anion is much slower than that for superoxide anion with NO or endogenous antioxidant enzymes.\textsuperscript{65} Oral intake only modestly increases plasma and tissue vitamin levels and is unlikely to affect events in the vascular wall, where it is not concentrated. Vitamin E is concentrated in the lipid bilayers and contributes little in the form of antioxidant protection in the cytoplasm, nucleus, or interstitial space. Finally, treatment may have been started too late to affect development of lesions while having little impact on plaque rupture and clinical events, although other therapeutic interventions showed significant benefit in some of the same trials (eg, HOPE and HPS).\textsuperscript{56,66} Equally important, conventional antiplatelet therapy has antioxidant effects by virtue of its ability to limit production of ROS by activated platelets. The importance of oxidative stress in the pathogenesis of atherosclerosis makes clear that the limitations of current therapies should not conclude therapeutic interest in this area but foster investigation into new avenues of treatment.

**Smooth Muscle Cell Proliferation**

The vascular smooth muscle cell (SMC) plays a central role in atherosclerosis. Once activated by injury, growth factor, or cytokine, the SMC undergoes a phenotypic change that leads to a migratory and secretory cell that migrates into the neointima.\textsuperscript{67} Through growth factor and cytokine stimulation, SMCs proliferate and secrete matrix proteins and enzymes. Although complex atherosclerotic lesions contain SMCs, lipid-laden macrophages, and lymphocytes, the vascular SMC is the dominant cellular component of de novo and in-stent restenotic lesions. Recent experimental and randomized clinical trials have shown that inhibitors of inflammation and of SMC proliferation such as sirolimus and paclitaxel can significantly retard in-stent stenosis when delivered to the site of intervention on drug-eluting stents.\textsuperscript{58,69}

The atherosclerotic plaque is composed of cellular elements and extracellular matrix. The matrix comprises $>$50% of the volume of the lesion. The constituents of the plaque’s extracellular matrix include glycosaminoglycans, proteoglycans, collagen, elastin, fibronectin, laminin, vitronectin, and thrombospondin. Activated SMCs are largely responsible for production of these matrix proteins.\textsuperscript{70} Collagen provides the structural support and scaffold for the vessel wall. In atherosclerosis, collagen production and degradation are both increased.\textsuperscript{71} Whereas SMCs produce collagen, activated SMCs and macrophages secrete MMPs that degrade collagen and elastin.\textsuperscript{72} MMP activity is tightly controlled by tissue inhibitors; in atherosclerosis, the net balance of collagen metabolism favors deposition and results in significant fibrosis. Less commonly, plaques have reduced collagen and elastin with a thin and weakened arterial wall, resulting in aneurysm formation.

During development of atherosclerosis, the entire vessel can enlarge or constrict in size. This is often referred to as geometric remodeling and is a critical process in determining luminal patency. Glagov et al\textsuperscript{73} described compensatory remodeling in humans as an outward displacement of the arterial wall that compensates for the enlarging atheroma. Once the plaque enlarges to $>$40% of the vessel area, the artery no longer enlarges, and the lumen narrows as the plaque grows. In addition, the vessel often constricts rather than dilates, which additionally narrows the lumen. This is common in restenosis and accounts for most of the late lumen loss seen after balloon angioplasty.\textsuperscript{74} These lesions are usually associated with more collagen deposition than those vessels that are dilated; however, the mechanism of vascular remodeling is poorly understood. Current evidence supports the importance of hemodynamic factors as well as abnormalities in matrix metabolism.\textsuperscript{75} All medium-sized arteries have been shown to remodel, including the carotid, iliac, femoral, and coronary.

The progression of atherosclerotic disease has been described as moving from an early lesion (phase 1) to a more...
advanced fibrolipid lesion (phase 2) (Figure 3). The formation of thrombus or hematoma can advance into an acute phase (phase 3 and 4) or even to total occlusion (phase 5). Although there is substantial evidence for this process in the coronary circulation, it is highly likely that it also occurs in PAD. MRI has allowed better characterization of lesions and has shown the importance of plaque composition to subsequent clinical events.77

Vasa Vasorum and Collaterals
The vasa vasorum may contribute to atherosclerosis as well. As plaques thicken, there is an increase in the vasa vasorum visible on histologic section. Whether this is a response to plaque growth or contributes to plaque growth is unknown. Damage to the vasa vasorum, however, has been implicated in plaque formation.79 Although development of vasa vasorum in plaques involves angiogenesis and is similar to collateral formation, it is not the same as observed in collateral vessel development.79 Some evidence suggests that disruption of the vasa vasorum is implicated in the genesis of aneurysms as well.

Development of collateral vessels is an integral, although variable, response to muscle ischemia produced by obstructive PAD.80 Collateral vessels provide a variable amount of flow but can be developed well enough (under certain circumstances) to minimize or eliminate ischemic symptoms. In PAD, distal collateral vessels are less effective than proximal collateral vessels in preventing an ischemic response to exercise. There seem to be marked regional differences in collateral vessel development as well. The mechanism of collateral vessel development involves the release of angiogenic factors such as vascular endothelial growth factor and fibroblast growth factor, but much of the process is poorly defined.

Genetic Factors
Genetic predisposition represents an important risk factor for atherosclerosis. In some studies, as much as 50% of the risk for atherosclerosis is attributable to genetic predisposition.81 It is clear, however, that this is a multifactorial disease, and as such it is likely that many genes contribute to susceptibility. Two major experimental approaches are being used to identify and understand the role of these genes. The first is use of powerful genomic and proteomic technologies to examine the expression, functions, and interactions of genes in models of the atherosclerotic disease process.82 These technologies have progressed to the point where it is possible to examine the behavior of all 30,000 to 40,000 human genes and many of the proteins they encode. Thus, they can be applied to cells, animal models, and human tissue specimens to examine the molecular profiles associated with disease initiation and progression. These approaches have already begun to elucidate the molecular architecture of the vessel wall and to describe some of the alterations that accompany development of vascular disease.83

The second major approach is to examine human populations for genetic variations that correlate with (and may determine) differences in rates of atherosclerotic disease.84 These techniques involve analysis of naturally occurring variations within genes (typically manifested as single-nucleotide polymorphisms) that may be associated with alterations in risk within populations of developing atherosclerosis. An example of this kind of study was published recently.85 This study analyzed naturally occurring alterations in the Toll-like-receptor 4, which mediates many of the effects of lipopolysaccharide signaling, a proinflammatory component of the bacterial wall, and is thus important in the innate immune system. The presence of a variation in this receptor has been demonstrated to be less sensitive to stimuli and thus predicted to be less inflammatory. It occurred more frequently in patients who had less progression of atherosclerosis, ie, the presence of the less inflammatory allele correlated with a delay in progression of atherosclerosis when assessed by several methods. Although the role of this receptor in the pathogenesis of the disease is unknown, this report demonstrates the usefulness of these types of genetic epidemiology studies.

Over the next several years, understanding of atherosclerosis as a molecular disease will increase. The challenge will be to use these findings to identify better diagnostic and therapeutic strategies for these syndromes.

Microcirculatory Disturbances
When a hemodynamically significant stenosis is present, distal pressure and flow are reduced. There is a poor correlation, however, between the pressure drop across the limb and ischemic symptoms and function, particularly in lower-extremity PAD.86–88 This is largely attributable to the complex pathophysiology of claudication. Abnormalities in endothelial function, microthrombi, changes in blood viscosity, white blood cell and platelet activation, and generation of ROS all contribute to the reduced regional flow and ischemia.89,90 In patients with claudication, muscle injury from ischemia-reperfusion during walking and rest causes partial denervation of limb muscles and alterations of muscle fiber distributions that can reduce muscle function additionally.90,91 Skeletal muscle metabolism is also altered with accumulation of acylcarnitines (markers of abnormal muscle metabolism).
and impaired electron transport.\textsuperscript{92,93} Accumulation of acylcarnitine predicts reduced skeletal muscle performance.\textsuperscript{92} Local ROS generation with elevation of plasma peroxides additionally impairs muscle function and can contribute to muscle damage.\textsuperscript{94}

**Regional Disease States**

The clinical response to ischemia associated with obstructive atherosclerosis is highly dependent on the regional circulation. For instance, in lower-extremity PAD, claudication and critical limb ischemia are the principal clinical manifestations, whereas in the carotid arteries, transient ischemic attacks and stroke are the principal clinical presentations.\textsuperscript{95,96} Obstructive disease in the renal circulation can result in hypertension or ischemic nephropathy or both. Emerging evidence suggests that pathophysiological alterations occurring during renal artery stenosis increase risk of cardiovascular events.\textsuperscript{97} Abnormalities in renal function can also accelerate atherosclerosis elsewhere, particularly in the coronary arteries. Disease of the aorta can lead to obstruction or aneurysm formation; the latter is associated with rupture when the aneurysm reaches a critical size.\textsuperscript{98}

In atherosclerotic carotid artery disease, the primary pathophysiological mechanism is an embolism rather than hypoperfusion. Because both carotid and both vertebral arteries all contribute to cerebral blood flow and are interconnected via the circle of Willis, hypoperfusion is rare. When the circle of Willis is complete, adequate brain perfusion can be achieved from any of the extracranial vessels, making hypoperfusion events rare. Transient ischemic attacks and strokes are likely a result of carotid artery or aortic arch atherosclerotic plaque rupture or erosion causing formation and embolism of platelet-fibrin thrombi. Antiplatelet therapy is recommended for both acute treatment and long-term prevention of stroke in individuals at risk. Several major randomized trials have demonstrated that carotid endarterectomy is beneficial in both symptomatic and asymptomatic patients with stenoses >60\%.\textsuperscript{99-101} The benefit of percutaneous intervention for carotid artery disease is undergoing active investigation.

In contrast to carotid artery disease, the clinical findings in renal artery stenosis, including renal insufficiency and hypotension, result in hypoperfusion.\textsuperscript{97} The secondary pathophysiological effects process includes elevation of renin levels, diminished peripheral and renal angiotensin II receptor response,\textsuperscript{102} increased sympathetic tone,\textsuperscript{103} and reduced baroreceptor sensitivity.\textsuperscript{104} Elevation of renal vein renin is characteristic of renovascular hypertension, but the negative predictive value is low because phase III renovascular hypertension is associated with low renin levels.\textsuperscript{97} Embolization from the aorta may also play a role in the pathophysiology of end-stage renal disease and hypertension, although evidence is scarce.

**Abdominal Aortic Aneurysm**

Abdominal aortic aneurysm (AAA) is strongly associated with local aortic wall and systemic atherosclerosis, but a clear causal relationship is not established.\textsuperscript{105} Etiologic factors include genetic susceptibility; 15\% to 20\% of patients have a family history. AAA is much more common (2 to 5 times) in men than in women and more common in white than black populations. Cigarette smoking is a major risk factor for AAA. The natural history is a gradual enlargement of the aneurysm until it exceeds 5.5 cm, when risk of rupture rises exponentially.\textsuperscript{106} The primary pathophysiological process is chronic transmural inflammation with destruction of the media, including loss of elastic fibers and SMCs.\textsuperscript{106} Elevation of inflammatory markers such as interleukin-6 and elevation of proteins such as MMP9 and elastin are correlated with AAA and the expansion rate.\textsuperscript{106-108} Doxycycline inhibits MMP activity and can reduce AAA in experimental animals.\textsuperscript{109} Clinical trials have shown a reduction in MMP9 levels with doxycycline but not a reduction in expansion rate in preliminary studies.\textsuperscript{110} Other agents such as roxithromycin have shown modest effects as well.\textsuperscript{111}

**Future Directions**

There is considerable need for additional investigation into the basic mechanisms of atherosclerosis in PAD. It is important to clarify the differential role of HDL cholesterol metabolism and other lipid disturbances as well as the biomechanical and rheologic factors in development and progression of disease in the noncoronary circulations. The role of matrix-degrading enzymes and their inhibitors in atherosclerosis and geometric remodeling needs additional study. Greater research is needed in understanding regional differences in plaque formation and clinical manifestations of disease. Genetic variability across individuals and populations merits additional exploration using genomics and proteomics. Pathophysiological responses to changes in metabolic demand such as exercise and factors that determine development of collateral vessels and angiogenesis need greater attention. In particular, the interaction between reduced oxygen and substrate delivery and skeletal muscle, neurological, and metabolic function needs additional study. The mechanisms by which diabetes mellitus accelerates atherosclerosis and affects regional circulations are of particular importance, given the disturbing increase in diabetes in the United States. One of the limitations in our understanding of the disease in the noncoronary circulations is a need for improved functional imaging and biomarkers of disease progression and unstable patterns of atherosclerosis to assist in understanding of regional disease pathophysiology. The lack of research into these areas and many more unique aspects of the pathophysiology of PAD are of great concern, given its rising prevalence and its morbidity and mortality.

**Recommendations**

- Increased funding for basic science and translational research on regional atherosclerosis through collaborative efforts between the American Heart Association (AHA) and other voluntary health organizations and the National Institutes of Health (NIH) is recommended.
- Development of targeted research programs through the AHA and the NIH to stimulate additional research. Development of vascular research and vascular medicine fellowship programs is needed. The AHA should work with the NIH to enhance funding and program development. For-
mation of a research consensus panel and an annual meeting on PAD would stimulate research and increase awareness of the disease.

- Fostering relationships with the pharmaceutical and medical device industries as well as imaging and bioengineering companies to promote basic and translational research is important.

Much remains to be done in this area, and the AHA and its partners should take a leadership role in the advancement of science in this area.

References


Key Words: AHA Conference Proceedings ■ atherosclerosis ■ peripheral vascular disease ■ vasculature